Preferential Increase of Glutathione S-Transferase Class α Transcripts in Cultured Human Hepatocytes by Phenobarbital, 3-Methylcholanthrene, and Dithiolethiones

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Abstract

In rodents, a diversity of compounds are able to protect against acute and chronic toxicities of various xenobiotics including carcinogens, at least in part through induction of drug-metabolizing enzymes including glutathione S-transferase (GST) enzymes. We have posed the question as to whether or not these compounds also induce GSTs in human liver. Primary human hepatocyte cultures were exposed to phenobarbital, 3-methylcholanthrene, and two dithiolethiones [1,2-dithiole-3-thione and its 5-(2-pyrazinyl)-4-methyl derivative, oltipraz], and steady-state mRNA levels of GST classes α, μ, and π were determined by Northern blot analysis. After 3 daily treatments, the two dithiolethiones were the most potent inducers; phenobarbital was also effective but to a lesser extent and 3-methylcholanthrene increased GST mRNA in only 2 of the 6 samples, although it stimulated cytochrome P-450 1A2 mRNA in all cell preparations. Whatever the compound only GST A1 and/or A2 transcripts were induced. GST M1 mRNAs were not responsive or only slightly responsive, and GST P1 mRNAs, which were mostly undetectable in control cells, were not affected by treatment with any of the four chemicals. Large individual variations were observed in the level of induction of GST A1 and/or A2 mRNAs, and no sex difference could be demonstrated. These results clearly indicate that phenobarbital, 3-methylcholanthrene, and dithiolethiones are able to markedly increase mRNA levels of GST in human hepatocytes and that the GST α class is preferentially involved.

Introduction

Potent carcinogens and toxic compounds often show considerable species differences in their effects. As is the case for P-450 enzymes, the GSTs can be important in activation and detoxification reactions. Studies in animals have shown that GSTs are induced by compounds such as dithiolethiones, giving rise to substantial protection against carcinogenic and toxic effects of chemicals. For example, D3T and its 5-(2-pyrazinyl)-4-methyl derivative, OPZ, have been shown to be powerful chemoprotectors of AFBI hepatocarcinogenesis in the rat due to their ability to induce specific GSTs (1).

The principal GSTs are soluble and form a supergene family containing many enzymes that belong to four multigene families, α, μ, π, and θ (2, 3). The pattern of expression of these multiple forms of GSTs differs from one tissue to another, and conspicuous individual differences in the enzyme patterns have been demonstrated in humans (4, 5). The α class enzymes, which include GST A1-1, GST A1-2, and GST A2-2, are predominantly expressed in the liver and kidney. GST M1-1, a μ class enzyme, is a hepatic GST but is inherited in an autosomal dominant fashion and is virtually absent in 50% of the human population. Although the π class enzyme GST P1-1 is widely distributed, it is absent in human hepatocytes (6).

Variability in the expression of GSTs is considered to be an important factor in tissue and species susceptibility to toxins and carcinogens. Such variations may depend, at least in part, on the inducibility of GST enzymes by a diversity of compounds. These data have been mainly obtained from investigations in animals. However, huge variations in GST activities can be observed both in response to carcinogens and toxins between animals and humans and in humans, among individuals (7), indicating that meaningful extrapolation of animal data to the human situation is not necessarily possible. Since in vivo experimentation in humans is impossible for ethical reasons, primary human hepatocyte cultures represent a unique model system to investigate the expression and regulation of human GSTs.

The present study was designed to determine whether different GST classes in human liver are equally sensitive to a range of potential inducers. The results, based on a determination of steady-state mRNA levels, show that PB, 3-MC, and two dithiolethiones (i.e., D3T and OPZ) affect the expression of GSTs in primary cultures of human hepatocytes and that the degree of induction may vary greatly, depending on the individual from whom cells are derived.

Materials and Methods

Chemicals. Culture media and fetal calf serum were obtained from Gibco (Paisley, Scotland). Collagenase, 3-MC, PB, bovine albumin, and bovine insulin were products from Sigma Chemical Company (St. Louis, MO). The nick translation kit and [32P]dCTP were obtained from Amersham (Arlington Heights, IL). 1,2-Dithiole-3-thione was synthesized by Dr. T. W. Kensler, and Oltipraz was kindly supplied by Dr. G. Jolles (Rhône-Poulenc Rorer, Anthony, France).

