

Review article

CURCUMIN AND APOPTOSIS: RELATED TO OXIDATIVE STRESS, CARCINOGENESIS AND PROTEASOME INHIBITION

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Abstract Curcumin (diferuloylmethane) is an active ingredient in *Curcuma longa* Linn. It induces apoptosis in various cell types, e.g. human leukemic (HL-60) cells, breast cancer cells, endothelial cells and HeLa cells. On the contrary, it also inhibits programmed cell death in other systems, viz. breast cancer cells (MCF-7, MDA-MB-231), and T lymphocytes. Curcumin inhibits c-Jun NH₂-terminal kinase (JNK) activation, and alters the function of activator protein-1 (AP-1), nuclear factor-kappa B (NF-κB) and p53 functions. In the aspect of carcinogenesis, curcumin inhibits tumorigenesis of carcinogens in the steps of initiation and promotion. However, in the pathway of apoptosis, it also upregulates the proto-oncoprotein Bax and involves the release of cytochrome c and activation of caspases. In HL-60 cells, inhibition of proteasome mediated proteolysis by specific proteasomal inhibitors, such as curcumin, leads to induction of apoptosis, whereas, lactacystin (a proteasomal inhibitor) prolongs survival of nerve growth factor-deprived neuronal apoptosis. The production of free radicals or oxidative stress occurs in several systems of cells, such as HL-60 cells, treated with curcumin. Curcumin has many important roles such as anticarcinogenicity and antiangiogenicity. Its clinical usage for patients requires more information from human trials. **Chiang Mai Med Bull 2006;45(1):31-43.**

Keywords: curcumin, apoptosis, oxidative stress, carcinogenesis, proteasome inhibition

Curcumin

Originally cultivated in many tropical regions of Asia, *curcuma longa* Linn is a perennial herb from which dried rhizome is isolated from the spice, turmeric. It belongs to the family, Zingiberaceae, and has a long and distinguished human use in Eastern civilization. Its rhizome is used extensively for imparting color and fla-

vor to food. Turmeric, the powder from the dried rhizomes, is used for medicinal purposes and reportedly used as an antiseptic, a cure for poisoning, eliminating body waste products, treating dyspepsia and respiratory disorders, a cure for some skin diseases including wound healing, and a household remedy for treating sprains and swellings caused by injury.⁽¹⁾

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Curcumin, also known as diferuloylmethane: 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, is the major yellow pigment extracted from turmeric, used extensively in curries and identified as the active component in turmeric. Its properties as a coloring and flavoring agent have led to using it as a dietary additive in a variety of food. These include saffron, mustard and other spices, gelatins, puddings, ice creams, soups, pickles, margarine, and both alcoholic and nonalcoholic beverages. Extracts containing curcumin have also been used for generations in medicines in India and Southeast Asia, and according to tradition are useful in the treatment of inflammation, skin wounds, hepatic and biliary disorders, cough, and coryza, as well as certain tumors. As a result, dietary intake of curcumin is especially high in these areas of Asia, where adults consume up to >200 mg of curcumin/day or 7.8 $\mu\text{mol/kg}$ of body weight.⁽²⁾ However, curcumin exposure in France may be more representative of the typical world, where intake of as much as >3.4 $\mu\text{mol/kg/day}$ has been documented.⁽³⁾

Several studies in recent years have shown that curcumin is a potent inhibitor of the initiation and promotion of chemical carcinogen-induced tumor formation in animals. Besides its anti-carcinogenic effects, curcumin exhibits remarkable anti-inflammatory and anti-oxidant properties *in vivo*.⁽⁴⁾ The pharmacological safety of curcumin is demonstrated by its consumption for centuries at levels of up to 100 mg/day by people in certain countries.⁽⁵⁾

