**Comparison of Mouse and Human Colon Tumors with Regard to Phase I and Phase II Drug-metabolizing Enzyme Systems**

Liliane Massaad, Isabelle de Waziers, Vincent Ribrag, François Janot, Philippe H. Beaune, Jackie Morizet, Alain Gouyette, and Guy G. Chabot


**ABSTRACT**

Since human colorectal tumors are insensitive to most chemotherapeutic agents, there is a need for the discovery of new drugs that would show activity against this disease. In an attempt to better appreciate the relevance of a widely used mouse colon tumor (colonic adenocarcinoma Co38) as a screening model for human colorectal tumors, we compared the main phase I and phase II drug-metabolizing enzyme systems in both tumoral and nontumoral colon tissues. The following enzymes were assayed by Western blot: cytochromes P-450 (1A1/A2, 2B1/B2, 2C, 2E1, and 3A), epoxide hydrolase, and glutathione-S-transferases (GST-α, -μ, and -γ). The activities of the following enzymes or cofactors were determined by spectrophotometric or fluorometric assays: total cytochrome P-450, 1-chloro-2,4-dinitrobenzene-GST, selenium-dependent glutathione peroxidase, 3,4-dichloronitrobenzene-GST, ethacrynic acid-GST, total glutathione, epoxide hydrolase, UDP-glucuronosyltransferase, β-glucuronidase, sulfotransferase, and sulfatase. Results obtained by Western blot showed that mouse colon adenocarcinoma Co38 did not express any of the probed cytochromes P-450, whereas human colorectal tumors expressed only low levels of cytochrome P-450 3A. GST-α and GST-γ were detected in all tumoral and nontumoral tissues of both species. The neutral GST-μ enzyme was detected in all murine tissues investigated and was found to be polymorphic in human tissues. For human peritumoral and tumoral colorectal tissues there was no significant difference between GST isozyme levels, whereas mouse colon adenocarcinoma Co38 had a lower expression of GST-μ and GST-γ, compared to normal mouse colon. Enzymatic activities for glutathione peroxidase, 3,4-dichloronitrobenzene-GST, and ethacrynic acid-GST confirmed the Western blot results for GST-α, -μ, and -γ, respectively. Total GSH levels were similar between murine and human tumors but were 3-fold higher in human tumors than in peritumoral tissues, whereas they were 7-fold lower in mouse colon tumors compared to normal mouse colon. Epoxide hydrolase was not expressed in either mouse colon adenocarcinoma Co38 or normal mouse colon tissues, whereas it was expressed in human colon peritumoral and tumoral tissues at similar levels. No significant difference was observed between human tumors and peritumoral tissues for UDP-glucuronosyltransferase, β-glucuronidase, sulfotransferase, and sulfatase. For murine colon tissues, the conjugation pathways (UDP-glucuronosyltransferase and sulfotransferase) were lower in colon adenocarcinoma Co38, whereas the converse was observed for the corresponding hydrolytic enzymes (β-glucuronidase and sulfatase). In conclusion, similarities were noted between mouse colon adenocarcinoma Co38 and human colon tumors with regard to the absence of most forms of cytochromes P-450 except cytochrome P-450 3A, which was detected in human tumors only. However, for most phase II drug-metabolizing pathways the mouse model was qualitatively and quantitatively different from human colon tissues. These noteworthy interspecies differences may have implications with regard to drug-screening methodologies and preclinical evaluation of candidate anticancer drugs for the chemotherapy of human colorectal tumors.

**INTRODUCTION**

Unresectable or metastatic colorectal cancer is most often insensitive to chemotherapy and remains the second leading cause of all cancer deaths in the United States and in the European Community (1, 2). 5-Fluorouracil, the reference drug for this disease, produces a response rate of approximately 15%, and although its combination with leucovorin increases the response rate its impact on long term survival seems unchanged (3–5). Clearly, there is a need for novel anticancer drugs that would change the survival in this disease.

We believe that one of the reasons for the paucity of chemotherapeutic agents active against colon tumors could be that screening strategies did not use colon tumors in primary screening until the mid-1980s. Screening practices are now changing and rely on the use of either mouse or human tumor models of specific organ systems. Some of the mouse colon tumor models used have been characterized in terms of biological properties and drug responsiveness, and their resemblance to human colon tumors has strengthened their use as models for human disease (6, 7).

Drug-metabolizing enzymes play an important role in determining the susceptibility of organs or tissues to the toxic effects of drugs or other xenobiotics, and they may also influence tumor response to anticancer agents in vivo (8, 9). Although progress has recently been made in characterizing some enzymes involved in drug metabolism in human colorectal tumors (9–12), little information is presently available concerning many other important enzymatic systems of human tumors and, moreover, no information is available concerning the most frequently used mouse colon tumor models. It was, therefore, of interest to assess in these tumors the main phase I (P-4503) and phase II enzyme systems involved in drug metabolism (GST isoenzymes, EH, UDP-glucuronosyltransferase, and sulfotransferase), which correspond to functionalization and conjugation reactions, respectively. Moreover, in addition to GSH, the hydrolytic enzymes β-glucuronidase and sulfatase were assayed.

In this report, we present a comparison of the main xenobiotic-metabolyzing enzyme systems found in human colorectal tumors and in the widely used mouse colon adenocarcinoma Co38 model (13). We also compared the human colorectal tumor to its corresponding peritumoral tissue and compared the mouse colon adenocarcinoma Co38 to normal mouse colon. Our results showed similarities between colon tumors of the two species for some enzymatic pathways but also pointed to noteworthy qualitative and quantitative interspecies differences.