Cell Isolation and Culture. Human livers were obtained from seven surgical biopsies (Table 1). All experimental procedures were done in compliance with French laws and regulations and were approved by the National Ethics Committee. The procedure for the dissociation of liver cells has been described elsewhere (8). Cells obtained were seeded at a density of 10⁷ hepatocytes/80-cm² flask in 10 ml of a standard medium consisting of 75% minimum essential medium and 25% medium 199 supplemented with 10 µg bovine insulin/ml, 0.2% bovine serum albumin, and 10% fetal calf serum. The medium was renewed daily and supplemented with 7 × 10⁻⁵ M hydrocortisone hemisuccinate.

Inducers were added 36–48 h after cell seeding, then 24 and 48 h later as medium was renewed. 3-MC, D3T, and OPZ were dissolved in dimethyl sulfoxide before addition to the culture medium to give final concentrations of 5 µg for 3-MC and 50 µg for D3T and OPZ in 0.2% dimethyl sulfoxide (v/v). Phenobarbital was dissolved in phosphate-buffered saline and added to the culture medium at the final concentration of 3.2 mm. Control cultures received the same concentration of solvent.
Isolation of RNA and Northern Blot Analysis. Cells were washed with chilled phosphate-buffered saline and scraped in a solution containing 0.1 M chilled phosphate-buffered saline and scraped in a solution containing 0.1 M

complementary DNA probes: GST A1 (cross-hybridizing with GST A2), GST

the soluble supernatant fraction was obtained by centrifugation. Total GSTs

of Chirgwin

phase HPLC essentially as described by Meyer

filtration, filters were washed, dried, and autoradiographed at -80°C.

GST Subunit Analyses. Hepatocytes (10 × 10⁶) were homogenized, and the soluble supernatant fraction was obtained by centrifugation. Total GSTs were obtained by glutathione agarose affinity chromatography and by reverse-phase HPLC essentially as described by Meyer et al. (15), except that the column particle size was 5 μm and the flow rate was 1 ml/min. Analyses were carried out on control and inducer-treated cultures.

Results

GST Expression in Adult Hepatocytes during Culture. mRNAs encoding GSTs A1 and/or A2 (it is not possible to distinguish between these two transcripts) were demonstrated in freshly isolated human hepatocytes from the seven donors, but huge variations were observed between the different cell populations. GST M1 transcripts were not detected in two samples, and GST P1 was not expressed in any of the cell populations studied (with one exception, where it occurred at very low levels). GST A1 and/or A2 and GST M1 transcript levels decreased sharply during the first days of culture, dropping to 10-20% of initial values after 24 or 48 h, regardless of the cell population. Thereafter, mRNAs encoding GST M1 increased again and reached values close to those found in freshly isolated hepatocytes, but GST A1 and/or A2 mRNA levels remained quite low (Fig. 1).

Effects of Inducers on GST Expression in Human Hepatocyte Cultures. To determine whether GST forms were responsive to inducers, human hepatocytes were exposed to PB, 3-MC, D3T, or OPZ for 3 days. PB, D3T, and OPZ increased GST A1 and/or A2 transcripts in all cell cultures. The most consistent and highest increases were obtained with the two diethiolethiones. However, individual variations in the extent of induction were observed (Table 1; Fig. 1). 3-MC also augmented GST A1 and/or A2 transcripts, but only in 2 of 6 hepatocyte populations, while P-450 IA2, for which 3-MC is also an inducer, was markedly increased in all 6 cases studied. In contrast, GST M1 mRNA levels were little affected, if at all, by any of the inducers. GST P1 remained undetectable after treatment with these inducers. No sex differences have been observed. No marked changes were noted in GAPDH mRNA content, whatever the culture condition tested. Fig. 2 illustrates the results of a preliminary experiment in which hepatocytes from one individual were treated with D3T for 96 h and subjected to an analysis of GST by reverse-phase HPLC. A substantial increase in the levels of GST A1 and to a lesser extent GST A2 (and perhaps even a small increase of GST M1) was observed.

Discussion

The substantial protection against experimental carcinogenic effects in rodents achieved by the administration of GST inducers offers a new way to design cancer prevention strategies in humans. The results reported in this study show that compounds exhibiting structural diversity and acting as chemoprotectors in rodents are capable of inducing GST A1 and/or A2 mRNAs in human hepatocytes maintained in primary culture.

