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THE PHARMACOLOGY OF FUROSEMIDE IN THE HORSE
V. The Duration of Reduction of Urinary Concentration of Drugs

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SUMMARY

The administration of furosemide to horses in IVdoses of 0.5 mg/kg or less reduced drug concentrations in urine for less than 4 hours. The most prolonged reduction observed was that of the glucuronide metabolite of morphine, which required three hours post-dosing to return to control. Urinary concentrations of phenylbutazone were not significantly different from control by two hours post-dosing, while urinary concentrations of fentanyl appeared to return to normal within about two and one-half hours of dosing. Other experiments showed that blood levels of morphine were not significantly reduced by furosemide treatments.

INTRODUCTION

Furosemide (Lasix®)[a] is a potent, high ceiling diuretic which is the recommended treatment for epistaxis or exercise induced pulmonary hemorrhage (EIPH) in the horse. Since at least 43% of racing horses have some degree of EIPH, a number of racing jurisdictions have allowed the pre-race use of furosemide in horses.[1,5] However, the principal problem with the approval of furosemide for use in racing horses is that it acts to dilute out certain drugs and drug metabolites in equine urine. This dilution is important because it might reduce the efficacy of testing for some drugs.

Because furosemide is a very short acting drug in the horse, these diluting effects are quite transient. Therefore, one approach to the problem of the diluting effect of furosemide in the horse is to avoid taking urine samples during the period of the diluting effect.

Reviewing the problem in 1977, Drs. Gabel, Tobin, Ray, Maylin and the other members of the Veterinary Chemists Advisory Committee to the National Association of State Racing Commissioners concluded that "furosemide administered more than four hours before race time does not significantly reduce the ability to detect those drugs studied to date."[4] These are the only published recommendations on the duration of diluting effects of furosemide. However, since these workers based this 4-hour estimate on research done using 1.0 mg/kg doses of furosemide, we elected to investigate the duration of the diluting effect of furosemide after administration of smaller (0.5 mg/kg or less) doses of this drug.

It is important that the duration of the diluting effect of prophylactic doses of furosemide be determined. Although the diuretic effects of furosemide are relatively short-lived, the duration of other pharmacological actions of this drug may be more prolonged. Therefore, to establish the use of these doses in horses without undue interference with drug testing, the minimal period for which small, prophylactic doses of furosemide produce their diluting effects should be accurately known. Preliminary reports of this work have been communicated.[2]

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[a]W. A. Butler Co., Columbus, Ohio.

MATERIALS and METHODS

Animals: Mature thoroughbred and standardbred horses between 400 and 550 kg were used. The animals were kept at pasture until the day of an experiment when they were housed in box stalls and allowed hay and water *ad libitum.*

All drugs were injected intravenously into the left jugular vein. Blood samples were drawn from the right jugular vein and urine was obtained by bladder catheterization.

Experiment 1:

Phenylbutazone and Furosemide: Three mares were dosed with 4.4 mg phenylbutazone[b] per kg body weight. Urine samples were collected at intervals for 24 hours. The following week, the procedure was repeated on the same animals except that 0.385 mg furosemide per kg body weight was injected intravenously immediately after collection of the 2-hour sample. All samples were stored at 4° C until the analysis was performed.

Two hundred µl of each urine sample was mixed with 2 ml hexane:dichloromethane (2:1) and 2 ml sodium acetate buffer (pH 4.5). The tubes were rotoracked for 5 minutes and centrifuged at 1150 g, 2° C for 15 minutes. A 1 ml aliquot of the solvent phase was evaporated to dryness under nitrogen. The residue was redissolved in 50 µl hexane, 2 µl of which was injected into a Varian 2700 gas chromatograph equipped with a tritium on scandium electron capture detector. Separation was done on a 6-foot glass column packed with 3% OV-101 maintained at 249° C.

