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Investigation of the antinociceptive efficacy and relative potency of extended duration injectable 3-acylmorphine-6-sulfate prodrugs in rats

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Abstract

This investigation was designed to examine the antinociceptive activity in rats of 3-*O*-acyl prodrugs of M6S relative to the parent drug, after intravenous and intramuscular injection, using the tail flick latency test of antinociception. M6S, 3-acetylmorphine-6-sulfate (3AcM6S), 3-propionylmorphine-6-sulfate (3PrM6S), 3-butanoylmorphine-6-sulfate (3BuM6S) and 3-heptanoylmorphine-6-sulfate (3HpM6S) were administered by the IV route in a dose of 4.10 μmol/kg. Relatively high levels of antinociception ($>40\%$ Maximum Possible Effect) were achieved following administration of M6S, 3AcM6S and 3PrM6S, whereas insignificant antinociception ($<20\%$MPE) was achieved following administration of 3BuM6S or 3HpM6S. Although the mean duration of action for 3AcM6S (6 h) was longer than for M6S or 3PrM6S (4 h), the mean area ($\pm$S.E.M.) under the degree of antinociception versus time curve (AUC) for 3AcM6S ($151.6 \pm 6.9\%$MPE h) was not significantly different ($p < 0.05$) from that for M6S ($120.8 \pm 32.7\%$MPE h) or for 3PrM6S ($106.0 \pm 21.3\%$MPE h). The mean ED_{50} (range) doses for M6S, 3AcM6S and 3PrM6S were calculated to be 4.16 (3.61–4.48), 4.32 (3.55–5.09) and 4.54 (4.21–4.79) μmol/kg, respectively. Preliminary studies were conducted on potential long-acting formulations containing $8 \times ED_{50}$ doses of M6S and the 3-acetyl and 3-propionyl esters suspended in soybean oil. These showed that 3PrM6S gave a greater AUC (mean + S.E.M.) ($1087.4 \pm 97.4\%$MPE h) and longer duration of action (20 h) than did M6S ($613.1 \pm 155.9\%$MPE h; 10 h duration) or 3AcM6S ($379.3 + 114.2\%$MPE h; 8 h duration). Further studies are needed to more fully investigate these findings.

Keywords: Morphine; Morphine-6-sulfate; Analgesics; Prodrugs; Antinociception; Long-acting injection

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1. Introduction

The management of severe pain, especially in the terminally ill and in postsurgical patients, still presents many difficulties. Morphine is the most important potent opioid analgesic presently available. Nonetheless, its relatively low potency and short plasma elimination half-life of 1.5–2 h (Osborne et al., 1990) presents some difficulties in clinical pain management. Several long acting oral formulations of morphine have been developed and have greatly enhanced the management of chronic cancer pain. However, for patients unable to swallow or who have intractable nausea and vomiting, morphine may be administered either via a nasogastric tube or by continuous subcutaneous infusion, thereby greatly limiting patient comfort and mobility. Therefore, the availability of injectable depot preparations of morphine-like drugs may be of benefit to these patients. Several studies (Kim et al., 1993; Alvarez-Fuentes et al., 1996; Kim et al., 1996) have demonstrated a degree of success in the development of long-acting morphine formulations in the form of subcutaneous, oral controlled-release and epidural administrations, respectively.

Morphine is metabolized in animals and humans by a variety of metabolic pathways following oral and parenteral administration (Boerner et al., 1975), in particular glucuronidation of the 3-OH phenolic group and of the 6-OH alcoholic group to yield the major metabolites, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). M3G has no antinociceptive/analgesic activity and has been reported to be *antianalgesic*, in that following supraspinal administration, M3G attenuates morphine's antinociceptive effects (Smith et al., 1990; Gong et al., 1992). M6G has been reported to be considerably more potent than morphine as an antinociceptive agent, depending upon the species and the route of administration (Pasternak et al., 1987; Paul et al., 1989). That M6G is able to penetrate the blood–brain barrier (BBB), despite its apparent high polarity, has been proven unambiguously (Yoshimura et al., 1973).

Many compounds structurally similar to morphine have been prepared in efforts to minimize undesirable side-effects, such as nausea and vomiting, whilst retaining or even increasing the analgesic potency. A wide variety of substituents attached to the 3- and 6-positions of the morphine skeleton have been evaluated with a view to increasing antinociceptive potency. Some examples include normorphine-6-glucuronide (Oguri et al., 1989), acetyl and propionyl ester prodrugs of morphine (Whitehouse et al., 1990) and codeine-6-glucuronide (Srinivasan et al., 1996). Currently, there are no compounds that have been shown to have advantages over morphine in all aspects of the pharmacological profile.

