# Development of an injectable sustained-release formulation of morphine: antinociceptive properties in rats

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## Summary

The antinociceptive actions of morphine incorporated into an injectable chitosan-based gel were investigated in rats. Subcutaneous administration of 4.8 mg/kg morphine sulphate in a gel composed of N,O-carboxymethylchitosan (NOCC) and chitosan resulted in significant antinociception within 10 min that was maximal at 60 min and persisted for 6 h. In contrast, the same dose of morphine sulphate injected in sterile saline produced maximal responses at 30 min but only persisted for 2 h. NOCC/chitosan gel was easily injectable using a 22 guage needle and appears stable in long-term storage. No local or systemic adverse effects other than morphine-induced sedation were observed either at the time of injection or during the subsequent 48 h. We conclude that gels composed of chitosan and chitosan derivatives are effective matrices for sustained-release formulations of opioid analgesics capable of providing long-lasting antinociception.

**Keywords** Analgesia; opioid; NOCC; veterinary medicine; rat

The use of both opioid and non-opioid drugs for the provision of pain relief is becoming increasingly common in laboratory animal medicine (Flecknell 1984, Liles & Flecknell 1992). The primary indication for these drugs is in the provision of post-operative analgesia, which may be required for periods of up to 72 h (Flecknell 1984). Unfortunately, most commonly available drugs have a relatively short half life and duration of action in laboratory rodents (Flecknell 1996). This problem of limited duration of action is particularly relevant to the use of opioid drugs, and has been described as 'the most important problem ...' affecting the clinical use of opioid (narcotic) analgesics for post-operative care (Flecknell 1996). Because most of these drugs have poor bioavailability when administered in feed or water, they require parenteral administration, yet even the longer-acting agents (e.g. buprenorphine) have a duration of action of only 6–8 h in rats (Cowan *et al.* 1977, Dum & Herz 1981). Thus multiple injections are required if adequate analgesia is to be maintained. Multiple i.m. or s.c. injections are often beyond the capabilities of the care available in animal care facilities, particularly during the period from 17:00 to 08:00. Further, multiple injections or the use of drugs in combination to prolong the duration of action may be associated with an unacceptable risk of toxicity or may compromise experimental design.

In an effort to address this issue, we have embarked on a series of investigations designed to develop a sustained-release formulation of morphine that can be administered easily by s.c. injection. Our aim was to

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develop a product that would provide 12–24 h of effective analgesia, and would be inexpensive, non-irritating at the site of injection and devoid of side effects other than those normally associated with morphine. We report herein on the duration of morphine analgesia in rats following incorporation of the drug into a carboxymethylchitosan-based injectable gel. Chitosan is the N-deacetylated form of chitin, a naturally-occurring polysaccharide. These polymers are readily biodegradable (Miyazaki *et al.* 1981) and have been used previously to create oral sustained-release preparations in the form of either tablets (Sawayanagi *et al.* 1983, Inouye *et al.* 1988) or granules (Hou *et al.* 1985, Miyazaki *et al.* 1988a, Miyazaki *et al.* 1988b). Chitosan-based gels have also been used to create microspheres for targetted drug delivery (Ohya *et al.* 1993). Neither chitin nor chitosan are soluble at physiological pH unless the polymers are extensively depolymerized but, more importantly, it has been reported that chitosan glutamate is inflammatory when exposed to living tissue due to the presence of protonated amine centres (Johnson *et al.* 1992). Carboxymethylation of chitosan (Hayes 1986) however, produces a water-soluble, biocompatible (Elson *et al.* 1994) and bioabsorbable anionic polymer of high molecular weight (> 2 M daltons) with many of the characteristics of hyaluronic acid. This polymer, N,O-carboxymethylchitosan (NOCC), can be formulated into water-based gels and pastes by addition of cross-linking agents (Davies *et al.* 1989) or polycations. Further, the rate of degradation of these gels is generally dependent upon the degree of chemical or electrostatic cross-linking within the gel, making it theoretically possible to control the rate of drug delivery and the ease of injection by varying the gel composition. The current study describes the properties of morphine incorporated into a gel consisting of NOCC and chitosan polymers that have opposite charge at physiological pH and form a stable yet injectable complex.

## Materials and methods

### *Experimental animals*

Studies were conducted using conventionally-reared male Sprague-Dawley rats (250–450 g) obtained from Charles River Ltd (LaSalle, Quebec). Rats were group housed (4–5 per cage) in clear Plexiglas® boxes with heat treated hardwood chip bedding (PWI Industries, St Hyacinthe, Quebec). Food (constant formula, natural ingredient Purina Laboratory Chows 5001) and water were available *ad libitum* except during habituation and testing. Temperature in the restricted access colony room was maintained between 21 and 24°C and humidity was continually monitored (range 40–55%). Air supply was 100% fresh (non-recirculated) with an exchange rate of 14 exchanges per hour. Lighting was about 30 foot candles one metre from the floor and was maintained on a 13 h light/11 h dark cycle (06:00 and 19:00 h). All testing took place in a separate testing room between 08:00 and 17:00 h. Each rat was tested twice, one week apart to allow for drug clearance. Each data point represents the mean of a group of eight rats.

