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Sustained-release Morphine for Epidural Analgesia in Rats

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Background: Epidural opioid analgesia often requires either continuous infusion or repeated injections, which are inconvenient for patients, increase risk of infection, and consume expensive physician and nursing time. In addition, potential respiratory depression is a major safety concern. The authors studied whether a single dose of epidurally administered, sustained-release morphine could prolong analgesia and reduce toxic effects in rats.

Methods: Sustained-release morphine (DTC401) was prepared by encapsulating morphine sulfate in DepoFoam (Dep. Tech, San Diego, CA), a lipid-based, sustained-release drug delivery system. A standard hot-plate test for analgesia, pulse oximetry for hemoglobin oxygen saturation, corneal-reflex loss, and incidence of catalepsy were used to assess efficacy and toxicities. Cerebrospinal fluid and serum pharmacokinetic studies were performed after a single epidural dose, using a commercially available radioimmunoassay kit.

Results: Single epidural doses of DTC401 resulted in equivalent onset time to peak analgesia but significantly prolonged analgesia compared with morphine sulfate. Hemoglobin oxygen saturation was decreased minimally, and the incidences of catalepsy and corneal-reflex loss were minimal, even at large doses of DTC401. In contrast, the larger doses of morphine sulfate significantly decreased hemoglobin oxygen saturation, and caused catalepsy and loss of the corneal-reflex. The C_{max} for DTC401 was 32% in cerebrospinal fluid and 6% in serum, relative to morphine sulfate. The terminal half-life for DTC401 was increased 32 fold in the cerebrospinal fluid compared with morphine sulfate.

Conclusions: A single epidural dose of DTC401, compared with morphine sulfate, prolonged duration of analgesia, with minimal supraspinal toxic effects, in rats. (Key words: Phar-

macology, drug delivery: sustained-release. Analgesia: epidural. Analgesics, opioid: morphine.)

A lipid-based, injectable drug delivery system, DepoFoam™, was developed for sustained-release delivery of water-stable compounds.^{1-12*} DepoFoam is composed of spherical particles, each containing numerous nonconcentric aqueous chambers bound by a bilayer lipid membrane.¹³ The lipids used are identical to the lipids occurring naturally in the body. The active ingredient is encapsulated within the nonconcentric internal aqueous chambers and is released over an extended period of time. A phase I/II clinical trial in human patients with neoplastic meningitis showed that encapsulation of an antineoplastic agent, cytarabine, in DepoFoam maintains therapeutic cerebrospinal fluid concentrations over an extended period of time after a single intrathecal administration.¹⁴⁻¹⁶ A multicenter phase III clinical trial for this cytarabine formulation is in progress.

Injectable opioids are used widely as epidural analgesics in postoperative and postpartum settings.¹⁷⁻¹⁹ Postoperative and postpartum pain usually last several days, but injectable opioids have relatively short durations of action.²⁰⁻²⁴ Therefore, either continuous infusion or repeated injections are required to maintain adequate pain control.²⁵⁻²⁷ The use of continuous infusion or repetitive injections necessitates placement of indwelling catheter systems with or without attached infusion pumps, all of which consume physician and nursing time for care and maintenance. In addition, repeated bolus injections or continuous infusions can result in respiratory depression due to either vascular redistribution or rostral cerebrospinal fluid (CSF) movement of the drug. We developed a lipid-based, sustained-released formulation of morphine (DTC401)²⁸ and investigated whether a single-dose of epidurally administered DTC401 could provide sustained analgesia without causing supraspinal toxic effects in rats.

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* Kim S, Khatibi S, Howell SB, Scheerer S: Intratumoral chemotherapy with multivesicular liposomes containing cytosine arabinoside. *Regional Cancer Treatment* 1989; 2:170-3.

Materials and Methods

Materials

Morphine sulfate, triolein, and lysine were obtained from Sigma Chemical (St. Louis, MO); dipalmitoyl phosphatidylglycerol, dioleoyl lecithin, and cholesterol were purchased from Avanti Polar-Lipids, (Alabaster, AL); USP chloroform was procured from Spectrum Chemical (Gardena, CA). All of these materials were used without further purification.

