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Induction of apoptosis by curcumin: mediation by glutathione *S*-transferase P1-1 inhibition

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Abstract

Expression of glutathione *S*-transferase P1-1 (GSTP1-1) is correlated to carcinogenesis and resistance of cancer cells against chemotherapeutic agents. Curcumin, a natural compound extracted from *Curcuma longa*, has shown strong antioxidant and anticancer properties and also the ability to regulate a wide variety of genes that require activating protein 1 and nuclear factor κB (NF-κB) activation. In the present study, we examined the inhibitory effect of curcumin on the expression of GSTP1-1 mRNA as well as protein, and we correlated this inhibition with the apoptotic effect of curcumin on K562 leukemia cells. Curcumin efficiently inhibited the tumour necrosis factor α- and phorbol ester-induced binding of AP-1 and NF-κB transcription factors to sites located on the GSTP1-1 gene promoter. TNFα-induced GSTP1-1 promoter activity was also inhibited by curcumin as shown by reporter gene assay. In parallel, curcumin induced pro-caspases 8 and 9 as well as poly ADP ribose polymerase cleavage and thus leading to apoptosis in K562 cells. Our results overall add a novel role for curcumin as this chemoprotective compound could contribute to induce apoptosis by its ability to inhibit the GSTP1-1 expression at the level of transcription.

Keywords: GSTP1-1; Curcumin; Carcinogenesis; Drug resistance; Apoptosis; Leukemia

1. Introduction

GSTs (E.C. 2.5.1.18) are a multigene superfamily of enzymes that have been classified into eight distinct gene families coding for seven cytosolic isoforms (alpha, mu, pi, theta, omega, kappa and zeta) and one microsomal form. GSTP1-1, or GST pi, is expressed in most human tissues except in adult liver and in cancer cell lines like the Burkitt lymphoma Raji, HepG2 hepatoma and MCF7 breast cancer cells [1]. Its subunits are between 23 and 28 kDa and its gene has a length of 2.8 kbp with 7 exons and 6 introns. Studies of human GSTP1-1 promoter show that the necessary regulatory elements are situated in the region −80 to −8, containing AP-1 and Sp1 sites [2].

GSTs play an important role in detoxification by catalysing the conjugation of electrophilic compounds such as xenobiotic drugs, toxins and carcinogens [3,4] to glutathione (GSH) allowing the drug to be exported from the cell through the GS-X pump in an ATP-dependent way. However, GSTs also allow the development of resistance to chemotherapy and elevated levels of GSTP1-1 mRNA are found in cell lines resistant to a range of anticancer drugs. Indeed, MCF7, an estrogen-receptor positive breast cancer cell line, was found to develop a resistance to ethacrynic acid [5], doxorubicin and benzopyrene [6] when transfected with GSTP1-1 and multidrug resistant protein 1. Ovarian cancer cell lines overexpressing GSTP1-1 are resistant to doxorubicin or taxol [7]. COS cells become resistant to doxorubicin [8] and CHO cells

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Abbreviations: GST, glutathione *S*-transferase; NF-κB, nuclear factor κB; AP-1, activating protein 1; TNFα, tumour necrosis factor α; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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resist to cisplatine and carboplatine [9] after transfection with the GSTP1-1 gene. Moreover, carcinogenesis is found to be related in many cases to an overexpression of GSTs, in particular GSTP1-1. Indeed, in many human tumours, like prostate carcinoma [10], squamous-cell carcinoma [11], acute lymphoblastic leukemia [12] and chronic lymphoid leukemia [13], GSTP1-1 is overexpressed, even though in the corresponding normal tissues the protein is either absent or expressed at very low levels. GSTP1-1 can thus be used as a valuable prognostic tool in sarcoma [14] or gastric carcinoma [15].

AP-1 [16,17] and NF-κB activation is inhibited by curcumin (diferuloylmethane) [18,19], the yellow component of *Curcuma longa* that gives curry its colour and flavour. Curcumin has shown anti-inflammatory and antioxidant properties [20–26] due to the inhibition of cyclooxygenase 2 and lipoxygenase [27,28]. Curcumin inhibits tumour initiation (by benz[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene [29]) and promotion (by phorbol esters) thus showing anticarcinogenic properties [30,31].

In order to understand the relationship between apoptosis and transcriptional regulation of the GSTP1-1 gene, we examined the effect of curcumin on the GSTP1-1 expression correlated to cellular viability in cultured human leukemia cells. In this study, we provide evidence that curcumin significantly reduces GSTP1-1 mRNA and protein levels as well as TNFα- or phorbol ester-activated NF-κB and AP-1 transcription factor binding to consensus and GSTP1-1 promoter probes. This inhibition was also confirmed by reporter gene assays demonstrating the effect of curcumin at the molecular level and thus underlining its chemopreventive potential. Furthermore, the appearance of apoptotic cleavage products is correlated to a curcumin-induced reduction of GSTP1-1 expression.

