# 10 Curcumin — Biological and Medicinal Properties

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## 10.1 INTRODUCTION

The turmeric (*Curcuma longa*) plant, a perennial herb belonging to the ginger family, is cultivated extensively in south and southeast tropical Asia. The rhizome of this plant is also referred to as the "root" and is the most useful part of the plant for culinary and medicinal purposes. The most active component of turmeric is curcumin, which makes up 2 to 5% of the spice. The characteristic yellow color of turmeric is due to the curcuminoids.

Curcumin is an orange–yellow crystalline powder practically insoluble in water. The structure of curcumin ($C_{21}H_{20}O_6$) was first described in 1913 by Lampe and Milobedeska and shown to be diferuloylmethane (Aggarwal et al., 2003).

Turmeric is used as a dietary spice, coloring agent in foods and textiles, and a treatment for a wide variety of ailments (Figure 10.1). It is widely used in traditional Indian medicine to cure biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis. Turmeric paste in slaked lime is a popular home remedy for the treatment of inflammation and wounds. For centuries, curcumin has been consumed as a dietary spice at doses up to 100 mg/day. Extensive investigation over the last five decades has indicated that curcumin reduces blood cholesterol (Rao et al., 1970; Patil and Srinivasan, 1971; Keshavarz, 1976; Soudamini et al., 1992; Soni and Kuttan, 1992; Hussain and Chandrasekhara, 1992; Asai and Miyazawa, 2001) prevents LDL oxidation (Ramirez-Tortosa et al., 1999; Naidu and Thippeswamy, 2002; Patro et al., 2002), inhibits platelet aggregation (Srivastava et al., 1986,1995), suppresses thrombosis (Srivastava, 1985) and myocardial infarction (MI) (Dikshit et al., 1995; Nirmala and Puvanakrishnan, 1996a,b; Venkatesan, 1998), suppresses symptoms associated with type II diabetes (Srinivasan, 1972; Babu and Srinivasan, 1995,1997; Arun and Nalini, 2002), rheumatoid arthritis (Deodhar et al., 1980), multiple

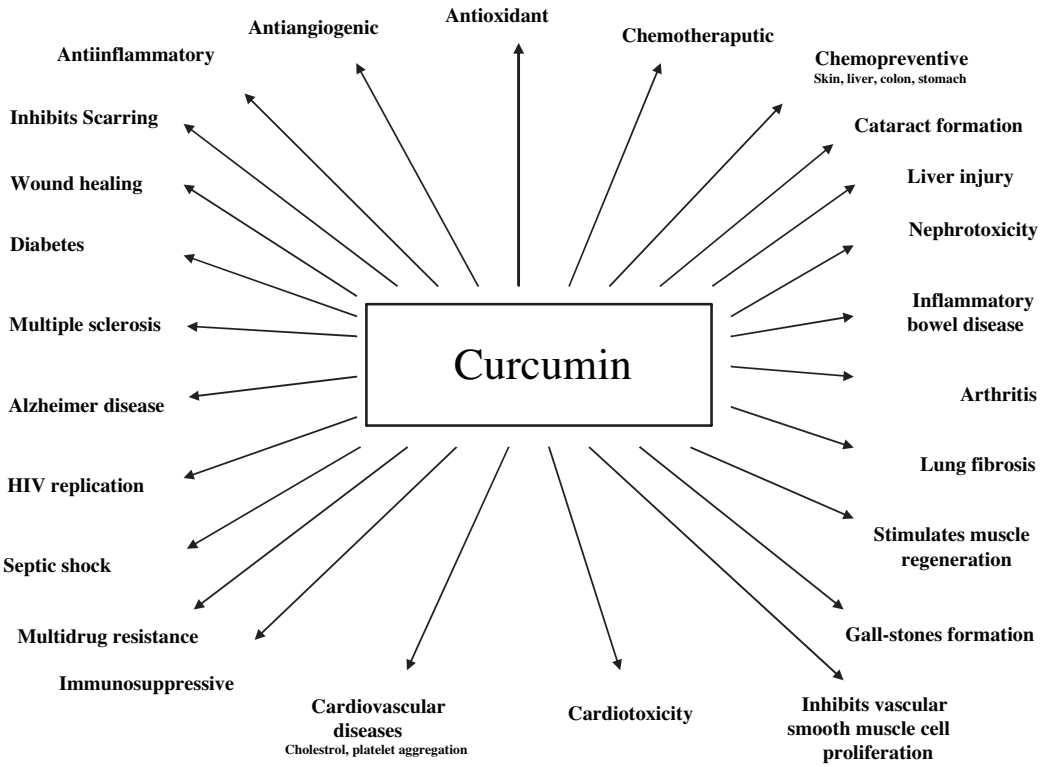


**FIGURE 10.1** Medicinal properties of curcumin.

sclerosis (MS) (Natarajan and Bright, 2002), and Alzheimer's disease (Lim et al., 2001; Frautschy et al., 2001), inhibits human immunodeficiency virus (HIV) replication (Sui et al., 1993; Li et al., 1993; Jordan and Drew, 1996; Mazumder et al., 1997; Barthelemy, 1998), enhances wound healing (Sindhu et al., 1998; Phan et al., 2001; Shahed et al., 2001), protects from liver injury (Morikawa et al., 2002), increases bile secretion (Ramprasad and Sirsi, 1956), protects from cataract formation (Awasthi et al., 1996), and protects from pulmonary toxicity and fibrosis (Venkatesan and Chandrakasan, 1995; Venkatesan et al., 1997; Venkatesan, 2000; Punithavathi et al., 2000), is an antileishmaniasis (Saleheen et al., 2002; Gomes Dde et al., 2002; Koide et al., 2002) and an antiatherosclerotic (Huang et al., 1992; Chen and Huang, 1998). Additionally, there is extensive literature that suggests that curcumin has potential in the prevention and treatment of a variety of other diseases (Figure 10.2).

## 10.2 CHEMICAL COMPOSITION OF TURMERIC

Curcumin was first isolated in 1815, obtained in crystalline form in 1870 (Vogel and Pelletier, 1818; Daube, 1870), and identified as 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E) or diferuloylmethane (Figure 10.3). The feruloylmethane skeleton of curcumin was subsequently confirmed in 1910 by the initial work and synthesis by Lampe (Lampe, 1910; Lampe and Milobedzka, 1913). Curcumin is a yellow-orange powder that is insoluble in water and ether but soluble in ethanol, dimethylsulfoxide, and acetone. Curcumin has a melting point of 183°C, molecular formula of $C_{21}H_{20}O_6$, and molecular weight of 368.37 g/mol.

Curcumin (also known as curcumin I) occurs naturally in the rhizome of *Curcuma longa*, which is grown commercially and sold as turmeric, a yellow-orange dye. Turmeric contains curcumin along with other chemical constituents known as the "curcuminoids" (Srinivasan, 1952). The major

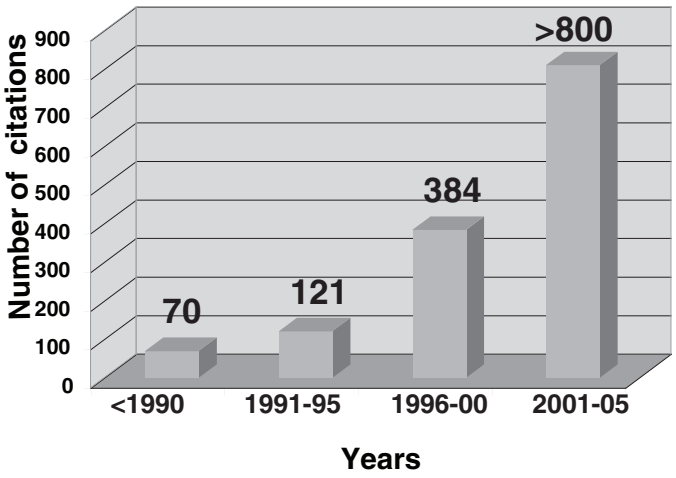


**FIGURE 10.2** Pubmed citations on curcumin.

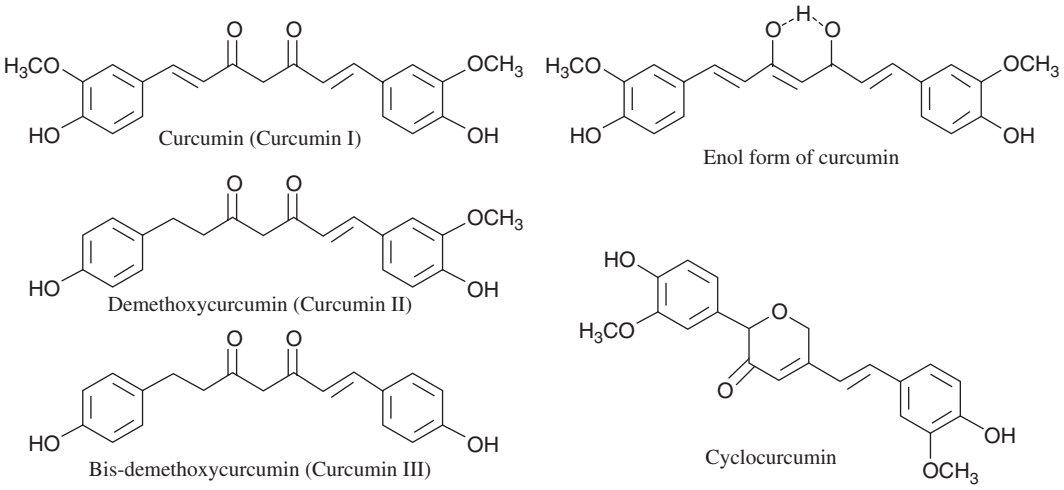


**FIGURE 10.3** Structure of curcumin.

curcuminoids present in turmeric are demethoxycurcumin (curcumin II), bisdemethoxycurcumin (curcumin III), and the recently identified cyclocurcumin (Kiuchi et al., 1993). Commercial curcumin contains curcumin I (~77%), curcumin II (~17%), and curcumin III (~3%) as its major components. The curcuminoid complex is also referred to as Indian saffron, yellow ginger, yellow root, *kacha haldi*, ukon, or natural yellow 3.

Spectrophotometrically, curcumin has a maximum absorption ($\lambda_{max}$) in methanol at 430 nm, with a Beer's law range from 0.5 to 5 µg/mL (Prasad, 1997). It absorbs maximally at 415 to 420 nm in acetone and a 1% solution of curcumin has 1650 absorbance units. Curcumin has a brilliant yellow hue at pH 2.5 to 7 and takes on a red hue at pH > 7. The spectral and photochemical properties of curcumin have been studied in different solvents by Chignell and coworkers (Chignell et al., 1994). In toluene, the absorption spectrum of curcumin contains some structure, which disappears in more polar solvents such as ethanol and acetonitrile. The fluorescence of curcumin occurs as a broad band in acetonitrile ($\lambda_{max}$ = 524 nm), ethanol ($\lambda_{max}$ = 549 nm), or micellar solution ($\lambda_{max}$ = 557 nm), but has some structure in toluene ($\lambda_{max}$ = 460, 488 nm) (Chignell et al., 1994). These workers also showed that the fluorescence quantum yield of curcumin is low in sodium

dodecyl sulfate solution (phi = 0.011) but higher in acetonitrile (phi = 0.104) (Chignell et al., 1994). In addition, curcumin was observed to produce singlet oxygen upon irradiation ($\lambda_{max}$ > 400 nm) in toluene or acetonitrile (phi = 0.11 for 50 μM curcumin). Curcumin quenched singlet oxygen in acetonitrile (kq = $7 \times 10^6$/M-s). Singlet oxygen production was about ten times lower in alcohols and was hardly detectable when curcumin was solubilized in an aqueous micellar solution of Triton X-100. However, in sodium dodecyl sulfate solution, no singlet oxygen phosphorescence could be observed for those micelles containing curcumin. Curcumin is also reported to be able to photogenerate superoxide in toluene and ethanol (Chignell et al., 1994).

The interactions between curcumin and biological radical stressors have been studied. For example, Iwunze and coworkers (Iwunze and McEwan, 2004) recently used both fluorescence and absorptiometric techniques to study the interaction between curcumin and peroxynitrite. Using both techniques, these workers observed that signals increased asymptotically until the concentration of peroxynitrite equaled that of the curcumin (held at a constant concentration of $1 \times 10^{-5}$ M). However, there was a shift in fluorescence wavelength after the initial oxidation of the hydroxyl group, which was attributed to the nitration of the phenoxyl group of curcumin. A second-order reaction rate for the nitration of curcumin by peroxynitrite was concluded with an association constant of $1.2 \times 10^6$/M-s and $3.6 \times 10^6$/M-s for the fluorescence and absorptiometric techniques, respectively (Iwunze and McEwan, 2004). In another report, Mishra et al. studied the reactions of superoxide–crown ether complex with curcumin (Mishra et al., 2004). Optical absorption spectra showed that on reaction with superoxide, curcumin forms a blue-colored intermediate (λmax = 560 nm), which subsequently decayed with the development of the absorption band corresponding to the parent curcumin. A 100% regeneration was observed at low superoxide concentrations (1:1–1:3, curcumin:superoxide) and a 60% regeneration at high superoxide concentration (>1:5, curcumin:superoxide). These researchers also determined the rate constant for the reaction of superoxide with curcumin. Based on their studies, the authors concluded that at low superoxide concentrations, curcumin effectively causes superoxide dismutation without itself undergoing any chemical change, but at higher concentrations of superoxide, curcumin inhibits superoxide activity by reacting with it. Toniolo and coworkers also investigated the action of curcumin on superoxide ions (Toniolo et al., 2002). They found that 1 mol of curcumin reacted with 6 mol of anion radical, which provides the perhydroxyl radical and further disproportionates to the anionic form of hydrogen peroxide and oxygen.

The free radical-scavenging mechanism of curcumin has been examined by Ohara and coworkers (Ohara et al., 2005). The second-order rate constants for the radical-scavenging reactions of curcumin and half-curcumin were measured by a stopped-flow spectrophotometer in several organic solvents (methanol, ethanol, acetonitrile, chloroform, and benzene) and in aqueous Triton X-100 (5.0%) micelle solutions at various pH values. The difference in the rate constants and solvent dependence between curcumin and half-curcumin suggested that the enol structure with the intramolecular hydrogen bond of curcumin (Figure 10.3) strongly enhances its radical-scavenging activity. Also, notable pH dependences were observed for the rate constants of both curcumin and half-curcumin in micelle solutions, suggesting that the acid–base dissociation equilibrium of phenol-protons in curcumin and half-curcumin affected their radical-scavenging activities (Ohara et al., 2005).

Priyadarsini and coworkers conducted studies to evaluate the relative importance of the phenolic hydrogens and the $-CH_2$ hydrogens on the antioxidant activity and free radical reactions of curcumin and dimethoxycurcumin (Priyadarsini et al., 2003). They showed that at equal concentrations, the efficiency to inhibit lipid peroxidation (LPO) changed from 82% with curcumin to 24% with dimethoxycurcumin. The kinetics of reaction of 2,2′-diphenyl-1-picrylhydrazyl (DPPH), a stable hydrogen-abstracting free radical, was tested with these two compounds using spectrophotometry. The authors concluded that although the energetics to remove hydrogens from both the phenolic hydrogens and the $-CH_2$ group of the diketo structure were very close, the phenolic hydrogen is essential for both antioxidant activity and free radical kinetics. This was further confirmed by density functional theory (DFT) calculations where it was shown that the phenolic hydrogen is more labile for abstraction, compared to the $-CH_2$ hydrogens in curcumin. Therefore, based on

both experimental and theoretical results, this report showed that the phenolic hydrogens play a major role in the antioxidant activity of curcumin.

The photophysical properties of curcumin have been investigated by Khopde et al. (2000), wherein a variety of spectroscopic techniques were used to investigate the photophysical properties of curcumin in different organic solvents and in Triton X-100 aqueous micellar media. The steady-state absorption and fluorescence characteristics of curcumin were found to be sensitive to the solvent characteristics. Curcumin was also found to be a weakly fluorescent molecule and its fluorescence decay properties in most of the solvents could be fitted well to a double-exponential decay function. The shorter component (lifetime in the range 50–350 ps) could be assigned to its enol form (Figure 10.3), whereas the longer component (lifetime in the range 500–1180 ps) was assigned to the diketo form of curcumin (Figure 10.3). These authors also conducted nuclear magnetic resonance (NMR) experiments in CDCl3 and dimethylsulfoxide-D6 and showed that the enol form of curcumin is present in the solution by more than about 95% in these solvents.

The stability of curcumin in aqueous media has been investigated by Bernabe-Pineda et al. (2004). They showed that the stability of curcumin was improved at high pH values (>11.7), fitting a model describable by a pseudo–zero-order rate equation with a rate constant k′ for the disappearance of the curcumin species of $1.39 \times 10^{-9}$/Mmin$^{-1}$. Three acidity constants (pKA) were measured for curcumin, as follows, pKA1 = 8.38 ± 0.04, pKA2 = 9.88 ± 0.02 and pKA3 = 10.51 ±0.01. Formation of quinoid structures played an important role in the tautomeric forms of curcumin in aqueous media, which made the experimental values differ from the theoretically calculated ones, depending on the conditions adopted in this study (Bernabe-Pineda et al., 2004). In a separate report, Souza and coworkers (Souza et al., 1997) also studied the influence of water activity on the stability of curcuminoid pigments in curcumin- and turmeric oleoresin–microcrystalline–cellulose model systems during storage at 21 ± 1°C. Samples were analyzed spectrophotometrically for curcuminoid pigments at specific time intervals and the degradation of the curcuminoids were observed to follow first-order reactions. Although the curcuminoid pigments were sensitive to light, the combined effects of air and light were the most deleterious (Souza et al., 1997). The authors did not observe any influence of water activity on the stability of curcuminoid pigments in the curcumin– and turmeric oleoresin–microcrystalline cellulose model systems. Tonnesen and coworkers (Tonnesen et al., 1986) also investigated the photodecomposition of curcumin on exposure to ultraviolet (UV)/visible radiation and identified the major degradation products. They also examined the photobiological activity of curcumin using bacterial indicator systems (Tonnesen et al., 1987). On irradiation with visible light, curcumin, at low concentrations, was phototoxic for *Salmonella typhimurium* and *Escherichia coli*. The authors concluded that the observed phototoxicity makes curcumin a potential photosensitizing drug that might find application in the phototherapy of psoriasis, cancer, and bacterial and viral diseases (Tonnesen et al., 1987). The same group also prepared cyclodextrin complexes of curcumin to improve the water solubility and the hydrolytic and photochemical stability of the compound (Tonnesen et al., 2002). Complex formation resulted in an increase in water solubility at pH 5 by a factor of at least $10^4$. The hydrolytic stability of curcumin under alkaline conditions was strongly improved by complex formation, while the photodecomposition rate was increased compared to a curcumin solution in organic solvents. The cavity size and the charge and bulkiness of the cyclodextrin side chains influenced the stability constant for complexation and the degradation rate of the curcumin molecule (Tonnesen et al., 2002). Wang et al. examined the degradation kinetics of curcumin under various pH conditions and the stability of curcumin in physiological matrices (Wang et al., 1997). When curcumin was incubated in 0.1 M phosphate buffer and serum-free medium at pH 7.2 at 37°C, about 90% decomposed within 30 min. The authors also tested curcumin stability from pH 3 to 10 and showed that the decomposition of curcumin was pH dependent and occurred faster at neutral to basic conditions. Curcumin was more stable in cell culture medium containing 10% fetal calf serum (FCS) and in human blood; less than 20% of curcumin decomposed within 1 h, and after incubation for 8 h, about 50% of curcumin still remained. *Trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal was pre-

dicted as the major degradation product and vanillin, ferulic acid, and feruloyl methane were identified as minor degradation products of curcumin (Wang et al., 1997).

The electrochemical behavior of curcumin has been recently investigated by Wu and coworkers (Wu et al., 2005). In 0.1 mol/L phosphate buffer solution at pH 3, the voltametry behaviors of curcumin at a glassy carbon electrode were studied. The adsorptive potential of curcumin was reported as +0.8V, while its peak potential was +0.386V (Wu et al., 2005).

## 10.3 ANTIOXIDANT PROPERTIES OF CURCUMIN

Sharma (1976), Ruby et al. (1995), and Sugiyama et al. (1996) studied the antioxidative properties of curcumin and its three derivatives (demethoxy curcumin, bisdemethoxy curcumin, and diacetyl curcumin). The authors demonstrated that these substances provide a protection of hemoglobin from oxidation at a concentration as low as 0.08 mM, except the diacetyl curcumin, which has little effect in the inhibition of nitrite-induced oxidation of hemoglobin. The effect of curcumin on LPO has also been studied in various models by several authors. Curcumin is a good antioxidant and inhibits LPO in rat liver microsomes, erythrocyte membranes, and brain homogenates. The LPO has a main role in the inflammation, in heart diseases, and in cancer.

The antioxidant activity of curcumin could be mediated through antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Curcumin has been shown to serve as a Michael acceptor, reacting with glutathione and thioredoxin (Adams et al., 2005). Reaction of curcumin with these agents reduces intracellular GSH in the cells. The suppression of LPO by curcumin could lead to the suppression of inflammation. In fact, curcumin has been found to be at least ten times more active as an antioxidant than even vitamin E (Khopde et al., 1999). In curcumin, the phenolic and the methoxy group on the phenyl ring and the 1,3-diketone system seem to be important structural features that can contribute to these effects. Another fact proposed in the literature is that the antioxidant activity increases when the phenolic group with a methoxy group is at the ortho position (Motterlini et al., 2000)

## 10.4 MOLECULAR TARGETS OF CURCUMIN

Various studies have shown that curcumin modulates numerous targets (Figure 10.4, Table 10.1, and Table 10.2). These include the growth factors, growth factor receptors, transcription factors, cytokines, enzymes, and genes regulating apoptosis.

### 10.4.1 Curcumin Downregulates the Activity of EGFR and Expression of Her2/Neu

HER2/neu and epithelial growth factor receptor (EGFR) activity represent one possible mechanism by which curcumin suppresses the growth of breast cancer cells. Almost 30% of the breast cancer cases have been shown to overexpress the HER2/neu protooncogene (Slamon et al., 1987), and both HER2 and EGF receptors stimulate proliferation of breast cancer cells. Overexpression of these two proteins correlates with progression of human breast cancer and poor patient prognosis (Slamon et al., 1987). Curcumin has been shown to downregulate the activity of EGFR and HER2/neu (Korutla and Kumar, 1994; Korutla et al., 1995) and to deplete the cells of HER2/neu protein (Hong et al., 1999). Additionally, we have recently found that curcumin can downregulate bcl-2 expression, which may contribute to its antiproliferative activity (Mukhopadhyay et al., 2001).

Like geldanamycin, curcumin has been shown to provoke the intracellular degradation of HER2 (Tikhomirov and Carpenter, 2003). HER2 mutations, however, limit the capacity of geldanamycin to disrupt the tyrosine kinase activity of HER2. Thus, these HER2 mutants are resistant to geldanamycin-induced degradation, but they maintain their sensitivity to curcumin through ErbB-2 degradation.

