Inhibition of CYP1A2 and CYP3A4 by Oltipraz Results in Reduction of Aflatoxin B₁ Metabolism in Human Hepatocytes in Primary Culture¹

Sophie Langouët,² Brian Coles,³ Fabrice Morel, Laurent Becquemont, Philippe Beaune, F. Peter Guengerich, Brian Ketterer, and André Guillouzo

INSEM U49, Centre Hospitalier Regional Universitaire Ponchaut, 35033 Rennes Cedex, France [S. L., F. M, A. G.]; Biochemistry and Molecular Biology Department, University College London, London WIP6DB, United Kingdom [B. C., B. K.]; INSEM U75, Centre Hospitalier Universitaire Necker, 75015 Paris, France [L. B., P. B.]; and Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, Tennessee 37232 [F. P. G.]

ABSTRACT

Dithiolethiones are thought to act as potent chemoprotective agents against aflatoxin B₁ (AFB₁)-induced hepatocarcinogenesis in the rat by inducing glutathione S-transferases (GSTs). To determine whether these antioxidants can be similarly effective in human beings, we have investigated metabolism of AFB₁, in primary human hepatocytes with or without pretreatment by oltipraz (OPZ), a synthetic derivative of the natural 1,2-dithiole-3-thione. Aflatoxin M₁ (AFM₁), glutathione conjugates of AFB₁, oxides (AFBSGs), and unchanged AFB₁ were quantitated in cultures derived from eight human liver donors. Parenchymal cells obtained from the three GST M₁-positive livers metabolized AFB₁ to AFM₁, and to AFBSGs derived from the isoemic exo- and endo-8,9-oxides, whereas no AFBSGs were formed in the GST M₁-null cells. Pretreatment of the cells with 3-methylcholanthrene or rifampicin, inducers of CYP1A2 and CYP3A4, respectively, caused a significant increase in AFB₁ metabolism. Although OPZ induced GST A2, and to a lesser extent GST A1 and GST M1, it decreased formation of AFM₁ and AFBSG, which involves CYP1A2 and CYP3A4. Inhibition by OPZ of AFB₁ metabolism by reducing CYP1A2 and CYP3A4 was also demonstrated by decreased activity of their monooxygenase activities toward ethoxyresorufin and nifedipine, respectively. The significant inhibition by OPZ of human recombinant yeast CYP1A2 and CYP3A4 was also shown. These results demonstrate that AFBSG can be formed by GST M₁-positive human hepatocytes only, and suggest that chemoprotection with OPZ is due to an inhibition of activation of AFB₁, in addition to a GST-dependent inactivation of the carcinogenic exo-epoxide.

INTRODUCTION

AFB₁,⁴ a product of the mold Aspergillus flavus, which infests grain and other food stuff stored under warm, moist conditions, is metabolized by certain CYPs to a powerful genotoxin, exo AFBO (1–3). AFB₁ is therefore a potential carcinogen; however, its carcinogeticity varies from one species to another (4). In the mouse, its hepatocarcinogenicity is negligible (5); this has been attributed to the particularly effective detoxication of exo AFBO by the GST Yc-Yc (6, 7). On the other hand, it is a powerful hepatocarcinogen in the rat apparently due to the absence, in any quantity, of a GST that utilizes detoxication in the human liver is not as great as in the rat liver because human liver has about 1 order of magnitude less GST activity toward exo AFBO (11) and no apparent homologue for rat GST M1. It is a powerful cocarcinogen in combination with hepatitis B virus, which is often endemic in communities exposed to AFB₁ (13).

The question arises whether inducers, so effective in the rat, might be of value for the chemoprevention of the AFB₁-associated hepatocellular carcinogenesis in humans. The potential for GST-dependent detoxication in the human liver is not so great as in the rat liver because human liver has about 1 order of magnitude less GST activity toward exo AFBO (11) and no apparent homologue for rat GST 10–10 and mouse GST Yc-Yc. The most active GST toward exo AFBO is human GST M1–1 (14), the benefit of which is limited by a genetic polymorphism involving a null allele, so abundant that about 50% of the human population has a GST M1-null phenotype (15).