2. Materials and methods

2.1. Cells and medium

K562 cells (human chronic myelogenous leukemia) were cultured in RPMI medium (Bio-Whittaker) containing 10% (v/v) fetal calf serum (FCS) (Bio-Whittaker) and 1% (v/v) antibiotic–antimycotic (Bio-Whittaker) at 37°, 5% of CO_2. Before treatments, cells were cultured in RPMI with 0.1% (v/v) FCS and 1% (v/v) antibiotic–antimycotic for 72 hr at 37°.

2.2. Compounds

TPA was obtained from ICN, TNFα and curcumin were purchased from Sigma Chemical Co. Curcumin and TPA were dissolved in 100% DMSO at 20, 1 mM, respectively. TNFα was dissolved at 10 μg/mL in 1× PBS supplemented with 0.5% (w/v) BSA according to the manufacturer instructions. All subsequent dilutions were made in cell culture media.

2.3. Northern blot analysis

Total RNA was isolated from approximately 10×10^6 cells using Nucleo-spin RNA II kit (Macherey-Nagel). Ten micrograms total RNA were separated on a 1% (w/v) denaturing agarose gel with 2% (v/v) formaldehyde and transferred on a Hybond XL membrane (AP-Biotech). Membranes were hybridised with a 0.72 kbp cDNA coding for human GSTP1-1 that was ^{32}P-labelled by random priming using RediprimeII kit (AP-Biotech). Pre-hybridisation and hybridisation were carried out in 50% (v/v) formamide, 6× SCC, 5 mM EDTA, 0.1% (v/v) SDS, 200 μg/mL herring sperm DNA, 100 μg/mL yeast tRNA and 5× Denhart's at 42°. Membranes were washed 5 min with 2× SSC, 0.5% (v/v) SDS at room temperature, 3× 5 min with 2× SSC, 0.1% (v/v) SDS at room temperature, and 3× 30 min with 1× SSC, 0.1% (v/v) SDS at 68°. Quantifications were performed by phosphorimaging the membranes on a Cyclone (Perkin-Elmer).

2.4. Western blot

Five micrograms total proteins, extracted with M-Per mammalian protein extraction reagent (Pierce) or with 1% SDS, were run on a 4% acrylamide concentration gel and a 10% acrylamide separation gel and transferred on a Hybond P membrane (AP-Biotech). Membranes were pre-hybridised in 1× PBS containing 0.1% (v/v) Tween 20 (PBS-T) and 5% fat free milk. Hybridisation was then carried out in PBS-T containing 5% fat free milk with primary antibody. Membranes were washed 3× for 5 min and 2× for 10 min in PBS-T before hybridisation in PBS-T containing 5% fat free milk with secondary antibody. Membranes were washed 3× for 5 min and 2× for 10 min in PBS-T. Proteins of interest were visualised with ECL-plus Western blot detection reagent (AP-Biotech). Polyclonal rabbit anti-pro-caspase 9 and anti-pro-caspase 8 antibodies (Pharmingen), a polyclonal mouse anti-GSTP1-1 antibody (Transduction Laboratories), a monoclonal mouse anti-β-actin (Sigma), a monoclonal mouse anti-PARP (Pharmingen) and secondary anti-rabbit and anti-mouse antibodies (Santa Cruz) were used.

2.5. Cytotoxicity assay

The percentages of cell death were determined using CytoTox 96 non-radioactive cytotoxicity assay kit (Promega). LDH activity is measured by an enzymatic assay that results in the conversion of a tetrazolium salt (INT) into a red formazan product detected at 490 nm. Percentage of cytotoxicity is calculated in relation with the 100% lysis control obtained by adding lysis buffer for 45 min at 37°, 5% CO_2 on the same number of cells.

2.6. Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared using the method described by Müller *et al.* [32] and stored at −80°.

The following oligonucleotides and their complementary sequences were used as probes: -73 (AP-1 site of human GSTP1-1 promoter) 5′-GCCGTGACTCAGCACTGGGG-3′, COL (consensus AP-1 site in the collagenase promoter) 5′-CGCTTGATGACTCAGCCGGAA-3′, consensus NF-κB site: 5′-AGTTGAGGGGACTTTCCCAGGC-3′ and NF-κB site of human GSTP1-1 promoter: 5′-TCTTAGGGAATTTCCCCCCGCGA-3′. They were hybridised and labelled as previously described [33].

