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The Usefulness of Saliva as a Biological Material for the Determination of Pharmacokinetics of Model Drugs (Antipyrine, Caffeine, Paracetamol) in Calves: Comparative Study

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Abstract: The aim of this study was to evaluate usefulness of saliva as a biological material for determination of pharmacokinetics of model drugs (antipyrine, caffeine and paracetamol) in calves at different age. For the experiment, 30 Black and White calves (BW) at the age of 10 and 40 days were divided into three groups. The calves of group I received antipyrine (10 mg kg^{-1} bw.), group II caffeine (5 mg kg^{-1} bw.) and group III paracetamol (10 mg kg^{-1} bw.). Samples of blood and saliva were collected from all examined animals. The concentration of antipyrine and paracetamol was measured spectrophotometrically. The concentration of caffeine was evaluated by EMIT method. The pharmacokinetics of antipyrine were estimated using one-compartment model whereas the pharmacokinetics of caffeine and paracetamol using non-compartment model. The following pharmacokinetic parameters were evaluated: volume of distribution, relative volume of distribution, mean residence time; biological half-life, metabolic clearance, relative metabolic clearance and level of model drugs-plasma proteins fractions. Results obtained in the experiment (not statistically significant differences in pharmacokinetic parameter values of antipyrine and caffeine and statistically significant according to paracetamol) determined on the basis of model drugs concentration in blood and saliva showed that saliva has potential to be used as a biological material from calves but only for evaluation of pharmacokinetics of antipyrine and caffeine. However, it cannot be used for assessment of pharmacokinetics of paracetamol.

Key words: Antipyrine, caffeine, paracetamol, pharmacokinetic, plasma, saliva, calves

INTRODUCTION

Activity of enzymes which catalyse biotrans-formation of xenobiotics can be evaluated under *in vivo* and *in vitro* conditions. Recently an increasing recognition of *in vivo* methods which involve implementationof model drugs is observed (Boothe *et al*., 1994; De Graves *et al*., 1995; Danielson and Golsteyn, 1996; Engelking *et al*., 1987; Higaki *et al*., 2003; Janus and Grochowina, 2006b).

Antipyrine is a model drug which is used for determination of rate of the first phase of hepaticbio-transformation (mostly oxidation); those reactions are catalysed by microsomal monooxygenases which form complex with cytochrome P450 (CYP450); an enzymatic complex which is crucial in oxidative metabolism of xenobiotics (Monshouwer *et al*., 1994; Depelchin *et al*., 1988; Janus and Suszycka, 1996). Major metabolites of antypirine are: 4-hydroxyandro-stenedione (4-OHA), 3-Hydroxymethylantipyrine (HMA) and Norantipyrine (NORA) (Monshouwer *et al*., 1994; Depelchin *et al*., 1988; Welch *et al*., 1975).

Caffeine (1, 3, 7-trimethylxanthine) is very common pharmalogical product which stimulates e.g., central nervous system and circulatory system (Zylber-Katz *et al*., 1984; Monshouwer *et al*., 1995; Peck *et al*., 1997). Isoenzymes involved in the caffeine metabolism belong to cytochrome P450 complex (mainly CYP1A2 isoform). Major metabolites which are produced as a result of caffeine demethylation in 1, 3 and 7 position are theobromine, paraxanthine and theophylline (Boothe *et al*., 1994; Zylber-Katz *et al*., 1984;

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De Graves *et al*., 1995; Danielson and Golsteyn, 1996). Caffeine is transformed by N-acetylotransferase (NAT) and xanthine oxidase (De Graves *et al*., 1995; Danielson and Golsteyn, 1996; Aramaki *et al*., 1991). This model drug is nearly completely transformed in the liver. Only 2-5% of caffeine after administration into the organism is excreted (in unchanged form) in urine (Boothe *et al*., 1994; Danielson and Golsteyn, 1996).

Paracetamol is derivative of aniline (Engelking *et al*., 1987; Adzu *et al*., 2001; Bannwarth and Pehourcq, 2003). Except its therapeutic use, it is also a tool in pharmacokinetic research as a model substance for examination factors effecting activity of enzymes involved in processes of second phase of hepatic biotrans-formation (Higaki *et al*., 2003; Janus *et al*., 2003; Li *et al*., 2004; Janus and Grochowina 2006a, b).

