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Mechanisms of Cancer Chemoprevention by Curcumin

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ABSTRACT

Curcumin is a major component of the Curcuma species, which is commonly used as a yellow coloring and flavoring agent in foods. Curcumin has shown anti-carcinogenic activity in animals as indicated by its ability to block colon tumor initiation by azoxymethane and skin tumor promotion induced by phorbol ester TPA. Recently, curcumin has been considered by oncologists as a potential third generation cancer chemopreventive agent, and clinical trials using it have been carried out in several laboratories. Curcumin possesses anti-inflammatory activity and is a potent inhibitor of reactive oxygen-generating enzymes, such as lipoxygenase/cyclooxygenase, xanthine dehydrogenase/oxidase and inducible nitric oxide synthase. Curcumin is also a potent inhibitor of protein kinase C, EGF-receptor tyrosine kinase and IκB kinase. In addition, curcumin inhibits the activation of NFκB and the expression of c-jun, c-fos, c-myc and iNOS. It is proposed that curcumin may suppress tumor promotion by blocking signal transduction pathways in the target cells. Curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin, and these compounds were subsequently converted into monoglucuronide conjugates. The experimental results suggest that curcumin-glucuronide, dihydrocurcumin-glucuronide, tetrahydrocurcumin-glucuronide and tetrahydrocurcumin are major metabolites of curcumin in mice.

Key Words: curcumin, curcuma species, curcumin-glucuronide, biotransformation, ROS, NFκB, iNOS, signal transduction, antioxidant, chemoprevention

I. Introduction

Curcumin (diferuloylmethane) is a major yellow pigment that has been isolated from the ground rhizome of the *Curcuma* species, Zingiberaceae (Table 1). Seven major species of *Curcuma* including *Curcuma longa* Linn., *C. xanthorrhiza* Roxb., C. *wenyujin* (Y.H. Chen et C, Ling); *C. sichuanensis*; *C. kwangsiensis*; *C. aeruginosa* Roxb.; and *C. elata* Roxb. have been cultivated in China and their composition of curcuminoids were analyzed (Chen and Fang, 1997). Three major curcuminoids namely curcumin, demethoxycurcumin and bisdemethoxycurcumin occur naturally in these *Curcuma* species. The contents of curcuminoids of these plants vary with the site and cultivation period as illustrated in Table 1. It seems that *C. longa* L. (turmeric) has the highest concentration of curcumin as compared to the other species.

Turmeric is widely used as a spice and coloring agent in several foods, such as curry, mustard, bean cake, cassava paste and potato chips, as well as in cosmetics and drugs. Another species *C. wenyujin* (Y.H. Chen et C. Ling) has been used for centuries in tradition Chinese medicine to treat a variety of inflammatory conditions, such as hepatitis and bile duct disorders.

Curcumin has been demonstrated to have potent antioxidant (Kunchandy and Rao, 1990; Subramanian *et al*., 1994; Sreejayan, 1994) and anti-inflammatory activity (Huang *et al*., 1988, 1991, 1997; Shih and Lin, 1993), and to inhibit the carcinogen-DNA adduct (Conney *et al*., 1991) and tumorigenesis in several animal models (Huang *et al*., 1992, 1994, 1995; Rao *et al*., 1995) as shown by the findings summarized in Table 2.

II. Biological Activities of Curcumin *In Vitro* and *In Vivo*

1. Scavenging of Reactive Oxygen Species (ROS)

Curcumin is a potent scavenger of a variety of ROS, including superoxide anion (Kunchandy and Rao, 1990), hydroxyl radical, singlet oxygen (Subramanian *et al*., 1994), ni-

