MOLECULAR BASIS FOR HEPATIC DETOXIFYING ENZYME INDUCTION BY 2-(ALLYLTHIO)PYRAZINE IN RATS IN COMPARISON WITH OLTIPRAZ: EFFECTS ON PROOXIDANT PRODUCTION AND DNA DEGRADATION

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ABSTRACT:

The expression of hepatic microsomal epoxide hydrolase (mEH) and glutathione S-transferases (GSTs) by 2-(allylthio)pyrazine (2-AP), an experimental chemopreventive agent, was investigated in rats. Northern blot analysis revealed that 2-AP caused increases in mEH, rGSTA2/3/5, and rGSTM1/2 mRNA levels. mEH and rGSTA2 proteins were also induced. Molecular basis of the enzyme induction by 2-AP was studied in comparison with oltipraz (Olt). Rats exposed to buthionine sulfoximine, a GST-depleting agent, before treatment with either 2-AP or Olt exhibited greater increases in the mRNA levels than the individual treatment. Conversely, increases of the mRNAs were prevented by cysteine treatment, indicating that metabolic intermediates or reactive oxides produced from the agents could be reduced by cysteine. Gel shift analysis revealed that nuclear factor-κB, which is associated with the altered cellular redox state, was not activated by the agents. Effects of these agents on the breakage of pX-174 DNA were compared in vitro. 2-AP effectively reduced the conversion of supercoiled DNA to the open circular form induced by benzenetriol and prevented benzenetriol- and iron-catalyzed degradation of DNA, whereas Olt failed to prevent strand breakage of DNA. These results provided evidence that: 1) 2-AP was effective in elevating the hepatic mEH and GST gene expression in rats, which might be mediated with the production of reactive oxygen species; 2) nuclear factor-κB activation was not involved in the induction of the detoxifying enzymes by either 2-AP or Olt in spite of their production of reactive oxides in vivo; and 3) the antioxidant effect of 2-AP in vitro differed from that of Olt.

2-(Allylthio)pyrazine (2-AP)1 was designed to develop a chemoprotective agent that potentially functions through selective modulation of cytochrome P-450 and other drug-metabolizing enzyme expression (Kim et al., 1997a). Previous studies have shown that 2-AP suppressed the constitutive and inducible cytochrome P-450 2E1 expression and was effective in blocking toxicant-induced liver injury (Kim et al., 1997a). A recent study demonstrated that 2-AP was active as a chemopreventive agent in reducing vinyl carbamate-induced tumorgenesis (Surh et al., 1998).

The anticarcinogenic effect of oltipraz (Olt) and dithiolethiones has been attributable to their induction of phase II detoxifying enzymes, e.g., microsomal epoxide hydrolase (mEH) and glutathione S-transferase (GST) as well as the inhibition of certain cytochrome P-450s (e.g., P-450 1A2 and 3A4; Davidson et al., 1990; Morel et al., 1993; or Langouet et al., 1995). The production of oxygen radicals by 1,2-dithiole-3-thiones has been proposed to play a role in the induction of the phase II enzymes (Hayes and Pulford, 1995). Olt and dithiolethiones mediate the conversion of molecular oxygen to reactive oxygen radicals in the presence of thiols, as monitored by the cleavage of DNA in vitro (Kim and Gates, 1997). Olt treatment elevates GSH levels in the liver of animals. Based on the observation that the biological thiols glutathione and cysteine were competent to elicit the cleavage of DNA by Olt, the role of sulfhydryl in the bioactivation of Olt and the subsequent conversion of oxygen to oxygen radicals has been raised (Kim and Gates, 1997). Nonetheless, the role of GSH in the bioactivation of Olt has not been demonstrated in vivo.

The present study was designed to establish whether 2-AP was effective in elevating mEH and GST mRNA and protein levels in the liver and to study the mechanistic basis of the detoxifying enzyme induction in vivo. We were interested in whether Olt and 2-AP were virtually bioactivated in animals and produced the reactive oxides in the presence of GSH in vivo. The role of in vivo bioactivation of Olt and 2-AP in the induction of detoxifying enzymes was assessed under the hypothesis that production of activated oxides may contribute to the antioxidant-responsive element (ARE)-mediated induction of anticarcinogenic phase II enzymes.