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3. The abbreviations used are: P-450, cytochrome P-450; GST, glutathione-S-transferase; CDNB, 1-chloro-2,4-dinitrobenzene; GPx, selenium-independent glutathione peroxidase; DCNB, 3,4-dichloronitrobenzene; EA, ethacrynic acid; GSH, glutathione; EH, epoxide hydrolase.
ences. These differences may have implications with regard to drug-screening methodologies and preclinical evaluation of candidate anticancer drugs for human colorectal tumors.

MATERIALS AND METHODS

Chemicals and Enzymes

5,5'-Dithiobis(2-nitrobenzoic acid), NADPH, 4-methylumbelliferone, 4-methylumbelliferone sulfate, 4-methylumbelliferone glucuronide, reduced GSH, 1-chloro-2,4-dinitrobenzene, 3,4-dichloronitrobenzene, ethacrynic acid, β-glucuronidase, and sulfatase were purchased from Sigma Chemical Co. (St. Louis, MO). Phenobarbital and 3-methylcholanthrene were purchased from Specia (Rhône-Poulenc, Paris, France) and from Aldrich (Milwaukee, WI), respectively. All other chemicals were of the highest purity available from standard commercial sources.

Human Tissues

Four liver samples were provided from the Necker Hospital Tissue Bank (Paris, France). Colon tissues were kindly provided by Dr. Cugnenc, Laennec Hospital Surgery Department (Paris, France). Seven colorectal tumors and their corresponding peritumoral tissues were obtained from seven different patients and are described briefly in Table 1. Peritumoral specimens were taken in a macroscopically noncancerous region of the resected pieces at >5 cm from the tumor. Tumoral tissues were dissected out from the middle of the tumor. Specimens were frozen within 30 min and stored at -80°C to allow preservation of cellular enzymes. Each tumor or peritumoral tissue was analyzed separately. Part of each tissue sample was reserved for histopathological examination and the remainder was used for this investigation.

Mouse Tissues

The chemically induced mouse colon adenocarcinoma Co38 is a well differentiated tumor (13) that is widely used in experimental chemotherapy. Colon adenocarcinoma Co38 tumors were transplanted in C57BL/6 mice, and non-necrotic tumors weighing approximately 1 g were kindly provided by Dr. M. C. Bissyery (Rhône-Poulenc Rorer Laboratory, Vitry-sur-Seine, France). Normal mouse colons and livers were obtained from non-tumor-bearing C57BL/6 mice (22–24 g) obtained from IFFA Credo (Lyon, France). Mouse tissues were immediately frozen and kept at -80°C until analysis. In some experiments, C57BL/6 mice were pretreated with phenobarbital as follows: one i.p. injection of 80 mg/kg every 24 h for 5 consecutive days, plus the addition of 0.1% phenobarbital in drinking water. Pretreatment of C57BL/6 mice with 3-methylcholanthrene dissolved in corn oil was accomplished as a single i.p. injection (25 mg/kg). Mice were sacrificed by decapitation 6 days after phenobarbital treatment or 2 days after 3-methylcholanthrene administration.

Preparation of Samples for Western Blot Analysis

Mouse or human livers, normal mouse colons, and colon adenocarcinoma Co38 tumors were quickly excised and rinsed in ice-cold 1.15% potassium chloride solution. Tissues were minced with scissors and homogenized in phosphate buffer (0.1 M, pH 7.4) containing EDTA (0.1 mM). Human tissues were prepared as follows. The lumen of the colon was flushed with ice-cold 0.9% sodium chloride solution. The tissues were then cut along the axis of the lumen and the epithelial cells were scraped off with the edge of a glass slide. After sonication of human and mouse tissues, microsomes and cytosols were prepared by differential centrifugation as previously described (14). Microsomal pellets were suspended in phosphate buffer (0.1 mM, pH 7.4) containing 20% glycerol and 10 mM MgCl2 and were then stored at -80°C.

Preparation of Samples for Enzymatic Assays

Human and mouse samples were homogenized in 2 ml Tris-HCl (pH 8.1), 230 mM mannitol, 70 mM sucrose, using a Polytron homogenizer. These homogenates were used for the determination of total GSH and of the following enzymatic activities: UDP-glucuronosyltransferase, β-glucuronidase, sulfotransferase, and sulfatase. CDNB-GST, DCNB-GST, EA-GST, and GPX activities were assayed in cytosols, and EH activity was assayed in microsomes.

Western Blot Analysis

Microsomal or cytosolic proteins (5–200 µg) were separated by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels as described by Laemmli (15). Resolved proteins were electrotransferred to nitrocellulose sheets, which were probed with antibodies and stained as described previously (16, 17). The absorbance of each stained band was determined by scanning with a densitometer (Venon, PHI, Paris). When the purified antigen was available, each gel contained a series of concentrations of this antigen. In the other cases, various concentrations of the same liver microsomes or cytosols were loaded on each gel. The amount of each isoenzyme was expressed as µg of microsomal antigen per mg of protein when the pure antigen was available (EH) or else as arbitrary units per mg of microsomal or cytosolic protein. Arbitrary units were comparable for the different tissues studied in the same species but were not comparable between species. The threshold of protein detection for all the antibodies used was about 1% of the concentration of each isoenzyme measured in the liver.