Table 1. Curcuminoid Contents in the Rhizome of *Curcuma* Species

Curcuma species	Origin (year)[a]	Curcuminoid (%)[b]			
		Total	Cur	Dcur	Bdcur
Curcuma longa Linn.	Nan-ning (1981)	3.97	1.84	1.09	1.01
Curcuma longa Linn.	Cheng-du (1979)	3.83	2.03	1.12	0.82
Curcuma longa Linn.	Beijing (1980)	3.82	1.79	1.11	0.75
Curcuma longa Linn.	Nan-Chang (1965)	1.41	0.70	0.35	0.25
Curcuma longa Linn.	Kwang-Chou (1979)	1.28	0.63	0.40	0.52
Curcuma xanthorrhiza Roxb.	Kwang-Chou (1980)	2.10	1.43	0.86	0.12
Curcuma wenyujin (Y.H. Chen et C. Ling)	Che-Chiang (1979)	0.20	0.13	0.07	0.02
Curcuma sichuanensis	Si-Chuan (1980)	0.04	0.01	0.01	<0.01
Curcuma Kwangsinensis	Yun-nan (1980)	1.54	0.89	0.57	0.23
Curcuma aeruginosa Roxb.	Si-Chuan (1980)	0.04	0.01	0.01	<0.01
Curcuma elata Roxb.	Kwang-see (1980)	0.01	<0.01	<0.01	<0.01

Source: Chen and Fang (1997).

[a] Origin, the site of cultivation or collection; year, the time of sample collection.

[b] Total, total curcuminoid; Cur, curcumin; Dcur, demethoxycurcumin; Bdcur, bisdemethoxycurcumin.

Table 2. Biochemical Actions of Curcumin

Biochemical action	Reference
Scavenges superoxide anion and hydroxyl radical	Kunchandy and Rao (1990)
Scavenges singlet oxygen	Subramanian *et al*. (1994)
Inhibits lipid peroxidation	Sreejayan (1994)
Inhibits TPA-induced ornithine decarboxylase (ODC) mRNA and activity	Huang *et al*. (1988)
Inhibits TPA-induced cellular 8-hydroxydeoxyguanosine	Shih and Lin (1993)
Inhibits TPA-induced skin inflammation	Huang *et al*. (1997)
Inhibits lipoxygenase and cyclooxygenase activities	Huang *et al*. (1991)
Inhibits arachidonic acid metabolism	Conney *et al*. (1991)
Inhibits the formation of carcinogen-DNA adducts	Conney *et al*. (1991)
Inhibits skin tumor initiation and promotion	Huang *et al*. (1992)
Inhibits BaP induced forestomach and lung tumorigenesis	Huang *et al*. (1994)
Inhibits ENNG-induced duodenal tumorigenesis	Huang *et al*. (1994)
Inhibits azoxymethane-induced colon tumorigenesis in mice and rats	Rao *et al*. (1995)

tric oxide and peroxynitrite. Curcumin has the ability to protect lipids, hemoglobin, and DNA against oxidative degradation. Pure curcumin has more potent superoxide anion scavenging activity than demethoxycurcumin or bisdemethoxycurcumin (Kunchandy and Rao, 1990). Curcumin is also a potent inhibitor of ROS-generating enzyme cyclooxygenase and lipoxygenase in mouse epidermis (Huang *et al*., 1991).

2. Inhibition of Chemical Carcinogenesis

Curcumin inhibited chemical carcinogenesis in different tissue sites in several experimental animal models as indicated by Table 2. Curcumin inhibited tumor initiation by benzo[a]pyrene (BaP) and 7,12-dimethylbenz[a]anthracene (DMBA) in mouse epidermis (Conney *et al*., 1991). Topical application of curcumin strongly inhibited tumor promotion in the skin of DMBA-initiated mice (Huang *et al*., 1988, 1992, 1995). Including 0.5% – 2.0% curcumin in the diet decreased BaP-induced forestomach tumors per mouse by 51% – 53% when it was administered during the initiation period and by 47% – 67% when it was administered during the postinitiation period (Huang *et al*., 1994). Including curcumin in the diet decreased the number of N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)-induced duodenal tumors per mouse (Huang *et al*., 1994). Administration of curcumin in the diet decreased the number of azoxymethane (AOM)-induced colon tumors in mice (Huang *et al*., 1994) and in rats (Rao *et al*., 1995).

