The bioavailability, pharmacokinetics, plasma and urinary "clearance times" of flunixin (Banamine®:Schering) in horses was investigated. After rapid intravenous (IV) injection of 1 mg/kg of flunixin, plasma levels of the drug peaked at 10 μg/ml 3 minutes after injection of the drug. Thereafter, plasma levels of the drug fell rapidly, with an apparent alpha phase half-life of about 11.8 minutes. This was followed by a slower beta phase half-life of about 1.6 hours. Evidence suggestive of a third phase of distribution was observed, but this putative third phase was too close to the detection limit of our method to allow its unequivocal identification.

After oral administration of flunixin, plasma levels of the drug peaked within 30 minutes and then declined at rates which closely matched those after IV administration of the drug. Flunixin was about 80% bioavailable after oral administration of the drug. Following IV administration urinary levels of flunixin and its alkaline releasable metabolites peaked at about 200 μg/ml and were detectable in urine levels of about 100 ng/ml for up to 48 hours after dosing.

The Pharmacology of Nonsteroidal Anti-inflammatory Drugs in the Horse: Flunixin Meglumine (Banamine®)

Sylvia Chay, B.Sc.; W.E. Woods, M.Sc; T. Nugent; J.W. Blake, Ph.D.; Thomas Tobin, M.R.C.V.S., Ph.D.
Kentucky Equine Drug Research and Testing Programs
Department of Veterinary Science
College of Agriculture
University of Kentucky
Lexington, Kentucky 40546-0076

Please address correspondence to Dr. Tobin.

Introduction

Flunixin meglumine (Banamine®:Schering) is a potent nonsteroidal anti-inflammatory agent which has been approved by the Food and Drug Administration for use in horses in the United States.[1,3] It is now widely used in equine medicine and some jurisdictions have approved its use in racing horses. Previous work on the pharmacokinetics of flunixin in the horse has suggested that it has a plasma half-life of about 1.6 hours.[3,6] This is a very short plasma half-life for a drug which produces pharmacological effects for up to 30 hours after a single dose. Consistent with this apparently very short plasma half-life, however, are reports that flunixin has little ability to interfere with the detection of other drugs in urine samples. Further, no good "detection" or "clearance" time studies for Banamine® in the horse are available.

Because of this lack of information concerning the mode of action of flunixin, its pharmacokinetics, its "detection" times in horses, and its relative lack of interference

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with the detection of other drugs, we elected to study the pharmacology and pharmacokinetics of flunixin in the horse in some detail.

Materials and Methods

DRUG ADMINISTRATION

Mature Thoroughbred and Standardbred mares (335-500 kg) were used in this study. Groups of four horses were used for each experiment. They were kept on pasture and brought into individual loose boxes preceding all experimental procedures. All drug administrations and fluid samplings were performed in the animals' boxes. Weights were determined with a weight tape and doses were calculated on a mg/kg basis.

The injectable form of flunixin meglumine as Banamine® was administered by rapid intravenous injection via the jugular vein. All subsequent blood samples were drawn from the opposite jugular vein used for dosing. For oral administration, the appropriate dose of the granular form of Banamine® was dissolved in water and administered through a stomach tube.

SAMPLE COLLECTION

Blood samples were drawn into evacuated heparinized collection tubes (Becton-Dickinson). Following centrifugation at 1150 x g, 5°C for 15 minutes, the plasma was removed and stored at -10°C until assayed. After oral administration of flunixin, blood samples were drawn at 30 minutes, 1, 2, 4, 6, 12 hours and then every 12 hours until the drug was no longer detectable. After intravenous administration of flunixin, blood samples were drawn at 5, 15, 30 minutes, 1, 2, 4, 8, 12 hours and then every 12 hours until the drug was no longer detectable. Following both routes of administration, urine samples were collected at 3, 6, 12 hours and then every 12 hours until the drug was no longer detectable. Ten ml aliquots were frozen at -10°C until assayed.

SAMPLE PREPARATION

Basic hydrolysis of urine: Prior to analysis, all urine samples were base-hydrolyzed to release the base-labile conjugate of flunixin; 3.2 ml of urine was hydrolyzed by diluting it with 1.6 ml of 0.5 M sodium hydroxide. The mixture was then gently mixed at room temperature for 15 minutes and centrifuged at 1150 x g, 5°C for 20 minutes. The supernatant was decanted and the hydrolyzed sample analyzed for flunixin.