Bonding of paracetamol with plasma proteins does not inhibit the rate of its elimination due to dissociation of the drug-protein complex (Mansor *et al*., 1991; Bannwarth and Pehourcq, 2003; Li *et al*., 2004). Paracetamol is completely metabolized in the liver (McNamara *et al*., 1991; Allegaert *et al*., 2004b; Bannwarth and Pehourcq, 2003; Li *et al*., 2004). It undergoes coupling reactions mostly with glucuronic acid by glucuronic transferase (Ali *et al*., 1996; Bock *et al*., 1987; Higaki *et al*., 2003; Janus *et al*., 2003) and with sulphite ions by phenylsulfotransferase (Wynne *et al*., 1990; Higaki *et al*., 2003; Janus *et al*., 2003).

Only 3-4% of paracetamol is oxidised into transitional form of N-acetylobenzochinoimine. The reaction is catalized by cytochrome P450 (Flouvat *et al*., 2004; Li *et al*., 2004). In humans adults humans, products of oxidative metabolism of paracetamol are <10% of all its metabolites however, in newborns reach 10-20% (Allegaert *et al*., 2004a, b).

Now a days, saliva is increasingly used as biological material to determine pharmacokinetics of model drugs in humans (Zylber-Katz *et al*., 1984; Posti, 1999; Linday *et al*., 1991; Babalola *et al*., 2004) and animals Lakin *et al*., 1997; Meffin *et al*., 1977; Janus *et al*., 2003; Kennedy *et al*., 2003). However, until now the possibility of using saliva for determination of the range of pharmacokinetic parameters of drugs which would allow for evaluation of both phases of hepatic biotransformation was not investigated.

The aim of this study was to evaluate usefulness of saliva as a biological material for determination of pharmacokinetics of model drugs in calves at different age.

MATERIALS AND METHODS

The experimental material: For the experiment (approved by the localethic committeefor scientific experiments on animals) 30 Black and White calves (BW) at the age of 10 and 40 days were divided into 3 groups: I-antipyrine group, II-caffeine group and III-paracetamol group. During the whole experiment, all animals were kept in the same standard environmental conditions. Before the experiment, external jugular vein catheterization was conducted on all animals. Any drugs which could interact pharmacokinetically and biochemically with antipyrine, caffeine and paracetamol were not administrated to animals during the experiment.

Experimental procedure

Antipyrine test: Calves of group I received intravenous antipyrine at dose of 10 mg kg^{-1} body weight (bw). Blood and saliva samples were collected before administration of antipyrine (0) and then after 1, 2, 4, 6, 8, 12 and 24 h after the drug was given.

Caffeine test: Calves of group I received intravenous caffeine at dose of 5 mg kg^{-1} bw. Samples of blood and saliva were collected before (0) administration of caffeine and then after 1, 2, 4, 6, 8, 12 and 24 h after the drug was given.

Paracetamol test: Calves of group III received intravenous paracetamol at dose of 10 mg kg^{-1} bw. Samples of blood and saliva were collected before (0) administration of paracetamol and then after 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 8 h after administration.

Doses of model drugs and time-points of blood sample collection were established on the basis of the previous experiments (Janus and Suszycka, 1996; Janus *et al*., 2003, 2007).

Blood samples were aseptically collected into tubes with heparin as a coagulant and then centrifuged (4000×g for 15 min) in order to obtain plasma. Simultaneously, saliva was centrifuged in order to obtain mucopolysaccharides. All samples were aliquoted and frozen at -20°C until further use.

The concentration of antipyrine and paracetamol was measured spectrophotometrically. The concentration of caffeine was evaluated by EMIT method (Enzyme Multiplied Immunoassay Technique) (Boothe *et al*., 1994; De Graves *et al*., 1995).

Pharmacokinetic calculations: The pharmacokinetic of antipyrine was estimated using open one-compartment model (Engelking *et al*., 1987). Calculations were carried out on the basis of elimination curves for that model substance. The determinations was made at free phase (β) elimination (Depelchin *et al*., 1988; Welch *et al*., 1975). The levels of pharmacokinetic parameters of caffeine and paracetamol were estimated using non-compartment

model (Aramaki *et al*., 1991; Janus *et al*., 2003). The following pharmacokinetic parameters were evaluated: volume of distribution V_d (l), relative volume of distribution V_d (kg^{-1}), mean residence time $T_{1/2\beta}$ (h); biological half-life (MRT), Metabolic clearance Cl_m (mL min^{-1}), relative metabolic clearance Cl_m (mL/min/kg) and level of model drugs-plasma proteins fractions (F_B).

