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Pharmacokinetics and protein binding of morphine in horses

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SUMMARY

Morphine could be detected in horses dosed with 0.1 mg of drug/kg of body weight for up to 48 hours in blood and 144 hours in urine. This dose of morphine elicited no observable effects and is a suggested analgesic dose. Computer analysis revealed that a 3-compartment open system was the best fitting model with a serum half life ($t_{1/2(\beta)}$) of 87.9 minutes and a urine $t_{1/2(\beta)}$ of 101.1 minutes. Binding to equine serum proteins was linear over a drug concentration range of $3.88 \times 10^{-5}M$ to $3.50 \times 10^{-8}M$ and averaged 31.6%. In RBC-partitioning experiments, 78.1% of the drug was found in the plasma fraction. The data indicated that a horse should not be given morphine closer than 1 week before a race.

Although morphine may be used to alleviate pain in a horse, its use in horses while racing is illegal.[1] Although the biological half-life ($t_{1/2}$) of morphine in the horse is of primary concern, other parameters, including the distribution rate constants need to be examined. The binding of some drugs to plasma proteins or RBC has a marked effect on the pharmacologic activity, distribution, excretion, and interactions with other drugs. Serum protein binding and RBC partitioning were determined under varying conditions to assess the effect of this phenomenon on morphine pharmacokinetics.

Materials and Methods

Thoroughbred and Standardbred mares ranging in age from 8 to 21 years and in weight from 415 to 577 kg were maintained on pasture, except during the experiments, when they were housed in box stalls and were given hay and water ad libitum. Four horses were used in each experiment. For the kinetic studies, morphine (0.1 mg/kg of body weight) was administered IV into the left jugular vein, and blood samples were drawn from the right vein into partial vacuum tubes[a] containing no anticoagulant. Urine samples were collected by bladder catheterization. For the protein-binding studies, blood was collected in similar partial vacuum tubes (with care taken not to allow contact of the blood with the rubber stoppers) and in glass syringes from horses that had been drug-free for 2 weeks. A similar procedure was used to obtain blood for the RBC partitioning study, except that heparinized tubes and syringes were used.

Morphine values were determined by a previously described method.[1] Briefly, samples to be hydrolyzed were incubated for 4 days at 37 C with 5,000 U of β-glucuronidase (Glucurase[b]) or for 3 hours at 65 C with 5,000 U of β-glucuronidase from *Patella vulgata*.[b] Liquid-liquid extraction and column chromatography was performed on all samples. The morphine was derivatized with pentafluorpropionic anhydride (PFPA).[c] The morphine-PFPA derivative was quantitated by gas chromatography, using a ^{63}Ni electron capture detector.

[^{3}H]Morphine[d] was used to compare the percentage of protein binding of the drug in serum collected in partial vacuum tubes with that collected in glass syringes. To determine the protein binding over a wide range of morphine values, samples were spiked with injectable morphine.[e] Plasma protein binding for all studies was determined by placing 5 ml of spiked serum in a dialysis bag[f] that was sealed and suspended in 50 ml of phosphate buffer (pH 7.4). After 24 hours' incubation at 37 C, morphine concentrations in the buffer and serum were determined. For experiments using [^{3}H]morphine, aliquots of the buffer and serum were dissolved in a scintillation cocktail consisting of 2 parts 2,5-diphenyloxazole and [*p-bis*-(o-methylstyryl)-benzene, in toluene (Triton X100) to 1 part nonionic octylphenozyethanol (3a20),[g] and counted for 10 minutes in a scintillation counter.[h]

The whole blood partitioning of morphine was measured by spiking 5 ml of whole blood with [^{3}H]morphine that was mixed gently during the 2-hour incubation at 37 C. Cellular and plasma fractions were separated by centrifugation, and the radioactivity of each was determined by liquid scintillation counting as described previously.