Nuclear factor-κB (NF-κB) activation by oxidative stress has been correlated with the cellular oxidation state, which may translate a redox sensor into a chemical signal that leads to transcriptional activation of the appropriate genes (Schreck et al., 1991; Primiano et

1 Abbreviations used are: 2-AP, 2-(allylthio)pyrazine; ARE, antioxidant-responsive element; BSO, buthionine sulfoximine; BT, benzenetriol; GST, glutathione S-transferase; mEH, microsomal epoxide hydrolase; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; Olt, oltipraz; SDS, sodium dodecylsulfate.

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Chemical structures of 2-AP and Olt.  

Fig. 1. Chemical structures of 2-AP and Olt.
experiments. Northern blot analysis for mEH and GST mRNA levels after 2-AP treatment.

The mRNA levels were determined in vehicle-treated rats (UN) or in rats at 6, 12, 24, 48, and 72 h after a single dose of 2-AP treatment (100 mg/kg po). The amount of RNA loaded in each lane was assessed by rehybridization of the stripped blots. Each point represents the mean ± S.D. with at least three independent experiments.

Northern blot analysis. Treatment of rats with 2-AP resulted in 2- to 12-fold increases in the mRNA levels for mEH, rGSTA2, rGSTA3, and rGSTA5 in a time-dependent manner at 6, 12, and 24 h after a single dose of 100 mg/kg of 2-AP (Fig. 2). Marked increases in the mRNAs were noted at 12 or 24 h. The mRNA level for rGSTM1 was 7-fold elevated by 2-AP at 24 h, relative to control, whereas rGSTM2 mRNA was increased less (i.e., 5-fold). The mRNA levels were reduced to 25–50% of the maximal increases at 48 h, followed by returning to that in untreated animals at 72 h.

We next determined the mRNA levels of mEH and rGSTA2 as a function of dose of 2-AP at 24 h because the magnitude of increases of the mRNAs was the greatest at the time point. Treatment of rats with 2-AP at single doses of 10, 30, 50, 100, and 200 mg/kg resulted in 1.5-, 3-, 6.5-, 12-, and 12-fold increases in mEH mRNA levels, respectively (Fig. 3). Change in rGSTA2 mRNA was comparable to that in mEH with an ~11-fold maximal increase being observed. The ED₅₀ value for the increases of mEH and rGSTA2 mRNAs was ~40 mg/kg.

mEH and GST Protein Expression. We tested the GSH conjugating activity in the liver cytosol and carried out immunoblot analyses to determine whether the increases in mRNAs were in parallel with those in proteins. A dose-response study showed that the cytosolic GSH conjugating activity toward 1-chloro-2,4-dinitrobenzene was significantly elevated after treatment of rats with 2-AP for 3 days at the dose of 10, 60, 100, and 200 mg/kg per day, resulting in 1.3-, 1.9-, 2.1-, and 3-fold increases relative to vehicle-treated animals (Table 1).

Immunoblot analysis revealed that both mEH and rGSTA1/2 proteins were induced in a dose-dependent manner (Fig. 4). Rats treated with 2-AP exhibited increases in mEH protein of 1.3 ± 0.1-, 1.5 ± 0.02-, 2.4 ± 0.2-, and 3.5 ± 0.2-fold at the above-mentioned doses, respectively, as compared with control (mean ± S.D., n = 3). rGSTA1/2 protein was also affected to similar extents, resulting in 1.2 ± 0.1-, 1.8 ± 0.1-, 2.3 ± 0.1-, and 2.7 ± 0.2-fold increases, respectively (Fig. 4).

Effects of BSO and Cysteine. A number of studies have shown that Olt induces several GST subunits with transcriptional activation (Davidson et al., 1990; Primiano et al., 1997). This is presumably mediated by ARE in the genes. In view of the potential role of reactive oxygens in the induction of mEH and rGSTA2, we were interested in establishing the molecular basis for the enzyme induction by 2-AP in comparison with that by Olt.

Studies have shown that administration of BSO, a GSH-depleting agent, resulted in a substantial decrease in the hepatic GSH level at 6 h or later times (Oguro et al., 1997). The mRNA levels of mEH and rGSTA2 were quantified in rats after treatment with Olt in combination with BSO. The mEH mRNA level was ~18-fold elevated 12 h after Olt treatment in rats that were pretreated with a single dose of BSO (4.5 mmol/kg i.p.) 4 h before, as compared with that in untreated animals (Fig. 5A). BSO or Olt alone resulted in a 12- to 14-fold increase in the mEH mRNA level (Table 2). Thus, the relative mRNA level was significantly greater than that after the individual treatment. BSO alone caused a 13-fold increase in the rGSTA2 mRNA level (Table 2). Additive increase of the mRNA was not noted in rats treated with both Olt and BSO.