Morphine-6-sulfate (M6S) has been shown to have several-fold higher antinociceptive potency than morphine when administered subcutaneously to rodents. Mori et al. (1972) reported that M6S had high antinociceptive potency and a long duration of action following subcutaneous injection into mice, similar to M6G. Other investigations (Brown et al., 1985; Houdi et al., 1996) also found a higher potency for M6S relative to morphine after intracerebroventricular (icv) injection in rats. In contrast to M6G, the high potency of M6S has not been closely investigated. M6S and its derivatives were chosen for the studies described herein as their syntheses are much simpler than those of the M6G derivatives, but the potency and duration of action appear to be similar (Mori et al., 1972). Therefore, the aims of this study were to investigate the antinociceptive properties and the duration of action of M6S and its 3-*O*-acyl ester prodrug derivatives with a view to developing long-acting injectable preparations of morphine-like analgesics.

2. Materials and methods

2.1. Materials

M6S and four 3-acyl prodrugs of M6S, i.e. 3-acetylmorphine-6-sulfate (3AcM6S), 3-propionylmorphine-6-sulfate (3PrM6S), 3-butanoylmorphine-6-sulfate (3BuM6S) and 3-heptanoylmorphine-6-sulfate (3HpM6S) were synthesized and purified in earlier work (Preechagoon et al., 1998). Disodium monohydrogen phosphate and phosphoric acid (HPLC grade) were bought from BDH, Australia. Soybean oil was purchased from Sigma, Australia.

2.2. Animals

The study protocol was approved by the University of Queensland Animal Experimentation Ethics Committee (Approval Number: Pharm/575/95/PhD). Adult male Sprague–Dawley rats (Animal Breeding Facility of the Faculty of Medicine, The University of Queensland) weighing 300–350 g were used. Upon arrival, pairs of animals were housed in standard cages and maintained in a temperature-controlled room (21 ± 2°C) maintained on a 12/12 h light–dark cycle starting at 06:00. Animals had free access to water and food (Norco, Norco-cooperative, NSW). Rats were allowed at least three days to acclimatize to their new surroundings prior to surgery and were used only once.

2.3. Surgery

Rats were anaesthetized with 3% isoflurane (Forthan®, Abbott) using a recently calibrated delivery unit (Tritec Cyprane, UK) with a slow flow of medical-grade oxygen (BOC, Brisbane, Australia). After 5 min, the back of the neck and the inside of the left hind leg were shaved. The left ileac artery was exposed and a fine polyethylene cannula (0.5 × 1.0 mm i.d., Critchley, Sydney, Australia) filled with heparinized saline (10 IU/ml, Astra, Sydney, Australia) was inserted. Subsequently, the right common jugular vein was exposed and a 2 cm soft cannula, with a second polyethylene cannula connected to its distal end, was inserted. Both cannulae were securely sutured and externalized through a subcutaneous tunnel from the neck incision to the interscapular area. The cannulae were held in place and protected by a stainless steel spring. The cannulae were continuously perfused with heparinized saline (10 IU/ml), using spring-operated syringe pumps (Springfusor 10, Go Medical, Perth, Australia) at a rate of 4 ml/24 h. After surgery, animals were placed in individual cages for 24 h, with free access to food and water, before commencing antinociception studies.

2.4. Dose preparation

Each compound was accurately weighed (± 5 μg) with a microbalance (Model M5P, Sartorius AG, Gottingen, Germany) and dissolved in 0.15 M phosphate buffer (pH adjusted to 7.4 with phosphoric acid) at a dose of 4.10 μmol/kg (equivalent to 1.5 mg/kg of M6S) for intravenous injection. In all studies, the compound was dissolved in the buffer immediately prior to injection. The injection volume was 0.4 ml, except for 3HpM6S, where it was 0.8 ml. Doses ranging from 3.55 to 6.02 μmol/kg were used to obtain dose-response curves for M6S, 3AcM6S and 3PrM6S. Control rats received 0.4 ml of phosphate buffer by IV injection.

2.5. Tail flick latency determination

The antinociceptive activity of all compounds administered was compared using the rat tail flick latency method of D'Amour and Smith (1941). Rats were placed inside restraining cages which permitted free movement of the tail. Radiant heat was applied to the dorsum of the lower third of the tail and the time to flick the tail out of the beam was recorded automatically. A maximum tail flick latency of 9 s was used to minimize tissue damage. Prior to antinociceptive testing, each animal was habituated to the equipment for 15 min. The mean baseline pre-dose latency, from three consecutive determinations at 5 min intervals, was then measured just before drug administration.