Acid extraction: 2 ml samples of hydrolyzed urine or 200 µl of plasma were added to a screw-cap culture tube containing 4 ml saturated aqueous monobasic potassium phosphate (pH adjusted to 3.0) and 4 ml dichloromethane. All samples were analyzed in duplicate. The tubes were gently mixed at room temperature for 15 minutes and then centrifuged at 1150 x g at 5°C for 20 minutes. The aqueous layer was aspirated off and discarded. Two ml of the dichloromethane sample was transferred to a clean screw-cap culture tube and evaporated to dryness under a stream of nitrogen. Fifty µl of N,O-bis (trimethylsilyl) acetamide (Pierce Chemical) was then added to the residue. The tubes were agitated for 5 seconds and incubated at room temperature for 15 minutes. Following evaporation to dryness under a stream of nitrogen, the samples were reconstituted with 50 µl of hexane. Two µl of this solution was then injected onto the gas chromatograph.

GAS CHROMATOGRAPHY

Gas chromatography of the urine samples taken up to 24 hours post-dosing was performed on a Perkin-Elmer 900 equipped with a nitrogen detector and a 6-foot 3% OV-101 column. Operating temperatures were as follows: injector temperature 275°C, column temperature 225°C, and manifold temperature 275°C. Gas chromatography of all plasma samples and urine samples taken later than 24 hours post-dosing were performed on a Varian Aerograph 700 equipped with a scandium tritide electron capture detector and a 6-foot 3% OV-101 column. Operating temperatures were as follows: injector temperature 260°C, column temperature 225°C, and detector temperature 270°C. Carrier gas (nitrogen) flow was 30 cc/minute.

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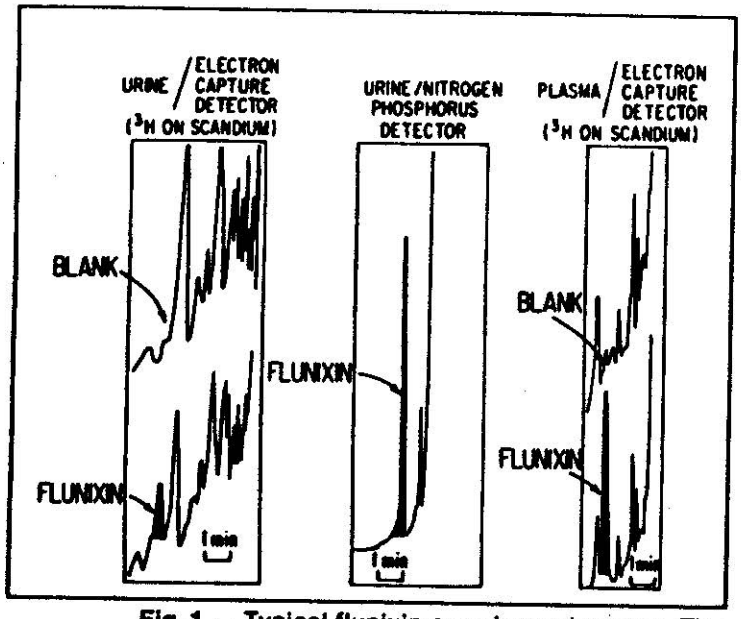

Fig. 1 — Typical flunixin gas chromatograms. The left-hand panel shows the response of an electron capture detector (^3H on scandium) to extracts of urine samples prior to dosing with flunixin (blank) and a urine sample collected 24 hrs post-dosing. The center panel shows the nitrogen detector response to extracts of urine samples collected 6 hrs post-dosing. The right-hand panel shows the electron capture detector (^3H on scandium) response to plasma extracts collected prior to dosing with flunixin (blank) and a plasma sample collected 30 min post-dosing.

