Doping control in horses: housing conditions and oral recycling of flunixin by ingestion of contaminated straw

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Nonsteroid anti-inflammatory drugs (NSAID) such as flunixin, phenylbutazone and naproxen are prohibited substances in competition horses, and they are subjected to regular doping controls. The aforementioned NSAIDs are also reported to be major contaminants present in the horse environment (Barker, 2008). Norgren et al. (2000) and Wennerlund et al. (2000) reported that untreated horses were excreting flunixin or naproxen for several days when housed in boxes previously used for horses treated with these drugs. Russell and Maynard (2000) have pointed out that contamination of the horses environment with isoxsuprine can lead to the presence of the drug up to 10 weeks after the end of treatment. An experimental study with dipyrone and chlorpheniramine (Duluard et al., 2006) has also drawn attention to the need to control the bedding conditions for horses, to avoid self- or cross-contamination. Spurious urinary excretion profiles in horses, owing to bedding contamination and drug recycling, were also observed with meclofenamic acid administered intravenously (IV) (Popot et al., 2007).

We report here on the flunixin excretion profiles in urine collected from 17 horses subjected to different housing conditions with respect to bedding and management. Flunixin is a NSAID marketed for equine use, either as an injectable at the concentration of 50 mg/mL or as an oral preparation at the concentration of 0.5 g/10 g (Finadyne™: Intervet/Schering-Plough Animal Health, Beaucouze, France), and for which the pharmacokinetics and metabolism have been intensively studied (Jaussaud et al., 1987; Johansson & Anler, 1988; Soma et al., 1988; Toutain et al., 1994; Lee et al., 2004; Pellegrini-Masini et al., 2004). Flunixin is extensively eliminated by urinary clearance (Soma et al., 1988) and as such, flunixin is a drug for which recycling from bedding ingestion could be expected.

In this observational survey, flunixin was administered either by the IV route at 1 mg/kg in one single administration or by the oral route at a dosing regimen of 0.5 mg/kg twice a day for 3 days. For the oral route, the paste was given outside the box. For the flunixin IV administration (Finadyne™), six thoroughbreds (two females, two geldings and two stallions) weighing from 450 to 600 kg and aged 2–16 years were used in the experiment. The study on oral flunixin involved six standard-bred horses and three thoroughbreds (four females, three geldings, two stallions) weighing from 450 to 600 kg and aged 2–17 years. A wash-out period of at least 1 month separated the two administration protocols for the horses selected more than once. The horses were accommodated in individual boxes and received a regular diet composed of manufactured feed and hay. Unlimited water was available. The horses were allowed either a moderate exercise daily (20 min of walking, 35 min of trotting, 5 min of galloping) or one hour per day in a paddock. Bedding conditions were the investigated factor and differed by the amount of straw in the box (about 20 vs. 45 kg), the daily cleaning (straw completely vs. not completely removed every day). Daily cleaning means the complete removal of the straw every day. In case of no daily cleaning, a complete cleaning was done once every week, and the messy straw was taken away the other days. For a group of three horses, the subjects were moved to another clean box 24 h after the single IV administration, while for the other horses, they stayed in the same box for the entire observational period. Table 1 details the bedding conditions for the different groups of observed horses. Urine was sampled from spontaneously urinating horses. Based upon results obtained by Sams et al., 1999: urine samples were collected before administration and after from at least 24 h up to 15 days. Urine samples were kept at −20 °C and thawed just before analysis. For quantification, flunixin in plasma and urine was extracted by solid-phase extraction according to the method previously described (Popot et al., 2007) in which an in-house preparation of clonixin was used as internal standard. Flunixin was quantified using a gas chromatography/mass spectrometry in the electronic impact ionization mode (GC/EI-MS) method employed for the screening of NSAID in urine, which was slightly modified for quantification purposes. Linearity was observed from 10 to 300 ng/mL; (when necessary, urine is diluted with blank urine negative for flunixin) the limit of detection was 2 ng/mL and the limit of quantification was 10 ng/mL.

Following a previous study (Jaussaud et al., 1987), urine concentrations of total flunixin (i.e. the sum of flunixin and flunixin glucuronide) were measured. Thus, it is the total...
flunixin vs. time after oral treatment (Fig. 1a) and after IV administration (Fig. 1b,c) that were plotted. Urine samples collected before administration were checked for the presence of flunixin and were all negative. After oral flunixin administration, visual inspection of the plots from postadministration samples after 1 or 2 days, until at least 9 days (Fig. 1a), showed a decrease in urinary flunixin for at least 5 days postadministration. However, spurious rebounds of urinary flunixin were observed (Table 1) for two of three horses kept in boxes bedded with 20 kg of straw that was changed completely every day (Fig. 1a). In this condition (Trial 1a), the maximal urine concentration associated to a rebound was up to 550 ng/mL.

