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Effect of chemopreventive agents on glutathione *S*-transferase P1-1 gene expression mechanisms via activating protein 1 and nuclear factor kappaB inhibition

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Abstract

Glutathione *S*-transferase P1-1 (GSTP1-1) is a phase II drug metabolism enzyme implicated in carcinogenesis and development of resistance to anti-cancer drugs. It was previously shown that both activating protein 1 (AP-1) and nuclear factor κB (NF-κB) are involved in its regulation. In the present study we examined the inhibitory effect of several chemopreventive agents on the tumor necrosis factor (TNF) α- or 12-O-tetradecanoylphorbol 13 acetate (TPA)-induced promoter activity of GSTP1-1, as demonstrated by transient transfection experiments in K562 and U937 leukemia cells. Our results provide evidence for a differential effect of chemopreventive agents such as β-lapachone, emodin, sanguinarine and capsaicin, which significantly inhibit reporter gene expression as well as TNFα- and TPA-induced binding of AP-1 and NF-κB, whereas *trans*-anethole and silymarin do not produce any inhibitory effect. Our results demonstrate the ability of selected chemopreventive agents to decrease GSTP1-1 gene expression mechanisms and could thus contribute to reduce the incidence of glutathione related drug resistance in human leukemia.

Keywords: GSTP1-1; Chemopreventive agents; Carcinogenesis; Drug resistance; Leukemia

1. Introduction

Glutathione *S*-transferase P1-1 (GSTP1-1) is a heterodimeric enzyme with subunits varying between 23 and 28 kDa. This enzyme is a member of the GST (EC 2.5.1.18) multigene superfamily which is classified into eight distinct gene families coding for seven cytosolic isoforms (alpha, mu, pi, theta, omega, kappa and zeta) and one microsomal form [1].

GSTP1-1 is a phase II drug metabolism enzyme playing an important role in cell detoxification by conjugating electrophilic compounds to glutathione, allowing their export through the GS-X pump [2]. However, it is also implicated in carcinogenesis [3–7] and development of anti-cancer drug resistance [8–11]. Indeed, GSTP1-1 over-expression is associated with cisplatin resistance in head and neck squamous cell carcinoma [12] and with poor prognosis in breast cancer [13].

Moreover, GSTP1-1 expression is found to be elevated in many gastric cancers and esophagus tumors even though in the corresponding normal tissues the protein is either absent or expressed at very low levels [14]. It was shown that GSTP1-1 over-expression in cholangiocarcinoma [15], ovary carcinoma [5,16–19] and leukemia [20] leads to resistance to chemotherapy.

At the molecular level, GSTP1-1 is regulated by a minimal promoter, containing one AP-1, two Sp-1 binding sites [21,22], as well as one NF-κB [23] and one GATA-1 binding site [24] which regulate GSTP1-1 gene expression under various physiological conditions including inflammation and erythroid differentiation.

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

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Chemoprevention is a novel anti-cancer approach with less secondary effects compared to classical chemotherapy [25,26]. Chemopreventive agents are natural or synthetic chemical products that allow suppression, delay or inversion of carcinogenesis [27]. They were described to act as anti-oxidants [28–30], anti-inflammatory [28,31] and anti-tumoral agents [32–35]. Some compounds showed anti-viral [36], anti-bacterial [37–39] or anti-fungal [40] properties and induced apoptosis in cancer cell lines [41–47].

In this study we used the natural agents β-lapachone, *trans*-anethole, emodin, sanguinarine, capsaicin and silymarin in order to confirm their effect on GSTP1-1 transcriptional control in leukemia cells. We showed recently that curcumin, a powerful chemopreventive agent, can induce apoptosis as well as inhibit GSTP1-1 expression through the inhibition of AP-1 and NF-κB binding on the GSTP1-1 promoter [48]. We extended our studies to other chemopreventive agents by investigating the cytotoxic effect of each agent on two leukemia cell lines, K562 and U937.

In opposition to *trans*-anethole and silymarin, we demonstrated that β-lapachone, emodin, sanguinarine and capsaicin were able to inhibit TNFα- or TPA-induced GSTP1-1 promoter induction using reporter gene assays. Results were confirmed by electromobility shift assays using consensus and GSTP1-1 NF-κB and AP-1 binding sites. Overall, this study underlines the differential effect of natural chemopreventive agents in different leukemia cell lines and their potential interest as inhibitors of GSTP1-1 expression.

2. Materials and methods

2.1. Cells and medium

K562 (human chronic myelogenous leukemia) and U937 (histiocytic lymphoma) cells 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 °C and 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 h at 37 °C.

2.2. Compounds

TNFα, β-lapachone, emodin, sanguinarine, capsaicin, *trans*-anethole and silymarin were purchased from Sigma, while 12-O-tetradecanoyl phorbol 13-acetate (TPA) was obtained from MP-Biomedicals. TNFα was dissolved at 10 μg/ml in 1X PBS supplemented with 0.5% (w/v) BSA according to the manufacturer instructions. TPA, β-lapachone, emodin and silymarin were dissolved in 100% DMSO at 1 mM, 10 mM, 30 mg/ml and 100 mM, respectively. Sanguinarine was dissolved at 10 mM in 100% methanol and capsaicin was dissolved at 150 mM in 100% ethanol. *Trans*-anethole was purchased as a 6.8 M solution. All subsequent dilutions were made in cell culture media.

2.3. Cytotoxicity assay

The percentage of cell death was determined using CytoTox 96 Non-Radioactive Cytotoxicity Assay kit (Promega).

2.4. Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared using the method described by Müller et al. [49] and stored at −80 °C. The following oligonucleotides and their complementary sequences were used as probes: AP-1 GST (AP-1 site of human GSTP1-1 promoter) 5′-GCCGTGACTCAGCACTGGGG-3′, AP-1 C (consensus AP-1 site in the collagenase promoter) 5′-CGCTTGATGACTCAGCCGGAA-3′, NF-κB C (consensus NF-κB site) 5′-AGTTGAGGGGACTTTCCCAGGC-3′, GSTP1-1 NF-κB (NF-κB site of human GSTP1-1 promoter) 5′-TCTTAGGGAATTTCCCCCCGCGA-3′ and mutated NF-κB D: 5′-TCTTACTCAATTTCCCCCCGCGA-3′. Probes were hybridized and labeled as previously described [22].

Five micrograms of cell nuclear extracts were incubated for 20 min at 4 °C with ^{32}Pγ-ATP labeled probe. In supershift/immunodepletion experiments, the nuclear extracts and labeled probes were incubated for 30 min on ice prior a 30 min incubation with 2 μg antibody on ice, for NF-κB antibodies, and after 25 min incubation with 2 μg antibody on ice, for AP-1 antibodies. In competition experiments nuclear extracts and 50-fold molar excess of unlabeled probes were pre-incubated in the reaction mixture for 20 min on ice.

2.5. Transient transfections

The promoter constructs used were described previously [23]. 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. Five micrograms luciferase reporter gene construct and 5 μg Renilla plasmid were used for each pulse. After 48 h cells were harvested and resuspended in growth medium (RPMI/FCS 10%) to a final concentration of 10^5 cells/ml with or without treatments. Before luciferase activity was measured, 75 μl Dual-Glo™ Luciferase Reagent were added to cells for a 10 min incubation at 22 °C. Then, 75 μl Dual-Glo™ Stop & Glo® Reagent were added for 10 min at 22 °C in order to assay Renilla activity. Luciferase and Renilla activities were measured using an Orion microplate luminometer (Berthold) by integrating light emission for 10 s. Results are expressed as a ratio arbitrary units of firefly luciferase normalized to Renilla luciferase. The plasmids

used were termed p-1175 representing the GSTP1-1 gene promoter between +8 and –1175 relative to the transcription start site, p5xNF-κB containing five repeats of a consensus NF-κB site and p5xAP-1 containing five repeats of a consensus AP-1 site.