Antigens and Antibodies

P-450. In this paper we used P-450 gene nomenclature based on the updated list published by Nebert et al. (18). For reference purposes, typical inducers of the different P-450 isoenzymes are as follow: P-450 1A1/1A2, 3-methylcholanthrene, polycyclic hydrocarbons; P-450 2B1, phenobarbital; P-450 2C8-10 (substrate, mephenytoin); P-450 2E1, ethanol, acetone; P-450 3A, steroids, macrolide antibiotics. Polyclonal anti-rat P-450 1A1/1A2 and anti-rat 2B1/2, phenobarbital; P-450 2C8-10 (substrate, mephenytoin); P-450 2E1, ethanol, acetone; P-450 3A, steroids, macrolide antibiotics. Polyclonal anti-rat P-450 1A1/1A2 and anti-rat 2B1/2 were prepared in Dr. Beaune’s laboratory (INSERM U 75, Paris, France) and were checked against Dr. F. P. Guengerich’s preparations of P-450 (Vanderbilt University, Nashville, TN). The polyclonal anti-rat P-450 2E1 was kindly provided by Dr. C. S. Yang (New Jersey Medical School, Newark, NJ) (19). Monoclonal anti-human P-450 3AA4 was prepared as described previously (20). Polyclonal anti-human P-450 2C8-10 was described by Shimada et al. (21).

EH. Human epoxide hydrolase and the corresponding polyclonal antibodies were previously described by Beaune et al. (22) and checked against Dr. Guengerich’s preparations.

Clinical data for the patients investigated

Table 1

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Tumor localization</th>
<th>Tumor diameter (cm)</th>
<th>Degree of differentiation of adenocarcinoma</th>
<th>Degree of invasion</th>
<th>Degree of invasion</th>
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<tr>
<td>1</td>
<td>F</td>
<td>67</td>
<td>Unknown</td>
<td>2.5</td>
<td>Well differentiated</td>
<td>B2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>76</td>
<td>Rectum</td>
<td>2.5</td>
<td>Well differentiated</td>
<td>B2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>75</td>
<td>Rectum</td>
<td>5</td>
<td>Well differentiated</td>
<td>B1</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>65</td>
<td>Right colon</td>
<td>2.5</td>
<td>Poorly differentiated</td>
<td>B1</td>
<td>A</td>
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<td>A</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
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<td>Right colon</td>
<td>8</td>
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<td>C1</td>
<td>C</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>58</td>
<td>Left colon</td>
<td>5</td>
<td>Well differentiated</td>
<td>C1</td>
<td>C</td>
</tr>
</tbody>
</table>

* F, female.
* M, male.
GST. The polyclonal anti-human GST-α, -β, and -γ were purchased from Biorep (Stillorgan, Dublin, Ireland).

Total Cytochrome P-450 Assay

Microsomal cytochrome P-450 content was determined spectrophotometrically (limit of detection, 0.001 absorbance units) using the reduced carbon monoxide versus reduced difference spectra, as described by Omura and Sato (23).

CDNB-GST Activity

GST was assayed as described by Habig et al. (24), using CDNB as substrate. Formation of the 1-chloro-2,4-dinitrobenzene-GSH conjugate by cytosols was measured continuously in a spectrophotometer at 340 nm. The results were expressed in nmol of CDNB conjugated per min per mg of cytosolic protein.

GPX Activity

GPX activity was measured spectrophotometrically (340 nm) in cytosols using NADPH as substrate, according to the method of Paglia and Valentine (25). The results were expressed in nmol of NADPH oxidized per min per mg of cytosolic protein.

DCNB-GST Activity

DCNB-GST activity was measured in cytosols using DCNB as substrate (24). The reaction was measured in a spectrophotometer at 345 nm. The results were expressed in nmol of DCNB conjugated per min per mg of cytosolic protein.

EA-GST Activity

EA-GST activity was measured in cytosols using EA as substrate (24). The reaction was measured in a spectrophotometer at 270 nm. The results were expressed in nmol of EA conjugated per min per mg of cytosolic protein.

Total GSH Concentration

The sum of the reduced and oxidized forms of glutathione was determined in tissue homogenates using the method of Akerboom and Sies (26). In this assay, the sum of the reduced and oxidized forms of glutathione are determined using a kinetic assay in which catalytic amounts of reduced or oxidized glutathione and glutathione reductase bring about the continuous reduction of 5,5'-dithiobis(2-nitrobenzoic acid) by NADPH. The reaction rate is proportional to the concentration of GSH below 2 μM. The formation of 5-thio-2-nitrobenzoate is followed spectrophotometrically at 412 nm. The results were expressed as nmol per mg of protein.

Epoxide Hydrolase Activity

EH activity was measured in microsomes using benzo(a)pyrene-4,5-oxide as substrate, according to the method of Jerina et al. (27).

UDP-Glucuronosyltransferase Activity

The activity of this enzyme was assayed in tissue homogenates using 4-methylumbelliferone as substrate. Homogenate aliquots were incubated in 20 μl of reagent containing 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.3), 0.1 mM dithiothreitol, 1 mM MgCl₂, 0.02% bovine serum albumin, and 0.5 mM 4-methylumbelliferone sulfate. The reaction was stopped by adding 1.25 ml of 0.04 mM sodium carbonate buffer (pH 10). The released methylumbelliferone was measured fluorometrically (9). The results were expressed as nmol per h per mg of protein.