3. Induction of Apoptosis

We have demonstrated that curcumin (30 μM) induces apoptosis in several tumor cell lines (Jiang *et al*., 1996). The curcumin-induced apoptosis is highly dependent on the origin and malignancy of cell lines. It appears that the typical apoptosis can only be induced in immortalized mouse embryo fibroblast NIH 3T3, erbB2 oncogene-transformed NIH 3T3, mouse Sarcoma 180, human colon cancer cell HT29, human kidney cancer cell 293, and human hepatocellular carcinoma HepG2 cells but not in primary cultures of mouse embryonic fibroblast C3H 10T1/2, rat embryonic fibroblast or human foreskin fibroblast cells (Jiang *et al*., 1996). Treatment of NIH 3T3 cells with the PKC inhibitor staurosporine, the tyrosine kinase inhibitor herbimycin A or arachidonic acid metabolism inhibitor quinacrine induces typical apoptosis. These findings suggest that blocking the cellular signal transduction in immortalized or transformed cells might trigger the induction of apoptosis.

We have also demonstrated that curcumin (3.5 μg/ml) induces human promyelocytic HL-60 cells. The apoptosis-inducing activity of curcumin occurred in a dose- and time-dependent manner. Flow cytometric analysis showed that the hypodiploid DNA peak of propidium iodide-stained nuclei

appeared 4 h after treatment with 7 μg/ml curcumin. The apoptotic effect of curcumin was not affected by cycloheximide, actinomycin D, EGTA, W7 (calmodulin inhibitor), sodium orthovanadate, or genistein whereas an endonuclease inhibitor, $ZnSO_4$, and a proteinase inhibitor, N-tosyl-L-lysine chloro-methyl-ketone (TLCK), could markedly abrogate curcumin-induced apoptosis. The antioxidants N-acetyl-L-cysteine (NAC), L-ascorbic acid, alpha-tocopherol, catalase and superoxide dismutase all effectively prevented curcumin-induced apoptosis. Furthermore, overexpression of bcl-2 in HL-60 cells delayed the entry of curcumin-treated cells into apoptosis, suggesting that bcl-2 plays an important role in the early stage of curcumin-triggered apoptotic cell death (Kuo *et al*., 1996).

III. Mechanisms of Cancer Chemoprevention by Curcumin

1. Suppression of c-jun and c-fos Expression

In 1991, we have made an interesting finding that the phorbol ester TPA induced transcriptional factor c-jun/AP-1 in mouse fibroblast cells was suppressed by curcumin (Huang *et al*., 1991). Elevated expression of genes transcriptionally induced by TPA is among the events required for tumor promotion. Functional activation of the transcriptional factor c-jun/AP-1 is believed to play an important role in signal transduction of TPA-induced tumor promotion. Suppression of c-jun/AP-1 activation by curcumin (10 μM) was observed in mouse fibroblast cells. The results of *in vitro* experiments indicate that inhibition of c-jun/AP-1 binding to its cognate motif, 5'-TGACTCAG-3', by curcumin may be responsible to the inhibition of c-jun/AP-1-mediated gene expression (Huang *et al*., 1991). These findings show for the first time that the effect of curcumin on TPA-induced inflammation/tumor promotion can be studied at the molecular level as illustrated in Table 3.

Curcumin also inhibits the TPA- and UVB light-induced expression of c-jun and c-fos in JB6 cells and in mouse epidermis (Lu *et al*., 1994).

Table 3. Modulation of Tumor Biomarkers by Curcumin

Tumor biomarker	Reference
Inhibition of TPA-induced c-jun and c-fos expression	Huang *et al*. (1991) and Lu *et al*. (1994)
Inhibition of TPA-induced protein kinase C (PKC)	Liu *et al*. (1993)
Inhibition of TPA-induced EGF-receptor tyrosine kinase (RTK)	Korutla *et al*. (1994)
Inhibition of inducible nitric oxide synthase (iNOS)	Chan *et al*. (1998)
Inhibition of NFκB and IκB kinase (IKK) activation	(unpublished, from Pan and Lin)
Inhibition of TPA-induced xanthine oxidase	Lin and Shih (1994)
Inhibition of p53 gene expression	Chen *et al*. (1996)
Induction of HSP70 gene expression	Chen *et al*. (1996)
Reduction of ER(+)PgR(+) mammary tumor	Inano *et al*. (1999)
Inhibition of TPA-induced transformation in mouse fibroblast cells	Lee and Lin (1997)
Inhibition of the invasion of hepatocellular carcinoma cells by suppressing matrix metalloproteinase-9	Lin *et al*. (1998)
Induction of apoptosis in NIH 3T3 fibroblast cells	Jiang *et al*. (1996)
Induction of apoptosis in human leukemia HL-60 cells	Kuo *et al*. (1996)