Human hepatocytes in primary culture have proved to be useful in past studies of GST induction (16). In the present work, the use of hepatocytes in culture has been extended to studies of the effects of inducers on the metabolism of AFB₁. Particular importance is attached to OPZ, a pharmaceutical derived from D3T, which has been reported to be a natural product occurring in cruciferous vegetables (17). In human hepatocytes, OPZ proves to be not only an inducer of GSTs but also an inhibitor of exo AFBO biosynthesis by CYP1A2 and CYP3A4. The mechanism of this effect has been investigated, and the significance of these findings for chemoprevention of AFB₁-dependent hepatocellular carcinoma in humans is discussed.

MATERIALS AND METHODS

Chemicals. Culture media and FCS were obtained from Gibco (Paisley, Scotland). Collagenase, MC, RIF, bovine albumin, bovine insulin, AFB₁, and AFBO, metabolites were products from Sigma Chemical Co. (St. Louis, MO). D3T was synthesized by Dr T. W. Kessler, and OPZ was kindly supplied by Dr C. G. Caillard (Rhône-Poulenc Rorer, Antony, France).

Cell Isolation and Culture. Human liver samples were obtained in France from eight patients undergoing liver resection for primary or secondary hepatomas (Table 1). Hepatocytes were isolated by a two-step collagenase perfusion procedure, and the experimental procedures used were approved by the National Ethics Committee (18). Cell viability was 70–85%, as estimated by the trypan blue exclusion test. Cell yields varied with the size of the liver fragment, and the design of each experiment (time points and treatments) depended on the number of viable cells obtained.

Liver parenchymal cells were seeded at a density of 10⁶ viable cells/35-cm² dish in 2 ml of a nutrient medium consisting of 75% MEM and 25% Medium 199, supplemented with 10 μg bovine insulin/ml, 0.2% BSA, and 10% FCS. This medium, supplemented with 0.1 μM dexamethasone but lacking serum, was renewed daily.

Inducers were added 36–48 h after cell seeding and then 24, 48, and 72 h later, with medium renewal. MC, D3T, OPZ, and RIF were dissolved in DMSO before addition to the culture medium to give final concentrations of 5

Received 6/1/95; accepted 10/2/95.

1 This work was supported by the Institut National de la Sante et de la Recherche Medicale, the ligue contre le cancer, the Association pour la Recherche contre le Cancer—cancer research, the BIOAVENIR program, the Cancer Research Campaign (United Kingdom), and USPHS Grants CA4433 and ES00267. S. Langouët was a recipient of a fellowship from the Ministère de la Recherche et de l'Espace and was granted a short-term visiting fellowship from the European Science Foundation to carry out this work in the London group.

2 To whom requests for reprints should be addressed.

3 Present address: National Center for Toxicological Research, Jefferson, AZ 72079-9502.

4 The abbreviations used are: AFB₁, aflatoxin B₁; AFBO, aflatoxin 8,9-epoxide; AFBSG, glutathione conjugate of AFB₁, oxides; CYP, cytochrome P450; GS, glutathione; GST, glutathione S-transferase; MC, 3-methylcholanthrene; D3T, 1,2-dithiole-3-thione; OPZ, oltipraz; RIF, rifampicin; HPLC, high-pressure liquid chromatography; EROD, 7-ethoxyresorufin-O-deethylase.
Table 1 Characteristics of human liver samples and AFB₁ biotransformation in corresponding primary hepatocyte cultures

