Comparative Metabolism of Thiabendazole in Cultured Hepatocytes from Rats, Rabbits, Calves, Pigs, and Sheep, Including the Formation of Protein-Bound Residues

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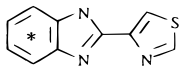
Cultured hepatocytes from rat, rabbit, calf, pig, and sheep were used to study metabolism and formation of protein-bound residues of thiabendazole ([^{14}C]TBZ), a benzimidazole anthelmintic and fungicide. In all investigated species, major pathways corresponded to 5-hydroxylation of TBZ and its further conjugation. However, marked interspecies differences in rates of TBZ disappearance and 5-hydroxy metabolite formation were demonstrated. Rabbit hepatocytes presented the fastest TBZ biotransformation and were the most extensive hydroxylators. By contrast, the lowest capacity of oxidation was observed for the rat. Two unidentified minor metabolites, designated M1 and M2, were particularly produced by sheep hepatocytes. Moreover, the protein-bound residues in these cells, which could be related to cytochrome P450-dependent oxidation, were formed in 4 times greater amounts than in the other animal cells. These findings substantiate hepatocytes as an in vitro model for prediction of hepatic metabolism in vivo.

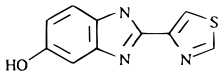
Keywords: *Thiabendazole; hepatocytes; food-producing animals; metabolism; residues*

INTRODUCTION

Thiabendazole [2-(4′-thiazolyl)-1*H*-benzimidazole (TBZ)] is used as a broad-spectrum anthelmintic in various animal species (Brown et al., 1961) and in humans (Hennekeuser et al., 1969). More recently, this compound has been used as a food preservative and an agricultural fungicide (Szeto et al., 1993; Arenas and Johnson, 1994). The metabolism of TBZ has been previously investigated. Oral administration of TBZ to sheep (Tocco, 1964), cattle and goats (Tocco et al., 1965), and humans and dogs (Tocco et al., 1966) resulted in a rapid absorption from the gastrointestinal tract and excretion in urine and feces. The major metabolite was determined to be 5-hydroxythiabendazole (5OH-TBZ), formed by aromatic ring hydroxylation, detected in both its free form and as its glucuronide and sulfate conjugates (Figure 1). 4-Hydroxythiabendazole, formed by hydroxylation of the benzimidazole ring at the 4-position, was reported as a minor metabolite of TBZ in cow urine (Gardiner et al., 1974). In rodents, both *N*-methylthiabendazole and 2-acetylbenzimidazole have been identified as minor urinary metabolites (Tsuchiya et al., 1987; Fujitani et al., 1991). Furthermore, TBZ has been said to bind irreversibly to tissue proteins in mouse embryos through a putative cytochrome P450-mediated mechanism (Yoneyama et al., 1985; Yoneyama and Ichikawa, 1986). Regarding its toxicology, TBZ has recently been recognized to be teratogenic (Ogata et al., 1984) and nephrotoxic in mice (Mizutani et al., 1990). By considering these toxicological properties, the acceptable daily intake of TBZ has recently been established at 100 μg/kg of body weight (FAO/WHO, 1993).



Thiabendazole (TBZ)



5-Hydroxy-Thiabendazole (5OH-TBZ)

Figure 1. Chemical structures of TBZ and 5OH-TBZ. Asterisk (*) indicates site of ^{14}C radiolabeling.

The basic pattern of drug metabolism involves several liver drug oxidizing and conjugating enzymes. The levels of these enzymes vary among species or individuals due to environmental as well as genetic factors. Basic knowledge on comparative biotransformation in relation to drug elimination is of major importance in the evaluation of the safety and efficacy of therapeutic agents and in the estimation of drug dosage regimen. In addition, pharmacotherapy of food-producing animals may result in the presence of residues of the drug and/or its metabolites in meat, milk, or eggs. Nevertheless, by comparison to the laboratory animals, these animal species are less used for biotransformation studies of veterinary drugs, owing to costs, time, and the requirement of special technical facilities, particularly in the

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case of experiments using radiolabeled compounds. The use of in vitro models such as cell fractions or cell cultures may be an alternative way to provide useful information. Primary cultures of hepatocytes have been shown to be a fruitful model for studying the biotransformation of veterinary drugs (Shull et al., 1987)). They are, in general, considered to be more relevant than liver microsomes since hepatocytes provide physiological intracellular conditions including compartmentation, cofactor supply, and molecular arrangement between biotransformation enzyme systems. In this context, the present study was designed to compare biotransformation and formation of protein-bound residues of TBZ by using cultured hepatocytes from rats, rabbits, sheep, calves, and pigs.