The mEH and rGSTA2 mRNA levels were also determined in rats after treatment with 2-AP in conjunction with BSO (Fig. 5A). The mEH and rGSTA2 mRNA levels were increased to greater extents in rats exposed to both 2-AP and BSO than those treated with either 2-AP or BSO alone (Table 2), providing evidence that 2-AP further elevated the mRNA levels in the GSH-depleting state.

Prior studies showed that cysteine prevents toxicant-induced liver injury partly by supplementing the intracellular GSH levels (De Ferreyra et al., 1979). In the subsequent experiment, we determined the effect of cysteine on the increases in the mEH and rGSTA2 mRNA levels by Olt or 2-AP. Concomitant cysteine treatment (1 g/kg po) completely inhibited increases of hepatic mEH and rGSTA2 mRNAs by Olt, although cysteine alone did not alter the constitutive mRNA expression (Fig. 5B, Table 3). 2-AP-inducible mEH and
Northern blot analysis was performed to determine the mRNA levels in rats at 24 h after treatment with 2-AP (10–200 mg/kg). Increases in mRNA levels were plotted as a function of dose of 2-AP, relative to vehicle-treated rats (UN). Each point represents the mean ± S.D. with at least three independent experiments.

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GST Activity</th>
<th>Percent Increase</th>
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<tr>
<td>Control</td>
<td>0.94 ± 0.09</td>
<td>0</td>
</tr>
<tr>
<td>2-AP 10 mg/kg</td>
<td>1.22 ± 0.03</td>
<td>30</td>
</tr>
<tr>
<td>2-AP 60 mg/kg</td>
<td>1.74 ± 0.04**</td>
<td>85</td>
</tr>
<tr>
<td>2-AP 100 mg/kg</td>
<td>1.92 ± 0.06**</td>
<td>105</td>
</tr>
<tr>
<td>2-AP 200 mg/kg</td>
<td>2.81 ± 0.06**</td>
<td>200</td>
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</table>

The GSH conjugating activity toward 1-chloro-2, 4-dinitrobenzene was monitored in the liver cytosol after 2-AP treatment (10–200 mg/kg per day for 3 days). The values represent the mean ± S.D. with three separate experiments. Significant as compared to control, **p < .01.

**Fig. 3. Dose-dependent increases in mEH and rGSTA2 mRNA levels by 2-AP.**

**Fig. 4. Western blot analyses for mEH and rGSTA1/2.**

Immunoblot analyses were carried out with hepatic microsomal and cytosolic fractions produced from vehicle (UN)- or 2-AP- treated rats (10–200 mg/kg per day for 3 days). Four micrograms of microsomal or cytosolic proteins was used for mEH and rGSTA1/2 immunoblottings, respectively.

rGSTA2 mRNA levels were also suppressed by cysteine treatment (Fig. 5B, Table 3). Cysteine might scavenge activated oxygens derived from the agents by supplementing the intracellular GSH and/or reduce reactive metabolites through conjugative metabolism.

**Effect on NF-κB Activation.** The intracellular redox potential has been implicated in the regulation of certain gene expression in association with the activation of NF-κB. Olt activated the NF-κB at 30 min to 1 h in primary-cultured hepatocytes, which has been claimed to be responsible for the induction of NAD(P)H:quinone oxidoreductase and for manganese-dependent superoxide dismutase (Yao and O’Dwyer, 1995; Antras-Ferry et al., 1997). The effects of 2-AP and Olt on the activation of NF-κB were determined in the rat liver by gel shift assays using the NF-κB consensus sequence. The level of NF-κB failed to be activated at 1 h after administration of 2-AP at the dose of 300 mg/kg (Fig. 6). Similar results were obtained with Olt (data not shown). Multiple analyses of three separate animal experiments showed that the agents at the doses of 100 through 300 mg/kg did not activate NF-κB in the liver even at various time points (e.g., 30 min to 3 h). The agents were incapable of activating NF-κB in rats pretreated with BSO (4.5 mmol/kg i.p.). Whereas treatment of rats with LPS at the dose of 1 μg/kg resulted in an increase in the nuclear p65/p50 NF-κB complex, either Olt or 2-AP failed to enhance or reduce the LPS-inducible NF-κB activation.