Five micrograms of cell nuclear extracts were incubated 20 min at 4° with ^{32}P-ATP labelled probe in 20 μL reaction mixture containing 4% glycerol, 1 mM $MgCl_2$, 0.5 mM EDTA, 509 mM NaCl, 10 mM Tris–HCl (pH 7.5), 0.5 mM DTT and 0.25 μg/mL poly(dI-dC). Each reaction mixture was then loaded on a 5% polyacrylamide gel containing 0.5× TBE. Electrophoresis was performed at 16 mA per gel for 2 hr at room temperature. The gel was then dried and autoradiographed.

2.7. Transfections

Transfections of K562 cells were performed by electroporation using a BioRad gene Pulser. For each experiment 3.75×10^6 cells at a concentration of 1.5×10^7 cells/mL were electroporated at the following settings: 250 V and 500 μF. Ten micrograms reporter gene construct has been used for each pulse. After 48 hr the cells were harvested and resuspended in 300 μL reporter lysis buffer (Promega). Light emission resulting from luciferase activity was measured in a Turner luminometer by integration of peak light emission over 15 s at 25°. Luciferase activity was measured in 1× reporter lysis buffer (Promega) using 10 μL supernatant.

The ratio between arbitrary luciferase light units and total protein content was normalised relatively to the cells transfected with pGL3-control (Promega). All results were expressed as a fold increase or decrease over the standardised luciferase activity of this pGL3-control defined as 100%. Each value is the average of the results of at least three independent transfection experiments. Assays were repeated twice.

3. Results

3.1. Inhibition of the basal GSTP1-1 expression by curcumin

K562 cells were treated with curcumin (0, 1, 5 or 10 μM) for 24 hr at 37°. Figure 1A shows that 10 μM curcumin strongly inhibits (−25%) the expression of the 1.2 kbp transcript corresponding to the human GSTP1-1 mRNA.

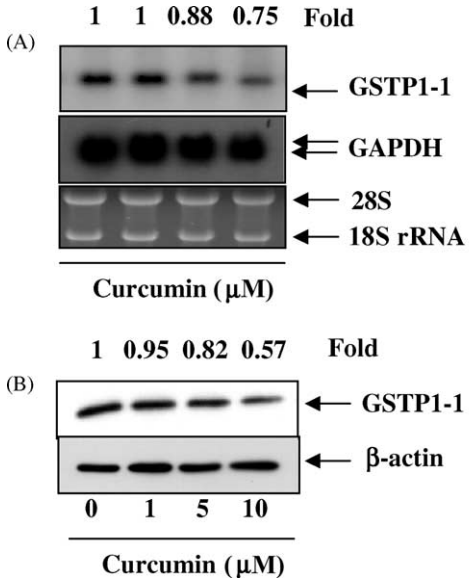

Fig. 1. Effect of curcumin on GSTP1-1 mRNA and protein expression. Cells were treated with curcumin for 24 hr (A) GSTP1-1 mRNA expression was analysed by Northern blot technique. Results shown are representative of three independent experiments. (B) GSTP1-1 protein expression was analysed by Western blot technique. Results shown are representative of three independent experiments.

This inhibition was not due to an unspecific downregulation of RNA synthesis, since curcumin treatment did not significantly modify the expression of GAPDH mRNA. Figure 1B shows that GSTP1-1 protein expression was decreased as well with 10 μM curcumin under low serum conditions.

3.2. Inhibition of TNFα-induced GSTP1-1 mRNA expression by curcumin

K562 chronic myelogenous leukemia cells expressed detectable levels of a 1.2 kbp transcript corresponding to human GSTP1-1 mRNA, which were increased after TNFα treatment (Fig. 2). The 1.8-fold induction of GST gene expression was observed at 0.5 ng/mL TNFα after 8 hr treatment and did not increase at higher concentrations of TNFα. This induction was not due to an unspecific upregulation of RNA synthesis, since TNFα treatment did not significantly modify the levels of GAPDH mRNA expression.

K562 cells were incubated with 10 μM curcumin for 2 hr before the addition of TNFα (0.5 ng/mL). This pretreatment prevented the previously observed increase in GSTP1-1 transcript synthesis suggesting that curcumin is able to interfere with the signal transduction mechanisms leading to GSTP1-1 expression at the transcriptional level.

