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Trichostatin A improves the anticancer activity of low concentrations of curcumin in human leukemia cells

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Received October 6, 2005, accepted November 18, 2005

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Pharmazie 61: 710–716 (2006)

Curcumin (Cur), a promising anticancer drug, kills tumor cells through either diminishing or promoting reactive oxygen species (ROS) generation. In this study, it was investigated whether trichostatin A (TSA), a specific histone deacetylase (HDAC) inhibitor and a new anticancer drug, could improve the anticancer activity of low concentrations of Cur in human leukemia cells (HL-60). HL-60 cells were treated with Cur, TSA or their combinations; cell proliferation arrest, lactate dehydrogenase (LDH) release and cell viability were measured as indicators of cell damage. Reactive oxygen species (ROS) accumulation and the acetylation of histones were also measured. The cytotoxicity of Cur and TSA increased in a time and dose-dependent manner. Low Cur (no more than 20 μM) diminished the ROS generation in HL-60 cells, while high Cur (50 and 100 μM) promoted that. In contrast, TSA showed no influence on ROS generation. When their effects on histone acetylation were determined, low Cur showed no effect, while TSA significantly increased that. As expected, combinations of low Cur and TSA could not only diminish ROS generation, but also increase histone acetylation, and hence showed a more significant cytotoxicity in HL-60 cells. Since the extra ROS generation may also harm normal cells, instead of using high Cur, combining low Cur with TSA is obviously a better strategy to improve the anticancer activity of Cur.

1. Introduction

Curcumin (diferuloylmethane, Cur), a polyphenol derived from the plant *Curcuma longa*, is recognized as a promising anticancer drug due to its efficient induction of proliferation arrest and cell death (including apoptosis and necrosis) in a variety of tumor cells (Aggarwal et al. 2003; Anto et al. 2002; Roy et al. 2002; Kim et al. 2001; Hadi et al. 2000; Bhaumik et al. 1999). Like most polyphenols, although Cur is a naturally occurring antioxidant, it exhibits prooxidant properties under certain conditions (Kim et al. 2001; Bhaumik et al. 1999; Sakano and Kawanishi 2002; Galati et al. 2002; Nogaki et al. 1998; Wang et al. 1996). Interestingly, both antioxidant and prooxidant properties are found to be involved in the anticancer activity of Cur (Aggarwal et al. 2003; Hadi et al. 2000; Bhaumik et al. 1999; Khar et al. 2001; Chen et al. 2005). Generally, high concentrations of Cur (such as at no less than 50 μM) promoted ROS generation (Kim et al. 2001; Bhaumik et al. 1999; Sakano and Kawanishi 2002; Galati et al. 2002; Chen et al. 2005), while low Cur (such as at no more than 25 μM) diminished that (Chen et al. 2005; Chan et al. 2003; Joe and Lokesh 1994). Considering the possible damage of extra ROS generation to normal cells, instead of using high Cur, trying to improve the anticancer activity of low Cur is useful.

Histone acetylation plays important roles in gene transcription regulation, cell differentiation and carcinogenesis. Specifically, histone deacetylation contributes to a 'closed' chromatin state and transcriptional repression (Archer and Hodin 1999; Klochendler-Yeivin and Yaniv 2001; Lehrmann et al. 2002), while histone acetylation contributes to the formation of an 'opening' chromatin and permits access of transcription factors to DNA (Fry and Peterson 2002; Grunstein 1997). The histone acetylation-deacetylation balance, accurately maintained in normal cells through a balance of histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzyme activities (Archer and Hodin 1999; Klochendler-Yeivin and Yaniv 2001), favors hypoacetylation in tumor cells (Archer and Hodin 1999), and this histone hypoacetylation in tumor cells generally disrupt the transcriptional initiation of genes for differentiation and apoptosis, such as p53, APC, $p14^{ARF}$, and K-ras (Fry and Peterson 2002; Baylin et al. 2001). Thus increasing the histone acetylation in tumor cells has been recognized as an efficient strategy to combat cancer, and HDAC inhibitors are believed to be useful in cancer chemotherapy (Marks et al. 2000; Hendersona and Brancolini 2003; Yamashita et al. 2003; Donadelli et al. 2003; Rosato and Grant 2003; Marks et al. 2001; Kim et al. 2003). Among the HDAC inhibitors, trichostatin A has been well studied and found to be toxic in different tumor cells (Yamashita et al. 2003; Donadelli et al. 2003; Rosato and Grant 2003; Marks et al. 2001).

Since Cur and TSA exert their anticancer activity through different mechanisms, we speculate that combining with

TSA may significantly improve the anticancer activity of Cur. To address this hypothesis, the cytotoxicity of Cur, TSA and the combinations of Cur and TSA was studied in human leukemia HL-60 cells, and their effects on ROS generation and histone acetylation were also evaluated. Both Cur and TSA showed cytotoxicity to HL-60 cells, and combining with TSA significantly improved the cytotoxicity of Cur under its antioxidant conditions. These results thus suggested a new approach, combining with TSA or other HDAC inhibitors, to improve the chemotherapy effect of Cur.

