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 EMT 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 biotransformation of xenobiotics can be evaluated under in vivo and in vitro conditions. Recently an increasing recognition of in vivo methods which involve implementation of model drugs is observed (Boothe et al., 1994; De Graves et al., 1995; Danielson and Golsteyn, 1996; Engellking 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 hepatic biotransformation (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; Depechlin et al., 1988; Janus and Suszycka, 1996). Major metabolites of antipyrine are 4-hydroxyandro-stenedione (4- OHA), 3-Hydroxymethylandipyrine (HMA) and Norantipyrine (NORA) (Monshouwer et al., 1994; Depechlin et al., 1988; Welch et al., 1975).

Caffeine (1, 3, 7-trimethylxanthine) is very common pharmacological 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 isofrom). 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;
De Graves et al., 1995; Danielson and Golsteyn, 1996). Caffeine is transformed by N-acetyltransferase (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; Adjau et al., 2001; Bannwarth and Pehourec, 2003). Except its therapeutic use, it is also a tool in pharmacokinetic research as a model substance for examining factors affecting activity of enzymes involved in processes of second phase of hepatic biotransformation (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 Pehourec, 2003; Li et al., 2004). Paracetamol is completely metabolized in the liver (McNamara et al., 1991; Allegaert et al., 2004b; Bannwarth and Pehourec, 2003; Li et al., 2004). It undergoes coupling reactions mostly with glucuronic acid by glucuronide transferase (Ali et al., 1996; Bock et al., 1987; Higaki et al., 2003; Janus et al., 2003) and with sulphite ions by phenylsulphotransferase (Wynne et al., 1990; Higaki et al., 2003; Janus et al., 2003).

Only 3-4% of paracetamol is oxidised into transitional form of N-acetylbenzochinolinone. The reaction is catalysed 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; Lindsay et al., 1991; Babalola et al., 2004) and animals (Lakin et al., 1997; Meehan 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 local ethical committee for 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 (b) elimination (Depelchin et al., 1988; Woleh 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 $V_d/\text{kg}$), mean residence time $T_{\text{res}}$ (h), biological half-life (MRT), Metabolic clearance $Cl_{\text{m}}$ (mL/min/kg), relative metabolic clearance $Cl_{\text{r}}$ (mL/min/kg), and level of model drugs-plasma proteins fractions ($F_{\text{p}}$).

**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.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Antipyrine</th>
<th>Caffeine</th>
<th>Paracetamol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Saliva</td>
<td>Plasma</td>
</tr>
<tr>
<td>$V_d$ (l)</td>
<td>28.80±3.10</td>
<td>28.00±2.50</td>
<td>27.20±2.90</td>
</tr>
<tr>
<td>$V_d$ (kg)$^{-1}$</td>
<td>0.72±0.06</td>
<td>0.70±0.08</td>
<td>0.68±0.05</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>720.00±60.0</td>
<td>700.00±80.0</td>
<td>685.00±59.0</td>
</tr>
<tr>
<td>$T_{\text{res}}$ (min)</td>
<td>705.00±54.0</td>
<td>693.00±62.0</td>
<td>667.00±63.0</td>
</tr>
<tr>
<td>$Cl_{\text{m}}$ (mL/min)$^{-1}$</td>
<td>30.70±3.30</td>
<td>32.50±3.90</td>
<td>34.20±4.10</td>
</tr>
<tr>
<td>$Cl_{\text{r}}$ (mL/min/kg)</td>
<td>0.77±0.09</td>
<td>0.81±0.08</td>
<td>0.85±0.10</td>
</tr>
<tr>
<td>$F_{\text{p}}$ (%)</td>
<td>1.90</td>
<td>2.50</td>
<td>7.42±0.84</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Antipyrine</th>
<th>Caffeine</th>
<th>Paracetamol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Saliva</td>
<td>Plasma</td>
</tr>
<tr>
<td>$V_d$ (l)</td>
<td>39.00±4.20</td>
<td>36.60±2.90</td>
<td>37.20±3.30</td>
</tr>
<tr>
<td>$V_d$ (kg)$^{-1}$</td>
<td>0.65±0.06</td>
<td>0.61±0.08</td>
<td>0.62±0.07</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>630.00±55.0</td>
<td>619.00±51.0</td>
<td>615.00±63.0</td>
</tr>
<tr>
<td>$T_{\text{res}}$ (min)</td>
<td>615.00±54.0</td>
<td>603.00±59.0</td>
<td>604.00±57.0</td>
</tr>
<tr>
<td>$Cl_{\text{m}}$ (mL/min)$^{-1}$</td>
<td>53.20±4.70</td>
<td>54.70±2.10</td>
<td>55.10±4.90</td>
</tr>
<tr>
<td>$Cl_{\text{r}}$ (mL/min/kg)</td>
<td>0.87±0.07</td>
<td>0.91±0.09</td>
<td>0.92±0.11</td>
</tr>
<tr>
<td>$F_{\text{p}}$ (%)</td>
<td>2.70</td>
<td>3.50</td>
<td>17.90</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Antipyrine</th>
<th>Caffeine</th>
<th>Paracetamol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vd (l)</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Vd (l/kg)</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>T1/2 (min)</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Cl (ml/min/kg)</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

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 $V_d - 1\, \text{kg}^{-1}$ and an increase of $V_d - 1$ 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 Grochowinska, 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 (1 kg$^{-1}$) volume of distribution also changes in MRT, T$_{1/2}$ and Cl$_n$ 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 (Depechini et al., 1988; Janus and Suszycka, 1996; Kearns and Reed, 1989; Kawalek and El-Said, 1990; Janus and Grochowinska, 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 80/80%, 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-acetyltransferase 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 glucuronyltransferase 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 (Zyber-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 secretting (Lakin et al., 1997; Lindsey 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;
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; Lindsey 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; Lindsey et al., 1991; Kennedy et al., 2003).

It is known that only 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; Lindsey 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.

REFERENCES


