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Antiproliferative Effects of Curcumin Analogues

— Comparative antiproliferative activities of curcumin, tetrahydrocurcumin, dimethoxycurcumin and bis-demethoxycurcumin in human leukemia HL-60 cells —

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Abstract

Curcumin and its analogues(tetrahydrocurcumin THC, demethoxycurcumin ; BDMC and dimethoxycurcumin DiMC) were compared for their ability to inhibit the growth of human leukemia HL-60 cells. The growth of HL-60 cells was inhibited by curcumin, DeMC and DiMC, but not by THC lacking α,β-unsaturated carbonyl groups thus suggesting that α,β-unsaturated carbonyl groups are crucial for antiproliferative activity. The order of antiproliferative activity was DiMC, curcumin and BDMC indicating that the number of methoxy groups on the aromatic rings of the active compounds plays an important role in enhancing anti-proliferating activity. In comparison with cellular uptake of the active compounds, uptake capacity was found to be highest with DiMC, followed by curcumin and BDMC. Therefore, it is most likely that the differential antiproliferative activities of DiMC, curcumin and BDMC are associated with their capacities of cellular uptake resulting in building up of enough concentration inside the cells.

Key words : curcumin, dimethoxycurcumin, bis-demethoxycurcumin, tetrahydrocurcumin, antiproliferation

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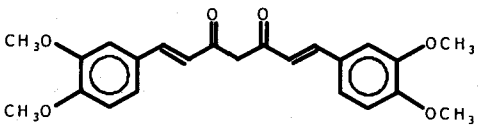
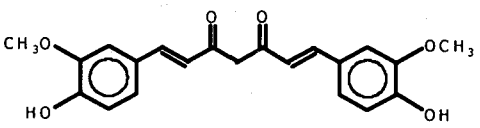
I. Introduction

Despite significant advances in medical technology for diagnosis and treatment, cancer is still widely posing a threat of mortality. Considerable attention has been focused on identifying naturally occurring anticancer substances capable of inhibiting cancer growth or killing cancer cells. A wide spectrum of phenolic substances, particularly those present in dietary and medicinal plants, have been reported substantial anticancer activity. The majority of these substances retain antioxidant and anti-inflammatoryproperties, which appear to contribute to their anticancer activity [1]. Curcumin is one of the major components of turmeric, the dried rhizome of *Curcuma longa* L, which has been used for centuries as a herbal remedy for treatment of inflammatory disorders as well as many other diseases [2]. Curcumin has been shown to be an antioxidant that has antiproliferative and anti-carcinogenic properties [3]. It has been reported that the induction of apoptosis by curcumin is responsible such an antiproliferative effect [4].

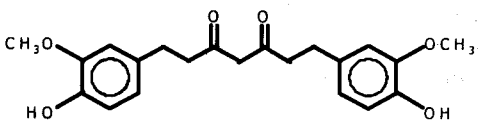
Several curcumin-related compounds are found in medicinal plants or biometabolites, and the original form of curcumin is chemically modified for special purposes. Bisdemethoxycurcumin(BDMC), which lacks two methoxy groups on the aromatic rings in comparison with curcumin(Fig. 1), is contained in turmeric extract [5]. Tetrahydrocurcumin



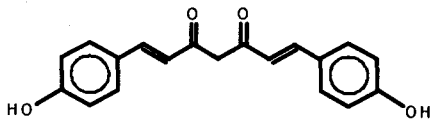
Dimethoxycurcumin (DiMC)



Curcumin



Tetrahydrocurcumin (THC)



Bis-Deethoxycurcumin (BDMC)

Figure 1. Chemical structures of curcumin analogues.

(THC), which lacks the conjugated bonds in the central seven-carbon chain(Fig. 1), isknown to be an active metabolite of curcumin [6]. Dimethoxycurcumin(DiMC), which possesses four methoxy groups on aromatic rings by substitution of two hydroxylgroups of curcumin with two methoxy groups(Fig. 1), has been reported to exert anticancer activity and to have increased metabolic stability in comparison with curcumin [7]. How the different forms of curcumin differ in their ability to exert antiproliferative activity is so far not investigated.

In this study, we compared antiproliferative activity of curcumin analogues in human leukemia HL-60 cells. We found that DiMC, curcumin and BDMC differentially inhibited the growth of HL-60 cells.

II. Materials and methods

Chemicals. Curcumin and BDMC were isolated from the rhizomes of turmeric, as described earlier [5]. THCwas prepared from curcumin by hydrogenating the two double bonds conjugated to the β-diketone, as described previously [6]. DiMC was synthetically prepared as described [8] at the College of Pharmacology, Wonkwang University(Iksan, Republic of Korea). Purity of each compound was detected by high-performance liquid chromatography to be > 90%. All solventsused in this study were liquid-chromatography-mass grade and purchased from Sigma-Aldrich(St. Louis, MO).