Drug Preparation

DTC401 was prepared according to a previously described method.²⁸ Before epidural injection, standard DTC401 preparations containing approximately 25 mg/ml morphine were diluted with normal saline so that an appropriate dose was in a volume of 50 μ l. Stock solutions of unencapsulated morphine sulfate initially prepared in normal saline were diluted in the same manner. The volume of injection was chosen in anticipated study of larger doses of DTC401. The exception was the 2,000 μ g dose of DTC401 used for toxicity studies where an injection volume of 75 μ l was required. The morphine concentration in DTC401 was determined by dissolving 50 μ l DTC401 with 1 ml isopropyl alcohol, followed by dilution in water and assay by a published high-pressure liquid chromatography method.²⁹ Analyses of DTC401 supernatants revealed that as much as 10% of total morphine was external to DepoFoam particles and therefore immediately bioavailable. For the placebo control, blank DepoFoam was made by substituting with 4% (weight-to-volume ratio) glucose in place of morphine sulfate.

Animals

Six- to eight-week-old male Sprague-Dawley rats that weighed 205–254 g were purchased from Harlan Sprague-Dawley (San Diego, CA). The animals were housed, 1 or 2 per cage, in a temperature-controlled environment with an alternating 12-h light and darkness cycle and were given unrestricted access to food and water. Before each study, animals were habituated to the environment for at least 2 days. Each animal was studied only once. All animals were maintained in accordance with guidelines of the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Epidural Catheterization

Epidural catheterization of rats was performed during halothane anesthesia, with the animal secured in ste-

reotaxic recumbency, 7 cm in height. The head was flexed, taking care that the animal maintained normal breathing. A short-beveled 19-gauge needle was inserted at an angle of approximately 170° to the spine, just caudad to the occipital crest in the midline, with needle bevel facing down. The needle was advanced caudad toward the C1 vertebra until the needle tip touched the spinous process or posterior lamina of C1. The needle tip was advanced carefully to the ventral edge of the posterior lamina. At this point, a slight loss of resistance was felt, and the needle was advanced 1–2 mm further. Care was taken not to let the needle penetrate the dura. Accidental penetration of the dura was confirmed by the appearance of CSF through the hub of the needle or through the subsequently placed catheter. A polyethylene catheter (PE-10; length: 12 cm; internal diameter: 0.28 mm; outside diameter 0.61 mm; volume: 7.4 μ l; Becton Dickinson, Sparks, MD) was threaded through the needle into the dorsal epidural space. The catheter was advanced slowly through the needle and stopped at the approximate level of L1, 8 cm from C1. The exposed portion of the catheter was tunneled subcutaneously under the scalp and fixed with a purse-string 3-0 silk suture. Finally, the catheter was flushed with 10 μ l normal saline and plugged with a stainless steel wire. The procedure from induction of anesthesia to suture placement generally lasted 10–15 min. Animals were allowed to recover overnight from the anesthesia and catheter placement. We used only those animals that completely recovered from the procedure with normal hot plate latency values and without any signs of neurologic deficit.

The epidural catheter implantation was verified by x-ray studies and dissection. After catheter implantation, animals were killed by halothane overdose and injected with 5 μ l Omnipaque (350 mg/ml). Radiograms were obtained with the x-ray machine set at 52 kVp, 300 mA, $\frac{1}{30}$ second, and 40-inch distance. Dissections were performed by first making a midline skin incision over the spinous process of the lumbar and thoracic vertebrae. The superficial muscles were dissected from the lumbar and lower thoracic vertebrae and retracted laterally. Posterior laminectomy was done from T11 to L2. The epidural position of the catheter was confirmed by visualization of dura mater beneath the catheter, and photographs were taken.