Experiment 2:

Fentanyl and Furosemide: Four horses were given 0.001 mg fentanyl[c] per kg body weight and urine samples were collected at intervals (Figure 2) during the next 24 hours. The following week, the same horses were again dosed with fentanyl. Immediately after the 30-minute urine sample was obtained, the animals were given 0.5 mg furosemide per kg body weight. All samples were stored at -20° C for later analysis.

Levels of fentanyl in the urine were determined by a radioimmunoassay method utilizing a commercially available kit.[d] The procedure has been described elsewhere.[1] Briefly, 0.05-0.5 ml urine was incubated with radioactive tracer and antiserum for 2 hours at pH 7.4. Fentanyl in the sample competed with the ^{3}H-fentanyl for binding sites on the fentanyl antibody. After formation of

[b]National Laboratories Corp., Somerville, N.J.
[c]McNeil Laboratories, Fort Washington, PA.
[d]Institut National des Radioelements, Fleurus, Belgium.

EFFECT OF FUROSEMIDE TREATMENT ON URINARY PHENYLBUTAZONE CONCENTRATION

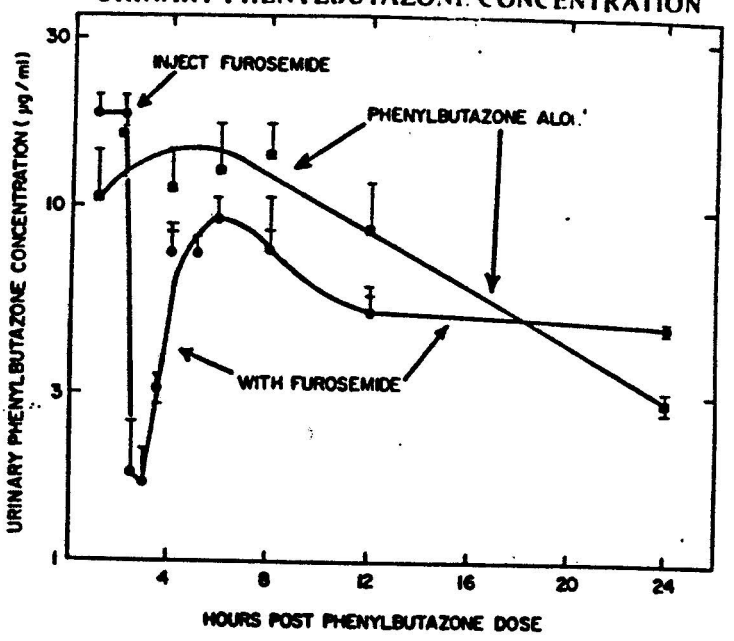

Figure 1. Effect of furosemide treatment on urinary phenylbutazone concentration. Urinary phenylbutazone concentrations following IV dosing with phenylbutazone alone are shown by the solid squares (■-■) and following IV dosing with phenylbutazone and furosemide (0.385 mg/kg) are shown by the solid circles (●-●). Vertical bars represent ± SEM. (Reproduced with permission from "Drugs and the Performance Horse," Charles C. Thomas, Publishers, Springfield, Illinois 62717, 1981).

the immunocomplex was completed, bound and free fentanyl were separated by selective adsorption onto dextran-coated charcoal. The charcoal complex was removed by centrifugation and the radioactivity in the supernatant, due to bound ^{3}H-fentanyl, was measured in a liquid scintillation counter.

Experiment 3:

Morphine and Furosemide: Mares were injected IV with 0.1 mg morphine[e] per kg body weight. Blood and urine samples were collected (Figure 3-5) during the next 6 hours. Six months later, the same horses were again dosed with the same amount of morphine. Following collection of the 1-hour samples, 0.4 mg furosemide/kg body weight was injected. Blood samples were allowed to clot and serum was removed. Serum and urine samples were stored at -20° C until the analysis was performed.