2. Investigations and results

2.1. Cytotoxicity of antioxidants and TSA in HL-60 cells

Treating cells with Cur or TSA resulted in a concentration- and time-dependent arrest in the proliferation of HL-60 cells (Fig. 1). Under the same conditions, LDH leakage, the indicator of plasmatic membrane damage (Del Raso 1992), increased (Fig. 2), while the viability of cells significantly decreased (Fig. 3), proving the cytotoxicity of Cur and TSA in HL-60 cells.

2.2. Effect of Cur and TSA on the ROS generation and histone acetylation

To evaluate the ROS generation in our system, HL-60 cells preloaded with DCFH-DA, commonly used to detect the generation of ROS in cells (LeBel et al. 1992), were exposed to Cur and TSA for 8 h. Low concentrations of Cur (at no more than 20 μM) diminished the ROS generation, while high Cur (40, 80 μM) significantly increased that (Fig. 4A and 4B). In contrast, TSA showed no influence on the ROS generation in HL-60 cells (Fig. 4A). At the same time, the state of histone acetylation was also evaluated in differently treated cells. Cur had no obvious influence on the histone acetylation, while TSA significantly increased that in HL-60 cells via a dose-dependent manner (Fig. 4C and 4D). These results suggest that Cur and TSA kill HL-60 cells via different mechanisms, i.e., Cur via its antioxidant or prooxidant activity, while TSA via its HDAC inhibition activity.

2.3. Effects of TSA on the cytotoxicity of low Cur in HL-60 cells

To test whether TSA could improve the anticancer activity of low Cur, effects of low Cur and TSA combinations on the generation of ROS and the status of histone acetylation in HL-60 cells were first detected. Low Cur and TSA combinations showed similar activities with Cur or TSA in diminishing the ROS generation (Fig. 5A) or increasing the histone acetylation (Fig. 5B), respectively, indicating that neither the ROS scavenging activity of Cur nor the HDAC inhibition activity of TSA was affected by the combinations. In other words, the combinations possessed both the antioxidative characteristic of low Cur and the activity of TSA. Considering the important roles of scavenging ROS and increasing histone acetylation in cancer therapy, these results suggest that combinations with TSA may be able to improve the anticancer activity of low Cur. To test this hypothesis, the cytotoxicity of the low Cur and TSA combination in HL-60 cells was studied. As we expected, combining with TSA significantly improved the effect of low Cur on the proliferation arrest (Fig. 5C), and the LDH release (Fig. 5D) and cell death induction (Fig. 5E), indicating the cooperative anticancer activity of low Cur and TSA.

3. Discussion

Both antioxidants and TSA are promising anticancer drugs, and capable of improving the efficiency of chemotherapy when combined with other anticancer drugs (Rosato and Grant 2003; Marks et al. 2001; Kim et al.

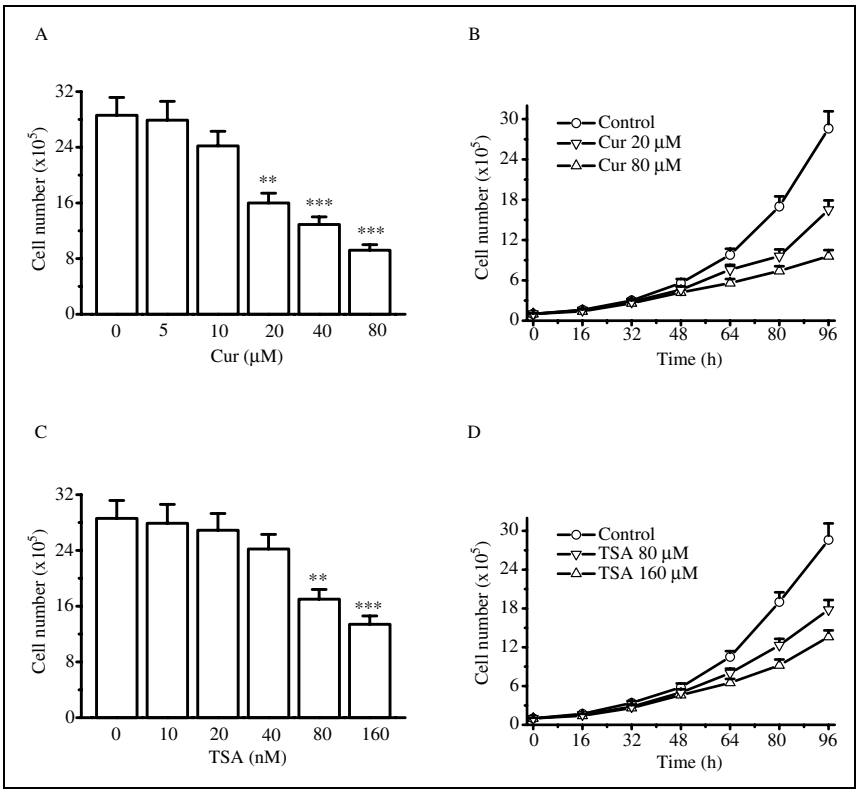

Fig. 1:
Effects of Cur and TSA on the proliferation of HL-60 cells. Cells were incubated with different concentrations of Cur and TSA for 48 h in A and C, with the indicated concentrations of Cur and TSA for different times in B and D. Means ± SD of three parallel experiments was indicated, $n = 3 \times 3$ cultures per conditions, **, *** indicate $P < 0.01$ and 0.001 vs. the control group