The kinetic data were analyzed by computer, using the general linear models procedure of the Statistical Analysis System.[i]

Results

Analysis of free morphine values indicated the drug could be detected for up to 48 hours in serum after IV dosing with 0.1 mg/kg (Fig 1). Computer analysis indicated that the data were consistent with a 3-compartment open model ($r = 0.9995$) with an α phase $t_{1/2}$ of 2.5 minutes, β phase $t_{1/2}$ of 87.9 minutes, and γ phase $t_{1/2}$ of 3,377 minutes. The drug concentration in the plasma immediately after

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[a] Vacutainer, Becton, Dickinson & Co, Rutherford, NJ.

[b] Sigma Chemical Co, St Louis, Mo.

[c] Pierce Chemical Co, Rockford, Ill.

[d] Amersham Corp, Arlington Heights, Ill.

[e] Eli Lilly & Co, Indianapolis, Ind.

[f] Spectrapor membrane No. 4 tubing, molecular weight cutoff 12,000 to 14,000, Spectrum Medical Industries Inc, Los Angeles, Calif.

[g] 3a20, Triton X100 Research Products, International Corp, Elk Grove Village, Ill.

[h] LS-315OT, Beckman Instruments Inc, Irvine, Calif.

[i] Third revised edition of the 1979 version, SAS Institute Inc, Cary, NC.

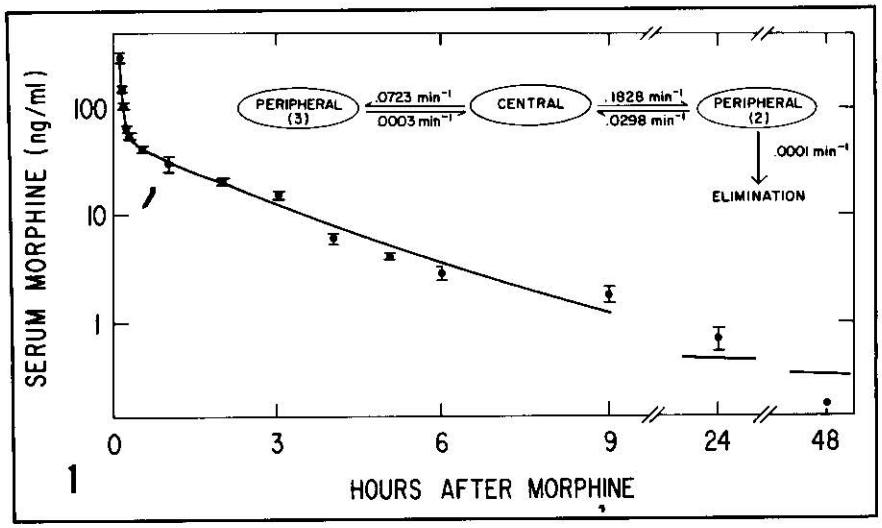

Fig 1—The disposition kinetics of morphine in 4 horses dosed IV with 0.1 mg of morphine/kg. The solid circles (●) show the mean serum values (± SEM) of free morphine. Computer analysis determined the $t_{1/2(\alpha)}$ was 2.5 minutes, $t_{1/2(\beta)}$ was 87.9 minutes, and $t_{1/2(\gamma)}$ was 3,377 minutes.

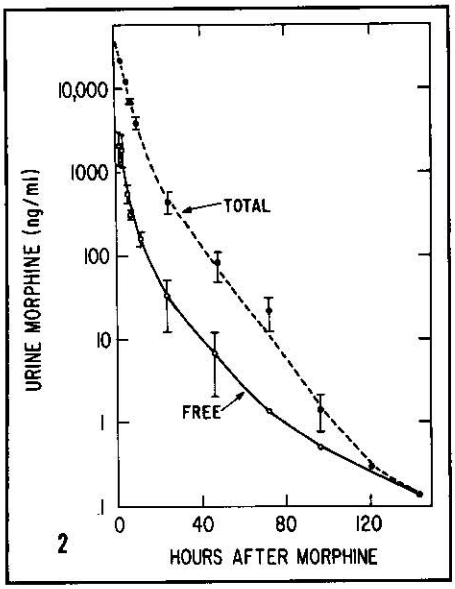

Fig 2—The dotted line indicates urinary concentrations of total morphine and the solid line indicates free morphine concentrations in urine (± SEM) after IV administration of 0.1 mg of morphine/kg. Urinary elimination could be divided into 2 phases, with $t_{1/2}$ of 1.68 and 5.98 hours.

