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Prodrugs for improved oral nalbuphine bioavailability: inter-species differences in the disposition of nalbuphine and its acetylsalicylate and anthranilate esters

Bruce J. Aungst, Melvyn J. Myers, Eli Shefter and Elie G. Shami

Du Pont Pharmaceuticals, Medical Products Department, Experimental Station, Wilmington, DE 19898 (U.S.A.)

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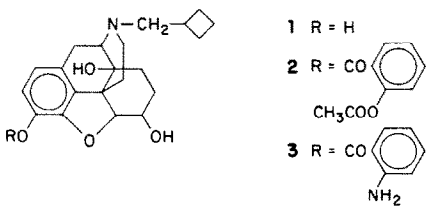
Summary

Two prodrugs of nalbuphine (**1**), the acetylsalicylate (**2**) and anthranilate (**3**) esters, were prepared with the objective of increasing oral nalbuphine bioavailability. Hydrolysis rates in rat, monkey, dog, and human plasma, human whole blood, and rat and dog liver and intestine homogenates were measured. Nalbuphine bioavailability after oral nalbuphine doses was determined, also in rats, monkeys, and dogs, and compared to a published value for man. There were large inter-species variations in prodrug hydrolysis rates and in oral nalbuphine bioavailability, and the dog bore closest resemblance to man. Oral nalbuphine bioavailability in dogs was increased 3–4-fold after administration of **2** and approximately 9-fold after administration of **3**. Levels of conjugated nalbuphine in plasma were reduced. This demonstrates the utility of the prodrug approach to reducing first-pass nalbuphine metabolism.

Introduction

The narcotic agonist/antagonist, nalbuphine (Nubain, Du Pont Pharmaceuticals), is an effective analgesic for relief of moderate to severe pain. Nalbuphine (**1**) undergoes extensive first-pass metabolism after oral administration. Oral bioavailability of nalbuphine in man was reported to be less than 10% of the dose (Lo et al., 1984). The major route of first-pass metabolism of nalbuphine and other phenolic opioids and opioid antagonists is conjugation of the phenolic hydroxyl group. This probably occurs in both the intestines and liver (Iwamoto and Klaassen, 1977).

There are a number of examples of the use of prodrugs to reduce first-pass metabolism and increase oral bioavailability. Bioavailability of methyldopa in rats and man was increased and sulfate conjugation was reduced when administered as the pivaloyloxyethyl ester (Vickers et al., 1978; Vickers et al., 1984). As a consequence of greater oral



Correspondence: B.J. Aungst, Du Pont Pharmaceuticals, Medical Products Department, Experimental Station, Building 400, Wilmington, DE 19898, U.S.A.

methyldopa bioavailability, the variability in bioavailability was also significantly reduced when administered as this prodrug (Dobrinska et al., 1982). Prodrugs improved the oral bioavailability of other phenols and catechols including etilefrine (Wagner et al., 1980), L-DOPA (Bodor et al., 1977), and terbutaline (Hörnblad et al., 1976). However, there are few published reports discussing prodrugs of phenolic opioids for improved oral bioavailability. Acetate and sulfate esters of the opioid antagonist, naloxone, were synthesized and preliminary pharmacologic activity in rats was reported (Linder and Fishman, 1973). However, oral naloxone bioavailability was not determined. Heroin is similar to a prodrug since it is metabolized to morphine after parenteral and oral administration, but oral morphine bioavailability is not improved when administered as heroin (Inturrisi et al., 1984).

We hypothesized using the prodrug approach to reduce first-pass metabolism and increase oral nalbuphine bioavailability. This hypothesis is diagrammed in Fig. 1. A prodrug with a labile substituent at the phenolic hydroxyl position might be completely absorbed intact, or at least to a greater extent than nalbuphine, and then be hydrolyzed in the blood to release nalbuphine. We wanted to test the proposed hypothesis and to screen prodrug candidates using methods most predictive of man. In addition, it was necessary to develop a methodology by which screening could proceed most rapidly. Rather than developing analytical methods for each prodrug candidate, we chose to use techniques in which only nalbuphine concentrations were determined. This paper describes the evaluation of two nalbuphine prodrugs using various criteria and the inter-species variability of those criteria, and demonstrates that two prodrugs substantially increased oral nalbuphine bioavailability.

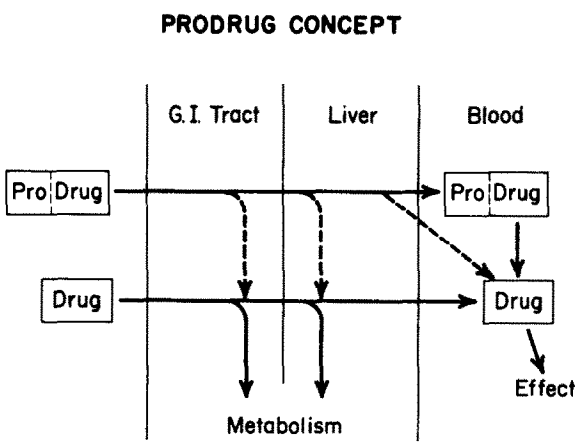


Fig. 1. Schematic illustration of the prodrug approach to reducing first-pass metabolism. The prodrug might be metabolized on passage through the intestinal membrane and liver, but hopefully to a lesser extent than the drug. By definition the prodrug must be converted to the drug to exert the desired pharmacologic effect.