The methodology for the determination of morphine levels in equine biological samples has been previously described. Urine samples were split, 1 portion analyzed for free morphine, while the other portion was subjected to hydrolysis with β-glucuronidase to allow measurement of total morphine. Samples were cleaned by a combination of liquid-liquid extraction and column chromatography. A strong electron capturing compound was formed by the derivatization of the extracted morphine with pentafluoropropionic anhydride (PFPA). Separation was performed on a SP 2250-DB on 100/120 Supelcoport[f] column at 235° C in a Varian 3700 equipped with a ^{63}Ni electron capture detector.

RESULTS

Urinary levels of phenylbutazone were reduced by an average of 10-fold during peak diuresis.

Experiment 1 — Urinary concentrations of phenylbutazone quickly recovered, there being no significant difference (paired data t-test, $t = 2.370$, $\alpha = 0.05$) between the treated and untreated horses from 2 hours after the horses had been given the diuretic.

Experiment 2 — Horses were dosed with 0.001 mg/kg of fentanyl and this dose was followed 30 minutes later by either furosemide, 0.5 mg/kg, or normal saline. Urine samples were collected at the indicated periods and analyzed for fentanyl equivalents by RIA. Administration of furosemide caused urinary levels of "fentanyl" to fall about 18-fold within 30 minutes of dosing but urinary "fentanyl" levels had returned to control within about 2½ hours of dosing.

Experiment 3 — Morphine (0.1 mg/kg) was administered to 6 horses and followed 1 hour later with 0.4 mg furosemide/kg body weight. Free morphine plus β-glucuronidase releasable morphine (referred to here as total morphine) fell 13-fold within 15 minutes after the diuretic had been given (Figure 3). Total morphine found in the urine rose rapidly from this low point until the levels were not significantly different (paired data t-test, t

[e]Eli Lilly and Co., Indianapolis, Ind.
[f]Supelco, Inc., Bellefonte, PA.

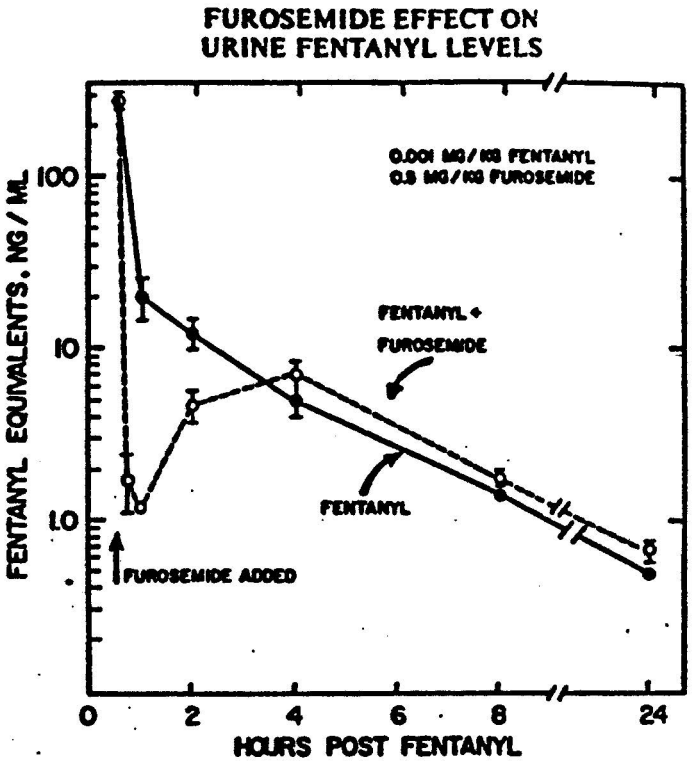

Figure 2. Furosemide effect on urine fentanyl levels. Urinary fentanyl levels were determined by RIA in horses given fentanyl alone (shown by the solid circles, ●–●) and given fentanyl and furosemide (shown by the open circles, ○–○). Vertical bars represent ± SEM. (Reproduced with permission from "Drugs and the Performance Horse," Charles C. Thomas, Publishers, Springfield, Illinois 62717, 1981).