2. Inhibition of Protein Kinase C (PKC) and EGFR Tyrosine Kinase

When mouse fibroblast cells (NIH 3T3) were treated with TPA alone, PKC translocated from the cytosolic fraction to the particulate (membrane) fraction. Treatment with 15 or 20 μM curcumin for 15 min inhibited TPA-induced PKC activity in particulate fractions by 26 or 60% and did not affect the level of PKC protein. Curcumin also inhibited the PKC activity in both cytosolic and particulate fractions in vitro by competing with phosphatidylserine. However, the inhibitory effect of curcumin was reduced following preincubation with thiol compounds (Liu *et al*., 1993).

Curcumin (10 μM) inhibits EGF receptor kinase activity by up to 90% in a dose- and time-dependent manner and also inhibits EGF-induced tyrosine phosphorylation of EGF-receptors in A431 cells (Korutla and Kumar, 1994). Treatment of NIH 3T3 cells with a saturating concentration of EGF for 5 – 15 min induced increased EGF-R tyrosine phosphorylation by 4 to 11-folds and this effects was inhibited by curcumin, which also inhibited the growth of EGF-stimulated cells (Korutla *et al*., 1995).

Recent studies in our laboratory have demonstrated that curcumin blocks NFκB activation by down-regulating IκB kinase activity in macrophages (unpublished results). Curcumin has been shown to suppress the expression of inducible nitric oxide synthase (iNOS) *in vivo* (Chan *et al*., 1998).

3. Suppression of Colonic Aberrant Crypt Foci through Inhibiting iNOS

It has been demonstrated that iNOS is overexpressed in colonic tumors of humans and also in rats treated with a colon carcinogen. INOS appears to regulate cyclooxygenase-2 (COX-2) expression and the production of pro-inflammatory prostaglandins, which are known to play a key role in colon tumor development. Experiments were designed to study the inhibitory effects of curcumin on the formation of azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF) in male F344 rats. Both iNOS activity and colonic ACF formation were significantly inhibited by curcumin (Rao *et al*., 1999).

4. Inhibition of COX-2 by Curcumin in Bile Acid and PMA-Treated Cells

The inhibition of PMA- or chenodeoxycholate (CD)-induced COX-2 by curcumin in several human gastrointestinal cell lines (SK-GT-4, SCC450, IEC-18 and HCA-7) was studied (Zhang *et al*., 1999). Treatment with curcumin suppressed CD- and PMA-mediated induction of COX-2 m-RNA as well as protein level and synthesis of prostaglandin E2 in these cell lines. Nuclear run-offs revealed increased rates of COX-2 transcription after treatment with CD or PMA , and these effects were inhibited by curcumin. Treatment with CD or PMA increased the binding of AP-1 to DNA. This effect was also inhibited by curcumin (Huang *et al*., 1991; Zhang *et al*., 1999). Furthermore, the activity of COX-2 was found to be directly inhibited by curcumin *in vitro* (Zhang *et al*., 1999). These findings may provide new insightes into the cancer chemoprevention properties of curcumin.

5. Inhibition of Xanthine Oxidase

Treatment of NIH 3T3 cells with the tumor promoter TPA results within 30 min in a 1.8 fold elevation of xanthine oxidase activity, an enzyme capable of generating ROS, such as superoxide and hydrogen peroxide. Simultaneous administration of 2 and 10 μM curcumin with 100 ng/ml TPA was found to inhibit TPA-induced increases in xanthine oxidase activity measured 30 min later by 22.7% and 36.5% respectively (Lin and Shih, 1994). The TPA-induced conversion of xanthine dehydrogenase into xanthine oxidase is reduced by curcumin to the basal level found in untreated cells. Activity of xanthine oxidase is remarkably inhibited by curcumin *in vitro*, but not by its structurally related compounds caffeic acid, chlorogenic acid and ferulic acid. Based on these findings, the induction of xanthine oxidase activity is seemed to be one of the major causative elements in TPA-mediated tumor promotion, and the major inhibitiory mechanism by which curcumin inhibits TPA-induced increases in xanthine dehydrogenase/oxidase enzyme activities is considered to occur through direct inactivation in the protein level (Lin and Shih, 1994).