3.3. Inhibition of TNFα-activated AP-1 binding by curcumin

It was previously shown that curcumin is a potent inhibitor of NF-κB [34] and AP-1 [35]. Whether curcumin can suppress constitutive and TNFα-induced AP-1 and

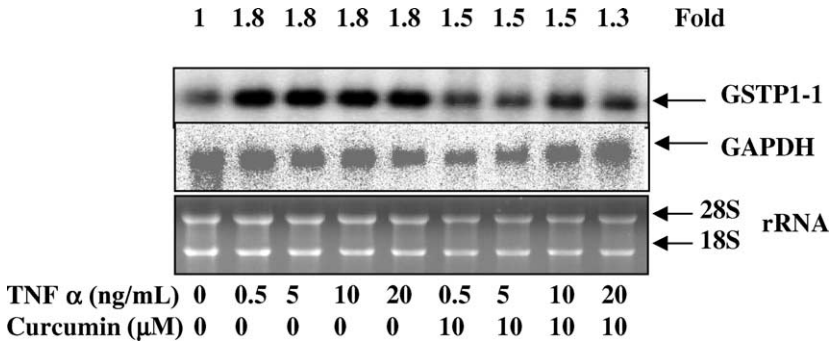

Fig. 2. Inhibition of TNFα-dependent activation of GSTP1-1 mRNA expression by curcumin on K562 cells. Cells were cultured for 8 hr with TNFα with or without a 2 hr pre-treatment with 10 μM curcumin. GSTP1-1 mRNA expression was analysed by Northern blot technique. Results shown are representative of three independent experiments.

NF-κB binding on human GSTP1-1 gene promoter in K562 cells was investigated. Cells were pre-incubated for 2 hr with different concentrations of curcumin before treatment with TNFα (0.5 ng/mL). Nuclear extracts were then examined for AP-1 binding by EMSA using two probes: a consensus AP-1 site from the collagenase promoter (COL), and the AP-1 site located near the transcription initiation site of GSTP1-1 (-73). Ten micromolars curcumin completely suppressed TNFα-induced AP-1 binding on GSTP1-1 probe after a 2 hr pre-treatment in K562 cells (Fig. 3A). The use of a consensus AP-1 probe (Fig. 3B) confirmed that curcumin was able to suppress the TNFα-induced activation of AP-1 in K562 cells.

3.4. Inhibition of TPA-activated AP-1 binding by curcumin

The phorbol ester TPA, known to induce AP-1 binding in several cell types [36,37], was included in these experiments

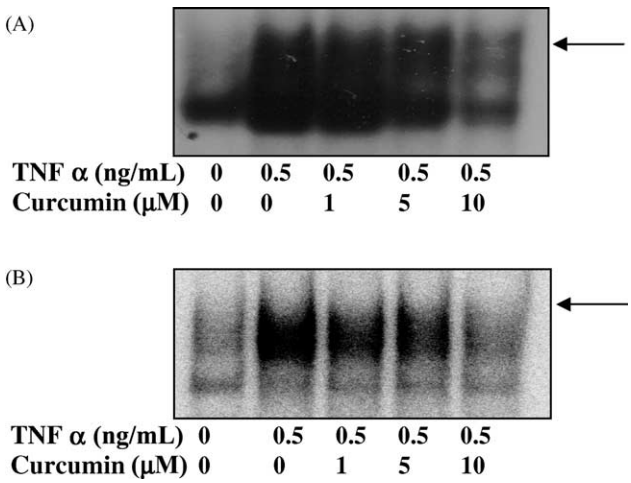

Fig. 3. Effect of curcumin and TNFα on AP-1 binding in K562 cells. K562 cells were treated for 4 hr with 0.5 ng/mL TNFα with or without a 2 hr curcumin pre-treatment. (A) Nuclear extracts were analysed by EMSA with AP-1 (-73) probe or (B) with AP-1 collagenase probe (arrows indicate specific binding).

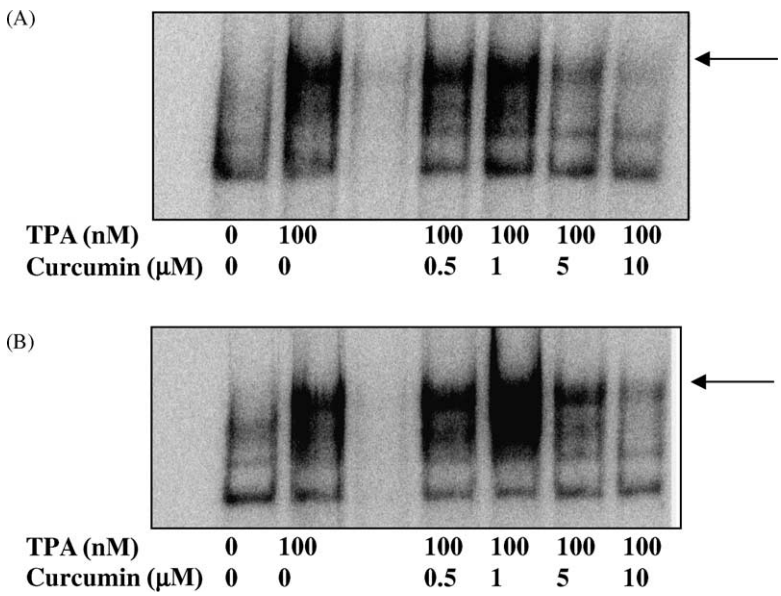

Fig. 4. Effect of curcumin and TPA on AP-1 binding in K562 cells. K562 cells were treated for 2 hr with 100 nM TPA with or without a 2 hr curcumin pre-treatment. (A) Nuclear extracts were analysed by EMSA with AP-1 (-73) probe or (B) with AP-1 collagenase probe (arrows indicate specific binding).