The degree of antinociception was quantified by the percent maximum possible effect (%MPE) (Dewey and Harris, 1971), which was calculated from the tail flick latencies using the following equation:

$$\%\mathrm{MPE} = 100 \times \frac{\text{test latency} - \text{baseline latency}}{\text{maximum latency (9 s)} - \text{baseline latency}}$$

The duration of action was assessed from the time needed for the %MPE value to decay to the mean value for the controls plus three standard deviations. This corresponded to 10.5%MPE.

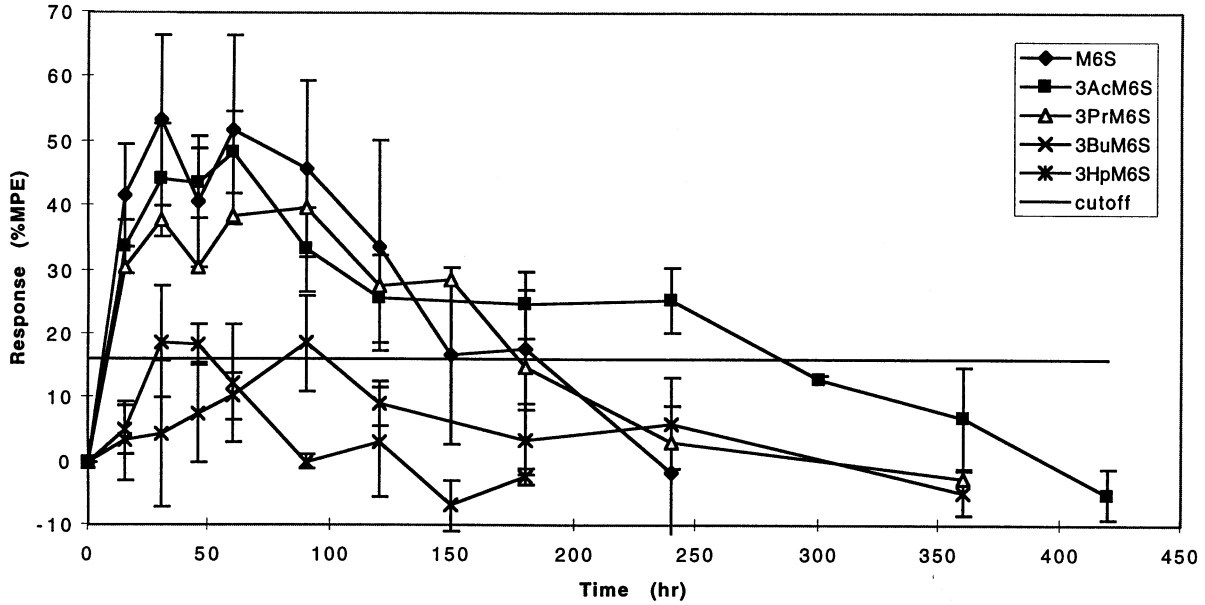

Fig. 1. Mean (± S.E.M.) degree of antinociception (%MPE) versus time curve for M6S and its 3-acyl prodrugs. The drugs were given intravenously in a dose of 4.10 μmol/kg ($n = 5$, M6S, 3AcM6S, 3PrM6S; $n = 4$, 3BuM6S, 3HpM6S). Control data has been omitted for clarity. The cutoff line marks the %MPE value which defined the duration of action.

2.6. Statistical analyses

Statistical comparisons between groups of rats were performed using the non-parametric Wilcoxon rank sums test. The statistical significance criterion was $p < 0.05$.

2.7. Antinociception studies

2.7.1. Intravenous single dose administration

To evaluate and compare the relative i.v. antinociceptive potencies of the 3-*O*-acyl derivatives of M6S, the administered dose of each compound was the same on a mol/kg basis (4.10 μmol/kg). The drug solution or phosphate buffer (controls) was administered through the jugular vein cannula over approximately 20–25 s, then flushed with 0.3 ml heparinized saline (10 IU/ml). Tail flick latencies were then determined at the following times post-dosing; 15, 30, 45, 60 min, 1.5, 2.0, 3.0, 4.0, 6.0 and 7.0 h (until antinociception had reached baseline values). At the completion of the experiment, the rats were euthanased with 100% carbon dioxide and the integrity of the venous cannula was verified by injection of concentrated red dye solution. At least four rats received each drug dose or phosphate buffer. For each drug administered, a graph of the mean (± S.E.M.) %MPE achieved was plotted as a function of time, Fig. 1. The area under the %MPE versus time curve (AUC) was calculated using the trapezoidal rule.