The exposure of curcumin to populations worldwide, and its many uses, have led to studies aimed at elucidating some of its activities. Curcumin and related compounds inhibit free radical generation, and act as free radical scavengers⁽⁶⁾ and antioxidants,⁽⁷⁾ inhibiting lipid

peroxidation⁽⁸⁾ and oxidative deoxyribonucleic acid (DNA) damage.⁽⁹⁾ Inhibition of cyclooxygenase and lipooxygenase resulting in decreased activation of NF- κ B, may contribute to the anti-inflammatory activity of this compound. Another property ascribed to curcumin is the inhibition of c-jun/AP-1 function and JNK activation.⁽¹⁰⁾ Curcumin has been noted as a potent inhibitor of cytochrome P450 and possesses the ability to induce glutathione S-transferase, and as such, has been proposed as a potential chemoprotective agent. Because curcumin inhibits tumor formation in several murine tissues, and antagonizes both initiation and promotion of tumors in rodent epithelial and colon cancer models, interest has been raised in this compound as a chemopreventive agent.⁽¹¹⁾

Most recently, curcumin has demonstrated antiangiogenic properties in several laboratory and *in vivo* model systems.⁽¹²⁻¹⁴⁾ These properties of curcumin have led to several phase I human trials that have shown this agent to be well tolerated,^(15,16) and their successful completion suggests that curcumin may be increasingly used in the future.

Apoptosis

Apoptosis, a mode of cell death, plays a crucial role in embryonic development, metamorphosis, hormone-dependent atrophy, and tumor growth as a physiological event, regulating cell number and eliminating damaged cells.⁽¹⁷⁾ Apoptosis is the most common form of eukaryotic cell death. It is a physiological suicide mechanism that preserves homeostasis, in which cell death naturally occurs during tissue turnover.⁽¹⁸⁾ In general, cells undergoing apoptosis display profound structural changes, including a rapid blebbing of the plasma membrane and nuclear disintegration.

The nuclear collapse is associated with extensive damage to chromatin and DNA cleavage into oligonucleosomal-length DNA fragments after activation of calcium-dependent endogenous endonucleases.⁽¹⁹⁾ Apoptosis is essential in many physiological processes, including turnover of skin, epithelium of the gastrointestinal system and the maturation of the immune response.⁽²⁰⁾

Curcumin-induced apoptosis

The apoptosis-inducing activity of curcumin appears in a dose- and time- dependent manner. It has been found that curcumin can induce apoptotic cell death in promyelocytic leukemia HL-60 cells at a concentration as low as 3.5 µg/mL. The apoptosis-inducing activity of curcumin is not affected by cycloheximide, actinomycin D, EGTA W7 (calmodulin inhibitor), sodium orthovanadate, or genistein. By contrast, the endonuclease inhibitor, ZnSO₄, and proteinase inhibitor, *N*-tosyl-L-lysine chloro-methyl ketone (TLCK), markedly abrogate apoptosis induced by curcumin, whereas 12-*O*-tetradecanoylphorbol-13-acetate (TPA) has a partial effect.⁽²¹⁾

Treatment of DNA from the plasmid, pBR322, and calf thymus, with curcumin plus copper ion, causes strand scission and the formation of 8-hydroxy-2'-deoxyguanosine in DNA. The addition of catalase protects DNA from curcumin-dependent injuries, indicating that the hydroxyl radical may participate in DNA damage. Curcumin can generate reactive oxygen species as a prooxidant in the presence of transition metals in cells, resulting in DNA injuries and HL-60 apoptotic cell death. The prooxidant action of curcumin may be related to the conjugated β-diketone structure of this compound.⁽²²⁾

Curcumin induces cell death in HL-60 cells, both sensitive and with the MDR phenotype, which is classified as caspase-3-dependent apoptosis, together with cytochrome c release, activation of caspase-3 and oligonucleosomal DNA fragmentation. No active caspase-8 is detected. There is no correlation between P-gp expression and resistance to caspase-3-dependent apoptosis induced by curcumin in HL-60 cells.⁽²³⁾

It has also been found that when HL-60 cells are treated with curcumin, the expression level of Mcl-1 is down-regulated, but that of Bax and Bak up-regulates time-dependently. Curcumin can increase the peak of sub-G1. The expression of P27Kipl, P21waf1 and pRbp is elevated and that of cyclin D3 decreases in the presence of curcumin. These findings suggest that the Bcl-2 gene family indeed participates in the regulatory process of apoptosis induced by curcumin in HL-60 cells. Curcumin can disturb cell cycle progression of HL-60 cells. The mechanism appears to be mediated by perturbing G0/G1 phases checkpoints.⁽²⁴⁾