3. Results

3.1. Cytotoxic effect of chemopreventive agents on human leukemia cell lines

In order to study the potential therapeutic effects of different chemopreventive agents, we measured the survival of K562 and U937 leukemic cell lines. Cells were treated with different concentrations of β-lapachone, *trans*-anethole, emodin, sanguinarine, capsaicin or silymarin for 24 h. As shown in Fig. 1A, cytotoxicity of β-lapachone increased after 5 μM to reach a maximum of 60% cell death in K562 cells but had no effect in U937 cells. Cytotoxicity of *trans*-anethole was similar for both cell lines and increased in a concentration-dependent manner to reach 77 and 82% in K562 and U937, respectively at 10 mM *trans*-anethole (Fig. 1B). Emodin did not have a strong cytotoxic effect as only 43 and 26% of cell death were obtained in K562 and U937, respectively, after a 250 μg/ml treatment (Fig. 1C). Similarly to *trans*-anethole, sanguinarine had a cytotoxic effect on both cell lines in a

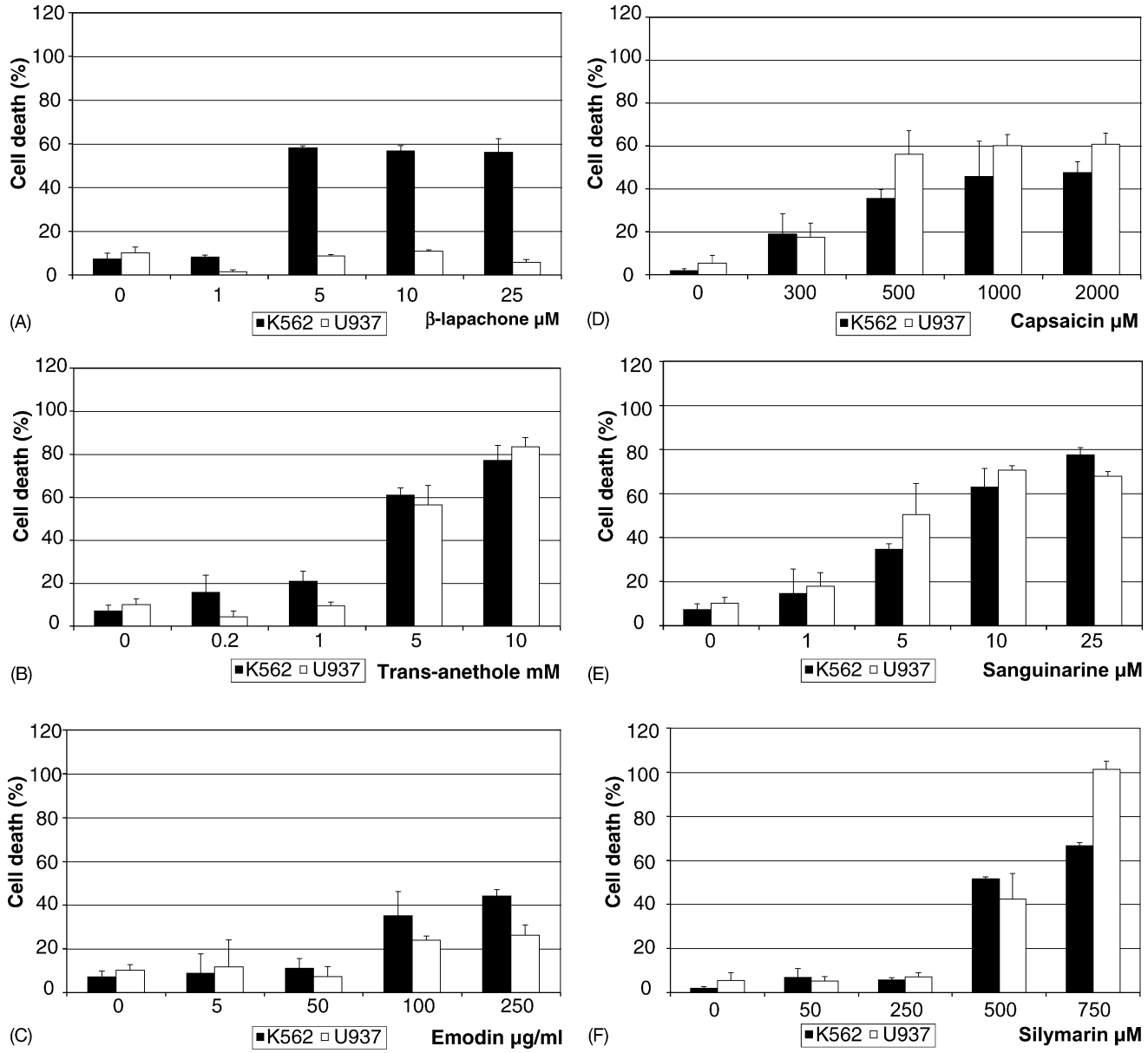


Fig. 1. Effect of chemopreventive agents on K562 and U937 cell death. Cells were treated with β-lapachone (A), *trans*-anethole (B), emodin (C), sanguinarine (D), capsaicin (E) and silymarin (F) for 24 h. Cell death was determined using LDH release measurement. Assays were repeated three times.

concentration-dependent manner as 78% and 68% of cell death were obtained in K562 and U937, respectively, after a 25 μM treatment (Fig. 1E). Capsaicin induced 45% cell death at 1 mM in K562 whereas 60% of cell death was obtained in U937 (Fig. 1D). Silymarin induced cellular death at concentrations above 500 μM in both cell lines (Fig. 1F).

3.2. Inhibition of TNFα- or TPA-activated transcription by chemopreventive agents

We next examined the effect of the different chemopreventive agents on TNFα-induced GSTP1-1 gene promoter activity. A human GSTP1-1 genomic fragment of 1175 bp was ligated upstream of the firefly luciferase reporter gene (p-1175) and was assessed for luciferase activity in K562 cells. Results were compared to a reporter gene under the control of five repeats of a previously identified TNFα-inducible NF-κB site within the promoter [23]. In order to demonstrate the inhibitory potential of the natural compounds used in this study, we pretreated K562 cells with various concentrations of chemopreventive agents before challenging with TNFα at 0.5 ng/ml. Results show that β-lapachone, emodin, sanguinarine or capsaicin induced inhibition of a TNFα-induced reporter gene controlled by the GSTP1-1 NF-κB site whereas pretreatments with *trans*-anethole or silymarin did not produce any inhibiting effect (Fig. 2A). Active compounds achieved a concentration-dependent inhibition of the reporter gene activity under the control of the full GSTP1-1 promoter (Fig. 2B). For sanguinarine and capsaicin strong transcriptional inhibition could only be obtained by increasing the concentration of these chemopreventive agents.

Similarly, we published that AP-1 plays a crucial role in the regulated expression of GSTP1-1 and that the phorbol ester TPA significantly increases reporter gene activity as well as AP-1 and NF-E2 binding in K562 cells [22]. In order to generalize our results, we compared inhibitory effects of chemopreventive agents on reporter genes under the control of both a consensus AP-1 repeat (Fig. 3A) and the AP-1 site within the GSTP1-1 promoter (Fig. 3B) both challenged by TPA at 100 nM. As for TNFα, induction by TPA was concentration-dependently inhibited by chemopreventive agents β-lapachone, emodin, sanguinarine or capsaicin whereas *trans*-anethole did not induce any inhibition. Silymarin interestingly induced a significant 1.4-fold induction of luciferase activity (Fig. 3A).