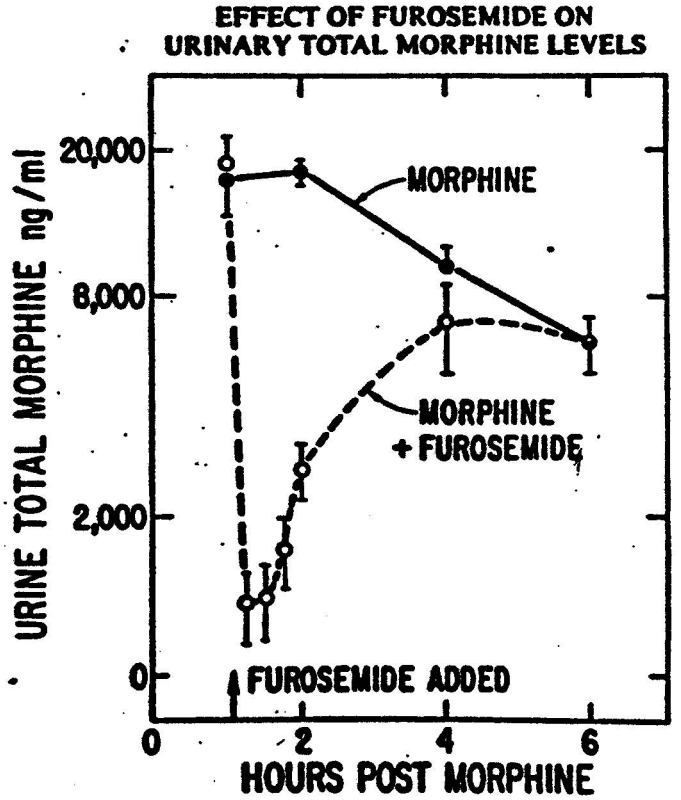

Figure 3. Effect of furosemide on urinary total morphine levels. Horses were dosed with 0.1 mg morphine per kg body weight and urine levels of morphine were analyzed following hydrolysis with β-glucuronidase (shown by the solid circles ●–●). Later, the experiment was repeated but 0.4 mg furosemide/kg body weight was injected 1 hour after the horses were given morphine (shown by the open circles, ○–○). Vertical bars

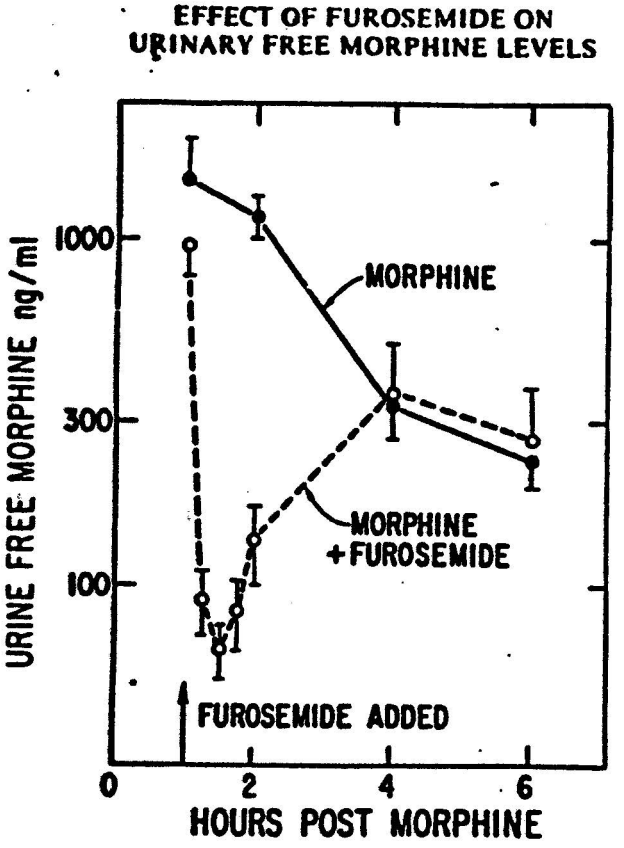

Figure 4. Effect of furosemide on urinary free morphine levels. Urine concentrations of free morphine in horses dosed with morphine alone are shown by the closed circles (o-o) and in the same horses later dosed with morphine and furosemide are shown by the open circles (o-o). Vertical bars indicate ± SEM.