Doxorubicin induces HL-60 cells to undergo apoptosis based on a morphological criterion. Doxorubicin is used in the range of 5-100 µg/mL. This implicates the mode of cell death via the mutagenic effect of doxorubicin in an *in-vitro* system.⁽²⁵⁾ Curcumin affects the number of HL-60 apoptotic deaths induced by doxorubicin. It has been shown that at a curcumin dose of 0.27 nM-0.27 µM (but not in a dose response) inhibits HL-60 cell apoptotic cell death, whereas, a dose of 2.72-27.2 µM with doxorubicin causes HL-60 apoptotic death synergistically. The HL-60 cells treated with curcumin alone at various doses causes HL-60 cell apoptosis in a dose dependent manner (correlation coefficient, R², =0.46). Furthermore, curcumin (27.2 µM) increases more

HL-60 necrotic cells in synergy with doxorubicin, when compared with the cells treated with doxorubicin alone.⁽²⁶⁾

Because reactive oxygen species (ROS) have been found to play important roles in drug-induced apoptosis, curcumin has been shown as an antioxidant and free radical scavenger, which inhibits the ability of chemotherapeutic drugs to induce apoptosis. Recently, it was also demonstrated that production of ROS may be the cause of tumor cell apoptosis as a result of curcumin treatment.⁽²⁷⁾

Moreover, it was found that curcumin concentration-dependently and time-dependently decreases the proliferation and viability of HL-60 cells via ROS generation (as indicated by the level of malondialdehyde, MDA), which means that low concentrations of curcumin diminish the ROS generation, while high concentrations of promote it. Combining the opposite effect of 50 μM H_2O_2 on low or high concentrations of curcumin induces MDA alteration, cell proliferation arrest and cell death. These results prove that low concentrations curcumin exerts its anticancer activity through diminishing it in HL-60 cells, while a high concentrations does so by promoting ROS generation. Further studies show that all water-soluble antioxidants (ascorbic acid, N-acetylcysteine, glutathione) significantly enhance both the antioxidant and anticancer activity of low curcumin concentrations. Considering that the extra accumulation of ROS is harmful to normal cells, the data suggest that instead of using high doses, combining low doses of curcumin with water-soluble antioxidants is a better strategy for improving the anticancer activity of curcumin.⁽²⁸⁾

This strategy is currently the subject of intense research, partially because it is recognized that tumor cells are susceptible to death

by apoptosis in response to drugs and/or radiation treatment. Several studies have demonstrated that apoptosis may be involved in cell death induced by chemotherapeutic agents including cisplatin, camptothecin, etoposide, and teniposide.⁽²⁹⁻³¹⁾ Curcumin's chemopreventive activity in animal model systems has led investigators to study its potential impact upon tumor cell growth and apoptosis. It has been found that curcumin contains an antiproliferative effect on cultured cells such as colon and breast cancer cells.^(32,33) In the past few years, the interest in using apoptosis as a possible measure of radiosensitivity has increased substantially with regard to both the possibilities of using the extent of apoptosis as a biological dosimeter and for estimating the radiosensitivity of cancer cells before radiotherapy.⁽³⁴⁻³⁶⁾ The status and level of protein expression, which regulates apoptosis, have even been proposed to serve as radiation exposure indicators or sensors. Curcumin has diverse effects on various kinds of cells. It has been found to reduce the number of cells with chromosomal aberrations, but protect normal cells from chromatid breaks, due to γ -irradiation exposure.⁽³⁷⁾