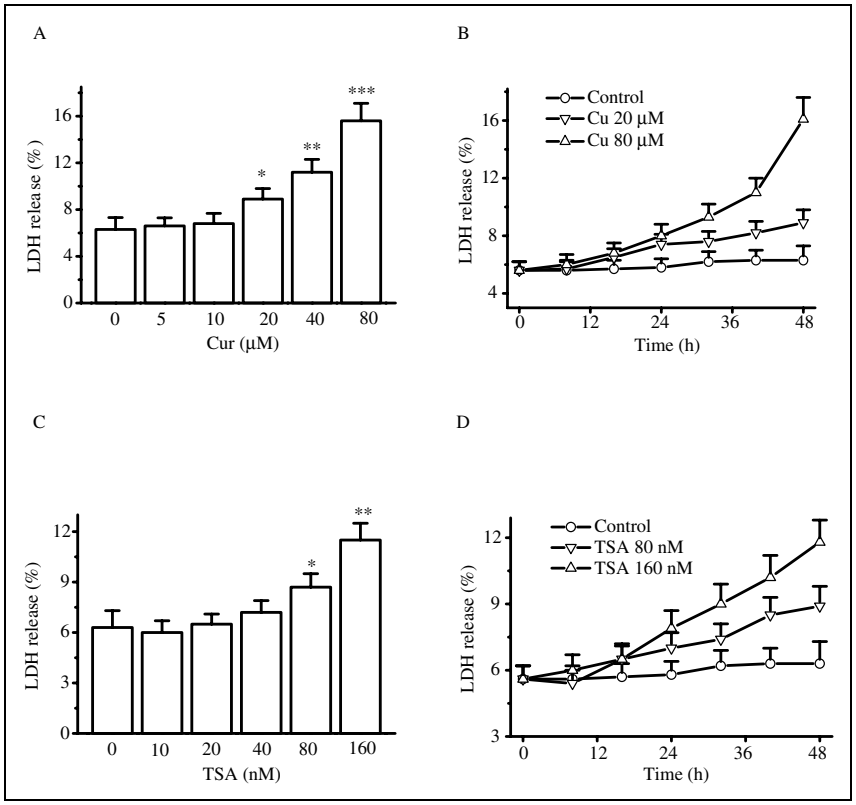

Fig. 2:
Effects of Cur and TSA on the LDH release in HL-60 cells. Cells were incubated with different concentrations of Cur and TSA for 48 h in A and C, with the indicated concentrations of Cur and TSA for different times in B and D. Means ± SD of three parallel experiments was indicated, $n = 3 \times 3$ cultures per conditions, *, **, *** indicate $P < 0.05$, 0.01 and 0.001 vs. the control group

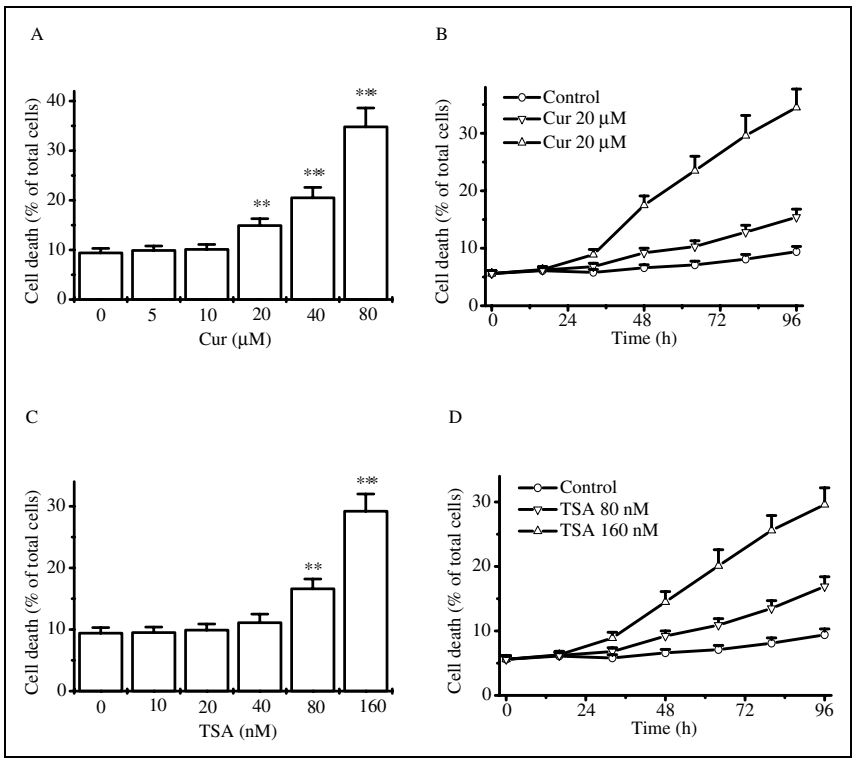

Fig. 3:
Effects of Cur and TSA on the viability of HL-60 cells. Cells were incubated with different concentrations of Cur and TSA for 48 h in A and C, with the indicated concentrations of Cur and TSA for different times in B and D. Means ± SD of three parallel experiments was indicated, $n = 3 \times 3$ cultures per conditions, **, *** indicate $P < 0.01$ and 0.001 vs. the control group