Sulfotransferase Activity

Sulfotransferase was assayed in tissue homogenates using a 3'-phosphate-adenosine-5'-phosphosulfate-generating system plus 125 μl of 4-methylumbelliferone to obtain 4-methylumbelliferone sulfate and 250 mM sodium sulfite to inhibit hydrolysis of the formed sulfate conjugate. After 30 min at 37°C, the reaction was stopped by adding the tubes in ice-cold water and adding 200 μl of 0.05 mM sodium acetate buffer (pH 5). Unreacted 4-methylumbelliferone was removed by three successive extractions with toluene:butanol (3:1). Finally, 200 μl of 10 mM sodium acetate buffer (pH 5) containing 0.02% bovine serum albumin and sulfatase (1000 IU/ml) were added to the aqueous phase to hydrolyze the sulfate conjugate. After a 75-min incubation at 37°C, complete hydrolysis of 4-methylumbelliferone sulfate was achieved and 1 ml of 0.04 mM sodium carbonate buffer (pH 10) was added. The liberated 4-methylumbelliferone was measured fluorometrically as described by El Mouelhi et al. (9). The results were expressed as nmol per h per mg of protein.

Sulfatase Activity

Sulfatase was measured in the presence of 4-methylumbelliferone sulfate. Tissue homogenate aliquots were incubated for 30 min at 37°C in 20 μl of reagent containing 75 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.3), 5 mM MgCl₂, 0.02% bovine serum albumin, and 200 mM 4-methylumbelliferone sulfate. The reaction was stopped by adding 1.25 ml of 0.04 mM sodium carbonate buffer (pH 10). The released 4-methylumbelliferone was measured fluorometrically as described by El Mouelhi et al. (9). The results were expressed as nmol per h per mg of protein.

Protein Assay

Protein concentrations of microsomes, cytosols, and homogenates were determined by the bicinchoninic acid assay (28) using a commercial preparation (Pierce BCA protein assay reagent).

Statistical Analysis

The level of significance was determined using nonparametric tests. The Mann and Whitney U test was used between normal mouse colon and colon adenocarcinoma Co38, and the Wilcoxon t test was used in view of the above results, we decided to use a more sensitive and specific method to determine the different P-450 isoenzyme levels.

RESULTS

Total P-450 Content

We first determined by spectrophotometric assay the total cytochrome P-450 content in colon adenocarcinoma Co38. The microsomal cytochrome P-450 was undetectable in colon adenocarcinoma Co38. In view of the above results, we decided to use a more sensitive and specific method to determine the different P-450 isoenzyme levels.
Cross-Reactivity of Rat and Human Antibodies with Human and Mouse Antigens

In preliminary experiments, we first investigated whether antibodies directed against rat or human antigens could recognize the corresponding mouse liver proteins. The antibodies against purified rat P-450 2B1/B2 and human P-450 3A4, GST-α, GST-π, and EH recognized a single protein band that comigrated in human and mouse samples. The polyclonal anti-rat P-450 1A1/1A2 recognized two protein bands for P-450 1A1 and P-450 1A2 in mouse liver pretreated with 3-methylcholanganthrene. However, in noninduced mouse liver and human liver only P-450 1A2 was detected. Polyclonal anti-human P-450 2C8–10, anti-rat P-450 2E1, and anti-human GST-μ reacted with a single protein band in liver microsomes of both species, but the human proteins had a higher molecular weight for P-450 2C and P-450 2E1 and a lower molecular weight for GST-μ, compared to the corresponding mouse proteins (data presented below).

Phase I Drug-metabolizing Enzymes in Human and Mouse Colon Tumors and Nontumoral Tissues

Western blot immunoquantitation of the main P-450 isoenzymes was performed in human and mouse tumoral and nontumoral tissues and also in liver samples as reference. Table 1 describes the clinical specimens used.

P-450 1A1

In human samples, P-450 1A1 was not detected in any of the tissues studied. In mouse tissues, P-450 1A1 was absent from noninduced mouse liver and colon adenocarcinoma Co38 but was expressed in the four normal mouse colons investigated (2.8 ± 0.8 arbitrary units/ng of protein).

P-450 1A2

Cytochrome P-450 1A2 was present in the three human livers studied (28 ± 7 arbitrary units/ng of protein) but was not detected in human colon peritumoral and tumoral tissues. This P-450 was detected in the four mouse livers (23 ± 4 arbitrary units/ng of protein) and in the four normal colons (3 ± 0.8 arbitrary units/ng of protein) tested but was not found in mouse colon tumor (5 ± 0.5 arbitrary units/ng of protein). The concentrations of this isoenzyme in normal mouse colon were about 5-fold lower than the corresponding liver levels.

P-450 2B1/B2

This cytochrome P-450 was barely detectable in the liver of the two species, and it was not expressed in either human or mouse colon tumoral or nontumoral tissues.

P-450 2C

Cytochrome P-450 2C was detected in the four human livers (34 ± 4 arbitrary units/ng of protein) and in the four mouse livers (68 ± 13 arbitrary units/ng of protein) investigated, but it was not expressed in human or mouse colon tumoral or nontumoral tissues.

P-450 2E1

Cytochrome P-450 2E1 was present in both mouse and human livers (61 ± 9 and 27 ± 5 arbitrary units/ng of protein, respectively, for four samples from each species). It was not detected in human colon peritumoral or tumoral tissues or in mouse tumoral tissue but was detected in the three normal mouse colons investigated (5 ± 0.5 arbitrary units/ng of protein). The concentrations of this isoenzyme in normal mouse colon were about 5-fold lower than the corresponding liver levels.

P-450 3A

Cytochrome P-450 3A4 was detected in all human tissues investigated: five livers (1620 ± 239 arbitrary units/ng of protein), four peritumoral colon tissues (34 ± 15 arbitrary units/ng of protein), and four colon tumors (18 ± 7 arbitrary units/ng of protein), although colon tumors expressed this P-450 to a lesser extent (P < 0.05) than did the corresponding peritumoral tissue (Fig. 1). In mouse tissues, P-450 3A was detected in liver (319 ± 114 arbitrary units/ng of protein; five samples) and normal colon (21 ± 3 arbitrary units/ng of protein; six samples) but was not detectable in mouse colon adenocarcinoma Co38 (with 500 μg of protein).