Materials and Methods

Synthesis

2 was prepared as follows. To a round-bottom flask containing 35 ml methylene chloride was added and dissolved 3.57 g (0.01 mol) of **1**. Then 1.11 g (0.011 mol) triethylamine (Aldrich Chemical Co.) was added and the solution was cooled to 0–5 °C. A solution of 2.18 g (0.011 mol) of acetylsalicyloyl chloride (Aldrich Chemical Co.) in 25 ml methylene chloride was added dropwise with vigorous stirring under nitrogen at 0–5 °C. After the addition was completed, the ice-bath was removed and the reaction was stirred at ambient temperature for 5 h. TLC on silica gel using ethyl acetate/hexane (3/1) showed the desired product with a trace of nalbuphine remaining. The reaction mixture was washed once with 10% sodium carbonate and once with water, dried over sodium sulfate, filtered, and evaporated. The remaining solid was triturated with ether, filtered and air dried to yield 4.6 g nalbuphine-3-acetylsalicylate, with a melting point of 165–168 °C. TLC showed the product at R_f 0.22 and impurities at R_f 0.01 and 0.09. This material was purified using column chromatography at 40 psi (house air) and 15–25 μm silica gel in a 5 × 30 cm column. The product was dissolved in methylene chloride and added onto the column. Elution with hexane/acetone (70 : 30) produced 3.6 g of pure product, m.p. 172–173 °C. On TLC there was one spot, R_f 0.22. Analytical HPLC using a mobile phase of 0.05 M trishydroxymethyl-

aminomethane at pH 7.5 and acetonitrile (50 : 50) on a 4.6 mm × 25 cm column showed purity > 99%. No bis-acylation was detected at either the 6α-OH, or the 14β-OH positions by HPLC, TLC, high resolution mass spectrophotometry, and NMR. NMR (DMSO-d_6) 2.25 ppm (singlet, 3H, CH_3CO); 4.05 ppm (broad, 1H, 6β-H); 4.29 ppm (singlet, 1H, 6α-OH, J = 5.00 Hz); 4.52 ppm (singlet, 1H, 14β-OH); 6.79 ppm (AB quartet, 2H, nalbuphine 1- and 2-aromatic protons, J = 7 Hz); 7.29 ppm (doublet of doublets, 1H, ortho to OAc, J = 7 Hz, J_{meta} = 1.0 Hz); 7.49 ppm (triplet of doublets from meta coupling, 1H, para to OAc, J = 7 Hz, J_{meta} = 1.5 Hz); 7.77 ppm (triplet of doublets, 1H, para to –C=O, J = 7 Hz, J_{meta} = 2 Hz); 8.14 ppm (doublet of doublets, 1H, ortho to –C=O, J = 7 Hz, J_{meta} = 2 Hz). The phenolic 3-OH of nalbuphine is gone. Anal.: calculated for $C_{30}H_{33}NO_7$: C, 69.21%; H, 6.58%; N, 2.69%. Found: C, 69.50%; H, 6.55%, N, 2.61%.

For **3** synthesis the following were added to a 50 ml round-bottom flask; 3.57 g (0.01 mol) of **1**, 1.96 g (0.023 mol) of isatoic anhydride (Aldrich Chemical Co.), 0.12 g (0.0001 mol) of 3-dimethylaminopyridine (Aldrich Chemical Co.), and 25 ml dimethylformamide. The reaction mixture was then heated under nitrogen in an oil bath at 55–60 °C for 5 h. The flask was removed from the oil bath and 25 ml water added. The product precipitated out as dense crystals. After being at room temperature for 1 h, the product was collected, washed with water and air-dried. The yield was 4.2 g with m.p. 199–202 °C. TLC on silica gel using ethyl acetate/hexane (3 : 1) showed product at R_f 0.6 and an impurity at R_f 0.16. The product was dissolved in methylene chloride and treated with charcoal to remove the tan color. Evaporation of the methylene chloride gave white crystals. One recrystallization from ethyl acetate gave 4.0 g of nalbuphine-3-anthranilate, m.p. 205–206 °C. TLC showed product at R_f 0.6. Analytical HPLC as described for **2** showed the product was 99% pure. NMR (DMSO-d_6) 4.03 ppm (broad, 1H, 6β-H); 4.33 ppm (singlet, 1H, 6α-OH, J = 3.3 Hz); 4.53 ppm (singlet, 1H, 5β-H, J = 3.3 Hz); 4.82 ppm (singlet, 1H, 14β-OH); 6.56–6.94 ppm (complex multiplet, 4-aromatics and $-NH_2$); 7.33 ppm (triplet, 1H, aromatic, 4′); 7.92 ppm (doublet, 1H-aromatic, 6′, deshielded by the carbonyl of the ester); the phenolic-3-OH of nalbuphine is gone. Anal.: calculated for $C_{28}H_{32}N_2O_5$: C, 70.57%; H, 6.77%; N 5.88%. Found: C, 70.67%; H, 6.86%; N, 5.81%. The base was converted to the monohydrochloride by dissolving it in 10.0 ml tetrahydrofuran and adding 2.0 g of a 20% solution of HCl in ethanol dropwise. The monohydrochloride precipitated as white crystals. It was collected by filtration, washed with ether and air dried. The yield was 4.1 g with m.p. 254 °C.