MATERIALS AND METHODS

Materials. Thiabendazole, uniformly radiolabeled with ^{14}C in the benzene ring (specific radioactivity of 74 mCi/mmol; radiochemical purity >96%) was purchased from Isotopchim (Ganagobie-Peyruis, France). 5-Hydroxythiabendazole and 2-acetylbenzimidazole were kindly provided by Dr W. L. Henckler (Merck Sharp & Dohme, Rahway, NJ) and Dr T. Fujitani (Laboratory of Public Health, Tokyo, Japan), respectively. Thiabendazole, dimethyl sulfoxide, HEPES, Waymouth, Ham's F12 and William's E culture media, collagen, collagenase, and bovine serum albumin, were obtained from Sigma (St. Quentin Fallavier, France). Culture dishes were from Nunclon (Roskilde, Denmark). Fetal calf serum was from Seromed (Berlin, Germany). Acetonitrile and ethyl acetate, RS grade for HPLC, were obtained from Carlo Erba (Milan, Italy). All other chemicals used were of the highest purity commercially available, and distilled deionized water was used in all studies.

Hepatocyte Cultures and Treatments. Livers were obtained from 1-month-old male rabbits (New Zealand, 1.5 kg), 6-month-old sheep (Lacaune, 45 kg), 6−11-month-old calves (Holstein Friesian, 100 kg), 6−8-month-old pigs (Dutch Landrace × Yorkshire, 100 kg), and male rats (Sprague Dawley, 180−250 g). Hepatocytes from rabbit, pig, rat, sheep, and calf were isolated as previously described by Daujat et al. (1987), Hoogenboom et al. (1989), Gomez-Lechon et al. (1984), and Van't Klooster et al. (1992), respectively. Cell suspensions, containing 80−95% trypan blue-excluding cells, were diluted to a density of 3×10^6 viable cells/2.5 mL of medium and seeded into 60 mm diameter Petri dishes coated (collagen = 50 μg/dish, rabbit; fibronectin = 100 μg/dish, rat) or uncoated (sheep, pig, and calf). Culture medium was Ham's F12/Leibovitz L15 (1:1 v/v) for rats and William's E for the other animal species. Fetal calf serum (5%), penicillin (50 IU/mL), streptomycin (50 μg/mL), and insulin (0.5 μg/mL) were supplemented to the culture medium. Plates were then incubated in a 37 °C incubator under a humidified 5% CO_2 atmosphere. Four hours after plating, the culture medium was renewed in the absence of serum (with the exception of pigs) and thereafter at 24 h in the presence of the drug (1 μCi/mL of culture medium). Experiments were initiated by the addition of [^{14}C]-TBZ, dissolved in DMSO (0.01% v/v), to cells cultured for 24 h to achieve a final concentration of 25 μM. The concentration of 25 μM was selected after preliminary investigations, since it did not provoke any morphological alteration or lactate dehydrogenase leakage over a 24 h period. Incubation was stopped at 0, 0.5, 1, 2, 4, 8, and 24 h post-treatment. Media samples were then collected, and cells were washed twice with phosphate-buffered saline (PBS) and scraped in a cold mixture of PBS/methanol (1:1 v/v) using a rubber policeman. Media and cells were quickly frozen and stored at −20 °C until analysis.

Biotransformation Studies. The parent drug TBZ and its metabolites were measured in culture media by an adaptation of the previously published method of Alvinerie et al. (1991). Culture medium samples were analyzed before and after glusulase treatment as follows. Samples (100 μL) were extracted twice with 1 mL of ethyl acetate. While the aqueous phase was collected to measure unextractable radioactivity by scintillation counting (Kontron Beta V spectrometer), the organic phase was evaporated to dryness under nitrogen. Subsequently, the dry residue was dissolved in 100 μL of the mobile phase (see below). An 80 μL aliquot was injected for HPLC analysis. To hydrolyze glucuronides and sulfates, culture medium samples (100 μL) were mixed with 100 μL of 0.1 M sodium acetate buffer and 10 μL of glusulase type H-2 from *Helix pomatia* (Sigma, St. Louis, MO) with β-glucuronidase and sulfatase activities (97 900 and 3400 IU/mL, respectively). Samples were incubated for 2 h at 37 °C. Further handling was similar to the medium samples for biotransformation studies. HPLC analysis was performed with a Waters model 590 equipped with an automatic injector (Waters 710B Wisp). Separation was performed on a pkb 100 Suplex column (granulometry 5 μm, 4.6 × 150 mm, Supelco France). The mobile phase consisting of acetic acid (0.2% in water) and acetonitrile (95/5 v/v) was pumped at a flow rate of 1 mL/min. TBZ and its metabolites were detected by using both a diode array detector (Kontron M440) with a fixed wavelength at 305 nm and a radiometric detector (Bertholdt LB 507A). The limit of quantification was 10 ng/mL, and the detection limit of the method was 3 ng/mL for TBZ and 5OH-TBZ. The effluent radioactivity was detected using a solid scintillator cell. The limit of detection was 300 dpm, corresponding to 7 pmol of thiabendazole. Under the above conditions, retention times for 5OH-TBZ and TBZ were 5 and 8 min (± 0.5 min), respectively. 2-Acetylbenzimidazole was characterized by a retention time of 28 min in the same experimental conditions.