Antinociception

Antinociception studies were performed after placement of the epidural catheters by subjecting animals

to standard hot plate ($52.5 \pm 0.5^\circ\text{C}$) testing.³⁰ Response latencies (in seconds) to nociception was measured from the time when the animals were placed on the hot plate to the time when they either licked their hind paw or jumped. The baseline (pretreatment) response latency value was defined as 0% of the maximum possible effect (MPE) in each experimental animal. After the completion of baseline testing, 50 μl DTC401, unencapsulated morphine sulfate, or blank DepoFoam was injected epidurally. After the injection, the catheter was flushed with 10 μl 0.9% sodium chloride and plugged with a stainless steel wire. The animals were then subjected to hot plate testing again at specific time points (0.5, 1, 2, 3, 4, 6, 12, and 24 h after morphine sulfate injection and 0.5, 1, and 6 h and 1, 2, 3, 4, 5, 6, 7, and 8 days after DTC401 injection) for measurement of antinociceptive effect. The doses of epidural morphine sulfate and DTC401 were 10, 50, 175, and 250 μg . Antinociception was determined in 5–6 animals at each dose. To prevent tissue damage to the footpads, a cutoff time of 60 s was used. Accordingly, 100% MPE was defined as response latency of 60 s. The latency interval of 10 ± 2 to 60 s corresponding to 0% to 100% MPE was sensitive for demonstrating dose-response in the studied dose range.

Toxicity Studies

Hemoglobin oxygen saturation was quantified by pulse oximetry. The animals were removed from their cages, placed in polystyrene rat restraints (Plas Labs, Lansing, MI) and allowed to acclimate for 5 min. Oxygen saturation was determined at baseline and at specific time points after a single epidural bolus of morphine sulfate or DTC401 by placing a pulse oximeter probe on the right hind paw (model 3740, Ohmeda Medical Systems, Madison, WI). The doses of DTC401 and morphine sulfate were 10, 50, 175, 1,000, and 2,000 μg . Pulse oximetry was performed on 5 animals at each data point except for the 50- μg doses of DTC401 and morphine sulfate, where $n = 3$. The pulse oximetry values of percent hemoglobin oxygen saturation (SpO_2) were monitored continuously for 3 min. The maximum value obtained during this recording period was defined as the oxygen saturation value.

Catalepsy and presence or absence of corneal reflex also were recorded as indicators of supraspinal toxicity. Catalepsy was defined as the failure of the animal to move within 15 s after placement of the forepaw on a horizontal bar 4 cm in height.

Pharmacokinetic Studies

The pharmacokinetic studies were done by measuring morphine concentrations in serum and CSF at appropriate time points (0.5 and 1 h, and 1, 3, 5, and 8 days for DTC401 and 0.5, 1, 3, 6, 12, and 24 h for morphine sulfate) after a single, 250- μg epidural dose of DTC401 or morphine sulfate. At each time point, 3 or 4 animals were restrained and 1-ml blood samples were collected *via* tail vein. Serum was separated from blood by centrifugation at 3,000 revolutions per minute for 10 min after letting the blood clot for 30 min at room temperature. The animals were then anesthetized with halothane and secured in a stereotaxic recumbency. A midline cutaneous incision was made from the occipital crest to just behind the ears, approximately 1 cm in length. The muscle ligament along the occipital crest was detached at the skull for 5 mm on either side of the midline. Gently freeing the muscle from the occipital bone and the Atlas/Occipital membrane, a retractor was locked in place to have a clear view of the membrane. Fifty-microliter CSF samples were then collected by cisternal tap. The animals were then killed by an overdose of halothane. Serum and CSF samples were stored at -20°C until analysis by radioimmunoassay.

Morphine concentrations in serum and CSF were determined using a commercially available radioimmunoassay kit highly specific for morphine (Coat-A-Count Serum Morphine, Diagnostic Products, Los Angeles, CA) according to the manufacturer's suggestions. All measurements were done in duplicate. The cross-reactivities of morphine-3-glucuronide and morphine-6-glucuronide were 0.19 and 0.27%, respectively. The limit of detection of the assay was 1 ng/ml, and the interassay coefficient of variation was 11%.