Statistical analysis: All statistical analysis were conducted with Statistica 6.0 software. The statistical significance of the differences between pharmacokinetic parameters of antipyrine, caffeine and paracetamol in plasma versus saliva in calves at age of 10 and 40 days was determined by Student's t-test.

RESULTS

Results obtained in the experiment are shown in Table 1 and 2. The statistical significance of the differences between pharmacokinetic parameters of antipyrine, caffeine and paracetamol in plasma versus saliva in calves at age of 10 and 40 days are shown in Table 3.

It was reported that levels of pharmacokinetic parameters of all model drugs in calves were changing significantly in an age dependent manner, irrespectively of the type of material (blood or saliva) used for analyzes.

Volume of distribution of antipyrine, caffeine and paraceta mol between 10th and 40th day of experiment increased of 35.4, 30.7; 38.6, 34.3, 40.0 and 44.4%, respectively for plasma and saliva. The different pattern was obtained for distribution coefficient which was decreasing with age (antipyrine: -9.8 and -12.9%; caffeine: -8.9 and -10.5%; paracetamol: -20.1 and -14.5%). Mean residence time was shortening with animals age (12.5 and 12.6% antipyrine; 11.0 and 10.1% caffeine; 25.0 and 22.1% paracetamol, respectively). Similar changes were also observed for half-life parameter: ($T_{1/2\beta}$): -12.8 and -13.0%; -9.5 and -8.5%; -21.0 and -22.5%, respectively. Changes of MRT and $T_{1/2\beta}$ values effected in an increased of absolute and relative metabolic clearance of antipyrine, caffeine and paracetamol in an age-depending pattern (absolute clearance antipyrine 73.3 and 68.3%; caffeine: 61.1 and 58.9%; paracetamol: 100.3 and 95.0% and also relative clearance 13.0 and 12.4%; 8.2 and 6.8%; 14.6 and 9.9%, respectively).

Percentage differences in values of pharmacokinetic parameters calculated on the basis of antipyrine, caffeine and paracetamol concentration in blood and saliva were: (10th day) antipyrine -V_d (l)+2.8%, V_d (l kg^{-1})+2.9%, MRT +1.8%, $T_{1/2\beta}$+1.7%, Cl_m (mL min^{-1}) -5.5%, Cl_m (mL/min/kg) -4.9%; caffeine -V_d (l)+1.5%, V_d (kg^{-1})+1.5%, MRT+1.6%, $T_{1/2\beta}$+2.6%, Cl_m (mL min^{-1}) -4.5%, Cl_m (mL/min/kg) -4.6%; paracetamol -V_d (l) -11.2%, V_d (l kg^{-1}) -8.0%, MRT -15.1%, $T_{1/2\beta}$ -16.9%, Cl_m (mL min^{-1}) +13.2%, Cl_m (mL/min/kg) +11.6% and at 40th day: +6.6, +6.5, +1.2, +1.9, -2.8 and -4.4% (antipyrina); +3.3, +3.3, +1.5, +1.5, -3.2 and -3.1% (caffeine); -14.1, -14.2, -18.5, -18.1, +16.3 and +16.1% (paracetamol), respectively.

Coefficient of distribution of the investigated model drugs in plasma and saliva of calves was between antipyrine 0.973-0.981; caffeine 0.965-0.975; paracetamol 0.821-0878, respectively. The volumes of bound fraction used in the model drug experiment with plasma proteins were: 1.9-2.7% (antipyrine); 2.5-3.5% (caffeine)

Table 1: Pharmacokinetics of antipyrine, caffeine, paracetamol in plasma and saliva of 10 days old calves (x±s)

Pharmacokinetic parameters	Antipyrine		Caffeine		Paracetamol	
	Plasma	Saliva	Plasma	Saliva	Plasma	Saliva
V_d (l)	28.80±3.10	28.00±2.50	27.20±2.90	26.80±2.70	28.00±3.00	31.50±3.30
V_d (l kg^{-1})	0.72±0.06	0.70±0.08	0.68±0.05	0.67±0.07	0.70±0.06	0.76±0.09
MRT (min)	720.00±60.0	707.00±68.0	685.00±59.0	674.00±65.0	100.00±9.00	118.00±15.0
$T_{1/2\beta}$ (min)	705.00±54.0	693.00±62.0	667.00±63.0	650.00±49.0	89.00±7.00	107.00±19.0
Cl_m (mL min^{-1})	30.70±3.30	32.50±3.90	34.20±4.10	35.80±3.20	296.80±30.2	262.30±31.3
Cl_m (mL/min/kg)	0.77±0.09	0.81±0.08	0.85±0.10	0.89±0.07	7.42±0.84	6.65±0.79
FB (%)	1.90		2.50		12.20	