Determination of Protein-Bound Residues. Protein-bound residues were measured after various incubation times (0, 0.5, 1, 2, 4, 8, and 24 h). The precipitate obtained after addition of cold PBS/methanol on hepatocytes was collected by centrifugation (6000*g*; 10 min), washed with ice-cold methanol (two times), ethanol (two times), and diethyl ether (two times) and dissolved in 1 mL of a mixture of 0.8 N NaOH and 5% sodium dodecyl sulfate (50:50 v/v) for 15 min at 50 °C, according to the method of Hoogenboom et al. (1991). An aliquot of 0.25 mL was used for protein determination with bicinchoninic acid using bovine serum albumin as protein standard (Smith et al., 1985). Another 0.75 mL aliquot was mixed with scintillation cocktail and counted in a liquid scintillation counter. The residue formation was expressed as picomoles of protein-bound residues (TBZ equivalent) per milligram of protein.

Data Analysis. All experiments were carried out in triplicates of two different cultures from each animal species. TBZ disappearance and 5OH-TBZ formation rates were measured during the 8 h of incubation in all investigated species with the exception of cultured rabbit hepatocytes, for which only the first hour of incubation was considered. Correlation analyses between TBZ disappearance and TBZ metabolite formation rates were performed by linear regression using Instat 2.01 program (graph PAD software, San Diego, CA).

RESULTS

Characteristics of Cultured Hepatocytes. As judged by the exclusion of trypan blue, cultured hepatocytes from rats, rabbits, calves, sheep, and pigs were shown to possess a viability >80%. Cells attached within 4 h to culture dishes to form a monolayer of continuous granular cells within 24 h. No morphological change or cell detachment was observed in any of these cultures over a 24 h incubation period in the presence of 25 μM of TBZ.

HPLC Profiles of Culture Media. Figure 2 displays HPLC radiochromatograms of ethyl acetate extracts of deconjugated media from 24 h cultures of hepatocytes from rats, rabbits, calves, pigs, and sheep