2003; Park et al. 1980; Park and Kimler 1991; Prasad et al. 1994; Conklin 2000; Chinery et al. 1997). Our recent study found that combinations with TSA could significantly improve the anticancer activity of antioxidants (Kang et al. 2004). Cur is a well-known antioxidant and anticancer drug (Aggarwal et al. 2003; Anto et al. 2002; Roy et al. 2002; Kim et al. 2001; Hadi et al. 2000; Bhaumik et al. 1999), but high concentrations of Cur (such as at no less than 50 μM) were found to promote ROS generation in different studies (Kim et al. 2001; Bhaumik et al. 1999; Sakano and Kawanishi 2002; Galati et al. 2002; Chen et al. 2005). Our present study found that TSA could significantly improve the cytotoxicity of Cur at the concentrations where Cur diminished ROS generation in HL-60 cells. Thus instead of using high Cur, these results provide new approaches for us to improve the anticancer activity of Cur, especially under its antioxidant concentrations.

Because both Cur and TSA have been found to be cytotoxic in human leukemia cells (Chen et al. 2005; Marks et al. 2001; Kang et al. 2004; He et al. 2001), human leu-

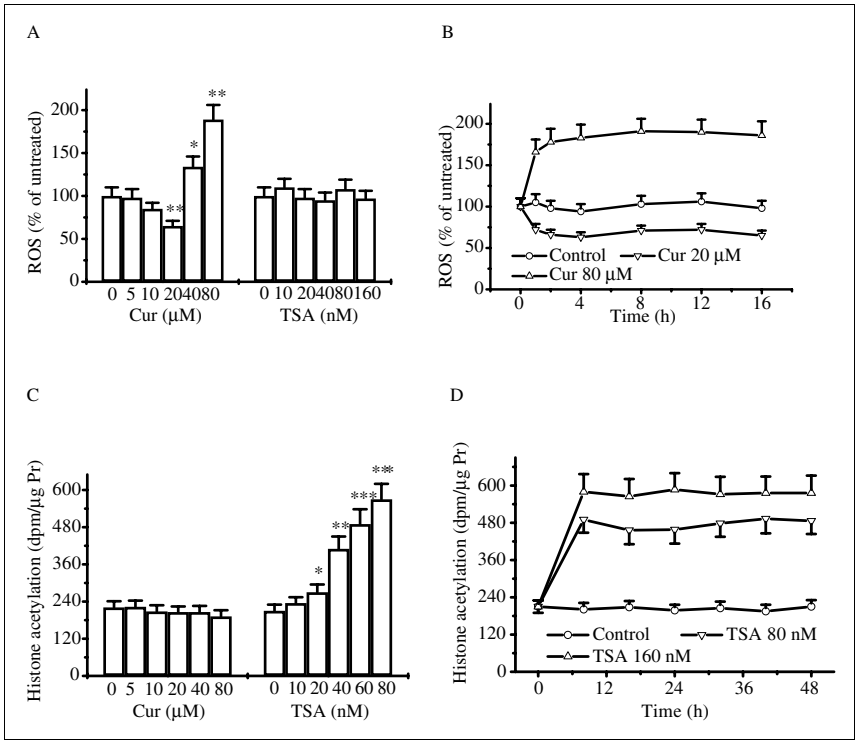

Fig. 4:
Effects of Cur and TSA on ROS generation and histone acetylation. Relative ROS generation in HL-60 cells exposed to the indicated agents for 8 h was measured in A, with the indicated concentrations of Cur for different times in B. In the presence of 10 μCi/ml [^{3}H] acetate, HL-60 cells were incubated with the indicated agents for 48 h in C, with the indicated concentrations of TSA for different times in D. The radioactivities of the [^{3}H] acetylated histones isolated from the cells were determined by liquid scintillation counting. All data represent mean values of six independent measurements ± SD. *, **, *** indicate $p < 0.05$, 0.01 and 0.001 as compared with the control group

kemia cells (HL-60) were used as model cells. Treating HL-60 cells with Cur or TSA resulted in a dose- and time-dependent increase of cell proliferation arrest, LDH release and cell death, proving the cytotoxicity of Cur and TSA in human leukemia cells.

Previous studies indicate that ROS are not only involved in carcinogenesis, but also in the prevention and cure of cancer (Wedgwood and Black 2003; Deshpande and Irani 2002; Kang et al. 2000; Kang et al. 1999). On one hand, ROS act at different stages of carcinogenesis (Deshpande and Irani 2002); on the other hand, either diminishing (Wedgwood and Black 2003; Kang et al. 2000; Kang et al. 1999; Chen et al. 2004) or enhancing (Bhaumik et al. 1999; Khar et al. 2001; Kang et al. 2001) ROS generation can lead to the proliferation arrest, re-differentiation, apoptosis or necrosis of tumor cells, thus both antioxidant and prooxidant therapy are potential anticancer strategies. In fact, the balance between ROS generation and scavenging *in vivo* is critical for the proliferation and viability of cells in all-aerobic animals (Martindale and Holbrook 2002; McCord 1998; Burdon 1995). Different studies showed that both antioxidant and prooxidant activity are involved in the anticancer activity of Cur (Aggarwal et al. 2003; Hadi et al. 2000; Bhaumik et al. 1999; Khar et al. 2001). Interestingly, in this study, Cur exerts its anticancer activity in the same system through diminishing ROS at low concentrations or increasing ROS at high concentrations. Although the anticancer activity of high Cur is obviously higher than the low Cur, considering the possible damage to normal cells caused by extra ROS accumulation *in vivo*, to improve the anticancer activity of low Cur is obviously helpful.

