XENOBOLIC BIOTRANSFORMATION IN LIVERS AND LUNGS OF ADULT BLACK-TAILED DEER: COMPARISON WITH DOMESTIC GOAT AND SHEEP

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Abstract—1. The capacity of liver and lung tissue of black-tailed deer (Odocoileus hemionus columbianus) to biotransform xenobiotics was compared in vitro to the domestic sheep and goat. Donor animals were all females of varying ages. Tissues from the black-tailed deer were collected in the wild. A variety of biotransformation enzymes were measured in both microsomal and cytosolic fractions.
2. Deer liver was lower in total cytochrome P450 concentration, but mono-oxygenase activities were greater compared to sheep and goat. The opposite was true for the lung.
3. Epoxide hydrolase activities were significantly different in deer vs sheep and goat.
4. In general, both hepatic and pulmonary activities were more similar between sheep and goat than either species compared to the deer, however, the magnitude of the hepatic differences did not exceed 5-fold.
5. Based on these limited results, there is no reason to discredit the sheep or goat as a toxicity testing model for deer.

INTRODUCTION

Many organisms in the world are inadvertently exposed to chemical agents intended for other targets. However, many of these non-target species are not available for toxicity testing. Consequently, data from one or more test species must be extrapolated to the species at risk to derive estimates of the hazards associated with a specific level of toxicant exposure. Humans and wildlife species are frequent beneficiaries of this process. Unfortunately, interspecies extrapolation of toxicity data can be very inaccurate because of the many factors or combinations of factors that result in the varying sensitivities of different species to chemical agents. Calabrese (1983) has cited numerous examples of sensitivity differences among mammals for a wide variety of chemicals. In fact, it is generally recognized that species differences represent the most serious drawback of all toxicological tests procedures (Zbinden, 1984). There is substantial evidence that, of the many underlying factors causing species differences, varying rates and routes of xenobiotic biotransformation are of foremost importance (Caldwell, 1981; Calabrese, 1984; Paulson, 1984).

Numerous reports have provided comparative in vitro data on a wide variety of hepatic phase I and II biotransformation pathways in laboratory species. In addition, a few recent reports have appeared comparing domestic livestock species (Smith et al., 1984; Smith and Watkins, 1984; Watkins and klaassen, 1986; Wisniewski et al., 1987). Marked differences between cattle and sheep in enzymatic activities for certain substrates were noted in these reports.

The best animal models for deer have been assumed to be other ruminants of similar dietary behavior and body weight, namely the domestic sheep and goat, but no direct comparisons of the biotransformation capabilities in these species have been done. In the present study, we compared several hepatic and pulmonary phase I and II enzyme activities in vitro using selected chemical substrates, in the domestic goat doe (Capra hircus), domestic sheep ewe (Ovis aries), and black-tailed deer doe (Odocoileus hemionus columbianus).

MATERIALS AND METHODS

Animals

The sheep (Ovis aries) were cross-bred ewes with predominately Rambouillet Suffolk breeding. All the ewes were greater than three years of age and had completed at least one gestation. Body wt ranged from 60–80 kg. The goats (Capra hircus) were purebred Alpine does from the University of California dairy goat herd. These goats ranged in body wt from 50–70 kg and all had completed at least one gestation. Adult female black-tailed deer (Odocoileus hemionus columbianus) 3–6 years of age and weighing 32–38 kg were field sacrificed with permission from the California State Department of Fish and Game.

Collection and storage of tissue samples

Tissues from both the goats and sheep were collected post mortem at the slaughter facility (Department of Animal Science, University of California, Davis, CA). Animals were stunned with a captive bolt gun followed immediately by exsanguination. The liver and lungs were removed, weighed, and cut into 3–4 cm cubes and quickly placed into ice-cold...

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1.15% KCl. The liver and lungs were ground through the fine plates of a manually operated meat grinder. The ground tissues were then quickly frozen and stored in liquid nitrogen. The black-tailed deer were shot in the wild with buckshot from a helicopter and then quickly exsanguinated. Approximately 50 g of liver and lung were removed immediately and placed in ice-cooled 1.15% KCl. A previous study in our laboratory demonstrated that biotransformation enzyme activities are uniformly distributed within the livers of cattle, sheep and goats (Wieniedi et al., 1987); therefore, random tissue samples were collected in all three species. The liver and lungs were blotted of excess KCl, quick-frozen, and stored in liquid nitrogen until used. The time from collection until freezing of the tissue was less than two hours for all species.