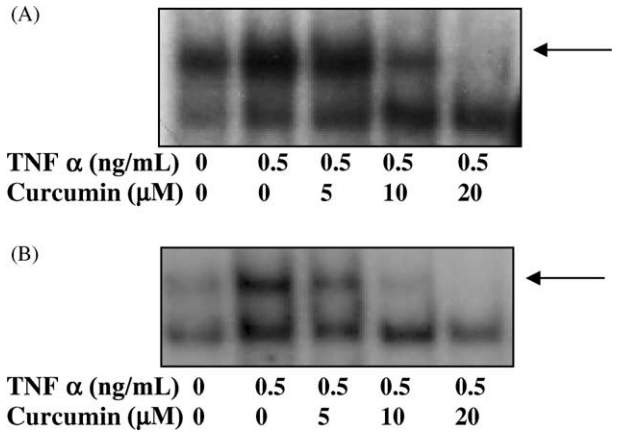

Fig. 5. Effect of curcumin and TNFα on NF-κB binding in K562 cells. K562 cells were treated for 4 hr with 0.5 ng/mL TNFα with or without a 2 hr curcumin pre-treatment. (A) Nuclear extracts were analysed by EMSA with GSTP1-1 NF-κB probe or (B) with NF-κB consensus probe (arrows indicate specific binding).

for comparison with TNFα. Cells were pre-incubated for 2 hr with different concentrations of curcumin and then treated with TPA (100 nM) for 2 hr at 37°. As previously shown [33], TPA induces AP-1 binding on both sites in K562 cell lines. The results in Fig. 4A and B indicate that 10 μM curcumin inhibits TPA activation on both the consensus site (−50%) and the GSTP1 site (−60%).

3.5. Inhibition of TNFα-dependent NF-κB binding by curcumin

TNFα being a well-characterised activator of NF-κB, we studied the effect of curcumin on NF-κB activation in K562 cells by EMSA. K562 cells were treated for 4 hr at 37° with 0.5 ng/mL TNFα with or without a 2 hr pre-treatment with 5, 10 or 20 μM curcumin. Figure 5A and B show that TNFα activates NF-κB binding on both the consensus site and the GSTP1-1 NF-κB site. Pre-treatments with curcumin lead to inhibition of the NF-κB binding on both sites showing the effect of curcumin on the NF-κB signalling pathway.

3.6. Inhibition of TNFα activated transcription by curcumin

We next examined the effect of TNFα alone, or in combination with curcumin, on the activity of the human GSTP1-1 gene promoter. Human GSTP1-1 genomic fragments of different length (97, 396 and 1175 bp relative to the transcriptional start site) were ligated upstream of the firefly luciferase reporter gene and were assessed for luciferase activity in K562 cells. An additional construct harbouring five repeats of the GSTP1-1 NF-κB site (pGSTNFκB) was also used for this study (Fig. 6). A comparison of luciferase activities generated by transfection of those constructs between unstimulated and TNFα-stimulated cells showed that the GST promoter activity was enhanced following TNFα treatment, indicating that this human promoter contains TNFα-responsive sequences that might account for the TNFα-mediated induction of GSTP1-1 in these cells.

TNFα treatment on K562 cells transfected with pGSTNFκB (Fig. 7A) strongly induced the expression of luciferase gene (125-fold) showing the functionality of the GSTP1-1 NF-κB site located at -323. The same treatment on cells transfected with the other plasmids led to an average 5-fold increase of the luciferase expression (Fig. 7B). Moreover, there was no difference between transfection of the plasmid p-97 and plasmids p-396 and p-1175 containing the NF-κB site. Another site located downstream of p-97 can thus bind a transcription factor reacting to TNFα. Curcumin treatments had a significant inhibitory effect on luciferase activity whatever the plasmid transfected. Moreover, TNFα-dependent induction