Data Analysis

Analgesic efficacy and SpO_2 depression curves were plotted as a function of time for each dose administered. Hot plate latencies were first calculated as a percentage of the maximum possible effect (%MPE)³⁰:

$$\% \text{MPE} = \frac{\text{Postdrug latency} - \text{Predrug latency}}{\text{Cutoff latency} - \text{Predrug latency}} \times 100$$

All areas under the curves were calculated by the trapezoidal rule to the last data point, using the RSTRIP computer program (Micromath, Salt Lake City, UT). Half-lives were calculated by fitting the pharmacokinetic curves to a biexponential function. The RSTRIP program was used to perform the curve fittings.

Kruskal-Wallis tests were run to separately determine dose dependency for the different drug formulations and routes, whereas analysis of covariance was used for comparison between formulations. *Post hoc* F tests were performed on all hypotheses, using analysis of covariance. A statistical significance level of 0.05 was used for all tests. All data are displayed as mean \pm SEM, unless otherwise stated.

Results

Epidural Catheterization

Sixty-five percent of the animals in whom an epidural catheter was implanted were used for further study; the remaining animals were killed because of neurologic deficit or dura penetration. Of the implantation failures, approximately 70% experienced neurologic deficit, and the remaining animals had penetration of the dura. Radiograms revealed the catheter tip resting in the vicinity of L1. Dissections confirmed the location of the catheter tip and showed the catheter lying outside the dura.

Radiograms taken after injection of 50 μ l Omipaque with 10 μ l saline flush revealed maximum spread of this injection volume to be from L2 to T1 in one animal (data not shown). However, all the remaining radiograms showed spread up to T6–T8. The spread of drug seemed to originate at the catheter tip and then extend along the length of the catheter after injection, following the expected path of least resistance. Also, diffusion of injected drug appeared to depend on the speed of injection.

Antinociception

The epidural administration of DTC401 resulted in equivalent onset to peak analgesia of 1 h, but the duration of analgesia was significantly prolonged compared with an equivalent dose of morphine sulfate (fig. 1). Injection of blank DepoFoam or normal saline produced no demonstrable antinociceptive or other behavioral effects (data not shown). Figure 2a shows that the peak analgesic effects of epidural DTC401 and morphine sulfate were dose dependent, with the peak-analgesic potency of epidural morphine sulfate greater than epidural DTC401 ($P < 0.05$).

Substantial prolongation of analgesia in animals given epidural DTC401 is seen readily by inspection of figure 1, as well as by the significant difference in total analgesic effect shown in figure 2b ($P < 0.05$). At the