**Microsomal and cytosolic enzyme analysis**

Ten grams of either liver or lung was homogenized in 20 ml of 19.8 mM Tris hydrochloride in 1.15% KCl (pH 7.4) using a Polytron® homogenizer (Brunswick, Westburg, New York). The homogenates were centrifuged at 10,000 g for 20 min at 0–4°C then the supernatants were filtered through two layers of cheesecloth and ultracentrifuged at 105,000 g for 60 min at 0–4°C. The 105,000 g supernatant was collected and stored frozen (−20°C) for subsequent measurement of cytosolic enzyme activities. The microsomal pellet was washed in 15 ml of 0.4 M sucrose: 77 mM tetrassodium pyrophosphate (pH 7.5) and centrifuged at 105,000 g at 0–4°C for 60 min. The supernatants were discarded and the microsomal pellet was suspended in 1.15% KCl. Microsomal protein concentrations were determined colorimetrically by the biuret method using a bovine serum albumin standard (Gornall et al., 1949). Cytochrome P450 and b2 concentrations were determined spectrophotometrically using the procedure of Omura and Sato (1964). The protein concentration in 100 mM Tris hydrochloride (pH 7.4) was 1 mg/ml.

Microsomal activities of aminopyrine N-demethylase (APND) and ethoxycoumarin O-deethylase (ECOD) were determined as previously described (Shull et al., 1982). Substrate concentrations were 40 mM for aminopyrine and 0.80 mM for ethoxycoumarin. Aldrin epoxidase (AE) activity was measured by a modification (Gillet et al., 1987) of the method of Krieger and GEE (1976). The reaction mixture was the same as was employed for APND and ECOD (Shull et al., 1982). The aldrin concentration was 0.55 mM. Aldrin epoxidase assays were terminated by the addition of 5.0 ml of n-hexane and immediate cooling of the reaction mixture on ice. Samples were extracted (Fisher Kotorack®) for 20 min. Dielectroconcentrations were quantified using a Varian 1200 gas chromatograph (Varian, Walnut Creek, CA) equipped with a 3 m glass column packed with 0V101 and a tritium foil electron capture detector. All monooxygenase assays were validated for time and protein linearity. The microsomal protein concentration was 0.8 mg/ml and the reaction time was 20 min of APND, AE and ECOD. NADPH cytochrome P450 reductase activities were measured spectrophotometrically (Pederson et al., 1973) using concentrations of 0.1 mg assay microsomal protein and 0.257 mM cytochrome c.

Microsomal epoxide hydrolase (mEH), cytosolic epoxide hydrolase (cEH) and glutathione S-transferase (GST) activities were assayed by a single step radiometric partition assay (Gill et al., 1983). In brief, microsomes or cytosol were incubated with [3H] cis-stilbene oxide (CSO), pH 9.0, and assayed for diol formation. Cytosol was incubated with [3H] trans-stilbene oxide and assayed for diol formation. GST was assayed as the formation of diol conjugated from [3H] CSO, pH 7.4 in 5 mM glutathione (Gill et al., 1983; Ham-mock and Hasagawa, 1983). For cytosolic epoxide hydrolase, diluted cytosol were routinely preincubated with 0.5 mM diethylmaleate for 10 min at room temperature to deplete endogenous glutathione (Moody et al., 1986). Cytosolic protein concentration was quantified using the method of Bradford (1976) as modified by Moody et al. (1985).

**Statistical analysis**

Statistical comparisons between means were calculated using one-way analysis of variance procedure and Duncan’s multiple range test to locate differences among means (Steele and Torrie, 1960).

**RESULTS**

The gross morphology and visual appearance of the livers from all the species were regarded as normal. Liver microsomal protein concentrations were as follows: goats 23.4 ± 3.7 (SD); sheep 16.4 ± 4.9 and deer 17.7 ± 1.8 mg of wet tissue; the concentrations for goats were significantly greater than sheep and deer. Hepatic cytochrome P450 and b2 concentrations and biotransformation enzyme activities for the three species are given in Table 1. There were no significant differences among the species in hepatic cytochrome b2 concentrations or NADPH cytochrome P450 reductase activities. Deer cytochrome P450 content was significantly lower than that of the sheep and goat. APND activities in sheep were approximately 50% less than either goat or deer. AE