Analytical

Nalbuphine concentrations in extracts of plasma and tissue homogenates were determined by HPLC using a method similar to that reported by Lo et al. (1984). A 25 cm octylsilane (Zorbax, Du Pont) column and a mobile phase containing 10–15% acetonitrile and 0.2% tetrahydrofuran in 0.055 M phosphate buffer (pH 3–4) were used. Electrochemical detection at a potential of +0.98 V was employed with a glassy carbon electrode. Naltrexone (Du Pont Pharmaceuticals), which was used as an internal standard, and a volume of 1 M carbonate buffer (pH 9.3) equal to the sample volume were added to plasma and tissue homogenate samples. These were then doubly extracted into toluene/ethyl acetate/isopropanol (70 : 29 : 1) and back-extracted into 0.2 ml of 0.3 M phosphoric acid which was injected onto the HPLC. For 0.2–0.5 ml samples, two 4 ml volumes of organic phase were used, and for 1 ml samples, two 8 ml volumes of organic phase were used. The linear detector response range was 0.001–0.28 nmol, and the sensitivity assaying 0.2 ml samples was 0.014 μM.

Prodrug hydrolysis in plasma, blood, and tissue homogenates

Rates of **2** and **3** hydrolysis to nalbuphine in human, monkey, dog and rat plasma were determined within 24 h of plasma collection. Plasma from each species was anticoagulated with heparin. Human plasma was obtained from a local blood bank. The other species used were Cynomolgus monkey, beagle dog, and Sprague–Dawley rat. Monkey and dog blood was collected by venipuncture. Rat blood was collected by cardiac

puncture. Prodrug hydrolysis was performed in 0.5 ml plasma aliquots containing 0.14 μM naltrexone in extraction test tubes in a 37 °C bath. At time-zero the prodrug (0.28 μM) was added and after various times of incubation, the plasma was extracted as previously described. Enzymatic hydrolysis of the prodrugs was stopped by the addition of the extraction solvent. Prodrug stability during the extraction procedure and prior to HPLC injection was confirmed by the absence of nalbuphine in samples extracted at time zero.

Specimens of dog liver and small intestine were obtained after thiopental overdose. Rat liver and small intestine specimens were obtained after ether overdose. Prodrug hydrolysis experiments were begun within 24 h of tissue collection. Tissues were rinsed with saline, blotted dry, chopped with a pair of scissors, and an amount was weighed. The tissue was diluted with 3 or 9 vols. of saline to yield 25% or 10% (w/v) specimens, which were then homogenized. The rate of **3** hydrolysis was measured in tissue homogenates as previously described for plasma.

The rate of **3** hydrolysis in dog and human whole blood was also determined. **3** was added to 50 ml of fresh blood at 37 °C at a concentration of 0.28 μM. Blood was anticoagulated with heparin. At various times of incubation a 4 ml aliquot of blood was centrifuged and plasma collected and extracted for determination of nalbuphine concentration.

For plasma, blood, and tissue homogenates, the percentage of **2** or **3** remaining to be hydrolyzed was calculated using eqn. 1, where the nalbuphine concentration at time ∞ was assumed to be the initial prodrug concentration (μM).

$$\% \text{ Remaining} = \frac{(\text{Nalbuphine Conc})_\infty - (\text{Nalbuphine Conc})_t}{(\text{Nalbuphine Conc})_\infty} \times 100 \quad (1)$$

Bioavailability

Oral nalbuphine (**1**) bioavailability after administration of the parent drug (**1**) was determined in rats, dogs, and monkeys. Rats and dogs were also administered **2** and **3** orally, and **1** bioavailability was calculated. All animals were fasted overnight prior to dosing. Five separate groups of rats received one of the following treatments; 1.4 μmol/kg **1** i.v. (1.4 mM in saline), 5.6 μmol/kg **1** i.v. (5.6 mM in saline), or 56 μmol/kg of **1**, **2**, or **3** p.o. (56 mM in water or 0.1 N HCl). Dosing was by cardiac puncture under light ether anesthesia or by gastric intubation.

Thirteen beagle dogs were administered **1** i.v. or orally, or **2** or **3** orally. Some dogs received several treatments as summarized in Table 1. For i.v. dosing, **1** was dissolved in saline (2.8 mM) and the dose was 2.8 μmol/kg injected via the cephalic vein. **1** was administered orally as a powder packed into gelatin capsules or as a solution (11.2 mM) in water. The dose in each case was 11.2 μmol/kg. **2** was administered as the HCl salt packed into gelatin capsules or as the free base dissolved in 0.1 N HCl. The dose was 11.2 μmol/kg. **3** was administered at 2.8 μmol/kg and 11.2 μmol/kg doses dissolved in water using 1 ml/kg dosing volumes.

TABLE 1

Design of dog bioavailability experiments

Dog no.	**1**, i.v. [a]	**1**, p.o. [b]	**2**, p.o. [c]	**3**, p.o. [d]
1	×	C	C, HCl	
2	×	C	C, HCl	
3	×	C	C, HCl	
4	×	C	C, HCl	
5	× [e]	S	S, Base	S, High
6	×	S		S, High
7	×	S		S, High
8			S, Base	S, Low
9			S, Base	S, High
10				S, Low
11				S, Low
12				S, High
13				S, High

[a] × indicates which dogs received an i.v. dose.

[b] Oral doses were administered in powder-packed capsules (C) or in solution (S).