2.7.2. Dose-response relationships

Dose-response relationships were determined for M6S and two of the more promising 3-*O*-acyl prodrugs, 3AcM6S and 3PrM6S, following single-dose intravenous administration of three or four doses of each compound to rats in the dose range 3.55–6.02 μmol/kg and determination of tail flick latencies in a manner analogous to that already described in Section 2.5. %MPE versus time curves were plotted for each drug dose administered to individual rats and the AUC calculated. Dose-response curves were prepared using the 2 major methods commonly described in the literature viz. (i) plotting %MPE achieved at T_{max} versus dose and (ii) plotting AUC versus dose

(Fig. 2). The mean (± S.E.M.) ED_{50} doses (doses at which a half-maximal response would be expected) and their 95% confidence limits were estimated from the dose response relationships using Sigmaplot®, ver. 4.11 (Jandel, Corte Madera, CA, USA).

2.8. Preliminary evaluation of long-acting injectable formulations of M6S, 3AcM6S and 3PrM6S

This study was designed as a preliminary investigation of the antinociceptive effects of each of three compounds (M6S, 3AcM6S and 3PrM6S) suspended in soybean oil and administered to groups of four rats. Each potential long-acting formulation contained $8 \times ED_{50}$ doses of the individual drug (for M6S, 3AcM6S and 3PrM6S, doses were 33.3, 34.6 and 36.3 μmol/kg, respectively). After weighing sufficient compound (finely ground using an agate mortar and pestle for 5 min), the calculated volume of soybean oil required was added using a 1.0 ml syringe (Hamilton, Reno, NV, USA) and the formulations suspended using vortex mixing and sonication. The required volume of the suspension was drawn up into a 100 μl syringe with a sterile, disposable 25 gauge needle immediately following sonication. A maximum injection volume of 100 μl was administered into the left thigh muscle of the rat under light anaesthesia (50/50%, O_2/CO_2) over a 10–15 s interval. Following injection, the anaesthesia was quickly reversed with 100% oxygen. Antinociceptive testing was performed immediately pre-dosing and at the following post-dosing times: 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 30.0 and 36.0 h. The results were compared with pre-dosing tail flick latencies and %MPE values were calculated. In the control rats ($n = 3$), soybean oil only was administered IM. At the completion of the experiment, all rats were euthanased using 100% CO_2.

3. Results and discussion

3.1. Intravenous single dose administration

Following single-dose intravenous administration of M6S and the prodrugs in a dose of 4.10

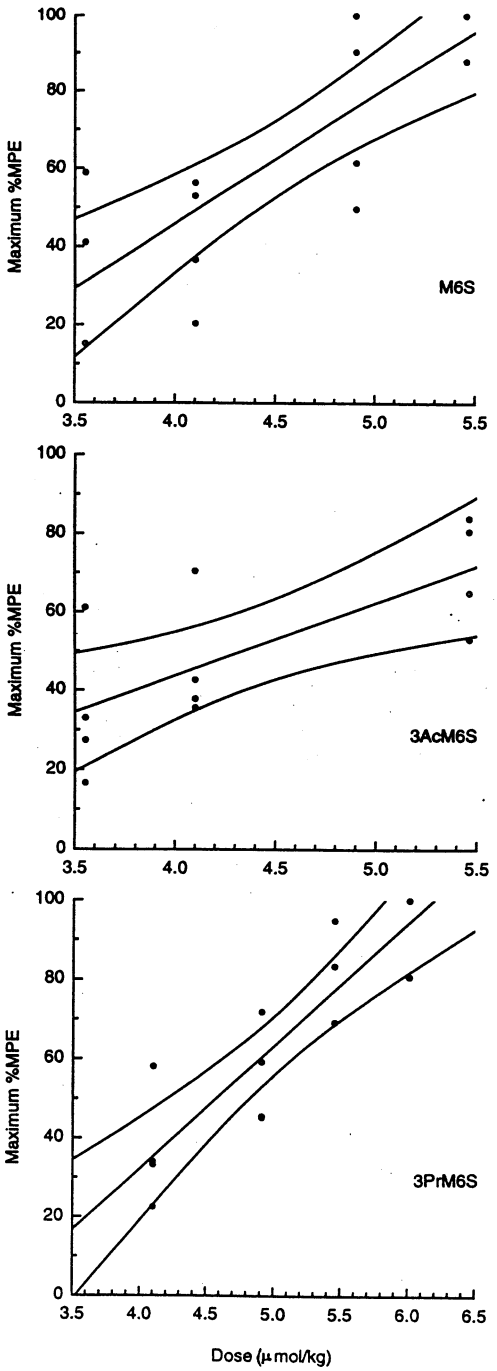

Fig. 2. Dose-response plots using maximal %MPE values after IV administration of M6S, 3AcM6S and 3PrM6S to rats ($n = 4$ for each compound). Curved lines are 95% confidence limits.