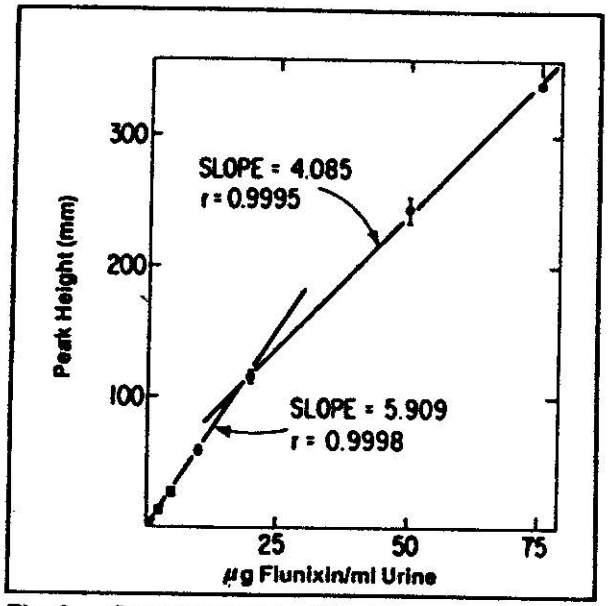

Fig. 2 — Standard curve of flunixin extracted from horse urine. Urine was "spiked" with the indicated amounts of flunixin, and the solid circles (● — ●) represent the detector response to the added amounts of flunixin. The data are best fitted by two individual regression lines as indicated, with an apparent break in the curve at 20 μg/ml.

THIN-LAYER CHROMATOGRAPHY

The acidic extraction procedure used for the gas chromatography samples was followed in preparing samples for thin-layer chromatography with these exceptions:

1. One ml of urine was extracted.
2. The samples were not derivatized with N,O-bis (trimethylsilyl) acetamide. Instead, the residue was reconstituted with several drops of dichloromethane to be spotted on silica gel 60 F-254-high performance thin-layer plates (Merck).

The basic extraction was performed the same as the acidic extraction except that the sample pH was adjusted with four drops of concentated ammonium hydroxide instead of the saturated potassium phosphate solution. The plates spotted with acid-extracted samples were developed in a thin-layer chromatography tank containing chloroform: cyclohexane:acetic acid (60:40:15). The basic extracted samples were developed in a chloroform:methanol (9:1) solvent system. The acidic-extracted samples were visualized with Dragendorff's and copper chloride. All solvents used for the gas chromatography were of nanograde quality; all solvents used for the thin-layer chromatography were of ChromA (Mallinckrodt) quality.

Results

Flunixin is unusual among nonsteroidal anti-inflammatory drugs in that it appears to be pharmacologically effective at relatively low plasma and urinary concentrations.[3] Urinary concentrations of flunixin, however, were sufficiently high for 24 hours after dosing to be detectable by use of a nitrogen-phosphorus detector. A typical chromatogram for urinary flunixin detected by the nitrogen phosphorus detector is presented in Figure 1, center panel, and a standard curve for this detection method is presented in Figure 2. However, for plasma or low urinary concentrations of these drugs, electron capture detection was necessary (Fig. 1, left and right panels) and for this detector a logit-log standard curve was used (Fig. 3).

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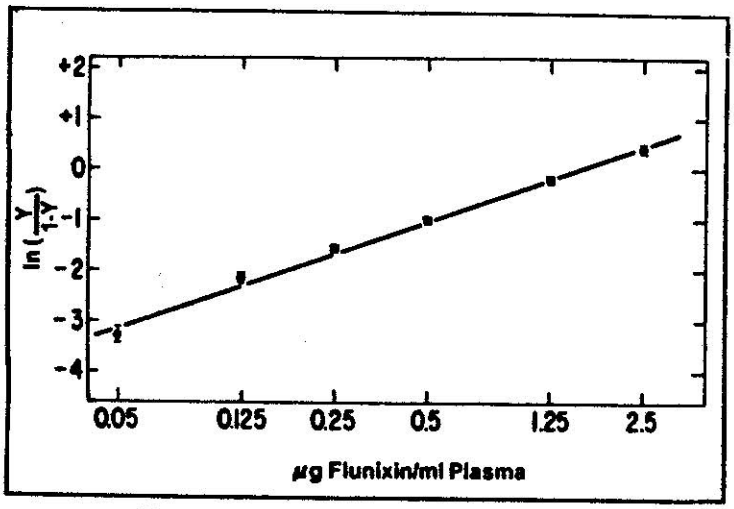

Fig. 3 — Standard curve of flunixin extracted from horse plasma. Plasma was spiked with indicated amounts of flunixin represented by solid circles (● — ●). The electron capture detector's response was measured by peak area and expressed in logit-log form. The solid line represents a least squares regression line fit to all the data points with a correlation coefficient of 0.9978 and a slope of 2.120.