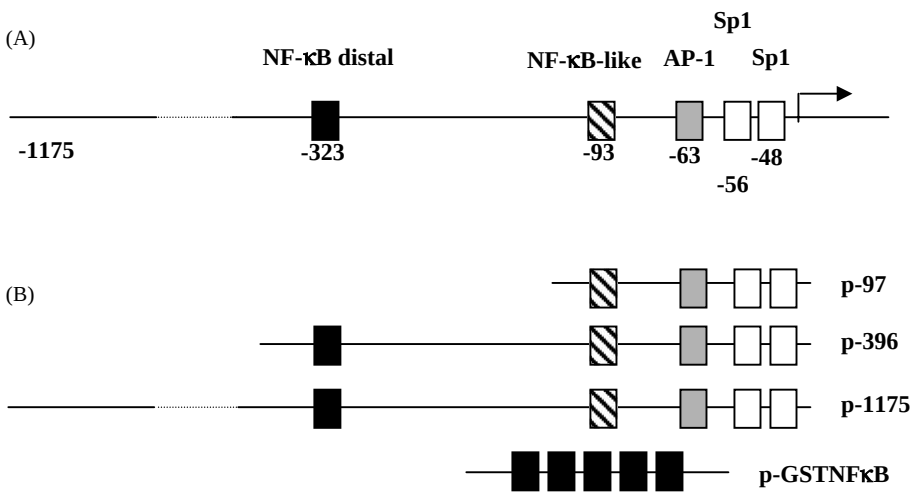

Fig. 6. Transfection constructs of the human GSTP1-1 promoter. (A) The position of the different transcription factor binding sites are indicated related to the transcription start site. (B) Promoter constructs used in transfection experiments: p-97, p-396 and p-1175 are constructs derived from the GSTP1-1 gene promoter. pGSTNFκB is a construct with five repeats of the NF-κB site located at -323 on the GSTP1-1 gene promoter.

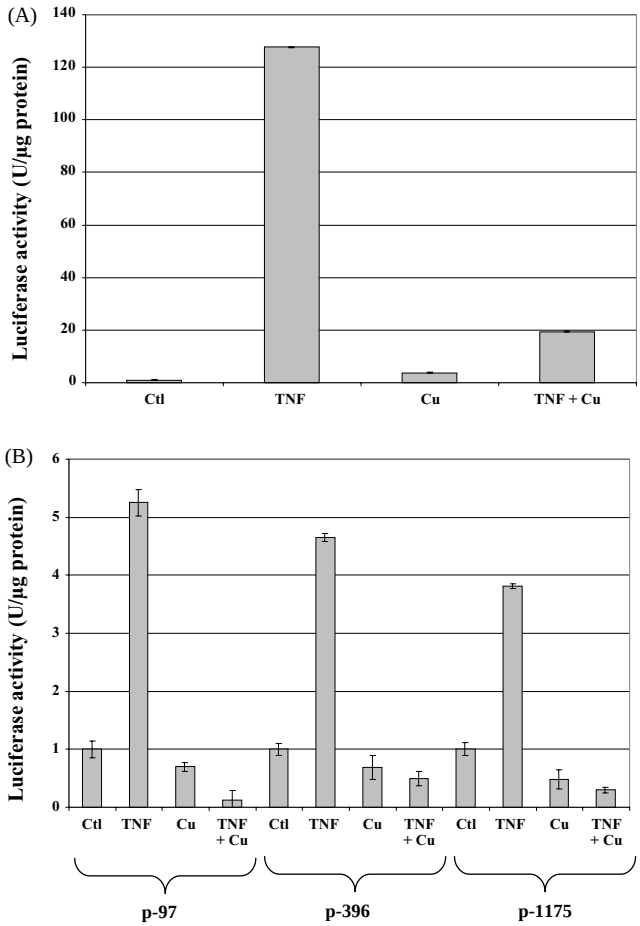

Fig. 7. Transfections experiments. K562 cells were transfected with the different constructs and treated with TNFα 0.5 ng/mL (TNF), curcumin 10 μM (Cu) or TNFα + curcumin (TNF + Cu) or without treatment (Ctl). (A) Transfection with pGSTNFκB. (B) Transfection with plasmids p-97, p-396 and p-1175.

was reduced by 80% by curcumin when cells were transfected with pGSTNFκB (Fig. 7A). Inhibition was even stronger (98, 90 and 93%) when the cells were transfected with the three promoter constructs p-97, p-396 and p-1175, respectively.

3.7. Decreases of leukemia cell survival by inducing apoptosis by curcumin

Whether or not the treatment by curcumin leads to an increase of cellular death was then investigated. Cells were treated with curcumin for 24 hr at 37°. As shown in Fig. 8A, cytotoxicity of curcumin increased in a concentration-dependent manner reaching a maximum at 20 μM. To determine whether curcumin-induced cell death was due to apoptotis, we tested the ability of curcumin to activate caspases 8 and 9. K562 cells were treated with 20 μM curcumin for various times. Western blot analysis (Fig. 8B) showed that curcumin reduced pro-caspase 9 levels reaching a maximum inhibition at 24 hr (80%) demonstrating its apoptotic effect on K562 cells. This was confirmed by the decrease of pro-caspase 8 levels after 24 hr treatment with curcumin. Finally curcumin also induced caspase-dependent cleavage of poly ADP ribose polymerase (PARP) in a time-dependent manner with the appearance of 85 kDa cleavage product at 24 hr of treatment.