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Goat</th>
<th>Sheep</th>
<th>Deer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P450</td>
<td>0.62</td>
<td>0.69</td>
<td>0.44</td>
</tr>
<tr>
<td>Cytochrome b2</td>
<td>0.21</td>
<td>0.40</td>
<td>0.28</td>
</tr>
<tr>
<td>NADPH cytochrome P450 reductase</td>
<td>103.2</td>
<td>108.60</td>
<td>102.10</td>
</tr>
<tr>
<td>Aminopyrine N-demethylase</td>
<td>4.22</td>
<td>2.11</td>
<td>5.57</td>
</tr>
<tr>
<td>Aldrin epoxidase</td>
<td>0.59</td>
<td>0.35</td>
<td>1.44</td>
</tr>
<tr>
<td>Ethoxycoumarin O-deethylase</td>
<td>0.36</td>
<td>0.23</td>
<td>0.51</td>
</tr>
<tr>
<td>Epoxide hydrolase (microsomal)</td>
<td>84.7</td>
<td>75.60</td>
<td>39.90</td>
</tr>
<tr>
<td>Epoxide hydrolase (cytosolic)</td>
<td>1.60</td>
<td>1.22</td>
<td>2.50</td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td>4.58</td>
<td>18.27</td>
<td>10.76</td>
</tr>
</tbody>
</table>

*Four animals/group.
†Units are nmol/mg microsomal protein.
Units are nmol/min per mg microsomal protein.
§Units are nmol/min per mg microsomal protein.
||Not significantly different (P < 0.05) from other means in the same row.
**Not different from goat value but is different (P < 0.05) from sheep value.
activities were approximately three-fold greater in the deer than either the sheep or goat. FCOD activities in the deer were significantly greater than the sheep, whereas activities in the goat and sheep were not significantly different. Both cytosolic and microsomal EH were significantly greater in the deer compared to sheep or goat. GST activities in the goat were less than 50% that of the sheep or deer. Hepatic activities in the deer and sheep were significantly different on all parameters except NADPH cytochrome P450 reductase and GST activities.

Lung

The gross morphology and visual appearance of the lungs from all the species were regarded as normal. Lung microsomal protein concentrations were not significantly different among the three species and were as follows: goats 3.94 ± 0.24 (SD); sheep 3.63 ± 0.40 and deer 4.22 ± 0.19 mg/g of wet tissue. Lung cytochrome P450 and b concentrations and activities of microsomal and cytosolic biotransformation enzymes are given in Table 2. Deer cytochrome P450 concentrations were twice that of sheep and goats, whereas NADPH cytochrome P450 reductase activities in the goat was nearly twice that of either the sheep or deer. APND was about 3-fold greater in the goat and sheep compared to the deer, whereas AE and ECOD activities were greatest in the sheep. AE and ECOD activities were about 6- and 10-fold greater, respectively, in sheep compared to deer. Microsomal EH activities were similar in the sheep and goat, but deer had activities that were approximately 40% less. Cytosolic activities of EH and GST in the sheep and goat were less than half the activities measured in the deer.

**DISCUSSION**

In this study, a comparison was made of phase I and II xenobiotic biotransformation activities in hepatic and pulmonary tissue of wild black-tailed deer, domestic sheep and domestic goats. There is substantial evidence that both qualitative and quantitative differences in these enzyme activities frequently correlate very closely with sensitivity differences among species to toxicants (Calabrese, 1983, 1984; Caldwell, 1983; Paulson, 1984). Consequently, for the sheep or goat to serve as valid model species for wild deer, biotransformation activities should be similar. Moreover, differences in phase I and II activities are of practical value when predicting differences in biological half-lives of xenobiotics in vivo (Walker, 1978). For most wildlife species, it is difficult, if not impossible, to collect phase I and II metabolic data directly, especially for endangered species. Yet wildlife are exposed to environmental toxicants, the effects of which are generally unknown (Bunyan and Stanley, 1982). Therefore, it is advantageous to collect information using metabolically representative species when possible. Sheep and goats were selected to compare to deer because of similar body weight and digestive physiology (all the species are ruminants). In addition, based on feeding behavior, goats and deer are browsers and sheep are grazers. Since non-ruminants it is well documented that the composition of diet plays an important role in the biotransformation of xenobiotics (Campbell and Hayes, 1976; Campbell, 1979, 1982) we reasoned that differences in dietary behavior, such as browsing vs grazing, and dietary composition might subsequently lead to enzyme activity differences in these three ruminants.