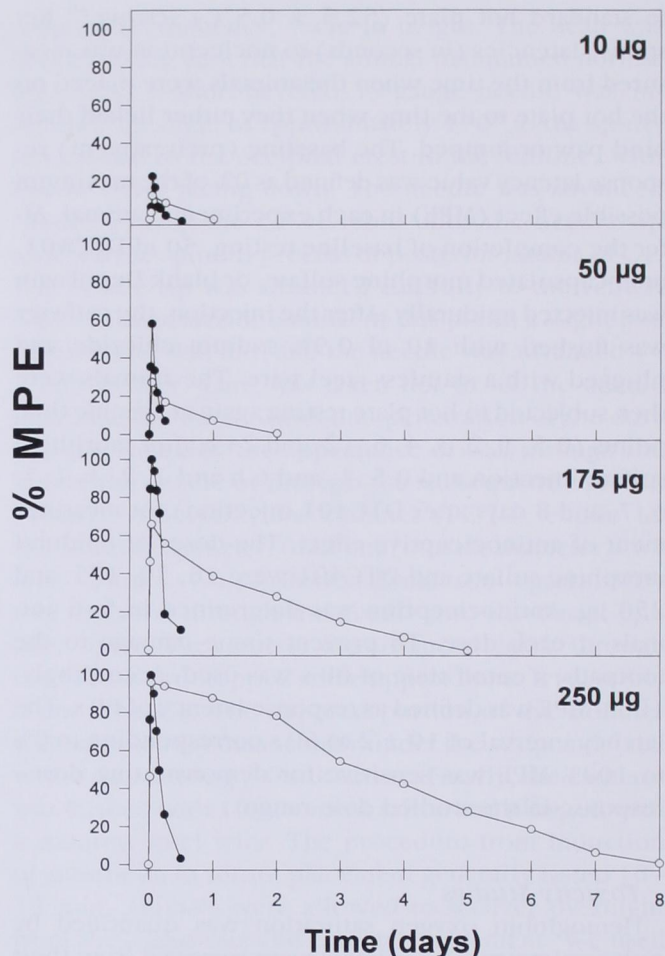


Fig. 1. Analgesic effect as a function of time after a single epidural dose of DTC401 (open circles) or morphine sulfate (closed circles). The dose given to each animal was, from the top panel to the bottom panel, 10, 50, 175, and 250 μ g, respectively. The intensity of analgesia is expressed as "percent of maximum possible effect (%MPE)." Each data point represents the average from 5 or 6 animals. Standard error bars have been deleted for clarity.

dose of 250 μ g, which produced peak effects close to 100% MPE for DTC401 and morphine sulfate, the duration of analgesia (time to decrease to 50% MPE) was 3.4 days for DTC401 compared with 0.17 day for morphine sulfate (fig. 1).

Toxicity

Figure 3 depicts the time course of SpO_2 as measured by pulse oximeter after epidural injection of various doses of DTC401 and morphine sulfate. There was a dose-dependent decrease in SpO_2 with increasing doses of morphine sulfate ($P < 0.05$), whereas no significant