All procedures were approved in advance by the UPEI Animal Care Committee and procedures and housing facilities were in compliance with the guidelines of the Canadian Council on Animal Care.

### *Preparation of morphine gel*

Gels for delivery of morphine sulphate were prepared from solutions of NOCC chemically combined with purified chitosan (NovaChem, Halifax, Nova Scotia).

Gels were prepared by first dissolving an appropriate quantity (usually about 0.25 g) of high viscosity NOCC in warm 0.1 M phosphate buffered saline (PBS)(pH 7.3) to create a clear, viscous (3.1% w/v) solution that was 'flash' sterilized in an autoclave (5 min at 121°C). To 8.0 ml of the NOCC solution was added sterile morphine sulphate solution (1.0 ml of 44 mg/ml)(Morphine HP50, Sabex Inc., Montreal, Quebec) that had been adjusted to pH 7.0 using PBS. The NOCC/morphine solution was mixed thoroughly and then combined with 2.0 ml of low viscosity chitosan in sterile PBS (25 mg/ml; pH 6.0). Gels were then loaded into sterile 5.0 ml syringes and stored at ambient temperature until use. The final concentration of morphine sulphate in all gels was 4.0 mg/ml.

*Experimental protocol*

To measure the relative efficacy and duration of action of NOCC/chitosan gel, a total of 32 rats were randomly divided into four groups ($n = 8$ in each). The two 'experimental' groups were administered either conventional morphine sulphate solution or morphine gel at a dose of 4.8 mg/kg morphine sulphate, whereas the animals in the 'control' groups received either saline or 'blank' gel (see above). Rats were tested using the tail-flick test (D'Armour & Smith 1941), a common measure of antinociception in rodents. Rats were habituated to the testing room for 4 h on the day prior to each testing session and handled for 10 min each during this session. Further, each rat was habituated to the testing room for an additional hour immediately before testing. Each rat was handled for 10 min during the last habituation session. Testing consisted of loosely wrapping each rat in a towel and placing the distal third of the tail into water maintained at 50°C. The time (s) taken to either flick or withdraw the tail was measured. A maximum response latency of 12 s was observed in order to prevent tissue damage and following each immersion the tail was wiped with a dry towel. Two baseline latencies were recorded for each rat at 30 min and 10 min prior to testing, followed by injection (1.2 ml/kg s.c.) of morphine or vehicle. Testing took place at 10, 30, 60, 120, 180, 240, 360 and 480 min following drug or vehicle administration.

*Data analysis*

Analgesia data were converted to per cent maximum possible effect (% MPE) values at each time point by comparing test response latencies following morphine administration to the average of the two pre-morphine baseline latencies according to the following equation:

$$\%\text{MPE} = \frac{100 \times (\text{test latency} - \text{baseline latency})}{(12 - \text{baseline latency})}$$

The value of '12' represents the maximum allowable latency (12 s) and baseline latencies were typically about 4 s. The Trapezoid Rule was used to calculate areas under the %MPE *vs* time curve (AUC) for each rat. Comparisons of %MPE and AUC between each type of morphine formulation and the appropriate control groups were made using a one-way ANOVA. Similarly, comparisons between the two formulations were made using a one-way ANOVA and post hoc analysis was conducted using orthogonal contrasts where indicated.

## Results

Injection of the NOCC/chitosan gel containing morphine did not result in any observable local or systemic abnormalities in any of the rats at the time of drug administration, other than sedation, which is commonly observed following administration of morphine at this dose in rats.

*Analgesic properties of morphine in rats*

A comparison of the analgesia observed following injection of either morphine or vehicle control for both formulations is presented in Fig 1 for conventional morphine and morphine gel respectively. When compared to their control groups both regular injectable morphine and morphine in NOCC/chitosan gel produced significant analgesia based on AUC values ($F = 35.85$, $P < 0.0001$ and $F = 18.53$, $P < 0.001$; respectively). Analysis of these data at each time point, revealed that conventional morphine resulted in significant analgesia during the first 2 h following injection only (Fig 1), whereas morphine in the NOCC/chitosan gel produced significantly elevated withdrawal latencies at all time points up to and including 360 min; (6 h) (Fig 1).