Cells. The human leukemia cell line HL-60(American Type Culture Collection, VA) was cultured in RPMI-1640 medium(Sigma-Aldrich) containing 10% fetal bovine serum and antibiotics(100units/mL of penicillin-G and 100g/mL of streptomycin). Cultures were maintained at 37℃ in a humidified 5% CO_2 atmosphere.

Proliferation of HL-60 cells. Cells were preincubated for 1h at a density of 5×10^4cells/mL on a 24-well plate. The test compound or vehicle was added to the cell culture, which was incubated for another 48h. Cell proliferation was measured using a cell counting kit(Dojin Laboratories, Kumamoto, Japan) according to the protocol of the manufacturer. Cells incubated only with the vehicle were used as a control and corrected to 100% of cell growth.

Annexin V assay. One of the early indicators of apoptosis is the rapid translocation of the membrane phospholipid phosphatidylserine from the cytoplasmic interface to the extracellular surface andits accumulation there, producing a loss of membrane symmetry that can be detected using annexin V. Briefly, 1×10^6cells were pretreated with 10μM of agents for indicated time points and then subjected to annexin V staining. Cells were washed, stained with FITC-onjugated anti-annexin V antibody, and then analyzed with a flow cytometer(FACSCalibur ; BD Biosciences).

Assessment of cellular uptake. After incubation of HL-60 cells with DiMC, curcumin or BDMC for 1h, cells were washed twice with cold PBS and spinned down at 1,000rpm for 5min. The pellet was dried and

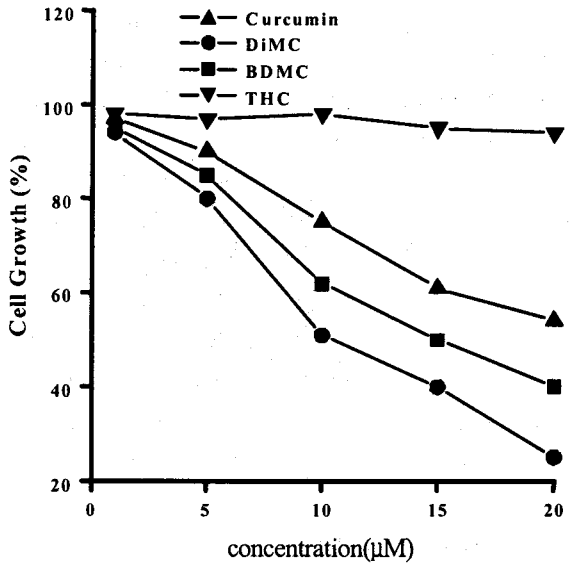


Figure 2. Effects of DiMC, curcumin, BDMC, and THC on the growth of HL-60 cells. Cells were incubated for 48 h in the absence or presence of variousconcentrations of DiMC, curcumin, BDMC, or THC. Cell proliferation was measured as described in Materials and Methods. Results represent the mean of three independent experiments.

suspended in 1mL of methanol and sonicated for 5min, so that agent is extracted into the methanol fraction. The lysate was centrifuged at 10,000rpm for 5min and absorption spectra of supernatant containing methanolic DiMC, curcumin or BDMC was recorded. From the molar absorption coefficient of curcumin in methanol, the amount of DiMC, curcumin or DiMC loaded to cells was estimated. The results were normalized to 1nmol of substrate added/million cells/mL and cell uptake has been expressed as pmole/million cells.

III. Results

Whether the different forms of curcumin inhibit cell growth to a similar extent was investigated. Results in Fig. 2 indicate that treatment of HL-60 cells with curcumin, DiMC, or BDMC inhibited HL-60 cell growth in a dose-dependent manner, but the potency varied. At 10M, DiMC was the most potent, followed by curcumin and BDMC. THC contains both methoxy groups but lacks conjugated bonds in the central seven-carbon chain. Whether THC could also inhibit cell growth to a similar extent as curcumin was investigated. The results showed that THC had no apparent effect on HL-60 cell growth (Fig. 2).