[c] **2** was administered as either the HCl salt or the free base dissolved in 0.1 N HCl.

[d] **3** was administered at a dose of 11.2 μmol/kg (high) or 2.8 μmol/kg (low).

[e] Underlining indicates that conjugated nalbuphine concentrations were also determined.

All oral doses were followed by oral administration of 50 ml water. Blood (4 ml) was collected by jugular venipuncture in evacuated tubes containing EDTA for anticoagulation. Plasma was immediately separated and frozen until analyzed. At least 1 week separated treatments if animals received more than one treatment.

Nalbuphine bioavailability was determined in two Cynomolgus monkeys administered **1** i.v. and orally. Intravenous dosing and blood sample collections were via cephalic cannulae. The monkeys were fasted overnight prior to dosing. The doses were 2.8 μmol/kg i.v. and 11.2 μmol/kg p.o., the dosing volumes being 1 ml/kg. Plasma was immediately separated and frozen until analyzed.

Oral nalbuphine bioavailability (F) was calculated using area under the plasma nalbuphine concentration vs time curve (AUC) as in Eqn. 2.

$$F = \frac{AUC^{\text{p.o.}} \times Dose^{\text{i.v.}}}{AUC^{\text{i.v.}} \times Dose^{\text{p.o.}}} \times 100 \qquad (2)$$

AUC was calculated using the trapezoidal method. For rats, individual values of $AUC_{0-4h}^{\text{p.o.}}$ were calculated and compared to an average, dose-normalized $AUC_{0-4h}^{\text{i.v.}}$ from 1.4 μmol/kg and 5.6 μmol/kg i.v. doses. The truncated AUC was preferred in rats rather than estimating the terminal decay slope after oral doses. In monkeys and dogs, however, $AUC_{0-\infty}$ values were calculated, with the residual area calculated using the terminal decay slope and the last measured plasma concentration. For the two monkeys individual $AUC^{\text{p.o.}}$ and $AUC^{\text{i.v.}}$ values were used. For dogs which were crossed-over, oral and i.v. individual $AUC^{\text{p.o.}}$ and $AUC^{\text{i.v.}}$ values were used. If dogs did not receive **1** i.v., F was calculated using the group mean $AUC_{0-\infty}^{\text{i.v.}}$. Statistical comparisons of F values were done using t-tests. For animals dosed with prodrugs, these bioavailability calculations represent nalbuphine in plasma derived from prodrug absorbed and metabolized to nalbuphine, plus nalbuphine absorbed subsequent to prodrug hydrolysis by the gut or liver.

Conjugate levels

To provide further evidence of the extent of first-pass metabolism, the levels of conjugated nalbuphine in some of the dog plasma samples from the bioavailability studies were also determined. To 0.5 ml plasma was added 1000 U β-glucuronidase (Sigma Chemical Co., type LII) in 0.2 ml of 0.1 M sodium acetate buffer. Controls, to which enzyme-free buffer was added, were also run for most plasma samples to measure non-enzymatic hydrolysis of conjugated nalbuphine. Samples were then incubated at 37 °C for 16–20 h, and then extracted and analyzed for nalbuphine concentration. The concentration of conjugated nalbuphine was taken as the nalbuphine concentration after β-glucuronidase hydrolysis minus the nalbuphine concentration in samples without added enzyme.

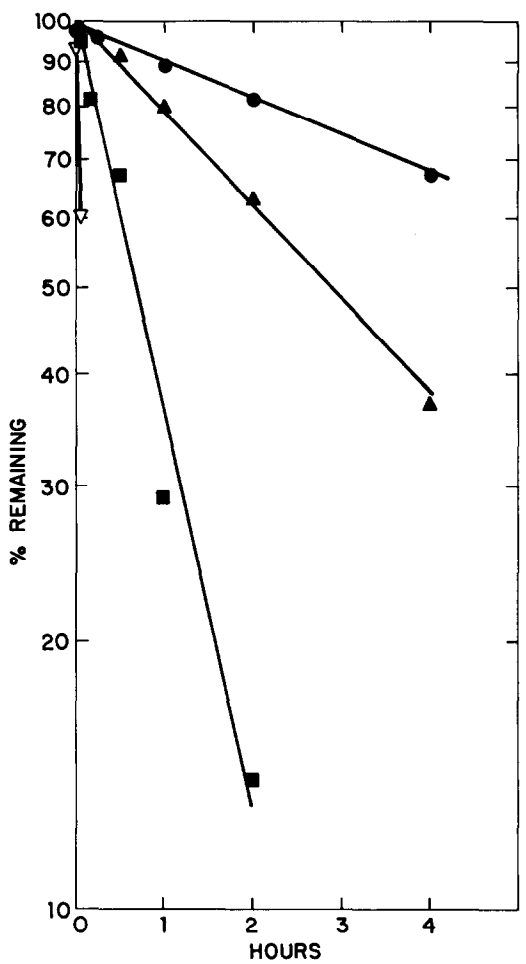


Fig. 2. Representative profiles of in vitro **2** hydrolysis in plasma from human (●), dog (▲), monkey (■), or rat (▽).