Maximum analgesia was obtained at 30 min following conventional sulphate whereas peak effect was observed at 60 min following the NOCC/chitosan gel (Fig 1). Statistical comparisons between the two formulations revealed that the morphine-NOCC gel resulted in significantly less analgesia at the two earliest time points ($P < 0.01$), but was significantly more effective at all time points from 120 to 480 min, inclusive ($P < 0.01$). Interestingly, the AUC value for data obtained with the NOCC/chitosan gel (15975 ± 3723) was significantly

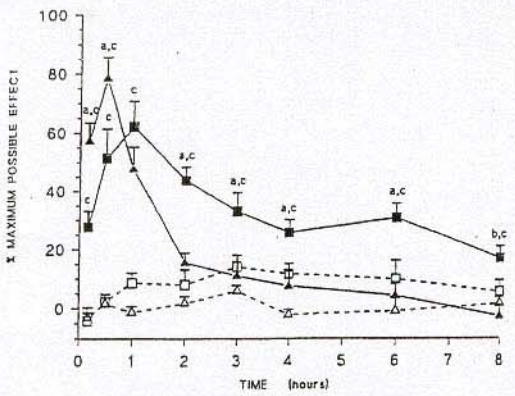


**Fig 1 Antinociception (% maximum possible effect) versus time profiles for groups of rats administered 4.8 mg/kg s.c. morphine sulphate either in sterile saline (▲) or in NOCC/chitosan gel (■).** Data for the respective control groups are shown using open symbols. All data are obtained in separate groups of rats (n = 8). Statistical analysis symbols are: [a] ($P < 0.01$) and [b] ($P < 0.05$) relative to the other treatment group, and [c] indicates points that were significantly different from the respective vehicle control groups

greater than that obtained using conventional morphine ($7775 \pm 4854$)($F = 19.60$; $P < 0.01$).

## Discussion

Administration of conventional morphine sulphate produced significant antinociception for only 2 h (Fig 1). This contrasts with the response following injection of morphine sulphate in the NOCC/chitosan gel matrix, which resulted in significant antinociception relative to vehicle control for 6 h (Fig 1) and was significantly more effective than conventional morphine for up to 8 h following injection. Maximal analgesia was seen 30 min following conventional morphine compared to 60 min following morphine gel (Fig 1). Because there is no reason to believe that the elimination of morphine would be different between the two experimental groups, these differences presumably reflect changes in absorption. Certainly, the results of pharmacokinetic trials conducted in our laboratory on these formulations in dogs indicate that absorption of morphine from the NOCC gel matrix occurs over about 2 h in this species (Tasker *et al.* 1997).

While the NOCC/chitosan matrix appears to cause prolonged absorption it does not appear to limit bioavailability, assuming that the total response AUC values are related to the amount of morphine in the systemic circulation. In fact, the analgesia versus time AUC for morphine gel was significantly higher than that observed for conventional morphine. The reason for this difference is unclear because there is no reason to expect reduced bioavailability with morphine sulphate solution following subcutaneous injection. More likely explanations include (a) underestimating the conventional morphine AUC, and (b) the contribution of active metabolites to the observed analgesia. It is possible that the experimental paradigm employed in this study may have underestimated the analgesia AUC for conventional morphine. Testing was conducted over a prolonged time frame and was therefore biased in favour of later time points. It is reasonable to expect that increased testing during the 0–60 min window might reveal enhanced antinociception in the conventional morphine group, and correspondingly greater AUC values. It is equally possible, however, that morphine metabolites contribute to greater antinociception following injection of the NOCC gel. Morphine-6-glucuronide has been shown to be analgesic in a variety of species including rats (Shimomura *et al.* 1971, Osbourne *et al.* 1990, Gong *et al.* 1992). Prolonged absorption and elimination of morphine from the gel matrix may lead to increased accumulation of active metabolites and hence, increased antinociception.

Finally, it is interesting to note that injection of morphine in an NOCC/chitosan gel did not result in any additional side effects either at the time of injection or during the subsequent 48 h when compared with rats receiving conventional morphine. None of the rats receiving either blank or morphine-containing gels showed signs of inflammation or sensitivity to palpation at the site of injection. Although biopsies were not performed on these animals, a previous study evaluated the inflammatory response using tissues harvested from rats 7 days following subcutaneous implantation of NOCC; no evidence of local inflammation was found

(Elson 1996). The observations in the current study, therefore, confirm previous reports using both rats and other species, that carboxymethyl derivatives of chitosan are both biodegradable and non-toxic. While the results reported herein on this particular formulation are quite promising we believe that even more prolonged durations of analgesic action using different drugs and/or different gels are possible. Preliminary studies in our laboratory have produced a number of NOCC-based gels that are easily injectable via a 22 guage needle and appear stable when stored either at ambient temperature or at 4°C. The potential exists, therefore, for the greater use of these compounds in sustained-release drug formulations. In the current study, the duration of action of morphine in rats was prolonged 3–4 fold. If NOCC-based gels are equally effective at prolonging the release of longer-acting agents such as buprenorphine, it may be possible to produce an injectable opioid formulation that provides 24–48 h of safe and effective analgesia in laboratory rodents.