4. Discussion

Curcumin is a compound known for its antioxidant and anticarcinogenic properties [20–26]. It has also been shown to have an inhibitory effect on AP-1 and NF-κB activation. We investigated here its effects on GSTP1-1 expression in leukemia cell lines. Indeed, GSTP1-1 was found to be related to carcinogenesis and resistance to anticancer drugs [38]. The implication of NF-κB and AP-1 [3,39] in GSTP1-1 regulation was demonstrated and the use of curcumin in leukemia could lead to a valuable therapeutic tool.

We show here that treatment by the chemopreventive agent curcumin of K562 cells leads to increased cell death compared to cells cultured without curcumin. Apoptosis is characterised by caspase activation [40]. Mukhopadhyay *et al.* [41] previously showed that curcumin induced apoptosis in human androgen-dependent LNCaP as well as androgen-independent DU145 human prostate cancer cell lines by down-regulating the expression of Bcl-2 and Bcl-X_L and the activation of the pro-caspases 3 and 8. Similar results were obtained by Bush *et al.* [42] by using a melanoma cell model. Our results agree with the data obtained by Barthi *et al.* [43] where in multiple myeloma cells curcumin induces caspases 7 and 9. We show here that apoptosis can be induced by curcumin in K562 cells through activation of caspases 8 and 9 indicating that both the mitochondrial and death receptor pathways are activated.

K562 cells carry the Philadelphia chromosome with the Bcr-Abl fusion gene. The Bcr-Abl oncoprotein activates several pathways implicated in carcinogenesis such as Myc, Ras, c-Raf, MAPK/ERK, SAPK/JNK, Stat, NF-κB, PI-3 kinase and c-Jun due to a constitutively activated tyrosine kinase activity. In cells carrying such a translocation, apoptosis is decreased and cell proliferation is upregulated. In K562 cells, classical apoptosis can only be induced in cells by inhibiting Ras, Raf, PI3K, Akt, Jun and Myc [44]. In our hands, inhibition of constitutively active AP-1 and NF-κB in K562 by curcumin leads to caspase 8 and 9-dependent cell death. Similar results were obtained by Barthi *et al.* [43] in human prostate cancer cells and Piwocka *et al.* in Jurkat T cells [45].

As AP-1 and NF-κB binding activities are correlated to GSTP1-1 expression, inhibition of those transcription factors by curcumin should induce a reduction of GSTP1-1 expression at the mRNA level. Singhal *et al.* [46] previously showed a weak induction by curcumin of the alpha-class hGST 5.8 isozyme, indicated by an increased activity toward 4-hydroxynonenal. We show here a substantial