<table>
<thead>
<tr>
<th>Human liver samples</th>
<th>Pathology</th>
<th>GST M1</th>
<th>Days of culture</th>
<th>μM AFB₁</th>
<th>Exo/endo ratio</th>
<th>μM AFM₁</th>
<th>μM AFB₁ remaining</th>
<th>Metabolism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-1 (69; M)</td>
<td>Carcinoid of the small bowel</td>
<td>+</td>
<td>2</td>
<td>0.04</td>
<td>1.0</td>
<td>0.060</td>
<td>1.17</td>
<td>76.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>0.43</td>
<td>1.0</td>
<td>0.360</td>
<td>1.17</td>
<td>76.00</td>
</tr>
<tr>
<td>HL-2 (unknown)</td>
<td>unknown</td>
<td>Null</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>0.036</td>
<td>1.70</td>
<td>65.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>0.089</td>
<td>0.24</td>
<td>95.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>0.094</td>
<td>1.37</td>
<td>72.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>0.194</td>
<td>1.60</td>
<td>67.95</td>
</tr>
<tr>
<td>HL-3 (69; M)</td>
<td>Colon adenocarcinoma</td>
<td>Null</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>0.250</td>
<td>3.17</td>
<td>36.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>0.064</td>
<td>1.55</td>
<td>69.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>0.060</td>
<td>0.73</td>
<td>85.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>0.125</td>
<td>0.99</td>
<td>80.20</td>
</tr>
<tr>
<td>HL-4 (59; M)</td>
<td>Colon adenocarcinoma</td>
<td>Null</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>0.065</td>
<td>4.50</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>0.115</td>
<td>1.68</td>
<td>66.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>0.047</td>
<td>0.61</td>
<td>87.80</td>
</tr>
<tr>
<td>HL-5 (68; F)</td>
<td>Hepatic metastasis</td>
<td>+</td>
<td>3</td>
<td>0.017</td>
<td>1.0</td>
<td>0.075</td>
<td>1.78</td>
<td>64.50</td>
</tr>
<tr>
<td>HL-6 (1; M)</td>
<td>None</td>
<td>+</td>
<td>3</td>
<td>0.02</td>
<td>1.0</td>
<td>0.100</td>
<td>2.92</td>
<td>41.60</td>
</tr>
<tr>
<td>HL-7 (48; F)</td>
<td>Necrotic areas</td>
<td>Null</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>0.012</td>
<td>3.99</td>
<td>20.20</td>
</tr>
<tr>
<td>HL-8 (49; M)</td>
<td>Colon adenocarcinoma</td>
<td>Null</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>0.040</td>
<td>4.66</td>
<td>7.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>0.047</td>
<td>4.07</td>
<td>18.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>0.033</td>
<td>3.22</td>
<td>35.60</td>
</tr>
</tbody>
</table>

--- not detected

*Age in years; sex.*

μM for MC and 50 μM for D3T, OPZ, and RIF (in 0.2% DMSO, v/v). Control cultures received the same concentration of solvent.

After different times of treatment with the inducers, the cells were exposed to 5 μM AFB₁, for 8 h. At the end of the incubation with the mycotoxin, both media and cells were collected and stored at −80°C until use.

**Analysis of AFB₁ Metabolites.** AFB₁ metabolites were separated by HPLC by direct injection of cell culture medium or the hepatocyte-soluble supernatant fraction onto a Waters μ-bondapak C₁₈ reverse-phase column with elution by a linear gradient of 10—75% methanol in water buffered with 10 mM ammonium acetate (pH 6.5). To separate AFM₁ from OPZ metabolite(s) that eluted at the same time, we used the same gradient conditions with a Alltech C₁₈ column, 5-μm particle size. Metabolites were identified by comparison to reference standards of known concentration using fluorescence excitation with a narrow-pass filter (370 nm) and a wide-pass emission filter (430 nm) with a low wavelength cutoff. Typically, 50—100 μl of solution were analyzed; the injected volume was adjusted to keep peak height within the fluorescence calibration window. To identify some metabolites, HPLC was also run at pH 4.5 using an Econosphere C18 reverse-phase column. This last column was also used to separate the two stereoisomeric GSH conjugates, essentially as described by Raney et al. (3).