Table 2: Pharmacokinetics of antipyrine, caffeine, paracetamol in plasma and saliva of 40 days old calves (x±s)

Pharmacokinetic parameters	Antipyrine		Caffeine		Paracetamol	
	Plasma	Saliva	Plasma	Saliva	Plasma	Saliva
V_d (l)	39.00±4.20	36.60±2.90	37.20±3.30	36.00±3.80	39.20±3.90	45.50±4.40
V_d (l kg^{-1})	0.65±0.06	0.61±0.08	0.62±0.07	0.60±0.09	0.56±0.05	0.65±0.07
MRT (min)	630.00±55.0	619.00±51.0	615.00±63.0	606.00±69.0	75.00±7.00	92.00±10.0
$T_{1/2\beta}$ (min)	615.00±54.0	603.00±59.0	604.00±57.0	595.00±46.0	69.00±8.00	84.00±9.00
Cl_m (mL min^{-1})	53.20±4.70	54.70±5.10	55.10±49.0	56.90±5.30	595.00±60.1	511.50±49.3
Cl_m (mL/min/kg)	0.87±0.07	0.91±0.09	0.92±0.11	0.95±0.08	8.50±0.92	7.31±0.89
FB (%)	2.70		3.50		17.90	

Table 3: The statistical significance of the differences between pharmacokinetic parameters of antipyrine, caffeine and paracetamol in plasma versus saliva in calves at age of 10 and 40 days

Pharmacokinetic parameters	Antipyrine	Caffeine	Paracetamol
	----------Plasma vs. Saliva----------		
V_d (l)	p>0.05	p>0.05	p>0.05
V_d (l kg^{-1})	p>0.05	p>0.05	p>0.05
MRT (min)	p>0.05	p>0.05	p>0.01
$T_{1/2\beta}$ (min)	p>0.05	p>0.05	p>0.01
Cl_m (mL min^{-1})	p>0.05	p>0.05	p>0.01
Cl_m (mL/min/kg)	p>0.05	p>0.05	p>0.01

and 12.2-17.9% (paracetamol) of the level of the present concentration of those drugs in plasma, respectively. The bound fraction values were increasing with the age of animals.

DISCUSSION

A decrease of V_d - l kg^{-1} and an increase of V_d - l for antipyrine, caffeine and paracetamol were observed in humans and different species of animals (Hahn *et al*., 2000; Allegaert *et al*., 2004a, b). The results obtained in this experiment are similar to the previous findings (Janus and Suszycka, 1996; Janus and Grochowina, 2006a; Janus *et al*., 2007) and also are consistent with other experiments performed in pigs (Monshouwer *et al*., 1995), sheep (Danielson and Golsteyn, 1996), rabbits (McNamara *et al*., 1991), camels (Ali *et al*., 1996, Wasfi *et al*., 2000), horses (Engelking *et al*., 1987; Peck *et al*., 1997; Aramaki *et al*., 1991), donkeys (Peck *et al*., 1997) and dogs (Boothe *et al*., 1994).

Results of the experiment: Significant shortening of MRT and biological half-life used in the model drug research had the effect on the values of metabolic clearance of antipyrine, caffeine and paracetamol in calves (significantly higher values obtained in older calves 40 days old, compared to calves at neonatal stage 10 days old). Similarly to absolute and relative (l kg^{-1}) volume of distribution also changes in MRT, $T_{1/2\beta}$ and Cl_m parameters are agree with previous findings from the experiments carried out in other animal species (Boothe *et al*., 1994; Monshouwer *et al*., 1995; Ali *et al*., 1996; Danielson and Golsteyn, 1996; Engelking *et al*., 1987; Peck *et al*., 1997; McNamara *et al*., 1991; Wasfi *et al*., 2000; Aramaki *et al*., 1991). Many studies proof that humans and animals' newborns have a lower metabolic efficiency of liver (Depelchin *et al*., 1988; Janus and Suszycka, 1996; Kearns and Reed, 1989; Kawalek and El-Said, 1990; Janus and Grochowina, 2006a). It is a consequence of reduced activity of several enzymatic complexes (Kearns and Reed, 1989; Kawalek and El-Said, 1990). However, many factors cause the increase of the ability of hepatocytes to biotransformation e.g., in the case of CYP450 complex, it is a change in proportion of cytochrome reductase and phospholipids. With age that proportion changes in favor of RED CYP450 (Kawalek and El-Said, 1990). In very young animals and newborns, it is 20/80%, however full metabolic efficiency of that complex is observed at ratio 50/50% (Kearns and Reed, 1989). Similar effect was reported also for other enzymatic systems that catalyze biotransformation of other model drugs: caffeine caused an increased in molar ratio of N-acetylotransferase to xanthine oxidase (De Graves *et al*., 1995; Danielson and Golsteyn, 1996) and paracetamol (Engelking *et al*., 1987; McNamara *et al*., 1991; Janus *et al*., 2003). Coupling of paracetamol and sulfate is a major metabolic pathway for that drug in young organisms which have not fully active glukuronylotransferse system (De Wildt *et al*., 1999; Miners *et al*., 1990; Janus *et al*., 2003). Previous studies have also found that an increase in intensity of glucuronidation processes follows a decrease in intensity of sulfation and vice versa (Wynne *et al*., 1990; Higaki *et al*., 2003; Allegaert *et al*., 2004a; Li *et al*., 2004).