6. Modulation of Ca^{+2} and Cellular p53 Protein

When COLO205 colorectal carcinoma cells were treated with curcumin (60 μM), the appearance of apoptotic DNA ladders was delayed about 5 h and G1 arrest was detected (Chen *et al*., 1996). Further analysis of the endonuclease activities in these cells revealed that the activity of Ca^{+2}-dependent endonuclease in COLO205 cells was profoundly inhibited, and that the extent of inhibition depended on the degree of calcium depletion. The reduction of p53 gene expression was accompanied by the induction of HSP70 gene expression in the curcumin-treated cells. These findings suggest that curcumin may induce the expression of the HSP70 gene through the initial depletion of intracellular Ca^{+2}, followed by the suppression of the p53 gene function in the target cells (Chen *et al*., 1996).

7. Reduction of ER(+)PgR(+) Mammary Tumor

The chemopreventive effects of curcumin on diethylstilbestrol (DES)-induced tumor promotion in rat mammary glands initiated with radiation have been evaluated (Inano *et al*., 1999). The administration of dietary curcumin significantly reduced the incidence (28%) of mammary tumors. Multiplicity and Iball's index of mammary tumors were also decreased by curcumin. Rats fed a curcumin diet showed a reduced incidence of the development of both mammary adenocarcinoma and ER(+)PgR(+) tumors in comparison with a control group. These findings suggest that curcumin has a potent preventive activity during the DES-dependent promotion stage of radiation-induced mammary tumorigenesis (Inano *et al*., 1999). Curcumin has also been found to suppress TPA-induced transformation in mouse fibroblast cells (Lee and Lin, 1997).

8. Curcumin acts as Inducer of Phase-2 Detoxification Enzymes

Several possible mechanisms of the observed anti-tumor effects of curcumin have been suggested. Among these, its antioxidant and anti-inflammatory properties have received much attention as noted above. On the other hand, curcumin may be a natural chemoprotective agent, since it also elevates the activities of Phase-2 detoxification enzymes of xenobiotic metabolism, such as glutathione transferase, epoxide hydrolase and NADPH: quinone reductase, while inhibiting procarcinogen activating Phase-1 enzymes, such as cytochrome p450 1A1 (Ciolino *et al*., 1998).

Based on compelling evidence that coordinate induction of Phase-2 enzymes is a critical and sufficient condition for protection against toxicity and carcinogenicity (Talalay *et al*., 1995), a series of naturally-occurring as well as synthetic structural analogs of the dietary constituent curcumin were examined to elucidate which portions of the molecule are critical for its ability to induce Phase-2 detoxification enzymes in murine hepatoma cells, and to assess the chemoprotective potential of these compounds (Dinkova-Kostova and Talalay, 1999). Two groups of compounds were studied: (1) classical Michael reaction receptors such as curcumin and (2) related β-diketones such as dibenzoylmethane which lack direct Michael reactivity. Two structural elements was found to be required for high inducer potency: (1) hydroxy groups at the *ortho*-position on the aromatic rings and (2) the β-diketone functionality.