The anticancer activity of TSA obviously could not be explained by the ROS scavenging activity, since TSA did not scavenge ROS at all. Consistent with the previous reports (Hendersona and Brancolini 2003; Yamashita et al. 2003; Donadelli et al. 2003; Rosato and Grant 2003; Marks et al. 2001), TSA maybe induce leukemia cell death by increasing the histone acetylation of HL-60 cells. Recent studies including ours found that Cur specifically inhibited the activity of p300, one HAT protein, and hence led to histone hypoacetylation in cultured cells (Balasubramanyam et al. 2004; Kang et al. 2005), but Cur showed no obvious influence on the histone acetylation in this study. This may be because different cells were used in our study. Although additional work is still needed to clarify whether p300 was inhibited in HL-60 cells and why Cur did not induce histone hypoacetylation in these cells, the anticancer activity of Cur is obviously not related to the modulation on histone acetylation in HL-60 cells. As we have noticed, the dose-dependent effect of Cur and TSA on the histone acetylation and cell viability was measured after cells were treated for 48 h, while the ROS generation was detected after the treatment of 8 h, this is mainly because the significant cell death caused by treating cells for 48 h may inevitably result in the decrease of ROS generation, and hence influence the evaluation on the effect of Cur and TSA. Treating cells for 8 h did not lead to the obvious cell death.

TSA has been found to be able to improve the anticancer activity of the antioxidants ascorbic acid and *N*-acetyl-cysteine. Combining with TSA should improve the anticancer activity of Cur in its antioxidant concentrations. As we expected, the combination of low Cur with TSA possessed both the ROS scavenging activity of low Cur and the histone acetylation increasing activity of TSA, and hence significantly improved the anticancer activity of either of them. Although further studies are needed to clarify the mechanisms involved in the cytotoxicity of antioxidants, TSA and their combinations in human leukemia cells, or the nature of antioxidants and TSA-induced cell death, we proved that both scavenging ROS and increasing histone acetylation played important roles in the induction of HL-60 cell death, and TSA could markedly improve the anticancer activity of Cur under its antioxidant concentrations in these cells.

In summary, we conclude that in HL-60 cells, low Cur exerts its anticancer activity through diminishing ROS, while high Cur through increasing that. Different from that, TSA induces HL-60 cell death through increasing histone acetylation. Combined with TSA, perhaps other