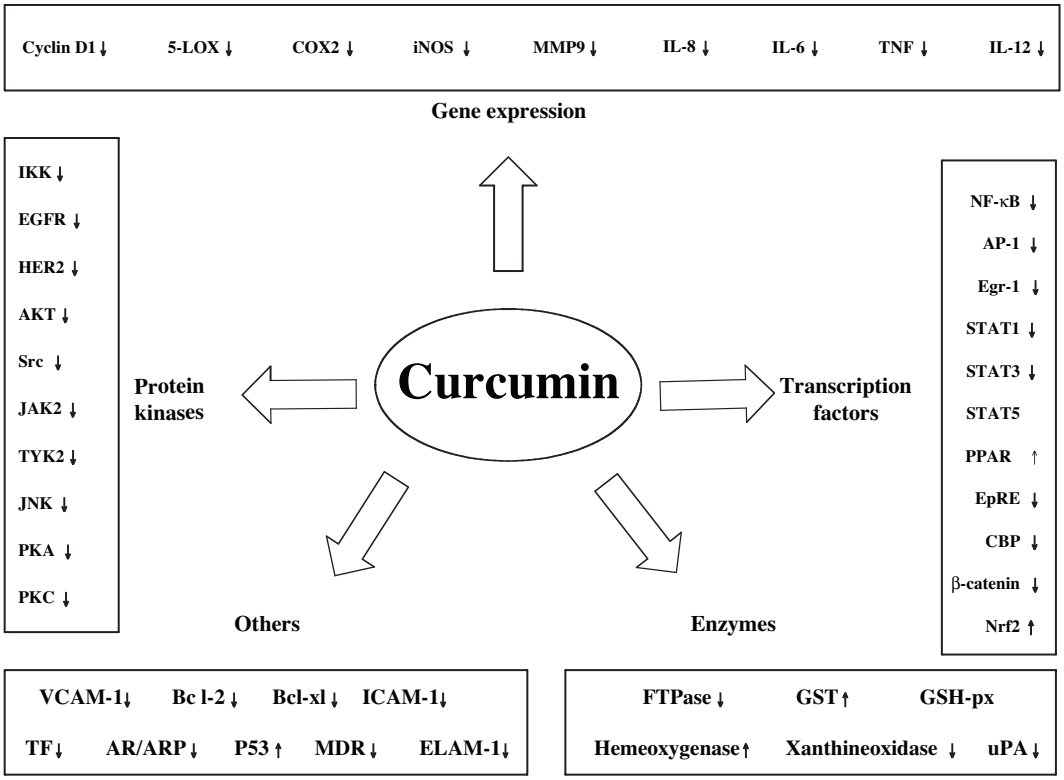


**FIGURE 10.4** Molecular targets of curcumin.

### 10.4.2 Curcumin Downregulates the Activation of NF-κB

Curcumin may also operate through the suppression of nuclear factor-κB (NF-κB) activation. NF-κB is a nuclear transcription factor required for the expression of genes involved in cell proliferation, cell invasion, metastasis, angiogenesis, and resistance to chemotherapy (Baldwin, 2001). This factor is activated in response to inflammatory stimuli, carcinogens, tumor promoters, and hypoxia, which is frequently encountered in tumor tissues (Pahl, 1999). Several groups, including ours, have shown that activated NF-κB suppresses apoptosis in a wide variety of tumor cells (Wang et al., 1996; Lee et al., 1995; Giri and Aggarwal, 1998), and it has been implicated in chemoresistance (Wang et al., 1996). Furthermore, the constitutively active form of NF-κB has been reported in human breast cancer cell lines in culture (Nakshatri et al., 1997), carcinogen-induced mouse mammary tumors (Kim et al., 2000), and biopsies from patients with breast cancer (Sovak et al., 1997). Our laboratory has shown that various tumor promoters, including phorbol ester, tumor necrosis factor (TNF), and $H_2O_2$, activate NF-κB and that curcumin downregulates the activation (Singh and Aggarwal, 1995). Subsequently, others showed that curcumin-induced downregulation of NF-κB is mediated through suppression of IκBα kinase activation (Jobin et al., 1999; Plummer et al., 1999). Recently, we have shown that curcumin downregulated cigarette smoke-induced NF-κB activation through inhibition of IκBα kinase in human lung epithelial cells (Shishodia et al., 2003). We also found that curcumin suppresses the constitutively active NF-κB activation in mantle cell lymphoma (Shishodia et al., 2005). This led to the downregulation of cyclin D1, COX-2, and matrix metalloproteinase (MMP)-9 by curcumin. Philip and Kundu (2003) have recently reported that curcumin downregulates osteopontin (OPN)-induced NF-κB–mediated promatrix metalloproteinase-2 activation through IκBα/IKK signaling (Philip and Kundu, 2003). Zheng et al. demonstrated that curcumin arrested cell growth at the G(2)/M phase and induced apoptosis in human

**TABLE 10.1**
**Effect of Curcumin on Different Cell Signaling Pathways**

| | Refs. |
|---|---|
| **Inhibition of NF-κB Signaling Pathway** | |
| Suppresses the activation of transcription factor NF-κB | Singh and Agarwal, 1995 |
| Inhibits IL-1α and TNF-induced NF-κB | Xu et al., 1997 |
| Inhibits TPA-induced activation of NF-κB | Surh et al., 2000; Han et al., 2002 |
| Inhibits anticancer drug-induced activation of NF-κB | Chuang et al., 2002 |
| Inhibits TNF production and release | Chan, 1995; Jang et al., 2001 |
| Inhibits inflammatory cytokine production by peripheral blood monocytes and alveolar macrophages | Abe et al., 1999 |
| Regulation of proinflammatory cytokine expression | Literat et al., 2001 |
| Blocks NF-κB activation and proinflammatory gene expression by inhibiting IκB kinase activity | Jobin et al., 1999 |
| Downregulates chemokine expression and release | Xu et al., 1997; Cipriani et al., 2001; Hidaka et al., 2002 |
| Inhibit the angiogenic response stimulated by FGF-2, including expression of MMP-9 | Mohan et al., 2000; Lin et al., 1998 |
| Inhibits IL-1–stimulated NF-κB and downregulates MMP gene expression | Liacini et al., 2002; Onodera et al., 2002 |
| Inhibits TNF-mediated cell surface expression of adhesion molecules and of NF-κB activation | Kumar et al., 1998; Gupta & Ghosh, 1999 |
| Reduces endothelial tissue factor gene expression | Bierhaus et al., 1997 |
| Inhibits COX-2 transcription and expression | Plummer et al., 1999; Zhang et al., 1999; Goel et al., 2001; Surh et al., 2001 |
| Inhibits NOS expression and nitrite production | Pan et al., 2000; Surh et al., 2001; Chan et al., 1998; Brouet and Ohshima, 1995; Chan et al., 1995; Onoda and Inano, 2000 |
| Induces p21 (WAF1/CIP1) and C/EBPβ expression | Hour et al., 2002 |
| **Inhibition of AP-1 Signaling Pathway** | |
| Suppresses PMA-induced c-Jun/AP-1 activation | Han et al., 2002 |
| Inhibits TNF-induced expression of monocyte chemoattractant JE via fos and jun genes | Hanazawa et al., 1993 |
| Inhibits TPA-induced expression of c-fos, c-jun, and c-myc proto-oncogenes mRNAs | Kakar and Roy, 1994 |
| Inhibits TPA- and UV-B light-induced expression of c-Jun and c-Fos | Lu et al., 1994 |
| Reduces endothelial tissue factor gene expression | Bierhaus et al., 1997 |
| Inhibits IL1α and TNF-induced AP-1 | Xu et al., 1997 |
| Inhibits TPA-induced activation of AP-1 | Surh et al., 2000 |
| Inhibits thrombin-induced, AP-1–mediated, plasminogen activator inhibitor 1 expression | Chen et al., 2000 |
| Inhibits release of MIP-1α, MIP-1β, and RANTES, and AP-1 | Cipriani et al., 2001 |
| Inhibits IL-1–stimulated AP-1 and downregulates MMP gene expression | Liacini et al., 2002 |
| Suppresses transcription factor Egr-1 | Pendurthi and Rao, 2000 |
| Downregulates transactivation and gene expression of androgen receptors | Nakamura et al., 2002 |
| **Inhibition of MAPK Pathway** | |
| Inhibits JNK signaling pathway | Chen and Huang, 1998 |

*Continued*

**TABLE 10.1 *(Continued)***
**Effect of Curcumin on Different Cell Signaling Pathways**

| | Refs. |
|---|---|
| Inhibits IL-1-stimulated MAP kinases and downregulates MMP gene expression | Liacini et al., 2002 |
| **Inhibition of Growth Factor Pathway** | |
| Inhibits EGF receptor kinase activity | Korutla and Kumar, 1994 |
| Inhibits ligand-induced activation of EGF receptor tyrosine phosphorylation | Korutla et al., 1995 |
| Inhibits PTK activity of p185neu and also depletes p185neu | Hong et al., 1999 |
| Inhibits PTK activity of EGF receptor and depletes the protein | Dorai et al., 2000 |
| Inhibition of serine protein kinase pathway | |
| Inhibits protein kinase C activity induced by PMA | Liu et al., 1993 |
| Inhibits phosphorylase kinase | Reddy and Aggarwal, 1994 |
| Inhibits cyclic AMP-dependent protein kinase | Hasmeda and Polya, 1996 |
| **Others** | |
| Inhibits LOX and COX activities | Huang et al., 1991; Ramsewak et al., 2000; Skrzypczak-Jankun et al., 2000 |
| Induces GST activity | Susan and Rao, 1992; Oetari et al., 1996; Awasthi et al., 2000 |
| Inhibits HIV-1 and HIV-2 proteases | Sui et al., 1993 |
| Inhibits PMA-induced xanthine dehydrogenase/oxidase | Lin et al., 1994 |
| Modulates brain $Na^+/K^+$ ATPase activity | Kaul and Krishnakanth, 1994 |
| Modulates cytochrome P450 activity | Oetari et al., 1996; Thapliyal et al., 2001; Ciolino et al., 1998 |
| Inhibits the $Ca^{2+}$-ATPase of sarcoplasmic reticulum | Logan-Smith et al., 2001; Logan-Smith et al., 2002 |
| Increases the rate of accumulation of $Ca^{2+}$ | Logan-Smith et al., 2001 |
| Inhibits SERCA $Ca^{2+}$ pumps | Bilmen et al., 2001; Sumbilla et al., 2002 |
| Inhibits mammalian phospholipase D activity | Yamamoto et al., 1997 |
| Inhibits of IL-12 production in LPS-activated macrophages | Kang et al., 1999; Kang et al., 1999 |
| Blocks TGF-β1–induced uPA expression | Santibanez et al., 2000 |
| Induces cell migration in nontumorigenic murine colon epithelial cells through MT-MMP expression | Fenton et al., 2002 |
| Inhibits heme oxygenase-1 | Motterlini et al., 2000; Scapagnini et al., 2002 |
| Modulates aryl hydrocarbon receptor | Ciolino et al., 1998 |
| Modulates *P*-glycoprotein in primary cultures of rat hepatocytes | Romiti et al., 1998 |
| Intercalates in DNA and poison Topo II isomerase | Snyder and Arnone, 2002 |
| Stimulates the stress-induced expression of stress proteins | Kato et al., 1998 |

*Abbreviations*: AP-1, activating protein-1; NF-κB, nuclear-factor kappa B, LPS, lipopolysaccharide; IL, interleukin; MMP, matrix metalloproteinase; EGF, epidermal growth factor; PTK, protein tyrosine kinase; COX, cyclooxygenase; LOX, lipoxygenase; GST, glutathione S-transferase; NOS, nitric oxide synthase; TNF, tumor necrosis factor; ATPase, adenosine triphosphatase; FGF, fibroblast growth factor; JNK, c-Jun N-terminal kinase.

**TABLE 10.2**
**Proteins/Enzymes That Physically Interact with Curcumin**

| Protein/Enzyme | $IC_{50}$ | Refs. |
|---|---|---|
| Xanthine oxidase | — | Lin and Shih, 1994 |
| Lipooxygenase | — | Skrzypczak-Jankun et al., 2000 |
| Cyclooxygenase-2 | — | Ramsewak et al., 2000 |
| IκBα kinase | — | Bharti et al., 2003 |
| *P*-Glycoprotein | — | Anuchapreeda et al., 2002; Romiti et al., 1998 |
| Glutathione *S*-transferase | 1.79–2.29 μM | Oetari et al., 1996 |
| Protein kinase A | — | Reddy and Aggarwal, 1994 |
| Protein kinase C | — | Reddy and Aggarwal, 1994 |
| Protamine kinase | — | Reddy and Aggarwal, 1994 |
| Phosphorylase kinase | — | Reddy and Aggarwal, 1994 |
| Autophosphorylation-activated protein kinase | — | Reddy and Aggarwal, 1994 |
| pp60c-src tyrosine kinase | — | Reddy and Aggarwal, 1994 |
| $Ca^{2+}$dependent protein kinase | 41 μM | Hasmeda and Polya, 1996 |
| $Ca^{2+}$-ATPase of sarcoplasmic reticulum | — | Logan-Smith et al., 2001 |
| Aryl hydrocarbon receptor | — | Ciolino et al, 1998 |
| Rat liver cytochrome P450s | — | Oetari et al., 1996 |
| Topo II isomerase | — | Snyder and Arnone, 2002 |
| Inositol 1,4,5-triphosphate receptor | 10 μM | Dyer et al., 2002 |
| Glutathione | — | Awasthi et al., 2000 |

melanoma cells by inhibiting NF-κB activation and thus depletion of endogenous nitric oxide (Zheng et al., 2004). Kim et al. recently reported that curcumin inhibited lipopolysaccharide (LPS)-induced mitogen-activated protein kinase (MAPK) activation and the translocation of NF-κB p65 in dendritic cells (Kim et al., 2005).

### 10.4.3 Curcumin Downregulates the Activation of STAT3 Pathway

Numerous reports suggest that interleukin-6 (IL-6) promotes survival and proliferation of various tumors, including multiple myeloma (MM) cells, through the phosphorylation of a cell-signaling protein, signal transducers, and activators of transcription (STAT)-3. Thus, agents that suppress STAT3 phosphorylation have the potential for the treatment of MM (Bharti et al., 2003). Bharti et al. demonstrated that curcumin inhibited IL-6–induced STAT3 phosphorylation and consequent STAT3 nuclear translocation. Curcumin had no effect on STAT5 phosphorylation but inhibited the interferon (IFN)-α–induced STAT1 phosphorylation. The constitutive phosphorylation of STAT3 found in certain MM cells was also abrogated by treatment with curcumin. Curcumin-induced inhibition of STAT3 phosphorylation was reversible. Compared with AG490, a well-characterized Janus kinase (JAK)-2 inhibitor, curcumin was a more rapid (30 min vs. 8 h) and more potent (10 μM vs. 100 μM) inhibitor of STAT3 phosphorylation. Similarly, the dose of curcumin that completely suppressed proliferation of MM cells, AG490 had no effect. In contrast, STAT3-inhibitory peptide that can inhibit the STAT3 phosphorylation mediated by Src blocked the constitutive phosphorylation of STAT3 and also suppressed the growth of myeloma cells. TNF-α and lymphotoxin (LT) also induced the proliferation of MM cells, but through a mechanism independent of STAT3 phosphorylation. In addition, dexamethasone-resistant MM cells were found to be sensitive to curcumin. Overall, these results demonstrated that curcumin was a potent inhibitor of STAT3 phosphorylation and this plays a role in curcumin's suppression of proliferation of MM.

Kim et al. (2003) investigated the inhibitory action of curcumin on JAK-STAT signaling in the brain. Curcumin markedly inhibited the phosphorylation of STAT1 and 3 as well as JAK1 and 2

in rat primary microglia activated with gangliosides, LPS, or IFN-γ (Kim et al., 2003). Li et al. (2001) showed that curcumin suppressed oncostatin-M-stimulated STAT1 phosphorylation, Deoxy ribonucleic acid (DNA)-binding activity of STAT1, and c-Jun N-terminal kinase activation without affecting JAK1, JAK2, JAK3, ERK1/2, and p38 phosphorylation. Curcumin also inhibited OSM - induced MMP-1, MMP-3, MMP-13, and TIMP-3 gene expression.

Natarajan et al (2002) showed that treatment of activated T-cells with curcumin inhibited IL-12–induced tyrosine phosphorylation of JAK2, tyrosine kinase 2, and STAT3 and STAT4 transcription factors. The inhibition of the JAK–STAT pathway by curcumin resulted in a decrease in IL-12–induced T-cell proliferation and Th1 differentiation.

### 10.4.4 Curcumin Activate Peroxisome Proliferator–Activated Receptor-γ (PPARγ)

Activation of PPAR-γ inhibits the proliferation of nonadipocytes. The level of PPAR-γ is dramatically diminished along with activation of hepatic stellate cells (HSC). Xu et al. (2003) demonstrated that curcumin dramatically induced the gene expression of PPAR-γ and activated PPAR-γ in activated HSC. Blocking its *trans*-activating activity by a PPAR-γ antagonist markedly decreased the effects of curcumin on the inhibition of cell proliferation. Zheng et al. (2004) reported that curcumin stimulated PPAR γ activity in activated HSC *in vitro*, which was required for curcumin to reduce cell proliferation, induce apoptosis, and suppress extracellular matrix gene expression. Chen and Xu (2005) recently reported that curcumin activation of PPAR γ inhibited Moser cell (human colon cancer cell line) growth and mediated the suppression of the gene expression of cyclin D1 and EGFR.

### 10.4.5 Curcumin Downregulates the Activation of AP-1 and JNK

Activated protein-1 (AP-1) is another transcription factor that has been closely linked with proliferation and transformation of tumor cells (Karin et al., 1997). The activation of AP-1 requires the phosphorylation of c-jun through activation of stress-activated kinase JNK (Xia et al., 2000). The activation of JNK is also involved in cellular transformation (Huang et al., 1999). Curcumin has been shown to inhibit the activation of AP-1 induced by tumor promoters (Huang et al., 1991) and JNK activation induced by carcinogens (Chen and Tan, 1998). Bierhaus et al. (1997) demonstrated that curcumin caused inhibition of AP-1 due to its direct interaction with AP-1DNA binding motif (Bierhaus et al., 1997). Prusty and Das (2005) recently reported that curcumin downregulated AP-1–binding activity in tumorigenic HeLa cells.

Dickinson et al. (2003) have demonstrated that the beneficial effects elicited by curcumin appear to be due to changes in the pool of transcription factors that compose EpRE and AP-1 complexes, affecting gene expression of glutamate-cysteine ligase and other phase II enzymes (Dickinson et al., 2003). Squires et al have demonstrated that curcumin suppresses the proliferation of tumor cells through inhibition of Akt/PKB (protein kinase B) activation (Squires et al., 2003).

### 10.4.6 Curcumin Suppresses the Induction of Adhesion Molecules

The expression of various cell surface adhesion molecules such as intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and endothelial leukocyte adhesion molecule-1 on endothelial cells is absolutely critical for tumor metastasis (Ohene-Abuakwa and Pignatelli, 2000). The expression of these molecules is in part regulated by NF-κB (Iademarco et al., 1995). We have shown that the treatment of endothelial cells with curcumin blocks the cell surface expression of adhesion molecules, and this accompanies the suppression of tumor cell adhesion to endothelial cells (Kumar et al., 1998). We have demonstrated that downregulation of these adhesion molecules is mediated through the downregulation of NF-κB activation (Kumar et al., 1998). Gupta and Ghosh (1999) reported that curcumin inhibits TNF-induced expression of adhesion molecules on human

umbilical vein endothelial cells (HUVECs). Jaiswal et al. (2002) showed that curcumin treatment causes p53- and p21-independent G(2)/M phase arrest and apoptosis in colon cancer cell lines. Their results suggest that curcumin treatment impairs both Wnt signaling and cell–cell adhesion pathways, resulting in G(2)/M phase arrest and apoptosis in HCT-116 cells.

### 10.4.7 Curcumin Downregulates COX-2 Expression

Overexpression of COX-2 has been shown to be associated with a wide variety of cancers, including that of colon (Fournier and Gordon, 2000), lung (Hida et al., 1998), and breast (Harris et al., 2000) cancers. The role of COX-2 in the suppression of apoptosis and tumor cell proliferation has been demonstrated (Williams et al., 1999). Furthermore, celebrex, a specific inhibitor of COX-2, has been shown to suppress mammary carcinogenesis in animals (Reddy et al., 2000). Several groups have shown that curcumin downregulates the expression of COX-2 protein in different tumor cells (Plummer et al., 1999; Chen et al., 1999), most likely through the downregulation of NF-κB activation (Plummer et al., 1999), which is needed for COX-2 expression. Chun et al. (2003) reported that curcumin inhibited phorbol ester–induced expression of COX-2 in mouse skin through suppression of extracellular signal-regulated kinase activity and NF-κB activation. COX-2 has been implicated in the development of many human cancers.

Plummer et al. explored the inhibition of COX-2 activity as a systemic biomarker of drug efficacy, a biomarker of potential use in the clinical trials of many chemopreventive drugs known to inhibit this enzyme. They measured COX-2 protein induction and $PGE_2$ production in human blood after incubation with LPS. When 1 μM curcumin was added *in vitro* to blood from healthy volunteers, LPS-induced COX-2 protein levels and concomitant $PGE_2$ production were reduced by 24% and 41%, respectively (Plummer et al., 2001).

### 10.4.8 Curcumin Inhibits Angiogenesis

For most solid tumors, including breast cancer, angiogenesis (blood vessel formation) is essential for tumor growth and metastasis (Folkman, 2001). The precise mechanism that leads to angiogenesis is not fully understood, but growth factors that cause proliferation of endothelial cells have been shown to play a critical role in this process. Curcumin has been shown to suppress the proliferation of human vascular endothelial cells *in vitro* (Singh et al., 1996) and abrogate the fibroblast growth factor-2–induced angiogenic response *in vivo* (Mohan et al., 2000), thus suggesting that curcumin is also an antiangiogenic factor. CD13/aminopeptidase N (APN) is a membrane-bound, zinc-dependent metalloproteinase that plays a key role in tumor invasion and angiogenesis. Shim et al. (2003) observed that curcumin binds to APN and irreversibly inhibits its activity. Indeed curcumin has been shown to suppress angiogenesis *in vivo* (Arbiser et al., 1998). Dorai et al. (2001) also reported that curcumin inhibits angiogenesis of LNCaP prostate cancer cells *in vivo*.

To elucidate the possible mechanisms of antiangiogenic activity by curcumin, Park et al. (2002) performed cDNA microarray analysis and found that curcumin modulated cell-cycle–related gene expression. Specifically, curcumin induced G0/G1 and/or G2/M phase cell cycle arrest, upregulated CDKIs, p21WAF1/CIP1, p27KIP1, and p53, and slightly downregulated cyclin B1 and cdc2 in ECV304 cells. The upregulation of CDKIs by curcumin played a critical role in the regulation of cell cycle distribution in these cells, which may underlie the antiangiogenic activity of curcumin.

### 10.4.9 Curcumin Suppresses the Expression of MMP-9 and iNOS

The MMPs make up a family of proteases that play a critical role in tumor metastasis (Kumar et al., 1999). One of them, MMP-9 has been shown to be regulated by NF-κB activation (Lin et al., 1998), and curcumin has been shown to suppress its expression (Lin et al., 1998). Swarnakar et al. (2005) recently reported that curcumin attenuates the activity of MMP-9 during prevention and healing of indomethacin-induced gastric ulcer. Chan et al. (2003) reported that curcumin reduced

the production of iNOS mRNA in a concentration-dependent manner in *ex vivo* cultured BALB /c mouse peritoneal macrophages.

Curcumin has also been demonstrated to downregulate iNOS expression, also regulated by NF-κB and involved in tumor metastasis (Pan et al., 2000). These observations suggest that curcumin must have antimetastatic activity. Indeed, there is a report suggesting that curcumin inhibits tumor metastasis (Menon et al., 1999).

### 10.4.10 Curcumin Downregulates Cyclin D1 Expression

Cyclin D1, a component subunit of cyclin-dependent kinase (Cdk)-4 and Cdk6, is the rate-limiting factor in the progression of cells through the first gap (G1) phase of the cell cycle (Baldin et al., 1993). Cyclin D1 has been shown to be overexpressed in many cancers including breast, esophagus, head and neck, and prostate (Bartkova et al., 1994; Adelaide et al., 1995; Caputi et al., 1999; Nishida et al., 1994; Gumbiner et al., 1999; Drobnjak et al., 2000). It is possible that the antiproliferative effects of curcumin are due to inhibition of cyclin D1 expression. We found that curcumin can indeed downregulate cyclin D1 expression (Mukhopadhyay et al., 2001; Bharti et al., 2003; Mukhopadhyay et al., 2002) and this downregulation occurred at the transcriptional and posttranscriptional level. Choudhuri et al. (2005) recently reported that curcumin reversibly inhibits normal mammary epithelial cell cycle progression by downregulating cyclin D1 expression and blocking its association with Cdk4/Cdk6, as well as by inhibiting phosphorylation and inactivation of retinoblastoma protein.

### 10.4.11 Curcumin Inhibits Androgen Receptors and AR-Related Cofactors

Nakamura et al. (2002) have evaluated the effects of curcumin in cell growth, activation of signal transduction, and transforming activities of both androgen-dependent and -independent cell lines. The prostate cancer cell lines LNCaP and PC-3 were treated with curcumin, and its effects on signal transduction and expression of androgen receptor (AR) and AR-related cofactors were analyzed. Their results showed that curcumin downregulates transactivation and expression of AR, AP-1, NF-κB, and cAMP response element-binding protein (CREB)-binding protein (CBP). It also inhibited the transforming activities of both cell lines as evidenced by reduced colony-forming ability in soft agar. These studies suggest that curcumin has a potential therapeutic effect on prostate cancer cells through downregulation of AR and AR-related cofactors, AP-1, NF-κB, and CBP (Nakamura et al., 2002).

### 10.4.12 Curcumin Inhibits FPTase

Ras proteins must be isoprenylated at a conserved cysteine residue near the carboxyl terminus (Cys-186 in mammalian Ras p21 proteins) in order to extend their biological activity. Previous studies indicate an intermediate in the mevalonate pathway, most likely farnesyl pyrophosphate, is the donor of this isoprenyl group, and that using inhibitors of the mevalonate pathway could block the transforming properties of the ras oncogene. Chen et al. (1997) examined the effects of curcumin on farnesyl protein transferase (FPTase). They found that partially purified FPTase capable of catalyzing the farnesylation of unprocessed Ras p21 proteins *in vitro* was inhibited by curcumin and its derivatives. This is another potential mechanism by which curcumin could suppress cellular growth.