3.3. Inhibition of TNFα-induced NF-κB binding by chemopreventive agents

We previously demonstrated that the chemopreventive agent curcumin inhibits GSTP1-1 mRNA and protein expression as well as NF-κB and AP-1 binding activities [48]. In order to select novel inhibitors of glutathione-based drug resistance mechanisms, K562 cells were pretreated for 2 h with different concentrations of β-lapachone, emodin, sanguinarine or capsaicin before treatment with 0.5 ng/ml TNFα. Nuclear extracts were then examined for NF-κB binding by EMSA using two probes: the NF-κB site located at −323 on the GSTP1-1 promoter (GSTP1-1 NF-κB) compared to a consensus NF-κB site (NF-κB C). In K562, the four chemopreventive agents decreased the TNFα-induced NF-κB binding on both consensus (Fig. 4A) and GSTP1-1 NF-κB binding sites (Fig. 4B). β-lapachone and emodin reduced NF-κB binding to basal level at 1 μM and 50 μg/ml, respectively. However, sanguinarine and capsaicin completely abolished NF-κB binding from 5 and 300 μM, respectively.

3.4. Inhibition of TPA-induced AP-1 binding by chemopreventive agents

We next examined by EMSA the effect of the chemopreventive agents on TPA-induced AP-1 binding using both the consensus binding site from the collagenase promoter (AP-1 C) and the corresponding GSTP1-1 promoter site (AP-1 GST). K562 cells were pretreated with different concentrations of β-lapachone, emodin, sanguinarine or capsaicin before treatment with TPA (100 nM). As for NF-κB binding, in K562, β-lapachone and emodin were found to reduce the binding to basal level from 1 μM and 50 μg/ml, respectively, whereas sanguinarine and capsaicin completely abolished binding on both sites from and 300 μM, respectively (Fig. 4C and D).

3.5. Characterization of NF-κB and AP-1 binding

In order to characterize NF-κB and AP-1 binding activities, we realized immunodepletion assays as well as competition experiments. U937 cells were treated with 0.5 ng/ml TNFα for NF-κB and with 100 nM TPA for AP-1. Nuclear factors were analyzed by EMSA using the corresponding wild type and mutated sites. The use of NF-κB-specific antibodies demonstrates the presence of p50/p65 and p65/p65 dimers (Fig. 5A) whereas antibodies directed against AP-1 isoforms determined that the observed complexes include c-Jun, JunD and JunB (Fig. 5B). Competition assays show that cold GSTP1-1 NF-κB and AP-1 probes abolish binding whereas the mutated counterparts have no effect on its specificity (Fig. 5C and D).

4. Discussion

Glutathione *S*-transferases are a family of enzymes catalyzing cellular detoxification of xenobiotics. However, increased levels of the human pi class isoenzyme GSTP1-1 are correlated to carcinogenesis and resistance of cancer cells to oxidative stress and chemotherapeutic drugs. We recently demonstrated that the use of curcumin, a chemo-

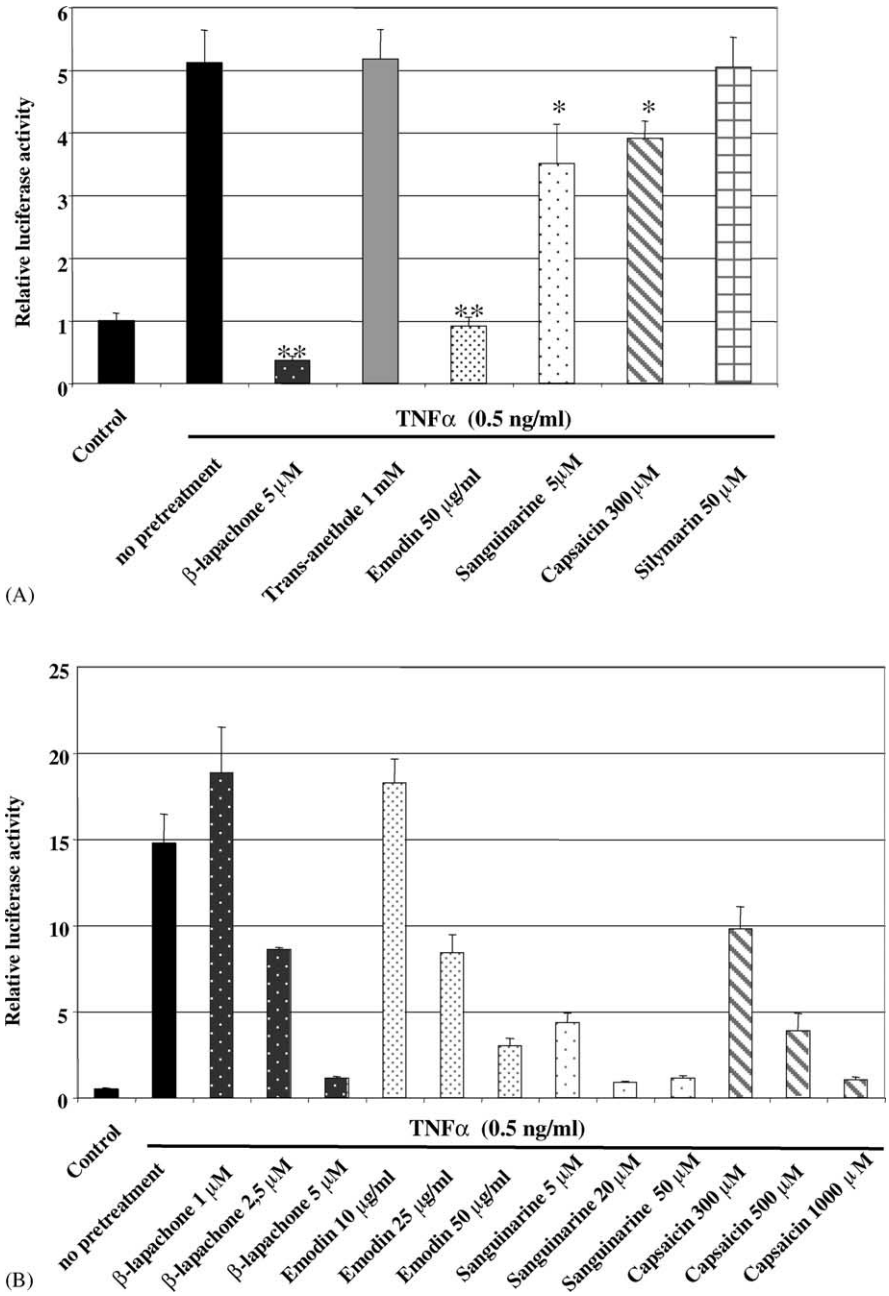
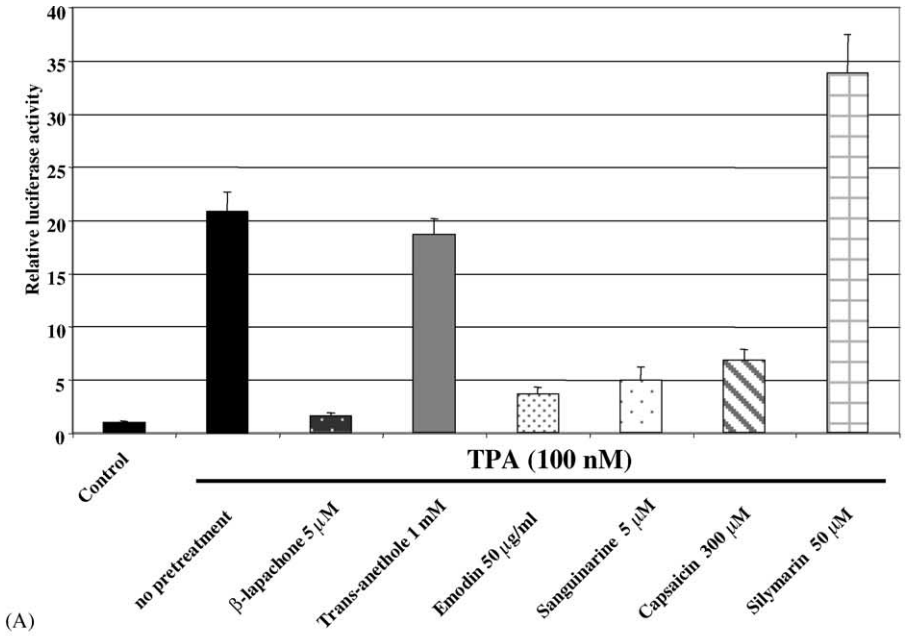