It is well established that both total and relative amounts of GST enzymes present in human liver show large interindividual differences and that GST M1 has a frequent allele (4, 5), all of which makes likely a wide range of susceptibility to carcinogens and response to inducers. The present work shows that GST A1 and/or A2 and GST M1 mRNAs in freshly isolated hepatocytes and the absence of GST M1 mRNA in some of them reflect the in vivo situation. When put in culture, human hepatocytes exhibit marked changes in GST mRNA levels. Both GST A1 and/or A2 and GST M1 transcript contents sharply decreased to ~10-20% of initial values within 1-2 days of culture. Similar observations have been reported for other functions, particularly P-450 enzymes (16), and can be explained by an increased turnover of these transcripts due to the conditions of liver preservation before dissociation and/or to an adaptation of isolated parenchymal cells to a new

<table>
<thead>
<tr>
<th>Human liver</th>
<th>Age (Years)</th>
<th>Sex</th>
<th>Primary pathology</th>
<th>GST A1 and/or A2 mRNAs</th>
<th>GST M1 mRNAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL1</td>
<td>63</td>
<td>F</td>
<td>Colon carcinoma</td>
<td>++ ++ ++ ND</td>
<td>- 0 0 0 0 0</td>
</tr>
<tr>
<td>HL2</td>
<td>37</td>
<td>M</td>
<td>Lymphoblastoma</td>
<td>++ ++ ++ ND</td>
<td>- 0 0 0 0 0</td>
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<tr>
<td>HL3</td>
<td>67</td>
<td>F</td>
<td>Esophagus carcinoma</td>
<td>++ ++ ++ ++</td>
<td>- - - - -</td>
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<tr>
<td>HL4</td>
<td>64</td>
<td>M</td>
<td>Rectal adenocarcinoma</td>
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<td>+ + ND +</td>
</tr>
<tr>
<td>HL5</td>
<td>59</td>
<td>F</td>
<td>Hepatic tumor</td>
<td>++ ++ ++ ++</td>
<td>0 0 0 0 0 0</td>
</tr>
<tr>
<td>HL6</td>
<td>66</td>
<td>F</td>
<td>Cholestasis</td>
<td>++ ++ ++ ++</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>HL7</td>
<td>60</td>
<td>M</td>
<td>Chronic lymphoid leukemia</td>
<td>+ ++ ++</td>
<td>0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

*ND, not determined; 0, not detected; --, no effect of the inducer; +, induction by 1.5- to 2-fold; ++, induction by more than 2-fold.*
The GST P1 was not expressed in either control or treated cells. In contrast we have demonstrated in recent studies that GST subunit 7 mRNA levels were highly induced in rat hepatocyte cultures after D3T treatment and slightly increased by 3-MC and PB.6

In contrast to GST α, GST M1 mRNAs were either unaffected or only slightly increased by the four inducers. Similar results have been obtained with rat GST 3 and/or 4 mRNAs after exposure of rat hepatocyte cultures to these inducers.5 These in vitro findings do not entirely repeat previous in vivo observations. Indeed, Meyer et al. (23) have shown that after administration of PB or D3T, GST 3 and 4 subunits were increased, although much less than GST subunits 1 and 7.

Our observation that several structurally distinct chemoprotective agents can induce GST in cultured human hepatocytes holds significant implications and encouragement for the development of preventive interventions in humans. One of these agents, OPZ, has been shown to protect against chemically induced carcinogenesis in over a dozen different animal models, including AFB₁-induced hepatocarcinogenesis in the rat (24). As a consequence, OPZ is currently undergoing phase I clinical trials in the United States to determine its toxicity and pharmacokinetics during low-dose, chronic administration (25). Buttressed by the availability of validated intermediate biomarkers, subsequent trials to assess the efficacy of OPZ are envisioned in populations at high risk for AFB₁ exposure and primary liver cancer (26). The rationale for conducting interventions with agents like OPZ is strengthened by recent studies with purified enzymes demonstrating that human GST A1, A2, and M1 all utilize AFB₁-exo-8,9-epoxide, the ultimate carcinogenic metabolite of AFB₁, as a substrate (27). Although GST μ-containing isozymes are the most active among hepatic GSTs in detoxifying AFB₁, they are not expressed in individuals with the homozygous null genotype, and if expressed, they are present in relatively low concentration. Thus, the major burden of AFB₁ epoxide detoxication may be taken by the GST A1- and A2-containing enzymes. The present finding demonstrates that both mRNA and protein levels of these enzymes can be significantly induced by 1,2-dithiole-3-thiones. It is probable that inducers such as OPZ, D3T, and PB would significantly increase the GST-dependent capacity to detoxify AFB₁-exo-8,9-epoxide and bring about an important reduction in susceptibility to AFB₁-induced hepatocarcinogenesis in humans.

References
4. O. Farrel, F. Morel, and A. Guillouzo, P-glycoprotein expression in human mouse, hamster and rat hepatocytes in primary culture, submitted for publication.
5. F. Morel et al., manuscript in preparation.


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