Curcumin (concentrations ranging from 0 to 200 μM) has been applied to human cancer cell cultures (human cervical cancer, HeLa cells), chronic myeloid leukemic (K-562) cells and human bone marrow multiple myeloma (IM-9) cells with or without X-irradiation (doses comprised between 0 and 8 Gy). It induces apoptosis at concentrations above 100 μM by the characteristics of morphology. Cells treated with curcumin exhibit a sub-G1 peak from which the magnitude is proportional to the concentration of curcumin. X-irradiation alone induces polyploidization and apoptosis of the three cell lines, which are proportional to

the doses of irradiation with a marked difference in radiation sensitivity between cell lines (IM-9<K-562<HeLa). However, when radiation and curcumin are applied together, the results show that in HeLa, K-562 and IM-9, curcumin has a radiation sensitizing effect only at the dose of 200 μ M. This result may open a perspective of synergical therapy at a condition to address the intrinsic toxicity of curcumin on tumor cells.⁽³⁸⁾ Curcumin can also induce normal cells to undergo apoptosis in vascular smooth muscle cells and it has an effect on cell cycle progression.⁽³⁹⁾

Curcumin inhibited apoptosis

In other systems, curcumin can inhibit apoptosis in T lymphocytes,⁽⁴⁰⁾ it protect rat lungs from injury by bleomycin⁽⁴¹⁾ and myocardium from adriamycin,⁽⁴²⁾ but its impact on the therapeutic applications of antineoplastic drugs has not been well studied.

Curcumin inhibits apoptosis in human breast cancer cell lines, i.e. MCF-7, MDA-MB-231, and BT-474 cells, in a time and dose dependent manner when induced with camptothecin, mechlorethamine, and doxorubicin. Under these conditions, curcumin exhibits antioxidant properties and inhibits both JNK activation and mitochondrial release of cytochrome c in a concentration-dependent manner. Using an *in-vivo* model of human breast cancer, dietary supplementation with curcumin is found to significantly inhibit cyclophosphamide-induced tumor regression. These findings support the hypothesis that dietary curcumin can inhibit chemotherapy-induced apoptosis through inhibition of ROS generation and blockade of JNK function. They suggest that additional studies are needed to determine whether breast cancer patients undergoing chemotherapy should avoid curcumin supplementation, and possibly

limit their exposure to curcumin-containing food.⁽¹¹⁾

Curcumin and its mechanism in apoptosis

Curcumin inhibits c-Jun NH₂-terminal kinase (JNK) activation,⁽¹⁰⁾ which has been associated with chemotherapy-mediated induction of apoptosis in tumor cells.⁽⁴³⁾ To regulate an array of cellular biochemical processes such as inhibition of nitric oxide synthase, receptor tyrosine kinase and protein kinase C activities,⁽⁴⁴⁻⁴⁶⁾ and alter transcription factors and nuclear factor κ B, p53 has been suggested.^(47,48) Modulation activities of these factors may be linked with the initiation of the apoptotic signal. Curcumin has also been reported to induce mitochondrial abnormalities, and promote p53-dependent apoptosis and activation of caspase-8 and caspase-3.⁽⁴⁹⁻⁵³⁾ However, the mechanism underlying the diverse effects of curcumin is not fully understood. One possible molecular mechanism that has been suggested is that curcumin can suppress the phorbol ester-induced transcriptional factor, c-jun/AP-1.⁽⁵⁴⁾ Recently, Korutla and Kumar⁽⁴⁶⁾ showed that curcumin (10 μ M) is capable of inhibiting the intrinsic kinase activity of epidermal growth factor of human skin squamous cell carcinoma (A431) cells.

Curcumin and carcinogenesis involved apoptosis

It has been demonstrated that topical application of curcumin inhibits benzo(a)pyrene-induced DNA adduct formation and development of skin tumors, as well as TPA-induced epidermal DNA synthesis and tumor promotion in mouse skin.^(55,56) In addition, a tumoricidal activity of curcumin has been observed in a wide range of cell lines like mouse fibroblast (NIH3T3) cells, mouse sarcoma S180,

human kidney cancer 293 cells,⁽⁴⁸⁾ Chinese hamster ovary,⁽⁵⁷⁾ rat histiocytoma (AK-5) tumor cells,^(27,58) B-cell lymphoma cells,⁽⁵⁹⁾ and human basal cell carcinoma.⁽⁵²⁾ Furthermore, curcumin has a strong inhibitory effect on cell proliferation in the HT-29 and HCT-15 human colon cancer cell lines.⁽³²⁾ Importantly, dietary administration of curcumin during initiation and/or postinitiation periods significantly suppresses development of chemically induced forestomach, duodenal, and colon tumors in CF-1 mice,⁽⁶⁰⁾ it also reduces formation of focal areas of dyspepsia and aberrant crypt foci in the colon, which are early preneoplastic lesions in rodents.^(56,61)

