Pharmacokinetics of oral caffeine in sows: a pilot study

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SUMMARY
Introduction - Caffeine (1,3,7 trimethylxanthine) is a natural alkaloid and one of the most widely employed psychoactive substances which induces several important stimulatory effects on central nervous system such as enhanced alertness, decreased fatigue and stimulation of respiratory center. Caffeine, which is currently employed for the treatment of neonatal hypoxia in humans, could also be beneficial against perinatal asphyxia in piglets, that is responsible of high mortality rates and poor weight gain.

Aim - The aim of this study was a preliminary assessment of caffeine pharmacokinetic parameters in sows. Moreover, this study could provide information on caffeine pharmacokinetics in pigs which could be of great help for the possible therapeutic use of this drug, as in vivo information regarding ADME (Absorption Distribution Metabolism Excretion) behaviour of the methylxanthine in this species are thus far lacking.

Materials and methods - In our experimental design, a single dose of 25 mg/kg of caffeine was given orally to six healthy sows, mixed with 200 g of lactation feed given before morning meal. Blood samples were collected at specific times and caffeine levels in plasma were measured by means of HPLC method. The dose was chosen according to a previously published study. The method was intra-laboratory validated according to EMA guidelines (2012).

Results and discussion - No adverse effects were observed in the animals after caffeine administration. Caffeine concentration reached a peak (Cmax = 20.02 ± 1.51 µg/mL) at 9.51 ± 1.17 h and declined displaying a bi-phasic behaviour. Our pharmacokinetic data were suggestive of a slower elimination compared to humans, monkeys, rabbits and rodents, while they were similar to those observed in other species like sheep, cattle and equines. An interesting clinical implication of our study could be suggested by the concentration-time curve of orally administered caffeine, showing plasma levels at 24 h after treatment of 13.77 ± 0.97 µg/mL. Such blood levels of caffeine are similar to those detected in human neonates treated for perinatal hypoxia. Since caffeine is known to freely cross the placenta and, therefore, to reach the same levels in fetal as in maternal blood, our data seem to support a previous study, in which oral caffeine administration to sows the day before parturition was able to improve the vitality of newborn piglets.

Conclusions - This study provides first data about pharmacokinetics of caffeine in pigs and suggests a possible clinical utility in swine. However, further experiments with diverse doses and routes of administration of caffeine, and in farrowing sows, are needed to enlighten its pharmacokinetic profile.

KEY WORDS
Pharmacokinetics, caffeine, pig.

INTRODUCTION
Caffeine (1,3,7 trimethylxanthine) is a natural alkaloid and one of the most widely employed psychoactive substances, since it is present in several foods (coffee, tea, chocolate, energy drinks) as well as in over-the-counter drug formulations or in combination with analgesics for mild to moderate pain. Caffeine induces several important stimulatory effects on central nervous system such as enhanced alertness, decreased fatigue and stimulation of respiratory centre. Moreover, analeptic effect of caffeine is enhanced by peripheral actions like increased respiratory muscle performance and mild bronchodilation, and this methylxanthine has indeed shown to be effective against neonatal apnoea in humans. Pharmacological effects of caffeine are mostly due to its antagonism at adenosine A1 and A2 receptors, although, at higher concentrations, it is also an inhibitor of phosphodiesterase, thus increasing AMPc intracellular content. Since adenosine reduces the release of several excitatory neurotransmitters in the brain, caffeine is able to enhance the effects of these mediators, thus inducing a complex array of central effects. Caffeine might be useful in the treatment of post-partum hypoxia in other species beside humans, like in pigs, which are very prone to suffer from perinatal asphyxia that is responsible of high mortality rates or poor weight gain. Indeed, oral caffeine administered in newborn piglets with perinatal hypoxia was shown to be effective in improving several metabolic parameters. Furthermore, in a previous study, a high
dose of caffeine was able to improve mammary gland development and milk yield in sows without affecting weight gain of the piglets. Since the administration of a single dose of caffeine to newborn piglets could be troublesome, an interesting option might be the administration of an appropriate dose of caffeine to parturient sows, as methylxanthines are able to easily cross the placental barrier. Indeed, a beneficial effect of oral administration of caffeine in parturient sows against neonatal hypoxia in piglets was recently demonstrated.

The aim of this study was a preliminary assessment of caffeine pharmacokinetic parameters in sows. Moreover, this study could provide information on caffeine pharmacokinetics in pigs which could be of great help for the possible therapeutic use of this drug, as in vivo information regarding ADME (Absorption Distribution Metabolism Excretion) behaviour of the methylxanthine in this species are thus far lacking.

MATERIAL AND METHODS

Six healthy sows (third to fifth parity order, average weight 200 kg) were used in the study. The animals did not receive any other medication for at least 15 days prior to or during the study. The study design was approved by the Local Ethics Committee according to national laws (Prot. N° 61/12).