μmol/kg, it was found that relatively high levels of antinociception were achieved after dosing with M6S itself or its 3-acetyl and 3-propionyl esters. The dose (4.10 μmol/kg) of M6S and the prodrugs was based on preliminary experiments intended to find a dose of M6S which gave a %MPE value of approximately 50. Very low levels of antinociception were achieved following administration of 3BuM6S or 3HpM6S (Fig. 1). The control groups showed no significant antinociception. Maximal mean peak antinociception for M6S, 3AcM6S and 3PrM6S occurred at 30, 60 and 90 min, respectively, and the mean maximum %MPE values were 53, 48 and 39%MPE, respectively. However, there was no statistically significant difference between these values ($p > 0.53$). The AUC values for the plots in Fig. 1 were 121.4 ± 32.6 (M6S), $152.9 + 6.7$ (3AcM6S) and 106.7 ± 21.0 (3PrM6S) %MPE h. Again, these values are not different ($p = 0.540$ for M6S and 3AcM6S; $p = 0.834$ for M6S and 3PrM6S; $p = 0.111$ for 3AcM6S and 3PrM6S). However, it was found that there were significant differences in the duration of action when comparing 3AcM6S (6 h) with M6S (4 h; $p = 0.036$) or with 3PrM6S (4 h; $p = 0.021$) (Fig. 1).

3.2. Dose-response relationships

Plots of the mean maximal %MPE as a function of the dose for M6S, 3AcM6S and 3PrM6S were linear (Fig. 2). The estimated ED_{50} values and relative potencies from these dose-response relationships are shown in Table 1. Although there is a trend which suggests that the rank order of potency is M6S > 3AcM6S > 3PrM6S, the ED_{50} values were not significantly different (Table 1). These ED_{50} values were used in the subsequent work on depot injections. Using the AUC as a function of dose to define the dose-response relationship (Fig. 3), 3AcM6S appeared to be more potent than M6S and 3PrM6S. This appeared to be due to a longer duration of antinociception following IV administration at higher doses. However, for the AUC data in Fig. 3, only one pair of points (M6S and 3AcM6S at a dose of 5.46 μmol/kg) were significantly different ($p = 0.02$). There was significant variability and dose-response relationships based on mean AUC were irregular, in that larger doses sometimes resulted in smaller AUC values. This could be due to the relative rates of processes such as in vivo hydrolysis, absorption across the BBB, metabolism and excretion, all of which may be dose-dependent.

Table 1
Relative antinociceptive potency (mean ± S.E.M.) of M6S and its 3-*O*-acyl derivatives following IV administration to rats

Compound	ED_{50} (μmol/kg)	Potency[a]
M6S	4.16 (3.61--4.48)[b]	1.00
3AcM6S	4.32 (3.55--5.09)[b]	0.96
3PrM6S	4.54 (4.21--4.79)[b]	0.91

[a] Compared with M6S.
[b] Range from the 95% confidence limits.

Following IV morphine administration in a dose of 4.25 mg/kg to adult male Sprague–Dawley rats, the mean antinociceptive response using the Tail flick latency test was found to be 84%MPE (M. Lim and M.T. Smith, unpublished results). These experiments used the same equipment as in the present work and Sprague–Dawley rats from the same source. By inverse prediction from the M6S dose-response curve, an equipotent dose of M6S would be 1.5 mg/kg. This indicates that on IV administration, M6S is approximately three times more potent than morphine using the tail flick test. Additional studies recently performed in our laboratory (S. South and M.T. Smith, unpublished results) have shown that IV M6G is approximately five times more potent than IV morphine, using the same experimental paradigm. These findings are consistent with reports that M6G is a little more potent than M6S following subcutaneous injection in mice (Mori et al., 1972).

In vitro studies to determine the log *P* values of M6S and its 3-*O*-acyl derivatives (Preechagoon et al., 1998) showed that these compounds are very hydrophilic, with log *P* values of -3.0, -2.23 and -1.71 for M6S, 3AcM6S and 3PrM6S, re-

