Pharmacological basis for hepatic drug metabolism in sheep

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Review article

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Summary — Age-related changes in hepatic drug metabolizing activities of Lacaune ewes were determined in foetal, neonatal, growing, pregnant and adult animals. The ontogenic evolution of cytochrome P450 was compared to those of microsomal monooxygenases and some microsomal and cytosolic transferases. The involvement of two purified izoenzymes P4502B and P4503A was determined in the *N*-demethylation of various substrates and the hydroxylations of progesterone. An experimental fascioliasis, provoked by the oral administration of 150 metacercariae of *Fasciola hepatica* to sheep, was proposed as a pathological model. Its effect was measured on the pharmacokinetics of various hepatic tracers and veterinary drugs. The mean residence times of antipyrine, pentobarbital, albendazole and mebendazole were increased in infected lambs with consequences on the urinary excretion of 4-hydroxyantipyrine, prolongation of pentobarbital-induced sleeping time, elimination of albendazole sulfone and reduced mebendazole. The characteristic decrease in liver cytochrome P450 could be responsible for most of the pharmacokinetic and pharmacodynamic changes observed in fluke-infected ruminants.

sheep / liver / biotransformation / parasitism / benzimidazole

Résumé — Bases pharmacologiques du métabolisme hépatique des médicaments chez le mouton. *Les variations en fonction de l'âge des capacités hépatiques de biotransformation ont été déterminées chez la brebis Lacaune au stade fœtal, néonatal, jeune, gravide et adulte. L'évolution ontogénique du cytochrome P450 était comparée à celle des monooxygénases et transférases microsomales ou cytosoliques. L'implication des deux isoenzymes purifiés P4502B et P4503A était déterminée pour la* N*-déméthylation et l'hydroxylation de différents substrats et de la progestérone. Une fasciolose expérimentale provoquée par l'administration de 150 metacercaires de* Fasciola hepatica *a été utilisée comme modèle de pathologie pour reconnaître la perturbation des pharmacocinétiques de divers traceurs de la fonction hépatique et de médicaments vétérinaires. Les temps moyens de résidence de l'antipyrine, du pentobarbital, de l'albendazole et du mébendazole étaient prolongés chez les moutons infestés avec des conséquences sur l'excrétion urinaire de la 4-hydroxyantipyrine, un allongement du temps de sommeil*

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induit par le pentobarbital et sur l'élimination de la sulfone de l'albendazole ou du métaboliste réduit du mébendazole. La diminution caractéristique du cytochrome P450 hépatique pourrait être responsable de la plupart des modifications pharmacocinétiques et pharmacodynamiques observées chez les ruminants infestés par la douve.

mouton / foie / biotransformation / parasitisme / benzimidazole

INTRODUCTION

The mammalian liver is described as being the major site for the transformation of endogenous and exogenous compounds. The roles played by hepatic enzymes in endogenous pathways encompass the metabolism of steroids, eicosanoids, fatty acids and other biological molecules. They also play key roles in the pharmacology and toxicology of drugs, therapeutic agents and natural or pollutant xenobiotics.

The aim of the present paper is to review recent data obtained in sheep, concerning the ontogenic development of liver drug-metabolizing enzymes, the participation of cytochrome P4502B and P4503A subfamilies to the hepatic biotransformation of endogenous and exogenous substrates, and the incidence of pathological conditions on liver drug metabolism with the resulting consequences on drug pharmacokinetics.

ONTOGENIC DEVELOPMENT OF DRUG METABOLIZING ENZYMES IN SHEEP LIVER

In rodents and pigs, the activities of oxidative and conjugative liver enzymes involved in the biotransformation of xenobiotics have been described as attaining adult levels within the two first months after birth (Van Bezoïjen, 1984; Cresteil, 1987; Short and Stith, 1973; Peggins et al, 1984). Information concerning the development of hepatic enzyme activity in sheep was limited to comparative data involving liver enzymes in adult ewes and foetal lambs (Dvorchik et al, 1986; Wang et al, 1986) or between different ruminant species (Smith et al, 1984; Wisniewski et al, 1987).