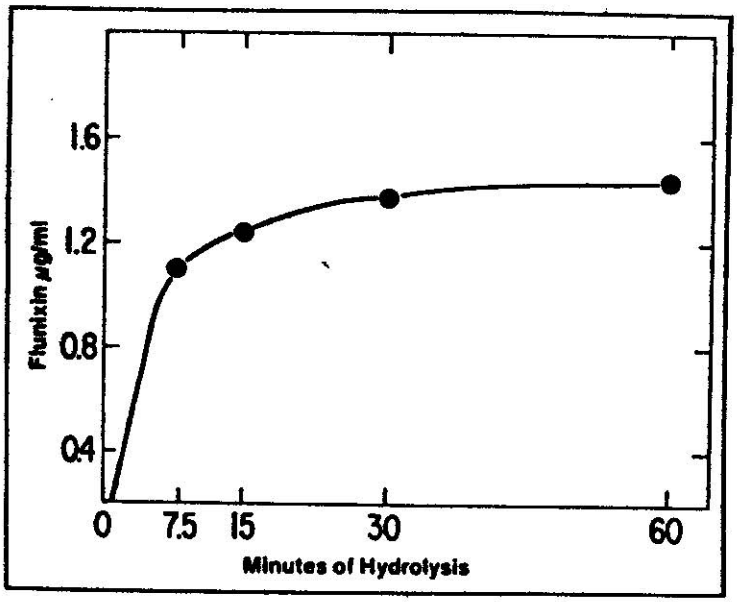

Fig. 4 — Time course of hydrolysis of flunixin "glucuronide." A 24-hr post-dosing (1 mg/kg flunixin) urine sample was base hydrolyzed and the reaction stopped at the indicated times. The solid circles (● — ●) represent the amount of free flunixin found in the urine after hydrolysis.

As with other nonsteroidal anti-inflammatory drugs, a considerable portion of the flunixin found in equine urine is in a conjugated form.[6] Mild alkaline hydrolysis rapidly increases the levels of free flunixin, as shown in Figure 4. All urine sample hydrolyses reported in this work were subjected to this treatment for 15 minutes at room temperature, which releases about 90% of the alkaline-releasable flunixin. While at least a portion of this material is likely to be a glucuronide metabolite, the nature of these alkaline-releasable metabolites remains to be determined.

Flunixin may be administered to horses either orally or by intravenous (IV) injection (Fig. 5). When 1 mg/kg of flunixin was administered by rapid IV injection, plasma levels of flunixin were initially about 10 µg/ml. Plasma levels fell rapidly at first, with an apparent half-life of about 12 minutes, to about 5 ng/ml. Thereafter, plasma levels fell more slowly, with an apparent half-life of about 1.6 hours, to a blood concentration of about 0.1 µg/ml, which was reached at about 12 hours after dosing. These data were well fitted by a two-compartment open model, as indicated in Figure 6. Thereafter, traces of flunixin were found in blood samples, but the pharmacokinetic significance of these low drug levels is unclear.

Since flunixin is available to horsemen in an oral dosing form, we also administered this agent by the oral route. When 1 mg/kg was administered orally, plasma levels of this drug peaked at about 3 µg/ml within about 30 minutes of dosing. Plasma levels of the drug quite closely matched those found after IV administration, falling with an apparent half-life of about 1.6 hours until levels of 0.8 µg/ml were reached. Thereafter, blood levels of the drug decayed with an apparently somewhat longer half-life than was observed after IV administration (Fig. 5).