Phase II Drug-metabolizing Enzyme Systems in Human and Mouse Colon Tumors and Nontumoral Tissues

Glutathione System

Western blot immunoquantitation of GST isoenzymes is presented in Table 2.

GST-α. This isoenzyme was expressed in all human and mouse tissues studied, but the levels in nontumoral and tumoral tissues were not statistically different in the two species.

GST-μ. GST-μ was expressed in three of the four human livers studied, confirming the polymorphism of this isoenzyme.

Fig. 1. Western blot of human and mouse microsomes developed with monoclonal anti-human P-450 3A. Lane 1, noninduced mouse liver (15 μg protein); lane 2, 3-methylcholanganthrene-induced mouse liver (20 μg protein); lane 3, phenobarbital-induced mouse liver (3 μg protein); lane 4, human liver 1 (4 μg protein); lane 5, human colon tumor (60 μg protein); lane 6, human colon peritumoral oral sample (60 μg protein); lane 7, mouse colon adenocarcinoma Co38 (40 μg protein); lane 8, mouse normal colon (80 μg protein); lane 9, human liver 2 (4 μg protein).
Table 2 Immunooquantitation of glutathione-S-transferase isoenzymes in human and mouse colon and liver tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>GST-α (AU/ng)</th>
<th>GST-μ (AU/ng)</th>
<th>GST-τ (AU/ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human tissues</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>623 ± 129a</td>
<td>117 ± 41b</td>
<td>12 ± 7</td>
</tr>
<tr>
<td>Colon peritumoral</td>
<td>10 ± 7</td>
<td>ND°</td>
<td>113 ± 32</td>
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<td>Colon tumor</td>
<td>9 ± 8</td>
<td>ND°</td>
<td>127 ± 33</td>
</tr>
<tr>
<td><strong>Mouse tissues</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>97 ± 8</td>
<td>338 ± 80</td>
<td>320 ± 61</td>
</tr>
<tr>
<td>Normal colon</td>
<td>15 ± 3</td>
<td>28 ± 4</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>Colon tumor Co38</td>
<td>13 ± 2</td>
<td>4 ± 2d</td>
<td>7 ± 1e</td>
</tr>
</tbody>
</table>

* Mean ± SD; number of observations in parentheses.
° Of the four livers assayed, only three expressed the enzyme. Results represent the mean of the three livers expressing the enzyme, excluding the liver with no detectable levels.
° ND, not detectable.
° P < 0.05, determined by the Mann Whitney U test between mouse tumors and normal mouse colon.

in humans. We did not find the GST-μ protein in the tumors or their corresponding peritumoral tissues for any patients studied (n = 6). In mouse, GST-μ was present in all tissues studied but was 7-fold lower (P < 0.05) in colon adenocarcinoma Co38, compared to normal colon.

GST-τ. This isoenzyme was more abundant in human colon tumoral and peritumoral tissues than in the liver. In contrast to human tissues, GST-τ expression was lower in mouse colon adenocarcinoma Co38, compared to normal colon and mouse liver. A typical Western blot depicting the qmmunoquantitation of GST-τ in mouse and human tissues is presented in Fig. 2.

GST Activities. The GST activities in human and mouse colon tissues with representative substrates for the different isoenzymes are presented in Table 3. There was no difference in CDNB-GST activity (representing total GST activity) between human colon and peritumoral tissues, whereas significantly lower activity was observed in mouse colon tumor versus normal mouse colon (P < 0.05). For GPX activity, representative of GST-α activity (29), there was no difference between nontumoral and tumoral tissues of either species. DCNB-GST activity, representative of GST-μ, was found in three of five human livers (0.75 ± 0.8 nmol/min/mg, n = 3) but was not detected in human colon tissues. This activity was observed in both mouse colon tissues, although it was lower in the colon tumor Co38 (P < 0.05), compared to normal colon. EA-GST, representative of GST-τ activity, was detected in human tissues at similar levels, but in mouse tissues EA-GST was 2-fold higher (P < 0.05) in normal mouse colon, compared to colon adenocarcinoma Co38.

DISCUSSION

In an attempt to better understand the relevance of a frequently used mouse colon tumor model for human colorectal tumors, we compared the main drug-metabolizing enzyme systems in both tumoral and nontumoral colon tissues. The drug-metabolizing enzyme systems studied can influence markedly the sensitivity of organs or tissues to the cytotoxicity and/or

**Total GST.** Concentrations of total GST in human and mouse colon tissues are presented in Table 3. In contrast to human tissues, where a significantly higher (3-fold) GST concentration was found in the tumor compared to peritumoral tissues, mouse tissue GST levels were 7-fold lower in mouse colon tumor Co38, compared to normal colon.

**Epoxide Hydrolase.**

EH was detected (Western blot) in human peritumoral tissues and colon tumors at levels representing 13 and 9% of liver levels (164 ± 27 μg of microsomal EH/mg of protein), respectively (Table 4). EH was also present in mouse livers (22 ± 3 μg of microsomal EH/mg of protein) but was not detected in either colon adenocarcinoma Co38 or normal mouse colon. To verify further the absence of the EH antigen in mouse colon tissues, EH activity was determined using benzopyrene-4,5-oxide as substrate, and results showed the absence of EH activity in these tissues.