More recently, liver microsomal and cytosolic preparations have been made from foetal, neonatal (one- and four-week-old), growing (seven-month-old), pregnant

(eleven-month-old) and adult (six-year-old) female Lacaune sheep selected from the same flock (Kaddouri et al, 1990). The development of hepatic monooxygenase was studied by measuring microsomal cytochrome P450-dependent monooxygenases whereas the rates of UDP-glucoronyltransferase, glutathione *S*-transferase and *N*-acetyltransferase were measured using their three respective substrates: *p*-nitrophenol, 1-chloro-2,4-dinitrobenzene and sulphamethazine. In a further study, the ontogenesis of the two purified sheep liver cytochrome subfamilies, P4502B and P4503A, was studied in the same animals (Kaddouri et al, 1992). The development of progesterone metabolism was also investigated by measuring both the 6β- and 16α-21-hydroxylations of the hormone and its reduction at the 20-position leading to 20α-hydroxyprogesterone. This was previously described as being a major circulating metabolite of progesterone in ewes (Paterson et al, 1983).

The main results of these studies are reported in table I. Although total cytochrome P450 was not detected spectrophotometrically in the liver microsomes of three-month-old foetuses, this enzyme doubled in concentration during the first seven months of life and remained constant thereafter. Cytochrome P4502B was present after birth but only at low levels. By contrast, isoenzyme P4503A was detectable in sheep foetal liver and represented respectively 82 and 48% of total P450 in one- and four-week-old lambs. This percentage decreased in older animals, in which it represented only about 20%. The evolution of monoxygenase activities was quite variable during animal growth. Some increased regularly to adult levels, including aminopyrine *N*-demethylase, ethylmorphine *N*-demethylase, ethoxycoumarin *O*-deethylase and benzopyrene hydroxylase. Benzphetamine *N*-demethylation remained constant and was correlated with cytochrome P4502B levels. Erythromycin *N*-demethylation and aniline hydroxylation levels decreased from four weeks of age to adulthood and their evolution paralleled that of isoenzyme P4503A.

The hepatic progesterone metabolites, formed by 17α- and 20β-hydroxylation, were generally present in concentrations below the limit of the analytical method. 6β-Hydroxylation was the major route of progesterone metabolism and represented 66% of its total biotransformation in adult ewes. This activity was present in foetuses, increased rapidly during the first month of life and remained constant from four weeks of age to adulthood. 16α- and 21-hydroxylations of progesterone appeared as constant, low-level metabolic pathways in ovine microsomes (1–2% of the total conversion). The reductive conversion to 20α-hydroxyprogesterone represented 30% of the total liver microsomal metabolism. This was measurable in the foetal liver and increased rapidly during the first month of life to reach adult levels. As with all investigated phase II enzymes, UDP-glucuronyltransferase activity towards *p*-nitrophenol was found to be present in foetal sheep liver. It increased at birth and decreased in adult animals. Interestingly this ontogenic pattern was similar to that of hepatic γ-glutamyltransferase measured in the same animals (Braun et al, 1992). The cytosolic glutathione *S*-conjugation of 1-chloro-2,4-dinitrobenzene was constant during the first seven months of life and rapidly underwent an eightfold increase during the course of pregnancy and thereafter. Cytosolic *N*-acetyltransferase, employing sulphamethazine as a substrate, was found to be present in all liver samples and reflected a large interindividual variability.

The two above-mentioned studies demonstrated both the similarities in ovine hepatic drug-metabolizing capabilities with those of other mammals and the early development of these enzyme systems in ovine species. The activities of the different bio-

Table I. Incidence of age on microsomal and cytosolic drug metabolizing enzymes in female sheep[a] (from Kaddouri et al, 1990, 1992).