Ample evidence exists to support the use of curcumin in cancer prevention for its antiproliferative and anticarcinogenic properties. Curcumin, *in vivo*, suppresses carcinogenesis of the skin,⁽⁵⁷⁾ stomach,⁽⁶⁰⁾ colon,^(60,62) breast⁽⁶³⁾ and liver⁽⁶⁴⁾ in mice and *in vitro* it has been shown to inhibit the growth of a wide variety of tumor cells.^(49,50,59)

It has also been found that nuclear factor-kappa B (NF- κ B) is constitutively active in all human multiple myeloma (MM) cell lines, and that curcumin down-regulates NF- κ B in the cell lines, as indicated by electrophoretic mobility gel shift assay, and prevents the nuclear retention of p65 as shown by immunocytochemistry. All MM cell lines show constitutively active I κ B kinase (IKK) and I κ B α phosphorylation. Curcumin suppresses the constitutive I κ B α phosphorylation through the inhibition of IKK activity. It also down-regulates the expression of NF- κ B-regulated gene products, including I κ B α , Bcl-2, Bcl-xL, cyclin D1, and interleukin-6. This leads to the suppression of proliferation and arrest of cells at the G(1)/S phase of the cell cycle. Suppression of NF- κ B complex by IKK γ /NF- κ B essential

modulator-binding domain peptide also inhibits the proliferation of MM cells. Curcumin also activates caspase-7 and caspase-9 and induces polyadenosine-5'-diphosphate-ribose polymerase (PARP) cleavage. Curcumin-induced downregulation of NF- κ B, a factor that has been implicated in chemoresistance, also induces chemosensitivity to vincristine and melphalan.⁽⁶⁵⁾

Curcumin induces Ehrlich's ascites carcinoma (EAC) cells to die via apoptosis. Probing further into the molecular signals leading to apoptosis of EAC cells, curcumin upregulates the proto-oncoprotein Bax, releases cytochrome c from the mitochondria, and activates caspase-3. The status of Bcl-2 remains unchanged in EAC, which would signify that curcumin is bypassing the Bcl-2 checkpoint and overriding its protective effect on apoptosis.⁽⁶⁶⁾

Treatment of human renal Caki cells with 50 μ M curcumin results in the activation of caspase-3, cleavage of phospholipase C- γ 1 and DNA fragmentation. Curcumin-induced apoptosis is mediated through the activation of caspase, which is specifically inhibited by the caspase inhibitor, benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone. Curcumin causes dose-dependent apoptosis and DNA fragmentation of Caki cells, which is preceded by the sequential dephosphorylation of Akt, down-regulation of the anti-apoptotic Bcl-2, Bcl-xL and IAP proteins, release of cytochrome c and activation of caspase-3. Cyclosporin A, as well as caspase inhibitor, specifically inhibit curcumin-induced apoptosis in Caki cells. Pretreatment with N-acetyl-cysteine, markedly prevents dephosphorylation of Akt, cytochrome c release, and cell death, suggesting a role for reactive oxygen species in this process.⁽⁶⁷⁾

Apoptosis and proteasomal function

The ubiquitin-proteasome pathway (UPP) is the cell's principle mechanism for controlled protein degradation. The pathway has been involved in the regulation of critical cellular processes such as transcription, cell cycle progression, oncogenesis, growth and development and selective elimination of abnormal proteins, and antigen processing.⁽⁶⁸⁾ Degradation of a protein by UPP involves two distinct and successive steps: (a) covalent attachment of multiple ubiquitin molecules to the target protein and (b) degradation of the targeted protein by 26S proteasome. The 26S proteasome is a 2.1 Mda complex of which approximately 65 subunits are divided into three sub-complexes: 20S, 19S and 11S. The 20S core catalytic complex is a cylindrical stack of four seven membered rings and is flanked on both sides by 19S regulatory complexes. Three distinct types of proteolytic activities have been defined for 20S proteasome: chymotrypsin-like (Tyr or Phe at P1), trypsin-like (Arg or Lys at P2) and post-glutamyl peptidyl hydrolytic-like (Glu at P1). Since UPP plays a crucial role in the degradation of many regulatory proteins, which are necessary for cell growth, it is not surprising that the altered function of this pathway affects cell survival. It is known that the inhibition of proteasome function induces apoptosis dependence on cell types and conditions.⁽⁶⁹⁻⁷⁶⁾