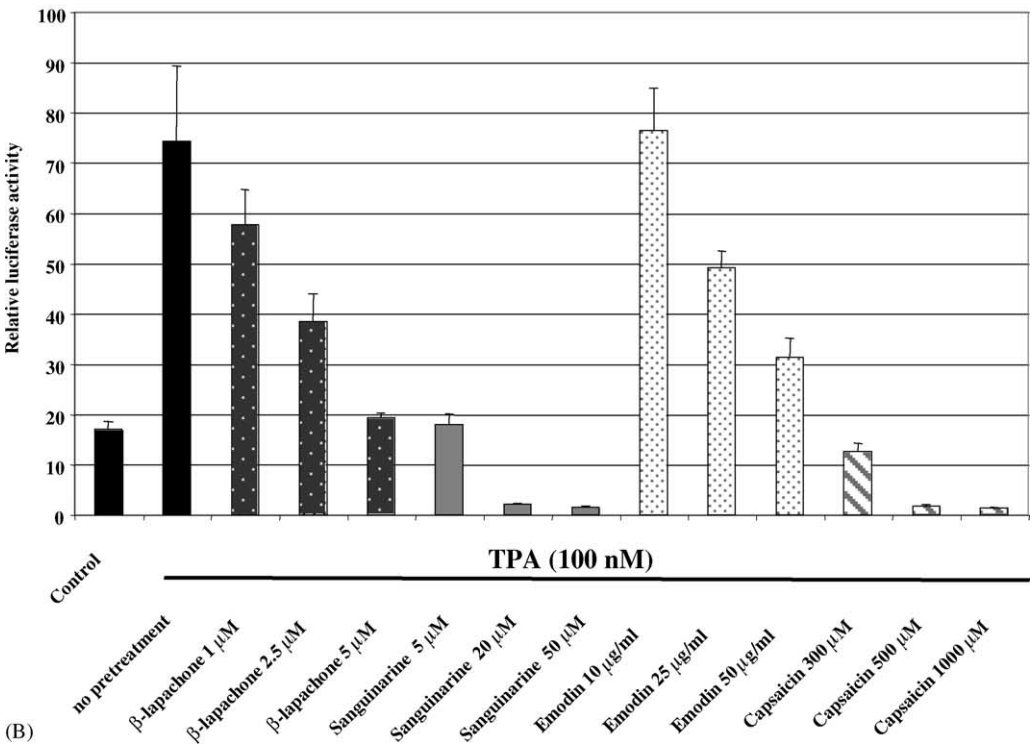
Fig. 2. Effect of chemopreventive agents on TNFα-induced *GSTP*1-1 promoter activity. (A) K562 cells were transiently transfected with 5 μg of the luciferase reporter pGSTNF-κB plasmid. Transfected cells were pretreated for 2 h with 5 μM β-lapachone, 1 mM *trans*-anethole, 50 μg/ml emodin, 5 μM sanguinarine, 300 μM capsaicin or 50 μM silymarin before treatment with 0.5 ng/ml TNFα for 8 h. (∗) $P < 0.05$; (∗∗) $P < 0.01$ (compared to TNFα treated control). (B) K562 cells were transiently transfected with 5 μg of the luciferase reporter p-1175 plasmid. Transfected cells were pretreated for 2 h with different concentrations of β-lapachone, emodin, sanguinarine or capsaicin before treatment with 0.5 ng/ml TNFα for 8 h. Luciferase activity was measured and results are the mean of three independent experiments.

preventive agent extracted from *Curcuma longa*, leads to reduction of GSTP1-1 expression through the inhibition of AP-1 and NF-κB binding onto the GSTP1-1 promoter [48]. In order to extend these results, we demonstrate that chemopreventive agents have a differential effect on TNFα- and TPA-induced GSTP1-1 expression as β-lapachone, emodin, sanguinarine and capsaicin significantly inhibit GSTP1-1 promoter activity whereas *trans*-anethole or silymarin do not.

Chemopreventive agents are considered to display anti-oxidant and anti-inflammatory properties but each component has its own characteristics. β-lapachone is extracted from the Lapacho tree and is known for inhibiting TNFα-induced NF-κB and AP-1 binding in U937 leukemic cells [31]. In this cell line, β-lapachone inhibits TNFα-induced apoptosis. On the other hand, activation of apoptosis was described in MCF7 and T47D breast cancer cells [44] and in HL-60 cells line [45]. Results obtained during this study



(A)



(B)

Fig. 3. Effect of chemopreventive agents on TPA-induced *GSTP*1-1 promoter activity. (A) K562 cells were transiently transfected with 5 μg of the luciferase reporter p5xAP-1 plasmid. Transfected cells were pretreated for 2 h with 5 μM β-lapachone, 1 mM *trans*-anethole, 50 μg/ml emodin, 5 μM sanguinarine, 300 μM capsaicin or 50 μM silymarin before treatment with 100 nM TPA for 4 h. Luciferase activity was measured and results are the mean of three independent experiments. (B) K562 cells were transiently transfected with 5 μg of the luciferase reporter p-1175 plasmid. Transfected cells were pretreated for 2 h with different concentrations of β-lapachone, sanguinarine, emodin or capsaicin before treatment with 100 nM TPA for 4 h. Luciferase activity was measured and results are the mean of three independent experiments.

confirm the cell-specific effect of β-lapachone on apoptosis since cytotoxic assays strongly differ between U937 and K562 cells. Indeed, U937 cells remain unaffected by growing β-lapachone concentrations, while a high cytotoxic effect could be observed in K562 cells treated at concentrations above 5 μM of β-lapachone.