Parameter	*Age*					
	Foetus	*1 wk*	*4 wk*	*7 mth*	*11 mth*	*6 y*
Cytochrome P450 (nmol/mg protein)						
Total P450	ND [b]	0.40 [c]	0.66 [c]	0.91 [c]	0.99	1.10
P4502B	ND	0.03	0.05	0.06	0.07	0.05
P4503A	0.06 [c]	0.33 [c]	0.32	0.18	0.23	0.20
Monooxygenases (nmol/min/mg)						
Aminopyrine *N*-demethylase	0.34 [c]	1.67 [c]	2.31 [c]	2.52 [c]	3.82 [c]	5.72
Benzphetamine *N*-demethylase	0.15 [c]	1.62	1.62	1.64	1.94 [c]	1.47
Ethylmorphine *N*-demethylase	0.08 [c]	1.88 [c]	2.17 [c]	1.77 [c]	3.32	3.64
Erythromycin *N*-demethylase	0.21 c	1.21 [c]	1.31 [c]	0.91	0.89	0.87
Ethoxycoumarin *O*-deethylase	ND	0.36 [c]	0.30 [c]	0.47	0.52	0.60
Benzopyrene hydroxylase	0.004 [c]	0.057 [c]	0.065 [c]	0.083 [c]	0.082 [c]	0.166
Aniline hydroxylase	ND	0.81 [c]	0.84 [c]	0.69 [c]	0.35	0.30
Progesterone hydroxylation (nmol/min/mg)						
6β	0.04	0.77	1.32	1.07	1.50	1.13
16α	ND	0.03	0.06 [c]	0.04	0.04	0.02
20α	0.03	0.15 [c]	0.57	0.51	0.62	0.51
21	ND	0.03	0.05	0.04	0.04	0.04
Transferases (nmol/min/mg)						
Glucuronyltransferase	0.67 [c]	2.27 [c]	2.44 [c]	2.89 [c]	2.44 [c]	1.48
Glutathione *S*-transferase	0.19 [c]	0.33 [c]	0.52 [c]	0.37 [c]	3.02	2.39
N-acetyltransferase	0.23	0.19	0.29	0.16	0.16	0.37

[a] Values are means from determinations in six different animals. [b] Not detected or below 0.01 nmol/mg or 0.01 nmol/mg/min. [c] Significantly different ($P < 0.05$) from corresponding adult value (6 years of age).

transformation reactions, however, do not increase at the same rate as has been previously described in rats (Cresteil et al, 1986), calves (Shoaf et al, 1987) and pigs (Short and Stith, 1973).

PARTICIPATION OF CYTOCHROMES P4502B AND P4503A IN SHEEP LIVER METABOLISM

Since no previous cytochrome P450 isolation has been carried out from sheep liver, the ovine P4502B and P4503A were isolated from hepatic microsomes of phenobabital- and troleandomycin-treated sheep respectively (Kaddouri et al, 1992; Pineau et al, 1990). The electrophoretic homogeneities were obtained following successive diethylaminoethylcellulose, carboxymethylcellulose and hydroxylapatite separations. Antibodies against these hemoproteins were raised in female rabbits.

Ovine P4502B and P4503A had apparent molecular weights of 50.5 and 52.0 kDa respectively, and their oxidized forms were essentially low spin. The comparison of their 20 NH_2-terminal amino acid sequence led to

the observation of strong homologies with corresponding isoenzymes from rabbit, rat and human livers. By contrast, there were only six amino acids common to the two sheep liver subfamilies. *N*-Demethylation of various substrates by the purified P450 was assayed in a reconstituted system. These experiments indicated high turnover rates in the cases of benzphetamine and erythromycin for P4502B and 3A respectively. This was in good agreement with the specific activities recognized for these two subfamilies in other animal species. A confirmation was obtained from the good correlation between these two demethylase activities and the level of corresponding P450 isoenzyme was determined by Western blot in 18 different ovine liver microsomal preparations.

The IgG fractions prepared from rabbit antisera raised against P4502B or P4503A were also examined for their capacity to inhibit the *N*-demethylation of various drugs and the metabolism of progesterone catalysed by microsomes from phenobarbital- or troleandomycin-treated sheep (table II). In the case of P4502B, no inhibition was observed against the *N*-dealkylation of ivermectin or spiramycin or against 6β-, 16α- or 20α-hydroxylations of progesterone, whereas significant inhibitions occurred in the demethylation of erythromycin, bromhexine, chlorpheniramine, chlorpromazine, ephedrine and more particularly of benz-

Table II. In vitro inhibition of various sheep liver microsomal activities by anti-sheep P4502B and P4503A (from Pineau et al, 1990; Kaddouri et al, 1992; Huan et al, 1995a)[a].

Activity	*Anti P4502B*		*Anti P4503A*	
	2.5 mg	*5 mg*	*2.5 mg*	*5 mg*
N-*Demethylase activities*				
Benzphetamine	34 [b]	67 [b]	9	0
Bromhexine	2	33 [b]	15	43 [b]
Chlorpheniramine	17	32 [b]	24	48 [b]
Chlorpromazine	30 [b]	56 [b]	29	45 [b]
Ephedrine	17	43 [b]	9	28 [b]
Erythromycin	1	46 [b]	35 [b]	61 [b]
Ivermectin	2	11	17	1
Spiramycin	2	1	1	14
Progesterone hydroxylation				
6β	9	0	20 [b]	51 [b]
16α	11	13	24 [b]	45 [b]
20α	13	5	18 [b]	38 [b]
21	28 [b]	42 [b]	14	14
Inhibition of senecionine conversion (%)				
Into dehydroretronecine	–	20	–	59 [b]
N-oxidation	–	4	–	13