After IV administration of flunixin, urinary concentrations of this drug and its conjugated metabolites peaked at about 200 µg/ml and thereafter fell rapidly for the first 12 hours (Fig. 7). From 12 hours post-dosing on, however, urinary concentrations of this drug

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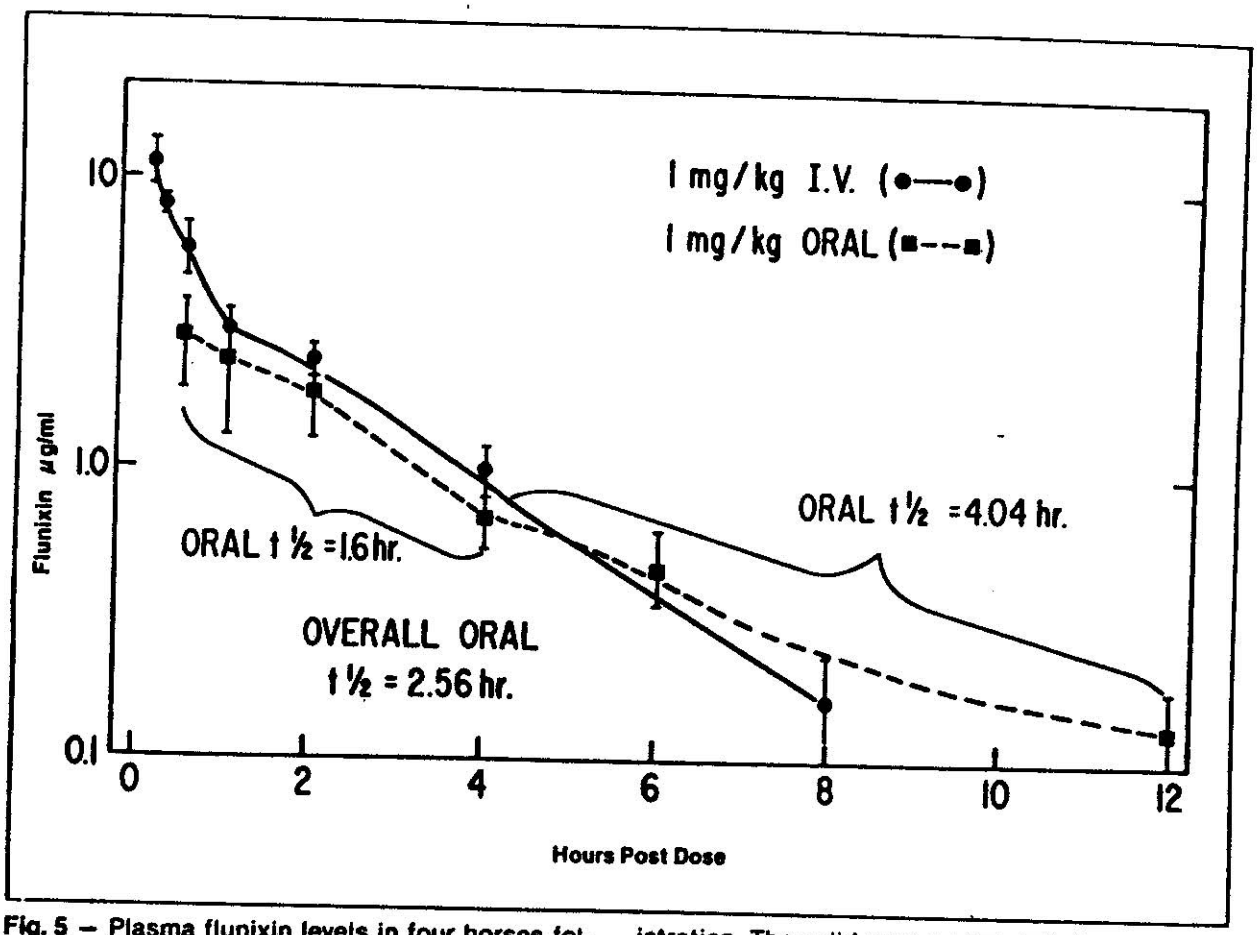

Fig. 5 — Plasma flunixin levels in four horses following oral and intravenous administration of a 1 mg/kg dose. The solid circles (● — ●) represent plasma levels of flunixin in µg/ml after oral administration. The solid squares (■ — ■) show plasma levels of flunixin after oral administration of 1 mg/kg flunixin meglumine as Banamine® granules.

declined more slowly, with an apparent half-life in the order of about 4 hours. Again, the urinary concentration of flunixin after oral administration closely followed those obtained after IV administration. Flunixin could not be detected in equine urine for more than 48 hours after administration of a single dose by the methodology used in this report.