Emodin, present in *Rumex hymenosepalus* [50], *Rheum palmatum* [46] and *Cassia siamea* [33], is a powerful anti-

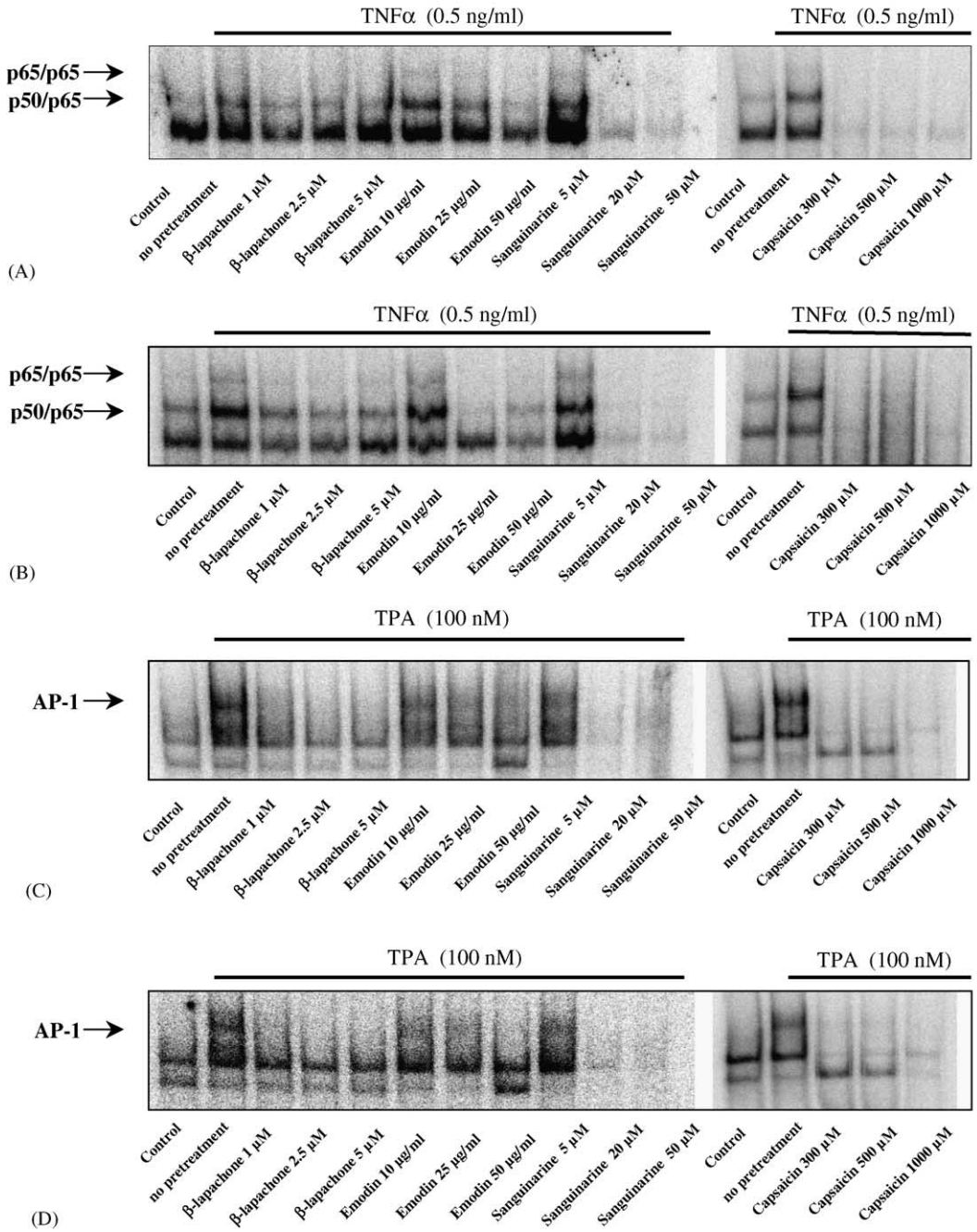


Fig. 4. Effect of chemopreventive agents on TNFα-induced NF-κB binding and TPA-induced AP-1 binding. K562 cells were treated for 1 h with 0.5 ng/ml TNFα or 100 nM TPA with or without a 2 h pretreatment with different concentrations of β-lapachone, emodin, sanguinarine or capsaicin at various concentrations. Nuclear extracts were analyzed by EMSA with (A) consensus NF-κB probe, (B) GSTP1-1 NF-κB probe, (C) consensus AP-1 probe and (D) GSTP1-1 AP-1 probe. Arrows designate specific binding activities.

inflammatory component, as it can dramatically inhibit TNFα-induced NF-κB in EC cells [51]. Its ability to block cancer progression in human cervical cancer [52] as well as in lung carcinoma [53] makes it a good candidate for novel therapies. Indeed, Fenig et al. showed that association of aloe-emodin with usual chemotherapeutic agents such as *cis*-platinol, doxorubicin and 5-fluorouracyl potentiates their ability to inhibit proliferation of Merkel cell carcinoma [54]. However, emodin displayed a weak cytotoxic action, compared to other chemopreventive agents used in this work, as percentage of cell death did not rise above 50% in K562 and U937 cell lines.

Sanguinarine is extracted from *Sanguinaria canadensis L.* and is widely used in toothpaste to reduce bacterial plaque [55]. It can inhibit TNFα-, TPA-, IL-1β- or okadaic acid-induced NF-κB activation [56] and induce apoptosis in low-level Bcl2 expressing cells, such as the K562 cell line. Moreover, it was found to be cytotoxic for human PC3