### 10.4.13 Suppression of Egr-1 by Curcumin

The transcription factor, early growth response-1 gene product (Egr-1), is a member of the family of immediate early response genes and regulates a number of pathophysiologically relevant genes

in vasculature, which are involved in growth, differentiation, immune response, wound healing, and blood clotting. Pendurthi et al. (2000) investigated the effect of curcumin on Egr-1 expression in endothelial cells and fibroblasts. Gel mobility shift assays showed that pretreatment of endothelial cells and fibroblasts with curcumin suppressed tumor promoting agent (TPA) and serum-induced Egr-1 binding to the consensus Egr-1 binding site and also to the Egr-1 binding site present in the promoter of the tissue factor gene. Western blot analysis revealed that curcumin inhibited TPA-induced *de novo* synthesis of Egr-1 protein in endothelial cells. Suppression of Egr-1 protein expression in curcumin-treated cells stemmed from the suppression of Egr-1 mRNA. Northern blot analysis showed that curcumin inhibited serum and TPA-induced expression of tissue factor and urokinase-type plasminogen activator receptor mRNA in fibroblasts. These results showed that curcumin suppresses the induction of Egr-1 and thereby modulates the expression of Egr-1–regulated genes in endothelial cells and fibroblasts. The downregulation of tissue factor by curcumin has also been demonstrated by another group (Bierhaus et al., 1997).

### 10.4.14 Suppression of MAPKs by Curcumin

Most inflammatory stimuli are known to activate three independent MAPK pathways, leading to activation of p44/42 MAPK (also called ERK1/ERK2), JNK, and p38 MAPK pathway. Chen et al. (1999) found that curcumin inhibits JNK activation induced by various agonists including PMA plus ionomycin, anisomycin, UV-C, gamma radiation, TNF, and sodium orthovanadate. Although both JNK and ERK activation by PMA plus ionomycin were suppressed by curcumin, the JNK pathway was more sensitive. The IC50 (50% inhibition concentration) of curcumin was between 5 and 10 μM for JNK activation and was 20 μM for ERK activation. In transfection assays, curcumin moderately suppressed mitogen-activated protein kinase kinase (MEKK)-1–induced JNK activation; however, it effectively blocked JNK activation caused by cotransfection of TAK1, GCK, or human progenitor kinase 1 (HPK1). Curcumin did not directly inhibit JNK, SEK 1 (SAPK/Erk kinase), MEKK1, or HPK1 activity. Although curcumin suppressed TAK1 and GCK activities at high concentrations, this inhibition cannot fully account for the JNK inhibition by curcumin *in vivo*. Thus, these results suggested that curcumin affected the JNK pathway by interfering with the signaling molecule(s) at the same level or proximally upstream of the mitogen-activated protein kinase kinase kinase (MAPKKK) level. The inhibition of the MEKK1–JNK pathway reveals a possible mechanism of suppression of AP-1 and NF-κB signaling by curcumin and may explain the potent anti-inflammatory and anticarcinogenic effects of this chemical.

### 10.4.15 Suppression of Protein Kinases by Curcumin

Curcumin could also mediate its effects through inhibition of various other serine/threonine protein kinases. Our group showed that treatment of highly purified protein kinase A (PKA), protein kinase C (PKC), protamine kinase (cPK), phosphorylase kinase (PhK), autophosphorylation-activated protein kinase (AK), and pp60c-src tyrosine kinase with curcumin inhibited all kinases. PhK was completely inhibited at low concentration of curcumin (Reddy and Aggarwal, 1994). At around 0.1 mM curcumin, PhK, pp60c-src, PKC, PKA, AK, and cPK were inhibited by 98, 40, 15, 10, 1, and 0.5%, respectively. Lineweaver–Burke plot analysis indicated that curcumin is a noncompetitive inhibitor of PhK, with a Ki of 0.075 mM.

Other investigators have shown suppression of PMA-induced activation of cellular PKC by curcumin (Liu et al., 1993). Treatment of cells with 15 or 20 μM curcumin inhibited TPA-induced PKC activity in the particulate fraction by 26 or 60%, respectively, and did not affect the level of PKC. Curcumin also inhibited PKC activity in both cytosolic and particulate fractions *in vitro* by competing with phosphatidylserine. However, the inhibitory effect of curcumin was reduced after preincubation with the thiol compounds. These findings suggested that the suppression of PKC activity may contribute to the molecular mechanism of inhibition of TPA-induced tumor promotion by curcumin.

Besides *in vitro* suppression, curcumin could also inhibit PKC in the cells (Hasmeda and Polya, 1996). Hasmeda et al. (1996) showed that curcumin inhibits $Ca^{2+}$- and phospholipid-dependent PKC and of the catalytic subunit of cyclic AMP-dependent protein kinase (cAK; IC50 values 15 and 4.8 μM, respectively). Curcumin inhibits plant $Ca^{2+}$-dependent protein kinase (CDPK) (IC50 41 μM), but does not inhibit myosin light chain kinase or a high-affinity 3′,5′-cyclic AMP–binding phosphatase. Curcumin inhibits cAK, PKC, and CDPK in a fashion that is competitive with respect to both ATP (adenosine triphosphatase) and the synthetic peptide substrate employed. The IC50 values for inhibition of cAK by curcumin are very similar when measured with kemptide (LRRASLG) (in the presence or absence of ovalbumin) or with casein or histone III-S as substrates. However, the presence of bovine serum albumin (0.8 mg $ml^{-1}$) largely overcomes inhibition of cAK by curcumin.

## 10.5 ANTICANCER PROPERTIES OF CURCUMIN

Several studies indicate that curcumin is a potent anticancer agent (Figure 10.5). The tumorigenesis of the skin, mammary gland, oral cavity, forestomach, oesophagus, stomach, intestine, colon, lung, and liver have been shown to be suppressed by curcumin (Huang et al., 1988; Kuttan et al., 1985, 1987; Rao et al., 1984; Lee et al., 2005; Chuang et al., 2000; Deshpande et al., 1998; Ushida et al., 2000; Limtrakul et al., 1997; Piper et al., 1998) (Table 10.3).

To explain the anticarcinogenic effects of curcumin on different tumors, a wide variety of mechanisms have been implicated, including inhibition of ROI, suppression of inflammation, down-regulation of ODC, inhibition of cell proliferation, inhibition of cytochrome P450 isoenzymes, induction of GSH, suppression of certain oncogenes (e.g., cHa-ras, c-jun, and c-fos), inhibition of transcription factors NF-κB and AP-1, suppression of COX2, inhibition of cell-cycle–related proteins (PCNA, cyclin E, p34 cdc2), inhibition of chromosomal damage, inhibition of oxidation of DNA bases, inhibition of malondialdehyde (MDA) DNA adduct formation, inhibition of tumor implantation, inhibition of protein tyrosine kinase and protein kinase C activity, inhibition of biotransformation of carcinogens, and induction of gluthathione S-transferase (GST) activity (Huang et al., 1988; Kuttan et al., 1985, 1987; Rao et al., 1984; Deshpande et al., 1998; Sharma et al., 2001; Chuang et al., 2002; Tanaka et al., 2004; Inano et al., 1999).

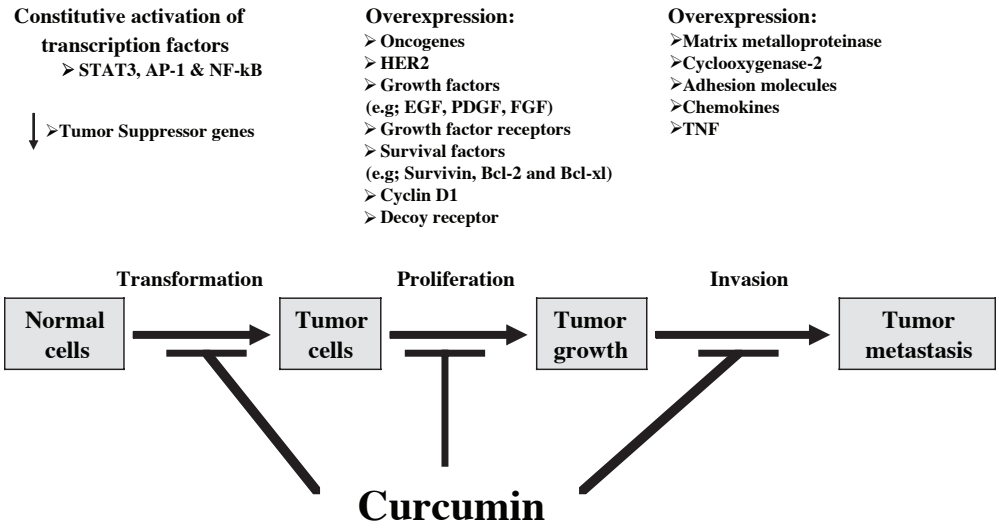


**FIGURE 10.5** Antitumor properties of curcumin.

**TABLE 10.3**
**Effects of Curcumin on Survival and Proliferation of Different Cell Types**

| Cell Type and Effect | Mechanism | Refs. |
|---|---|---|
| Inhibits proliferation of breast tumor cells | Inhibition of ODC; arrested cells at G2/S | Mehta et al., 1997; Ramachandran and You, 1999; Simon et al., 1998; Verma et al., 1997 |
| Induction of apoptosis in H-ras transformed MCF10A cells | P53-dependent bax induction | Kim et al., 2001; Choudhari et al., 2002 |
| Induces apoptosis of AK-5 cells | Production of ROI<br>Activation of caspase-3 | Bhaumik et al., 1999; Khar et al., 2001 |
| Inhibits proliferation of colon cancer (HT29; HCT-15) cells | Accumulation of cells in G2/M phase | Hanif et al., 1997 |
| Induces apoptosis in colon (LoVo) cancer cells | Accumulates in S, G2/M phase of cell cycle | Chen et al., 1999; Mori et al., 2001; Moragoda et al., 2001 |
| | Induction of HSP70 and p53 | Chen et al., 1996; Samaha et al., 1997 |
| Transformed cells | — | Gautam et al., 1998 |
| Fibroblast (NIH3T3), colon cancer (HT29) | Induces cell shrinkage | Ramsewak et al., 2000; Jiang et al., 1996; Nogaki et al., 1998; Pal et al., 2001 |
| Kidney cancer (293), hepatocellular Carcinoma (HepG2) | Chromatin condensation<br>DNA fragmentation | Park et al., 2005 |
| Induces apoptosis of leukemia cells | — | Kuo et al., 1996 |
| Induces apoptosis of T-cell leukemia (Jurkat) modulation by GSH | Independent of mitochondria and caspases | Piwocka et al., 1999; Piwocka et al., 2001 |
| Induces growth arrest and apoptosis of B-cell lymphoma | Downregulation of Egr-1 c-myc, bcl-xl, NF-κB and p53 | Han et al., 1999 |
| Induces apoptosis in HL-60 cells | Increases Sub-G1; activates caspase-3 | Bielak-Zmijewska et al., 2000 |
| Induces apoptosis of myeloid (HL-60) cells | Cytochrome C release; caspase-3 activation; loss of MMTP; caspase-9 activation | Pan et al., 2001 |
| Induces apoptosis of myeloid (HL-60) cells | Caspase-8 activation; BID cleavage; cytochrome C release | Anto et al., 2002 |
| Induces apoptosis of basal cell carcinoma cells | p53-dependent | Jee et al., 1998 |
| Inhibits proliferation of prostate cancer cells | Downregulation of bcl-2 and NF-κB | Mukhopadhyay et al., 2001 |
| Induces apoptosis of melanoma cells | Fas/FLICE pathway; p53-independent | Bush et al., 2001 |
| Inhibits the proliferation of HUVEC | Accumulation of cells in S-phase | Singh et al., 1996; Park et al., 2002 |
| Inhibits proliferation of oral epithelial cells | — | Khafif et al., 1998; Elattar and Virji, 2000 |
| Induces apoptosis of T-lymphocytes | Loss of mitochondrial membrane potential, plasma membrane asymmetry & permeability; GSH-independent | Sikora et al., 1997; Jaruga et al., 1998 |
| Induces apoptosis of γδT-cells | Increase in annexin V reactivity; nuclear expression of active caspase-3; cleavage of PARP; nuclear disintegration; translocation of AIF to the nucleus; large-scale DNA chromatolysis | Cipriani et al., 2001 |
| Induced apoptosis of breast cancer cells | P53-dependent<br>Bax induction | Chowdhuri et al., 2002 |

*Continued*

**TABLE 10.3 *(Continued)***
**Effects of Curcumin on Survival and Proliferation of Different Cell Types**

| Cell Type and Effect | Mechanism | Refs. |
|---|---|---|
| Induces apoptosis of osteoclasts | — | Ozaki et al., 2000 |
| Induces apoptosis in VSMC | Reduction in the S-phase; increase in G0/G1 phase; increase in TUNNEL-positive cells; DNA fragmentation; decrease in mRNA for c-myc and bcl-2 but not p53; inhibition of PKC and PTK | Chen and Huang, 1998 |
| Inhibits the PDGF-induced proliferation of VSMC | — | Huang et al., 1992a |
| Inhibits the PHA-induced proliferation of PBMC | — | Huang et al., 1992b |
| Induces apoptosis in hepatocytes | Increase MMTP, loss of MMP, mitochondrial swelling; inhibition of ATP synthesis, oxidation of membrane thiol | Morin et al., 2001; Gomez-Lechon et al., 2002 |

*Abbreviations*: ROI, reactive oxygen intermediates; HUVEC, human umbilical vein vascular endothelial cells; MMTP, mitochondrial membrane permeability transition pore; GSH, glutathione; VSMC, vascular smooth muscle cells; PARP, poly(ADP-ribose) polymerase; AIF, apoptosis-inducing factor; MMP, mitochondrial membrane potential; TUNEL, TdT-mediated dUTP nick end labeling; PKC, protein kinase C; PTK, protein tyrosine kinase; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin.

### 10.5.1 *IN VITRO* STUDIES

Curcumin has been shown to inhibit the proliferation of a wide variety of tumor cells, including B-cell and T-cell leukemia (Kuo et al., 1996; Ranjan et al., 1999; Piwocka et al., 1999; Han et al., 1999), colon carcinoma (Chen et al., 1999), epidermoid carcinoma (Korutla and Kumar, 1994), head and neck squamous cell carcinoma (Aggarwal et al., 2004), MM (Bharti et al., 2003), and mantle cell lymphoma (Shishodia et al., 2005). It has also been shown to suppress the proliferation of various breast carcinoma cell lines in culture (Mehta et al., 1997; Ramachandran and You, 1999; Simon et al., 1998).

Mehta et al. (1997) examined the antiproliferative effects of curcumin against several breast tumor cell lines, including hormone-dependent and -independent and multidrug-resistance (MDR) lines. Cell growth inhibition was monitored by [3H] thymidine incorporation, Trypan blue exclusion, crystal violet dye uptake, and flow cytometry. All the cell lines tested, including the MDR-positive ones, were highly sensitive to curcumin. The growth inhibitory effect of curcumin was time- and dose dependent, and correlated with its inhibition of ornithine decarboxylase activity. Curcumin preferentially arrested cells in the G2/S phase of the cell cycle.

Thioredoxin reductases (TrxR) have been found to be overexpressed by a number of human tumors. Fang et al. (2005) reported that rat TrxR1 activity in Trx-dependent disulfide reduction was inhibited by curcumin. The IC50 value for the enzyme was 3.6 μM after incubation at room temperature for 2 h *in vitro*. The inhibition occurred with enzyme only in the presence of NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) and persisted after removal of curcumin. By using mass spectrometry and blotting analysis, they showed that this irreversible inhibition by curcumin was caused by alkylation of both residues in the catalytically active site (Cys (496)/Sec (497)) of the enzyme. Inhibition of TrxR by curcumin added to cultured HeLa cells was also observed with an IC50 of around 15 μM. Modification of TrxR by curcumin provides a possible mechanistic explanation for its cancer-preventive activity.

Kang et al. reported that the exposure of human hepatoma cells to curcumin led to a significant decrease in histone acetylation. Curcumin treatment resulted in a comparable inhibition of histone acetylation in the absence or presence of trichostatin A and showed no effect on the *in vitro* activity of HDAC (histone deacetylase). Curcumin treatment significantly inhibited the HAT (histone acetyltrausferase) activity both *in vivo* and *in vitro*. Curcumin-induced hypoacetylation led to the loss of cell viability in human hepatoma cells (Kang et al., 2005).

The transcription factor AP-1 plays a central role in the transcriptional regulation of specific types of high-risk human papillomaviruses (HPVs) such as HPV16 and HPV18, which are etiologically associated with the development of cancer of the uterine cervix in women. Prusty et al. (2005) showed that curcumin can selectively downregulate HPV18 transcription as well as the AP-1–binding activity in HeLa cells. Most interestingly, curcumin can reverse the expression dynamics of c-fos and fra-1 in this tumorigenic cell line.

Curcumin synergized with the chemotherapeutic agent Vinorelbine in suppressing the growth of human squamous cell lung carcinoma H520 cells. Both the agents caused apoptosis by increasing the protein expression of Bax and Bcl-Xl while decreasing that of Bcl-2 and Bcl-X (L), releasing apoptogenic cytochrome c and augmenting the activity of caspase-9 and caspase-3. Curcumin treatment induced 23.7% apoptosis in the H520 cells, while Vinorelbine caused 38% apoptosis. Pretreatment with curcumin enhanced the Vinorelbine-induced apoptosis to 61.3%. These findings suggest that curcumin has the potential to act as an adjuvant chemotherapeutic agent and enhance chemotherapeutic efficacy of Vinorelbine in H520 cells *in vitro* (Sen et al., 2005).

Curcumin significantly inhibited the growth of human gastric carcinoma (AGS) cells in a dose- and time-dependent manner (Koo et al., 2004). Curcumin caused a 34% decrease in AGS proliferation at 5 μmol/L, 51% at 10 μmol/L, and 92% at 25 μmol/L after 4 d of treatment. When curcumin (10 μmol/L) was removed after a 24-h exposure, the growth pattern of curcumin-treated AGS cells was similar to that of control cells, suggesting reversibility of curcumin on the growth of AGS cells. Combining curcumin with 5-FU (5-fluorouracil) significantly increased growth inhibition of AGS cells compared with either curcumin or 5-FU alone, suggesting synergistic actions of the two drugs. After 4 d of treatment with 10 μmol/L of curcumin, the G2/M phase fraction of cells was 60.5% compared to 22.0% of the control group, suggesting a G2/M block by curcumin treatment. The curcumin concentrations (5 μmol/L) used in this study were similar to steady-state concentrations (1.77 ± 1.87 μmol/L) in human serum of subjects receiving chronic administration of a commonly recommended dose (8 g/d). Thus, curcumin may be useful for the treatment of gastric carcinoma, especially in conjunction with 5-FU.

Using time-lapse video and immunofluorescence-labeling methods, Holy (2004) demonstrated that curcumin significantly alters microfilament organization and cell motility in PC-3 and LNCaP human prostate cancer cells *in vitro*. Curcumin rapidly arrests cell movements and subsequently alters cell shape in the highly motile PC-3 cell line, but has a less noticeable effect on the relatively immobile LNCaP cell line. Stress fibers are augmented, and the overall quantity of f-actin appears to increase in both types of cells following curcumin treatment. At least some of the effects of curcumin appear to be mediated by protein kinase C (PKC), as treatment with the PKC inhibitor, bisindolylmaleimide, inhibits the ability of curcumin to block CB (cytochalasin B)-induced membrane blebbing. These findings demonstrate that curcumin exerts significant effects on the actin cytoskeleton in prostate cancer cells, including altering microfilament organization and function. This may represent an important mechanism by which curcumin functions as a chemopreventative agent, and as an inhibitor of angiogenesis and metastasis.

Chemoresistance is a major problem in the treatment of patients with MM due to constitutive expression of NF-κB and STAT-3. Bharti et al. (2003, 2004) showed that the suppression of NF-κB and STAT3 activation in MM cells by *ex vivo* treatment with curcumin resulted in a decrease in adhesion to bone marrow stromal cells, cytokine secretion, and the viability of cells.

*Helicobacter pylori* is a Group 1 carcinogen and is associated with the development of gastric and colon cancer. A methanol extract of the dried powdered turmeric rhizome and curcumin were

tested against 19 strains of *H. pylori*, including 5 cagA+ strains. Both the methanol extract and curcumin inhibited the growth of all strains of *H. pylori in vitro,* with a minimum inhibitory concentration range of 6.25 to 50 μg/ml (Mahady et al., 2002). These data demonstrate that curcumin inhibits the growth of *H. pylori* cagA+ strains *in vitro*, and this may be one of the mechanisms by which curcumin exerts its chemopreventative effects.

Chen et al. (2004) used microarray analysis of gene expression profiles to characterize the anti-invasive mechanisms of curcumin in highly invasive lung adenocarcinoma cells (CL1-5). Results showed that curcumin significantly reduces the invasive capacity of CL1-5 cells in a concentration range far below its levels of cytotoxicity (20 μM) and that this anti-invasive effect was concentration dependent ($10.17 \pm 0.76 \times 10^3$ cells at 0 μM; $5.67 \pm 1.53 \times 10^3$ cells at 1 μM; $2.67 \pm 0.58 \times 10^3$ cells at 5 μM; $1.15 \pm 1.03 \times 10^3$ cells at 10 μM; $p < 0.05$) in the Transwell cell culture chamber assay. Using microarray analysis, 81 genes were downregulated and 71 genes were upregulated after curcumin treatment. Below sublethal concentrations of curcumin (10 μM), several invasion-related genes were suppressed, including MMP14 (0.65-fold), neuronal cell adhesion molecule (0.54-fold), and integrins alpha6 (0.67-fold) and beta4 (0.63-fold). In addition, several heat-shock proteins (Hsp) (Hsp27 [2.78-fold], Hsp70 [3.75-fold], and Hsp40-like protein [3.21-fold]) were induced by curcumin. Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR), Western blotting, and immunohistochemistry confirmed these results in both RNA and protein levels. Curcumin (1 to 10 μM) reduced the MMP14 expression in both mRNA and protein levels and also inhibited the activity of MMP2, the downstream gelatinase of MMP14, by gelatin zymographic analysis.

Kim et al. (2002) evaluated the antiangiogenic activity of demethoxycurcumin (DC), a structural analog of curcumin, and investigated the effect of DC on genetic reprogramming in cultured HUVECs using cDNA microarray analysis. Of the 1024 human cancer-focused genes arrayed, 187 genes were upregulated and 72 genes were downregulated at least twofold by DC. Interestingly, nine angiogenesis-related genes were downregulated over fivefold in response to DC, suggesting that the genetic reprogramming was crucially involved in antiangiogenesis by the compound. MMP-9, the product of one of the angiogenesis-related genes downregulated over fivefold by DC, was investigated using gelatin zymography. DC potently inhibited the expression of MMP-9, yet showed no direct effect on its activity.

### 10.5.2 *In Vivo* Studies

Numerous studies have been performed to evaluate the chemopreventive properties of curcumin (Table 10.4). Kuttan et al (1985) examined the anticancer potential of curcumin *in vivo* in mice using Dalton's lymphoma cells grown as ascites. Initial experiments indicated that curcumin reduced the development of animal tumors. They encapsulated curcumin (5 mg/ml) into neutral and unilamelar liposomes prepared by sonication of phosphatidylcholine and cholesterol. An aliquot of liposomes (50 mg/kg) was given i.p. to mice the day after giving the Dalton's lymphoma cells and continued for 10 d. After 30 and 60 d, the surviving animals were counted. When curcumin was used in liposomal formulations at concentration of 1 mg/animal, all animals survived 30 d and only two of the animals developed tumors and died before 60 d.