[a] Values are mean percentages of inhibition of actual rates of metabolite formation calculated respectively in phenobarbital (2B) and troleadomycin (3A)-treated microsomes in the presence of preimmune IgG (triplicate determinations in three different animals). [b] Significant differences between incubations with antiP450 and preimmune fractions.

phetamine and 21-hydroxylation of progesterone. When anti-P4503A was used, the major differences were the lack of inhibition against benzphetamine *N*-demethylation and the high inhibition of the metabolism of erythromycin and the 6β- 16α- and 20α-hydroxylation of progesterone. These results demonstrated the strong participation of hepatic cytochrome P450 isoenzymes in the oxidation of drugs, namely bromhexine, chlorpheniramine, chlorpromazine or ephedrine, which had not previously been considered as substrates. By contrast, these hemoproteins might be less involved in the oxidative metabolism of ivermectin or spiramycin. Interestingly, cytochrome P4503A may contribute to the reductive conversion of progesterone to 20α-hydroxyprogesterone in sheep liver.

By using the same antisheep P450IgG, Huan et al (1995) demonstrated a major role of sheep P4503A in the toxicogenic conversion of the pyrolizidine alkaloid senecionine into dehydroretronecine. Flavin-containing monooxygenase (FMO) would be the major catalyst in the formation of senecionine-*N*-oxides, which are more water soluble and relatively non toxic.

INCIDENCE OF PATHOLOGIES ON SHEEP HEPATIC DRUG METABOLISM

As is now well established, most pathological states lead to significant decreases in the concentrations of liver drug-metabolizing enzymes. For ruminants, fascioliasis represents one of the more common liver parasitisms and is present throughout the world where the climatic conditions are suitable for snail development, as these are the intermediate hosts for *Fasciola hepatica* and *Fasciola gigantica*. Clinical manifestations of acute or chronic fascioliasis are primarily observed in sheep and cattle and produce significant production losses in apparently healthy animals. In spite of the frequent occurence of this disease, there is only limited information (Facino et al, 1984) about the effects of this infection on hepatic drug biotransformation systems in ruminants. A lamb model for fascioliasis has therefore been developed (Galtier et al, 1986a). The effects of an experimental fascioliasis provoked by oral administration of 150 metacercariae of *F hepatica* were measured in lambs at weeks 4, 8, 12 and 16 after administration and compared to animals without any liver parasitism. Hepatic microsomal cytochrome P450 was significantly decreased in all infected groups of animals. In the early stages of the parasitic disease, decreases in cytochrome b5 and ethoxycoumarin *O*-deethylase contents were observed, whereas aminopyrine *N*-demethylase and benzphetamine *N*-demethylase activities were significantly lowered by 8 and 16 weeks postinfection. A recent investigation (Galtier et al, 1993) demonstrated reduced *N*-dealkylation of veterinary drugs such as bromhexine, chlorpheniramine, ephedrine, erythromycin, imipramine or spiramycin in liver microsomes prepared from sheep infected for 12 and 16 weeks. Among the investigated transferases, glutathione transferase was only decreased by 28% in animals killed 16 weeks after the onset of the infestation; in these animals, a significant increase in microsomal α-glutamyltransferase was observed, probably related to the elevated plasma activity of this enzyme. At eight weeks postinfection, a simultaneous increase in cytosolic calcium (38%) and a decrease in cytosolic glutathione (22%) may correspond to oxidative cell injuries occuring in the course of fascioliasis.

These studies demonstrated the effects of an experimentally-induced subclinical fascioliasis on the hepatic drug-metabolizing system in ruminants. Since infested liver cells lost some of their capacity for drug metabolism, it was supposed that the capacity of the liver for handling drugs and xeno-

biotics could also be reduced with consequences affecting the drug retention in the animal's body. Therefore, a series of investigations was carried out in order to measure the effect of such an experimental fascioliasis on the pharmacokinetics of various tracers and veterinary drugs.