It is important to note that the boxes had been swept as thoroughly as those with the conditions of Trial 1b, but in condition 1b, the quantity of straw was higher (45 vs. 20 kg daily), which probably explains why the rebounds of concentrations that were observed for five of six of the investigated horses did not exceed 27 ng/mL. Similar results were observed in the case of the single IV injection. When the horses were housed in the same box for the entire period of observation after the drug administration, rebounds of flunixin concentration (about 100 ng/mL) were observed when the straw was not changed daily (Trial 2a) and also perhaps very low rebounds when the straw was removed daily. For this last bedding condition, we observed a blip in the depletion curve rather than a clear-cut rebound, and we cannot exclude that this shoulder in the curves was because of a lower dilution of the urine rather than some oral recycling of flunixin. In contrast, no rebound was seen in the horses given flunixin by the IV route when they were definitively removed from their administration box 24 h after the

**Table 1.** Design of the oral and IV flunixin experiment; the doses were 0.5 mg/kg twice a day for 3 days for the oral route and 1 mg/kg for the IV route. Daily cleaning (Trial 2a) means the complete removal of the straw. In the case of no daily cleaning*, a complete cleaning was done once every week and the messy straw has been taken away the other days.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Administration route</th>
<th>Number of horses</th>
<th>Straw quantity (kg)</th>
<th>Daily cleaning</th>
<th>Recourse to a clean box 24 h following administration</th>
<th>Rebounds present</th>
<th>Magnitude of rebounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Oral</td>
<td>3</td>
<td>20</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Large</td>
</tr>
<tr>
<td>1b</td>
<td>Oral</td>
<td>6</td>
<td>45</td>
<td>Yes</td>
<td>No*</td>
<td>Yes</td>
<td>Low</td>
</tr>
<tr>
<td>2a</td>
<td>IV</td>
<td>3</td>
<td>45</td>
<td>No*</td>
<td>No</td>
<td>Yes</td>
<td>Low</td>
</tr>
<tr>
<td>2b</td>
<td>IV</td>
<td>2</td>
<td>45</td>
<td>Yes</td>
<td>No</td>
<td>Perhaps Low if any</td>
<td></td>
</tr>
<tr>
<td>2c</td>
<td>IV</td>
<td>3</td>
<td>45</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

**Fig. 1.** Semi logarithmic plot of flunixin concentrations in urine samples collected from three horses after (a) oral administration of flunixin at the dosing regimen of 0.5 mg/kg twice a day for 3 days (Trial 1a) (A rebound of flunixin concentration of 550 ng/mL and 56 ng/mL was observed for exp 1 and exp 13 respectively 6.5 days after the last administration; no rebound was observed for exp 2); (b) IV administration of flunixin at the dose of 1 mg/kg (Trial 2a) (A rebound of flunixin concentration of 70 ng/mL was observed for exp 519, 5.5 days after administration). (c) IV administration of flunixin at the dose of 1 mg/kg (Trial 2c). Twenty-four hours after the flunixin administration, horses were placed in a clean box until the end of the sampling period. No rebounds of flunixin concentration were observed in this trial.
flunixin administration to be placed in a new and clean box until
the end of the sampling period (Trial 2c). From the present series
of observations, it can be concluded that an apparently good
daily cleaning (i.e. straw completely removed and ground swept)
cannot totally prevent flunixin rebounds but that a lack of daily
cleaning is associated with the largest rebound. In the present
experiment, the only condition in which we did not observe any
spurious rebound is the one where the horses were not housed in
the same box beyond 24 h after flunixin administration. The
explanation is as follows: flunixin is mainly eliminated by renal
clearance (Soma et al., 1988), and a large amount of flunixin is
eliminated in urine within the first 24 h following administra-
tion. Then, for horses housed in unclean boxes, the conditions
exist for the possibility of prolonged recycling; the only way to
break recycling is to move the horse in another separate box
after the first 24 h of treatment, rendering unavailable the
flunixin excreted in the urine for the first 24 h. It is also
interesting to note that rebounds are more likely to be observed
when the urine concentration become relatively low i.e. after
some delay after administration (typically 3–4 days); this is
owing to the fact that re-ingested flunixin can increase urine
concentration only during the terminal phase of the flunixin
disposition, when flunixin concentrations are slowly decreasing.

In conclusion, exerting due care and proper stable hygiene
according to available specific guidelines such as recommenda-
tions made in Vogel’s book (Vogel, 2008) drastically decreases
the occurrence of spurious drug excretion profiles for a drug
extensively eliminated in urine as is the case for flunixin for
which urinary elimination is about 75% of the administered dose
(Soma et al., 1988).

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