**Effects on φx-174 DNA Topology.** The effects of 2-AP on the topology of φx-174 DNA was examined in the presence of β-mercaptoethanol. Although Olt completely converted supercoiled φx-174 DNA to the open circular form at the concentration of 30 μM in the presence of β-mercaptoethanol, 2-AP failed to alter φx-174 DNA topology at the concentration of 1 mM (Fig. 7A). Thus, in contrast to Olt, 2-AP was incapable of converting oxygen to reactive oxygens in the presence of thiol.

Supercoiled φx-174 DNA could be converted to the open circular form in the presence of 5 μM BT, a known radical-producing agent. Studies were extended to assess the ability of Olt or 2-AP in blocking DNA damage. Whereas 2-AP efficiently prevented BT-induced conversion of supercoiled DNA to the open circular form, Olt failed to inhibit the change of DNA topology (Fig. 7B). Hence, 2-AP was effective in protecting the DNA through scavenging oxygen-free radicals in vitro, although Olt was incapable of scavenging reactive oxygens produced from BT-induced autooxidation at the concentrations of 100 μM through 1 mM.

The effects of Olt and 2-AP on degradation of φx-174 DNA catalyzed by BT and ferrous sulfate were also monitored (Fig. 7C). Although the supercoiled φx-174 DNA was intact in the presence of ferrous sulfate at the concentrations of 20 through 50 μM, addition of 5 μM BT in combination with ferrous sulfate caused complete degradation of the supercoiled φx-174 DNA. 2-AP protected the breakdown of DNA at the concentrations of 300 μM or greater, whereas Olt failed to prevent the DNA degradation. These results showed that only

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2 Kim and Gates (1997) Olt was dissolved in acetonitrile, as described previously. Comparable result was observed by Olt dissolved in an aqueous solution.
2-AP was active in preventing DNA injury caused by autoxidation of BT in the presence of ferrous iron.

Discussion

2-AP exerts the hepatoprotective and chemopreventive effects through selective modulation of cytochrome P-450 2E1 and other detoxifying enzyme expression (Kim et al., 1997a). The present study demonstrated that 2-AP was efficacious in inducing the hepatic mEH and GSTs with marked increases in the mRNAs, as supported by the elevation of metabolic activity, immunoblot, and Northern blot analyses. 2-AP was efficacious in inducing mEH and major GSTs including rGSTA1/2, rGSTA3/5, rGSTM1, and rGSTM2, which was comparable to the response observed after Olt treatment (Nam et al., 1997; Kim et al., 1997b). An additional dose-response study showed that 2-AP was active in inducing the detoxifying enzymes even at the dose of 10 mg/kg.

BSO inhibits the heavy subunit of γ-glutamylcysteine synthase, which possesses all of the catalytic activity for GSH feedback (Mulcahy et al., 1995). Stilbene oxide induced heme oxygenase-1 mRNA with concomitant decreases in the hepatic GSH level and BSO augmented the increase in heme oxygenase-1 mRNA with GSH depletion (Oguro et al., 1997). Olt has been also shown to induce heme oxygenase-1 in rat tissues (Primiano et al., 1996). The present study revealed that both Olt and 2-AP further elevated the hepatic

Northern blot analyses were performed to determine the mEH and rGSTA2 mRNA levels in total RNA fractions isolated at 12 h after treatment with either Olt or 2-AP (100 mg/kg p.o.). BSO was intraperitoneally injected at the dose of 4.5 mmol/kg 4 h before Olt or 2-AP treatment (−, control; +, BSO). The relative mRNA levels were obtained by scanning densitometry of the Northern blots, followed by normalization with a labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe.