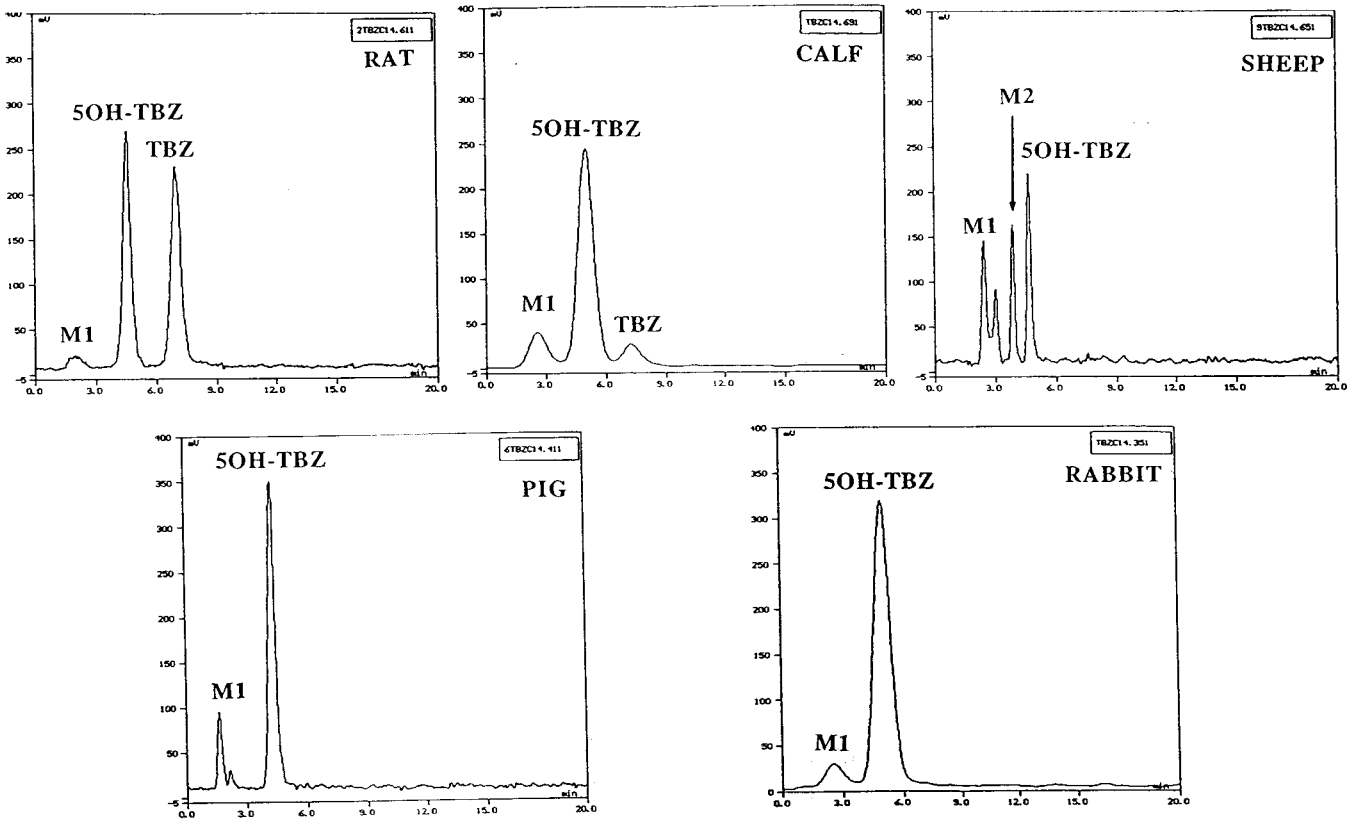

Figure 2. Radiochromatograms of ethyl acetate extracts of deconjugated media from rat, calf, sheep, pig, and rabbit hepatocytes incubated with 25 μM [^{14}C]TBZ for 24 h.

incubated with 25 μM [^{14}C]TBZ. On the basis of the retention times of the corresponding standards, peaks were assigned as follows: TBZ (thiabendazole); 5OH-TBZ (5-hydroxythiabendazole); M1 and M2, two unidentified more polar metabolites of TBZ.

The metabolic patterns were qualitatively similar among animal species: TBZ metabolism resulted in the formation of one predominant metabolite and one or two minor ones. The peak eluting at 8 min corresponded to the parent drug TBZ. After a 24 h incubation period, TBZ was detected only in media from rat and calf hepatocytes. The major peak of radioactivity was identified as 5OH-TBZ with a retention time of 5 min. Minor peaks M1 and M2 with retention times lower than 5 min were observed. M2 (eluting at 3.7 min) was produced only by sheep hepatocytes cultured for 24 h, whereas M1, and a shoulder peak, M1b, in the case of pig and sheep, were present in all investigated hepatocytes with a retention time of 2.8 min. Although M1 and M2 are currently unidentified, these two derivatives possess the phenyl ring of benzimidazole on which the radiolabeling of [^{14}C]TBZ is located.

5-Hydroxylation of TBZ and Its Further Conjugation. The kinetics of TBZ disappearance and its 5-hydroxylation were determined over a 24 h exposure period of cultured hepatocytes from rats, pigs, calves, sheep, and rabbits to 25 μM [^{14}C]TBZ (Figure 3). In all investigated animal species, the level of TBZ declined, demonstrating the capacity of these hepatic cells to metabolize this drug. TBZ biotransformation rates (Table 1) were in good agreement with these results. Indeed, TBZ was shown to be metabolized 5−10 times more rapidly in cultured rabbit hepatocytes compared to hepatocytes from other species, paralleled by the 5-hydroxylation of TBZ, which appeared to be 10−40 times more rapid in rabbit hepatocytes. The major product, identified as 5OH-TBZ by its characteristic retention time at 5 min, was detected after a 1−2 h exposure period. Cultured rabbit hepatocytes presented the most rapid biotransformation of TBZ into 5OH-TBZ as compared to the other cells. By contrast, cultured rat and sheep hepatocytes appeared to be poor hydroxylators of the parent compound. As illustated in Table 2, 5OH-TBZ was intensely and immediately conjugated with the exception of calf hepatocytes, in which conjugation was complete only after a 4−8 h period. After 24 h, both free and conjugated 5OH-TBZ accounted for 59%, 53%, 45%, 25%, and 20% of the parent [^{14}C]TBZ initially introduced in cultured hepatocytes from rabbits, calves, pigs, rats, and sheep, respectively.