The pharmacokinetics of antipyrine following an intravenous administration of 25 mg/kg to similar young male sheep were determined before and each month following infestation with 150 metacercariae of *F hepatica*, and for eight weeks following a flukicidal treatment (Tufenkji et al, 1988). The parasitic pathology was ascertained by clinical observation of the animals and an increase in plasma antibodies directed against liver flukes. The biological half-life of the parent drug tended to increase (45% by week 12 postinfection) but this increase was not significant because of the large interindividual variability of this parameter. The plasma clearance of antipyrine, however, decreased from 4 to 16 weeks following the infection (by 22–42%) and a 1.7-fold increase in its mean residence time was observed by week 12 postinfection. The urinary excretion of antipyrine metabolites was determined before and eight weeks after the infestation. In sheep, the urinary excretion of the parent drug, norantipyrine or 3-hydroxymethylantipyrine, corresponded to only 1–3% of the administered dose. 3-Hydroxymethylantipyrine was 71–75% conjugated. These values did not differ significantly between healthy and infected groups. The major metabolite was 4-hydroxyantipyrine (36% of the dose) and the 4-hydroxylation of antipyrine was significantly decreased in the case of animals infected for eight weeks. This decrease was particularly significant within the period 2–4 h following drug administration. Of the three mean clearances for production of antipyrine metabolites, the clearance of 4-hydroxyantipyrine alone was significantly decreased in the groups of infected animals. A good correspondence existed between the lowered antipyrine clearance and the decrease in urinary excretion of 4-hydroxymetabolite in infected sheep. This is in agreement with the results of Huffman et al (1974) who recommended the determination of the rate of appearance of 4-hydroxyantipyrine in the urine as the most adequate method for evaluating antipyrin metabolism. Since there was no change in the elimination of either parent drug or its other derivatives, this revealed the selective incidence of the parasitism toward the hepatic 4-hydroxylation of the drug. This also supported the supposition that liver disease may affect different oxidative drug-metabolizing enzymes to differing extents as previously suggested by Farrell et al (1979). The impairment of antipyrine clearance in the course of fascioliasis could be related to a decrease in liver microsomal cytochrome P 450 levels and, more particularly, in the P4502C subfamily, as previously demonstrated in rats (Galtier et al, 1986b).

In a further study, the pharmacokinetics of two common veterinary drugs, ampicillin and pentobarbital, intravenously administered at 10 mg/kg were determined in similarly fluke-infected young male sheep (Tufenkji et al, 1991). If the pharmacokinetics of ampicillin were not significantly affected by the liver parasitism, the disposition of pentobarbital was affected. In the first stages of fascioliasis (weeks 4–12), there were significant increases in the elimination half-life (180%), volume of distribution (120–130%) and mean residence time (143–157%). In animals infected for 4, 8 and 12 weeks, the duration of narcosis was significantly longer (150%) than in control animals or in sheep infected for 17–21 weeks. The evolution of this pharmacodynamic parameter was correlated with that of the elimination half-life of the drug. These changes could have been caused by the inhibition of the liver oxidative metabolism which may have led to higher biological lev-

els of the unchanged parent drug. The prolonged duration of narcosis could be related to reduced in vivo metabolism of pentobarbital, as has been previously demonstrated in animals receiving enzyme inhibitors such as cimetidine (Matsubara et al, 1988), diphenylhydantoin or chlorcyclizine (Cooper and Hawk, 1978).

In another investigation (Galtier et al, 1991), the in vivo *S*-oxidation of albendazole (ABZ), a widely used nematocide drug, was measured from the pharmacokinetic profile of albendazole sulfoxide and albendazole sulfone. Studies were carried out in young male sheep receiving oral albendazole (1.9 mg/kg) considered before and each month after receiving a 150 metacercariae burden. In animals infected for 4–12 weeks, the maximum observed plasma concentrations were unchanged, whereas the corresponding times for plasma maxima were significantly increased, by 15–46% for albendazole sufloxide and by 35–55% for albendazole sulfone, with a consequent increase in the area under the curve of plasma sulfone concentration versus time. A 50–87% decrease in the rate of elimination from the sulfone compartment was correlated with an increased mean residence time for the drug (table III) in the same animals. The slower rate of sulfone elimination would correspond to a decrease in elimination due to cholestasis or the biotransformation of this metabolite. According to Gyurik et al (1981), albendazole sulfone would be hydroxylated with possible further conjugation. In the course of fascioliasis, such metabolic processes could be altered. In a complementary study, a 58% decrease in albendazole sulfonation was demonstrated in liver microsomal preparations obtained from sheep infected for eight weeks (table III), while there was no change in the flavine mooxygenase-directed sulfoxidation of albendazole. The hemoprotein cytochrome P450 is sensitive to fluke infection, and is known to be involved in albendazole sulfonation; its decreasing concentration in infected livers could explain the changes in the pharmacokinetics of albendazole.