A single dose (25 mg/kg b.w.) of caffeine (Sigma-Aldrich, Milan, Italy) was administered orally mixed with 200 g of standard lactation feed, given before morning meal. The dose was chosen according to a previous study.

After cleaning and disinfection of the neck region, catheter was placed into the right jugular vein, in order to make the collection of blood easier and less stressful for the animals. Blood (5 mL) was collected at 0 (pre-dose), 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 14, 20, 24, 32, and 48 h after treatment; it was centrifuged within one hour after collection at 2000x g.

For the obtained sample of blood, the methylxanthines were extracted by acidification with acetonitrile, and the mixture was placed in vortex mixer for 4.6 min. 100 µL of β-hydroxyethyl-theophylline, was added to 700 µL of plasma. The plasma proteins were precipitated by addition of 300 µL of acetonitrile, and the mixture was placed in vortex mixer for 30 sec and then centrifuged at 7500xg for 10 min. 100 µL of the supernatant were put in a vial and evaporated to dryness under a gentle stream of nitrogen and then reconstituted with 100 µL of water. 10 µL of the obtained sample were injected into the HPLC system.

The obtained mean recovery of caffeine and IS were 98.1 ± 3.6% and 97.5 ± 1.8%, respectively. The recovery was reproducible over seven replications performed over 7 different days. The method was intra-laboratory validated according to EMA guidelines (2012). The specificity of the method was tested by analyzing pig plasma samples before the administration of caffeine. No interfering peaks were observed at the elution times of caffeine or IS. Calibration graphs for caffeine (n = 6) were constructed over the concentration range of 0.1-100 mg/mL and showed an average correlation coefficient of 0.999. The repeatability was tested by analyzing samples of pig plasma spiked with caffeine. Samples were spiked at the levels of 1 mg/mL (low concentration), 20 mg/mL (medium concentration), and 100 mg/mL (high concentration). All samples were measured in triplicate on the same day. For the within-laboratory reproducibility test, each spiked level was tested in triplicate over seven days. The results of these experiments were used also for the determination of the recovery. The accuracy of the estimated caffeine concentration was more than 90% at three concentrations. The precision expressed as inter-day coefficient of variation (CV%) ranged from 3.8% to 6.5% and the intraday CV% ranged from 1.5% to 4.0%. The sensitivity of the method was expressed as the LOQ (Limit Of Quantification), which is the minimum concentration of caffeine in plasma that can be quantitatively determined with a peak height to baseline ratio of at least 10:1, and the LOD (Limit Of Detection), which has a peak height to baseline ratio of 3:1. Caffeine LOD and LOQ were found to be 0.01 and 0.1 mg/mL, respectively.

Caffeine plasma concentration-time curves were modelled for each subject using a mono, bi- or multi-compartmental open model. Comparison between competing models was made using the residual plots, visual inspection of the goodness of fit curves, and Akaike’s information criterion. A bi-compartmental model best fitted the plasma concentrations after oral administration in all the sows. As a result, the serum concentration-time curves of caffeine after a single oral dose were fitted to the following equation:

\[ C_{p.o.} = Ae^{-at} + Be^{-bt} - Ce^{-kat} \]

where \( C_{p.o.} \) is the concentration of caffeine at time \( t \) after oral administration; \( A \) and \( B \) are the zero-time plasma drug concentration intercepts of biphasic p.o. disposition curves; \( e \) is the base of the natural logarithm; \( C \) is the zero-time plasma drug concentration intercept of the absorption phase; \( a \) is the slope of distribution phase; \( b \) is the slope of elimination phase, and \( ka \) is the slope of absorption phase.

Pharmacokinetic analysis was performed using the software Phoenix WinNonlin ver.4.1 (Pharsight Co., St. Louis, USA). All data were expressed as mean ± SE.

Animals were evaluated daily by trained personnel up to 5 days after the completion of the study for visible adverse effects. Given the well-known strong central effects of methylxanthines, particularly at high doses, all the sows enrolled in the study were clinically evaluated for the occurrence of tremors, diarrhoea, anxiety, convulsions: moreover, a clinical examination was performed about heart rate and rhythm.

RESULTS

No adverse effects were observed in the animals after caffeine administration. Concentration-time curve is shown in Figure 1. Caffeine was already detected in plasma of sows 15
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pharmacokinetic parameters are summarized in Table 1. Residence Time) of 26.31 ± 2.50 h was calculated. Main where at 48 h it was 3.83 ± 0.66 µg/mL, and a MRT (Mean Residence Time) of 26.31 ± 2.50 h was calculated. Main pharmacokinetic parameters are summarized in Table 1.