Busquets et al. (2001) showed that systemic administration of curcumin (20 μg/kg body weight) for six consecutive days to rats bearing the highly cachectic Yoshida AH-130 ascites hepatoma resulted in an important inhibition of tumor growth (31% of total cell number). Interestingly, curcumin was also able to reduce by 24% *in vitro* tumor cell content at concentrations as low as 0.5 μM without promoting any apoptotic events. Although systemic administration of curcumin has previously been shown to facilitate muscle regeneration, administration of the compound to tumor-bearing rats did not result in any changes in muscle wasting, when compared with the untreated tumor-bearing animals. Indeed, both the weight and protein content of the gastrocnemius muscle significantly decreased as a result of tumor growth, and curcumin was unable to reverse

**TABLE 10.4**
**Chemopreventive Effects of Curcumin**

| Effects | Refs. |
|---|---|
| Inhibits tumor promotion in mouse skin by TPA | Huang et al., 1988 |
| Inhibits TPA-induced increase in mRNA for ODC in mouse epidermis | Lu et al., 1993 |
| Inhibits chemical carcinogen-induced stomach and skin tumors in Swiss mice | Azuine and Bhide, 1992 |
| Inhibits TPA- and UV-B light-induced expression of c-Jun and c-Fos in mouse epidermis | Lu et al., 1994 |
| Inhibits TPA-induced tumor promotion by curcumin analogues | Huang et al., 1995 |
| Inhibits UV-A-induced ODC induction and dermatitis induced by TPA in mouse skin | Ishizaki et al., 1996 |
| Inhibits TPA-induced tumor promotion and oxidized DNA bases in mouse epidermis | Huang et al., 1997 |
| Inhibits skin carcinogenesis in mice | Limtrakul et al., 1997 |
| Inhibits chemical carcinogenesis by curcumin | Soudamini and Kuttan, 1989 |
| Protection from fuel smoke condensate-induced DNA damage in human lymphocytes | Shalini and Srinivas, 1990 |
| Reverses aflatoxin-induced liver damage | Soni and Kuttan, 1992 |
| Inhibits BPDE-induced tumor initiation | Huang et al., 1992a |
| Inhibits azoxymethanol-induced colonic epithelial cell proliferation and focal areas of dysplasia | Huang et al., 1992b |
| Inhibits azoxymethane-induced aberrant crypt foci formation in the rat colon | Rao e al., 1993 |
| Inhibits forestomach, duodenal, and colon carcinogenesis in mice | Huang et al.,1994 |
| Prevents colon carcinogenesis | Rao et al., 1995 |
| Prevents 1,2-dimethylhydrazine initiated mouse colon carcinogenesis | Kim et al., 1998 |
| Inhibits the promotion/progression stages of colon cancer | Kawamori et al., 1999 |
| Induces genetic reprogramming in pathways of colonic cell maturation | Mariadason et al., 2000 |
| Inhibits PhIP-induced tumor formation in Apc(min) mice | Collett et al., 2001 |
| Inhibits GST and MDA–DNA adducts in rat liver and colon mucosa | Sharma et al., 2001 |
| Prevents familial adenomatous polyposis | Perkins et al., 2002 |
| Suppresses methyl (acetoxymethyl) nitrosamine-induced hamster oral carcinogenesis | Azuine and Bhide, 1992 |
| Inhibits 4-nitroquinoline 1-oxide-induced oral carcinogenesis | Tanaka et al., 1994 |
| Inhibits experimental cancer: in forestomach and oral cancer models | Azuine and Bhide, 1994 |
| Inhibits benzo[a]pyrene-induced forestomach cancer in mice | Singh et al., 1998 |
| Inhibits *N*-nitrosomethylbenzylamine-induced esophageal carcinogenesis in rats. | Ushida et al., 2000 |
| Prevents *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and NaCl-induced glandular stomach carcinogenesis | Ikezaki et al.,2001 |
| Inhibits azoxymethane-induced colon cancer and DMBA-induced mammary cancer in rats | Pereira et al., 1996 |
| Inhibits DMBA-induced mammary tumorigenesis and DMBA-DNA adduct formation | Singletary et al., 1996 |
| Prevents DMBA-induced rat mammary tumorigenesis | Deshpande et al., 1998 |
| Inhibits formation of DMBA-induced mammary tumors and lymphomas/leukemias in Sencar mice | Huang et al., 1998 |
| Inhibits the promotion stage of tumorigenesis of mammary gland in rats irradiated with γ-rays | Inano et al., 1999 |
| Prevents radiation-induced initiation of mammary tumorigenesis in rats | Inano and Onoda, 2002; Inano et al., 2000 |
| Inhibits DMBA-induced mammary tumorigenesis by dibenzoylmethane, a analogue of curcumin | Lin et al., 2001 |

*Continued*

**TABLE 10.4 *(Continued)***
**Chemopreventive Effects of Curcumin**

| Effects | Refs. |
|---|---|
| Inhibits mammary gland proliferation, formation of DMBA–DNA adducts in mammary glands and mammary tumorigenesis in Sencar mice by dietary dibenzoylmethane | Lin et al., 2001 |
| Inhibits B[a]P plus NNK-induced lung tumorigenesis in A/J mice | Hecht et al., 1999 |
| Inhibits the initiation stage in a rat multiorgan carcinogenesis model | Takaba et al., 1997 |
| Retards experimental tumorigenesis and reduction in DNA adducts | Krishnaswamy et al., 1998 |
| Induces glutathione linked detoxification enzymes in rat liver | Piper et al., 1998 |
| Inhibits TPA-induced ODC activity | Lee and Pezzuto, 1999 |
| Structurally related natural diarylheptanoids antagonize tumor promotion | Chun et al., 1999 |
| Inhibits aflatoxin B(1) biotransformation | Lee et al., 2001 |
| Inhibits B[a]P-induced cytochrome P-450 isozymes | Thapliyal et al., 2001 |
| Inhibits diethylnitrosamine-induced murine hepatocarcinogenesis | Chuang et al., 2000 |
| Inhibits diethylnitrosamine-induced hepatic hyperplasia in rats | Chuang et al., 2000 |
| Inhibits Purnark against benzo(a)pyrene-induced chromosomal damage in human lymphocytes | Ghaisas and Bhide, 1994 |
| Inhibits carcinogen induced c-Ha-ras and c-fos proto-oncogenes expression | Limtrakul et al., 2001 |
| Prevents intravesical tumor growth of the MBT-2 tumor cell line following implantation in C3H mice | Sindhwani et al., 2001 |
| Suppresses growth of hamster flank organs by topical application | Liao et al., 2001 |
| Inhibits Epstein–Barr virus BZLF1 transcription in Raji DR-LUC cells | Hergenhahn et al., 2002 |
| Prevents radiation-induced mammary tumors | Inano and Onoda, 2002 |
| Induces GST activity by curcumin in mice | Susan and Rao, 1992 |

*Abbreviations*: BPDE, benzo[a]pyrene diolepoxide; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; ODC, ornithine decarboxylase, DMBA, 7,12-dimethylbenz[a]anthracene; GST, glutathione *S*-transferase; MDA, malondialdehyde; PhIP, 2-amino 1-methyl-6-phenylimidazo[4,5-b]pyridine; BaP, benzo[a]pyrene; NNK, 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone.

this tendency. It was concluded that curcumin, in spite of having clear antitumoral effects, has little potential as an anticachectic drug in the tumor model used in the study.

Menon et al. (1995) reported curcumin-induced inhibition of B16F10 melanoma lung metastasis in mice. Oral administration of curcumin at concentrations of 200 nmol/kg body weight reduced the number of lung tumor nodules by 80%. The life span of the animals treated with curcumin was increased by 143.85% (Menon et al., 1995). Moreover, lung collagen hydroxyproline and serum sialic acid levels were significantly lower in treated animals than in the untreated controls. Curcumin treatment (10 μg/ml) significantly inhibited the invasion of B16F-10 melanoma cells across the collagen matrix of a Boyden chamber. Gelatin zymographic analysis of the trypsin-activated B16F-10 melanoma cells sonicate revealed no metalloproteinase activity. Curcumin treatment did not inhibit the motility of B16F-10 melanoma cells across a polycarbonate filter *in vitro*. These findings suggest that curcumin inhibits the invasion of B16F-10 melanoma cells by inhibition of MMPs, thereby inhibiting lung metastasis.

Curcumin decreases the proliferative potential and increases apoptotic potential of both androgen-dependent and androgen-independent prostate cancer cells *in vitro*, largely by modulating the apoptosis suppressor proteins and by interfering with the growth factor receptor signaling pathways as exemplified by the EGF receptor. To extend these observations, Dorai et al. (2001) investigated the anticancer potential of curcumin in a nude mouse prostate cancer model. The androgen-dependent LNCaP prostate cancer cells were grown, mixed with Matrigel, and injected subcutaneously. The experimental group received a synthetic diet containing 2% curcumin for up to 6 weeks. At the end point, mice were killed, and sections taken from the excised tumors were evaluated

for pathology, cell proliferation, apoptosis, and vascularity. Results showed that curcumin induced a marked decrease in the extent of cell proliferation as measured by the BrdU incorporation assay and a significant increase in the extent of apoptosis as measured by an *in situ* cell death assay. Moreover, a significant decrease in the microvessel density as measured by CD31 antigen staining was also seen. It was concluded that curcumin was a potentially therapeutic anticancer agent, as it significantly inhibited prostate cancer growth, as exemplified by LNCaP *in vivo*, and it had the potential to prevent the progression of this cancer to its hormone refractory state. Aggarwal et al. (2004) recently reported that curcumin inhibits growth and survival of human head and neck squamous cell carcinoma cells via modulation of NF-κB signaling.

The chemopreventive activity of curcumin was observed when it was administered prior to, during, and after carcinogen treatment, as well as when it was given only during the promotion/progression phase of colon carcinogenesis (Kawamori et al., 1999). Collett et al. (2001) investigated the effects of curcumin on apoptosis and tumorigenesis in male apc (min) mice treated with the human dietary carcinogen PhIP. The intestinal epithelial apoptotic index in response to PhIP treatment was approximately twice as great in wild type C57BL/6 APC (+/+) strain as in Apc (min) mice. Curcumin enhanced PhIP-induced apoptosis and inhibited PhIP-induced tumorigenesis in the proximal small intestine of Apc (min) mice. Mahmoud et al. (2000) investigated the effect of curcumin for the prevention of tumors in C57BL/6J-Min/+ (Min/+) mice that bear germline mutation in the apc gene and spontaneously develop numerous intestinal adenomas by 15 weeks of age. At a dietary level of 0.15%, curcumin decreased tumor formation in Min-/- mice by 63%. Examination of intestinal tissue from the treated animals showed the tumor prevention by curcumin was associated with increased enterocyte apoptosis and proliferation. Curcumin also decreased expression of the oncoprotein β-catenin in the erythrocytes of the Min/+mouse, an observation previously associated with an antitumor effect.

Recently, Perkins et al. (2002) also examined the preventive effect of curcumin on the development of adenomas in the intestinal tract of the C57BL/6J-Min/+ mouse, a model of human familial APC. These investigators explored the link between its chemopreventive potency in the Min/+ mouse and levels of drug and metabolites in target tissue and plasma. Mice received dietary curcumin for 15 weeks, after which adenomas were enumerated. Levels of curcumin and metabolites were determined by high-performance liquid chromatography in plasma, tissues, and feces of mice after either long-term ingestion of dietary curcumin or a single dose of [$^{14}$C] curcumin (100 mg/kg) intraperitoneally. Whereas curcumin at 0.1% in the diet was without effect, however, at 0.2 and 0.5%, it reduced adenoma multiplicity by 39 and 40%, respectively.

Odot et al. (2004) showed that curcumin was cytotoxic to B16-R melanoma cells resistant to doxorubicin. They demonstrated that the cytotoxic effect observed was due to the induction of programmed cell death. They examined the effectiveness of a prophylactic immune preparation of soluble proteins from B16-R cells, or a treatment with curcumin as soon as tumoral appearance, alone or in combination, on the murine melanoma B16-R. The combination treatment resulted in substantial inhibition of growth of B16-R melanoma, whereas each treatment by itself showed little effect. Moreover, animals receiving the combination therapy exhibited an enhancement of their humoral antisoluble B16-R protein immune response and a significant increase in their median survival time (> 82.8% vs. 48.6% and 45.7%, respectively for the immunized group and the curcumin-treated group).

## 10.6 CURCUMIN AND CHEMOSENSITIVITY

Chemosensitivity is the susceptibility of tumor cells to the cell-killing effects of anticancer drugs. Most of the chemotherapeutic agents frequently induce drug resistance. HER2, a growth factor receptor overexpressed in breast cancer, has been implicated in Taxol-induced resistance, probably through the activation of NF-κB. Acquired resistance to chemotherapeutic agents is most likely mediated through a number of mechanisms including the gene product MDR protein. Multidrug

resistance is a phenomenon that is often associated with decreased intracellular drug accumulation in the tumor cells of a patient, resulting from enhanced drug efflux. It is often related to the overexpression of P-glycoprotein on the surface of tumor cells, thereby reducing drug cytotoxicity. Curcumin has been shown to augment the cytotoxic effects of chemotherapeutic drugs, including doxorubicin (Harbottle et al., 2001), tamoxifen (Verma et al., 1998), cisplatin and camptothecin, daunorubicin, vincristine, and melphalan (Bharti et al., 2003). Taxol has a major disadvantage in its dose-limiting toxicity. Bava et al. (2005) reported that combination of Taxol with curcumin augments anticancer effects more efficiently than Taxol alone. This combination at the cellular level augments activation of caspases and cytochrome c release. Similarly, the combination of curcumin with cisplatin resulted in a synergistic antitumor activity in hepatic cancer HA22T/VGH cell line, which constitutively expresses activated NF-κB. Combination of curcumin with cisplatin led to an additive decrease in the expression of c-myc, Bcl-$X_L$, c-IAP-2, and XIAP (Notarbartolo et al., 2005)

Curcumin was found to be cytotoxic to B16-R melanoma cells resistant to doxorubicin either cultivated as monolayers or grown in three-dimensional (3D) cultures (spheroids). A prophylactic immune preparation of soluble proteins from B16-R cells, or a treatment with curcumin upon tumor appearance, alone or in combination, on the murine melanoma B16-R resulted in substantial inhibition of growth of B16-R melanoma, whereas each treatment by itself showed little effect. Moreover, animals receiving the combination therapy exhibited an enhancement of their humoral antisoluble B16-R protein immune response and a significant increase in their median survival time (Odot et al., 2004).

NF-κB has been implicated in the development of drug resistance in cancer cells. The basal level of NF-κB activity has been found to be heterogeneous in various cancer cells and roughly correlated with drug resistance. Curcumin has been shown to downregulate doxorubicin-induced NF-κB activation. (Chuang et al., 2002). Multidrug resistance (MDR) is a major cause of chemotherapy failure in cancer patients. One of the resistance mechanisms is the overexpression of drug efflux pumps such as P-glycoprotein and multidrug resistance protein 1 (MRP1, [ABCC1]). Upon treating the cells with etoposide in the presence of 10 μM curcuminoids, the sensitivity of etoposide was increased by several folds in MRP1 expressing HEK 293 cells (Limtrakul et al., 2004). Curcumin also decreased P-glycoprotein function and expression, and the promotion of caspase-3 activation in MDR gastric cancer cells. Gastric cancer cells treated with curcumin decreased the IC50 value of vincristine and promoted vincristine-mediated apoptosis in a dose-dependent manner. Curcumin reversed the MDR of the human gastric carcinoma SGC7901/VCR cell line (Tang et al., 2005). Curcumin decreased P glycoprotein expression in a concentration-dependent manner and was also found to have the same effect on MDR1 mRNA levels (Anuchapreeda et al., 2002; Romiti et al., 1998). The effect of curcumin on apoptosis in multidrug resistant cell lines has been reported. Piwocka et al. demonstrated that curcumin induced cell death in multidrug-resistant CEM(P-gp4) and LoVo(P-gp4) cells in a caspase-3–independent manner (Piwocka et al., 2002). Mehta et al. (1997) also examined the antiproliferative effects of curcumin against multidrug-resistant (MDR) lines, which were found to be highly sensitive to curcumin. The growth-inhibitory effect of curcumin was time- and dose dependent and correlated with its inhibition of ornithine decarboxylase activity. Curcumin preferentially arrested cells in the G2/S phase of the cell cycle.

Subtoxic concentrations of curcumin sensitize human renal cancer cells to TRAIL (TNF-related apoptosis inducing ligand)-mediated apoptosis. Apoptosis induced by the combination of curcumin and TRAIL is not interrupted by Bcl-2 overexpression. Treatment with curcumin significantly induces death receptor 5 expression accompanying the generation of the reactive oxygen species (ROS) (Jung et al., 2005). Curcumin causes cell death in melanoma cell lines with mutant p53. Since melanoma cells with mutant p53 are strongly resistant to conventional chemotherapy, curcumin overcomes the chemoresistance of these cells and provides potential new avenues for treatment (Bush et al., 2001).

## 10.7 RADIOSENSITIZING EFFECTS OF CURCUMIN

Radiotherapy plays an important role in the management of cancers. Whilst its role in achieving local control following surgery in patients with early stage cancer is well established, there is still unclear evidence to explain the factors governing radioresistance in patients who develop recurrences.

Chendil et al. (2004) investigated the radiosensitizing effects of curcumin in p53 mutant prostate cancer cell line PC-3. Compared to cells that were irradiated alone, curcumin at 2 and 4 μM concentrations in combination with radiation showed significant enhancement to radiation-induced clonogenic inhibition and apoptosis. In PC-3 cells, radiation upregulated TNFα protein, leading to an increase in NF-κB activity resulting, in the induction of Bcl-2 protein. However, curcumin in combination with radiation treated showed inhibition of TNFα-mediated NF-κB activity, resulting in bcl-2 protein downregulation. The results suggested that curcumin is a potent radiosensitizer, and it acts by overcoming the effects of radiation-induced prosurvival gene expression in prostate cancer.

Khafif et al. (2005) investigated whether curcumin can sensitize squamous cell carcinoma (SCC) cells to the ionizing effects of irradiation. Incubation with curcumin only (3.75 μM) for 48 h did not decrease the number of cells or the ability to form colonies in the absence of radiation. However, in plates that were exposed to 1 to 5 Gy of radiation, cell counts dropped significantly if pretreated with curcumin with a maximal effect at 2.5 Gy. The colonogenic assay revealed a significant decrease in the ability to form colonies following pretreatment with curcumin at all radiation doses. Thus, curcumin may serve as an adjuvant in radiotherapy.

### 10.7.1 Curcumin Can Induce Radioprotection

There are several studies that suggest that curcumin is radioprotective. Thresiamma et al. (1996) showed that curcumin protects from radiation-induced toxicity. They showed that whole body irradiation of rats (10 Gy as five fractions) produces lung fibrosis within 2 months as seen from increased lung collagen hydroxyproline and histopathology. Oral administration of curcumin (200 μmole/kg body weight) significantly reduced the lung collagen hydroxyproline. In serum and liver LPO increased by irradiation was reduced significantly by curcumin treatment. The liver superoxide dismutase (SOD) and GSH peroxidase activity increased was reduced significantly by curcumin. Curcumin also significantly reduced the whole body irradiation-induced increased frequency of micronucleated polychromatic erythrocytes in mice. In another study, Thresiamma et al. (1998) later investigated the protective effect of curcumin on radiation-induced genotoxicity. They showed that induction of micronuclei and chromosomal aberrations produced by whole body exposure of γ-radiation (1.5–3 Gy) in mice was significantly inhibited by oral administration of curcumin (400 μmol/kg body weight), inhibited micronucleated polychromatic and normochromatic erythrocytes, significantly reduced the number of bone marrow cells with chromosomal aberrations and chromosomal fragments, and inhibited the DNA strand breaks produced in rat lymphocytes upon radiation as seen from the DNA unwinding studies (Thresiamma et al., 1998). In contrast to these studies, Araujo et al. (1999) showed potentiation by curcumin of γ-radiation–induced chromosome aberrations in Chinese hamster ovary cells. They treated the cells with curcumin (2.5, 5, and 10 μg/ml), and then irradiated (2.5 Gy) during different phases of the cell cycle. Curcumin at 10 μg/ml enhanced the chromosomal damage frequency. Curcumin did not show protective effect against the clastogenicity of γ-radiation. Instead, an obvious increase in the frequencies of chromosome aberrations was observed with curcumin at 10 μg/ml plus γ-radiation during S and G2/S phases of the cell cycle. Why there is a difference in the results of Thresiamma et al. (1998) and that of Araujo et al. (1999) is unclear.

Inano and Onoda (2002) investigated the radioprotective action of curcumin on the formation of urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG), tumorigenesis, and mortality induced by gamma-ray irradiation. The evaluation of the protective action of dietary curcumin (1%, w/w) against the long-term effects revealed that curcumin (1%, w/w) significantly decreased the incidence

of mammary and pituitary tumors. However, the experiments on survival revealed that curcumin was not effective when administered for 3 d before and/or 3 d after irradiation (9.6 Gy). These findings demonstrate that curcumin can be used as an effective radioprotective agent to inhibit acute and chronic effects, but not mortality, after irradiation.

How curcumin exactly provides radioprotection is not fully understood. There are studies, however, that indicate that curcumin can inhibit the radiation-induced damage of specific proteins (Kapoor and Priyadarsini, 2001). Varadkar et al. (2001) examined the effect of curcumin on radiation-induced PKC activity isolated from the liver cytosol and the particulate fraction of unirradiated mice and mice irradiated at 5 Gy. Following irradiation, the PKC activity was increased in both cytosolic and particulate fractions. Curcumin was found to inhibit the activated cytosolic and particulate PKC at very low concentrations. Since activation of PKC is one of the means of conferring radioresistance on a tumor cell, suppression of PKC activity by curcumin may be one of the means of preventing the development of radioresistance following radiotherapy.

Another potential mechanism of radioprotection involves suppression of radiation-induced gene expression. Oguro and Yoshida (2001) examined the effect of curcumin on UVA-induced ODC and metallothionein gene expression in mouse skin. They showed that UVA induced metallothionein mRNA in mouse skin, and 1,4-Diazabicylo-[2,2,2]-octane (DABCO), a singlet oxygen scavenger, reduced UVA-mediated induction of MT mRNA (by 40%). UVA slightly enhanced TPA-mediated ODC mRNA induction, while it enhanced ODC enzyme activity by 70%. UVA additively intensified TPA-mediated MT mRNA induction. Curcumin dramatically inhibited both TPA- and TPA + UVA-induced expression of ODC and MT genes.

## 10.8 CARDIOVASCULAR DISEASES

### 10.8.1 Effect of Curcumin on Atherosclerosis and MI

The effect of curcumin on MI in the cat and the rat has been investigated (Dikshit et al., 1995; Nirmala and Puvanakrishnan, 1996; Nirmala et al., 1999). Dikshit et al. (1995) examined the prevention of ischemia-induced biochemical changes by curcumin in the cat heart. Myocardial ischemia was induced by the ligation of the left descending coronary artery. Curcumin (100 mg/kg, i.p.) was given 30 min before ligation. Cats were killed and hearts were removed 4 h after coronary artery ligation. Levels of GSH, malonaldelhyde (MDA), myeloperoxidase (MPO), SOD, catalase, and lactate dehydrogenase (LDH) were estimated in the ischemic and nonischemic zones. Curcumin protected the animals against decrease in the heart rate and blood pressure following ischemia. In the ischemic zone, after 4 h of ligation, an increase in the level of MDA and activities of MPO and SOD (cytosolic fraction) were observed. Curcumin pretreatment prevented the ischemia-induced elevation in MDA contents and LDH release but did not affect the increase in MPO activity. Thus, curcumin prevented ischemia-induced changes in the cat heart.

### 10.8.2 Curcumin Inhibits Proliferation of Vascular Smooth Muscle Cells

The proliferation of peripheral blood mononuclear cells (PBMC) and vascular smooth muscle cells (VSMC) is a hallmark of atherosclerosis. Huang et al. (1992a) investigated the effects of curcumin on the proliferation of PBMC and VSMC from the uptake of [$^3$H] thymidine. Curcumin dose dependently inhibited the response to phytohemagglutinin and the mixed lymphocyte reaction in human PBMC at dose ranges of 1 to 30 μM and 3 to 30 μM, respectively. Curcumin (1–100 μM) dose dependently inhibited the proliferation of rabbit VSMC stimulated by fetal calf serum. Curcumin had a greater inhibitory effect on platelet-derived growth factor-stimulated proliferation than on serum-stimulated proliferation. Analogues of curcumin (cinnamic acid, coumaric acid, and ferulic acid) were much less effective than curcumin as inhibitors of serum-induced smooth muscle

cell proliferation. This suggested that curcumin may be useful for the prevention of the pathological changes associated with atherosclerosis and restenosis.

Chen and Huang (1998) examined the possible mechanisms underlying curcumin's antiproliferative and apoptotic effects using the rat VSMC cell line A7r5. Curcumin (1–100 μM) inhibited serum-stimulated [$^3$H] thymidine incorporation of both A7r5 cells and rabbit VSMC. Cell viability, as determined by the trypan blue dye exclusion method, was unaffected by curcumin at the concentration range 1 to 10 μM in A7r5 cells. However, the number of viable cells after 100 μM curcumin treatment was less than the basal value. Following curcumin (1–100 μM) treatment, cell cycle analysis revealed a G0/G1 arrest and a reduction in the percentage of cells in S phase. Curcumin at 100 μM also induced cell apoptosis, as demonstrated by hematoxylin-eosin staining, TdT-mediated dUTP nick end labeling, DNA laddering, cell shrinkage, chromatin condensation, and DNA fragmentation. The membranous protein tyrosine kinase activity stimulated by serum in A7r5 cells was significantly reduced by curcumin (10–100 μM). On the other hand, PMA-stimulated cytosolic PKC activity was reduced by 100 μM curcumin. The levels of c-myc mRNA and bcl-2 mRNA were significantly reduced by curcumin but had little effect on the p53 mRNA level. These results demonstrate that curcumin inhibited cell proliferation, arrested cell cycle progression, and induced cell apoptosis in VSMC. These results may explain how curcumin prevents the pathological changes of atherosclerosis and postangioplasty restenosis.