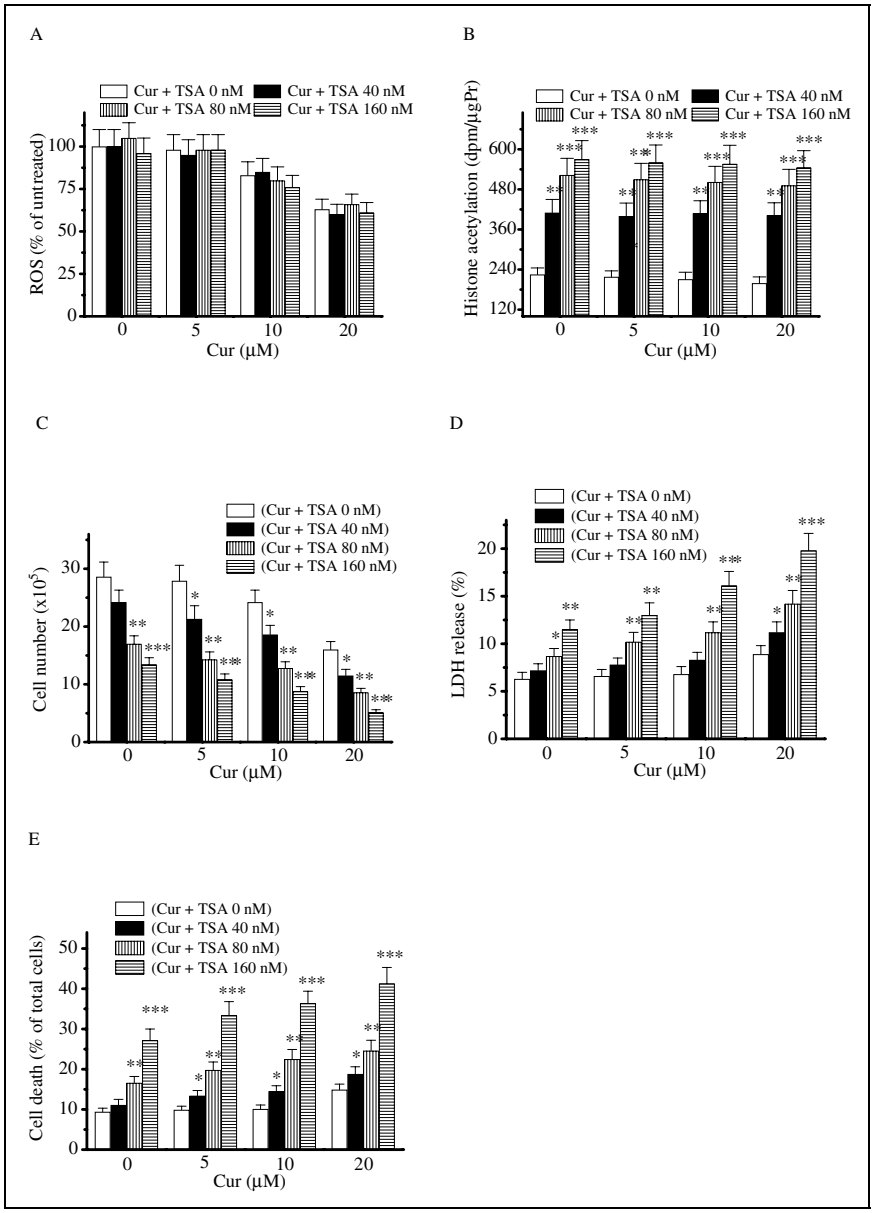

Fig. 5:
Effects of low Cur and TSA combinations on ROS generation, histone acetylation and cell viability. Cells were treated with different concentrations of Cur alone, or the combinations of Cur with different concentrations of TSA for 8 h in A or for 48 h in the others. The effect of Cur and TSA combinations on ROS generation (A), histone acetylation (B), cell proliferation (C), LDH release (D) and cell viability (E) was examined. Means of six (in A and B) or three (in others) parallel experiments are displayed (means ± SD), $n = 3 \times 3$ cultures per conditions, *, **, *** indicate $p < 0.05$, 0.01 and 0.001 as compared with the corresponding Cur alone group

HDAC inhibitors can significantly improve the anticancer activity of low Cur. Considering that the extra accumulation of ROS is harmful to normal cells, for example, high Cur (at 50 μM) has been found to cause cell death in both tumor and normal cells (Bielak-Zmijewska et al. 2000; Gautam et al. 1998), instead of using high Cur, combining low Cur with TSA is a better strategy for us to improve the anticancer activity of Cur. In addition, since polyphenols with similar structures possess similar antioxidant and pro-oxidant property, our present data may also suggest a good strategy to improve the antioxidant and anticancer activity of other polyphenols with similar structures to Cur.

4. Experimental

4.1. Reagents

Curcumin (Cur), trichostatin A (TSA), trypsin and trypan blue were purchased from Sigma (Sigma, St. Louis, MO), RPMI-1640 was purchased from Gibco (Santa Clara, CA). All other reagents are of analytical grade.

4.2. Cell culture and treatment

Human leukemia cells (HL-60) were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (equivalent to 100 units/ml and 100 mg/ml, respectively) at 37 °C in a humidified atmosphere containing 5% CO_2. After culturing the cells (1×10^5 cells/ml) for 24 h, the culture medium was replaced with new medium containing Cur, TSA and their combinations where indicated, after that, the different cultures were replaced with fresh medium containing the corresponding reagents every 24 h.

4.3. Determination of cell proliferation and viability

Cells at 1×10^5/ml were cultured for 24 h, then treated with Cur and/or TSA at the indicated concentrations, three dishes for each of differently treated cells were collected every 8 h in the first 2 days, the total and dead cells were counted using the trypan blue stain exclusion method under a phase-contrast microscope.

4.4. Measurement of lactate dehydrogenase (LDH)

LDH release was measured in 100 μl aliquot of cellular suspension using an assay, which monitors the decrease in absorbance at 340 nm during the reduction of pyruvate (Wroblewski and La Due 1995). LDH release was expressed as percentage of the total LDH released from cells treated with 10% Triton X-100.

4.5. Measurement of intracellular ROS generation

The level of intracellular ROS was measured by the alteration of fluorescence resulting from oxidation of 29,79-dichlorofluorescein diacetate (DCFH-DA, Molecular Probes, Eugene, OR) (LeBel et al. 1992). DCFH-DA was dissolved in DMSO to a final concentration of 20 mM before use. For the measurement of ROS, cells were incubated with 10 μM DCFH-DA at 37 °C for 30 min, then the excess DCFH-DA was washed with RPMI-

1640 media prior to the treatment with Cur and/or TSA for a time period as indicated. The intensity of fluorescence was recorded using a flow cytometry (Becton Dickenson), with an excitation filter of 485 nm and an emission filter 535 nm. The ROS level was calculated as a ratio: ROS = mean intensity of exposed cells: mean intensity of unexposed cells.

4.6. Histone purification and histone acetylation assay

Cells were plated at a density of 2×10^5 cells/ml, exposed to Cur and/or TSA as indicated in the presence of 10 μCi/ml [3 H] acetate (5.0 Ci/mmol) for the indicated times. Preparation of histones from HL-60 cells was done as previously reported (Cousens et al. 1979) with the following modifications: the washed cells were suspended in lysis buffer (Cousens et al. 1979) containing TSA (100 ng/ml) and PMSF (1 mM). After pipetting up and down for 20 times, the nuclei were washed three times in the lysis buffer and once in 10 mM Tris and 13 mM EDTA (pH 7.4). The histones were extracted from the pellet in 0.4 N H_2SO_4. After centrifugation, the histones in the supernatant were collected by cold-acetone precipitation, air-dried, then suspended in 4 M urea and stored at −20 °C before use. ^{3}H-labelled histones were determined by liquid scintillation counting.

4.7. Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA post-hoc Bonferroni), and p values less than 0.05, 0.01, or 0.001 were denoted as *, **, or ***, respectively.