<table>
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<th>Table 2</th>
<th>Effect of BSO pretreatment on Olt- or 2-AP-inducible mRNA levels</th>
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<tbody>
<tr>
<td>BSO</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>mEH</td>
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</table>
|         | 1       | 12.0 ± 2.8** | 14.0 ± 1.6** | 17.7 ± 1.6**
|         | rGSTA2  |     |     |
|         | 1       | 13.0 ± 1.1** | 9.1 ± 1.6**  | 13.1 ± 1.3**

The relative mRNA levels after treatment with Olt or 2-AP in combination with cysteine

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<th>Table 3</th>
<th>The relative mRNA levels after treatment with Olt or 2-AP in combination with cysteine</th>
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<tr>
<td>Cysteine</td>
<td>Control</td>
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<tr>
<td></td>
<td>mEH</td>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>rGSTA2</td>
</tr>
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Northern blot analyses showed the hepatic mRNA levels in rats pretreated with BSO (4.5 mmol/kg) 4 h before administration of Olt or 2-AP (100 mg/kg po). The RNA was isolated at 12 h after Olt or 2-AP treatment. UN, vehicle-treated rats. B, inhibition of inducible mEH and rGSTA2 mRNA expression by cysteine. RNA was isolated at 12 h after a single dose of Olt or 2-AP (100 mg/kg po) with or without concomitant cysteine administration (1 g/kg po). Amount of RNA loaded in each lane was confirmed by rehybridization of the stripped membrane with a labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe.
mEH and rGSTA2 mRNA levels in the GSH-depleting animals. The hypothesis that production of activated oxygens may be responsible for the enzyme induction by the agents was supported in part by the observation. Conversely, the mRNA levels inducible by the agents were prevented by cysteine administration. Reversal of increases in mEH and GST mRNA levels by concomitant treatment with cysteine confirmed the conclusion that transcriptional induction of anticarcinogenic enzymes by Olt or 2-AP might be mediated with the production of oxygen-free radicals. Cysteine is a diffusible thiol for thiol-protein mixed disulfides regulation (Simplicio et al., 1998). The level of cysteine is the major factor that regulates the metabolism of intracellular glutathione (Stipanuk et al., 1992). Cysteine, but not GSH or N-acetylcysteine, has been shown to rapidly restore GSH and to decrease thiol-protein mixed disulfides to basal levels (Stipanuk et al., 1992; Simplicio et al., 1998).

Metabolic conversion of Olt and/or 2-AP is likely to be coupled with the steady-state consumption of reduced GSH content in the cells. However, 2-AP increases the GSH content in the liver in a dose-related manner (Kim et al., 1997a). Increases in the GSH level were also observed in animals treated with Olt (Ansher et al., 1983; Bolton et al., 1993). mEH and rGSTA2 mRNAs were elevated to greater extents in the GSH-depleting animals. The level of cysteine is the major factor that regulates the metabolism of intracellular glutathione (Stipanuk et al., 1992). Cysteine, but not GSH or N-acetylcysteine, has been shown to rapidly restore GSH and to decrease thiol-protein mixed disulfides to basal levels (Stipanuk et al., 1992; Simplicio et al., 1998).

Gel shift analysis of hepatic nuclear extracts using the consensus sequence of NF-κB.

Nuclear extracts were isolated from rats 1 h after 2-AP treatment (300 mg/kg). Rats were intraperitonially injected with BSO (4.5 mmol/kg) 4 h before a single dose of 2-AP. Nuclear extracts were isolated 1 h after an LPS injection (1 μg/kg i.v.) to rats pretreated with 2-AP (300 mg/kg po) 2 h before. All lanes contained 5 μg of nuclear extract. Data were confirmed by at least three separate experiments.

Effects of Olt or 2-AP on the topology of dX-174 DNA.