**Glucuronide System.**

UDP-Glucuronosyltransferase. No significant difference in activity was observed for this enzyme in human colon tissues, but this activity was significantly lower (4-fold) in mouse colon tumors, compared to mouse normal colon (Table 4).

**β-Glucuronidase.** The activity of this hydrolitic enzyme was found to be similar in human peritumoral and tumoral tissues but was markedly higher (6-fold) in mouse tumors, compared to normal colon (Table 4).

**Sulfate System.**

Sulfotransferase. This conjugating enzyme activity was not different in human colon tissues but was about 8-fold lower in mouse tumor Co38, compared to normal mouse colon (Table 4).

Sulfatase. No difference was observed between human colon tissues for sulfatase activity, in contrast to the mouse colon adenocarcinoma Co38, which showed a 3-fold higher activity, compared to normal mouse colon (Table 4).

**Fig. 2.** Western blot of human and mouse colon cytosol proteins GST-α, GST-μ, and GST-τ (10-200 μg) were separated by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels. The resolved proteins were electrotransferred to nitrocellulose sheets, which were probed with antibodies as described in "Materials and Methods." The amount of each isoenzyme is expressed as optical units (AU) per ng of cytosolic protein.

**Fig. 2.** Western blot of human and mouse colon cytosol proteins GST-α, GST-μ, and GST-τ (10-200 μg) were separated by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels. The resolved proteins were electrotransferred to nitrocellulose sheets, which were probed with antibodies as described in "Materials and Methods." The amount of each isoenzyme is expressed as optical units (AU) per ng of cytosolic protein.

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carcinogenicity of drugs and other xenobiotics, and they could also determine tumor response to anticancer agents, by either detoxication or involvement in the toxic effects of these drugs (8, 9). We compared human colorectal tumors to the mouse colon adenocarcinoma Co38 because of its histological appearance and its widespread use in drug-screening programs, and its sensitivity to 5-fluorouracil, which is considered the reference drug for human colon tumors (6, 7, 13).

Cytochromes P-450, a multigene superfamily of microsomal hemoproteins, play a key role in the biotransformation of a wide variety of xenobiotics and are involved in their detoxication or toxic effects. P-450 isoenzymes are often involved in the biotransformation of anticancer agents, and these enzymes are sometimes required for the expression of anticancer activity, e.g., in the activation of cyclophosphamide (8, 30). In the present study, we did not detect P-450 1A1, 1A2, 2B1/2B2, 2C, or 2E1 in human peritumoral and tumoral tissues, whereas P-450 3A4 was present in human tumoral and peritumoral tissues, although it was reduced to about 50% in malignant tissues. This is in accordance with previous studies that also showed the presence of the isoenzyme P-450 3A in normal human colon, in peritumoral tissues, and in human colorectal adenocarcinomas (12).

In mouse tissues, none of the investigated P-450 isoenzymes could be detected in the chemically induced colon adenocarcinoma Co38, whereas P-450 1A1, 1A2, 2E1, and 3A were detected in normal mouse colon. The absence of P-450s in mouse colon adenocarcinoma Co38 indicates that this tumor would be unable to perform P-450-dependent oxidative phase I reactions, such as hydroxylation, epoxidation, or dealkylation, that may toxify or detoxify certain drugs. If drugs were not metabolized by other enzymes, e.g., phase II conjugating enzymes, then the retention of drugs within colon adenocarcinoma Co38 cells would be likely, thus favoring possible cytotoxic effects.

The low level of expression of P-450 isoenzymes in tumoral tissues was also observed in hyperplastic liver nodules from rats treated with chemical mutagens (31, 32) and in human primary hepatomas (9). The regularity of this decrease in multiple cytochrome P-450 enzymes in various neoplastic tissues might be interpreted as being the result of abnormalities in the function of regulatory genes, which could be related to other cellular functions such as cell growth, cell differentiation, and, in particular, p53 gene expression, which has been implicated in cell cycle regulation. Indeed, it has recently been shown that the p53 gene is frequently mutated or rearranged in human colorectal carcinomas, resulting in structural or functional loss of p53 (33).

Table 3 Enzymatic activities of the glutathione system and GSH concentration in human and mouse colon tumoral and nontumoral tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>CDNB-GST (nmol/min/mg)a</th>
<th>GPX (nmol/min/mg)b</th>
<th>DCNB-GST (nmol/min/mg)c</th>
<th>EA-GST (nmol/min/mg)d</th>
<th>GSH (nmol/mg)e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human tissues</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>337 ± 73f (4)</td>
<td>128 ± 189 (5)</td>
<td>0.75 ± 0.88 (3)</td>
<td>11 ± 5 (5)</td>
<td>NTf</td>
</tr>
<tr>
<td>Colon peritumoral</td>
<td>98 ± 16 (4)</td>
<td>26 ± 7 (5)</td>
<td>NDf (6)</td>
<td>6 ± 4 (6)</td>
<td>3.4 ± 0.6 (4)</td>
</tr>
<tr>
<td>Colon tumor</td>
<td>123 ± 33 (4)</td>
<td>35 ± 11 (5)</td>
<td>NDf (6)</td>
<td>7 ± 3 (6)</td>
<td>11.1 ± 2.2f (4)</td>
</tr>
<tr>
<td>Mouse tissues</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1504 ± 590 (4)</td>
<td>1011 ± 159 (4)</td>
<td>29 ± 5 (4)</td>
<td>83 ± 8 (4)</td>
<td>NTf</td>
</tr>
<tr>
<td>Normal colon</td>
<td>370 ± 135 (4)</td>
<td>82 ± 25 (4)</td>
<td>10 ± 0.4 (4)</td>
<td>20 ± 5 (4)</td>
<td>41.0 ± 1.6 (4)</td>
</tr>
<tr>
<td>Colon tumor Co38</td>
<td>140 ± 121f (4)</td>
<td>79 ± 39 (4)</td>
<td>0.2 ± 1.5f</td>
<td>9 ± 6f (4)</td>
<td>7.0 ± 2.3f (4)</td>
</tr>
</tbody>
</table>