**GST Subunit Analysis.** The cells were sonicated, and a total GST fraction was obtained from the supernatant by GSH agarose affinity chromatography and analyzed by reverse-phase HPLC using a 5-μm particle size column (Rainin Instrument Co., Inc., Woburn, MA) and a flow rate of 1 ml/min (19). GST subunits were quantified from the absorbance at 214 nm. Cellular protein content was estimated by the Bradford procedure (20).

**CYP1A2 and CYP3A4 Activities in Human Hepatocytes.** Activities were measured in cultured living hepatocytes immediately after treatment. EROD activity was estimated essentially as described by Burke and Mayer (21); reaction rates were determined under linear conditions with regard to incubation time and protein concentration. Oxidation of nifedipine to its pyridine oxidation product, a reaction catalyzed mainly by CYP3A4 (22), was measured in cultures refreshed with MEM without phenol red and containing nifedipine at a concentration of 20 μM. The pyridine was measured in the supernatant by HPLC according to Guengerich et al. (22).

**CYP1A2 and CYP3A4 Activities in Recombinant Yeasts.** CYP1A2 and CYP3A4 cDNAs were expressed in yeasts as described by Renaud et al. (23) and Truan et al. (24). Microsomal proteins were prepared, and protein contents were determined using the Bradford method (20). Total CYP concentrations were determined by Fe²⁺-CO versus Fe³⁺ difference spectrophotometry as described by Omura and Sato (25) using a molecular extinction coefficient of 91 μM⁻¹·cm⁻¹. EROD activity was determined by spectrofluorimetry according to the method of Prough et al. (26). Samples of yeast microsomes containing 50 pmol of CYP1A2 were incubated at 28°C with variable concentrations of ethoxyresorufin (0.2—1.6 μM) added in 0.1 M potassium phosphate buffer. The reaction was initiated by the addition of 125 μM NADPH. Fluorescence was measured directly in the cuvettes after stirring for 2 min. Calibration was made by addition of 53 pmol of resorufin to the reaction medium.

The 6β-hydroxylation of testosterone catalyzed by CYP3A4 was tested by incubating microsomal samples from the yeast expression system (containing 50 pmol of enzyme) in the presence of a NADPH-generating system for 30 min at 28°C. Testosterone was added at various concentrations (25—200 μM). Extraction was done with 2 volumes of ethyl acetate after addition of 10 μl of 50% trifluoroacetic acid. 6β-Hydroxytestosterone was quantitated by HPLC on a 5-μm particle Nucleosil C₁₈ column (150 × 4.6 mm). The mobile phase, at a flow rate of 1 ml/min, consisted of water containing 0.1% (v/v) glacial acetic acid/acetonitrile (1:1) for 20 min, followed by a linear gradient increasing to 0.1% glacial acetic acid in water/acetonitrile (7:3) in 5 min. The reaction was followed spectrophotometrically at 254 nm. Curve calibration were performed by HPLC analysis of increasing amounts of 6β-hydroxytestosterone.

In both experiments, OPZ (in 0.1% DMOSO, v/v) was added to the assay mixture at selected concentrations in the range 0.1—100 μM. Control incubations contained the same amount of DMOS (0.1%, v/v).

**RESULTS**

**AFB₁ Metabolism by Untreated Hepatocytes.** The use of fluorescence analysis allowed determination of AFB₁, unconjugated AFM₁, and AFP₁, together with exo and endo AFBSG, at less than 1 nm concentration in the presence of the many UV-absorbing constituents of the cell culture medium. AFP₁ levels were generally below the level of quantitation, and AFQ₁ was not sufficiently fluorescent to be detected at this low concentration. Several other fluorescent species were detected after long incubation times but were not identified as known AFB₁ metabolites and could not, therefore, be quantitated.