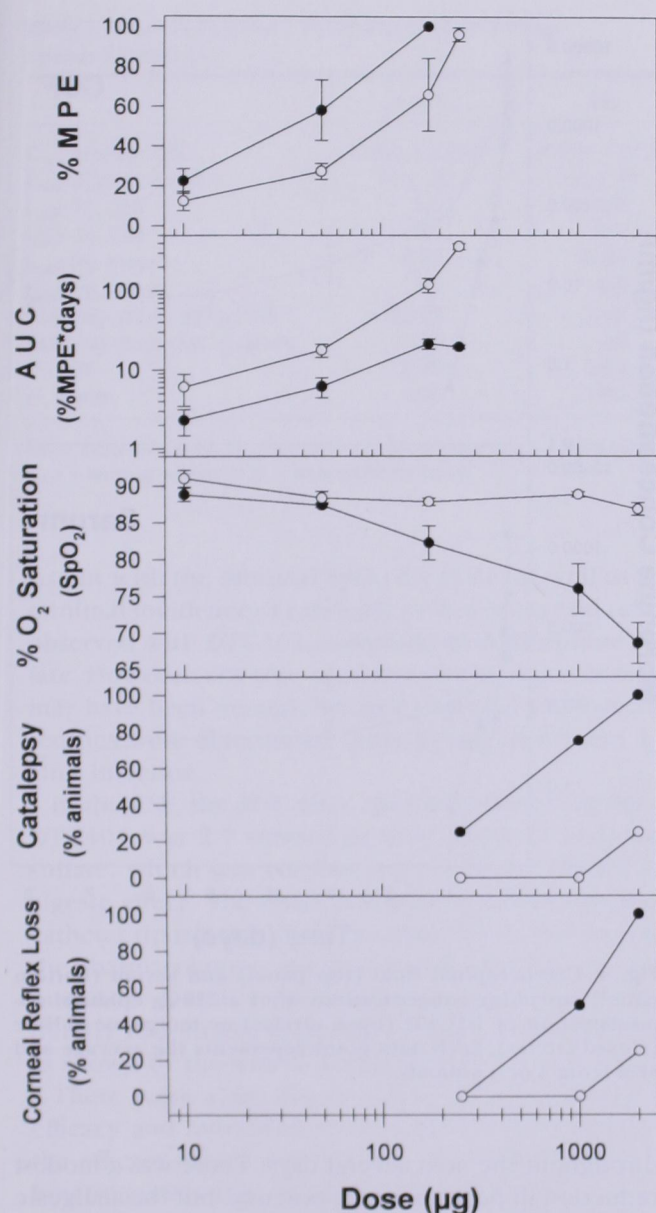


Fig. 2. Efficacy and toxicity dose response curves for (a) peak analgesia; (b) total analgesic effect as measured by the area under the analgesia-time curve (AUC); (c) percent hemoglobin oxygen saturation (SpO_2); (d) incidence of catalepsy; (e) loss of corneal reflex. All curves were generated after a single epidural dose of morphine sulfate (closed circles) or DTC401 (open circles). Each data point represents the average and SEM from 5–8 animals, except for the catalepsy and corneal reflex graphs, where only the average is shown, and 50 μg group for panel c, where $n = 3$.

decrease of SpO_2 was produced by the same doses of DTC401 (fig. 2c). The maximum decrease in SpO_2 was observed within 1 h after epidural administration of

morphine sulfate, and no delayed decrease in SpO_2 was observed with either formulation.

There was a dose-dependent increase in the percentage of animals that exhibited cataleptic behavior and corneal-reflex loss after injection of escalating doses of morphine sulfate (figs. 2d and 2e). Animals given equivalent doses of DTC401 showed no supraspinal toxic effects, except at the maximum dose of 2 mg, where one animal exhibited catalepsy and corneal reflex loss (figs. 2d and 2e).

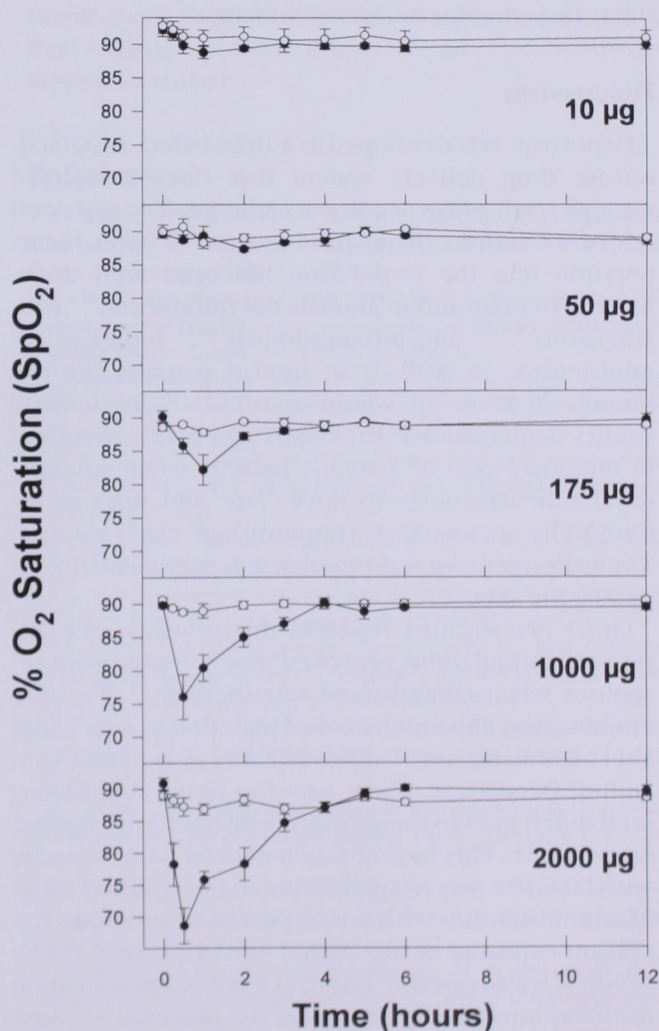


Fig. 3. Percent oxygen saturation of hemoglobin (SpO_2) as a function of time after single epidural doses of DTC401 (open circles) or morphine sulfate (closed circles). The dose given was, from the top panel to the bottom panel, 10, 50, 175, 1,000, and 2,000 μg , respectively. Each data point represents the average and SEM from 5 animals, except for the 50- μg group, where $n = 3$.