9. Suppression of Hepatocellualr Carcinoma Invasion by Inhibiting MMP-9

Recently, curcumin was further demonstrated to have

an anti-metastatic effect in mice. We attempted to investigate the possible mechanism of this effect. A highly invasive SK-Hep-1 cell line of human hepatocellular carcinoma was selected for this study. An *in vitro* assay, without or with the Matrigel matrix, was used to quantify cellular migration and invasion. Gelatin-based zymography was adopted to assay the secretion of matrix metalloproteinase-9 (MMP-9). We found that curcumin at 10 μM , inhibited by 17.4 and 70.6% cellular migration and invasion of SK-Hep-1 cells, respectively. Compared with a less invasive human hepatocellular carcinoma cell line, Huh 7, SK-Hep-1 showed a much higher level of MMP-9 secretion. Furthermore, parallel with its anti-invasion activity, curcumin inhibited MMP-9 secretion in SK-Hep-1 in a dose-dependent fashion. We conclude that curcumin has a significant anti-invasion activity in SK-Hep-1 cells, and that this effect is associated with its inhibitory effect on MMP-9 secretion (Lin *et al*., 1998).

IV. Biotransformations of Curcumin

1. Chemical Degradation of Curcumin

Curcumin is a yellow antioxidant substance. It seems that curcumin is sensitive to oxygen in aqueous solution, and that it is affected by UV under solar light exposure. The degradation kinetics of curcumin under various pH conditions and the stability of curcumin in physiological matrices were investigated in our laboratory (Wang *et al*., 1997). When curcumin was incubated in 0.1 M phosphate buffer and serum-free medium, pH 7.2 at 37°C, about 90% of the compound decomposed within 30 min. A series of pH conditions ranging from 3 to 10 were tested, and the results showed that decomposition was pH-dependent and occurred faster under neutral-basic conditions. Curcumin is more stable in cell culture medium containing 10% fetal calf cerum and in human blood; less than 20% of the curcumin decomposed within 1 h, and after incubation for 8 h, about 50% of the curcumin still remained (Wang *et al*., 1997). Based on mass and spectrophotometrical analysis, trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal was tentatively identified as a major degradation product while vanillin, ferulic acid and feruloylmethane were identified as minor degradation products (Fig. 1) (Wang *et al*., 1997).

Since curcumin decomposes rapidly in serum-free medium, precautions must be taken during cell culture experiments. In addition, the biological effects caused by the degradation products of curcumin, especially vanillin, must be taken into consideration. Vanillin, a naturally occurring flavoring agent, has been reported to inhibit mutagenesis in bacterial and mammalian cells. It may act as an antimutagen by modifying DNA replication and DNA repair systems after cellular DNA damage caused by mutagens occurs. Vanillin is also a powerful scavenger of superoxide and hydroxyl radicals.

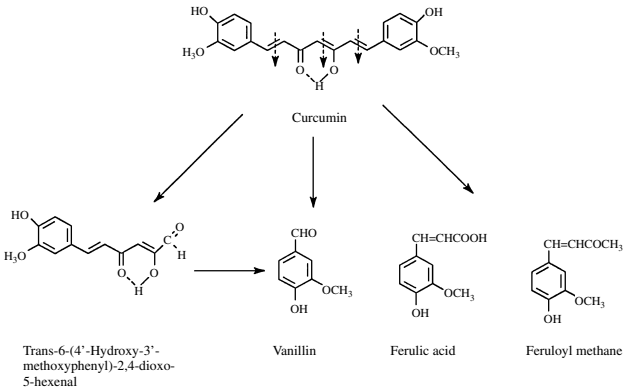

Fig. 1. Degradation of curcumin in aqueous solution. The major degradation product of curcumin in 0.1 M phosphate buffer at pH 7.2 was tentatively identified as trans-6-(4'-hydroxy-3'-methoxyphenyl)-2, 4-dioxo-5-hexenal while the minor products were identified as vanillin, ferulic acid and feruloylmethane. [Data from Wang *et al*. (1997)].