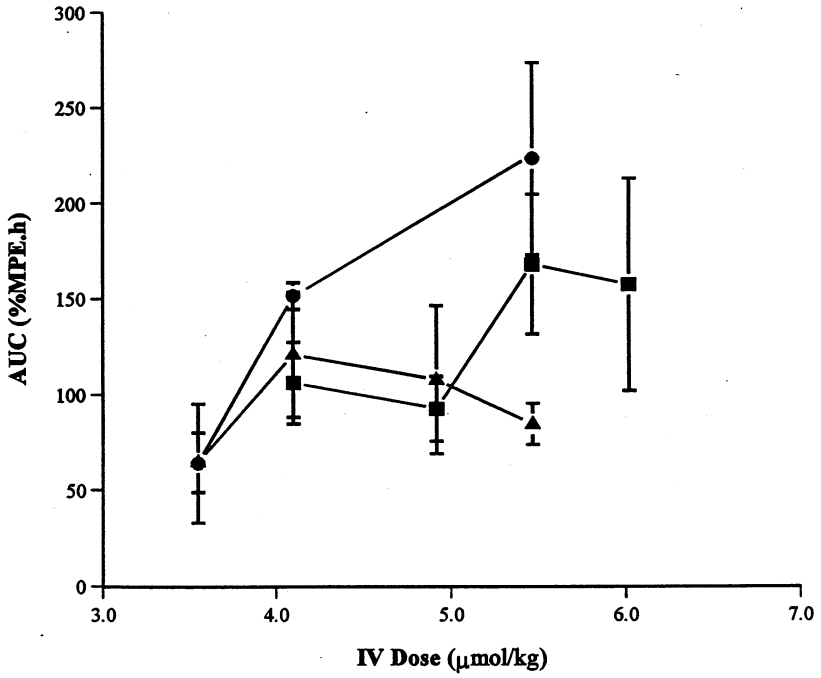

Fig. 3. Mean (±S.E.M.) dose-response curves based on AUC for M6S, 3AcM6S and 3PrM6S, after IV administration to rats.

spectively. As a consequence, one could predict that it would be difficult for these compounds to cross the BBB. However, M6S itself (at least) is similar to M6G, a polar glucuronide metabolize of morphine which has been shown to cross the BBB and elicit potent antinociception (Pasternak et al., 1987; Paul et al., 1989) following systemic administration in a manner analogous to that reported previously in preliminary form for M6S (Houdi et al., 1996). These workers reported that the antinociceptive effects of M6S and 3AcM6S, in an equimolar icv dose (0.22 μg of M6S), had durations of 60 and 100 min, respectively. This parallels the results from IV administration presented here.

Investigation of the hydrolysis in vitro (37°C; pH 7.4) of the prodrugs in 10% rat blood and 10% rat brain homogenate, both diluted with phosphate buffered saline (Preechagoon et al., 1998), showed that the hydrolysis rate for each compound was substantially faster in 10% brain homogenate, with half-lives of 25–36 min, than in 10% blood (half-lives, 70–110 min). One would expect the prodrugs to be hydrolyzed reasonably quickly in vivo, producing the potent antinociceptive agent, M6S, with half-lives about ten-fold less than those found for the in vitro experiments. From these results, it is reasonable to expect the prodrugs to be hydrolyzed on both sides of the BBB, and the overall pharmacodynarnic effect to result both from M6S formed in the blood before absorption into the CNS, and from M6S formed by hydrolysis after absorption of the prodrugs. The longer duration of action of 3AcM6S compared with M6S itself requires additional in vivo studies to determine the mechanisms underlying this observation.

The factors that influence the passage of a drug across the BBB and which determine its time-dependent concentration within the brain have been described (Rapoport, 1976; Greig et al., 1990; Greig, 1992; Pardridge, 1995). These factors include: (i) the unbound plasma concentration-time profile of the drug; (ii) the BBB permeability of the compound; and (iii) cerebral blood flow. In general, uptake into the brain occurs by passive diffusion, determined by factors such as lipophilicity, molecular size and degree of ioniza-

tion at physiological pH (Rapoport, 1976). Among these, lipophilicity is though to be the most important factor. Many studies have found a linear relationship between the cerebrovascular permeability of a compound and its lipophilicity (octanol:water partition coefficient, P) (Rapoport et al., 1979; Levin, 1980). Deviations from the linear relationship can be affected by other factors such as molecular size, steric and electronic parameters and specific interactions with cell membranes.

Maintenance of high plasma concentrations maximizes distribution of drug from the blood to the brain. From the concepts of plasma protein binding and drug transport, it is the unbound drug that diffuses through lipid membranes, which in turn is dependent on the equilibrium between unionized and ionized drug species in the blood. The binding of drugs to plasma proteins may affect the pharmacokinetics, as well as providing a reservoir for slow release of the drug into the circulation (Vallner, 1977). However, if plasma protein binding is too high, entry into the brain can be restricted (Greig, 1992).