Other Extracellular Metabolites. Figure 4 summarizes the relative quantities of metabolites of TBZ observed after a 24 h incubation period with 25 μM [^{14}C]-TBZ in culture media incubated with β-glucuronidase. In the case of rat hepatocytes, a high percentage of radioactivity was recovered as parent drug (44.5%), whereas 60−70% of the parent drug was metabolized to 5OH-TBZ and M1 in hepatocytes from calf, sheep, pig, and rabbit hepatocytes. M1 metabolite production represented generally 2% and 10% of the incubated [^{14}C]-TBZ, while metabolite M2 was detected only as traces, except in the case of sheep hepatocytes (26%). By contrast to 5OH-TBZ, M1 was not conjugated and M2 only partly (~50% in sheep). Indeed, the action of glusulase from *H. pomatia* provided an indication of corresponding conjugates, particularly the glucuronides, which are present in the aqueous phase. By comparing the values obtained before (data not shown) and after β-glucuronidase hydrolysis, conjugates accounted for 30% and 80% of the total radioactivity after a 24 h

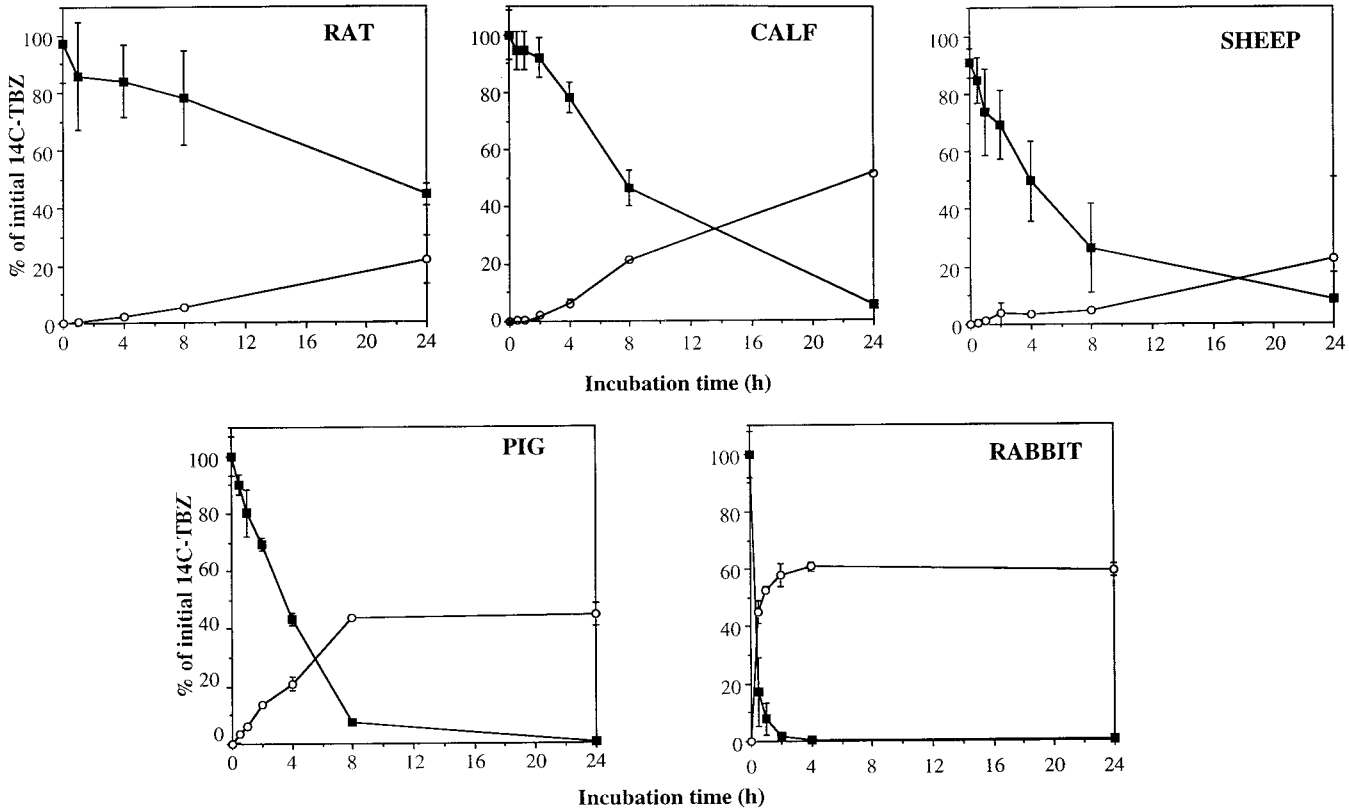

Figure 3. Interspecies variability of TBZ metabolism: extracellular kinetics of TBZ disappearance and total 5OH-TBZ formation. Each point represents the mean (± SD) of six determinations of two different cultures. Hepatocytes were exposed to 25 μM [^{14}C]-TBZ over a 24 h period: (■) TBZ; (○) total 5OH-TBZ.