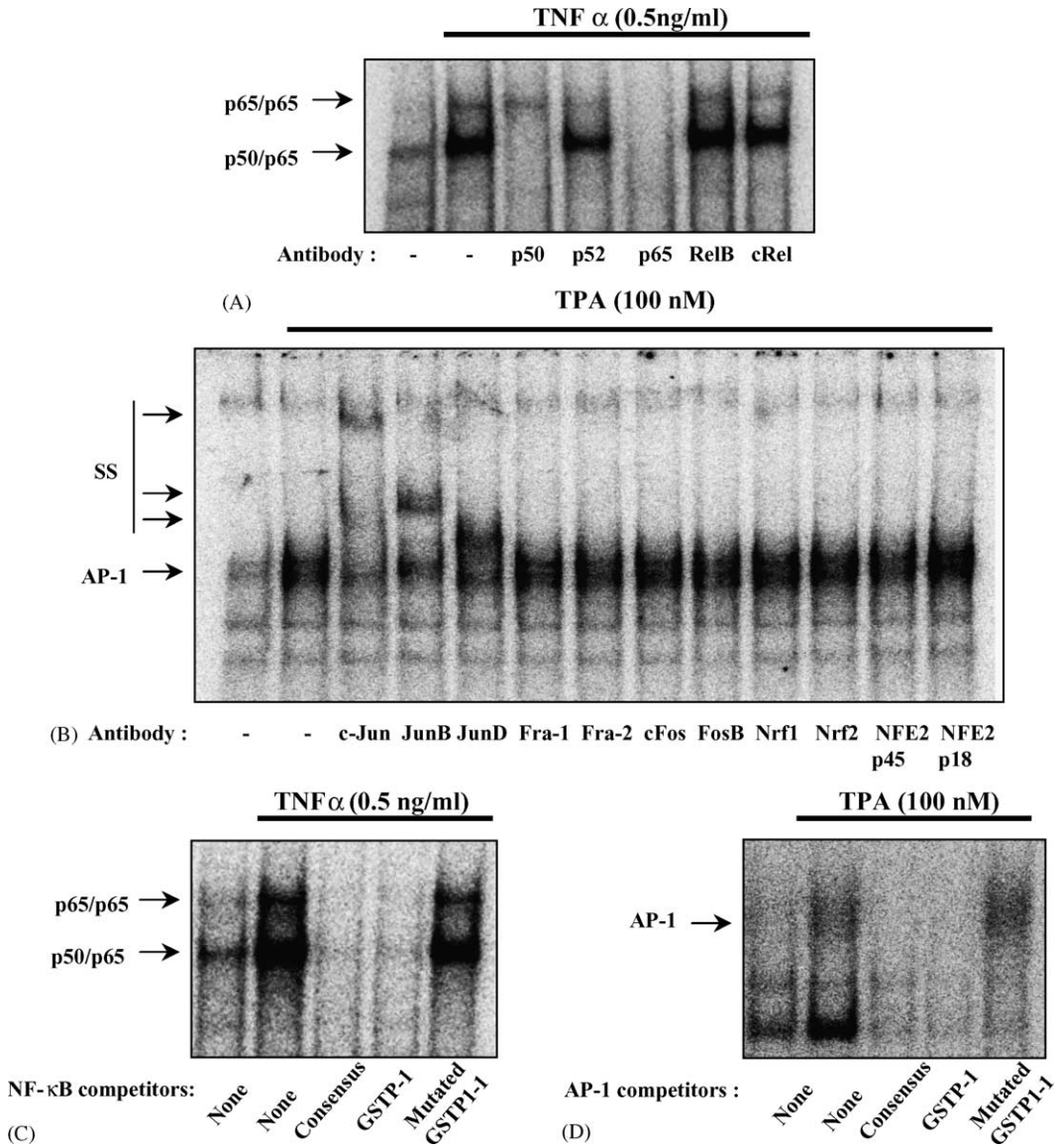


Fig. 5. Characterization of TNFα- and TPA-induced binding. U937 cells were treated for 1 h with 0.5 ng/ml TNFα (A and C) or for 2 h with 100 nM TPA (B and D). Nuclear extracts were analyzed by EMSA using radiolabeled NF-κB (A and C) and AP-1 (B and D) probes derived from the GSTP1-1 promoter. Supershift experiments were realized using antibodies directed against NF-κB (p50, p52, p65, cRel, RelB, Bcl3) (A) and AP-1 transcription factor families (c-Jun, JunB, JunD, Fra1, Fra2, c-Fos, FosB, Nrf1, Nrf2, NFE2 p45, NFE2 p18) (B) (SS designates supershifted binding activity). Competition experiments were realized using wild type GSTP1-1, mutated GSTP1-1 and consensus competitor probes for NF-κB (C) and AP-1 (D) as indicated.

prostate adenocarcinoma cells as well as for human epidermoid carcinoma (A431) cells and normal human epidermal keratinocytes (NHEKs) [57]. As for PC3 cells, sanguinarine induces high levels of death in K562 cells, thus confirming the results mentioned above for the same cell line [56]. Furthermore, our data allow us to extend this important cytotoxic effect to the U937 cell lineage.

The effect of the pepper extract capsaicin was previously discussed as this compound blocks the development of lung tumors [32] but at the same time initiates stomach tumors in mice [25]. It was shown that populations eating large quantities of pepper develop more stomach and liver cancer [58]. However, capsaicin inhibits TPA-induced NF-κB and AP-1 binding in numerous cancer cell lines [26,59–61]. Cytotoxicity studies show that capsaicin has a significant death-inducing effect in K562 and U937 cells, which is slightly higher in U937.

The extract of fennel and anise, *trans*-anethole, has strong anti-tumoral effects especially in colon cancers [35,62,63], and induces apoptosis in isolated rat hepatocytes [64]. In leukemic cell lines such as U937 and K562 we have observed a very strong mortality at concentrations above 5 mM.

Silymarin is a mixture of several components: silybin, isosilybin, silydianin and silychristin extracted from the thistle *Silybum marianum L. Gaertn.* Results obtained during this study show that U937 cells are extremely

sensitive to the presence of silymarin, as this treatment elicits complete cell death at a concentration of 750 μM. As for U937, K562 cells exhibit significant mortality for the highest concentrations used.

In order to assess the activity of the different chemopreventive agents on the TNFα- and TPA-induced transcriptional activity of the GSTP1-1 gene promoter, we performed transient transfection assays. As transfection efficiency was rather low in the case of U937 cells, we only present the results obtained for K562 cells. The data obtained during this study allow us to classify the different substances into two distinct groups. A first set of transfections was realized with a reporter gene under the control of five repeats of a TNFα-inducible NF-κB site within the promoter. Our results show that *trans*-anethole and silymarin did not have any significant effect on GSTP1-1 promoter activity after TNFα induction, whereas the four other compounds displayed an inhibitory activity. For the latter, a second set of transfections was performed with increasing concentrations of the various substances on a genomic fragment of 1175 bp of the human GSTP1-1 gene promoter driving the expression of a reporter gene. These results show that the chemopreventive agents capsaicin, β-lapachone, emodin and sanguinarine inhibit GSTP1-1 expression in a concentration-dependent manner on the genomic promoter fragment.

Transient transfection experiments were then performed on TPA-treated cells using reporter genes whose expression is driven either by an AP-1 consensus repeat or by the 1175 bp genomic fragment. These transfections displayed similar results as those obtained with TNFα induction, except in the case of silymarin where a further induction of the GSTP1-1 expression could be observed with the AP-1 consensus repeat. Interestingly, recent results show that resveratrol, a natural phytoalexin, induces the NF-κB and AP-1 pathways in K562 rather than inhibiting pro-inflammatory mechanisms (unpublished results). Data obtained by transfections of the genomic fragment and TPA treatment are analogous to those obtained with TNFα on the same construct. Furthermore, the results of the concentration-dependent transfections allow us to assess the effect of these chemopreventive compounds to the whole promoter instead of a single consensus sequence.

The concentration-dependent inhibition of GSTP1-1 expression parallels the increased cell death observed during cytotoxicity measurements. This tendency confirms the significant role of GSPT1-1 expression in leukemic as well as other cancer cell lines as previously shown [8–11]. Furthermore it can be noted that the inhibition of AP-1 and NF-κB transcriptional activity is not due to cell death, as the percentage of the latter is low as compared to the decrease in transcriptional activity. This result is confirmed by the Renilla luciferase control, whose expression did not exhibit significant changes during the different treatments performed in this work. Finally, for transfection experiments, no noticeable cell death could be observed after 4–8 h of treatment by TPA or TNFα following a 2 h pretreatment by the different chemopreventive agents.

As GSTP1-1 expression decreases after capsaicin, β-lapachone, emodin or sanguinarine treatment, we investigated their effect towards the TNFα- and TPA-induced binding of NF-κB and AP-1 transcription factors on the GSTP1-1 promoter. As expected, EMSA experiments performed on K562 nuclear extracts showed an inhibition of the NF-κB and AP-1 binding induced by TNFα and TPA, respectively. These results were obtained with either consensus as well as GSTP1-1 promoter NF-κB binding sites or with consensus or GSTP1-1 promoter AP-1 binding sites. These EMSA studies are consistent with the concentration-dependent inhibition of GSTP1-1 gene expression described in this paper. Nevertheless, it is noteworthy to mention that none of the chemopreventive agents completely annihilated reporter gene activity. It was previously shown that GSTP1-1 constitutive expression is partially under the control of two SP-1 sites located at −57 and −47 [65]. Previous results from our group revealed the involvement of GATA-1 in the regulated expression of the GSTP1 gene [24]. Those transcription factors were never described to be affected by chemopreventive agents and could contribute to the basal reporter gene expression levels.

Overall, numerous chemopreventive agents specifically regulate human GSTP1-1 transcriptional control mechanisms. Our results reveal that β-lapachone, emodin, sanguinarine and capsaicin are good candidates to be used in association with usual chemotherapeutic drugs in order to decrease the molecular mechanisms at the origin of resistance phenomenon due to GSTP1-1 activation in human leukemia.

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References

[1] Sheehan D, Meade G, Foley VM, Dowd CA. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 2001;360:1–16.

[2] Ishikawa T. The ATP-dependent glutathione S-conjugate export pump. Trends Biochem Sci 1992;17:463–8.