a CDNB-GST activity is expressed in nmol per min per mg of protein of glutathione conjugate formed using 1-chloro-2,4-dinitrobenzene as substrate.
b GPX activity is expressed in nmol of NADPH oxidized per min per mg of cytosolic protein.
c DCNB-GST activity is expressed in nmol of DCNB conjugated per min per mg of cytosolic protein.
d EA-GST is expressed in nmol of EA conjugated per min per mg of cytosolic protein.
e Glutathione content is expressed in nmol of total GSH per mg protein.

Table 4 Conjugating and hydrolytic enzymes in human and mouse colon tumoral and nontumoral tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>EH (µg/mg)a</th>
<th>UDP-glucuronosyltransferase (nmol/h/mg)b</th>
<th>β-Glucuronidase (nmol/h/mg)c</th>
<th>Sulfotransferase (nmol/h/mg)d</th>
<th>Sulfatase (nmol/h/mg)e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human tissues</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>164 ± 27c (3)</td>
<td>NTd</td>
<td>435 ± 58 (4)</td>
<td>0.6 ± 0.4 (4)</td>
<td>45 ± 20 (4)</td>
</tr>
<tr>
<td>Colon peritumoral</td>
<td>22 ± 13 (3)</td>
<td>4.4 ± 1.2 (4)</td>
<td>359 ± 54 (4)</td>
<td>0.4 ± 0.2 (4)</td>
<td>26 ± 12 (4)</td>
</tr>
<tr>
<td>Colon tumor</td>
<td>15 ± 4 (3)</td>
<td>1.3 ± 0.6 (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse tissues</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>22 ± 8 (4)</td>
<td>63 ± 10 (4)</td>
<td>611 ± 232 (4)</td>
<td>64 ± 44 (4)</td>
<td>22 ± 9 (4)</td>
</tr>
<tr>
<td>Normal colon</td>
<td>NDf (4)</td>
<td>38 ± 8 (4)</td>
<td>630 ± 54 (4)</td>
<td>26 ± 0.9 (4)</td>
<td>15 ± 4 (4)</td>
</tr>
<tr>
<td>Colon tumor Co38</td>
<td>ND (4)</td>
<td>9 ± 2f (4)</td>
<td>3566 ± 373 (4)</td>
<td>3.5 ± 1.3f (4)</td>
<td>45 ± 2.5f (4)</td>
</tr>
</tbody>
</table>

a Microsomal proteins (5–200 µg) were separated by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels. The resolved proteins were electrotransferred to nitrocellulose sheets, which were probed with EH antibody as described in "Materials and Methods." The amount of EH protein is expressed as µg of microsomal EH antigen per mg of protein.
b Enzymatic activities were assayed in tissue homogenates as described in "Materials and Methods." Enzymatic activity is expressed in nmol per h per mg protein.
c Mean ± SD.
d NT, not tested.
e ND, not detectable.
f P < 0.05, determined by the Mann and Whitney U test between mouse tumors and normal mouse colons.

NT, not tested.
ND, not detectable.
P < 0.05, determined by the Wilcoxon t test between human tumors and peritumoral tissues.
The glutathione system, another major enzymatic pathway involved in the detoxication of exogenous compounds (34), was also studied in tumoral and nontumoral colon tissues. The concentration of the various GST isoenzymes in normal and tumoral tissues is important because these enzymes play a central role in the detoxication of many electrophilic toxic compounds, including carcinogens and cytotoxic drugs. Given the great diversity of GST functions, several authors (35, 36) have postulated that the variability in the expression of these enzymes could be a factor in the susceptibility of various tissues to toxins and carcinogens, and they suggested that these enzymes may be used as potential prognostic markers in carcinogenesis. The cytosolic GST enzymes in humans have been divided into three distinct groups, which are commonly referred to as basic (α), neutral (μ), and acidic (σ) transferases, according to their isoelectric points (37). In agreement with others (38), our results showed the expression of the three classes of GST in human liver.

We observed that there was no difference between human or mouse tumoral and nontumoral colon tissues in the expression of GST-α as determined either by Western blot analysis or by GPX activity. Other studies have reported a significant elevation (about 2-fold) of the expression of GPX in tumoral tissues versus matched normal tissues of breast (29, 39), lung (40, 41), and colon and stomach (27). This apparent discrepancy may be due to our relatively small number of matched human colon tissues assayed (n = 5), because we also observed a tendency to an increase in GPX activity in tumors, although it did not reach statistical significance. Our data suggest that GPX may not play a predominant role in the detoxication of organic hydroperoxides in either human or mouse colon tissues.

Although information is available concerning the expression of extrahepatic GST-μ in normal and tumoral human tissues, to our knowledge no information is available for the mouse. GST-μ has been shown to be genetically polymorphic and is not expressed in 40–50% of the human population (37). In our human colon samples, we did not detect GST-μ or DCNB-GST in tumors or peritumoral tissues, but they were found in three of five livers studied, confirming the polymorphism of this isoenzyme. Since there was no difference between tumoral and peritumoral tissues, our results are in agreement with those of Peters et al. (11) for colon cancer, who suggested that the presence or absence of GST-μ does not seem to play an important role in the etiology of colorectal human cancer. In mouse, GST-μ and DCNB-GST were detected in all tissues investigated but were markedly decreased in mouse colon adenocarcinoma Co38, compared to normal colon. It is possible that GST-μ could play a key role in the malignant phenotype of this mouse tumor.