2. Biotransformations of Curcumin in Mice

We have investigated the pharmacokinetic properties of curcumin in mice (Pan *et al*., 1999). After intraperitoneal adminstration of curcumin (0.1 g/kg) to mice, about 2.25 μg/ml of the curcumin appeared in the plasma during the first 15 min. One hour after administration, the levels of curcumin in the intestine, spleen, liver and kidneys were 177, 26, 27, and 7.5 μg/g, respectively. Only traces (0.41 μg/g) were observed in the brain at 1 h. To clarify the nature of the metabolites of curcumin, the plasma was analyzed using reversed-phase HPLC, and two putative conjugates were observed. Further treatment of the plasma with β-glucuronidase resulted in a decrease in the levels of these two putative conjugates and in the concomitant appearance of tetrahydrocurcumin and curcumin, respectively. To investigate the nature of these glucurinide conjugates *in vivo*, the plasma was analyzed by means of electrospray. The chemical structures of these metabolites were determined by means of MS/MS analysis. The experimental results suggested that curcumin was first biotransformed into dihydrocurcumin and tetrahydrocurcumin and these compounds subsequently were converted to monoglucuronide conjugates as illustrated in Fig. 2. These results suggest that curcumin-glucuronide, dihydrocurcumin-glucuronide, tetrahydrocurcumin-glucuronide and tetrahydro-curcumin are major metabolites of curcumin *in vivo*.

V. A Proposal for the Action Mechanisms of Curcumin

Recent intensive studies on the action mechanisms of curcumin in various biological systems have indicated that this compound employs multiple anti-tumor promoting pathways (Lin *et al*., 1994). It has been demonstrated that TPA-induced tumor promotion is significantly inhibited by curcu-

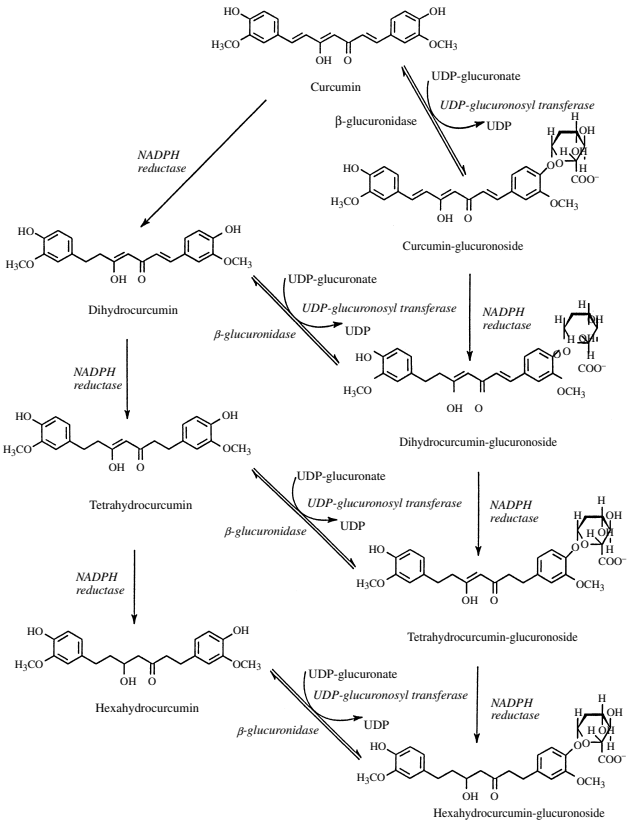

Fig. 2. Biotransformations of curcumin in mice. Two main transformation pathways, namely, reduction and glucuronidation of Curcumin, are depicted. Our preliminary results indicate that NADPH is required for reduction reaction but the nature of the reductase is unknown. Furthermore, most of the conjugated derivatives are hydrolyzed by β-glucosidase and has been identified as glucuronides in mouse plasma. [Data from Pan *et al.* (1999)].

min (Huang *et al*., 1988, 1997; Rao *et al*., 1995). TPA is a versatile biological active agent which induces several molecular biological processes, namely, enhanced expression of cellular oncogenes such as c-jun, c-fos and c-myc; induction of ornithine decarboxylase; elevation or translocation of PKC; induction of cyclooxygenase and lipoxygenase; and other processes. It seems that all of these biochemical processes are required for anabolic pathways and cell proliferation that lead to tumor promotion. It is noteworthy that all of these processes have been shown to be effectively inhibited by the presence of curcumin (Tables 2 and 3).