In contrast to morphine, one would expect M6S and its prodrugs to be fully ionized at physiological pH, due to the very strongly acidic sulfate ester group. It is therefore possible that M6S may cross the BBB in a similar manner to the potent analgesic/antinociceptive M6G (Shimomura et al., 1971). Both M6S and M6G are largely zwitterionic at physiological pH. Carrupt et al. (1991) proposed that M6G may cross the BBB by assuming folded conformations which mask part of the polar groups of the compound, thus increasing lipophilicity. Prankerd (1993) has proposed from molecular models that zwitterionic M6G (and M3G) may form energetically favoured dimers through ion–ion interactions (double ion-pairs). As these dimers may form in both aqueous and lipid solutions, M6G may cross the BBB in an electrically 'neutralized' form. As M6S is expected to behave in similar fashion to M6G, this model might also account for the passage of M6S across the BBB.

3.3. Preliminary evaluation of long acting injectable formulations of M6S, 3AcM65, 3PrM6S

Fig. 4 shows the mean degree of antinociception (%MPE) versus time curves for M6S and the two prodrugs given as oil suspensions by the IM route. The time to maximum response (T_{max}) was found to be the same for all compounds (4 h). It was found that 3PrM6S produced a greater extent and duration of antinociception (AUC) than M6S and 3AcM6S (Table 2). Specifically, the AUC for 3PrM6S was approximately two and three times greater than the corresponding AUC values of M6S and 3AcM6S, respectively. From the statistical analysis, there was a significant difference between 3PrM6S and both M6S and 3AcM6S ($p = 0.02$ and $p = 0.0016$, respectively), but no significant difference between M6S and 3AcM6S. The results differ from the single dose IV studies, where M6S produced the highest maximum response, and 3AcM6S had the longest duration of action, as described in Section 3.1. Clearly, physicochemical factors related to the dosage form, in addition to biopharmaceutical factors related to the agents alone, are of some importance.

It is usually assumed that the rate limiting step for release of a drug from an oily depot injection is the rate of diffusion of dissolved molecules from the triglyceride vehicle into tissue fluids. However, it may be that the rate at which the vehicle is hydrolyzed enzymatically is also important. It is possible that the rate of solution in tissue fluids of the solid drug or prodrug particles liberated from the vehicle is the rate-limiting step in determining the pharmacodynamic response. The AUC values (Table 2) are in the same rank order as the aqueous solubilities of 3PrM6S, M6S and 3AcM6S in 0.15 M pH 7.4 phosphate buffer at 37°C (9.8, 3.8 and 3.3 mg/ml, respectively) (Preechagoon et al., 1998). The solubilities of these high melting zwitterionic compounds in the soybean oil vehicle are very low and could only be estimated visually at < 50 μg/ml. Consequently, dissolution in the oil phase and diffusion to the aqueous boundary might be slow compared with the rate of enzymatic hydrolysis of the triglyceride.