A. Reaction mixtures contained 50 mM sodium phosphate (pH 7.0), 0.2 μg of supercoiled dX-174 DNA, β-mercaptoethanol and Olt or 2-AP. β-Mercaptoethanol was added at 5 equivalents based on the moles of either Olt or 2-AP. Incubations were carried out at 37°C for 1 h. Lane 1, no addition; lane 2, 5 mM β-mercaptoethanol alone; lane 3, 1 mM 2-AP + 5 mM β-mercaptoethanol; lane 4, 1 mM 2-AP alone; lane 5, 0.03 mM Olt + 0.15 mM β-mercaptoethanol. B. Protective effect of 2-AP against the cleavage of dX-174 DNA induced by autoxidation of BT. Reaction mixtures contained 50 mM sodium phosphate buffer (pH 7.0), 0.2 μg of supercoiled dX-174 DNA, and 5 μM BT in the absence or presence of 0.1 to 1 mM Olt or 2-AP. Incubations were carried out at 37°C for 1 h. Lane 1, no addition; lane 2, 5 μM BT alone; lane 3, 0.1 mM Olt + 5 μM BT; lane 4, 1 mM Olt + 5 μM BT; lane 5, 1 mM Olt alone; lane 6, 0.1 mM 2-AP + 5 μM BT; lane 7, 1 mM 2-AP + 5 μM BT; lane 8, 1 mM 2-AP alone. C. Effects on the degradation of dX-174 DNA induced by BT and ferrous iron. Incubation mixtures contained 50 mM sodium phosphate buffer (pH 7.0), 0.2 μg of supercoiled dX-174 DNA, 5 μM BT, and 20 μM ferrous sulfate in the presence or absence of Olt or 2-AP at the concentration of 1 mM. Lane 1, no addition; lane 2, 5 μM BT alone; lane 3, 20 μM Fe²⁺ + 5 μM BT; lane 4, 1 mM Olt + 20 μM Fe²⁺ + 5 μM BT; lane 5, 1 mM 2-AP + 20 μM Fe²⁺ + 5 μM BT. OC, open circular; SC, supercoiled DNA.

at later times after multiple treatments than at early times. Hence, the elevation of the intracellular GSH content by either Olt or 2-AP is unlikely to cause the transcriptional activation of phase II enzymes. Reduction in the GSH level caused by oxidative stress would subsequently feedback-stimulate the production of GSH as well as the induction of phase II enzymes.

NF-κB is activated by oxidative stress and the activation has been correlated with the cellular redox state. It has been proposed that the intracellular thiol level affects the expression of several genes after early activation of NF-κB (Schreck et al., 1992; Hecker et al., 1996). Transcriptional activation of the genes of NAD(P)H:quinone oxidoreductase and manganese-dependent superoxide dismutase has been implicated with NF-κB activation in cultured hepatocytes (Yao and O’Dwyer, 1995; Antras-Ferry et al., 1997). In this study, however, the level of NF-κB transcription factor failed to be altered by Olt or 2-AP in the rat liver, supporting the hypothesis that NF-κB activation might not be involved in the transcriptional activation of mEH and
GST genes by the agents in rats. This was also in accordance with our previous observation that LPS inhibited the constitutive and inducible mEH and GST expression irrespective of its activation of NF-κB (Choi and Kim, 1998). The transient activation of NF-κB by Olt in cultured hepatocytes might result from the altered signals in conjunction with other substances present in the culture media (e.g., serum-derived factors). Neither Olt or 2-AP activated the AP-1 nuclear factor complexes in the liver (data not shown).

The activated oxygen species seem to be potentially responsible for the transcriptional induction of anticarcinogenic enzymes (Kensler et al., 1992; Hayes and Pulford, 1995). Increases in the mRNA levels of mEH and GST by Olt and presumably by 2-AP may be mediated with ARE in the genes by transcriptional activation (Hayes and Pulford, 1995). The greater increases in the hepatic mEH and ϒGSTA2 mRNA levels in the GSH-depleting animals provide evidence that reactive oxygen species appeared not to be produced by nonenzymatic breakdown of the agents in the presence of thiol, but presumably by bioactivation. 2-AP was effective in scavenging the reactive oxygen species and in preventing iron-catalyzed DNA degradation in vitro, showing that 2-AP serves as an antioxidant in vitro. In contrast, Olt failed to scavenge oxygen species and to prevent the DNA damage. In spite of their different antioxidant effects in vitro, both agents produced reactive oxygen species in vivo, which would lead to the induction of anticarcinogenic enzymes. 2-AP, in contrast with Olt, failed to convert oxygen to activated oxygen radicals in the presence of thiol in vitro. Hence, the proposed thiol-dependent production of activated oxidents by Olt (Kim and Gates, 1997) is unlikely to be responsible for the production of reactive oxygen involved in the induction of detoxifying enzymes.

In summary, 2-AP was effective in elevating the mEH and major GST gene expression in the rat liver through production of activated oxidents with no NF-κB activation in vivo and that 2-AP differed from Olt in thiol-dependent conversion of DNA topology and prevented DNA damage caused by autooxidation of BT in vitro, although both agents produced reactive oxygen species in vivo.

References
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