Eight hepatocyte populations were investigated, of which three were found to be GST M₁ positive and five GST M₁ negative (Table 1). The cells were exposed to 5 μM AFB₁ for 8 h after 3, 4, 5, or 6 days of culture. Large quantitative variations in overall AFB₁ metabolism and the metabolites formed were observed depending on the cell population investigated and the time of culture (Table 1). The concentration of AFBSG varied from 0.017 to 0.43 μM, AFM₁ from 0.012 to 0.36 μM, and AFB₁ from 0.93 to 4.7 μM.
Table 2. Induction of the GST subunits by OPZ in human hepatocytes in primary culture after 48 h of treatment.

Human hepatocytes were maintained in the absence (C) or presence of OPZ for 48 h as described in “Material and Methods.” The soluble fractions of cellular homogenates were obtained, and the GST subunits were quantitated by glutathione affinity chromatography, followed by reversed-phase HPLC. The fold of induction is indicated in parentheses.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>GST subunits (μg/mg cytosolic protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M1a</td>
</tr>
<tr>
<td>HL-1</td>
<td>C</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>OPZ</td>
<td>0.80 (2.10)</td>
</tr>
<tr>
<td>HL-5</td>
<td>C</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>OPZ</td>
<td>1.38 (1.64)</td>
</tr>
<tr>
<td>HL-6</td>
<td>C</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>OPZ</td>
<td>0.33 (1.43)</td>
</tr>
</tbody>
</table>

The number of time points examined was determined by the size of the liver sample, smaller samples limiting the number. A 3-day culture period appeared to give a good representation of metabolism, and this was chosen for comparative studies.

Effect of GST M1 Polymorphism. Three metabolites were identified in the GST M1-positive liver cells (i.e., AFM1 and the GSH conjugates of endo and exo AFBO); these two isomers were produced in the same proportion (Table 1). In contrast, only AFM1 was formed in GST M1-negative hepatocyte cultures.

Effect of OPZ on AFB1 Metabolism and GST Expression. In all cases, OPZ-treated hepatocytes had lower AFB1 metabolism than their untreated counterparts after either 3, 4, or 5 days of culture. Less AFB1 was metabolized, and less AFBSG and AFM1 were produced after 48 h of treatment. In the three GST M1-positive samples, AFBSG was negligible in one case and reduced by 71 and 84% in the others. The production of AFM1 was negligible in 4 cases and decreased by 82–97% in the others (Fig. 1). A similar decrease in AFB1 metabolism was observed when hepatocytes were exposed to the parent D3T. To determine whether the decrease in AFB1 biotransformation observed in the culture medium was not due to the retention of metabolites or unmetabolized AFB1 in the hepatocytes, cell homogenates were also assayed. Only negligible amounts of AFB1 and its metabolites were detectable in either untreated or OPZ-exposed hepatocytes. Neither AFB1 nor its metabolites were adsorbed into the plastic culture dishes used.

We have already shown that OPZ increased human GST A1, A2, and to a lesser extent, GST M1 transcripts (16). This was reevaluated at the protein level for the three GST M1-positive hepatocyte populations used in the present study after a 48-h exposure to OPZ. As shown in Table 2, OPZ increased GST A1 subunit 2.0-, 1.8-, and 3.8-fold; GST A2 by 4.0-, 1.9-, and 2.1-fold; and GST M1 by 1.6-, 1.4-, and 2.0-fold in liver samples HL-1, HL-5, and HL-6, respectively.

Fig. 1. Effect of OPZ on AFB1 biotransformation and on formation of AFBSG and AFM1 in primary cultures of human hepatocytes derived from eight donors after 48 h of treatment. AFBSG, AFM1, and unmetabolized AFB1 levels in OPZ-treated cultures were calculated as percentages of the values measured in control cultures. Control values for AFB1 epoxidation, AFM1 formation, and AFB1 level in HL-1 to HL-8 were given in Table 1. * < 5% of the control value.
had a greater affinity for and was consequently a more potent competitive inhibitor of CYP1A2 in comparison with CYP3A4.