### 10.8.3 Curcumin Lowers Serum Cholesterol Level

Numerous studies suggest that curcumin lowers serum cholesterol levels (Rao et al., 1970; Patil and Srinivasan, 1971; Keshavarz, 1976; Soudamini et al., 1992; Hussain and Chandrasekhara, 1992; Soni and Kuttan, 1992; Hussain and Chandrasekhara, 1994). Soudamini et al. (1992) investigated the effect of oral administration of curcumin on serum cholesterol levels and on LPO in the liver, lung, kidney, and brain of mice treated with carbon tetrachloride, paraquat, and cyclophosphamide. Oral administration of curcumin significantly lowered the increased peroxidation of lipids in these tissues, produced by these chemicals. Administration of curcumin also significantly lowered the serum and tissue cholesterol levels in these animals, indicating that the use of curcumin helps in conditions associated with peroxide-induced injury, such as liver damage and arterial diseases. Soni and Kuttan (1992) examined the effect of curcumin administration in reducing the serum levels of cholesterol and lipid peroxides in ten healthy human volunteers receiving 500 mg of curcumin per day for 7 d. A significant decrease in the level of serum lipid peroxides (33%), an increase in high-density lipoproteins (HDL)-cholesterol (29%), and a decrease in total serum cholesterol (12%) were noted. Because curcumin reduced serum lipid peroxides and serum cholesterol, the study of curcumin as a chemopreventive substance against arterial diseases was suggested.

*Curcuma xanthorrhiza* Roxb., a medicinal plant used in Indonesia (known as *temu lawak* or Javanese turmeric), has been shown to exert diverse physiological effect. However, little attention has been paid to its effect on lipid metabolism. Yasni et al. (1993) investigated the effects of *C. xanthorrhiza* on serum and liver lipids, serum HDL cholesterol, apolipoprotein, and liver lipogenic enzymes. In rats given a cholesterol-free diet, *C. xanthorrhiza* decreased the concentrations of serum triglycerides, phospholipids, and liver cholesterol and increased the concentrations of serum HDL-cholesterol and apolipoproteins. The activity of liver fatty acid synthase, but not glycerophosphate dehydrogenase, was decreased by the medicinal plant. In rats on a high-cholesterol diet, *C. xanthorrhiza* did not suppress the elevation of serum cholesterol, although it did decrease liver cholesterol. Curcuminoids prepared from *C. xanthorrhiza* had no significant effects on the serum and liver lipids. These studies, therefore, indicate that *C. xanthorrhiza* contains an active principle other than the curcuminoids that can modify the metabolism of lipids and lipoproteins.

In later studies Yasni et al., (1994) identified the major component (approximately 65%) of the essential oil as alpha-curcumene. Addition of essential oils (0.02%), prepared by steam distillation, to a purified diet lowered hepatic triglyceride concentration without influencing serum triglyceride

levels, whereas addition of the hexane-soluble fraction (0.5%) lowered the concentration of serum and hepatic triglycerides. Rats fed with the essential oil and hexane-soluble fraction had lower hepatic fatty acid synthase activity. The fraction containing β-curcumene, prepared from the hexane-soluble fraction by silica gel column chromatography, suppressed the synthesis of fatty acids from [$^{14}$C]-acetate in primary cultured rat hepatocytes.

Skrzypczak-Jankun et al. (2003) showed the 3D structural data and explained how curcumin interacts with the fatty acid–metabolizing enzyme, soybean lipoxygenase. Curcumin binds lipoxygenase in a noncompetitive manner. Trapped in that complex, it undergoes photodegradation in response to x-rays, but utilizes enzyme catalytic ability to form the peroxy complex Enz-Fe-O-O-R as 4-hydroperoxy-2-methoxy-phenol, which later is transformed into 2-methoxycyclohexa-2, 5-diene-1,4-dione. However, when Rukkumani et al. (2002) compared the effects of curcumin and photo-irradiated curcumin on alcohol and polyunsaturated fatty acid-induced hyperlipidemia, they found that photo-irradiated curcumin was more effective than curcumin in treating the above pathological conditions.

### 10.8.4 Curcumin Inhibits LDL oxidation

The oxidation of low-density lipoproteins (LDL) plays an important role in the development of atherosclerosis. Atherosclerosis is characterized by oxidative damage, which affects lipoproteins, the walls of blood vessels, and subcellular membranes. Several studies suggest that curcumin inhibits oxidation of LDL (Asai et al., 2001; Ramirez-Tortosa et al., 1999; Naidu and Thippeswamy, 2002; Quiles et al., 1998). Naidu and Thippeswamy (2002) examined the effect of curcumin on copper ion-induced LPO of human LDL by measuring the formation of thiobarbituric acid reactive substance (TBARS) and relative electrophoretic mobility of LDL on agarose gel. Curcumin inhibited the formation of TBARS effectively throughout the incubation period of 12 h and decreased the relative electrophoretic mobility of LDL. Curcumin at 10 μM produced 40 to 85% inhibition of LDL oxidation. The inhibitory effect of curcumin was comparable to that of BHA (butylated hydroxyanisole), but more potent than ascorbic acid. Further, curcumin significantly inhibited both initiation and propagation phases of LDL oxidation.

Ramirez-Tortosa et al. (1999) evaluated the effect of curcumin on LDL oxidation susceptibility and plasma lipids in atherosclerotic rabbits. A total of 18 rabbits were fed for 7 weeks on a diet containing 95.7% standard chow, 3% lard, and 1.3% cholesterol, to induce atherosclerosis. The rabbits were divided into groups, two of which were also orally treated with turmeric extract at doses of 1.66 (group A) and 3.2 (group B) mg/kg body weight. A third group (group C) acted as an untreated control. Plasma and LDL lipid composition, plasma alpha-tocopherol, plasma retinol, LDL TBARS, LDL lipid hydroperoxides were assayed, and aortic atherosclerotic lesions were evaluated. The low but not the high dosage of turmeric extracts decreased the susceptibility of rabbit LDL to LPO. Both doses produced lower levels of total plasma cholesterol than the control group. Moreover, the lower dosage group had lower levels of cholesterol, phospholipids, and triglycerides than the 3.2-mg dosage.

Quiles et al. (1998) evaluated the antioxidant capacity of a turmeric extract on the LPO of liver mitochondria and microsome membranes in atherosclerotic rabbits. Male rabbits fed 3% (w/w) lard and 1.3% (w/w) cholesterol diet was randomly assigned to three groups. Two groups were treated with different dosages of a turmeric extract (A and B) and the third group (control) with a curcumin-free solution. Basal and in vitro 2,2′-azobis (2-amidinopropane) dihydrochloride–induced hydroperoxide and TBARS production in liver mitochondria and microsomes were analyzed. Group A had the lowest concentration of mitochondrial hydroperoxides. In microsomes, the basal hydroperoxide levels were similar in all groups but, after the induction of oxidation, group C registered the highest value; TBARS production followed the same trend in mitochondria. These findings suggest that active compounds in turmeric extract may be protective against lipoperoxidation of subcellular membranes in a dosage-dependent manner.

Asai et al. (2001) examined the effect of curcumin on lipid metabolism in rats fed a control, moderately high-fat diet (15 g soybean oil/100 g diet), and those given supplements of 0.2 g curcuminoids/100 g diet. Liver triacylglycerol and cholesterol concentrations were significantly lower in rats fed curcumin than in control rats. Plasma triacylglycerols in the very low-density lipoproteins fraction were also lower in curcumin-fed rats than in control ($P < 0.05$). Hepatic acyl-CoA oxidase activity of the curcumin group was significantly higher than that of the control. Furthermore, epididymal adipose tissue weight was significantly reduced with curcuminoid intake in a dose-dependent manner. These results indicated that dietary curcuminoids have lipid-lowering potency in vivo, probably due to alterations in fatty acid metabolism.

### 10.8.5 Curcumin Inhibits Platelet Aggregation

Shah et al. (1999) studied the mechanism of the antiplatelet action of curcumin. They found that curcumin inhibited platelet aggregation mediated by the platelet agonist's epinephrine (200 μM), ADP (4 μM), platelet-activating factor (PAF: 800 nM), collagen (20 μg/ml), and arachidonic acid (AA: 0.75 mM). Curcumin preferentially inhibited PAF- and AA-induced aggregation (IC50: 25-20 μM), whereas much higher concentrations of curcumin were required to inhibit aggregation induced by other platelet agonists. Pretreatment of platelets with curcumin resulted in inhibition of platelet aggregation induced by calcium ionophore A-23187 (IC50: 100 μM), but curcumin up to 250 μM had no inhibitory effect on aggregation induced by the PKC activator phorbol myrsitate acetate (1 μM). Curcumin (100 μM) inhibited the A-23187–induced mobilization of intracellular $Ca_2^+$ as determined by using fura-2 acetoxymethyl ester. Curcumin also inhibited the formation of thromboxane A2 (TXA2) by platelets (IC50: 70 μM). These results suggest that the curcumin-mediated preferential inhibition of PAF- and AA-induced platelet aggregation involves inhibitory effects on TXA2 synthesis and $Ca_2^+$ signaling, but without the involvement of PKC.

## 10.9 CURCUMIN STIMULATES MUSCLE REGENERATION

Skeletal muscle is often the site of tissue injury due to trauma, disease, developmental defects, or surgery. Yet, to date, no effective treatment is available to stimulate the repair of skeletal muscle. Thaloor et al. (1999) investigated the kinetics and extent of muscle regeneration in vivo after trauma, following systemic administration of curcumin to mice. Biochemical and histological analyses indicated faster restoration of normal tissue architecture in mice treated with curcumin after only 4 d of daily intraperitoneal injection, whereas controls required over 2 weeks to restore normal tissue architecture. Curcumin acted directly on cultured muscle precursor cells to stimulate both cell proliferation and differentiation under appropriate conditions. The authors suggested that this effect of curcumin was mediated through suppression of NF-κB; inhibition of NF-κB–mediated transcription was confirmed using reporter gene assays. They concluded that NF-κB exerts a role in regulating myogenesis, and that modulation of NF-κB activity within muscle tissue is beneficial for muscle repair. The striking effects of curcumin on myogenesis suggest therapeutic applications for treating muscle injuries.

## 10.10 CURCUMIN ENHANCES WOUND HEALING

Tissue repair and wound healing are complex processes that involve inflammation, granulation, and remodeling of the tissue. Perhaps, the earliest report that curcumin has wound-healing activity was reported by Gujral and coworkers (Srimal and Dhawan, 1973). Sidhu et al. (1998) examined the wound-healing capacity of curcumin in rats and guinea pigs. Punch wounds in curcumin-treated animals closed faster in treated than in untreated animals. Biopsies of the wound showed reepithelialization of the epidermis and increased migration of various cells including myofibroblasts, fibroblasts, and macrophages in the wound bed. Multiple areas within the dermis showed extensive neovascularization, and Masson's trichrome staining showed greater collagen deposition in cur-

cumin-treated wounds. Immunohistochemical localization showed an increase of transforming growth factor beta 1 (TGF-β1) in curcumin-treated wounds as compared with untreated wounds. *In situ* hybridization and PCR analysis also showed an increase in the mRNA transcripts of TGF-β1 and fibronectin in curcumin-treated wounds. Because TGF-β1 is known to enhance wound healing, it is possible that curcumin modulates TGF-β1 activity.

To further understand its therapeutic effect on wound healing, the antioxidant effects of curcumin on $H_2O_2$ and hypoxanthine-xanthine oxidase-induced damage to cultured human keratinocytes and fibroblasts were investigated by Phan et al. (2001). Cell viability was assessed by colorimetric assay and quantification of LDH release. Exposure of human keratinocytes to curcumin at 10 μg/mL significantly protected against the keratinocytes from $H_2O_2$-induced oxidative damage. Interestingly, exposure of human dermal fibroblasts to curcumin at 2.5 μg/ml showed significant protective effects against $H_2O_2$. No protective effects of curcumin on either fibroblasts or keratinocytes against hypoxanthine-xanthine oxidase-induced damage were found. These investigators thus concluded that curcumin indeed possessed powerful inhibitory capacity against $H_2O_2$-induced damage in human keratinocytes and fibroblasts and that this protection may contribute to wound healing.

Mani et al. (2002) investigated the effect of curcumin treatment by topical application in dexamethasone-impaired cutaneous healing in a full-thickness punch wound model in rats. They assessed healing in terms of histology, morphometry, and collagenization on the fourth and seventh days postwounding and analyzed the regulation of TGF-β1, its receptors type I (tIrc) and type II (tIIrc) and iNOS. Curcumin significantly accelerated healing of wounds with or without dexamethasone treatment as revealed by a reduction in the wound width and gap length compared to controls. Curcumin treatment enhanced expression of TGF-β1 and TGF-β tIIrc in both normal and impaired healing wounds. Macrophages in the wound bed showed an enhanced expression of TGF-β1 mRNA in curcumin-treated wounds as evidenced by *in situ* hybridization. iNOS levels were increased, following curcumin treatment in unimpaired wounds, but not so in the dexamethasone-impaired wounds. Their study indicated an enhancement in dexamethasone-impaired wound repair by topical curcumin and its differential regulatory effect on TGF-β1, its receptors, and iNOS in this cutaneous wound-healing model.

## 10.11 CURCUMIN SUPPRESSES SYMPTOMS ASSOCIATED WITH ARTHRITIS

Deodhar et al. (1980) were the first to report on the antirheumatic activity of curcumin in human subjects. They performed a short-term double blind crossover study in 18 patients with "definite" rheumatoid arthritis to compare the antirheumatic activity of curcumin (1200 mg/day) with phenylbutazone (300 mg/day). Subjective and objective assessment in patients who were taking corticosteroids just prior to the study showed significant ($P < 0.05$) improvements in morning stiffness, walking time, and joint swelling, following two weeks of curcumin therapy.

Liacini et al. (2002) examined the effect of curcumin in articular chondrocytes. IL-1, the main cytokine instigator of cartilage degeneration in arthritis, induces MMP-3 and MMP-13 RNA and protein in chondrocytes through the activation of mitogen-activated protein kinase (MAPK), AP-1, and NF-κB transcription factors. Curcumin achieved 48 to 99% suppression of MMP-3 and 45 to 97% of MMP-13 in human and 8 to 100% (MMP-3) and 32 to 100% (MMP-13) in bovine chondrocytes. Inhibition of IL-1 signal transduction by these agents could be useful for reducing cartilage resorption by MMPs in arthritis.

## 10.12 CURCUMIN REDUCES THE INCIDENCE OF CHOLESTEROL GALL STONE FORMATION

Hussain and Chandrasekhara (1992) studied the efficacy of curcumin in reducing the incidence of cholesterol gallstones induced by feeding a lithogenic diet in young male mice. Feeding a

lithogenic diet supplemented with 0.5% curcumin for 10 weeks reduced the incidence of gallstone formation to 26%, as compared to 100% incidence in the group fed with the lithogenic diet alone. Biliary cholesterol concentration was also significantly reduced by curcumin feeding. The lithogenic index, which was 1.09 in the cholesterol-fed group, was reduced to 0.43 in the 0.5% curcumin-supplemented group. Further, the cholesterol:phospholipid ratio of bile was also reduced significantly when 0.5% curcumin supplemented diet was fed. A dose–response study with 0.2%, 0.5%, and 1% curcumin-supplemented lithogenic diets showed that 0.5% curcumin was more effective than a diet with 0.2% or 1% curcumin. How curcumin mediates antilithogenic effects in mice was further investigated by this group (Hussain and Chandrasekhara, 1994). For this purpose, the hepatic bile of rats was fractionated by gel filtration chromatography, and the low-molecular weight (LMW) protein fractions were tested for their ability to influence cholesterol crystal growth in model bile. The LMW protein fraction from the lithogenic agent-fed control group's bile shortened the nucleation time and increased the crystal growth rate and final crystal concentration. But with the LMW protein fractions from the bile of rats given curcumin, the nucleation times were prolonged, and the crystal growth rates and final crystal concentrations were decreased. The LMW fractions were further purified into three different sugar-specific proteins by affinity chromatography. A higher proportion of LMW proteins from the control group bile was bound to Con-A, whereas higher proportions of LMW proteins from the groups fed with curcumin were bound to wheat germ agglutinin (WGA) and *Helix pomatia* lectin. The Con-A–bound fraction obtained from the control group showed a pronucleating effect. In contrast, the WGA-bound fraction obtained from curcumin group showed a potent antinucleating activity.

## 10.13 CURCUMIN MODULATES MS

MS is an inflammatory disease of the central nervous system (CNS), which afflicts more than 1 million people worldwide. The destruction of oligodendrocytes and myelin sheath in the CNS is the pathological hallmark of MS. MS is an inflammatory autoimmune disease of the CNS resulting from myelin antigen-sensitized T-cells in the CNS. Experimental allergic encephalomyelitis (EAE), a CD4+ Th1 cell-mediated inflammatory demyelinating autoimmune disease of the CNS, serves as an animal model for MS. IL-12 plays a crucial proinflammatory role in the induction of neural antigen-specific Th1 differentiation and pathogenesis of CNS demyelination in EAE and MS.

Natarajan and Bright (2002) investigated the effect of curcumin on the pathogenesis of CNS demyelination in EAE. *In vivo* treatment of SJL/J mice with curcumin significantly reduced the duration and clinical severity of active immunization and adoptive transfer EAE (Natarajan and Bright, 2002). Curcumin inhibited EAE in association with a decrease in IL-12 production from macrophage/microglial cells and differentiation of neural antigen-specific Th1 cells. *In vitro* treatment of activated T-cells with curcumin inhibited IL-12–induced tyrosine phosphorylation of Janus kinase 2, tyrosine kinase 2, and STAT3 and STAT4 transcription factors. The inhibition of Janus kinase–STAT pathway by curcumin resulted in a decrease in IL-12–induced T-cell proliferation and Th1 differentiation. These findings show that curcumin inhibits EAE by blocking IL-12 signaling in T-cells and suggest its use in the treatment of MS and other Th1 cell-mediated inflammatory diseases.

Verbeek and coworkers (2005) examined the effects of oral flavonoids as well as of curcumin on autoimmune T-cell reactivity in mice and on the course of experimental autoimmune encephalomyelitis (EAE), a model for MS. Continuous oral administration of flavonoids significantly affected antigen-specific proliferation and IFN-gamma production by lymph node–derived T-cells following immunization with an EAE-inducing peptide (Verbeek et al., 2005). The effects of curcumin on EAE were assessed using either passive transfer of autoimmune T-cells or active disease induction. In passive EAE, curcumin led to delayed recovery of clinical symptoms rather than to any reduction in disease. Oral curcumin had overall mild but beneficial effects.

## 10.14 CURCUMIN BLOCKS THE REPLICATION OF HIV

Transcription of type 1 HIV-1 provirus is governed by the viral long-terminal repeat (LTR). Drugs can block HIV-1 replication by inhibiting the activity of its LTR. Li et al. examined the effect of curcumin on HIV-1 LTR-directed gene expression and virus replication (Abraham et al., 1993). Curcumin was found to be a potent and selective inhibitor of HIV-1 LTR-directed gene expression, at concentrations that have minor effects on cells. Curcumin inhibited p24 antigen production in cells either acutely or chronically infected with HIV-1 through transcriptional repression of the LTR. Sui et al. (1993) examined the effect on the HIV-1 and HIV-2 proteases by curcumin and curcumin–boron complexes. Curcumin was a modest inhibitor of HIV-1 (IC50 = 100 μM) and HIV-2 ($IC_{50}$ = 250 μM) proteases. Simple modifications of the curcumin structure raised the IC50 value, but complexes of the central dihydroxy groups of curcumin with boron lowered the $IC_{50}$ to a value as low as 6 μM. The boron complexes were also time-dependent inactivators of the HIV proteases. The increased affinity of the boron complexes may reflect binding of the orthogonal domains of the inhibitor in intersecting sites within the substrate-binding cavity of the enzyme, while activation of the α, β-unsaturated carbonyl group of curcumin by chelation to boron probably accounts for time-dependent inhibition of the enzyme.

Mazumder et al. (1997) examined the effect of curcumin analogs with altered potencies against HIV-1 integrase. They reported that curcumin inhibited HIV-1 integrase activity. They also synthesized and tested analogs of curcumin to explore the structure–activity relationships and mechanism of action of this family of compounds in more detail. They found that two curcumin analogs, dicaffeoylmethane and rosmarinic acid, inhibited both activities of integrase, for IC50 values below 10 μM. They demonstrated that lysine 136 may play a role in viral DNA binding and that two curcumin analogs had equivalent potencies against both an integrase mutant and a wild-type integrase, suggesting that the curcumin-binding site and the substrate-binding site may not overlap. Combining one curcumin analog with the recently described integrase inhibitor NSC 158393 resulted in integrase inhibition that was synergistic, again suggesting that drug-binding sites may not overlap. They also determined that these analogs could inhibit binding of the enzyme to the viral DNA, but that this inhibition is independent of divalent metal ion. Furthermore, kinetic studies of these analogs suggest that they bound to the enzyme at a slow rate. These studies can provide mechanistic and structural information to guide the future design of integrase inhibitors.

The transcription of HIV-1 provirus is regulated by both cellular and viral factors. Various pieces of evidence suggest that Tat protein secreted by HIV1-infected cells may have additional activity in the pathogenesis of AIDS because of its ability to also be taken up by noninfected cells. Barthelemy et al. (1998) showed that curcumin used at 10 to 100 nM inhibited Tat transactivation of HIV1-LTR lacZ by 70 to 80% in He la cells. To develop more efficient curcumin derivatives, the researchers synthesized and tested in the same experimental system the inhibitory activity of reduced curcumin (C1), which lacks the spatial structure of curcumin; allyl-curcumin (C2), which possesses a condensed allyl derivative on curcumin that plays the role of metal chelator; and tocopheryl-curcumin (C3), whose structural alterations enhances the antioxidant activity of the molecule. Results obtained with the C1, C2, and C3 curcumin derivatives showed a significant inhibition (70 to 85%) of Tat transactivation. Despite the fact that tocopheryl–curcumin (C3) failed to scavenge $O^{2-}$, this curcumin derivative exhibited the most activity; 70% inhibition was obtained at 1 nM, whereas only 35% inhibition was obtained with the curcumin.

Acetylation of histones and nonhistone proteins is an important posttranslational modification involved in the regulation of gene expression in eukaryotes and all viral DNA that integrates into the human genome (e.g., the HIV). Dysfunction of histone acetyltransferases (HATs) is often associated with the manifestation of several diseases. In this respect, HATs are the new potential targets for the design of therapeutics. Balasubramanyam and coworkers (2004) report that curcumin is a specific inhibitor of the p300/CBP HAT activity but not of p300/CBP-associated factor, *in vitro* and *in vivo*. Furthermore, curcumin could also inhibit the p300-mediated acetylation of p53 *in vivo*.

It specifically represses the p300/CBP HAT activity-dependent transcriptional activation from chromatin but not a DNA template Balasubramanyam et al., 2004). It is significant that curcumin could inhibit the acetylation of HIV–Tat protein*in vitro* by p300 as well as proliferation of the virus, as revealed by the repression in syncytia formation upon curcumin treatment in SupT1 cells. Thus, nontoxic curcumin, which targets p300/CBP, may serve as a lead compound in combinatorial HIV therapeutics.

## 10.15 CURCUMIN AFFECTS ALZHEIMER'S DISEASE

Brain inflammation in Alzheimer's disease (AD) patients is characterized by increased cytokines and activated microglia. Epidemiological studies suggest reduced AD risk is associated with long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs). Whereas chronic ibuprofen suppressed inflammation and plaque-related pathology in an Alzheimer transgenic APPSw mouse model (Tg2576), excessive use of NSAIDs targeting cyclooxygenase I can cause gastrointestinal, liver, and renal toxicity. One alternative NSAID is curcumin. Lim et al. (2001) found that curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse model. To evaluate whether it could affect Alzheimer-like pathology in the APPSw mice, they tested the effect of a low (160 ppm) and a high (5000 ppm) dose of dietary curcumin on inflammation, oxidative damage, and plaque pathology. Low and high doses significantly lowered oxidized proteins and IL-1β, a proinflammatory cytokine usually elevated in the brains of these mice. With low-dose, but not high-dose, curcumin treatment, the astrocytic marker glial fibrillary acidic protein was reduced, and insoluble beta-amyloid (Aβ), soluble Aβ, and plaque burden were significantly decreased, by 43 to 50%. However, levels of amyloid precursor in the membrane fraction were not reduced. Microgliosis was also suppressed in neuronal layers but not adjacent to plaques. In view of its efficacy and apparent low toxicity, this Indian spice component has promise for the prevention of Alzheimer's disease.