It has been reported that proteolytic activities of the proteasome are essential in apoptosis or the programmed cell death of neurons. Nanomolar concentrations of several proteasome inhibitors, including the highly selective inhibitor, lactacystin, not only prolong survival of NGF-deprived neurons, but also prevent processing of poly(ADP-ribose) polymerase, which is known to be cleaved by an ICE/Ced-3 family member during apoptosis. These

results demonstrate that the proteasome is a key regulator of neuronal apoptosis and that, within this process, it is involved with upstream proteases of the ICE/Ced-family. This order of events is confirmed in macrophages, where lactacystin inhibits the proteolytic activation of the precursor, ICE, and the subsequent generation of active interleukin-1 β .⁽⁶⁹⁾

In human leukemic HL-60 cells, inhibition of proteasome-mediated proteolysis by specific proteasomal inhibitors leads to the rapid induction of apoptosis, as judged by morphological changes as well as nuclear condensation and DNA fragmentation. HL-60 apoptosis is due to activation of CPP32, a member of the ced-3/ICE family of cysteine proteases, and this appears to occur independently from ICE activity. HL-60 apoptosis is accompanied by an increase in the concentration of the cyclin-dependent kinase inhibitor, p27Kip1. Labeling of the cells by the TUNEL technique demonstrates that HL-60 cells undergoing apoptosis are primarily in the G1 phase of the cell cycle. Proteasomal activity, therefore, appears to be required in proliferating, but not in quiescent, HL-60 cells for cell survival or normal progression through the cell cycle.⁽⁷⁰⁾

The peptide aldehydes: carbobenzoxy-L-isoleucyl- γ -t-butyl-L-glutamyl-L-alanyl-L-leucinal (PSI) and MG115, which specifically inhibit the chymotrypsin-like activity of the proteasome, induce apoptosis of both rat (Rat-1) fibroblasts and rat pheochromocytoma (PC12) cells. In contrast, apoptosis is not induced by inhibitors of lysosomal proteases or an alcohol analog of PSI. The tumor suppressor, p53, rapidly accumulates in cells treated with proteasome inhibitors, as do the p53-inducible gene products, p21 and Mdm-2. In addition, apoptosis induced by proteasome inhibitors is inhibited by expression of dominant-negative

p53, whereas overexpression of wild-type p53 is sufficient to induce apoptosis of Rat-1 cells in transient transfection assays. Although other molecules may also be involved, these results suggest that stabilization and accumulation of p53 play a key role in apoptosis induced by proteasome inhibitors.⁽⁷¹⁾

The effects of proteasome inhibitors on primary neuronal (CA1) cultures has also been reported. When the two different types of proteasome inhibitors, carbobenzoxy-Leu-Leu-Leu-aldehyde or lactacystin, are used separately to suppress proteasome activity, it is observed that induction of apoptotic neuronal cell death occurs in both cases. During the apoptotic process, mitochondrial membrane potential is disrupted, cytochrome c is released from mitochondria into the cytosol, and caspase-3 like proteases are activated. Apoptosis is inhibited by pretreatment with acetyl-aspartyl-glutamyl-valyl-aspartyl-1-aldehyde or overexpression of Bcl-xL.⁽⁷²⁾