Results

Prodrug hydrolysis in vitro

The rates of hydrolysis of **2** and **3** in plasma in vitro were highly species-dependent. Plots of **2** and **3** hydrolysis are shown in Figs. 2 and 3, respectively. Both prodrugs exhibited apparent first-order decay kinetics. Hydrolysis half-lives are summarized in Table 2. The rank order among species of the hydrolysis rates was the same for both prodrugs, that being: rat > monkey > dog > human. In each species hydrolysis in plasma of **2** was several-fold faster than that of **3**. For both **2** and **3**, initial nalbuphine concentrations were negligible, indicating stability of these prodrugs through extraction and storage until HPLC injection. Our methods do not, however, exclude the possibility that nalbuphine acetylsalicylate (**2**) was deacetylated more rapidly than the hydrolysis to nalbuphine, since only nalbuphine appearance was measured.

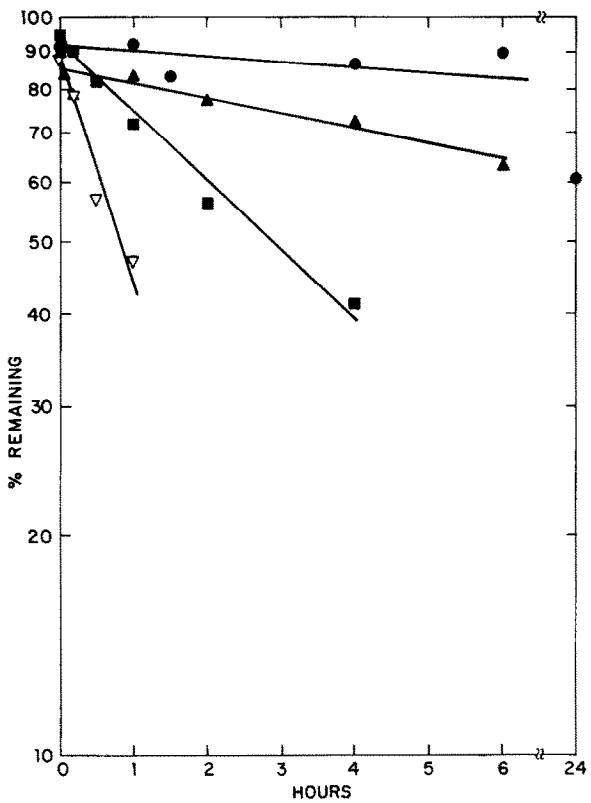


Fig. 3. Representative profiles of in vitro **3** hydrolysis in plasma from human (●), dog (▲), monkey (■), or rat (▽).

TABLE 2

Rates of hydrolysis of the acetylsalicylate (2) and anthranilate (3) esters to nalbuphine (1) in various species, determined by measuring nalbuphine appearance. Each value represents a separate experiment

Species	Tissue [a]	Hydrolysis half-life (h)	
		2	**3**
Rat	Plasma	0.08, 0.24, 0.08	2.0, 1.0
	Intestine (10% w/v)		1.6
	Liver (10% w/v)		0.7
Monkey	Plasma	0.6	3.3
Dog	Plasma	2.8, 3.7	14.6, 15.6, 16.5
	Intestine (25% w/v)		2.9
	Liver (25% w/v)		3.1
	Whole Blood		5.9, 2.2
Human	Plasma	9.6, 7.4	44.7 [a], 66.8
	Whole Blood		4.7 [a], 5.3

[a] Plasma and whole blood were from the same blood specimen.

As also shown in Table 2, **3** was hydrolyzed in dog and human whole blood more rapidly than in the plasma fraction, demonstrating the significant contribution of the erythrocytes. Hydrolysis rates in dog and human whole blood were quite similar. **3** was also hydrolyzed in diluted liver and small intestine homogenates from dog and rats much more rapidly (if corrected for the dilution factor) than in plasma from those species. It would therefore appear that the contribution of plasma to the hydrolytic metabolism of **3** in vivo is probably not as great as that of other tissues, and the in vitro half-life in plasma is not predictive of in vivo disposition.

Oral nalbuphine bioavailability

Rats were administered two i.v. nalbuphine doses. Plasma nalbuphine concentrations were proportional to the dose (Fig. 4) and systemic clearance was independent of the dose. Plots of dose-normalized plasma concentrations were superimposable. These data were therefore normalized for dose and pooled; pooled data were used

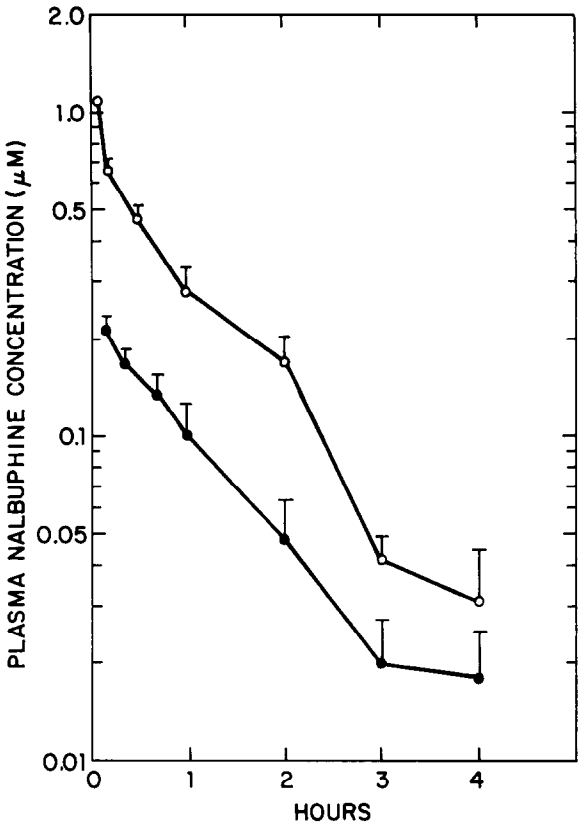


Fig. 4. Plasma nalbuphine concentration versus time profiles (mean ± S.E.M.) in rats administered nalbuphine-HCl i.v., 1.4 μmol/kg (●) or 5.6 μmol/kg (○).