One of the potential problems with the approval of any medication for use in performance horses is that the presence of the approved medication in a urine sample may interfere with the detection of other possibly unapproved medications. Since thin-layer chromatography is a commonly used screening method for drugs in race horses, we investigated the ability of flunixin to "interfere" with the routine TLC detection of drugs by the methods used in our routine testing laboratory. Figure 8 shows the extent of interference by flunixin with routine thin-layer screening for drugs. At 6 hours after dosing, a flunixin spot at R_f 0.4 was clearly visible, but by 24 hours post-dosing this spot was no

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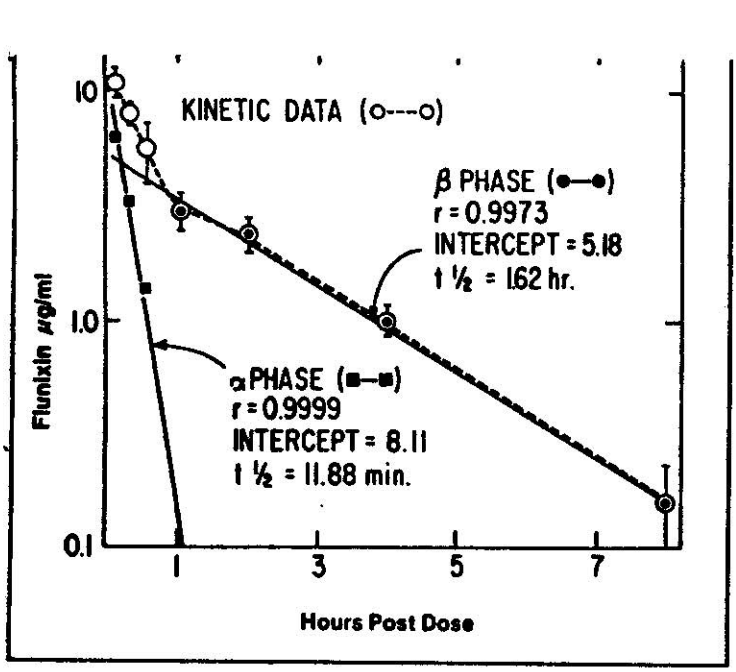

Fig. 6 — Pharmacokinetic analysis of plasma levels of flunixin after IV injection. The open circles (O—O) show plasma levels of flunixin after IV injection of 1 mg/kg. The time points from 1 hour to 8 hours, open circles filled by solid circles (◉—◉) were fitted by a beta phase with a t½ of 1.62 hours, while points prior to 30 minutes (■—■) were fitted by an alpha phase with a t½ of 11.88 minutes.

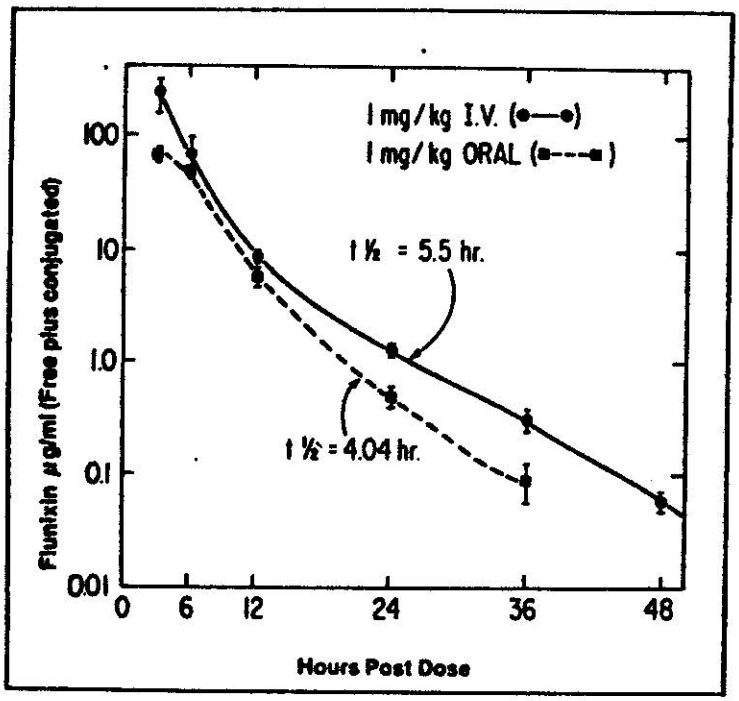

Fig. 7 — Urinary levels of flunixin in four horses. The solid circles (●—●) show urinary levels of flunixin after administration of 1 mg/kg orally, while the solid squares (■—■) show urinary levels of the drug after 1 mg/kg orally.