References

Aggarwal BB, Kumar A, Bharti AC (2003) Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res 23: 363–396.

Anto RJ, Mukhopadhyay A, Denning K, Aggarwal BB (2002) Curcumin (diferuloylmethane) induces apoptosis through activation of caspase-8, BID cleavage and cytochrome c release: its suppression by ectopic expression of Bcl-2 and Bcl-xl. Carcinogenesis 23: 143–150.

Archer SY, Hodin RA (1999) Histones acetylation and cancer. Curr Opin Gene Develop 9: 171–174.

Balasubramanyam K, Varier RA, Altaf M, Swaminathan V, Siddappa NB, Ranga U, Kundu TK (2004) Curcumin, a novel p300/CBP specific inhibitor of acetyltransferase, represses the acetylation of histones/nonhistone proteins and HAT dependent chromatin transcription. J Biol Chem 279: 51163–51171.

Baylin SB, Esteller M, Rountree MR, Bachman KE, Schuebel K, Herman JG (2001) Aberrant pattern of DNA methylation, chromatin formation and gene expression in cancer. Hum Mol Genet 10: 687–692.

Bhaumik S, Anjum R, Rangaraj N, Pardhasaradhi BVV, Khar A (1999) Curcumin mediated apoptosis in AK-5 tumor cells involves the production of reactive oxygen intermediates. FEBS Lett 456: 311–314.

Bielak-Zmijewska A, Koronkiewicz M, Skierski J, Piwocka K, Radziszewska E, Sikora E (2000) Effect of curcumin on the apoptosis of rodent and human nonproliferating and proliferating lymphoid cells. Nutr Cancer 38: 131–138.

Burdon RH (1995) Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radic Biol Med 18: 775–794.

Chan WH, Wu CC, Yu JS (2003) Curcumin inhibits UV irradiation-induced oxidative stress and apoptotic biochemical changes in human epidermoid carcinoma A431 cells. J Cell Biochem 90: 327–338.

Chen J, Da WM, Zhang DW, Liu Q, Kang JH (2005) Water-soluble antioxidants improve the antioxidant and anticancer activity of low concentrations of curcumin in human leukemia cells. Pharmazie 60: 57–61.

Chen J, Ou YX, Da WM, Kang JH (2004) Coadjustment of quercetin and hydrogen peroxide: the role of ROS in the cytotoxicity of quercetin. Pharmazie 59: 155–158.

Chinery R, Brockman JA, Peeler MO, Shyr Y, Beauchamp RD, Coffey RJ (1997) Antioxidants enhance the cytotoxicity of chemotherapeutic agents in colorectal cancer: a p53-independent induction of p21 WAF1/CIP1 via C/EBPβ. Nat Med 3: 1233–1241.

Conklin KA (2000) Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. Nutr Cancer 37: 1–18.

Cousens LS, Gallwitz D, Alberts BM (1979) Different accessibilities in chromatin to histone acetylase. J Biol Chem 254: 1716–1723.

Del Raso N (1992) *In vitro* methods for assessing chemical or drug toxicity and metabolism in primary hepatocytes. In: Watson RR (Ed.), *In Vitro* Methods of Toxicology. CRC Press Inc, Boca Raton, FL, pp. 176–201.

Deshpande SS, Irani K (2002) Oxidant signalling in carcinogenesis: a commentary. Hum Exp Toxicol 21: 63–64.

Donadelli M, Costanzo C, Faggioli L, Scupoli MT, Moore PS, Bassi C, Scarpa A, Palmieri M (2003) Trichostatin A, an inhibitor of histone deacetylases, strongly suppresses growth of pancreatic adenocarcinoma cells. Mol Carcinogen 38: 59–69.

Fry CJ, Peterson CL (2002) Unlocking the gates to gene expression. Science 295: 1847–1848.

Galati G, Sabzevari O, Wilson JX, O'Brien PJ (2002) Prooxidant activity and cellular effects of the phenoxyl radicals of dietary flavonoids and other polyphenolics. Toxicology 177: 91–104.

Gautam SC, Xu YX, Pindolia KR, Janakiraman N, Chapman RA (1998) Nonselective inhibition of proliferation of transformed and nontransformed cells by the anticancer agent curcumin (diferuloylmethane). Biochem Pharmacol 55: 1333–1337.

Grunstein M (1997) Histone acetylation in chromatin structure and transcription. Nature 389: 349–352.

Hadi SM, Asad SF, Singh S, Ahmad A (2000) Putative mechanism for anticancer and apoptosis-inducing properties of plant-derived polyphenolic compounds. IUBMB Life 50: 167–171.

He L, Tolentino T, Grayson P, Zhong S, Warrell RP, Rifkind RA, Marks PA, Richon VM, Pandolfi PP (2001) Histone deacetylase inhibitors induce remission in transgenic models of therapy-resistant acute promyelocytic leukemia. J Clin Invest 108: 1321–1330.

Hendersona C, Brancolini C (2003) Apoptotic pathways activated by histone deacetylase inhibitors: implications for the drug-resistant phenotype. Drug Resist Updates 6: 247–256.

Joe B, Lokesh BR (1994) Role of capsaicin, curcumin and dietary n-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. Biochim Biophys Acta 1224: 255–263.