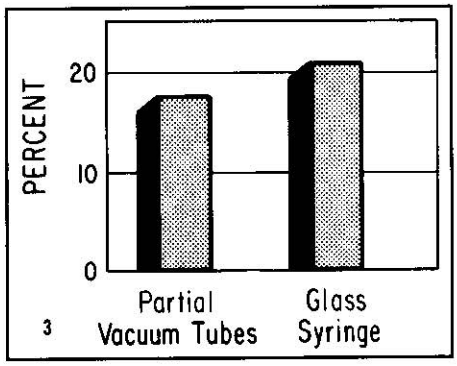

Fig 3—Serum protein binding of [^{3}H]morphine was determined on serum obtained from 4 horses. Blood was collected in partial-vacuum tubes (17.5% binding) and in all glass equipment (20.8% binding). Statistical analysis revealed a significant difference between the 2 methods.

the IV injection was 624 ng/ml (Table 1). The microconstants indicated a more rapid movement out of the central compartment into peripheral tissues than biotransformations and/or excretion. The intercepts of the serum distribution and clearance curves A, B, and C were 573, 51, and 0.61 ng/ml, respectively.

Modeling from the peak and using total morphine values in the urine, the computer analysis indicated a best fit to a 2-phase system with a β phase $t_{1/2}$ of 1.68 hours and a γ phase $t_{1/2}$ of 5.98 hours. Urine total morphine values peaked at 2 hours after dosing at an average of 21,894 ng/ml and decreased thereafter, permitting morphine detection in hydrolyzed and unhydrolyzed urine up to 144 hours after dosing (Fig 2).

The protein-binding study to determine the effect of the blood collection technique on this parameter determined that the average percentage of binding in serum from partial vacuum tubes was 17.5, whereas that from glass syringes was 20.8 (Fig 3). A t test (paired data) indicated that this was a significant difference ($t = 4.01$, $\alpha = 0.05$). Over a total drug concentration range of $1.36 \times 10^{-4}M$ to $8.41 \times 10^{-9}M$, protein binding was determined on 4 horses, using a constant-protein concentration. There was no significant change in the percentage of drug bound to the serum proteins over the range of $3.88 \times 10^{-5}M$ to $3.50 \times 10^{-8}M$ (1-way analysis of variance, $F = 2.080$, $\alpha = 0.05$). As indicated in Figure 4, there was a slight decrease in binding at extremely high, nontherapeutic morphine values, and an increase in binding at extremely low morphine values which bordered on the detection limit of the assay. Analysis of samples spiked with morphine in the concentration range commonly seen for several hours after dosing a horse with 0.1 mg of morphine/kg indicated that there was 35% to 37% protein binding of the drug.

There was no significant difference (paired data t test, $t = 0.97$, $\alpha = 0.05$) of RBC partitioning of morphine between running the assay at 21 C and 37 C (Fig 5). A paired data t test ($t = 2.88$, $\alpha = 0.05$) indicated that no significant difference in RBC partitioning could be attributed to the difference in the blood collection technique. In blood drawn in glass syringes and analyzed at 37 C, 78.1% of the drug was found in the plasma fraction. Correcting for the unequal volumes of plasma and packed cells found in whole blood, 0.56 U of morphine was sequestered in an equal volume of RBC for each unit of morphine found in 1 volume of plasma.