Table III. Changes in mean residence time (MRT) and associated parameters of benzimidazole anthelmintics in the course of experimental ovine fascioliasis (from Galtier et al, 1991, 1994) [a].

Parameter	*Control*	*Weeks post-infection*			
		4	*8*	*12–13*	*16–19*	*25*
Albendazole (oral, 1.9 mg.kg^{-1})						
MRT (h)	17.2	32.4	59.4 *	30.5	18.7	
ABZ sulfonation [b]	17	–	7 *	–	15	
Mebendazole (oral, 25 mg.kg^{-1})						
MRT (h)	17.9	20.9	21.9	27.2 *	48.6 *	30.8 *
MBZ reduction [c]	1.72			0.72 *		
Thiabendazole (oral, 50 mg.kg^{-1})						
MRT (h)	12.8	12.8	11.7	13.2	12.2	12.6

[a] Values are means of determinations in four or five different animals. [b] ABZ sulfonation: hepatic microsomal albendazole sulfonation (mmol/min•mg). [c] MBZ reduction: hepatic microsomal mebendazole ketone reduction (nmol/min•mg). * Significantly different ($P < 0.05$) from corresponding control values.

Recently, the pharmacokinetics of mebendazole (MBZ) and thiabendazole were determined before and 4–25 weeks following a similar experimental fascioliasis (Galtier et al, 1994). After oral administration of mebendazole (25 mg.kg^{-1}), the parent drug and especially its reduced metabolite were present in the animal's plasma. A significant 1.5–2.7-fold increase in the mean residence time occurred by weeks 13–25 postinfection (table III). This change was related to decreases both in the elimination from the pharmacokinetic compartment representing the reduced metabolite and in the area under the curve of plasma metabolite concentration versus time. A 59% decrease in MBZ reduction was demonstrated in liver microsomes prepared from 12-week-infected sheep. This reductase activity was characterized by NADPH dependency and a peak pH activity of 6.0. It was competitively inhibited by daunomycin. In sheep receiving a 50 mg.kg^{-1} oral dose of thiabendazole, fascioliasis provoked only decreased plasma concentrations of the metabolite 5-hydroxythiabendazole by weeks 4–25 postinfection. This change paralleled an increase in urinary excretion of free metabolites but this was of minor significance in the general fate of the drug because of the prevalence of conjugates in the excretion.

In the case of corticosteroids, the kinetics of intravenously-administered prednisone (0.5 mg/kg) and methylprednisolone hemisuccinate (4 mg/kg) were compared in two groups of sheep before and regularly after fluke infection (Alvinerie et al, 1989). In infected animals, prednisone clearance was significantly reduced by weeks 11 and 23 (21 and 31% respectively) without any change in the distribution of this drug. For the major metabolite prednisolone, both the area under the plasma concentration versus time curve and the mean residence time were increased at the same stages of fascioliasis. When methylprednisolone hemisuccinate was administered to infected sheep, the area under the curve, elimination half-life and mean residence time of its alcohol metabolite were shown to be decreased by weeks 9 and 13 following the infection. These results demonstrated that the parasitic disease would not influence the reductive biotransformation of prednisone into prednisolone but, more probably, it would decrease the further oxidative metabolism via 6β-hydroxylation and conjugation. The major difference with methylprednisolone consisted of the presence of a 6α-methyl group which would prevent a hydroxylation reaction occurring at the 6-position. Since such an oxidative pathway is probably directed by cytochrome P450, the decrease in the activity of such enzymes described in the course of fascioliasis could explain, here again, the alterations observed in both prednisone and prednisolone pharmacokinetics but would not affect methylprednisolone.

CONCLUSION

All these clinical pharmacokinetic studies demonstrated the transient influence of an experimentally-induced fascioliasis on the kinetics of drugs which are largely used in veterinary practice. In the case of natural fascioliasis however, the repetitive infestation of ruminant animals could also lead to more lasting changes in hepatic biotransformation activities. All these studies also demonstrated the need for molecular information about the enzymes of ruminant liver, especially in terms of cytochrome P450 isoenzymes other than P4502B and P4503A, flavine-containing monooxygenases, ketone reductases and glucoronyl- or glutathione-transferases.

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