Subconfluent, proliferating endothelial cells undergo carbobenzoxy-L-isoleucyl- γ -t-butyl-L-glutamyl-L-alanyl-L-leucinal (PSI)-induced apoptosis at low concentrations ($EC_{50}=24nM$), whereas at least 340-fold higher concentrations of PSI are necessary to obtain the same effect in confluent, contact-inhibited cells. PSI-mediated cell death can be blocked by a caspase-3 inhibitor (Ac-DEVD-H), but not a caspase-1 inhibitor (Ac-YVAD-H), suggesting that a caspase-3 like enzyme is activated during PSI-induced apoptosis. When applied to the embryonic chick chorioallantoic membrane (CAM), a rapidly expanding tissue, PSI induces massive apoptosis also *in vivo*. PSI treatment of CAM leads to the formation of areas devoid of blood flow, due to the induction of apoptosis in endothelial and other cells, and the collapse of capillaries and first order vessels. It can be

concluded that the proteasomal inhibitors such as PSI may prove effective as novel anti-angiogenic and anti-neoplastic substances.⁽⁷³⁾

The mechanism of pro- and anti-apoptotic action of proteasome inhibitors is also elucidated in U937 lymphoid and 293 human kidney tumor cells. Treatment with peptidyl aldehyde MG132 and other proteasome inhibitors leads to a steady increase in activity of the c-Jun N-terminal kinase, JNK1, which is known to initiate the apoptotic program in response to certain stresses. Dose dependence of MG132-induced JNK activation is parallel with that of apoptosis. Furthermore, inhibition of the JNK signaling pathway strongly suppresses MG132-induced apoptosis. The data indicate that JNK is critical for the cell death caused by proteasome inhibitors. An anti-apoptotic action of proteasome inhibitors can be revealed by a short incubation of cells with MG132 followed by its withdrawal. Under these conditions, the major heat shock protein, Hsp72, accumulates in cells and causes suppression of JNK activation in response to certain stresses. Accordingly, pretreatment with MG132 reduces JNK-dependent apoptosis caused by heat shock or ethanol, but it is unable to block JNK-independent apoptosis induced by TNF α . Therefore, **they induces** Hsp72, which suppresses JNK-dependent apoptosis. A balance between these two (proapoptotic and antiapoptotic) effects may define the eventual fate of cells exposed to the inhibitors.⁽⁷⁴⁾

Expansion of CAG repeats within the coding region of target genes is the cause of several autosomal dominant neurodegenerative diseases, including Huntington's disease (HD). A hallmark of HD is the proteolytic production of N-terminal fragments of huntingtin containing polyglutamine repeats that form ubiquitinated aggregates in the nucleus and

cytoplasm of the affected neurons. An ecdysone-inducible stable mouse neuro2a cell line that expresses truncated N-terminal huntingtin (tNhtt) with different polyglutamine lengths, along with mice transgenic for HD exon 1, are used to demonstrate that the ubiquitin-proteasome pathway is involved in the pathogenesis of HD. The proteasomal 20S core catalytic component is redistributed to the polyglutamine aggregates in both the cellular and transgenic mouse models. The proteasome inhibitor dramatically increases the rate of aggregate formation caused by the tNhtt protein with 60 glutamine (60Q) repeats, but it has very little influence on aggregate formation by the tNhtt protein with 150Q repeats. Both normal and polyglutamine-expanded tNhtt proteins are degraded by proteasome, but the rate of degradation is inversely proportional to the repeat length. The shift of the proteasomal components from the total cellular environment to the aggregates, as well as the comparatively slower degradation of tNhtt with longer polyglutamine, decreases the proteasome's availability for degrading other key target proteins, such as p53. This altered proteasomal function is associated with disrupted mitochondrial membrane potential, released cytochrome c from mitochondria into the cytosol and activated caspase-9 and caspase-3-like proteases. These results suggest that the impaired proteasomal function plays an important role in polyglutamine protein-induced cell death.⁽⁷⁵⁾

It has been reported that curcumin-induced apoptosis is mediated through the impairment of the ubiquitin-proteasome system. Exposure of curcumin to the mouse neuro2a cells causes a dose-dependent decrease in proteasome activity and an increase in ubiquitinated proteins. Curcumin exposure also decreases the turnover of the destabilized enhanced green fluo-

rescence protein, a model substrate for the proteasome and cellular p53 protein. Like other proteasome inhibitors, curcumin targets proliferative cells more efficiently than differentiated cells and induced apoptosis via mitochondrial pathways. The addition of curcumin to the neuro2a cells induces a rapid decrease in mitochondrial membrane potential and the release of cytochrome c into cytosol is followed by activation of caspase-9 and caspase-3.⁽⁷⁶⁾