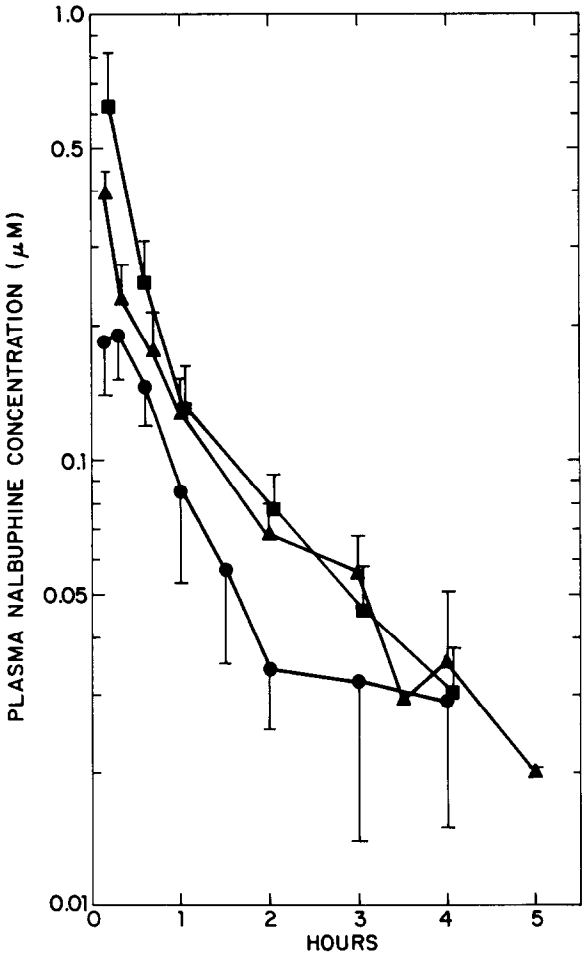


Fig. 5. Plasma nalbuphine concentrations (mean ± S.E.M.) in rats administered equimolar oral doses of **1** (●), **2** (▲), or **3** (■).

to estimate oral nalbuphine bioavailability. The plasma concentration vs time profiles in rats which were administered **1**, **2**, or **3** orally are shown in Fig. 5. Oral nalbuphine bioavailability when administered as nalbuphine-HCl was quite low (Table 3). This is consistent with extensive first-pass metabolism, a trait shared with other phenolic opioids. When **2**, the acetylsalicyloyl nalbuphine ester, was administered nalbuphine bioavailability was slightly, but not significantly, improved. Oral nalbuphine bioavailability was increased almost two-fold when administered as **3**, the anthranilate ester.

TABLE 3

Oral nalbuphine bioavailability (F) in rats

Compound administered	*F* (% dose) [a]	Number of rats	Difference from **1**
1	2.7 ± 0.4	12	
2	3.9 ± 0.5	6	$0.05 < P < 0.1$
3	5.1 ± 1.1	5	$P < 0.05$

[a] Results are mean ± S.E.M.

1 was administered intravenously and orally in two Cynomolgus monkeys and oral bioavailability (*F*) was determined. Plasma nalbuphine concentration vs time profiles are shown in Fig. 6. In these two monkeys *F* was 0.9 and 1.6% of the dose. Since these values were even less similar to man than the rat was, as were the rates of **2** and **3** hydrolysis in plasma in vitro, the monkey was judged to not be a good animal model in which to evaluate bioavailability with **2** and **3**.

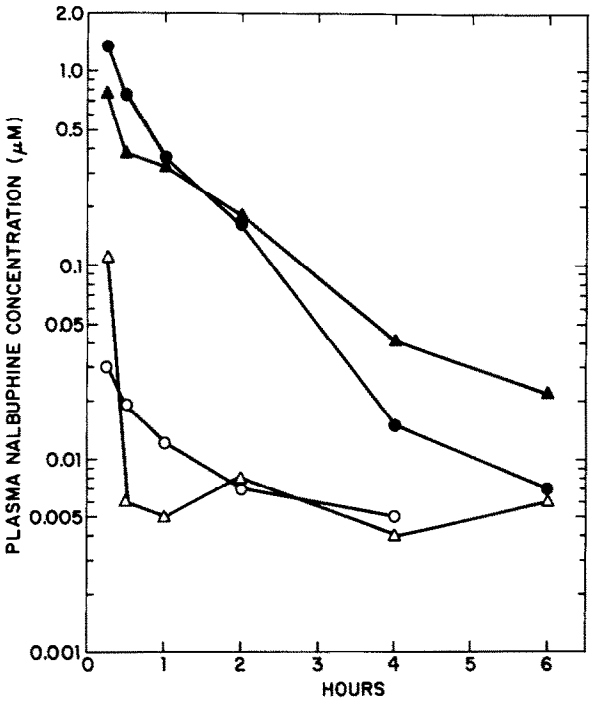


Fig. 6. Plasma nalbuphine concentration versus time profiles in monkeys administered **1**, 2.8 μmol/kg i.v. or 11.2 μmol/kg p.o. Key: monkey no. 1 i.v. (●) and p.o. (○); monkey no. 2 i.v. (▲) and p.o. (△).