The findings on the differential effectiveness of equal concentrations of the active compounds on cell proliferation prompted us to use 10μM in flow cytometric studies to be

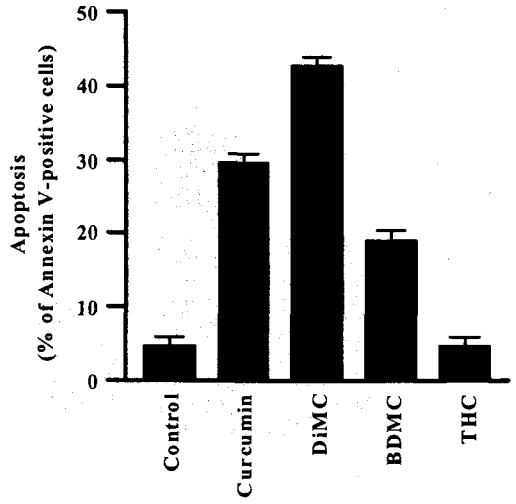


Figure 3. Effects of DiMC, curcumin, BDMC, and THC on apoptosis HL-60 cells. Cells were incubated for 24h in the absence or presence of 10μM of DiMC, curcumin, BDMC, or THC. Apoptosis(% of Annexin V-positive cells) was measured as described in Materials and Methods. Results represent the mean±SD of three independent experiments.

able to examine whether the active compound could induce apoptosis of HL-60 cells. As shown in Fig. 3, DiMC, curcumin and BDMC differentially induced apoptosis ; the rank order of apoptosis being DiMC > curcumin > BDMC.

The cellular uptakes of the active compounds were compared. Curcumin undergoes extensive metabolic reduction [9] and DiMC is more stable than curcumin in cells [7]. To overcome these limitations, HL-60 cells were exposed to the active compounds at 10μM only for 1h, so that an extensive loss of each compound should be prevented. Uptake was estimated by absorbance measurement of methanol extracted cell lysates as a function of the total amount of the active compounds added in the incubation medium. Fig. 4 shows relative cellular uptake by three agents. It can be seen that the uptake of DiMC was significantly higher, followed by curcumin and BDMC.

IV. Discussion

Although it is well established that curcumin can inhibit the growth of cancer cells, how curcumin analogues differ in their ability to exert antiproliferative activity is not known. Our results indicate that DiMC is most active in suppressing HL-60 cell growth the order of antiproliferative activity was DiMC, curcumin and BDMC. Interestingly, THC was found to be inactive.

One of the notable findings was that THC, lacking the α,β-unsaturated carbonyl groups present in the active compounds, was virtually inactive. Compounds carrying this

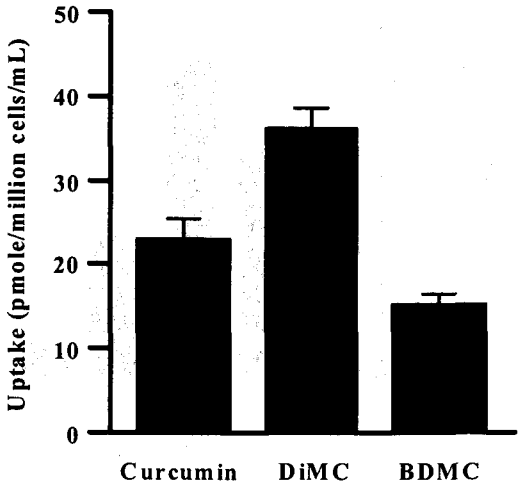


Figure 4. Cellular uptake. HL-60 cells were incubated for 1h with 10μM of DiMC, curcumin, or BDMC. Total cellular uptake was determined by measuring the absorbance of each compound extracted into the methanol fraction as described under Materials and methods. Data shown are mean SD of three independent experiments.

reactive group have been shown to exhibit versatile biological activities, e.g., inhibition of tumor cell growth [10], induction of differentiation [11], apoptosis [12], and anti-inflammatory activity [13]. Modifications of cysteine residues and consumption of intracellular glutathione have been implicated as the essential action mechanisms of α,β-unsaturated carbonyl agents [12]. We assume that curcumin, DiMC and BDMCare effective, at least in part, on account of bearing the α, β-unsaturated carbonyl groups. However, such an assumption could not explain differential antiproliferative activities of curcumin, DiMC and BDMC since these compounds possess the same functionality(α,β-unsaturated carbonyl groups). Curcumin contains two methoxy groups, DiMC contains four, and BDMC contains none. Since the order of antiproliferative activity is DiMC, curcumin and BDMC, this indicates that the number of methoxy group would be associated with differential antiproliferative activities of the active compounds. How the methoxy group can enhance antiproliferative activity, however, is unclear. One of possible explains is that the differential antiproliferative activities of the active compounds may be caused by their differential permeability into cell membrane, mainly due to the number of hydrophobic methoxy group. This is supported by our finding that the relative potency for cellular uptake was DiMC > curcumin > BDMC and casually correlating with the order of antiproliferative activity. Therefore, it is most likely that the differential antiproliferative activities of the active compounds are associated with their capacities of cellular uptake resulting in building up of enough concentration inside the cells.