Conclusion

Curcumin has many functional activities: it causes and inhibits apoptosis depending on cell types and doses. It contains antiproliferative and tumoricidal effect in the process of chemical-induced carcinogenesis. It is a potential inhibitor of the initiation and promotion of carcinogen-induced tumor formation in animals. The mechanism of action involves cell cycle progression, Bcl-2 family protein expression, cytochrome c release, caspase activation and proteasome inhibition. The signal transduction might also involve the regulation of NF- κ B, AP-1, and JNK transcription factor. However, in different cell types and with different concentrations of curcumin, the cellular response is unique for each environment and system, as described above.

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เคอร์คิวมินและการตายอะพอพโทซิส: สัมพันธ์กับภาวะเครียดออกซิเดชัน การเกิดมะเร็งและการยับยั้งโปรตีนเอโซม

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บทคัดย่อ เคอร์คิวมิน (ไดเฟอรูโลอีลมิเชน) เป็นสารประกอบสำคัญในขมิ้น หรือ *Curcuma longa* Linn. เคอร์คิวมินกระตุ้นให้เซลล์ตายแบบอะพอพโทซิสในเซลล์มะเร็งเม็ดเลือดขาว (HL-60 cells) เซลล์มะเร็งเต้านม เซลล์บุผนังหลอดเลือด และเซลล์ฮีลา (เซลล์มะเร็งปากมดลูก) ในทางตรงกันข้าม เคอร์คิวมินยับยั้งการตายในแบบที่โปรแกรมไว้ในระบบอื่นๆ คือ ในเซลล์มะเร็งเต้านม (MCF-7, MDA-MB-231) และในทีลิมโฟไซต์ เคอร์คิวมินยับยั้งการกระตุ้น c-Jun NH2-terminal kinase (JNK) เปลี่ยนแปลงการทำงานของเอพี-1 (activator protein-1) การทำงานของนิวเคลียร์แฟกเตอร์แคปปา β (NF- κ B) และการทำงานของพี53 ในเชิงขบวนการเกิดมะเร็ง เคอร์คิวมินยับยั้งขบวนการเกิดมะเร็งในขั้นตอนก่อตัวและขั้นตอนส่งเสริม อย่างไรก็ตามในวิถีการตายอะพอพโทซิส เคอร์คิวมินเพิ่มการแสดงออกของโปรโต-อองโคโปรตีนแบ็กซ์และเกี่ยวข้องกับการหลั่งไซโตโครมซีและการกระตุ้นคาสเพส ในเซลล์ HL-60 การยับยั้งการเกิดโปรตีนเอโซมผ่านทางโปรตีนเอโซม โดยสารยับยั้งโปรตีนเอโซมที่จำเพาะ เช่น เคอร์คิวมินนำไปสู่การกระตุ้นการตายแบบอะพอพโทซิส ขณะที่แอสเพน-ซิสติน (สารยับยั้งโปรตีนเอโซมชนิดหนึ่ง) ทำให้เซลล์ประสาทรอดชีวิตนานขึ้น ในการตายแบบอะพอพโทซิสที่กระตุ้นโดยการขาดสารที่ทำให้เจริญเติบโตของเซลล์ในระบบประสาท การผลิตสารอนุมูลอิสระ หรือภาวะเครียดออกซิเดชันเกิดขึ้นในเซลล์หลายระบบ เช่น เซลล์ HL-60 เมื่อได้รับเคอร์คิวมิน เคอร์คิวมินมีบทบาทสำคัญหลายประการ เช่น การต้านการเกิดมะเร็งและต้านการเกิดหลอดเลือดใหม่ การให้เคอร์คิวมินสำหรับผู้ป่วยจำเป็นต้องมีข้อมูลในการทดสอบกับมนุษย์เพิ่มมากขึ้นก่อน เชียงใหม่ เวชสาร 2549;45(1):31-43.

คำสำคัญ: เคอร์คิวมิน การตายแบบอะพอพโทซิส ภาวะเครียดออกซิเดชัน การเกิดมะเร็ง การยับยั้งโปรตีนเอโซม