[3] Kodate C, Fukushi A, Narita T, Kudo H, Soma Y, Sato K. Human placental form of glutathione *S*-transferase (GST-pi) as a new immunohistochemical marker for human colonic carcinoma. Jpn J Cancer Res 1986;77:226–9.

[4] Peters WH, Nagengast FM, Wobbes T. Glutathione *S*-transferases in normal and cancerous human colon tissue. Carcinogenesis 1989; 10:2371–4.
[5] Moscow JA, Townsend AJ, Cowan KH. Elevation of pi class glutathione *S*-transferase activity in human breast cancer cells by transfection of the GST pi gene and its effect on sensitivity to toxins. Mol Pharmacol 1989;36:22–8.
[6] Carmichael J, Forrester LM, Lewis AD, Hayes JD, Hayes PC, Wolf CR. Glutathione *S*-transferase isoenzymes and glutathione peroxidase activity in normal and tumour samples from human lung. Carcinogenesis 1988;9:1617–21.
[7] Toffoli G, Viel A, Tumiotto L, Giannini F, Volpe R, Quaia M, et al. Expression of glutathione-*S*-transferase-pi in human tumours. Eur J Cancer 1992;28A:1441–6.
[8] Whelan RD, Hosking LK, Townsend AJ, Cowan KH, Hill BT. Differential increases in glutathione *S*-transferase activities in a range of multidrug-resistant human tumor cell lines. Cancer Commun 1989;1:359–65.
[9] Hao XY, Bergh J, Brodin O, Hellman U, Mannervik B. Acquired resistance to cisplatin and doxorubicin in a small cell lung cancer cell line is correlated to elevated expression of glutathione-linked detoxification enzymes. Carcinogenesis 1994;15:1167–73.
[10] Cowan KH, Batist G, Tulpule A, Sinha BK, Myers CE. Similar biochemical changes associated with multidrug resistance in human breast cancer cells and carcinogen-induced resistance to xenobiotics in rats. Proc Natl Acad Sci U S A 1986;83:9328–32.
[11] Batist G, Tulpule A, Sinha BK, Katki AG, Myers CE, Cowan KH. Overexpression of a novel anionic glutathione transferase in multidrug-resistant human breast cancer cells. J Biol Chem 1986;261: 15544–9.
[12] Cullen KJ, Newkirk KA, Schumaker LM, Aldosari N, Rone JD, Haddad BR. Glutathione *S*-transferase pi amplification is associated with cisplatin resistance in head and neck squamous cell carcinoma cell lines and primary tumors. Cancer Res 2003;63:8097–102.
[13] Su F, Hu X, Jia W, Gong C, Song E, Hamar P. Glutathion *S*-transferase pi indicates chemotherapy resistance in breast cancer. J Surg Res 2003;113:102–8.
[14] Mohammadzadeh GS, Nasseri Moghadam S, Rasaee MJ, Zaree AB, Mahmoodzadeh H, Allameh A. Measurement of glutathione *S*-transferase and its class-pi in plasma and tissue biopsies obtained after laparoscopy and endoscopy from subjects with esophagus and gastric cancer. Clin Biochem 2003;36:283–8.
[15] Nakajima T, Takayama T, Miyanishi K, Nobuoka A, Hayashi T, Abe T, et al. Reversal of multiple drug resistance in cholangiocarcinoma by the glutathione *S*-transferase-pi-specific inhibitor O1-hexadecyl-gamma-glutamyl-S-benzylcysteinyl-D-phenylglycine ethylester. J Pharmacol Exp Ther 2003;306:861–9.
[16] Morrow CS, Smitherman PK, Townsend AJ. Combined expression of multidrug resistance protein (MRP) and glutathione *S*-transferase P1-1 (GSTP1-1) in MCF7 cells and high level resistance to the cytotoxicities of ethacrynic acid but not oxazaphosphorines or cisplatin. Biochem Pharmacol 1998;56:1013–21.
[17] Miyazaki M, Kohno K, Saburi Y, Matsuo K, Ono M, Kuwano M, et al. Drug resistance to *cis*-diamminedichloroplatinum (II) in Chinese hamster ovary cell lines transfected with glutathione *S*-transferase pi gene. Biochem Biophys Res Commun 1990;166: 1358–64.
[18] Puchalski RB, Fahl WE. Expression of recombinant glutathione *S*-transferase pi, Ya, or Yb1 confers resistance to alkylating agents. Proc Natl Acad Sci U S A 1990;87:2443–7.
[19] Masanek U, Stammler G, Volm M. Messenger RNA expression of resistance proteins and related factors in human ovarian carcinoma cell lines resistant to doxorubicin, taxol and cisplatin. Anticancer Drugs 1997;8:189–98.
[20] Tidefelt U, Elmhorn-Rosenborg A, Paul C, Hao XY, Mannervik B, Eriksson LC. Expression of glutathione transferase pi as a predictor for treatment results at different stages of acute nonlymphoblastic leukemia. Cancer Res 1992;52:3281–5.
[21] Xia C, Hu J, Ketterer B, Taylor JB. The organization of the human GSTP1-1 gene promoter and its response to retinoic acid and cellular redox status. Biochem J 1996;313(Pt 1):155–61.
[22] Borde-Chiche P, Diederich M, Morceau F, Wellman M, Dicato M. Phorbol ester responsiveness of the glutathione *S*-transferase P1 gene promoter involves an inducible c-jun binding in human K562 leukemia cells. Leuk Res 2001;25:241–7.
[23] Morceau F, Duvoix A, Delhalle S, Schnekenburger M, Dicato M, Diederich M. Regulation of glutathione *S*-tranbsferase P1-1 gene expression by NF-kappaB in tumor necrosis factor alpha-treated K562 leukemia cells. Biochem Pharmacol 2004.
[24] Schnekenburger M, Morceau F, Duvoix A, Delhalle S, Trentesaux C, Dicato M, et al. Expression of glutathione *S*-transferase P1-1 in differentiating K562: role of GATA-1. Biochem Biophys Res Commun 2003;311:815–21.
[25] Agrawal RC, Wiessler M, Hecker E, Bhide SV. Tumour-promoting effect of chilli extract in BALB/c mice. Int J Cancer 1986;38:689–95.
[26] Surh YJ, Han SS, Keum YS, Seo HJ, Lee SS. Inhibitory effects of curcumin and capsaicin on phorbol ester-induced activation of eukaryotic transcription factors NF-kappaB and AP-1. Biofactors 2000;12:107–12.
[27] Kelloff GJ, Boone CW, Steele VE, Fay JR, Lubet RA, Crowell JA, et al. Mechanistic considerations in chemopreventive drug development. J Cell Biochem Suppl 1994;20:1–24.
[28] Chainy GB, Manna SK, Chaturvedi MM, Aggarwal BB. Anethole blocks both early and late cellular responses transduced by tumor necrosis factor: effect on NF-kappaB, AP-1, JNK, MAPKK and apoptosis. Oncogene 2000;19:2943–50.
[29] Katiyar SK. Treatment of silymarin, a plant flavonoid, prevents ultraviolet light-induced immune suppression and oxidative stress in mouse skin. Int J Oncol 2002;21:1213–22.
[30] Wellington K, Jarvis B. Silymarin: a review of its clinical properties in the management of hepatic disorders. Bio Drugs 2001;15:465–89.
[31] Manna SK, Gad YP, Mukhopadhyay A, Aggarwal BB. Suppression of tumor necrosis factor-activated nuclear transcription factor-kappaB, activator protein-1, c-Jun N-terminal kinase, and apoptosis by beta-lapachone. Biochem Pharmacol 1999;57:763–74.
[32] Jang JJ, Kim SH, Yun TK. Inhibitory effect of capsaicin on mouse lung tumor development. In Vivo 1989;3:49–53.
[33] Koyama J, Morita I, Tagahara K, Nobukuni Y, Mukainaka T, Kuchide M, et al. Chemopreventive effects of emodin and cassiamin B in mouse skin carcinogenesis. Cancer Lett 2002;182:135–9.
[34] Lahiri-Chatterjee M, Katiyar SK, Mohan RR, Agarwal R. A flavonoid antioxidant, silymarin, affords exceptionally high protection against tumor promotion in the SENCAR mouse skin tumorigenesis model. Cancer Res 1999;59:622–32.
[35] Reddy BS. Chemoprevention of colon cancer by dietary administration of naturally-occurring and related synthetic agents. Adv Exp Med Biol 1997;400B:931–6.
[36] Stanberry LR. Capsaicin interferes with the centrifugal spread of virus in primary and recurrent genital herpes simplex virus infections. J Infect Dis 1990;162:29–34.
[37] De M, De AK, Sen P, Banerjee AB. Antimicrobial properties of star anise (Illicium verum Hook f). Phytother Res 2002;16:94–5.
[38] Cullinan MP, Powell RN, Faddy MJ, Seymour GJ. Efficacy of a dentifrice and oral rinse containing sanguinaria extract in conjunction with initial periodontal therapy. Aust Dent J 1997;42:47–51.
[39] Godowski KC. Antimicrobial action of sanguinarine. J Clin Dent 1989;1:96–101.
[40] Kubo I, Fujita K, Lee SH. Antifungal mechanism of polygodial. J Agric Food Chem 2001;49:1607–11.
[41] Weerasinghe P, Hallock S, Liepins A. Bax, Bcl-2, and NF-kappaB expression in sanguinarine induced bimodal cell death. Exp Mol Pathol 2001;71:89–98.