Data for bioavailability in dogs are summarized in Table 4. Nalbuphine bioavailability after oral nalbuphine-HCl (**1**) administration was low, indicative of extensive first-pass metabolism. Nalbuphine oral bioavailability in the dog was more similar to the 10% value reported for man (Lo et al., 1984), than were the values in monkeys and rats. This supports using bioavailability in dogs as the primary criterion for prodrug evaluation.

Solution formulations were examined in the initial bioavailability studies to avoid any potential dissolution problems. A solid dosage form was also studied for **2**, since this would eventually be required for further development, and a similar formulation of **1** was used as a control. There was no significant difference in F between the groups administered **1** in solution or as a solid.

Both nalbuphine prodrugs (**2** and **3**) provided significant increases in oral nalbuphine bioavailability in dogs. **2** was administered in solution at a low dose and as the solid HCl salt in capsules at a higher dose. Nalbuphine bioavailability was similar for both groups. **3** provided greater oral nalbuphine bioavailability than **2**, and bioavailability was much greater after **3** than after **1**. There was no significant difference in nalbuphine bioavailability between the low and high **3** doses. Thus, for both **2** and **3**, lower doses could be used to achieve similar plasma nalbuphine concentrations than a higher **1** dose. The prodrug approach, as depicted in Fig. 1, was successfully used to improve oral nalbuphine bioavailability.

In rats, monkeys, and dogs the terminal slopes of the plasma concentration vs time profiles appeared less steep after oral nalbuphine than after i.v. dosing. Nalbuphine is rapidly absorbed from the gastrointestinal tract. Prolonged plasma levels after oral administration are most likely a consequence of enterohepatic cycling, since nalbuphine is extensively conjugated. It was previously shown

TABLE 4

Oral nalbuphine bioavailability (F) in dogs administered nalbuphine (1) or nalbuphine prodrugs (2 or 3)

Compound	Formulation	Dose (μmol/kg)	Number of dogs	F (% dose) [a]
1	HCl salt in capsule	11.2	4	7.0 ± 1.0
1	Solution of HCl salt in H_2O	11.2	3	5.6 ± 1.7
2	Solution of free base in 0.1 N HCl	3.9	3	17.0 ± 3.8
2	HCl salt in capsule	11.2	4	23.8 ± 4.9
3	Solution of HCl salt in H_2O	2.8	3	46.6 ± 9.8
3	Solution of HCl salt in H_2O	11.2	6	51.3 ± 4.4

[a] Data are mean ± S.E.M.

that naltrexone, which is structurally similar to nalbuphine and is also extensively conjugated, had a relatively flat plasma concentration vs time profile after oral dosing in monkeys, but linear and rapid decay after i.v. doses (Shepard et al., 1985).

Plasma levels of conjugated nalbuphine

If the primary route of first-pass nalbuphine metabolism is conjugation, the reduction of first-pass metabolism using the prodrug approach could also be reflected in the levels of conjugated nalbuphine in plasma. Plasma concentrations of conjugated nalbuphine in dogs administered **1** i.v. or p.o., or **2** or **3** p.o. are shown in Fig. 7. For dogs administered **1** orally, the maximum concentration of conjugated nalbuphine was approximately 50-fold greater than the maximum plasma nalbuphine concentration. However, in dogs administered **2** the ratio of $C_{max}^{conjugate}/C_{max}^{nalbuphine}$ was 2.5. After **3** the maximum conjugate concentration was less than the maximum nalbuphine concentration.

The area under the plasma conjugated nalbuphine concentration versus time curve ($AUC^{conj.}$) provides an indication of the amount

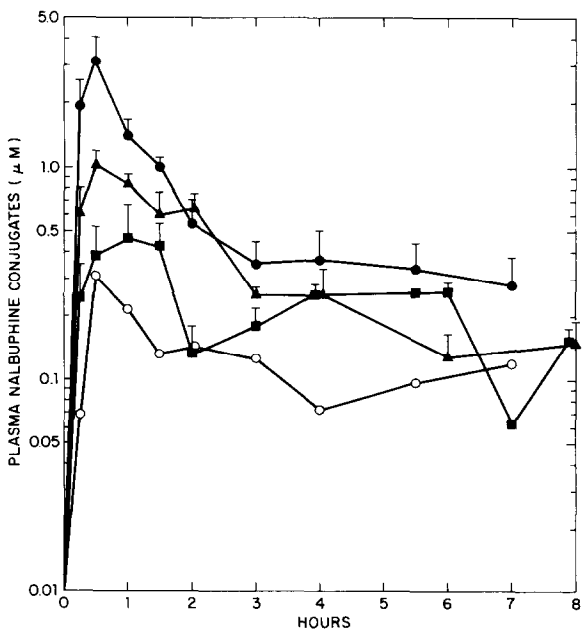


Fig. 7. Levels (mean ± S.E.M.) of conjugated nalbuphine in plasma of dogs administered **1** i.v. (○, $n = 2$) or p.o. (●, $n = 3$), **2** p.o. (▲, $n = 3$) or **3** p.o. (■, $n = 5$). The i.v. dose was 2.8 μmol/kg and each oral dose was 11.2 μmol/kg.