It has been suggested that cancer cell growth inhibition is associated with the promotion of apoptosis [4]. Indeed, we found that curcumin, DiMC or BDMC, but not THC, induced apoptosis of HL-60 cells. Interestingly, the potency of apoptosis was DiMC > curcumin > BDMC, which correlates well with the order of antiproliferative activity.

In summary, DiMC, curcumin and BDMC differentially inhibited the growth of HL-60 cells ; the order of antiproliferative activity being DiMC > curcumin > BDMC. In comparison with cellular uptakes of the active compounds, uptake capacity was found to be highest with DiMC, followed by curcumin and BDMC.

V. Acknowledgements

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VI. References

1. Cooper R, Morre DJ, Morre DM. Medicinal benefits of green tea : part II. review of anticancer properties. J. Altern. Complement Med. 2005 ; 11(4) : 639-652.
2. Thangapazham RL, Sharma A, Maheshwari RK. Multiple molecular targets in cancer chemoprevention by curcumin. AAPS J. 2006 ; 8(3) : E443-E449.
3. Rahman I, Biswas SK, Kirkham PA. Regulation of inflammation and redox signaling by dietary polyphenols. Biochem. Pharmacol. 2006 ; 72(11) : 1439-1452.
4. Shankar S, Srivastava RK. Bax and Bak genes are essential for maximum apoptotic response by curcumin, a polyphenolic compound and cancer chemopreventive agent derived from turmeric, Curcuma longa. Carcinogenesis. 2007 ; 28(6) : 1277-1286.
5. Jeong GS, Oh GS, Pae HO, Jeong SO, Kim YC, Shin MK, Seo BY, Han SY, Lee HS, Jeong JG, Koh JS, Chung HT. Comparative effects of curcuminoids on endothelial heme oxygenase-1 expression : ortho-methoxy groups are essential to enhance heme oxygenase activity and protection. Exp. Mol. Med. 2006 ; 38(4) : 393-400.
6. Pae HO, Jeong SO, Jeong GS, Kim KM, Kim HS, Kim SA, Kim YC, Kang SD, Kim BN, Chung HT. Curcumin induces pro-apoptotic endoplasmic reticulum stress in human leukemia HL-60 cells. Biochem. Biophys. Res. Commun. 2007 ; 353(4) : 1040-1045.
7. Tamvakopoulos C, Dimas K, Sofianos ZD, Hatziantoniou S, Han Z, Liu ZL, Wyche JH, Pantazis P. Metabolism and anticancer activity of the curcumin analogue, dimethoxycurcumin. Clin.Cancer Res. 2007 ; 13(4) : 1269-1277.
8. Chen WF, Deng SL, Zhou B, Yang L, Liu ZL. Curcumin and its analogues as potent inhibitors of low density lipoprotein oxidation : H-atom abstraction from the phenolic groups and possible involvement of the 4-hydroxy-3-methoxyphenyl groups. Free Radic. Biol. Med. 2006 ; 40(3) : 526-535.
9. Pan MH, Huang TM, Lin JK. Biotrans-

formation of curcumin through reduction and glucuronidation in mice. Drug Metab. Dispos. 1999 ; 27(4) : 486-494.

10. Zhao G, Liu C, Wang R, Song D, Wang X, Lou H, Jing Y. The synthesis of alpha, beta-unsaturated carbonyl derivatives with the ability to inhibit both glutathione S-transferase P1-1 activity and the proliferation of leukemia cells. Bioorg. Med. Chem. 2007 ; 15(7) : 2701-2707.
11. Honn KV, Marnett LJ. Requirement of a reactive alpha, beta-unsaturated carbonyl for inhibition of tumor growth and induction of differentiation by "A" series prostaglandins. Biochem. Biophys. Res. Commun. 1985 ; 129(1) : 34-40.
12. Murakami A, Takahashi D, Kinoshita T, Koshimizu K, Kim HW, Yoshihiro A, Nakamura Y, Jiwajinda S, Terao J, Ohigashi H. Zerumbone, a Southeast Asian ginger sesquiterpene, markedly suppresses free radical generation, pro-inflammatory protein production, and cancer cell proliferation accompanied by apoptosis : the alpha, beta-unsaturated carbonyl group is a prerequisite. Carcinogenesis. 2002 ; 23(5) : 795-802.
13. Na HK, Surh YJ. Peroxisome proliferator-activated receptor gamma(PPARgamma) ligands as bifunctional regulators of cell proliferation. Biochem. Pharmacol. 2003 ; 66(8) : 1381-1391.