[42] Weerasinghe P, Hallock S, Tang SC, Liepins A. Sanguinarine induces bimodal cell death in K562 but not in high Bcl-2-expressing JM1 cells. Pathol Res Pract 2001;197:717–26.
[43] Weerasinghe P, Hallock S, Tang SC, Liepins A. Role of Bcl-2 family proteins and caspase-3 in sanguinarine-induced bimodal cell death. Cell Biol Toxicol 2001;17:371–81.
[44] Pink JJ, Wuerzberger-Davis S, Tagliarino C, Planchon SM, Yang X, Froelich CJ, et al. Activation of a cysteine protease in MCF-7 and T47D breast cancer cells during beta-lapachone-mediated apoptosis. Exp Cell Res 2000;255:144–55.
[45] Li CJ, Wang C, Pardee AB. Induction of apoptosis by beta-lapachone in human prostate cancer cells. Cancer Res 1995;55:3712–5.
[46] Chen YC, Shen SC, Lee WR, Hsu FL, Lin HY, Ko CH, et al. Emodin induces apoptosis in human promyeloleukemic HL-60 cells accompanied by activation of caspase 3 cascade but independent of reactive oxygen species production. Biochem Pharmacol 2002;64:1713–24.
[47] Lee YS, Nam DH, Kim JA. Induction of apoptosis by capsaicin in A172 human glioblastoma cells. Cancer Lett 2000;161:121–30.
[48] Duvoix A, Morceau F, Delhalle S, Schmitz M, Schnekenburger M, Galteau MM, et al. Induction of apoptosis by curcumin: mediation by glutathione *S*-transferase P1-1 inhibition. Biochem Pharmacol 2003;66:1475–83.
[49] Müller MM, Schreiber E, Schaffner W, Matthias P. Rapid test for in vivo stability and DNA binding of mutated octamer binding proteins with 'mini-extracts' prepared from transfected cells. Nucleic Acids Res 1989;17:6420.
[50] Buchalter L. Isolation and identification of emodin (1,3,8-tri-hydroxy-6-methyl anthraquinone) from Rumex hymenosepalus, family polygonaceae. J Pharm Sci 1969;58:904.
[51] Kumar A, Dhawan S, Aggarwal BB. Emodin (3-methyl-1,6,8-trihydroxyanthraquinone) inhibits TNF-induced NF-kappaB activation, IkappaB degradation, and expression of cell surface adhesion proteins in human vascular endothelial cells. Oncogene 1998;17:913–8.
[52] Srinivas G, Anto RJ, Srinivas P, Vidhyalakshmi S, Senan VP, Karunagaran D. Emodin induces apoptosis of human cervical cancer cells through poly(ADP-ribose) polymerase cleavage and activation of caspase-9. Eur J Pharmacol 2003;473:117–25.
[53] Lee HZ. Effects and mechanisms of emodin on cell death in human lung squamous cell carcinoma. Br J Pharmacol 2001;134:11–20.
[54] Fenig E, Nordenberg J, Beery E, Sulkes J, Wasserman L. Combined effect of aloe-emodin and chemotherapeutic agents on the proliferation of an adherent variant cell line of Merkel cell carcinoma. Oncol Rep 2004;11:213–7.
[55] Wolinsky LE, Cuomo J, Quesada K, Bato T, Camargo PM. A comparative pilot study of the effects of a dentifrice containing green tea bioflavonoids, sanguinarine or triclosan on oral bacterial biofilm formation. J Clin Dent 2000;11:53–9.
[56] Chaturvedi MM, Kumar A, Darnay BG, Chainy GB, Agarwal S, Aggarwal BB. Sanguinarine (pseudochelerythrine) is a potent inhibitor of NF-kappaB activation, IkappaBalpha phosphorylation, and degradation. J Biol Chem 1997;272:30129–34.
[57] Ahmad N, Gupta S, Husain MM, Heiskanen KM, Mukhtar H. Differential antiproliferative and apoptotic response of sanguinarine for cancer cells versus normal cells. Clin Cancer Res 2000;6:1524–8.
[58] Archer VE, Jones DW. Capsaicin pepper, cancer and ethnicity. Med Hypotheses 2002;59:450–7.
[59] Han SS, Keum YS, Chun KS, Surh YJ. Suppression of phorbol ester-induced NF-kappaB activation by capsaicin in cultured human promyelocytic leukemia cells. Arch Pharm Res 2002;25:475–9.
[60] Han SS, Keum YS, Seo HJ, Chun KS, Lee SS, Surh YJ. Capsaicin suppresses phorbol ester-induced activation of NF-kappaB/Rel and AP-1 transcription factors in mouse epidermis. Cancer Lett 2001;164:119–26.
[61] Singh S, Natarajan K, Aggarwal BB. Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a potent inhibitor of nuclear transcription factor-kappa B activation by diverse agents. J Immunol 1996;157:4412–20.
[62] Reddy BS. Chemoprevention of colon cancer by minor dietary constituents and their synthetic analogues. Prev Med 1996;25:48–50.
[63] Reddy BS, Rao CV, Rivenson A, Kelloff G. Chemoprevention of colon carcinogenesis by organosulfur compounds. Cancer Res 1993;53:3493–8.
[64] Nakagawa Y, Suzuki T. Cytotoxic and xenoestrogenic effects via biotransformation of *trans*-anethole on isolated rat hepatocytes and cultured MCF-7 human breast cancer cells. Biochem Pharmacol 2003;66:63–73.
[65] Moffat GJ, McLaren AW, Wolf CR. Sp1-mediated transcriptional activation of the human Pi class glutathione *S*-transferase promoter. J Biol Chem 1996;271:1054–60.