Table 1. Interspecies Variability in the Rate of [^{14}C]TBZ Metabolism in Cultured Hepatocytes from Rabbit, Pig, Sheep, Calf, and Rat

species	pmol min^{-1} (mg of protein)$^{-1}$ [a] disappearance of TBZ	pmol min^{-1} (mg of protein)$^{-1}$ [a] production of 5OH-TBZ	production/ disappeaance ratio (%)
rat	10.48 ± 1.35	3.05 ± 0.67	29.10
calf	13.37 ± 2.01	5.43 ± 0.08	40.61
sheep	25.78 ± 3.94	6.84 ± 1.74	26.53
pig	29.04 ± 1.30	11.37 ± 0.25	39.15
rabbit	157.45 ± 2.99	125.75 ± 10.12	79.87

[a] Data for each species represent the mean of six determinations of two different cultures.

Table 2. Interspecies Comparison of the Conjugation Kinetics of 5OH-TBZ in Cultured Hepatocytes from Rat, Calf, Sheep, Pig, and Rabbit[a]

	conjugation of 5OH-TBZ (% of total 5OH-TBZ				
time (h)	rat	calf	sheep	pig	rabbit
0.5	100.0	0	0	0	0
1	100.0	4.7	68.1	89.0	91.2
2	87.2	79.7	87.2	91.7	95.3
4	90.9	86.2	93.6	97.4	98.4
8	88.3	95.5	94.3	96.7	99.3
24	90.9	99.4	96.1	99.0	99.0

[a] Values represent the mean percentage of total 5OH-TBZ (six determinations of two different cultures).

incubation period. In the investigated species, all of the radioactivity present in the aqueous phase represented between 18% and 22% of the total radioactivity after β-glucuronidase hydrolysis. This level, which was almost identical in all animals, should correspond to polar metabolites of TBZ, which were not extractable by ethyl acetate. By considering the differences between the parent [^{14}C]TBZ initially introduced and the total

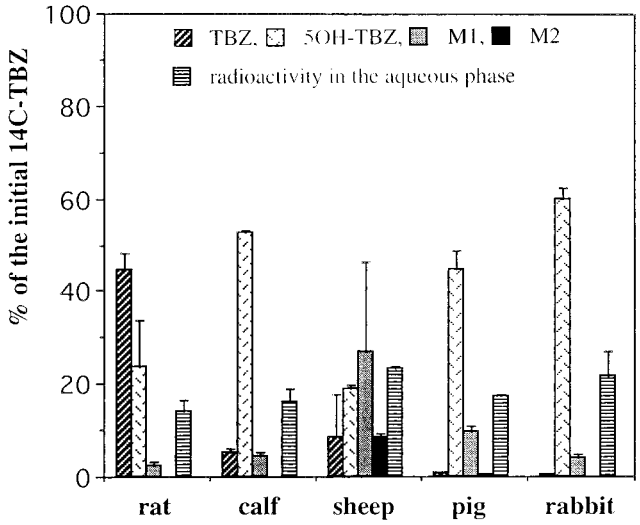

Figure 4. Metabolic profiles of TBZ in cultured hepatocytes from rat, calf, sheep, pig, and rabbit after a 24 h incubation period with 25 μM [^{14}C]TBZ after glusulase treatment of culture media. Values are expressed as the relative percentages of the parent drug TBZ initially introduced.

measured extracellular radioactivity, it appears that a significant radioactivity level would be present within hepatocytes, particularly in cases of rat, calf, and pig.