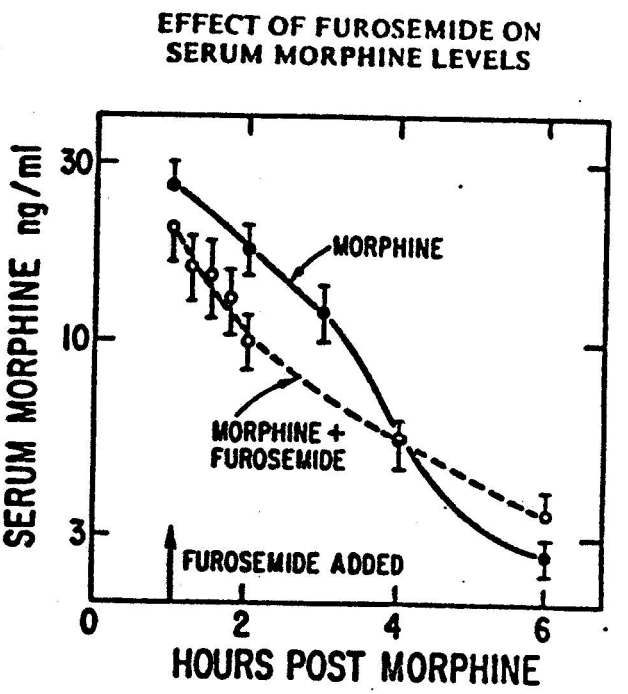

Figure 5. Effect of furosemide on serum morphine levels. Serum morphine levels of 6 horses given 0.1 mg morphine/kg body weight are shown by the closed circles (o-o). Later, the same animals were given the same amount of morphine but were also treated with furosemide (0.4 mg/kg). These serum levels are shown by the open circles (o-o). Vertical bars represent ± SEM.

= 1.026, α =0.05) from control values of furosemide by 3 hours after furosemide administration. Furosemide appeared to have broadly similar effects on total and free morphine levels as seen by comparing Figures 3 and 4.

Serum samples from these same horses were analyzed for morphine and the results are presented in Figure 5. Throughout the entire 5-hour period immediately following furosemide administration, there was no significant difference (2 sample t-test, t = 1.354, α = 0.05) in serum levels of morphine whether or not the horses had received furosemide.

DISCUSSION

Epistaxis, or bleeding from the nose, following exercise has occurred since the earliest days of thoroughbred racing. Historically, the incidence of epistaxis has been low (less than 2%),[3] and a variety of treatments for this condition have been recommended. In the early 1970's, equine practitioners and horsemen began recommending furosemide for the treatment of epistaxis. Since then, it has become the treatment of choice for this condition.

In the late 1970's, work by Pascoe and his associates showed that the classical 2% incidence of bleeding from the nose was a manifestation of a much more extensive problem.[4] Studying horses post-race with a fiberoptic endoscope, Pascoe showed that up to 40% of horses showed evidence of blood in the larynx or trachea post-race. Pascoe therefore renamed the syndrome exercise-induced pulmonary hemorrhage (EIPH)[5] and other work since then has supported Pascoe's observations and conclusions.[6]

The horse appears to be unusually prone to epistaxis, and this predisposition has been attributed to the architecture of the horse's lungs. As a species with poor collateral ventilation at the alveolar level, blockade of the direct entry of air to an alveolus has been theorized to cause blocked alveoli to rupture when the lung expands.[9] Furosemide may act to alleviate EIPH by improving the entry of air into a partially blocked alveolus, either by reducing pulmonary edema or by dilating bronchioles.[9] Alternatively, it may act to reduce epistaxis by reducing congestion.[9] While scientific studies on the efficacy of furosemide for EIPH are not available, there is a clear medical rationale and anecdotal clinical experience to justify its use in EIPH.