Kang J, Chen J, Shi Y, Jia J, Zhang Y (2005) Curcumin-induced histone hypoacetylation: the role of reactive oxygen species. Biochem Pharmacol 69: 1205–1213.

Kang J, Chen J, Zhang D, Da W, Ou Y (2004) Synergistic killing of human leukemia cells by antioxidants and trichostatin A. Cancer Chemother Pharmacol 54: 537–545.

Kang JH, Shi YM, Zheng RL (1999) Effects of ascorbic acid on human hepatoma cell proliferation and redifferentiation. Acta Pharmacol Sin 20: 1019–1024.

Kang JH, Shi YM, Zheng RL (2000) Effects of ascorbic acid and DL-alpha-tocopherol on human hepatoma cell proliferation and redifferentiation. Acta Pharmacol Sin 21: 348–352.

Kang JH, Wei YM, Zheng RL (2001) Effects of diethyldithiocarbamate on proliferation, redifferention, and apoptosis in human hepatoma cells. Acta Pharmacol Sin 22: 785–792.

Khar A, Ali AM, Pardhasaradhi BV, Varalakshmi CH, Anjum R, Kumari AL (2001) Induction of stress response renders human tumor cell lines resistant to curcumin-mediated apoptosis: role of reactive oxygen intermediates. Cell Stress Chaperones 6: 368–376.

Kim MS, Blake M, Baek JH, Kohlhagen G, Pommier Y, Carrier F (2003) Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. Cancer Res 63: 7291–7300.

Kim MS, Kang HJ, Moon A (2001) Inhibition of invasion and induction of apoptosis by curcumin in H-ras-transformed MCF10A human breast epithelial cells. Arch Pharm Res 24: 349–354.

Klochendler-Yeivin A, Yaniv M (2001) Chromatin modifiers and tumor suppression. Biochim Biophys Acta 1551: M1–M10.

LeBel CP, Ischiropoulos H, Bondy SC (1992) Evaluation of the probe 29,79-dichlorofluorescein as an indicator of reactive oxygen species formation and oxidative stress. Chem Res Toxicol 5: 227–231.

Lehrmann H, Pritchard LL, Harel-Bellan A (2002) Histone acetyltransferases and deacetylases in the control of cell proliferation and differentiation. Adv Cancer Res 86: 41–65.

Marks PA, Richon VM, Rifkind RA (2000) Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. J Nat Cancer Inst 92: 1210–1216.

Marks PA, Richon VM, Breslow R, Rifkind RA (2001) Histone deacetylase inhibitors as new cancer drugs. Curr Opin Oncol 13: 477–483.

Martindale JL, Holbrook NJ (2002) Cellular response to oxidative stress: signaling for suicide and survival. J Cell Physiol 192: 1–15.

McCord JM (1998) The importance of oxidant-antioxidant balance. In: Montagnier L et al. (ed.) Oxidative stress in cancer, AIDs and neurodegenerative diseases, New York, p. 1–6.

Nogaki A, Satoh K, Iwasaka K, Takano H, Takahama M, Ida Y, Sakagami H (1998) Radical intensity and cytotoxic activity of curcumin and gallic acid. Anticancer Res 18: 3487–3491.

Park CH, Amare M, Savin MA, Hoogstraten B (1980) Growth suppression of human leukemic cells in vitro by L-ascorbic acid. Cancer Res 40: 1062–1065.

Park CH, Kimler BF (1991) Growth modulation of human leukemic, preleukic, and myeloma progenitor cells by L-ascorbic acid. Am J Clin Nutr 54: 1241S–1246S.

Prasad KN, Hernandez C, Edwards-Prasad J, Nelson J, Borus T, Robinson WA (1994) Modification of the effect of tamoxifen, cisplatin, DTIC and interferon-2b on human melanoma cells in culture by a mixture of vitamins. Nutr Cancer 22: 233–245.

Rosato RR, Grant S (2003) Histone deacetylase inhibitors in cancer therapy. Cancer Biol Ther 2: 30–37.

Roy M, Chakraborty S, Siddiqi M, Bhattacharya RK (2002) Induction of apoptosis in tumor cells by natural phenolic compounds. Asian Pac J Cancer Prev 3: 61–67.

Sakano K, Kawanishi S (2002) Metal-mediated DNA damage induced by curcumin in the presence of human cytochrome P450 isozymes. Arch Biochem Biophys 405: 223–230.

Wang P, Kang J, Zheng R, Yang Z, Lu J, Gao J, Jia Z (1996) Scavenging effects of phenylpropanoid glycosides from Pedicularis on superoxide anion and hydroxyl radical by the spin trapping method. Biochem Pharmacol 51: 687–691.

Wedgwood S, Black SM (2003) Induction of apoptosis in fetal pulmonary arterial smooth muscle cells by a combined superoxide dismutase/catalase mimetic. Am J Physiol Lung Cell Mol Physiol 285: L305–312.

Wroblewski F, La Due J (1995) Lactic dehydrogenase activity in blood. Proc Soc Exp Biol Med 90: 210 –213.

Yamashita Y, Shimada M, Harimoto N, Rikimaru T, Shirabe K, Tanaka S, Sugimachi K (2003) Histone deacetylase inhibitor trichostatin A induces cell-cycle arrest/apoptosis and hepatocyte differentiation in human hepatoma cells. Int J Cancer 103: 572 –576.