Ono's group (2004) reported previously that nordihydroguaiaretic acid (NDGA) inhibits fAbeta formation from Abeta(1–40) and Abeta(1–42) and destabilize preformed fAbeta(1–40) and fAbeta(1–42) dose dependently *in vitro*. Using fluorescence spectroscopic analysis with thioflavin T and electron microscopic studies, they examined the effects of curcumin on the formation, extension, and destabilization of fAbeta(1-40) and fAbeta(1-42) at pH 7.5 at 37°C *in vitro*. They next compared the antiamyloidogenic activities of curcumin with NDGA. Curcumin dose dependently inhibited fAbeta formation from Abeta(1-40) and Abeta(1-42) as well as their extension. In addition, it dose dependently destabilized preformed fAbetas. The overall activities of curcumin and NDGA were similar. The effective concentrations EC50 of curcumin and NDGA for the formation, extension, and destabilization of fAbetas were in the order of 0.1 to 1 μM. Although the mechanism by which curcumin inhibits fAbeta formation from Abeta and destabilize preformed fAbeta *in vitro* remains unclear; they could be a key molecule for the development of therapeutics for AD (Ono et al., 2004).

Yang and coworkers (2005) investigated whether its efficacy in AD models could be explained by effects on Abeta aggregation. Under aggregating conditions *in vitro*, curcumin inhibited aggregation IC50 = 0.8 μM] as well as disaggregated fibrillar Abeta40 (IC50 = 1 μM)], indicating favorable stoichiometry for inhibition. Curcumin was a better Abeta40 aggregation inhibitor than ibuprofen and naproxen, and prevented Abeta42 oligomer formation and toxicity between 0.1 and 1.0 μM. Under EM, curcumin decreased dose dependently Abeta fibril formation beginning with 0.125 μM. The effects of curcumin did not depend on Abeta sequence but on fibril-related conformation. AD and Tg2576 mice brain sections incubated with curcumin revealed preferential labeling of amyloid plaques. *In vivo* studies showed that curcumin injected peripherally into aged Tg mice crossed the blood–brain barrier and bound plaques. When fed to aged Tg2576 mice with advanced amyloid accumulation, curcumin labeled plaques and reduced amyloid levels and plaque burden. Hence, curcumin directly binds small beta-amyloid species to block aggregation and fibril formation *in vitro* and *in vivo*. These data suggest that low-dose curcumin effectively disaggregates Abeta as

well as prevents fibril and oligomer formation, supporting the rationale for curcumin use in clinical trials preventing or treating AD.

## 10.16 CURCUMIN PROTECTS AGAINST CATARACT FORMATION IN LENSES

Age-related cataractogenesis is a significant health problem worldwide. Oxidative stress has been suggested to be a common underlying mechanism of cataractogenesis, and augmentation of the antioxidant defenses of the ocular lens has been shown to prevent or delay cataractogenesis. Awasthi et al. (1996) tested the efficacy of curcumin in preventing cataractogenesis in an *in vitro* rat model. Rats were maintained on an AIN-76 diet for 2 weeks, after which they were given a daily dose of corn oil alone or 75 mg curcumin/kg in corn oil for 14 d. Their lenses were removed and cultured for 72 h in vitro in the presence or absence of 100 μmol 4-hydroxy-2-nonenal (4-HNE)/L, a highly electrophilic product of LPO. The results of these studies showed that 4-HNE led to opacification of cultured lenses as indicated by the measurements of transmitted light intensity using digital image analysis. However, the lenses from curcumin-treated rats were resistant to 4-HNE-induced opacification. Curcumin treatment significantly induced the GST isozyme rGST8-8 in rat lens epithelium. Because rGST8-8 utilizes 4-HNE as a preferred substrate, we suggest that the protective effect of curcumin may be mediated through the induction of this GST isozyme. These studies suggest that curcumin may be an effective protective agent against cataractogenesis induced by LPO.

Suryanarayana and coworkers (2003) investigated the effect of curcumin on galactose-induced cataractogenesis in rats. The data indicated that curcumin at 0.002% (group C) delayed the onset and maturation of cataract. In contrast, even though there was a slight delay in the onset of cataract at the 0.01% level (group D), maturation of cataract was faster when compared to group B. Biochemical analysis showed that curcumin at the 0.002% level appeared to exert antioxidant and antiglycating effects, as it inhibited LPO, AGE (advanced glycated end products)-fluorescence, and protein aggregation. Though the reasons for faster onset and maturation of cataract in group D rats were not clear, the data suggested that, under hyperglycemic conditions, higher levels of curcumin (0.01%) in the diet may increase oxidative stress, AGE formation, and protein aggregation. However, feeding of curcumin to normal rats up to a 0.01% level did not result in any changes in lens morphology or biochemical parameters. These results suggest that curcumin is effective against galactose-induced cataract only at very low amounts (0.002%) in the diet. On the other hand, at and above a 0.01% level, curcumin seems to not be beneficial under hyperglycemic conditions, at least with the model of galactose-cataract.

Suryanarayana and coworkers (2005) reported that turmeric and curcumin are effective against the development of diabetic cataract in rats. Although, both curcumin and turmeric did not prevent streptozotocin-induced hyperglycemia, as assessed by blood glucose and insulin levels, slit lamp microscope observations indicated that these supplements delayed the progression and maturation of cataract. The present studies suggest that curcumin and turmeric treatment appear to have countered the hyperglycemia-induced oxidative stress, because there was a reversal of changes with respect to LPO, reduced glutathione, protein carbonyl content, and activities of antioxidant enzymes in a significant manner. Also, treatment with turmeric or curcumin appears to have minimized osmotic stress, as assessed by polyol pathway enzymes. Most important, aggregation and insolubilization of lens proteins due to hyperglycemia was prevented by turmeric and curcumin. Turmeric was more effective than its corresponding levels of curcumin. Further, these results imply that ingredients in the study's dietary sources, such as turmeric, may be explored for anticataractogenic agents that prevent or delay the development of cataract.

Padmaja's group investigated that Wistar rat pups treated with curcumin before being administered with selenium showed no opacities in the lens. TheLPO, xanthine oxidase enzyme levels in the lenses of curcumin, and selenium-cotreated animals were significantly less when compared to selenium-treated animals. The superoxidase dismutase and catalase enzyme activities of curcumin

and selenium-cotreated animal lenses showed an enhancement. Curcumin cotreatment seems to prevent oxidative damage and found to delay the development of cataract (Padmaja et al., 2004).

## 10.17 CURCUMIN PROTECTS FROM DRUG-INDUCED MYOCARDIAL TOXICITY

Cardiotoxicity is one of the major problems associated with administration of many chemotherapeutic agents. Venkatesan (1998) examined the protective effect of curcumin on acute Adriamycin (ADR) myocardial toxicity in rats. ADR toxicity, induced by a single intraperitoneal injection (30 mg/kg), was revealed by elevated serum creatine kinase (CK) and LDH. The levels of the LPO products, conjugated dienes, and malondialdehyde were markedly elevated by ADR. ADR also caused a decrease in myocardial glutathione content and glutathione peroxidase activity and an increase in cardiac catalase activity. Curcumin treatment (200 mg/kg) 7 d before and 2 d following ADR significantly ameliorated the early manifestation of cardiotoxicity (ST segment elevation and an increase in heart rate) and prevented the rise in serum CK and LDH exerted by ADR. ADR-treated rats that received curcumin displayed a significant inhibition of LPO and augmentation of endogenous antioxidants. These results suggest that curcumin inhibits ADR cardiotoxicity and might serve as novel combination of chemotherapeutic agent with ADR to limit free-radical-mediated organ injury.

## 10.18 CURCUMIN PROTECTS FROM ALCOHOL-INDUCED LIVER INJURY

Because induction of NF-κB–mediated gene expression has been implicated in the pathogenesis of alcoholic liver disease (ALD) and curcumin inhibits the activation of NF-κB, Nanji et al. (2003) determined whether treatment with curcumin would prevent experimental ALD and elucidated the underlying mechanism. Four groups of rats (six rats/group) were treated by intragastric infusion for 4 weeks. One group received fish oil plus ethanol (FE) and a second group received fish oil plus dextrose (FD). The third and fourth groups received FE or FD supplemented with 75 mg/kg/day of curcumin. Liver samples were analyzed for histopathology, LPO, NF–κB binding, TNFα, IL-12, monocyte chemotactic protein-1, macrophage inflammatory protein-2, COX-2, iNOS, and nitrotyrosine. Rats fed FE developed fatty liver, necrosis, and inflammation, which was accompanied by activation of NF-κB and the induction of cytokines, chemokines, COX-2, iNOS, and nitrotyrosine formation. Treatment with curcumin prevented both the pathological and the biochemical changes induced by alcohol. Because endotoxin and the Kupffer cell are implicated in the pathogenesis of ALD, they also investigated whether curcumin suppressed the stimulatory effects of endotoxin in isolated Kupffer cells. Curcumin blocked endotoxin-mediated activation of NF-kappaB and suppressed the expression of cytokines, chemokines, COX-2, and iNOS in Kupffer cells. Thus curcumin prevented experimental ALD, in part, by suppressing induction of NF-κB-dependent genes.

Hepatic fibrogenesis occurs as a wound-healing process after many forms of chronic liver injury. Hepatic fibrosis ultimately leads to cirrhosis if not treated effectively. During liver injury, quiescent HSC, the most relevant cell type, become active and proliferative. Oxidative stress is a major and critical factor for HSC activation. Activation of PPAR-γ inhibits the proliferation of nonadipocytes. The level of PPAR-γ is dramatically diminished along with the activation of HSC during liver injury. Xu et al. (2003) examined the effect of curcumin on HSC proliferation. They hypothesized that curcumin inhibits the proliferation of activated HSC by inducing PPAR-γ gene expression and reviving PPAR-γ activation. Their results indicated that curcumin significantly inhibited the proliferation of activated HSC and induced apoptosis *in vitro*. They also demonstrated, for the first time, that curcumin dramatically induced the expression of the PPAR-γ gene and activated PPAR-γ in activated HSC. Blocking its transactivating activity by a PPAR-γ antagonist markedly abrogated the effects of curcumin on inhibition of cell proliferation. These results provided a novel insight

into mechanisms underlying the inhibition of activated HSC growth by curcumin. The characteristics of curcumin, including antioxidant potential, reduction of activated HSC growth, and no adverse health effects, make it a potential candidate for prevention and treatment of hepatic fibrosis. Recently, Van der Logt et al. (2003) demonstrated that curcumin exerted its anticarcinogenic effects in gastrointestinal cancers through the induction of UDP-glucuronosyltransferase enzymes.

## 10.19 CURCUMIN PROTECTS FROM DRUG-INDUCED LUNG INJURY

Cyclophosphamide causes lung injury in rats through its ability to generate free radicals with subsequent endothelial and epithelial cell damage. Venkatesan and Chandrakasan (1995) examined the effect of curcumin on cyclophosphamide-induced early lung injury. In order to observe the protective effects of curcumin on cyclophosphamide-induced early lung injury, healthy, pathogen-free male Wistar rats were exposed to 20 mg/100 g body weight of cyclophosphamide, given intraperitoneally as a single injection. Prior to cyclophosphamide intoxication, curcumin was administered orally daily for 7 d. At various times (2, 3, 5, and 7 d after insult), serum and lung samples were analyzed for angiotensin-converting enzyme (ACE), LPO, reduced glutathione, and ascorbic acid. Bronchoalveolar lavage fluid (BALF) was analyzed for biochemical constituents. The lavage cells were examined for LPO and glutathione content. Excised lungs were analyzed for antioxidant enzyme levels. Biochemical analyses revealed increased lavage fluid total protein, albumin, ACE, LDH, *N*-acetyl-beta-D-glucosaminidase (NAG), alkaline phosphatase, acid phosphatase, lipid peroxide, GSH, and ascorbic acid levels 2, 3, 5, and 7 d after cyclophosphamide intoxication. Increased levels of LPO and decreased levels of GSH and ascorbic acid were seen in serum, lung tissue, and lavage cells of cyclophosphamide-treated groups. Serum ACE activity increased, which coincided with the decrease in lung tissue levels. Activities of antioxidant enzymes were reduced with time in the lungs of cyclophosphamide-treated groups. A significant reduction in the lavage fluid biochemical constituents and in LPO products in the serum, lung and lavage cells occurred concomitantly with an increase in antioxidant defense mechanisms in curcumin-fed cyclophosphamide rats. Therefore, the study indicated that curcumin is effective in moderating the cyclophosphamide-induced early lung injury.

In another study, Venkatesan et al. (1997) investigated the effect of curcumin on bleomycin (BLM)-induced lung injury. The data indicated that BLM-mediated lung injury resulted in increases in lung lavage fluid biomarkers such as total protein, ACE, LDH, NAG, LPO products, SOD, and catalase. BLM administration also increased the levels of malondialdehyde in BALF and bronchoalveolar lavage (BAL) cells and greater amounts of alveolar macrophage (AM) SOD activity. By contrast, lower levels of reduced GSH were observed in lung lavage fluid, BAL cells, and AM. Stimulated superoxide anion and $H_2O_2$ release by AM from BLM-treated rats were higher. Curcumin treatment significantly reduced lavage fluid biomarkers. In addition, it restored the antioxidant status in BLM rats. These data suggested that curcumin treatment reduces the development of BLM-induced inflammatory and oxidant activity. Therefore, curcumin offers the potential for a novel pharmacological approach in the suppression of drug- or chemical-induced lung injury.

Punithavathi et al., (2000) also evaluated the ability of curcumin to suppress BLM-induced pulmonary fibrosis in rats. A single intratracheal instillation of BLM (0.75 U/100 g, sacrificed 3, 5, 7, 14, and 28 d post-BLM) resulted in significant increases in total cell numbers, total protein, and ACE, and in alkaline phosphatase activities in BALF. Animals with fibrosis had a significant increase in lung hydroxyproline content. AM from BLM-treated rats elaborated significant increases in TNF-$\alpha$, and superoxide and nitric oxide production in culture medium. Interestingly, oral administration of curcumin (300 mg/kg) 10 d before and daily thereafter throughout the experimental time period inhibited BLM-induced increases in total cell counts and biomarkers of inflammatory responses in BALF. In addition, curcumin significantly reduced the total lung hydroxyproline in BLM-treated rats. Furthermore, curcumin remarkably suppressed the BLM-induced AM production

of TNFα, SOD, and nitric oxide. These findings suggest curcumin is a potent anti-inflammatory and antifibrotic agent against BLM-induced pulmonary fibrosis in rats. Punithavathi et al. (2003) also examined whether curcumin prevented amiodarone-induced lung fibrosis in rats. They found that curcumin had a protective effect on amiodarone-induced pulmonary fibrosis. Curcumin inhibited the increases in lung myeloperoxidade activity, TGF-β1 expression, lung hydroxyproline content, and expression of type I collagen and c-Jun protein in amiodarone-treated rats.

Paraquat (PQ), a broad-spectrum herbicide, can cause lung injury in humans and animals. An early feature of PQ toxicity is the influx of inflammatory cells, releasing proteolytic enzymes and oxygen free radicals, which can destroy the lung epithelium and cause pulmonary fibrosis. Suppressing early lung injury before the development of irreversible fibrosis is critical to effective therapy. Venkatesan (2000) showed that curcumin confers remarkable protection against PQ-induced lung injury. A single intraperitoneal injection of PQ (50 mg/kg) significantly increased the levels of protein, angiotensin converting enzyme (ACE), alkaline phosphatase, NAG, and TBARS, and neutrophils in the BALF, while it decreased GSH levels. In PQ-treated rats BAL cells, TBARS concentration was increased at the same time as glutathione content was decreased. In addition, PQ caused a decrease in ACE and glutathione levels and an increase in levels of TBARS and myeloperoxidase activity in the lung. Interestingly, curcumin prevented the general toxicity and mortality induced by PQ and blocked the rise in BALF protein, ACE, alkaline phophatase, NAG, TBARS, and neutrophils. Likewise, it prevented the rise in TBARS content in both BAL cell and lung tissue and MPO activity of the lung, reduced lung ACE, and abolished BAL cell and lung glutathione levels. These findings indicate that curcumin has important therapeutic potential in suppressing PQ lung injury.

Nicotine, a pharmacologically active substance in tobacco, has been identified as a major risk factor for lung diseases. Kalpana's group (Kalpana and Menon, 2004) showed that curcumin exerted its protective effect against nicotine-induced lung toxicity by modulating the biochemical marker enzymes, LPO, and augmenting antioxidant defense system. They evaluated the protective effects of curcumin on LPO and antioxidants status in BALF and BAL of nicotine-treated Wistar rats. Lung toxicity was induced by subcutaneous injection of nicotine at a dose of 2.5 mg/kg body weight (5 d a week, for 22 weeks) and curcumin (80 mg/kg body weight) was given simultaneously by intragastric intubation for 22 weeks. Measurements of biochemical marker enzymes ó alkaline phosphatase, LDH, LPO, and antioxidants ó were used to monitor the antiperoxidative effects of curcumin. The increased biochemical marker enzymes as well as lipid peroxides in BALF and BAL of nicotine treated rats was accompanied by a significant decrease in the levels of glutathione, glutathione peroxidase, superoxide dismutase, and catalase. Administration of curcumin significantly lowered the biochemical marker enzymes, LPO, and enhanced the antioxidant status.

They also showed that curcumin exerts its protective effect against nicotine-induced lung toxicity by modulating the extent of LPO and augmenting antioxidant defense system. They evaluated the protective effects of curcumin on tissue LPO and antioxidants in nicotine-treated Wistar rats. Lung toxicity was induced by subcutaneous injection of nicotine at a dose of 2.5 mg/kg (5 d a week, for 22 weeks). Curcumin (80 mg/kg) was given simultaneously by intragastric intubation for 22 weeks. The enhanced level of tissue lipid peroxides in nicotine-treated rats was accompanied by a significant decrease in the levels of ascorbic acid, vitamin E, reduced glutathione, glutathione peroxidase, superoxide dismutase, and catalase. Administration of curcumin significantly lowered the level of LPO and enhanced the antioxidant status.

## 10.20 CURCUMIN PROTECTS FROM DRUG-INDUCED NEPHROTOXICITY

Nephrotoxicity is another problem observed in patients given chemotherapeutic agents. Venkatesan et al. (Venkatesan, 1998; Venkatesan et al., 2000) showed that curcumin prevents ADR-induced nephrotoxicity in rats. Treatment with curcumin markedly protected against ADR-induced pro-

teinuria, albuminuria, hypoalbuminemia, and hyperlipidemia. Similarly, curcumin inhibited ADR-induced increase in urinary excretion of NAG (a marker of renal tubular injury), fibronectin and glycosaminoglycan, and plasma cholesterol. It restored renal function in ADR-treated rats, as judged by the increase in glomerular filteration rate (GFR). The data also demonstrated that curcumin protected against ADR-induced renal injury by suppressing oxidative stress and increasing kidney glutathione content and glutathione peroxidase activity. In like manner, curcumin abolished ADR-stimulated kidney microsomal and mitochondrial LPO. These data suggest that administration of curcumin is a promising approach in the treatment of nephrosis caused by ADR.

## 10.21 CURCUMIN INHIBITS SCARRING

Keloid and hypertrophic scars commonly occur after injuries. Overproliferation of fibroblasts, overproduction of collagen, and contraction characterize these pathologic scars. Current treatment of excessive scars with intralesional corticosteroid injections used individually or in combination with other methods often have unsatisfactory outcomes, frustrating both the patient and the clinician. Phan et al. (2003) investigated the inhibitory effects of curcumin on keloid fibroblasts (KF) and hypertrophic scar-derived fibroblasts (HSF) by proliferation assays, fibroblast-populated collagen lattice contraction, and electron microscopy. Curcumin significantly inhibited KF and HSF proliferation in a dose- and time-dependent manner. Curcumin seemed to have potent effects in inhibiting proliferation and contraction of excessive scar-derived fibroblasts.

## 10.22 CURCUMIN PROTECTS FROM INFLAMMATORY BOWEL DISEASE

Inflammmatory bowel disease (IBD) is characterized by oxidative and nitrosative stress, leucocyte infiltration, and upregulation of proinflammatory cytokines. Ukil et al. (2003) recently investigated the protective effects of curcumin on 2,4,6- trinitrobenzene sulphonic acid–induced colitis in mice, a model for IBD. Reports showed that curcumin could prevent and improve experimental colitis in murine model with inflammatory bowel disease (IBD) and could be a potential target for the patients with IBD (Jian et al., 2004; 2005).

Intestinal lesions were associated with neutrophil infiltration, increased serine protease activity (may be involved in the degradation of colonic tissue), and high levels of malondialdehyde. Dose–response studies revealed that pretreatment of mice with curcumin at 50 mg/kg daily i.g. for 10 d significantly ameliorated diarrhea and the disruption of colonic architecture. Higher doses (100 and 300 mg/kg) had comparable effects. In curcumin-pretreated mice, there was a significant reduction in the degree of both neutrophil infiltration and LPO in the inflamed colon as well as decreased serine protease activity. Curcumin also reduced the levels of NO and $O_2^-$ associated with the favorable expression of Th1 and Th2 cytokines and inducible NO synthase. Consistent with these observations, NF-κB activation in colonic mucosa was suppressed in the curcumin-treated mice. These findings suggested that curcumin exerts beneficial effects in experimental colitis and may, therefore, be useful in the treatment of IBD.

Salh et al. (2003) also showed that curcumin is able to attenuate colitis in the dinitrobenzene (DNB) sulfonic acid-induced murine model of colitis. When given before the induction of colitis, it reduced macroscopic damage scores and NF-κB activation, reduced myeloperoxidase activity, and attenuated the DNB-induced message for IL-1β. Western blotting analysis revealed a reproducible DNB-induced activation of p38 MAPK in intestinal lysates detected by a phosphospecific antibody. This signal was significantly attenuated by curcumin. Furthermore, the above workers showed that the immunohistochemical signal is dramatically attenuated at the level of the mucosa by curcumin. Thus they concluded that curcumin attenuates experimental colitis through a mechanism that also inhibits the activation of NF-κB and effects a reduction in the activity of p38 MAPK. They proposed that this agent may have therapeutic implications for human IBD.

## 10.23 CURCUMIN ENHANCES THE IMMUNOSUPPRESSIVE ACTIVITY

Chueh et al. (2003) have demonstrated that curcumin enhances the immunosuppressive activity of cyclosporine in rat cardiac allografts and in mixed lymphocyte reactions. Their study demonstrated for the first time the effectiveness of curumin as a novel adjuvant immunosuppressant with cyclosporine both *in vivo* and *in vitro*. The immunosuppressive effects of curcumin were studied in rat heterotopic cardiac transplant models, using Brown–Norway hearts transplanted to WKY hosts. In the Brown–Norway-to-WKY (Winstar-Kyoto) model, curcumin alone significantly increased the mean survival time, to 20.5 to 24.5 d as compared to 9.1 d in nontreated controls. The combination of curcumin and subtherapeutic doses of cyclosporine further prolonged the mean survival time to 28.5 to 35.6 d, better than that of curcumin or cyclosporine alone. Cytokine analysis revealed significantly reduced expression of IL-2, IFNγ and granzyme B in the day 3 specimens of the curcumin and curcumin plus cyclosporine-treated allografts compared with the nontreated allograft controls.

## 10.24 CURCUMIN PROTECTS AGAINST VARIOUS FORMS OF STRESS

Curcumin has been identified as a potent inducer of hemoxygenase-1 (HO-1), a redox-sensitive inducible protein that provides protection against various forms of stress. Curcumin stimulated the expression of Nrf2, an increase associated with a significant increase in HO-1 protein expression and HO-1 activity (Balogun et al., 2005). Chan et al. (2005) reported that curcumin prevented MG-induced cell death and apoptotic biochemical changes such as mitochondrial release of cytochrome-c, caspase-3 activation, and cleavage of poly [ADP-ribose] polymerase (PARP). Using the cell-permeable dye 2′,7′-dichlorofluorescein diacetate (DCF-DA) as an indicator of ROS generation, curcumin abolished MG-stimulated intracellular oxidative stress. The study demonstrates that curcumin significantly attenuates MG-induced ROS formation, and suggest that ROS triggers cytochrome-c release, caspase activation, and subsequent apoptotic biochemical changes. Besides, curcumin effectively blocks the detrimental effects of RTV, which is associated with many cardiovascular complications and causes vascular dysfunction through oxidative stress (Chai et al., 2005).