TABLE 1—Pharmacokinetic parameters for disposition of morphine in horses after IV injection of 0.1 mg/kg

Kinetic parameter	Value
C_p°	624 ng/ml
A	573 ng/ml
α	0.2772 Min^{-1}
$t_{1/2(\alpha)}$	2.5 Min
B	51 ng/ml
β	0.0079 Min^{-1}
$t_{1/2(\beta)}$	97.9 Min
G	0.61 ng/ml
γ	0.0002 Min^{-1}
$t_{1/2(\gamma)}$	3,377 Min
k_{12}	0.1828 Min^{-1}
k_{21}	0.0298 Min^{-1}
k_{13}	0.0723 Min^{-1}
k_{31}	0.0003 Min^{-1}
k_{20}	0.0001 Min^{-1}

Discussion

The pharmacokinetics of morphine in horses were similar to those determined in human studies. Computer analysis of equine serum free morphine concentrations established a 3-compartment open system as the best-fitting model (Fig 1). The $t_{1/2(\alpha)}$ of 2.5 minutes falls within the range determined on human subjects of 0.9 minutes,[2] 2.5 minutes,[3]

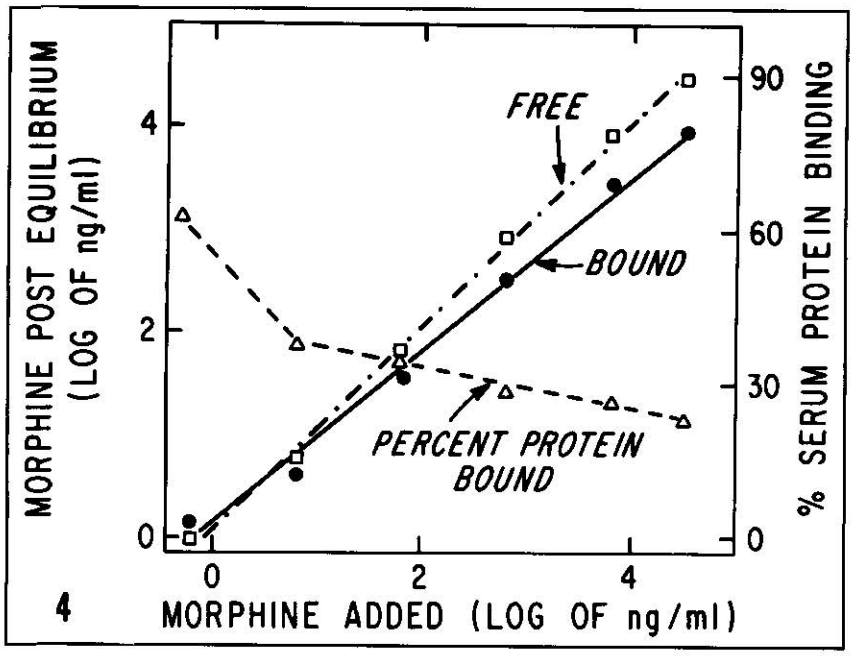

Fig 4—Binding of morphine to serum proteins was determined for 4 horses. Protein binding was independent of drug concentration between 3.88 × 10^{-5}*M* and 3.50 × 10^{-8}*M*. The concentration of morphine added to the system is plotted against the amounts of bound (●) and free (□----□) morphine after dialysis. △ = percentage of protein bound.

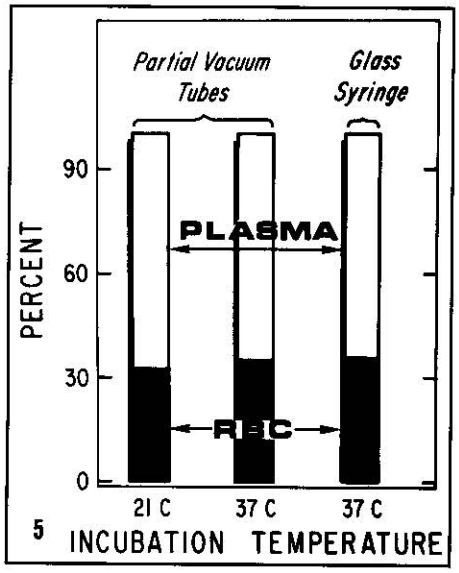

Fig 5—A lack of effect of temperature or collection method on RBC partitioning of morphine was found.