Table 1 - Main pharmacokinetic parameters of caffeine in sows after a single oral dose of 25 mg/kg (n = 6).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SE</th>
</tr>
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<tbody>
<tr>
<td>AUC 0-inf (µg.h/mL)</td>
<td>669.9 ± 12.75</td>
</tr>
<tr>
<td>AUC 0-last (µg.h/mL)</td>
<td>601.0 ± 29.07</td>
</tr>
<tr>
<td>Cl/F (mL/h/kg)</td>
<td>40.37 ± 0.74</td>
</tr>
<tr>
<td>Vc (L/kg)</td>
<td>0.44 ± 0.17</td>
</tr>
<tr>
<td>V/F (L/kg)</td>
<td>0.77 ± 0.18</td>
</tr>
<tr>
<td>t1/2abs (h)</td>
<td>1.24 ± 0.55</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>13.33 ± 3.13</td>
</tr>
<tr>
<td>t1/2max (h)</td>
<td>5.37 ± 1.55</td>
</tr>
<tr>
<td>K12 (h⁻¹)</td>
<td>0.98 ± 0.81</td>
</tr>
<tr>
<td>K21 (h⁻¹)</td>
<td>0.47 ± 0.10</td>
</tr>
<tr>
<td>K10 (h⁻¹)</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>Ka (h⁻¹)</td>
<td>0.17 ± 0.05</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>9.51 ± 1.17</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>20.02 ± 1.51</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>26.31 ± 2.50</td>
</tr>
<tr>
<td>R²</td>
<td>0.92 ± 0.02</td>
</tr>
</tbody>
</table>

AUC 0-inf area under the curve from time 0 to infinity; AUC 0-last, area under the curve from time 0 to the last sample drawn; Cl/F, apparent plasma clearance; Vc, volume of the central compartment; V/F, apparent volume of distribution; t1/2abs, distribution half-life; t1/2, elimination half-life; Ka, rate constant compartment 1 to 2; K10, rate constant compartment 2 to 1; K12, rate constant compartment 1 to 0; Cmax, absorption rate constant; T1/2, time to peak concentration; Cmax, peak concentration; MRT, mean residence time; R², correlation coefficient.

DISCUSSION AND CONCLUSIONS

To our knowledge, there are no published information about the pharmacokinetics of caffeine in pigs. In our experimental conditions, caffeine seemed to be absorbed rather slowly, reaching peak concentration at 9.5 h. This data is consistent with the calculated absorption rate constant (Kabs) of 0.2 h⁻¹, and with an absorption half-life (t1/2abs) of 5.4 h. However, we cannot exclude an interference on caffeine absorption by feed coadministration.

Caffeine is demethylated in the liver mainly by CYP1A2 and CYP2E1 enzymes to mono and dimethylxanthines paraxanthine, theophylline and theobromine. However, marked interspecies differences were detected in caffeine metabolism pathways, which could influence the elimination pharmacokinetic parameters. As a matter of fact, the obtained elimination half-life (t1/2) of caffeine in sows was higher (13.3 h) than those previously reported in humans, monkeys, rabbits, rats or mice (4.2, 3.2, 1.6, 0.8, 0.7 h, respectively). By contrast, in ruminants like sheep and cattle, calculated t1/2 values (8.9-15.7 h and 8.1 h, respectively) are closer to that found in our study.

Moreover, MRT of caffeine in sows (26.3 h) was quite similar to those calculated in equines.

A recent study in microminipigs reported a t1/2 for oral caffeine of 11 or 7.5 h in male and female subjects, respectively, which are lower values compared with our results. This difference in the elimination times could be due to the higher dose employed in our experimental design, since caffeine pharmacokinetics was seen to be non-linear, and clearance was significantly reduced by increasing caffeine dose. As reported in this study by Kaplan et al., it has been evidenced that caffeine pharmacokinetics in humans is dose-dependent. It is therefore possible that the difference with the results obtained in microminipigs could be due to the higher dose administered.

However, a strict comparison between our data and those by Mogi et al. cannot be made, due to the fact that a simultaneous administration of multiple drugs was performed, and also that a peculiar variety of pig was employed.

Further experiments with other doses of caffeine will be however necessary in order to better clarify the pharmacokinetic behaviour of this substance in the pig. It would be also of great interest to assess the blood concentrations of caffeine by other routes of administration, and to detect which are the main metabolites in this species.

An interesting clinical implication of our study could be suggested by the concentration-time curve of orally administered caffeine, showing high plasma levels 24 h after treatment. Indeed, caffeine is known to freely cross the placenta and, therefore, to reach the same levels in fetal as in maternal blood. Our data seem to corroborate a previous study, in which oral caffeine administration to sows the day before parturition was able to improve the vitality of newborn piglets against hypoxia.

Moreover, a previous study evidenced that blood levels of caffeine in humans treated for apnea of prematurity, were...
consistent with caffeine levels measured in the sows at 24 h; therefore, it is hypothesizable that, during labor and in the moment of birth, the piglets might have analeptic blood levels of caffeine. However, further studies about pharmacokinetics of diverse doses of caffeine in farrowing sows are needed to better clarify the feasibility of a prenatal treatment of peripartum hypoxia in piglets.

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