Predominance of one of those two main metabolic pathways (glucuronidation and sulfation) for that drug is species dependent (Monshouwer *et al*., 1994; Ali *et al*., 1996; De Wildt *et al*., 1999; Adzu *et al*., 2001; Janus *et al*., 2003; Flouvat *et al*., 2004). Higher activity of enzymes involved in glucuronidation compared to sulfation was reported in ruminates versus monogastric animals (Monshouwer *et al*., 1994; Ali *et al*., 1996). Also of not is the observation that in young organism processes of sulfation proceed about two times faster than glucuronidation (Miller *et al*., 1976; Janus *et al*., 2003; Li *et al*., 2004). Non-invasiveness of sample collection is a very important advantage of using saliva as a biological material during the pharmacokinetic research (Meffin *et al*., 1977; Posti, 1999).

Concentration of drug in saliva is a resultant of many factors (Zylber-Katz *et al*., 1984; Lakin *et al*., 1997). One of the major quantitative parameter of substance transportation (including model drugs) from blood to saliva is the transfer coefficient which represents the ratio of substance concentration in saliva to its concentration in plasma (Posti, 1999). The levels of that coefficient are close to value one, observed when the rate of drug transfer is higher or equal with rate of saliva secreting (Lakin *et al*., 1997; Linday *et al*., 1991). It was showed that majority of drugs diffuse to saliva and their ability to penetrate is correlated positively with lipophilicity and correlated negatively with particles size (Posti, 1999;

Kennedy *et al*., 2003). It was also observed that ionized form of substance (drug) can infiltrate biological barriers at lower ratio then neutral form (Zylber-Katz *et al*., 1984; Lakin *et al*., 1997; Meffin *et al*., 1977). It should be emphasized that particular attention is needed when comparing results obtained in experiments carried out in humans and different animal species an infiltration of pharmacological substances from blood to saliva is species dependent (Lakin *et al*., 1997; Posti, 1999; Linday *et al*., 1991; Welch *et al*., 1975). Comparison of drug concentration values in saliva and plasma allows for determination of range of free fraction of the particular pharmacological substance (Posti, 1999; Linday *et al*., 1991; Kennedy *et al*., 2003).

It is known thatonly free fractions of drugs are able to penetrate biological membranes, bond with receptors and be transformed (Lakin *et al*., 1997; Posti, 1999). The range of free fraction of drugs depends on various different factors like total drug concentration, affinity to proteins, protein concentration, presence of endo and exogenic substances (Lakin *et al*., 1997; Babalola *et al*., 2004; Welch *et al*., 1975).

Obtained results showed that antipyrine and caffeine were bonded by plasma proteins at a very low level and paracetamol at a moderate level. Slightly higher values according to antipyrine (3.3-4.8%) and caffeine (3.5-5.2%) were observed in humans (Posti, 1999; Linday *et al*., 1991; Welch *et al*., 1975).

CONCLUSION

In this study, the results obtained in the experiment (not statistically significant differences in pharmacokinetic parameter values of antipyrine and caffeine and statistically significant according to paracetamol) determined on the basis of model drugs concentration in blood and saliva showed that saliva has potential to be used as a biological material from calves but only for evaluation of pharmacokinetics of antipyrine and caffeine. However, it cannot be used for assessment of pharmacokinetics of paracetamol.

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