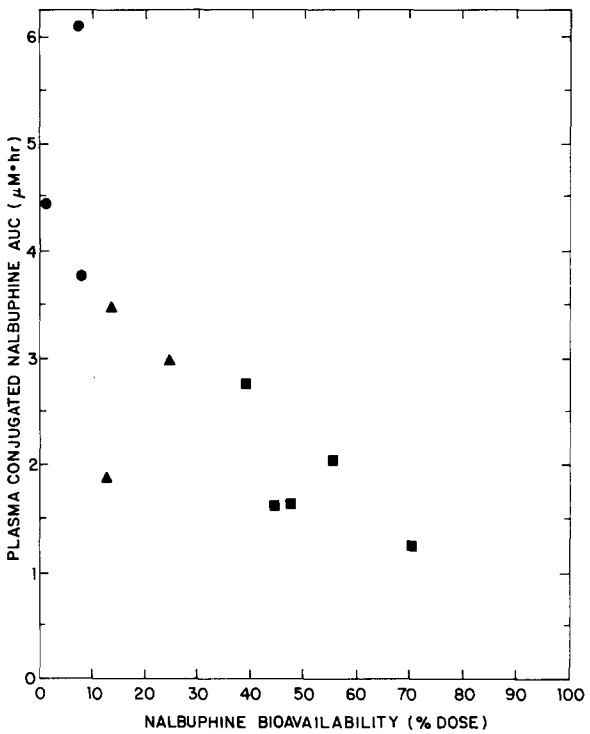


Fig. 8. Relationship between the *AUC* for plasma conjugated nalbuphine and oral nalbuphine bioavailability in dogs dosed orally with **1** (●), **2** (▲), of **3** (■).

of conjugate formed, assuming that clearance of conjugated nalbuphine is independent of the treatment. Individual values of ($AUC^{conj.}$) from 0 to 7 or 8 h were determined and are plotted versus the individual values of oral nalbuphine bioavailability which were previously determined. This relationship is shown in Fig. 8. Clearly there was a trend for increased bioavailability to be associated with decreased formation of conjugated nalbuphine. This supports the initial hypothesis that prodrugs which effectively block conjugation can increase oral nalbuphine bioavailability. For dogs administered the low dose of **3**, nalbuphine conjugate levels were very low. The decrease in $AUC^{conj.}$ implies that nalbuphine may be eliminated via a mechanism other than conjugation (otherwise $AUC^{conj.}$ should be independent of the treatment, if absorption was complete).

Nalbuphine concentrations in samples incubated with buffer as controls in determining conjugate concentrations were compared to the

previously determined nalbuphine concentrations in the same samples (but not incubated with buffer). For dogs administered **1** or **2**, these values were quite similar, indicating negligible **2** in plasma which would have been hydrolyzed during incubation. However, plasma from dogs dosed with **3**, when incubated with buffer, had higher nalbuphine concentrations than when initially determined. This indicates that plasma contained some unchanged prodrug which was hydrolyzed during incubation with buffer. This was confirmed in subsequent studies which will be presented separately.

Discussion

Prodrugs have been frequently used with the intent of improving physicochemical or pharmacokinetic properties of drugs; for example, modifying solubility, stability, taste, toxicity, elimination half-life, or bioavailability. The criteria by which prodrugs are evaluated depend on the intended application. Our goal is to improve oral nalbuphine bioavailability in man. Since it was not feasible to screen prodrug candidates by measuring bioavailability in man, it was necessary to establish testing criteria in the most similar animal model. We wanted a prodrug which would be absorbed intact to a greater extent than nalbuphine through the sites of nalbuphine first-pass metabolism (the intestinal wall and liver), and which would then be rapidly hydrolyzed to release nalbuphine. It was necessary, then, that the animal model be similar to man with regard to: (1) nalbuphine disposition (bioavailability) after oral dosing; and (2) prodrug hydrolysis rates. Of rat, monkey, and dog, the latter was most like man according to these criteria. The possibility remains that the dog, or any animal model, could be unlike man in ways we have not evaluated. Other factors, which could be species-dependent, include: (1) the site of nalbuphine first-pass metabolism (relative contributions of liver and intestines); and (2) relative contributions to nalbuphine clearance of metabolic routes other than conjugation.

Oral nalbuphine bioavailability was significantly improved by administration as prodrugs **2** and **3** and the levels of conjugated nalbuphine in plasma were correspondingly reduced. Based on in vitro hydrolysis experiments, it would appear that these prodrugs are at least partially hydrolyzed to nalbuphine by the gut and liver prior to reaching the systemic circulation. The hydrolysis half-life in plasma does not appear to be indicative of in vivo half-life, although in vitro half-life is a useful measure of relative stability to enzymatic attack.

A major problem limiting the chronic use of phenolic opioids for control of moderate to severe pain is that frequent injections are required; oral bioavailability is generally too low to make this route of administration efficacious (Gourlay and Cousins, 1984). Opioid antagonists are similarly subject to extensive first-pass conjugation at the 3-phenolic hydroxyl position. Nalbuphine has been shown to be equipotent to morphine when administered parenterally, and has the advantage of lower abuse potential, since it also has antagonist properties (Errick and Heel, 1983). Prodrugs appear to represent a feasible way to more effectively deliver nalbuphine orally.

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