Formation of Protein-Bound Residues of TBZ. As shown in Figure 5, biotransformation of 25 μM [^{14}C]-TBZ by cultured hepatocytes from all different animal species resulted in the formation of protein-bound residues. This radioactivity was not extracted from the protein fraction by subsequent treatments with methanol, ethanol, and diethyl ether, and it corresponded to 1−2% of the total [^{14}C]TBZ incubated for 24 h. Between

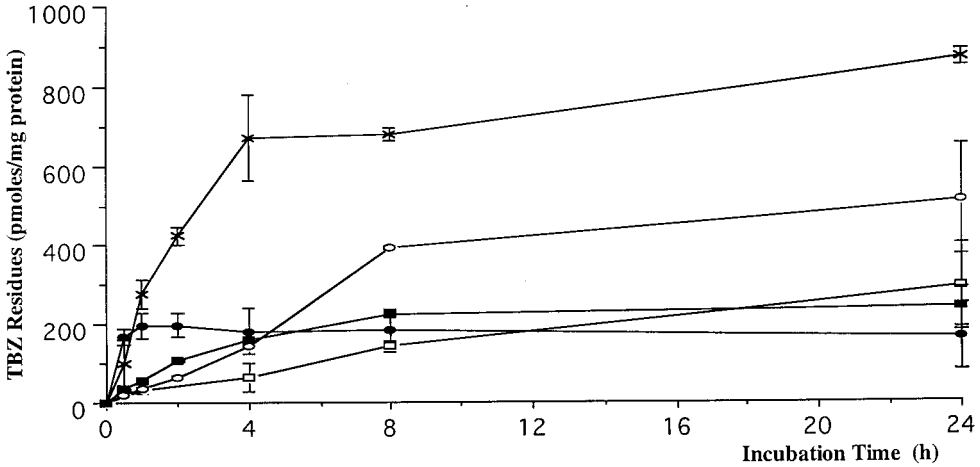

Figure 5. Interspecies differences in formation of protein-bound residues in cultured hepatocytes from rabbit, rat, sheep, pig, and calf incubated with 25 μM [^{14}C]TBZ for various time intervals: (●) rabbit; (□) rat; (×) sheep; (○) calf; (■) pig.

Table 3. Correlation Coefficients Relating the Formation of TBZ Protein-Bound Residues (BR) to TBZ Disappearance and 5OH-TBZ and Metabolite M1 Formations

species	BR/TBZ disappearance	BR/5OH-TBZ	BR/M1
rat	0.9616[a]	0.9420[a]	0.4494
calf	0.9685[a]	0.9467[a]	0.5962
sheep	0.9075[a]	0.9199[a]	0.6377[a]
pig	0.9839[a]	0.9170[a]	0.9423[a]
rabbit	0.1651	0.4669	0.3477

[a] Significant correlation was obtained ($p < 0.05$).

1 and 4 h of incubation, there was a time-related increase in the levels of these residues. After a 8−24 h period of incubation, 200−400 pmol of protein-bound residues/mg of protein was formed in hepatocytes of rabbit, pig, rat, or calf. In the case of sheep hepatocytes, these residues were produced more significantly (800 pmol/mg).

Except for rabbit hepatocytes, significant correlations were obtained between protein-bound residue formation and either TBZ disappearance or 5OH-TBZ formation (Table 3). By contrast, the formation of residues was correlated only to that of metabolite M1 in the cases of sheep and pig.

DISCUSSION

As demonstrated by both the low values of LDH leakages and the high fraction of trypan blue excluding cells obtained from the livers of all investigated animal species, the cells used in the present work appear to be suitable for studying the metabolic pathways of TBZ, including the formation of protein-bound residues. Since levels of LDH leakages in TBZ-treated hepatocytes were unchanged during incubation, this drug seems devoid of cytotoxic effects at the concentration of 25 μM. This result is consistent with previous data demonstrating the absence of any cytotoxicity of TBZ at doses ranging from 30 to 200 μM in cultured rabbit hepatocytes (Aix et al., 1994).