longer discernible. By 24 hours post-dosing, therefore, interference by flunixin with acidic thin-layer screening tests for drug detection was trivial. Similarly, basic extraction of flunixin, which apparently results in a rearrangement of the molecule, also resulted in no significant interference with drug testing at 24 hours after dosing.

Discussion

After IV injection of flunixin, plasma levels of the drug peaked at about 10 µg/ml, and then declined with a half-life of about 12 minutes. Thereafter, plasma levels of the drug fell more slowly, with an apparent half-life of about 1.6 hours. Based on this data, the most conservative model at this time for the pharmacokinetics of flunixin in horses is a two-compartment open model. On this basis, the data points up to 8 hours post-dosing are well fitted by a model with an alpha phase of about 12 minutes and a beta phase of about 1.6 hours. This proposed beta phase half-life is in good agreement with reports of Maylin (cited by Tobin, 1981), which show that plasma levels of flunixin in horses decline with an apparent half-life of about 1.6 hours between 1 and 8 hours post-dosing.

Some evidence suggestive of a third pharmacokinetic compartment for flunixin in the horse was obtained, but these data points were very close to the detection limit of this method. These data points, however, appear to be in agreement with recent reports of a possible third compartment for flunixin distribution in the horse, and these authors report that flunixin remains detectable in equine urine for up to 15 days.[5] This evidence for a third pharmacokinetic compartment and long plasma half-life of this drug may explain the relatively long pharmacologic action of flunixin reported at 24 to 30 hours, which seems at variance with the apparently short (1.6 hours) plasma half-life of this drug. Existence of a third kinetic compartment also suggests that flunixin is likely to distribute widely in the body and have a large volume of distribution.

As well as being widely distributed in the

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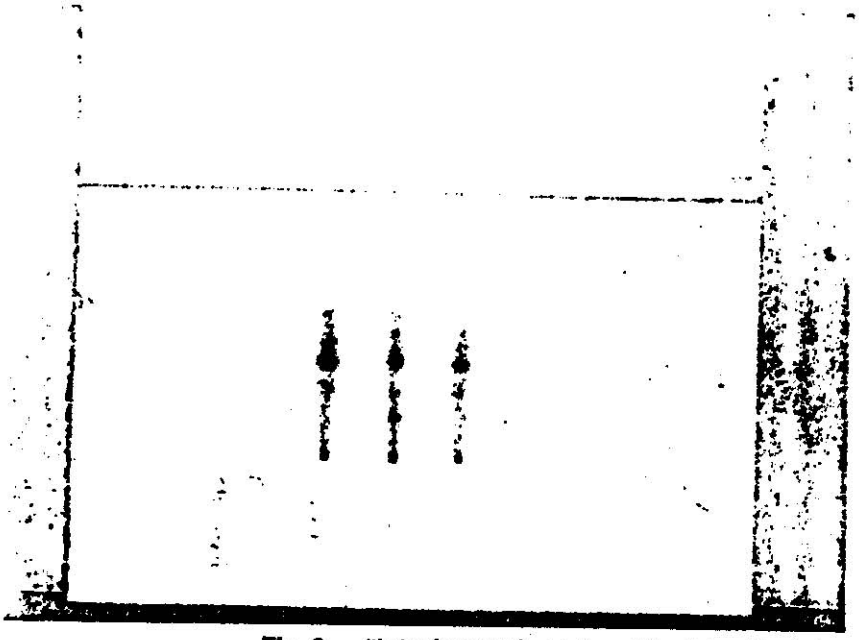