## 10.25 CURCUMIN PROTECTS AGAINST ENDOTOXIN SHOCK

Madan and Ghosh (2003) have demonstrated that curcumin exerts protective effects in high-dose endotoxin shock by improving survival and reducing the severity of endotoxin shock symptoms such as lethargy, diarrhea, and watery eyes following a challenge with lipopolysaccharide. They demonstrated that curcumin inhibits the transmigration and infiltration of neutrophils from blood vessels to the underlying liver tissue and, hence, inhibits the damage to the tissue. Curcumin blocks the induced expression of ICAM-1 and VCAM-1 in liver and lungs.

## 10.26 CURCUMIN PROTECTS AGAINST PANCREATITIS

Gukovsky et al. (2003) reported that curcumin ameliorates pancreatitis in two rat models. In both, cerulein pancreatitis and pancreatitis induced by a combination of ethanol diet and low-dose curcumin, curcumin decreased the severity of the disease. Curcumin markedly inhibited NF-κB and AP-1, IL-6, TNFα, and iNOS in the pancreas. Based on these studies, Gukovsky et al. (2003) suggested that curcumin may be useful for the treatment of pancreatitis. Pathologic activation of both digestive zymogens and the transcription factor NF-κB are early events in acute pancreatitis; these pathologic processes are inhibited in experimental pancreatitis by curcumin and the pH modulator chloroquine (Nagar and Gorelick, 2004).

## 10.27 CURCUMIN CORRECTS CYSTIC FIBROSIS DEFECTS

Cystic fibrosis is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). The most common mutation, DeltaF508, results in the production of a misfolded CFTR protein that is retained in the endoplasmic reticulum and targeted for degradation. Curcumin is a nontoxic Ca-adenosine triphosphatase pump inhibitor that can be administered to humans safely. Egan et al. (2004) demonstrated that oral administration of curcumin to homozygous DeltaF508 CFTR mice in doses comparable, on a weight-per-weight basis, to those well tolerated by humans corrected these animals' characteristic nasal potential difference defect. These effects were not observed in mice homozygous for a complete knockout of the CFTR gene. Curcumin also induced the functional appearance of DeltaF508 CFTR protein in the plasma membranes of transfected baby hamster kidney cells. Thus, curcumin treatment may be able to correct defects associated with the homozygous expression of DeltaF508 CFTR.

## 10.28 CURCUMIN BIOAVAILABILITY, PHARMACODYANAMICS, PHARMACOKINETICS, AND METABOLISM

Numerous studies have been performed on the biotransformation of curcumin (Table 10.5). Lin et al. (2000) showed that curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin and that these compounds subsequently were converted to monoglucuronide conjugates. Thus, curcumin–glucuronide, dihydrocurcumin–glucuronide, tetrahydrocurcumin–glucuronide, and tetrahydrocurcumin are major metabolites of curcumin in mice.

Since the systemic bioavailability of curcumin is low, its pharmacological activity may be mediated, in part, by its metabolites. To investigate this possibility, Ireson et al. (2002) compared curcumin metabolism in human and rat hepatocytes in suspension with that in rats *in vivo*. Analysis by high-performance liquid chromatography with detection at 420 and 280 nm permitted characterization of metabolites with both intact diferoylmethane structure and increased saturation of the heptatrienone chain. Chromatographic inferences were corroborated by mass spectrometry. The major metabolites in suspensions of human or rat hepatocytes were identified as hexahydrocurcumin and hexahydrocurcuminol. In rats, *in vivo*, curcumin administered i.v. (40 mg/kg) disappeared from the plasma within 1 h of dosing. After p.o. administration (500 mg/kg), parent drug was present in plasma at levels near the detection limit. The major products of curcumin biotransformation identified in rat plasma were curcumin glucuronide and curcumin sulfate, whereas hexahydrocurcumin, hexahydrocurcuminol, and hexahydrocurcumin glucuronide were present in small amounts (Ireson et al*.,* 2002). To test the hypothesis that curcumin metabolites resemble their progenitor in that they can inhibit COX-2 expression, curcumin and four of its metabolites at a concentration of 20 μM were compared in terms of their ability to inhibit phorbol ester–induced prostaglandin E2 (PGE2) production in human colonic epithelial cells. Curcumin reduced PGE2 levels to preinduction levels, whereas tetrahydrocurcumin, hexahydrocurcumin, and curcumin sulfate had only weak PGE2 inhibitory activity, and hexahydrocurcuminol was inactive. The results suggested that (1) the major products of curcumin biotransformation by hepatocytes occurred only at low abundance in rat plasma after curcumin administration and (2) metabolism of curcumin by reduction or conjugation generates species with reduced ability to inhibit COX-2 expression. Because the gastrointestinal tract seems to be exposed more prominently to unmetabolized curcumin than any other tissue, the results support the clinical evaluation of curcumin as a colorectal cancer chemopreventive agent.

Curcumin has very poor bioavailability. In Ayurveda, black pepper (*Piper nigrum*), long pepper, (*Piper longum*) and ginger (*Zingiber officinalis*) are collectively termed Trikatu, and are essential ingredients of numerous prescriptions, used for a wide range of disorders. Numerous studies suggest that Trikatu possesses bioavailability enhancing effect (Johri and Zutshi, 1992). Since curcumin belong to the same family as ginger, it has similar enhancer activity (Chuang et al*.,* 2002).

**TABLE 10.5**
**Phamacokinetics, Biotransformation, Tissue Distribution, and Metabolic Clearance Rates of Curcumin**

| Animal | Route | Dose | Remarks | Refs. |
|---|---|---|---|---|
| Mice | i.p. | 0.1 g/kg | 2.25 μg/ml in plasma in first 15 min<br>At 1 h, intestine, spleen, liver, and kidney are 177, 26, 27, 8 μg/kg; 0.4 μg/g brain<br>Biotransformed from DHC to THC, and then converted to monoglucuronide conjugates | Pan et al., 1999 |
| Mice | i.p. | 100 mg/kg[a] | 39–240 nmol/g tissue small intestine | Perkins et al., 2002 |
| Rats | Oral | 1 g/kg | 75% excreted in the feces<br>Negligible in urine<br>Poorly absorbed in the gut<br>No toxicity at 5 g/kg | Whalstrom and Blennow, 1978 |
| | i.v. | — | Transported into bile<br>Major part metabolized | |
| Rats | Oral, i.v., and i.p. | a | Mostly fecal excretion<br>Excreted in the bile<br>Major biliary metabolite THC/HHC glucuronides | Holder et al., 1978 |
| Rats | Oral | 400 mg | 60% absorbed<br>None in urine; conjugated glucuronides and sulfates<br>None in heart blood<br>Less than 5 μg/ml in portal blood<br>Negligible in liver /kidney (<20 μg/tissue) for 24 h<br>At 24 h, 38% in lower part of the gut | Ravindranath and Chandrashekara, 1980 |
| Rats | — | 2 g/kg | Low serum levels<br>Piperine increased bioavailability by 154% | Shoba et al., 1998 |
| Rats | Oral, i.g. | 2% of diet | Plasma levels 12 nM<br>Liver (0.1–0.9 nmol/g; colon (0.2–1.8 μmol/g)<br>Increased hepatic GST (16%)<br>Decreased colon MDH–DNA adduct (36%)<br>More in plasma, less in colon | Sharma et al., 2001 |
| Rats | i.v.<br>p.o. | 40 mg/kg<br>500 mg/kg | Disappeared from plasma in 1 h<br>Detectable in plasma<br>Biotransformed to curcumin glucuronide, and sulfate | Ireson et al., 2001 |
| Human | Oral | 2 g | Serum level not detectable<br>Piperine increased bioavailability by 2000% | Shoba et al., 1998 |
| Human | Oral | 375 mg × 3/d | Well tolerated for 12 weeks<br>Significant benefit | Lal et al., 1999 |
| Human | Oral | 375 mg × 3/d | Well tolerated for 6–22 months<br>Significant benefit | Lal et al., 1999 |
| Human | Oral | 1–12 g/d | Well tolerated up to 8 g/d up to 3 months<br>Serum levels peaked at 1–2 h and declined at 12 h<br>Serum levels 0.51 ± 0.11; 0.63 ± 0.06; 1.77 ± 1.87 μM | Cheng et al., 2001 |
| Human | Oral | 36–180 mg/d | Most in feces, none in blood or urine<br>59% decrease in lymphocytic GST after 14 d | Sharma et al., 2001 |

[a] Combined with [$^3$H] curcumin.

*Abbreviations*: DHC, dihydrocurcumin; THC, tetrahydrocurcumin; HHC, hexahydrocurcumin; i.v., intravenous; i.p., intraperitoneal; i.g., intragastric; p.o., post-oral; d, day; GST, glutathione S-transferase.

Shoba et al. (1998) found that curcumin has a poor bioavailability due to its rapid metabolism in the liver and intestinal wall. In this study, the effect of combining piperine, a known inhibitor of hepatic and intestinal glucuronidation, was evaluated on the bioavailability of curcumin in rats and healthy human volunteers. When curcumin was given alone, in the dose 2 g/kg to rats, moderate serum concentrations were achieved over a period of 4 h. Concomitant administration of piperine 20 mg/kg increased the serum concentration of curcumin for a short period of 1 to 2 h. Time to maximum was significantly increased, while elimination half-life and clearance significantly decreased, and the bioavailability was increased by 154%. On the other hand, in humans, after a dose of 2 g curcumin alone, serum levels were either undetectable or very low. Concomitant administration of piperine 20 mg produced much higher concentrations from 0.25 to 1 h later, and the increase in bioavailability was 2000%. The study shows that in the dosages used, piperine enhances the serum concentration, extent of absorption, and bioavailability of curcumin in both rats and humans with no adverse effects (Shoba et al., 1998).

In the studies by Kumar et al. (2002), natural biodegradable polymers, namely bovine serum albumin and chitosan, were used to encapsulate curcumin to form a depot drug delivery system. Microspheres were prepared by emulsion–solvent evaporation method coupled with chemical cross-linking of the natural polymers. As much as of 79.49 and 39.66% of curcumin could be encapsulated into the biodegradable carriers with albumin and chitosan, respectively. *In vitro* release studies indicated a biphasic drug release pattern, characterized by a typical burst-effect followed by a slow release, which continued for several days. It was evident from Kumar's study that the curcumin biodegradable microspheres could be successfully employed as prolonged release drug delivery system for better therapeutic management of inflammation as compared to oral or subcutaneous administration of curcumin. Kumar et al. (2000) synthesized bioconjugates of curcumin to improve its systemic delivery. Di-O-glycinoyl curcumin (I) and 2′-deoxy-2′-curcuminyl uridine (2′-cur-U) (IV) were quite potent against multiresistant microorganisms. These bioconjugates served dual purpose of systemic delivery as well as therapeutic agents against viral diseases.

Garcea et al. recently measured curcumin levels in normal and malignant human liver tissue after oral administration of the compound. In total, 12 patients with hepatic metastases from colorectal cancer received 450 to 3600 mg of curcumin daily, for one week prior to surgery (Garcea et al., 2004). The levels of curcumin and its metabolites in portal and peripheral blood, bile, and liver tissue of patients with hepatic metastases from colorectal cancer were measured. Curcumin was poorly available, following oral administration, with low nanomolar levels of the parent compound and its glucuronide and sulphate conjugates found in the peripheral or portal circulation. While curcumin was not found in liver tissue, trace levels of products of its metabolic reduction were detected. In patients who had received curcumin, levels of malondialdehyde–DNA (M(1)G) adduct, which reflect oxidative DNA changes, were not decreased in posttreatment normal and malignant liver tissue when compared to pretreatment samples. The results suggest that doses of curcumin required to furnish hepatic levels sufficient to exert pharmacological activity are probably not feasible in humans.

## 10.29 CLINICAL EXPERIENCE WITH CURCUMIN

Twelve different studies of the safety and efficacy of curcumin in humans have been reported (Figure 10.6). For example, Deodhar et al. (1980) performed a short-term, double blind, cross-over study in 18 patients (22 to 48 yr) to compare the antirheumatic activity of curcumin and phenylbutazone (Deodhar et al., 1980). They administered 1200 mg curcumin/day or 300 mg phenylbutazone/day for 2 weeks. These investigators reported that curcumin was well tolerated, had no side effects, and showed comparable antirheumatic activity.

Lal et al. (1999) administered curcumin orally to patients suffering from chronic anterior uveitis (CAU) at a dose of 375 mg three times a day for 12 weeks. Of 53 patients enrolled, 32 completed the 12-week study (Lal et al., 2000). They were divided into two groups: one group of 18 patients

**Table 10.6 Clinical studies with curcumin in human subjects**

| Study | Patients | Dose | Comments | Ref. |
|---|---|---|---|---|
| Double blind, cross-over study | 18 pts. (22-48 yrs) | 1200 mg /day x 2wks | Antirheumatic | Deodar et al (1980) |
| | 46 male pts. (15-68) | 400 mg; 3x/day x5 days | Inguinal hernia | Satosakar et al (1986) |
| | 10 volun. | 500 mg/day x7 days | Serum cholesterol & LPO | Soni & Kuttan (1992) |
| | 40 pts. | 625 mg; 4x/day x 8 wks | well-tolerated | James (1994) |
| | 53 pts. | 375 mg; 3x/day x12 wks | Chronic anterior uveitis | Lal etal (1999) |
| | 8 pts. | 375 mg; 3x/day 6-22 months | Idiopathic inflamm. orbital pseudotumors | Lal etal (2000) |
| Prospective Phase I | 25 pts. | 500 mg-12,000mg/day x3 months | H&N cancers | Cheng etal (2001) |
| | 15 pts. | 36-180 mg 4 months | Colorectal Serum GST-down | Sharma etal (2001) |
| | 12 pts. | 450-3600 mg/day x1 wks | Hepatic metastasis of colorectal cancer | Garcea et al. (2004) |
| | 15 pts. | 450-3600 mg/day x4 months | Advanced colorectal cancer | Sharma et al. (2004) |
| | 12 pts. (47-72 yrs) | 3.6, 1.8 or 0.45 g/day 7 days | Colorectal cancer | Garcea et al. (2005) |
| | 10 (20-40 yrs) | 18 mg | Colon cancer chemoprevention | Plummer (2001) |

received curcumin alone, whereas the other group of 14 patients, who had a strong PPD reaction, in addition, received antitubercular treatment. The patients in both the groups started improving after two weeks of treatment. All the patients who received curcumin alone improved, whereas the group receiving antitubercular therapy along with curcumin had a response rate of 86%. Follow-up of all the patients for the next 3 yr indicated a recurrence rate of 55% in the first group and of 36% in the second group. Four of 18 (22%) patients in the first group and 3 of 14 patients (21%) in the second group lost their vision in the follow-up period because of various complications, e.g., vitritis, macular edema, central venous block, cataract formation, and glaucomatous optic nerve damage, etc. None of the patients reported any side effects. The efficacy of curcumin and recurrences following treatment are comparable to corticosteroid therapy, which is at present considered the only available standard treatment for this disease. The lack of side effects with curcumin is its greatest advantage compared with corticosteroids. A double blind multicenter clinical trial of this drug for CAU is highly desirable to further validate the results of the study.

Satoskar et al. (1986) evaluated the anti-inflammatory properties of curcumin in patients with postoperative inflammation. They studied 46 male patients (between the ages of 15 and 68 years) having inguinal hernia and/or hydrocoele. After the hernia operation, spermatic cord edema and tenderness were evaluated. Either curcumin (400 mg) or placebo (250 mg lactose) or phenylbutazone (100 mg) was administered three times a day for a period of 5 d from the first postoperative day. Curcumin was found to be quite safe, and phenylbutazone and curcumin produced a better anti-inflammatory response than placebo (Satoskar et al., 1986).

Soni and Kuttan (1992) examined the effect of curcumin on serum levels of cholesterol and lipid peroxides in ten healthy human volunteers. A dose of 500 mg of curcumin per day for 7 d significantly decreased the level of serum lipid peroxides (33%), increased HDL cholesterol (29%), and decreased total serum cholesterol (11.63%). The results suggest curcumin as a chemopreventive substance against arterial diseases.

James (1996) led a New England clinical trial of curcumin's effectiveness as an antiviral agent in 40 participants**.** Two dropped out; 23 were randomized to high-dose group (four capsules, four times a day) and 15 to lose-dose (three capsules, three times a day) for 8 weeks. Though it had no antiviral effects, curcumin was well tolerated, and most participants liked taking curcumin and felt better.

Lal et al. (2000) described for the first time the clinical efficacy of curcumin in the treatment of patients suffering from idiopathic inflammatory orbital pseudotumors. Curcumin was administered orally at a dose of 375 mg/three times/d for a period of 6 to 22 months in eight patients. They were followed up for a period of 2 yr at three-monthly intervals. Five patients completed the study, of which four recovered completely. In the remaining patient, the swelling regressed completely, but some limitation of movement persisted. No side effect was noted in any patient, and there was no recurrence. Thus curcumin could be used as a safe and effective drug in the treatment of idiopathic inflammatory orbital pseudotumors.

Cheng et al. (2001) examined the toxicology, pharmacokinetics, and biologically effective dose of curcumin in humans. This prospective phase I study evaluated curcumin in patients with one of the following five high-risk conditions: (1) recently resected urinary bladder cancer; (2) arsenic Bowen's disease of the skin; (3) uterine cervical intraepithelial neoplasm (CIN); (4) oral leucoplakia; and (5) intestinal metaplasia of the stomach. Curcumin was taken orally for 3 months. Biopsy of the lesion sites was done immediately before and 3 months after starting curcumin treatment. The starting dose was 500 mg/day. If no toxicity of grade II or higher was noted in at least three successive patients, the dose was escalated to 1000, 2000, 4000, 8000, or 12000 mg/d in order. The concentration of curcumin in serum and urine was determined by high-pressure liquid chromatography (HPLC). A total of 25 patients were enrolled in this study. There was no treatment-related toxicity for doses up to 8000 mg/day. Beyond 8000 mg/day, the bulky volume of the drug was unacceptable to the patients. The serum concentration of curcumin usually peaked at 1 to 2 h after oral intake of curcumin and gradually declined within 12 h. The average peak serum concentrations after taking 4000 mg, 6000 mg, and 8000 mg of curcumin were $0.51 \pm 0.11$ μM, $0.63 \pm 0.06$ μM, and $1.77 \pm 1.87$ μM, respectively. Urinary excretion of curcumin was undetectable. One of four patients with CIN and one of seven patients with oral leucoplakia developed frank malignancies in spite of curcumin treatment. In contrast, histologic improvement of precancerous lesions was seen in one of two patients with recently resected bladder cancer, two of seven patients with oral leucoplakia, one of six patients with intestinal metaplasia of the stomach, one of four patients with CIN, and two of six patients with Bowen's disease. In conclusion, this study demonstrated that curcumin is not toxic to humans at doses up to 8000 mg/day when taken by mouth for three months. These results also suggested a biologic effect of curcumin in the chemoprevention of cancer.

Sharma et al. (2001) examined the pharmacodynamics and pharmacokinetics of curcumin in humans in a dose-escalation pilot study. A novel standardized turmeric extract in proprietary capsule form was given at doses between 440 and 2200 mg/day, containing 36 to 180 mg of curcumin (Sharma et al., 2001). Fifteen patients with advanced colorectal cancer refractory to standard chemotherapies received turmeric extract daily for up to 4 months. The activity of GST and levels of a DNA adduct (M(1)G) formed by malondialdehyde, a product of LPO and prostaglandin biosynthesis, were measured in patients' blood cells. Oral turmeric extract was well tolerated, and dose-limiting toxicity was not observed. Neither curcumin nor its metabolites were detected in blood or urine, but curcumin was recovered from feces. Curcumin sulfate was identified in the feces of one patient. Ingestion of 440 mg of turmeric extract for 29 d was accompanied by a 59% decrease in lymphocytic GST activity. At higher dose levels, this effect was not observed. Leukocytic M(1)G levels were constant within each patient and unaffected by treatment. Radiologically stable disease was demonstrated in five patients for 2 to 4 months of treatment. The results suggested that: (1) tumeric extract can be administered safely to patients at doses of up to 2.2 g daily, equivalent to 180 mg of curcumin; (2) curcumin has low oral bioavailability in humans and may undergo intestinal metabolism; and (3) larger clinical trials of curcuma extract are merited.

Sharma et al. (2004) designed a dose-escalation study to explore the pharmacology of curcumin in humans. Fifteen patients with advanced colorectal cancer refractory to standard chemotherapies consumed capsules compatible with curcumin doses between 0.45 and 3.6 g daily for up to 4 months. Levels of curcumin and its metabolites in plasma, urine, and feces were analyzed by HPLC and mass spectrometry. Three biomarkers of the potential activity of curcumin were translated from preclinical models and measured in patient blood leukocytes: GST activity, levels of deoxyguanosine adduct M (1) G, and PGE (2) production induced ex vivo. Dose-limiting toxicity was not observed. Curcumin and its glucuronide and sulfate metabolites were detected in plasma in the 10 nmol/l range and in urine. A daily dose of 3.6 g curcumin engendered 62% and 57% decreases in inducible PGE (2) production in blood samples taken 1 h after dose on days 1 and 29, respectively, of treatment compared with levels observed immediately predose ($P < 0.05$). A daily oral dose of 3.6 g of curcumin is advocated for Phase II evaluation in the prevention or treatment of cancers outside the gastrointestinal tract. PGE (2) production in blood and target tissue may indicate biological activity. Levels of curcumin and its metabolites in the urine can be used to assess general compliance (Sharma et al., 2004).

Garcea et al. (2004) investigated whether oral administration of curcumin results in concentrations of the agent in normal and malignant human liver tissue, which are sufficient to elicit pharmacological activity. In total, 12 patients with hepatic metastases from colorectal cancer received 450 to 3600 mg of curcumin daily, for one week prior to surgery. Curcumin was poorly available, following oral administration, with low nanomolar levels of the parent compound and its glucuronide and sulphate conjugates found in the peripheral or portal circulation. The results suggest that doses of curcumin required to furnish hepatic levels sufficient to exert pharmacological activity are probably not feasible in humans (Garcea et al., 2004).

Garcea et al. (2005) also tested the hypothesis that pharmacologically active levels of curcumin that can be achieved in the colorectum of humans as measured by effects on levels of M(1)G and COX-2 protein. Patients with colorectal cancer ingested curcumin capsules (3,600, 1,800, or 450 mg daily) for 7 d. Biopsy samples of normal and malignant colorectal tissue, respectively, were obtained at diagnosis and at 6 to 7 h after the last dose of curcumin. Blood was taken 1 h after the last dose of curcumin. Curcumin and its metabolites were detected and quantitated by high-performance liquid chromatography with detection by UV spectrophotometry or mass spectrometry. The concentrations of curcumin in normal and malignant colorectal tissue of patients receiving 3600 mg of curcumin were $12.7 \pm 5.7$ and $7.7 \pm 1.8$ nmol/g, respectively. Curcumin sulfate and curcumin glucuronide were identified in the tissue of these patients. Trace levels of curcumin were found in the peripheral circulation. M(1)G levels were 2.5-fold higher in malignant tissue as compared with normal tissue. Administration of curcumin (3600 mg) decreased M(1)G levels from $4.8 \pm 2.9$ adducts per 107 nucleotides in malignant colorectal tissue to $2.0 \pm 1.8$ adducts per 107 nucleotides. COX-2 protein levels in malignant colorectal tissue were not affected by curcumin. The results suggest that a daily dose of 3.6 g curcumin achieves pharmacologically efficacious levels in the colorectum with negligible distribution of curcumin outside the gut (Garcea et al., 2005).

A Phase I study of curcumin for the chemoprevention of colon cancer has recently been concluded (http://clinicaltrials.gov/ct/gui/show/NCT00027495?order=5). This study aimed at determining the maximum tolerated dose of curcumin as a chemopreventive agent of colon cancer in healthy subjects.