The only technical problem with the approval of furosemide for use in racing horses is its ability to dilute out certain drugs in equine urine.[7,8] The maximum duration of this dilution effect must, therefore, be determined to allow evaluation of its potential for use in racing. In this investigation the duration of the diluting effect after small doses of furosemide was determined, and the amount of apparent dilution of post-race urine samples associated with pre-treatment with furosemide was determined.

These experiments were based on the hypothesis that

both the duration and extent of the diluting effects of furosemide would be reduced if the dose of furosemide was reduced. Thus, one might expect that the duration of the dilution effect would be considerably less after a dose of furosemide in the order of 0.4 mg/kg, i.e. the dose commonly used in the treatment of epistaxis, than after 1 mg/kg, the manufacturer's recommended dose for pulmonary edema as was reported earlier.[7,8] In general, this expectation has been borne out by the results obtained.

Both the duration and extent of the dilution after the lower doses of furosemide (less than 0.5 mg/kg) were less than those reported in earlier experiments. Thus, 1 mg/kg of furosemide IV produced about a 40 to 50-fold dilution of the urinary concentrations of both phenylbutazone and the major glucuronide metabolite of pentazocine in a previously reported work.[8] Reducing the dose, however, produced a correspondingly smaller maximal dilution effect, about 10-fold for phenylbutazone, about 18-fold for fentanyl, and about 13-fold for morphine.

Similarly, the duration of the dilution effect was markedly reduced. While the effect lasted for longer than 4 hours when the dose of furosemide was 1.0 mg/kg,[7,8] reducing the dose of furosemide reduced the duration of the effect to about 3 hours or less. For example, the dilution effect when phenylbutazone was used about two hours, and three hours or less when morphine or fentanyl were the test drugs. Four hours is therefore a conservative time pre-race to administer a prophylactic dose of furosemide. In fact, these data suggest that the drug could be administered in less than 0.5 mg doses as close as three hours prior to sampling with little effect on drug detection in post-race urine samples.

These data further suggest that the diluting effect of furosemide on urinary drug concentrations in actual practice may be relatively small. In other experiments, we measured and compared the concentration of phenylbutazone and its metabolites in the post-race urines of horses with and without furosemide pre-treatment.[9] These data show that the apparent dilution of phenylbutazone and its metabolites in equine urine amounts to less than a 50% reduction in drug concentration in equine urine. Since all practical analytical methods have a margin of safety of much greater than 50%, this reduction in concentration is unlikely to be forensically significant.[9]

It is highly unlikely that less than 0.5 mg furosemide per kg body weight will significantly affect the detection of drugs in blood. This is because small doses of furosemide lead to the elimination of only about 4 liters of fluid from the horse, which is between 1 and 1.5% of the body water of a horse. In general, therefore, furosemide at less than 0.5 mg/kg is unlikely to lead to the elimination of more than 1.5% of the drug in a horse, which is unlikely to be a forensically significant amount.

Further, the fraction of the dose of drug eliminated in the urine in response to furosemide will generally be a lot less than 1.5%. This is because most drugs, either acidic or basic, have an apparent volume of distribution in the horse of much greater than total body water. For example, the tranquilizer acepromazine distributes in the horse in a manner equivalent to its distribution in a volume of over 800,000 liters. The proportion of the total amount of drug eliminated after a dose of furosemide will, therefore, in many cases be only a fraction of 1% of the dose present in the horse at the time of treatment with furosemide.

As a practical matter, therefore, the results reported here show that the diluting effects of doses of furosemide less than 0.5% mg/kg administered IV are essentially over within about three hours of dosing. These data, therefore, support the original conclusions of Gabel, Tobin, Ray and Maylin that dilution is not likely to be significant if the drug is administered four hours before post-time.[4] These results further suggest that four hours prior to post-time may be a relatively conservative time restraint to put on administration of this drug if the dose administered is equal to or less than 0.5 mg/kg.

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