Pharmacokinetics

Figure 4 shows the cisternal CSF and serum morphine concentrations as a function of time in animals that received 250 μ g morphine sulfate or DTC401. The pharmacokinetic parameters are summarized in Table 1. The peak CSF and serum morphine concentrations after epidural administration of DTC401 were, respectively, 32% and 6% of that after morphine sulfate. The terminal CSF half-life (β) for DTC401 was 82 h compared with 2.6 h for morphine sulfate. The CSF area under the curve for DTC401 was 2.7 times that for morphine sulfate, but the serum AUC for DTC401 was 0.91 times that for morphine sulfate.

Discussion

DepoFoam was developed as a lipid-based, sustained-release drug delivery system that can be injected through small gauge needles or catheters. The sustained release of various therapeutic agents^{1-16,7} after incorporation into the DepoFoam, has been well documented *in vitro* and in animals *via* intrathecal,¹⁻⁴ subcutaneous,^{5-9,28} and intraperitoneal¹⁰⁻¹² routes of administration, as well as in human patients *via* the intrathecal route of administration.¹⁴⁻¹⁶ Toxicology studies demonstrated that DepoFoam itself causes no, or minimal, local or systemic toxicity when administered subcutaneously to mice, rats, and dogs or intrathecally to monkeys (unpublished observations). Topically administered DepoFoam is nonsensitizing to guinea pig skin.

Other investigators reported that other types of liposome formulations provoked touch-evoked pain responses when administered intrathecally.³¹ We have not observed any touch-evoked agitation or squeaking after administration of either DTC401 or nondrug-containing DepoFoam. There were no noticeable behavioral differences to those subjects injected with normal saline alone. This lack of touch-evoked pain response with DTC401 may simply be due to the epidural route of administration, which is expected to preclude significant exposure of the central nervous system to the lipids. It is also possible that the DepoFoam formulation itself has intrinsically decreased the potential to cause touch-evoked agitation vis-a-vis other types of liposomes.

The data presented in this report show that a single dose of DTC401 results in prolonged duration of analgesia, with the peak analgesia occurring 60 min after a single epidural dose and then gradually decreasing

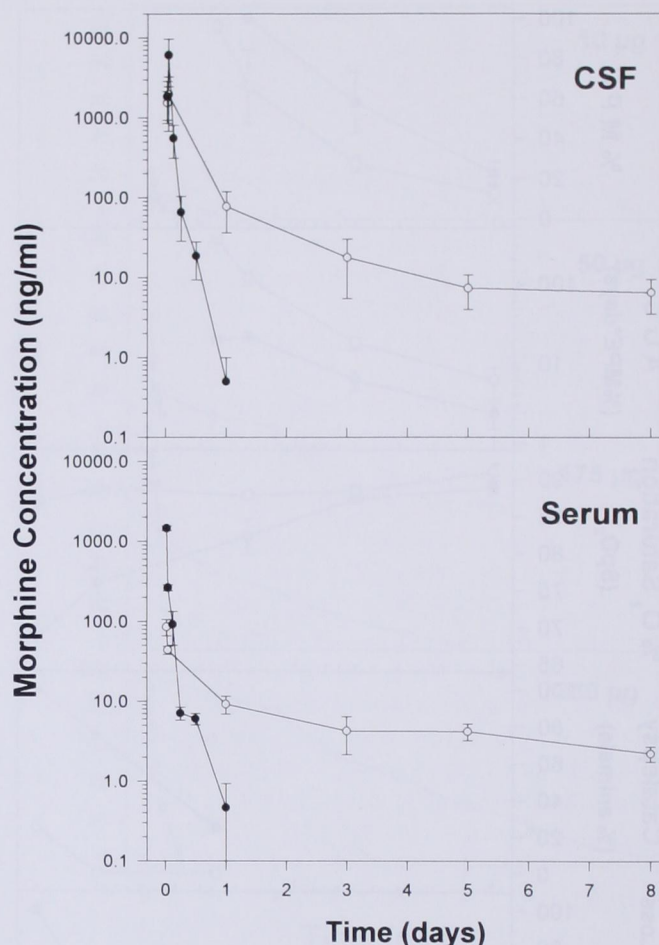


Fig. 4. Cerebrospinal fluid (top panel) and serum (bottom panel) morphine concentrations after a 250- μ g epidural administration of DTC401 (open circles) or morphine sulfate (closed circles). Each data point represents the average and SEM from 3 or 4 animals.

throughout the next several days. There was a modest reduction in peak analgesic potency, but the analgesic AUC was increased 3- to 19-fold compared with morphine sulfate.