## 10.30 NATURAL ANALOGUES OF CURCUMIN

Natural curcumin contains three major curcuminoids, namely curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Figure 10.6). Several analogues of curcumin have been identified from other plant sources. These include 6- and 8-gingerol, 6-paradol, cassumunin, galangals, diarylheptanoids, yakuchinones, isoeugenol, and dibenzoylmethane. Like curcumin, gingerol, paradol, cassumunin, shogaol, and diarylheptanoids are also derived from the roots of the plant (Table 10.7)

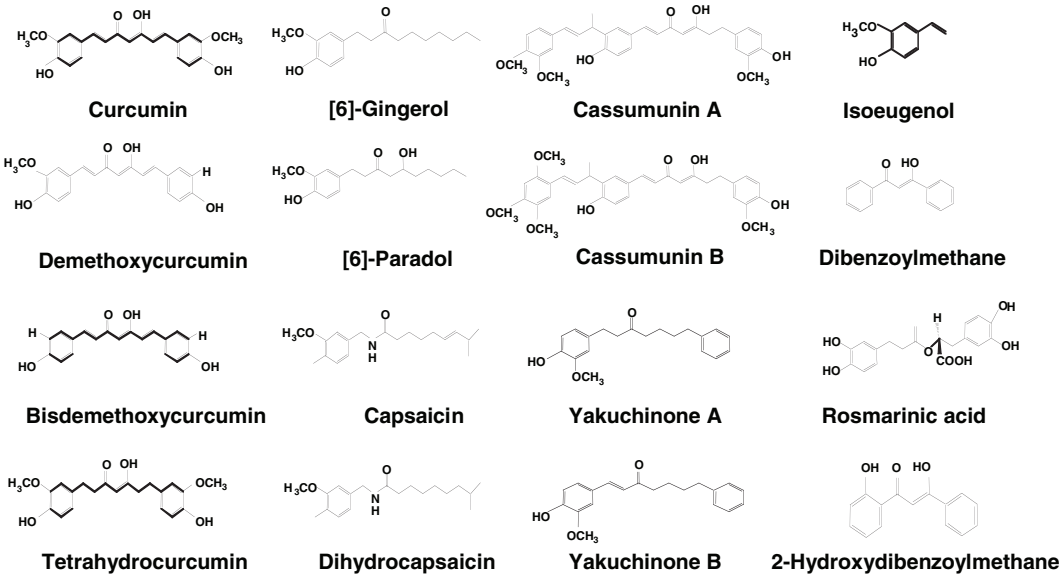


**FIGURE 10.6** Natural analogues of curcumin.

**TABLE 10.7**
**Sources and Site of Action of Natural Analogues of Curcumin**

| Analogues | Source | Target | Refs. |
|---|---|---|---|
| 6-Gingerol | Ginger (*Z. officinale* Roscoe) | TNF, NF-κB, AP-1, COX2, ODC, iNOS, p38MAPK, antifungal | Kim et al., 2005 |
| 8-Gingerol | Ginger (*Z. officinale* Roscoe) | | Kim et al., 2005 |
| 6-Paradol | Ginger (*Z. officinale* Roscoe) | Caspase activation | Keum et al., 2002 |
| Shogaol | Ginger (*Z. officinale* Roscoe) | Helicobacter pylori | Mahady et al., 2003 |
| Cassumunin A and B | Ginger (*Z. cassumunar*) | Antioxidant | Mosuda et al., 1998 |
| Diarylheptanoids | Ginger (*Zingiber* sps.) | PGE2 and LT | Hong et al., 2004 |
| Dibenzoylmethane | Licorice (*Glycyrrhiza echinata*) | COX1-1, LOX, HIF, VEGF | Hong et al., 2004 |
| Galangals A and B | Zingiber (*Zingiber mioga* Roscoe) | Caspase 3, Bcl-2 | Miyoshi et al., 2003 |
| Garcinol | Kokum (*Garcinia indica*) | NF-κB, COX-2, iNOS, HAT | Pan et al., 2001 |
| Isoeugenol | Cloves (*Eugenia caryophyllus*) | NF-κB, antioxidant β-amyloid | Chainy et al., 2000 |
| Yakuchinone A and B | Galanga (*Alpinia officinarum*) | PG synthetase, COX1-1, iNOS, NF-κB, insecticidal, adhesion molecules, TNF, AP-1, 5-HETE | Chun et al., 2002 |

*Note:* For structure of these analogues, see Figure 10.6.

(Kim et al., 2005; Keum et al., 2002; Mahady et al., 2003; Masuda et al., 1998; Hong et al., 2004; Miyoshi et al., 2003; Pan et al., 2001; Chainy et al., 2000; Chun et al., 2002). Although most of these analogues exhibit activities very similar to curcumin, whether they are more potent or less potent than curcumin has not been established. Yakuchinones have been shown to be more potent inhibitors of 5-HETE production than curcumin (Flynn et al., 1986). Synthetic cassumunins also show stronger protective activity than curcumin against oxidative cell death induced by hydrogen peroxide (Masuda et al., 1998). Garcinol is more potent than curcumin in inhibiting tumor cells (Pan et al., 2001). The anticancer potential of galangals, however, is comparable to that of curcumin (Miyoshi et al., 2003). Curcumin has been shown to be more cytotoxic than isoeugenol, bis-eugenol, and eugenol (Fujisawa et al., 2004).

## 10.31 SYNTHETIC ANALOGS OF CURCUMIN

Commercial curcumin isolated from the rhizome of *Curcuma longa* Linn. contains three major curcuminoids (approximately 77% curcumin, 17% demethoxycurcumin, and 3% bisdemethoxycurcumin) (Figure 10.7). Commercial curcumin, pure curcumin, and demethoxycurcumin are about equipotent as inhibitors of TPA-induced tumor promotion in mouse skin, whereas bisdemethoxycurcumin is somewhat less active (Huang et al., 1997). Besides curcumin, several analogues of curcumin have been synthesized and tested (Ishida et al., 2002; Dinkova-Kostova and Talalay, 1999). Tetrahydrocurcumin, an antioxidative substance, which is derived from curcumin by hydrogenation, has been shown to have a protective effect on oxidative stress in cholesterol-fed rabbits (Naito et al., 2004). Kumar et al. (2003) have developed an analogue of curcumin, 4-hydroxy-3-methoxybenzoic acid methyl ester (HMBME), which targets the Akt/NF-

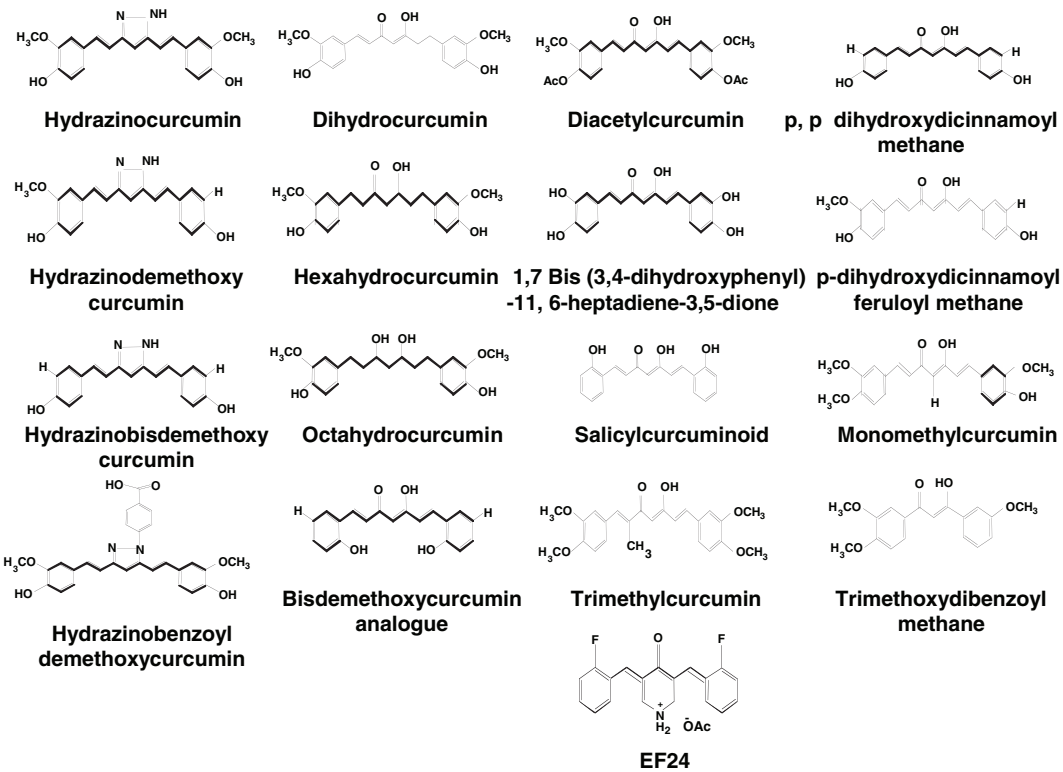


**FIGURE 10.7** Synthetic analogues of curcumin.

κB signaling pathway (Kumar et al., 2003). They demonstrated the ability of this novel compound to inhibit the proliferation of human and mouse PCA cells. Overexpression of constitutively active Akt reversed the HMBME-induced growth inhibition and apoptosis, illustrating the direct role of Akt signaling in HMBME-mediated growth inhibition and apoptosis. HMBME-mediated inhibition of Akt kinase activity may have a potential in suppressing/decreasing the activity of major survival/antiapoptotic pathways.

Using an *in vitro* SVR assay, Robinson et al. (2003) have demonstrated potent antiangiogenic properties in aromatic enone and dieneone analogues of curcumin. Based on a simple pharmacophore model, the aromatic enone and aromatic dienone analogues of curcumin were prepared using standard drug design concepts.

Devasena et al. (2002) examined the protective effect of a curcumin analogue [bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene-3,5-dione] on hepatic LPO and antioxidant status during 1,2-dimethylhydrazine-induced colon carcinogenesis in male Wistar rats. They observed that the curcumin analog exerted chemopreventive effects against cancer development at extrahepatic sites by modulating hepatic biotransformation enzymes and antioxidant status. The effect was comparable with that of curcumin. They proposed that the hydroxyl group in the aromatic ring is responsible for the protective effect rather than the methoxy group. Mishra et al. (2002) synthesized a novel curcumin conjugate viz. 1,7-bis (4-O-glycinoyl-3-methoxyphenyl)-1,6- heptadiene-3, 5, dione (I), which was attached to the deoxy-11 mer, 5′-GTT AGG GTT AG-3′, a complementary sequence of telomerase RNA template. This novel anticancer prodrug has the potential to target the telomerase sequence.

The antitumor properties of metal chelates of synthetic curcuminoids (John et al., 2002) have also been investigated. John et al. (2002) examined four synthetic curcuminoids, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione (curcumin1), 1,7-bis(piperonyl)-1,6-heptadiene-3, 5-dione (piperonyl curcumin), 1, 7-bis(2-hydroxy naphthyl)-1, 6-heptadiene-2, 5-dione (2-hydroxy naphthyl curcumin), 1,1-bis(phenyl)-1, 3, 8, 10-undecatetraene-5, 7-dione (cinnamyl curcumin), and their copper(II) complexes were investigated for their possible cytotoxic and antitumor activities. Copper chelates of synthetic curcuminoids showed enhanced antitumor activity. In addition to these, a novel curcumin derivative, named hydrazinocurcumin (HC), was synthesized and examined for its biological activities by Shim et al. (2002). HC potently inhibited the proliferation of bovine aortic endothelial cells at nanomolar concentrations (IC50 = 520 nM without cytotoxicity. Snyder et al. (2002) reported the synthesis of several different structural analogues of curcumin and examined their antitumor and antiangiogenic properties. They found analogues that are more potent than native curcumin.

Limtrakul et al. (2004) investigated natural curcuminoids, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin, isolated from turmeric, for their potential ability to modulate the human MDR-1 gene expression in multidrug-resistant human cervical carcinoma cell line, KB-V1. Multidrug resistance (MDR) is a very important phenomenon that is often associated with decreased intracellular drug accumulation in patient's tumor cells resulting from enhanced drug efflux (Limtrakul et al., 2004). It is related to the overexpression of a membrane protein, P-glycoprotein (Pgp-170), thereby reducing drug cytotoxicity. They found that all the three curcuminoids inhibited MDR-1 gene expression, and bisdemethoxycurcumin produced maximum effect. The commercial grade curcuminoid (approximately 77% curcumin, 17% demethoxycurcumin, and 3% bisdemthoxycurcumin) decreased MDR-1 gene expression in a dose-dependent manner and had about the same potent inhibitory effect on MDR-1 gene expression as natural curcuminoid mixtures. Their results indicate that bisdemethoxycurcumin is the most active of the curcuminoids present in turmeric for modulation of MDR-1 gene. Treatment of drug resistant KB-V1 cells with curcumin increased their sensitivity to vinblastine, which was consistent with a decreased MDR-1 gene product, a P-glycoprotein, on the cell plasma membrane.

In a recent study, Selvam et al. (2005), isolated curcuminoids from turmeric and their pyrazole and isoxazole analogues were synthesized. They compared for antioxidant and COX-1/COX-2 inhibitory and anti-inflammatory activities. The designed analogues exhibited significant

COX-2/COX-1 selectivity and also better antioxidant activity. Molecular docking studies revealed that these compounds were able to dock the active site of COX-2/COX-1. This approach would help in designing novel potent inhibitors. Furness et al. (2005) showed the efficacy of different curcumin analogs to exhibit antiangiogenic properties.

Gafner et al. (2004) investigated curcumin and 22 of its derivatives for their chemopreventive potential. Based on COX-2 inhibition, curcumin (IC50 = 15.9 μM), 1,7-bis (3-fluoro-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (IC50 = 23.7 μM) and 2,6-bis (3-fluoro-4-hydroxybenzylidene) cyclohexanone (IC50 = 5.5 μM) were found to be most potent. Tricyclic derivatives 2,6-bis (4-hydroxy-3-methoxybenzylidene)cyclohexanone, 2,6-bis (4-hydroxy-3,5-dimethoxybenzylidene) cyclohexanone and 2,5-bis (4-hydroxy-3,5-dimethoxybenzylidene)cyclopentanone inhibited LPS-induced COX-2 and iNOS gene expression in murine macrophages with potency equal to curcumin. RT-PCR experiments demonstrated suppression of COX-2 and iNOS gene expression occurred at the transcriptional level (Gafner et al., 2004).

Kalpana and Menon (2004) showed that curcumin and its analogues inhibited nicotine-mediated imbalances in oxidant–antioxidant status in male Wistar rats. In this study, nicotine was injected subcutaneously at a dose of 2.5 mg/kg of body weight (5 d a week, for 22 weeks). The enhanced circulatory lipid peroxides in nicotine-treated rats was accompanied by a significant decrease in the levels of ascorbic acid, vitamin E, reduced glutathione, glutathione peroxidase, superoxide dismutase, and catalase. Administration of curcumin and curcumin analogue significantly lowered the LPO and enhanced the antioxidant status with modulation in the levels of zinc, copper, and ferritin. However, the effect was more significant in curcumin analogue-treated rats than in curcumin-treated rats.

The inhibitory mechanism of curcumin and its derivative (CHC007) against beta-catenin/T-cell factor (Tcf) signaling in various cancer cell lines was investigated by Park et al. (2005). β-Catenin presents two different facets. One aspect is that it contributes to the cell–cell adhesion in cooperation with E-cadherin. The other aspect is that it possesses transcriptional activity in cooperation with T-cell factor (Tcf)/lymphoid enhancer factor (Lef) transcription factor in a nucleus. β-catenin gene is mutated in many cancer cells including colorectal cancer, melanoma, hepatocellular carcinoma, and gastric carcinoma; the transcriptional activity of β-catenin is upregulated in these cancer cells. Therefore, if β-catenin's transcriptional activity can be markedly downregulated, tumor growth will be suppressed. However, there exist few β-catenin inhibitors and one of them is aspirin. Curcumin and its derivatives showed excellent inhibition of β-catenin/Tcf signaling in different cancer cell lines and the reduced β-catenin/Tcf transcriptional activity is due to the decreased nuclear β-catenin and Tcf-4.

Synthetic curcumin analogs inhibited complex formations between Fos-Jun heterodimer and activator protein-1 (AP-1) DNA as reported by Hahm et al. (2002). These curcumin analogs have been observed to repress the AP-1 transcription in AP-1-transfected cells, and they also inhibited the increased expression of Jun/AP-1 protein by 12-O-tetradecanoylphorbol-13-acetate (TPA) in the same cells. Curcumin analogs downregulated the expression of MMP-9 (gelatinase-B), correlating with inhibition of cellular invasion and migration in conditions such as tumor invasion and metastasis.

## 10.32 STRUCTURE–ACTIVITY RELATIONSHIP OF CURCUMIN

To elucidate which portion of the molecule is critical for the activity, a large number of structural analogs of curcumin have been synthesized. Some analogues are more active than native curcumin, while others are less active (Table 10.7), (Bartherlemy et al., 1998; Gomes Dde et al., 2002; Tonnesen et al., 2002; Punithavathi et al., 2003; Dinkova-Kostova and Talalay, 1999; Kumar et al., 2003; Mishra et al., 2002; John et al., 2002; Shim et al., 2002; Selvam et al., 2005; Khopde et al., 1999; Rao et al., 1982; Hahm et al., 2002; Douglas, 1993; Rukkumani et al., 2004; Vajragupta et al., 2004). It was found that the phenolic analogues were more active than the nonphenolic analogues

**TABLE 10.8**
**Relative Potency of Curcumin and Its Synthetic Analogues**

| Effects | Refs. |
|---|---|
| **Analogues More Potent than Curcumin** | |
| THC: lipid peroxidation under aqueous condition by pulse radiolysis technique | Khopde et al., 1999 |
| THC: preventing nitrite-induced oxidation of hemoglobin | Venkatesan et al., 2003 |
| NaC: carrageenin-induced rat hind paw edema | Rao et al., 1982 |
| HMBME: inhibition of prostate cancer | Kumar et al., 2003 |
| BJC005, CHC011, and CHC007: formation of Fos-/Jun- DNA complex | Hahm et al., 2002 |
| Tocopheryl curcumin: inhibiting Tat transactivation of HIV-LTR | Barthelemy et al., 1998 |
| 4, 4′-DAC: histamine blocking activity | Douglas, 1993 |
| Copper chelates of 2-hydroxynapthyl curcumin: antitumor activity | John et al., 2002 |
| Hydrazinocurcumin: BAECs proliferation | Shim et al., 2002 |
| *o*-hydroxy substituted analog: inhibiting alcohol and PUFA induced oxidative stress | Rukkumani et al., 2004 |
| Di-*O*-glycinoyl curcumin and 2′-deoxy-2′-curcuminyl uridine: antiviral activity | Mishra et al., 2002 |
| Pyrazole and isoxazole analogues: Cox-2 inhibitory activity | Selvam et al., 2005 |
| 1,7-*bis*-(2-hydroxy-4-methoxyphenyl)-1,6-heptadiene-3,5-dione): AL activity | Gomes et al., 2002 |
| Salicylcurcuminoid: antioxidant | |
| **Analogues Less Potent than Curcumin** | |
| THC: lipid peroxidation under aerated condition by pulse radiolysis technique | Khopde et al., 2000 |
| THC: TPA-induced mouse ear edema and skin carcinogenesis | Dinkova-koztova and Talalay, 1999 |
| **Analogues as Potent as Curcumin** | |
| 5-hydroxy-1,7-diphenyl-1,4,6-heptatriene-3-one: scavenge hydroxyl radicals | Tonnesen and Greenhill, 1992 |
| Manganese complexes of curcumin and diacetylcurcumin: scavenge hydroxyl radicals | Vajragupta et al., 2004 |

*Abbreviation*s: THC, tetrahydrocurcumin; NaC, sodium curcuminate; HMBME, 4-hydroxy-3-methoxybenzoic acid methyl ester; DAC, diacetylcurcumin; BAEC, bovine aortic endothelial cells; PUFA, poly-unsaturated fatty acids; Cox-2: cycloxygenase-2; AL, anti-leishmanial.

(Venkatesan, 2000). The highest antioxidant activity was obtained when the phenolic group was sterically hindered by the introduction of two methyl groups at the ortho position. The phenolic group is essential for the free-radical scavenging activity, and the presence of the methoxy group further increases the activity (Sreejayan and Rao, 1996). Curcumin shows both antioxidant and pro-oxidant effects. Ahsan et al. have shown that both these effects are determined by the same structural moieties of the curcuminoids (Ahsan et al., 1999).

Dinkova–Kostova showed that the presence of hydroxyl groups at the ortho position on the aromatic rings and that beta-diketone functionality was required for high potency in inducing Phase 2 detoxification enzymes (Dinkova–Kostova and Talalay, 1999). Curcumin is a noncompetitive inhibitor of rat liver microsomal delta 5 desaturase and delta 6 desaturase. Kawashima et al. (1996) have shown that only half the structure is essential for the desaturase inhibition. A 3-hydroxy group of the aromatic ring is essential for the inhibition and a free carboxyl group at the end opposite to the aromatic ring interferes with the inhibitory effect.

Simon et al. found that the presence of the diketone moiety in the curcumin molecule seems to be essential for its ability to inhibit the proliferation of MCF-7 human breast tumor cells (Simon et al., 1998). The aromatic enone and dienone analogs of curcumin have been demonstrated to have potent antiangiogenic property in an *in vitro* SVR assay (Robinson et al., 2003).

**TABLE 10.9**
**Cancer Incidence in India and the U.S.**

| Cancer | U.S. | | India | |
|---|---|---|---|---|
| | Cases | Deaths | Cases | Deaths |
| Breast | 660 | 160 | 79 | 41 |
| Prostate | 690 | 130 | 20 | 9 |
| Colon/rectum | 530 | 220 | 30 | 18 |
| Lung | 660 | 580 | 38 | 37 |
| Head and neck SCC | 140 | 44 | 153 | 103 |
| Liver | 41 | 44 | 12 | 13 |
| Pancreas | 108 | 103 | 8 | 8 |
| Stomach | 81 | 50 | 33 | 30 |
| Melanoma | 145 | 27 | 18 | 1 |
| Testis | 21 | 1 | 3 | 1 |
| Bladder | 202 | 43 | 15 | 11 |
| Kidney | 115 | 44 | 6 | 4 |
| Brain, nervous system | 65 | 47 | 19 | 14 |
| Thyroid | 55 | 5 | 12 | 3 |
| Endometrial cancers | 163 | 41 | 132 | 72 |
| Ovary | 76 | 50 | 20 | 12 |
| Multiple myeloma | 50 | 40 | 6 | 5 |
| Leukemia | 100 | 70 | 19 | 17 |
| Non-Hodgkin lymphoma | 180 | 90 | 17 | 15 |
| Hodgkin's disease | 20 | 5 | 7 | 4 |

Showing cases per 1 million persons calculated on the basis of current consensus: Endometrial cancers include Cervix uteri and Corpus uteri.

*GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0. IARC Cancer Base No. 5. Lyon, IARC Press, 2001.*

## 10.33 CONCLUSION

All evidences accumulated so far clearly indicate that curcumin protects against cancer, cardiovascular diseases, and diabetes, the major ailments in the U.S. This drug has also shown preventive as well as therapeutic effects against Alzheimer's disease, MS, cataract formation, AIDS, and drug-induced nonspecific toxicity in the heart, lung, and kidney. Several of the studies establishing curcumin's potential were carried out in animals. Further testing of curcumin in humans is underway to confirm these observations. A clinical development plan for using curcumin to treat cancer was recently described by the NCI. Studies also show that in countries such as India, which consume curcumin, the profile of cancer incidence is very different than those that do not, such as in the U.S. (Table 10.9). How curcumin produces its therapeutic effects is not fully understood, but they are probably mediated in part through the antioxidant and anti-inflammatory action of curcumin. It is quite likely that curcumin mediates its effects through other mechanisms as well. Over a dozen different cellular proteins and enzymes have been identified to which curcumin binds. High-throughput ligand-interacting technology and microarray technology have begun to reveal more molecular targets and genes affected by curcumin.

Some of the sources of curcumin are given in Table 10.10.

**TABLE 10.10**
**Sources of Curcumin**

**Human use:**

Sabinsa (http:www.sabinsa.com/products/circumin_book.htm; Piscataway, NJ)
Synthite Industrial Chemicals (www.synthite.com/health.html)
Kalsec (http://www.kalsec.com/products/turmeric_over.cfm; Kalamazoo, MI)
Life Extension (http://www.lef.org/newshop/items/item00552.html?source=WebProtProd)
Turmeric Curcumin (http://www.turmeric-curcumin.com/)
Iherb (http://www.iherb.com/curcuminl.html)
Club Natural (http://www.clubnatural.com/curex9550180.html, Irvine, CA)
American Nutrition (www.AmericanNutrition.com)
Amerifit (www.amerifit.geomerx.com/items/categories.cfm?categoryid=2, Bloomfield, CT)
XKMS (www.xkms.org/Webvitamins-32/Curcumin-Power-60C.htm)
Immune Support (https://www.Immunesupport.com/shop/prodlisting.cfm?NOTE=NOC)
Nature's (www.naturesnutrition.com/SKU/55114.htm)
Big Fitness (www.bfwse.com/jr-021.html)
Power house Gym (http://store.yahoo.com/musclespot/curcumin95.html), MMS
MMS Pro (http://www.mmspro.com/)
Herbal Fields (http://www.herbalfields.com/curcumin.html)
Amazon.com

**Research use:**

Sigma Aldrich (http://www.sigmaaldrich.com/cgibin/hsrun/Distributed/HahtShop/HAHTpage/HS_CatalogSearch)
Calbiochem (http://www.calbiochem.com/Products/ProductDetail_CBCB.asp?catNO=239802)
LKT laboratories (www.lktlabs.com)

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