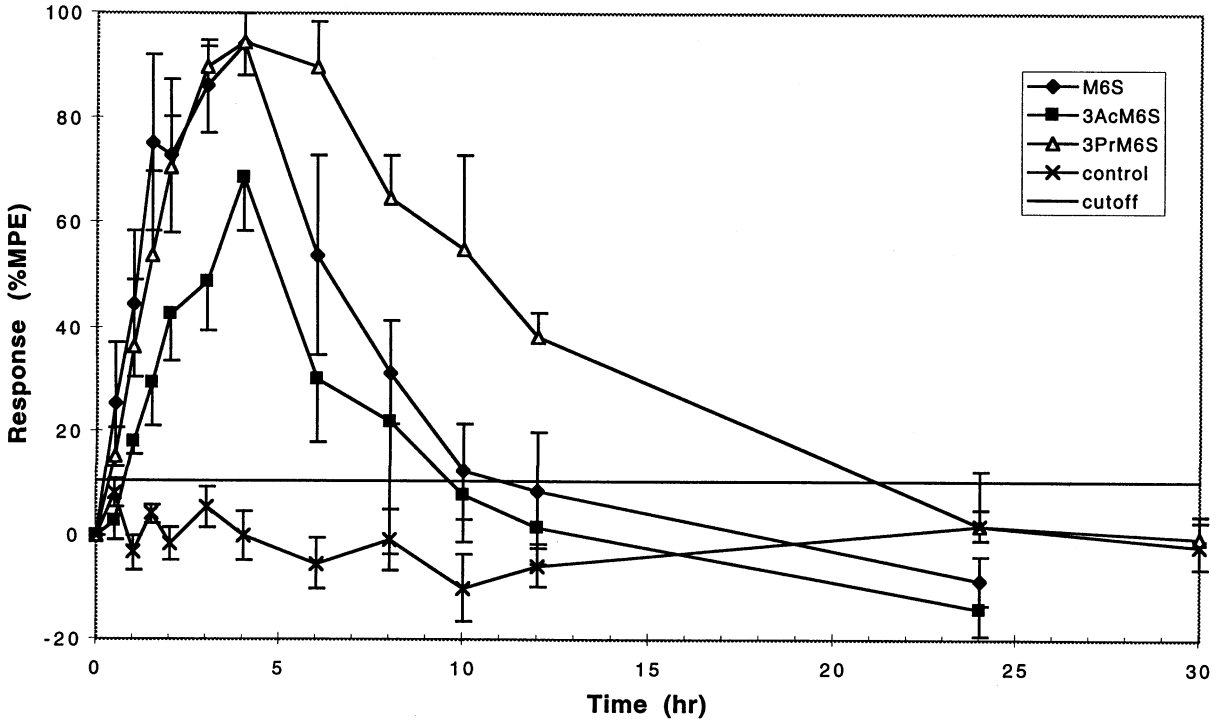

Fig. 4. Mean (± S.E.M.) %MPE versus time curve after IM injection of $8 \times ED_{50}$ doses of M6S, 3AcM6S and 3PrM6S suspended in soybean oil to rats ($n = 4$ for each compound). The cutoff line marks the %MPE value which defined the duration of action.

An additional factor that needs investigation is the concomitant rate of antinociceptive tolerance development that may have taken place during the 48 h study period. Nothing is known of the magnitude of this effect for M6S or the prodrugs following systemic dosing. Further studies are required to investigate this issue.

3.4. Do the prodrugs have intrinsic antinociceptive/analgesic effects?

It has recently been claimed that 3AcM6S is itself centrally acting (Houdi et al., 1996), based on competitive receptor binding assays and organ bath experiments. However, the experimental conditions did not exclude the possibility that the results may have been distorted by the presence of minor amounts of highly active contaminants exhibiting high receptor binding affinity. These could arise from incomplete purification after synthesis or from hydrolysis of the prodrug to M6S. It has been shown previously that trace contamination of components with high receptor binding affinity can significantly distort the results of equilibrium binding assays in rat brain homogenate preparations, e.g., morphine-3-glucuronide containing 0.5% of morphine had an apparent affinity for μ_1 opioid binding sites (K_i, 116 ± 25 nM) which was significantly diminished (K_i, 766 ± 30 nM) when the content of morphine was reduced to 0.08% (Bartlett and Smith, 1995).

In general, 3-*O*-substituted morphine derivatives bind poorly to opioid receptors. Codeine is the archetypal example of this behaviour, and much of its μ-opioid agonist activity derives from its *O*-demethylation in vivo to morphine (Foye et al., 1995). M3G is devoid of analgesic/antinociceptive activity (Yoshimura et al., 1973) and it has very low binding affinity ($K_i \gg 1$ μM) for opioid receptors of any class (Bartlett et al., 1994; Bartlett and Smith, 1995; Loser et al., 1996). Recently, Mignat et al. (1996) have demonstrated

Table 2
Mean ($\pm$S.E.M.) area under the degree of antinociception versus time curve (AUC), time of maximum response (T_{max}), maximum response (R_{max}) and duration of antinociceptive action for M6S, 3AcM6S and 3PrM6S after intramuscular administration of equiactive $8 \times ED_{50}$ doses suspended in soybean oil

Compound	T_{max} (h)	R_{max} (%MPE)	AUC (%MPE h)	Duration (h)
M6S	4	94.2	613.1 ± 155.9	10
3AcM6S	4	68.7	379.3 ± 114.6	8
3PrM6S	4	94.4	1087.4 ± 97.4	20

a loss in binding affinity of morphine for μ-, δ- and κ-opioid receptors by esterification at the 3-position using homogenate preparations from guinea pig brain. Therefore, it is currently unclear whether the 3-*O*-acyl esters of M6S have intrinsic antinociceptive properties, as extrapolation of the marked loss of activity when morphine is substituted at the 3-position to M6S suggests that 3-substituted derivatives of M6S should also have little or no intrinsic antinociceptive activity. Clearly, further studies are needed to clarify this issue.

4. Conclusions

This investigation has shown that, following both intravenous and intramuscular depot injection, M6S is a potent antinociceptive agent when administered to adult male Sprague–Dawley rats and using the tail flick latency test of antinociception. These findings suggest that M6S crosses the BBB following systemic administration. Pharmacodynamic evaluation of 3-*O*-acyl prodrug derivatives of M6S showed that both 3AcM6S and 3PrM6S elicited significant antinociception, presumably due to ester hydrolysis to the parent drug, M6S. Preliminary investigation of potential long-acting formulations of M6S, 3AcM6S and 3PrM6S suspended in soybean oil indicated that the duration of the antinociceptive effect was significantly extended for the 3PrM6S formulation compared with M6S or 3AcM6S. These results are sufficiently promising as to warrant further investigation.

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