Fig. 8 — "Interference" or "masking" by flunixin. Horses were dosed with 1 mg/kg flunixin as Banamine® IV. Urine samples were taken prior to drug administration and at 6 and 24 hours after drug administration. One ml aliquots of each urine sample were taken, hydrolyzed and subjected to TLC as described in Methods. From left to right, the chromatograms show respectively: 1. — flunixin standard (1 μg/ml); 2. — 24-hour urine sample; 3 — 6-hour urine sample; and 4. — pre-Banamine® sample.

horse, flunixin is very rapidly and relatively completely absorbed from the GIT. When administered orally, peak plasma levels of the drug were seen within 30 minutes of administration, suggesting very rapid absorption. Thereafter, plasma levels of the drug were very similar to and declined in parallel with levels observed after IV injection. This pharmacological pattern suggests that flunixin is relatively rapidly and completely absorbed after oral administration, with an apparent bioavailability after oral administration of about 80%.

This rapid and relatively complete absorption of flunixin after its oral administration may be of considerable importance to horsemen. It means that a horseman can administer flunixin orally and be confident of getting good absorption of the drug, reproducible and reliable blood levels and a good pharmacological effect. For a horseman facing a regulatory stipulation such as, for example, a certain blood level of a drug at a certain time, an agent which performs as reliably as flunixin appears to have considerable advantages. For example, with phenylbutazone it has been shown that blood levels obtained after oral administration are quite variable,[2] which renders it a less satisfactory drug to use if a specific blood level regulation has to be met.

The rapid and complete absorption of flunixin after oral administation may also be of considerable importance to the horse. Recently, adverse responses to phenylbutazone in horses have been reported if the dosage of phenylbutazone is increased or if horses are maintained on phenylbutazone for longer periods of time.[4,10] On postmortem examination of these horses, it has been noted that gastrointestinal signs of toxicity have been dominant. These observations have led one investigator to suggest that the delayed absorption resulting in high levels of phenylbutazone contribute to the GIT toxicity of this agent (Snow, DH, University of Glasgow, personal communication). The apparently very rapid absorption of flunixin after oral administration could therefore be associated with the lesser likelihood of GIT toxicity with flunixin as compared with phenylbutazone.

After either oral or IV administration of flunixin, urinary concentrations of this drug were relatively high, although somewhat less of the drug or its metabolites appeared in the urine after oral administration than after IV administration. The apparent urinary half-life of the drug, however, was slower than the apparent terminal plasma half-life of flunixin in these horses, supporting suggestions of a slower third phase of elimination of this drug from plasma.

Flunixin is unusual among nonsteroidal anti-inflammatory drugs in that it is both a relatively potent agent and has an unusually large volume of distribution. Also, its plasma and urinary concentrations decline relatively

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rapidly after either oral or IV administration. For these reasons, flunixin appears to have little ability to interfere with testing for other drugs, and a series of experiments designed to test this hypothesis was performed.

The most critical interference with drug testing occurs during thin-layer screening. Thin-layer tests are rapid, sensitive and flexible and as such constitute the preliminary screening mechanism in most drug testing systems. Interference with drug testing usually occurs when the presence of drugs or drug metabolites interfere with the ability to detect other drugs on thin-layer plates. Most commonly, this occurs as a simple overlying effect or, as it is sometimes popularly described, the presence of one drug "masks" the presence of another.

The data presented in Figure 8 show that by 24 hours after dosing with flunixin, interference is minimal. In fact, on inspection under UV light, acidic extracts of urine from treated and untreated horses could not be distinguished. Similarly, if basic extracts of urine samples from treated horses were examined it was again difficult to distinguish between the urines from treated and untreated horses.

In summary, flunixin is a potent nonsteroidal anti-inflammatory drug which is well absorbed after oral or IV administration in the horse. It appears to distribute widely in the horse, and pharmacological effects are associated with relatively low plasma or urinary levels of this drug. Blood and urinary levels of this drug are relatively low, consistent, and reproducible when administered either orally or IV. Because of the potency and low effective blood levels of this agent, its ability to interfere with testing for other drugs is minimal. Its tendency to accumulate in the blood stream is also likely to be minimal. Its rapid and complete absorption from the GIT may also be associated with the apparently lower GIT toxicity of this agent. Because of these characteristics, flunixin appears remarkably suited for use in horses close to events which require that a certain blood or urinary level of drug not be exceeded. ■

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