In agreement with other reports, GST-σ was the predominant GST isoenzyme in human colon, compared to liver (11, 12, 42, 43). In our studies, human peritumoral and tumoral tissues had similar levels of the GST-σ protein, and this may be due to our relatively small number of human colon tissues assayed (n = 6). These results could be in contrast to those of others (12, 44, 45) who showed an increase in the expression of GST-σ in tumoral tissues, but they are in agreement with those of Peters et al. (46) who did not find an increase in GST-σ in human colon adenocarcinoma. Moreover, CDNB-GST activity did not increase statistically in tumoral versus peritumoral human tissues. It is, therefore, likely that CDNB-GST activity in tumoral tissues is well correlated with GST-σ. In mouse tissues, GST-σ was found to be more abundant in liver than in normal colon and was decreased in colon adenocarcinoma Co38. High levels of GST-σ were also reported in mouse liver by Hatayama et al. (47). In addition, DCNB-GST and EA-GST activities and GSH levels were all decreased in mouse colon adenocarcinoma Co38. These low GST activities in mouse colon adenocarcinoma Co38 could be responsible for a different sensitivity of Co38 to cytotoxic drugs that would otherwise be inactivated via the glutathione system. Therefore, it is possible that drugs inactivated by this enzymatic pathway could be more active in mouse colon adenocarcinoma Co38 than in human colon tumors.

Epoxide hydrolase is thought to play a protective role with respect to epoxides metabolically produced from many pharmaceutical agents and other industrial compounds involved in carcinogenesis and cytotoxicity (48). EH is increased in hepatic preneoplastic and hyperplastic nodules and is considered a good marker for chemically induced hepatocarcinogenesis (49, 50). Our results obtained in human colorectal adenocarcinoma appear different from those studies (49, 50) but are comparable to results observed in human primary hepatic tumors, in which EH tended to be lower in the nodular regions (9). EH was decreased in human colorectal adenocarcinoma, compared to peritumoral tissues, as previously shown (12). Since mouse tissues did not express EH in either colon adenocarcinoma Co38 or normal colon, it is expected that these tissues would be more vulnerable to epoxides.

In human tissues, no difference was observed between colon peritumoral and tumoral tissues for UDP-glucuronosyltransferase, β-glucuronidase, sulfotransferase, or sulfatase activities. Our results obtained for β-glucuronidase are in accordance with the findings of Mekhall-Ishak et al. (10) for colon cancer. The importance of certain activities, such as β-glucuronidase, involved in the metabolism of many xenobiotics is uncertain, since the metabolism of many antineoplastic drugs has not been completely elucidated. However, colon β-glucuronidase could be responsible for the reactivation of a drug that had been detoxified by conjugation to glucuronic acid, as has been shown for doxorubicin analogues and mitoxantrone (51, 52). The presence of β-glucuronidase in both normal and malignant colon mucosa, in addition to the bacterial content of the intestine, probably has important implications for the activation of carcinogens as well as antineoplastic drugs (9, 51, 53).

In contrast to human tissues, the phase II drug-metabolizing enzymes involved in both glucuronidate and sulfate pathways were significantly different in mouse normal colon versus colon adenocarcinoma Co38. As noted above for the GSTs, since UDP-glucuronosyltransferase and sulfotransferase were decreased in mouse Co38 this could favor drug retention in this tissue. Also noteworthy was the high hydrolytic activities...
jugating enzymes in tumors (54, 55). Although this work was phase II enzyme levels or activities which were markedly different between mouse and human tumors, with the exception of P-450 3A being detected in colon tumors and have pointed out some similarities but also differences between mouse and human. Other differences included the absence of EH in mouse colon tissues and a higher GSH content in human colon tumors included the absence of EH in mouse colon tissues and a higher GSH content in human colon tumors versus peritumoral tissues, whereas the opposite was observed in mouse. It is of interest to note that similar changes in drug-metabolizing enzyme systems are observed in multidrug-resistant tumors, i.e., decreased P-450s and increased activities of several drug-conjugating enzymes in tumors (54, 55). Although this work was primarily aimed at finding major similarities and/or differences between normal and tumoral colon tissues of both humans and mice, it should be pointed out that it is possible that tissue heterogeneity may have played a role in the distribution of the various enzymatic systems studied. It should also be kept in mind that, although the s.c. growth of the mouse colon adenocarcinoma Co38 tumors may have influenced their enzymatic systems, compared to the same tumor grown in situ in the colon, this tumor implantation site was chosen because s.c. grown tumors are used in drug-screening strategies.

In conclusion, although similarities were observed for some drug-metabolizing enzymes between mouse colon adenocarcinoma Co38 and human colon tumors, these tumors were different for many of the drug-metabolizing enzyme systems studied. These interspecies differences could, therefore, influence the susceptibility of colon tumors to candidate anticancer drugs and may have important implications for drug-screening methodologies and preclinical evaluation of anticancer drugs for human colorectal tumors. It should be kept in mind, however, that a single mouse colon tumor may not be representative of the inherent diversity of human colon tumors and that future studies should include other mouse colon adenocarcinomas to assess their variability in terms of drug-metabolizing enzymes.

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Comparison of Mouse and Human Colon Tumors with Regard to Phase I and Phase II Drug-metabolizing Enzyme Systems

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