and 6 to 17 minutes.[4] This rapid phase probably represents the distribution of the alkaloid between blood and readily perfused tissue.[5] In human beings, a biological $t_{1/2}$ between 78 and 204 minutes has been reported.[1,4-7,9] The $t_{1/2(\beta)}$ of 87.9 minutes in the present study falls within this range and represents metabolism and redistribution, and excretion of the morphine. The $t_{1/2(\gamma)}$ of 56.3 hours is longer than the $t_{1/2(\gamma)}$ which ranged between 10 and 44 hours in a study of human volunteers.[5] It has been proposed that this prolonged presence of morphine may represent continued metabolism (although the present study examined only free morphine concentrations), release of the drug from tissues, and enterohepatic recirculation.[5] Because the $t_{1/2}$ after a 1-mg/kg dose in human beings were similar to those reported after 10-fold smaller doses, it is believed that the elimination mechanisms are nonsaturable,[2] at least over the therapeutic range.

Blood concentrations of morphine were similar between human studies and the present equine study. Four hours after IV dosing with approximately 0.15 mg of morphine/kg, Dahlstrom et al[3] and Brunk and Delle[7] reported average plasma values of 7 and 8.5 ng/ml, respectively. Hug,[4] after dosing with 0.1 mg of morphine/kg, recovered < 5 ng/ml at 4 hours, whereas in the present study the average morphine concentrations in horses 4 hours after dosing with 0.1 mg of morphine/kg were 6.2 ng/ml.

The short α phase was not detected in the urinary data. These data could be separated into 2 phases corresponding to the β and γ phases of the plasma data. The $t_{1/2}$ of the β phase of urinary data ranged from 101 minutes for total morphine, to 106 minutes for free morphine, to 113 minutes for the glucuronic acid conjugate. These are somewhat longer than the $t_{1/2(\beta)}$ of 87.9 minutes for free serum morphine, probably because analysis of larger urine volumes than serum volumes and hydrolysis permitted detection of slightly lower urine drug concentrations than serum drug concentrations. Furthermore, the urine data are based on absolute values, rather than cumulative excretion because of the difficulty in obtaining total urine volumes from a horse. Free morphine concentrations in the urine exhibited a longer γ phase $t_{1/2}$ (5,089 minutes) than the serum $t_{1/2(\gamma)}$ (3,377 minutes), again probably for reasons similar to those postulated for the longer urinary β phase $t_{1/2}$.

Because only a small fraction (8% to 12%)[8,9] of a morphine dose is recovered as the parent drug, renal clearance is an unimportant contribution to total body clearance. Hepatic biotransformation, mainly to morphine glucuronide, accounts for most of the total metabolic clearance.[2] The horses in the present study were maintained on pasture and had a more alkaline urine than would be expected for racing horses maintained on a high-protein diet. It should be remembered that racing horses with a lower urine pH may exhibit a different biological $t_{1/2}$ of morphine than indicated by the present study.

Work done with cannulated and normal dogs indicates that the slow terminal phase of urinary excretion is due to recycling of drug secreted into the bile.[10] Biliary excretion accounts for about 10% of the dose in human beings,[8] but there is evidence that this varies widely with the species.[3] Up to 63% of a dose of morphine given to a rat has been recovered from the bile.[11] Data are not available on biliary excretion of morphine in the horse.

The bulk of the morphine is excreted rapidly, despite a prolonged low-level excretion (Fig 2). Earlier studies done in this laboratory found that 51.3% of the total dose of morphine was recovered in the first 8 hours,[12] compared with 77% of the recovered amount from urine of human volunteers in the same period.[9]

Any drug that is bound to protein is usually pharmacologically inactive, ie, it is unavailable for membrane and renal transport. Although

the drug-protein complex is generally rapidly dissociable and thus has little effect on active transport processes (tubular secretion), protein binding of a drug can alter passive transport rates, including glomerular filtration and diffusion into the liver and across the blood-brain barrier.