In general, hepatic measurements among the three ruminant species were relatively similar compared to differences known to exist among mammalian species. Compared to sheep, deer had significantly different microsomal protein concentrations as well as microsomal and cytosolic enzyme activities whereas cytochrome P450 reductase and GST activities were similar. The deer and goat were similar in microsomal protein content, cytochrome b5, cytochrome P450 reductase, APND and ECOD. Deer liver had the lowest cytochrome P450 content compared to the other species, however, APND and AE (both cytochrome P450-dependent reactions) were more active in the deer. The phase I and II enzyme activities observed for sheep and goat liver were generally comparable and were similar to other data from our laboratory for these species (Wisniewski et al., 1987). Thus, selected metabolic pathways in the goat, and to a lesser extent the sheep, could be used as representative of deer. However, because biotransformation measurements are sufficiently dependent on experi-
mental conditions such that quantitative data comparisons among studies can be risky (Fouts, 1976), perhaps only trends are acceptable.

Variation in the activities of both hepatic and pulmonary enzymes can be attributed to quantitative and qualitative differences which are under genetic control. Also, as noted by Smith et al. (1984) this variation depends on the substrates used. They compared hepatic activities in cattle, sheep, swine and rats using approximately 30 test substrates and determined, for example, that with certain substrates, rats had up to a 1000-fold greater capacity to conjugate with glutathione than sheep. In the present study, we did not note any differences greater than 4-fold for either oxidative, hydrolytic or conjugative activities indicating that there was some but not a great deal of variation among the three ruminant species. Also, some of the variation might be attributed to different dietary patterns. Whether the diet of the deer, which was undetermined, was an important modulating factor of the enzymes measured is unknown.

In the lung, as observed in the liver, there was significant variation in some enzyme activities. Lung cytochrome P450 was greatest in the deer, yet the enzyme activities and APND and AE were highest in the sheep and goat. This response is exactly the opposite to that observed in the liver, but the activities of APND and AE (both cytochrome P450-type reactions) were highest in the deer. Lung cytochrome P450 was greatest in the deer, yet enzyme activities of APND and AE were highest in the sheep and goat lung. The reason for this difference between the lung and liver in APND and AE activities per nmole cytochrome P450 is unclear.

Little is known about biotransformation processes in the lungs of ruminant species. There are examples of pulmonary toxicoses in ruminants that indicate a direct involvement of metabolic enzymes (Breeze and Carlson, 1982). A well known example is acute pulmonary emphysema or 'frog fever'; 3-methyl indole which is produced from dietary tryptophan by rumen micro-organisms is subsequently bioactivated in the lung to a reactive form (Breeze and Carlson, 1982). This activation has been implicated as cytochrome P450 catalyzed (Carlson and Breeze, 1984). Relative to the liver, the lungs of the three ruminant species in this study contain greater concentrations of both cytochrome P450 and b, compared to the rat (Table 3). The unusually high concentrations of cytochrome b, are especially interesting in view of its suggested role in the toxicity of 4-ipomeanol in the lung (Boyle, 1980).

The results of this study indicate that while a certain degree of prediction of xenobiotic biotransformation is possible between the goat (and to a lesser extent the sheep) and black-tailed deer, caution should be taken when prediction is carried to novel environmental chemicals. Only a limited number of biotransformation pathways were examined in this study. Additional research should be conducted to establish a more complete baseline, and to ascertain more fully the significance of the ruminant lung in xenobiotic biotransformation.

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REFERENCES


Table 3. Percentage of lung and liver concentrations (cytochromes) and activities (cytochrome P450 reductase) in ruminants compared to the rat

<table>
<thead>
<tr>
<th>(Lung/liver) × 100 = %</th>
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</thead>
<tbody>
<tr>
<td>Rat*</td>
</tr>
<tr>
<td>Goat</td>
</tr>
<tr>
<td>Sheep</td>
</tr>
<tr>
<td>Deer</td>
</tr>
<tr>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>6.3</td>
</tr>
<tr>
<td>33.7</td>
</tr>
<tr>
<td>35.2</td>
</tr>
<tr>
<td>135.1</td>
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<tr>
<td>Cytochrome P450*</td>
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<tr>
<td>8.3</td>
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<tr>
<td>263.0</td>
</tr>
<tr>
<td>177.0</td>
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<tr>
<td>201.1</td>
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<tr>
<td>Cytochrome P450*</td>
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<tr>
<td>23.2</td>
</tr>
<tr>
<td>189.0</td>
</tr>
<tr>
<td>105.8</td>
</tr>
<tr>
<td>100.5</td>
</tr>
</tbody>
</table>

*Percentages were calculated from values presented in a review paper by Wolf (1984) who summarized average of liver and lung data published in 15 different reports between 1973 and 1983.

†Units of values used in percentage calculation are nM/mg microsomal protein.

‡Units of values used in percentage calculations are nM of cytochrome c reduced/min per mg microsomal protein.