Preincubation of OPZ with microsomes in presence of a NADPH-generating system for 5 min before adding the substrate in the same conditions as above enhanced inhibition. The percentage of inhibition was 71 ± 6%, with 1.6 μM ethoxyresorufin and 10 μM OPZ without preincubation, whereas it reached 85 ± 7% after a 5-min preincubation of the microsomes with OPZ (results not shown). In the presence of 50 pmol expressed CYP3A4 microsomes/ml, 100 μM testosterone, and 100 μM oltipraz, the percentage of inhibition varied from 6 ± 1% without preincubation to 47 ± 6% after a preincubation with OPZ. These results support the conclusion that OPZ is not only a competitive inhibitor of CYP1A2 and CYP3A4 substrates but also acts partly as an irreversible inhibitor.

**Effect of Exposure to OPZ on Cellular Activities of CYP1A2 and CYP3A4.** OPZ given to cells in primary culture inhibited EROD, two activities catalyzed by CYP1A2 and CYP3A4, respectively (Fig. 3), whereas 4-fold inductions of both EROD by MC and nifedipine oxidation, two activities catalyzed by CYP1A2 and CYP3A4, two major CYPs involved in the biotransformation of AFB1, in humans.

When compared with other species, the activity of GSTs toward AFB1 in human liver cytosols is apparently so low that some other workers have not been able to detect it (27). For the first time, we show that human hepatocytes are able to produce both AFB1, *exo-* and *endo*-oxide GSH conjugates in appreciable quantity. Values published for the specific activity of purified human hepatic GSTs in the conjugation of *exo* AFBO are GST A1–1, 0.04 nmol
tively. In cultures from these three livers, GST M1–1, the most effective enzyme with *exo* AFBO (14), was induced the least by OPZ.

**Effect of MC and RIF on AFB1 Metabolism in the Presence or Absence of OPZ.** The decreased metabolism of AFB1, in OPZ-treated culture suggested that OPZ had a direct effect on biotransformation by CYP1A2 and CYP3A4. To address this hypothesis, hepatocyte cultures were exposed to MC and RIF, specific inducers of CYP1A2 and CYP3A4, respectively. As shown in Fig. 2, MC treatment resulted in a marked increase in both metabolism of AFB1, and formation of AFBSG and AFM1. RIF also increased AFBSG levels but did not affect AFM1 formation, and had only a moderate positive effect on overall AFB1 metabolism. When coincubated with MC, OPZ partly suppressed formation of the two metabolites, whereas coincubation with RIF caused almost complete inhibition of increase of AFB1 metabolism seen with RIF alone.

**Effect of OPZ on the Enzyme Kinetics on Heterologously Expressed CYP1A2 and CYP3A4.** OPZ given to cells in primary culture inhibited EROD and nifedipine oxidation, two activities catalyzed by CYP1A2 and CYP3A4, respectively (Fig. 3), whereas 4-fold inductions of both EROD by MC and nifedipine oxidation by RIF were observed after 2 days. Simultaneous incubation with OPZ resulted in a marked decrease in both CYP-related enzyme activities.

**Effect of OPZ on the Enzyme Kinetics on Heterologously Expressed CYP1A2 and CYP3A4.** CYP1A2 and CYP3A4 were expressed in yeasts and microsomal preparations from the recombinants used to study the inhibition kinetics of OPZ. Dixon plots were constructed that showed that during the 30-min incubation period, OPZ behaved as a competitive inhibitor of both enzymes (Fig. 4). Respective *K*ₘs, calculated from the reciprocal plots of velocity versus OPZ concentration, were 10 and 80 μM for CYP1A2 and CYP3A4, respectively. The 8-fold lower *K*ₘ for CYP1A2 indicates that OPZ
excreted because after an 8-h incubation, no intracellular accumula-

oxide has been shown to react DNA very efficiently and cause

carcinogenic effects, if any (30). The major role played by GST M1—1

detoxication product and is known to have low acute toxic and

GST M1-positive individuals. The major role played by GST M1—1

of DNA damage in populations with high aflatoxin exposure. OW has

with activity toward exo AFBO and a possible decrease in the extent

of AFBSG in our cells, whereas by inducing CYP3A4, RIF

enhancement of AFBSG.