It is conceivable that the molecular mechanism of action of curcumin is quite complicated and dispersed. The locations of targets of its action vary from genome (DNA) level, to the messenger (RNA) level, to the enzyme (protein) level (Lin *et al*., 1994). The action of curcumin may proceed simultaneously or sequentially through these different levels. Accordingly, we propose the following pathways for the action of curcumin (Fig. 3). The primary target of curcumin could be the plasma membrane where the activity of PKC is first inhibited (Liu *et al*., 1993). At the same time, the activity of EGF receptor tyrosine kinase is also inhibited (Korutla and Kumar, 1994). Some PKC-mediated nuclear protein factors, such as IκB kinase and NFκB, are then inhibited through various signal transduction pathways. The TRE binding activity of c-jun/AP-1 is then repressed (Huang *et al*., 1991), and finally the transcriptions of genes essential for cell proliferation are suppressed as indicated by the inhibition of related enzymes, such as ornithine decarboxylase, PKC, cyclooxygenase and lipoxygenase (Table 2).

It appears that activation of calcium-dependent protein kinases (such as PKC) or inhibition of protein phosphatases results in tumor promotion (Haystead *et al*., 1989). In the case of tumor promoters, it appears that a common final effect is an increase in phosphorylation of the protein substrate on serine or threonine residues. The nature of these substrates

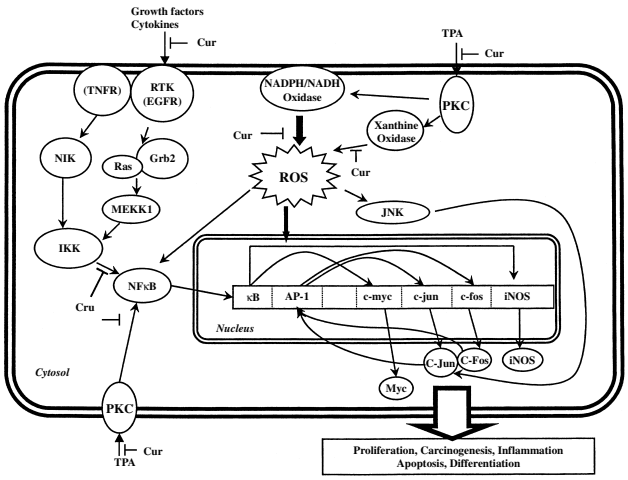

Fig. 3. A proposal model illustrating the proposed action mechanism of curcumin for the inhibition of carcinogenesis and inflammation. Extracellular growth factors, cytokines, or tumor promoter 12-O-tetradeca-noylphorbol-13-acetate (TPA) binds to membrane receptors, such as epidermal growth factor receptor (EGFR), tumor necrosis factor receptor (TNFR), or protein kinase C (PKC), resulting in the activation of a number of serine, threonine or tyrosine kinases, which include ras, NFκB inducing kinase (NIK), mitogen-activated protein kinase (MAPK), extracellular response kinase (ERK), MAPK/ERK kinase kinase ($MEKK_1$), IκB kinase (IKK) and c-jun N-terminal kinase (JNK). JNK is activated by MAPK kinase (MKK_4), causing activation of the c-jun protein which forms a heterodimer with the c-fos protein thus enhancing the activity of the transcription factor AP-1. Recent studies indicate that both IKK and PKC are important for activation of NFκB which leads to enhancement of the expression of c-myc, iNOS and other cellular proliferation genes. Reactive oxygen species (ROS) are considered to be endogenous mitogenic factors (or apoptotic factors under certain conditions) that can activate NFκB and other transcription factors in the nucleus. Ultimately, activation of the MAPK family members causes activation of specific transcription factors, such as NFκB, AP-1, serum response factor (SRF) and others which help determine the fate of cell such as proliferation, carcinogenesis,inflammation or apoptosis. Curcumin (Cur) has been demonstrated to block several sites of these multiple signal transduction pathways as indicated by the blockade sympbol (⊥).

has not been completely characterized, but the convergence of the actions of disparate tumor promoters on specific phosphorylation of protein substrate on serine and threonine residues strongly implicates signal transduction in tumor promotion. It appears that when any essential component of a signal transduction pathway is rendered hyperactive or autonomous, it may acquire the ability to drive the cell into unchecked proliferation leading to tumor promotion. Curcumin may attenuate or suppress the hyperactivity of these components of signal transduction and maintain simultaneously the normal cell function.

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