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## References

Cowan A, Doxey JC, Harry EJR (1977) The animal pharmacology of buprenorphine, an oripavine analgesic agent. *British Journal of Pharmacology* **60**, 547–54

D'Amour FE, Smith DL (1941) A method for determining loss of pain sensation. *Journal of Pharmacology and Experimental Therapeutics* **72**, 74–9

Davies DH, Elson C, Hayes ER (1989) N,O-carboxymethylchitosan, a new water soluble derivative. In: *Chitin & Chitosan* (Skjak-Braek G, Anthonsen T, Sanford P, eds). Amsterdam: Elsevier Applied Science, pp 467–72

Dum JE, Herz A (1981) *In vivo* receptor binding of the opiate partial agonist, buprenorphine, correlated with its agonistic actions. *British Journal of Pharmacology* **74**, 627–33

Elson CM (1996) Agricultural and medical applications of N,O-carboxymethylchitosan, a derivative of shrimp processing wastes. *Bulletin of the Aquaculture Association of Canada* 96(4), 39–44

Elson CM, Davies P, Hauschka B, Zaharian B, Drohan W (1994) Biocompatibility of N,O-carboxymethylchitosan. In: *Chitin & Chitosan. Proceedings of the Sixth International Conference*. Gydnia, Poland

Flecknell PA (1984) The relief of pain in laboratory animals. *Laboratory Animals* **18**, 147–60

Flecknell PA (1996) *Laboratory Animal Anaesthesia* (2nd edn). London: Academic Press

Gong Q-L, Hedner J, Bjorkman R, Hedner T (1992) Morphine-3-glucuronide may functionally antagonize morphine-6-glucuronide induced antinociception and ventilatory depression in the rat. *Pain* **48**, 249–55

Hayes ER (1986) N,O-carboxymethylchitosan and preparative method thereof. US Patent 4,619,995

Hou W-M, Miyazaki S, Takada M, Komai T (1985) Sustained release of indomethacin from chitosan granules. *Chemical and Pharmaceutical Bulletin (Tokyo)* **33**, 3986–92

Inouye K, Machida Y, Sannan T, Nagai T (1988) Buoyant sustained release tablets based on chitosan. *Drug Design and Delivery* **2**, 165–75

Johnson RS, Lewis TW, Lampecht EG (1992) *In vivo* tissue response to implanted chitosan glutamate. In: *Advances in Chitin and Chitosan* (Brine C, Sanford P, Zikakis J, eds). Amsterdam: Elsevier, pp 3–8

Liles JA, Flecknell PA (1992) The use of non-steroidal anti-inflammatory drugs for the relief of pain in laboratory rodents and rabbits. *Laboratory Animals* **26**, 241–55

Miyazaki S, Ishii K, Nadai T (1981) The use of chitin and chitosan as drug carriers. *Chemical and Pharmaceutical Bulletin (Tokyo)* **29**, 3067–9

Miyazaki S, Yamaguchi H, Yokkouchi C, Takada M, Hou W-M (1988a) Sustained-release and intragastric-floating granules of indomethacin using chitosan in rabbits. *Chemical and Pharmaceutical Bulletin (Tokyo)* **36**, 4033–8

Miyazaki S, Yamaguchi H, Yokouchi C, Takada M, Hou W-M (1988b) Sustained release of indomethacin from chitosan granules in beagle dogs. *Journal of Pharmacy and Pharmacology* **40**, 642–3

Ohya Y, Kobayashi H, Ouchi T (1993) Release behaviour of 5-fluorouracil from chitosan-gel microspheres immobilizing 5-fluorouracil derivative coated with polysaccharides and their cell specific recognition. *Journal of Microencapsulation* **10**, 1–9

Osbourne R, Joel S, Trew D, Slevin M (1990) Morphine and metabolite behaviour after different routes of morphine administration: demonstration of the importance of the active metabolite morphine-6-glucuronide. *Clinical Pharmacology and Therapeutics* **47**, 12–19

Sawayanagi Y, Nambu N, Nagai T (1983) Dissolution properties and bioavailability of phenytoin from ground mixtures with chitin or chitosan.

*Chemical and Pharmaceutical Bulletin (Tokyo)* **31**, 2064–8

Shimomura K, Kamata O, Ueki S, Ida S, Oguri K, Yoshimura H, Tsukamoto H (1971) Analgesic effects of morphine glucuronides. *Tohoku Journal of Experimental Medicine* **105**, 45–52

Tasker RAR, Ross SJ, Dohoo SE, Elson CM (1997) Pharmacokinetics of an injectable sustained-release formulation of morphine for use in dogs. *Journal of Veterinary Pharmacology and Therapeutics* **20**, 362–7