The inhibitory effect of OPZ on both CYP1A2 and CYP3A4 was

confirmed by two other approaches: (a) in hepatocytes, EROD and

nifedipine oxidation, which are catalyzed by CYP1A2 and CYP3A4,

respectively, were decreased in OPZ-treated cells; and (b) experiments

with recombinant enzymes expressed in yeasts show that this

inhibition is partly due to a competition between the AFB1, and OPZ

for CYP1A2 and CYP3A4, and also partly to an irreversible inhibition

by OPZ of these two CYPs. There may, however, be another element

inhibition observed in hepatocytes, particularly with respect to

CYP3A4, because OPZ reduced its activity to very low levels whether

the substrate was AFB1 or nifedipine, and much lower than would be

expected for a Kᵢ as high as 80 µM. The possibility that the hepatocy-

cytes metabolize OPZ to a more potent inhibitor is under study. These

effects have also been observed with D3T, a natural compound, which

is present in extracts of cruciferous vegetables, indicating that various

dithiolthiones can have the same inhibitory effect.

To our knowledge, this is the first demonstration of an inhibitory

effect of OPZ on human CYP activities. Such an effect had been

evoked by Pult et al. (34) when studying metabolism of AFBI in

OPZ-treated rats. It could be concluded that any shortcomings that

might arise from low activities of GSTs or, even in GST M1-null

individuals, the absence of any GST-dependent detoxication could be

compensated for in terms of chemoprotection by inhibition of activa-

tion by Phase I enzymes. It must be borne in mind, however, that

without Phase I enzymes, AFBI cannot undergo appreciable detoxi-

cation by CYP, although reduction to aflatoxicol followed by gluco-

ronidation is an alternative mechanism.

REFERENCES

1. Garner, R. C., Miller, E. C., and Miller, J. A. Liver microsomal metabolism of

aflatoxin B1 to a reactive derivative toxic to Salmonella rhyphiuma TA1535. Cancer


3. Raney, K. D., Coles, B., Guengerich, F. P., and Harris, T. H. The endo-8,9-epoxide


4. O'Brien, K., Moss, E., Judah, D., and Neal, G. Metabolic basis of species difference


813—821, 1983.


on hepatic in vivo DNA binding and in vitro biotransformation of aflatoxin B1 in the


6. Ramsdell, H. S., and Eaton, D. L. Mouse liver glutathione S-transferase isoenzyme

activity toward aflatoxin B1-8,9-epoxide and benzo[a]pyrine-7,8-dihydriodiol-9,10-


7. Hayes, J. D., Judah, D. J., McEllan, L. I., and Neal, G. E. Contribution of the

glutathione S-transferases to the mechanisms of resistance to aflatoxin B1. Pharmaco-


Modification of aflatoxin B1, binding to DNA in vivo in rats fed phenolic antioxidants,


Potent inhibition of aflatoxin-induced hepatic tumorgenesis by the monofunc-

tional enzyme inducer 1,2-dithiole-3-thione. Carcinogenesis (Lond.), 13: 95—100,


glutathione S-transferases in rat liver microsomes. J. Biol. Chem., 267: 15059—15065,


13. Qian, G. S., Ross, R. K., Yu, M. C., Yaun, J. M., Gao, Y. T., Henderson, B. E.,

and Wogan, G. N., and Groopman, J. D. A follow-up study of urinary markers of aflatoxin

exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer


14. Raney, K. D., Meyer, D. J., Kettherer, B., Harris, T. M., and Guengerich, F. P.

Langouit et al., manuscript in preparation.

5578


Inhibition of CYP1A2 and CYP3A4 by Oltipraz Results in Reduction of Aflatoxin B₁ Metabolism in Human Hepatocytes in Primary Culture

Sophie Langouët, Brian Coles, Fabrice Morel, et al.