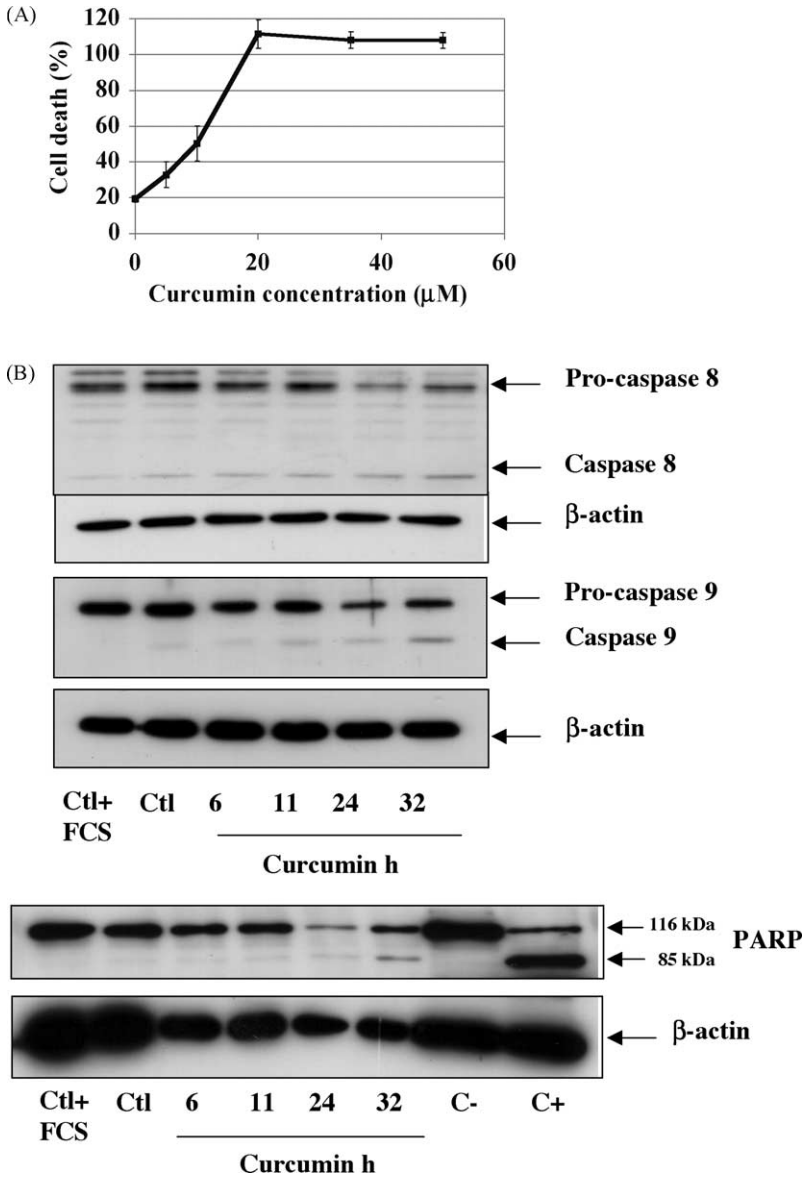

Fig. 8. Effect of curcumin on K562 cell death. Cells were treated with curcumin for 24 hr. (A) Toxicity was measured. (B) Cells were treated with 20 μM of curcumin. Pro-caspases 8 and 9 as well as PARP activation was detected by Western blot technique. Ctl + FCS designates control K562 cells cultured at 10% FCS, Ctl designates control K562 cells cultured at 0.1% FCS. C− designates untreated HL-60 cells and C+ designates etoposide-treated HL-60 cells as negative and positive PARP cleavage controls respectively.

reduction of GSTP1-1 mRNA expression in human K562 chronic myelogenous leukemia cells. It is of interest to note that with 66%, GSTP1-1 is the major GST isoform expressed by K562 and that the expression of this isoform is correlated to chemoresistance in leukemia [47], breast and ovary cancer [5–9].

Morales *et al.* [48] previously demonstrated the effect of the proinflammatory cytokine TNFα [49] on the expression of another key enzyme of the glutathione pathway, gamma-glutamyl cysteine synthetase (γGCS). TNFα was able to induce the γGCS mRNA expression by 70% in a rat hepatocyte model. In this model, TNFα was able to induce NF-κB as well as AP-1 transcription factors. As we and other groups recently showed the importance of both AP-1 [33] and NF-κB sites in the regulation of the GSTP1-1 expression, inhibition of DNA bindings of both transcription factors by curcumin should be responsible for a decrease in GSTP1-1 gene expression and thus could contribute to reduce drug resistance and carcinogenesis due to GSTP1-1 activity.

Our results show an inhibition of both constitutive and TNFα-induced GSTP1-1 mRNA expression in K562 cells. This transcriptional inhibition was paralleled by a progressive reduction of GSTP1-1 protein expression. In order to understand the molecular mechanisms responsible for these variations we examined the effect of curcumin on promoter activity as well as AP-1 and NF-κB binding onto the GSTP1-1 promoter.

Barthi *et al.* [43] previously showed that curcumin inhibits IKK complex activity and thus IκBα phosphorylation as well as the p65 nuclear translocation. Those data are in agreement with our results obtained by gel shift assays using GSTP1-1 promoter and consensus NF-κB probes. Moreover, luciferase activities generated by constructs associating GSTP1-1 promoter fragments of increasing length to a luciferase reporter gene were strongly inhibited by curcumin as was a similar construct harbouring five NF-κB repeats. In 1995, Singh and Aggarwal [18] using a ML-1a myelomonoblastic leukemia cell line showed that curcumin inhibits AP-1 binding onto a consensus AP-1 site. These results are in agreement with our data, where curcumin strongly inhibits TNFα- and TPA-induced bindings onto GSTP1-1 and consensus AP-1 probes. Inhibition of both NF-κB and AP-1 bindings thus accounts for the observed transcriptional inhibition.

Moreover, Gilot *et al.* [50] noticed that overexpression of ASK-1 restored caspase-3 activation and apoptosis in primary cultures of hepatocytes. Co-transfections of GSTM1/2, GSTA1/2 or GSTP1-1 isoforms with ASK-1 reduced apoptosis by antagonising ASK-1 activity and thus demonstrating the antiapoptotic function of the GSTs.

We confirm these data as reduction of GSTP1-1 expression by curcumin is paralleled by an onset of apoptosis as shown by caspases 8 and 9 activation as well as Poly ADP Ribose Polymerase cleavage.

In conclusion, these results add a novel role for curcumin: this chemoprotective compound could contribute to induce apoptosis by its ability to inhibit the GSTP1-1 expression at the level of transcription.

Acknowledgments

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