Before starting the study of protein binding of morphine to serum proteins, a potential in vitro problem was examined. A compound eluted from the stoppers of partial vacuum tubes (tris butoxyethyl phosphate ester, TBPE) has been shown to inhibit drug protein binding and allow increased distribution of certain drugs into the RBC.[13-15] Therefore, an experiment was done to determine whether the blood-collection technique would affect the whole blood partitioning or protein binding of morphine in equine blood samples. Significant influence by the blood-collection technique was not found on whole blood partitioning, but a significant effect was found on the protein binding. These findings support the theory that the TBPE-induced displacement phenomenon may be general for basic lipophilic drugs, particularly those that are highly protein-bound.[13-16] Unlike the drugs found to be affected by TBPE, which include quinidine, alprenolol, and propranolol,[13-15] morphine is neither as highly protein bound nor as lipid-soluble.

Equilibrium dialysis, the most commonly used technique to determine the degree of protein binding, was used in the present study. The protein and drug solutions were enclosed within a membrane that was permeable to the free drug, but not to the protein and drug-protein complexes. These sealed membranes, suspended in a solution at physiologic pH, were agitated gently at 37 C until equilibrium had been reached (24 hours).[17] The protein solution contained bound and free drug, whereas the buffer contained only free drug. Disadvantages of this method included the dilution of the protein solution due to osmotic pressure differences across the membrane, a prolonged time to reach equilibrium, and the adherence of some drugs to the dialysis membrane.[18]

Due to its lack of specificity, high affinity, and high concentration in serum, albumin is responsible for the major portion of serum protein binding for most drugs.[19] However, studies in which purified albumin is used as the protein are not fully predictive of the extent of binding in vivo. Some drugs bind to other serum proteins and some endogenous substances may compete with drugs for protein binding sites. Furthermore, the albumin concentration will affect the percentage of binding.[17,20,21] Therefore, whole serum was used in this protein-binding experiment.

For some drugs, there is a wide range in protein binding between species (50-fold between rat and rabbit protein binding of salicylate)[22] as a result of differences in functional groups on the albumin molecules involved in drug-protein interactions. However, this wide variability does not appear to hold true for morphine binding. In a study involving 13 species, the percentage of binding of morphine only varied between 10.6% (swine) and 23% (ox).[23] Individuals within the same species may show a wide range in protein binding (25% to 80% phenobarbital found in a human study).[24] This probably is due partly to disease processes and drug interactions, as well as genetic variability. The lack of variability seen among the horses in this study may have been partially attributed to the good health of all the horses, the absence of other drugs, the close breeding in this equine population, and the low percentage of morphine that was bound.

The present study showed that protein binding of morphine was independent of drug concentration over a wide range. Protein binding of morphine in human beings at a concentration of $3 \times 10^{-6}M$ to $4 \times 10^{-6}M$ has been reported as 20.08%[17] to 35.2%,[20] which is comparable with the average of 28.2% found in the present study at the same drug level.

The binding of drugs to RBC has received little attention. Drugs are known to penetrate the RBC at a rate related to their lipid solubility.[25] The fraction of a drug bound to the blood cells should be determined when examining the distribution of a drug in circulating blood. Although this procedure should be done at 37 C to help approximate in vivo conditions, there will seldom be a significant difference in results if the test is performed at room temperature, because lipid solubility of a substance, in this narrow temperature range, will be relatively unaffected. Twenty-two percent of the morphine was found sequestered in the RBC fraction. When corrected for the portion that RBC make up of the whole blood, it was found that 36% of the morphine was in the RBC.

Morphine remains detectable in the plasma and urine of horses for 48 and 144 hours, respectively. These doses of morphine, at about 50 mg/454-kg horse, are less than those that produce a locomotor response and are comparable with the dose required to produce analgesia in horses. These results, therefore, indicate that morphine should not be administered to a horse < 1 week before a race.

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