The metabolite profiles obtained by HPLC analysis of the culture media show that, except for rat liver cells, TBZ was almost entirely metabolized during a 24 h period of incubation. This observation is consistent with in vivo pharmacokinetics of TBZ, demonstrating that the unchanged TBZ in urine represented only 0−5% of the administered dose in sheep, calf, or rat (Tocco, 1964; Tocco et al., 1965, 1966). The same authors demonstrated that 5OH-TBZ was the major urinary metabolite existing either as free form or as glucuronide or sulfate conjugates. These results are consistent with our data since TBZ 5-hydroxylation appeared to be a predominant metabolic pathway in cultured hepatocytes of the species used in this study. The marked quantitative differences in the rates of TBZ 5-hydroxylation observed among the various animal species are in agreement with a preliminary investigation (Alvinerie et al., 1992) based on the measurement of in vitro biotransformation of TBZ by liver microsomes from sheep, pig, cow, rabbit, and rat. In this study, the microsomal 5-hydroxylation of TBZ was characterized by maximal rates (V_{max}) varying between 2.47 (rabbit), 0.23 (cow), 0.17 (pig), 0.11 (sheep), and 0.07 nmol min^{-1} mg^{-1} (rat). Since TBZ 5-hydroxylation has recently been described to be mediated by cytochrome P450 1A subfamily (Rey-Grobellet et al., 1996), interspecies differences in TBZ oxidation might be related to variability in the constitutive expression of this subfamily in the liver of the investigated animal species. On the other hand, the observed low capacity of cultured rat hepatocytes to hydroxylate TBZ in the 5-position might partly be explained by the low maintainance of cytochrome P450 in 24-h-old monolayer cultures of rat hepatocytes as compared to those from other animal species including ruminants (Van't Klooster et al., 1994). The fact that rabbit hepatocytes appeared as the most efficient cells to hydroxylate TBZ in the 5-position is in good agreement with in vivo pharmacokinetic data previously obtained by using the same analytical methodology. When TBZ was orally administered (50 mg/kg), a ratio of the area under the curve of plasma 5OH-metabolite to that of the unchanged TBZ was calculated as high as 2.42 ± 0.75 (Rey-Grobellet et al., personal communication). On the contrary, a corresponding ratio of only 0.15 ± 0.06 was extrapolated from previously published data in sheep receiving a similar oral dosage (Galtier et al., 1994). Such a difference demonstrates both the higher in vivo TBZ oxidation in rabbit by comparison to ruminant species and the good representativeness of hepatocyte models to depict such an interspecies variability in drug oxidation. Since 5OH-TBZ has been recognized to be devoid of any toxicological effects, this rapid conversion in rabbits could also explain the relative insensitivity

of this animal species to developmental toxicity of orally administered TBZ by comparison to the rat (Lankas and Wise, 1993). Concerning 5OH-TBZ conjugation, the present study supports the same conclusions as previous in vivo metabolic investigations. Thus, the lowest conjugation rate of 5OH-TBZ observed in calf hepatocytes compared to the other cultured cells could be related to the low urinary excretion of the glucuronide or sulfate conjugate in calf (Tocco et al., 1965) by comparison to sheep (Tocco, 1964) or rat (Tocco et al., 1966).

HPLC analysis of the culture media indicated that irrespective of the animal species, a radiolabeled metabolite of TBZ, M1, was produced by all investigated hepatocytes. On the other hand, the metabolite M2 was more specifically produced in sheep hepatocytes. Neither of these two unidentified metabolites corresponds to 2-acetylbenzimidazole, which has been characterized in our analytical conditions by a retention time of 28 min. These bioproducts could be similar to the polar unknown metabolites recovered by Tocco et al. (1966) in the urine of sheep receiving a similarly radiolabeled [^{14}C]TBZ.

Exposure of cultured hepatocytes to [^{14}C]TBZ was associated with the appearance of radioactivity irreversibly bound to hepatic proteins, suggesting that an electrophilic reactive intermediate could be formed. This result is in agreement with a previous study describing the presence of bound radioactivity in liver microsomal proteins of mice receiving uniformly radiolabeled [^{14}C]-TBZ (Yoneyama and Ichikawa, 1986). Such hepatic residues could correspond to the previously observed radioactivity recovered in livers from calves killed 34−59 days after treatment with 110−200 mg/kg of radiolabeled TBZ (Tocco et al., 1965). Regarding their long half-lives, further studies are needed to assess the health significancy of such residues. However, the present study clearly indicates that they can be formed in all investigated hepatocytes. Rabbit hepatocytes were found to be the more potent cells to hydroxylate TBZ but not to form protein-bound residues to a high extent. In this animal species, the early and extensive 5-hydroxylation of TBZ could compete with the metabolic pathways leading to the formation of residues. In the case of hepatocytes of other animal origin, significant correlations were obtained between the rates of 5-hydroxylation and formation of protein-bound residues, confirming the involvement of P450 monooxygenase system in both of these pathways (Rey-Grobellet et al., 1996; Yoneyama and Ichikawa, 1986).

In conclusion, as such good correspondence was obtained with previous in vivo studies describing metabolic pathways of TBZ in various animal species, including the formation of liver protein-bound residues, the present investigations clearly demonstrate that cultured hepatocytes from various animal species can be used as models for identification of possible metabolites that might be formed in vivo.

ABBREVIATIONS USED

DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; TBZ, thiabendazole; 5OH-TBZ, 5-hydroxythiabendazole.

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