Supraspinal toxic effects (*i.e.*, catalepsy or loss of corneal reflex) were not observed after injection of DTC401, except in one animal given the maximum dose of 2,000 μ g. To accommodate this dose of DTC401, the volume of injection had to be increased to 75 μ l, which may have increased the rostral spread.

Pharmacokinetic data showed that the peak cisternal CSF and serum morphine concentrations after epidural DTC401 were 32% and 6%, respectively, of that following epidural morphine sulfate. The lower peak concentrations in cisternal CSF and in serum are con-

SUSTAINED-RELEASE MORPHINE

Table 1. Pharmacokinetic Parameters after 250- μ g Epidural Injection

	DTC401	MS
C_{max} (ng/ml), CSF	1,960 \pm 1,280	6,060 \pm 3,590
C_{max} (ng/ml), serum	86 \pm 20	1,460 \pm 97
$t_{1/2\alpha}$ (h), CSF	5.0	0.85
$t_{1/2\beta}$ (h), CSF	82	2.6
$t_{1/2\alpha}$ (h), serum	0.48	0.68
$t_{1/2\beta}$ (h), serum	49	5.0
AUC (ng \cdot days \cdot ml $^{-1}$), CSF	1,170	432
AUC (ng \cdot days \cdot ml $^{-1}$), serum	53	58
r^2 , CSF	0.98	1.00
r^2 , serum	1.00	1.00

MS = morphine sulfate; C_{max} = maximum concentration; $t_{1/2\alpha}$ = initial half-life; $t_{1/2\beta}$ = terminal half-life; AUC = area under the curve.

sistent with the minimal SpO_2 decrease, as well as the minimal incidence of catalepsy or loss of corneal reflex observed with DTC401 compared with morphine sulfate. However, the true peak morphine concentrations may have been missed, because cisternal CSF concentrations were determined initially only at 0.5 and 1 h after injection.

In the CSF, the AUC after epidural administration of DTC401 was 2.7 times that after epidural morphine sulfate, which was consistent with the prolonged analgesic effect. The exact CSF kinetics at the epidural catheter tip is not known, because the CSF samples for the pharmacokinetic studies were obtained by cisternal puncture. DTC401 and morphine sulfate given epidurally had almost identical systemic bioavailability, as shown by the similar serum AUCs.

There were some discrepancies between analgesic efficacy and morphine concentrations. In comparing the efficacy-time curve (fig. 1) *versus* the pharmacokinetic curves (fig. 4) of DTC401, the time to reach half-maximal analgesic effect was 2.5 days, whereas the CSF terminal half-life ($t_{1/2\beta}$) was 3.4 days. Also, from day 3 to day 8, serum and CSF morphine concentrations remained relatively constant, whereas antinociception continued to decline. The development of opiate tolerance could explain these discrepancies. Spinal opioid tolerance during chronic spinal morphine administration has been well documented in both animals^{32,†} and in humans.³³ In humans experiencing

severe pain, there is clinical evidence that presence of pain could antagonize the development of opioid tolerance.^{34,35}

Other investigators examined the intrathecal use of other lipid-based formulations of opioids.^{30,36–38} However, neither their pharmacokinetics nor pharmacodynamics were sufficiently different from those of the free drug to warrant their use in clinical practice. To our knowledge, no comparable sustained-release formulations of opioids given *via* the epidural route have been reported previously.

In conclusion, a single epidural dose of DTC401 was shown to prolong the duration of analgesia, with minimal supraspinal toxic effects, in rats, compared with morphine sulfate.

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