Biovector™ Nanoparticles Improve Antinociceptive Efficacy of Nasal Morphine

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Received January 20, 2000 accepted March 9, 2000

Purpose. We have studied the antinociceptive activity and blood and brain delivery of nasal morphine with or without Biovector™ nanoparticles in mice.

Methods. A tail flick assay was used to evaluate the antinociceptive activity. The kinetics of morphine were evaluated in blood and brain, using tritiated morphine as tracer.

Results. These nanoparticles were shown to increase the duration of the antinociceptive activity of morphine after nasal administration. This effect was not due to an increase of morphine in the blood; and the analgesic activity of morphine in association with nanoparticles was reversed by naloxone. The ED_{50} value was 33.6 ± 15.6 mg/kg for morphine alone and 14.4 ± 7.6 mg/kg in presence of nanoparticles. They were only effective at low doses (1.5 to 2.5 μg), a higher or a lower dose had no effect. No interaction was found between nanoparticles and morphine. NaDOC, a permeation enhancer, was unable to improve nasal morphine activity.

Conclusions. These results show the presence of nanoparticles only at a very specific dose increases the antinociceptive activity of nasal morphine in mice. The occurrence of a direct transport of morphine from the nasal mucosa to the brain is discussed.

KEY WORDS: nasal; Biovector™; nanoparticles; morphine; antinociception; tail-flick test; mouse.

INTRODUCTION

Morphine is an important pain reliever which is widely used, particularly, in cases of severe and chronic pain associated with heart attacks, serious injury, post-operative discomfort, and terminal illnesses such as cancer. For patients with chronic pain, oral treatment is generally thought to be the most convenient and feasible protocol. However, due to difficulties with swallowing, nausea, vomiting, and gastrointestinal obstruction, the oral route cannot always be used. Nasal administration offers a promising alternative. The advantages of nasal administration for the systemic delivery of drugs include a high degree of absorption, since the nasal surface area is quite significant, and the mucosa highly vascularized. Thus, the nasal route represents an attractive alternative to the oral or parenteral administration of morphine. This route also avoids the first-pass hepatic metabolism and may give direct access to the brain. A weak opioid analgesic, butorphanol (1), has been studied in humans after intranasal administration and it has been shown that the kinetics of butorphanol are not altered after repeated nasal administration. Studies performed with oxycodone (2) have shown that it is rapidly absorbed by the nasal mucosa, but large interindividual differences were observed in the study.

A direct pathway between the olfactory mucosa and the central nervous system (CNS) has been observed with a number of tracer molecules (3). For example: albumin conjugated to Evans blue (4), wheat germ agglutinin conjugated to horseradish peroxidase (5), or tritium labeled dihydroergotamine (6) have been shown to by-pass the blood brain barrier using a direct nose-cerebrospinal fluid (CSF) pathway. Since these initial reports, some viruses (7), metals (8,9), or drugs (10,11) have also been reported to be directly transported from the nasal mucosa to the CNS. Although evidence clearly suggests the olfactory epithelium and its olfactory cells play a major role, little is known about the mechanism of direct transport of solutes into the brain.

The possibility that the intranasal route might be useful for a variety of centrally acting drugs has received a great deal of attention and a number of strategies has been developed to improve its efficacy. One such strategy consists in the use of enhancing compounds such as bile salts, synthetic surfactants, chelators, phospholipids, and cyclodextrins. The mechanisms by which these enhancers lead to an increase in nasal drug absorption are quite diverse and poorly understood. However, it has been postulated that the enhancing effect of bile salts and other surfactants stems from their ability to erode epithelial cells and permanently alter the structural integrity of the mucosal membrane (12). Furthermore, these permeation enhancers cannot be used for human administration due to their poor tolerability.

The nanoparticles consist of cationic 60 nm spherical nanoparticles surrounded by a lipid bilayer (13–15). They have been shown to be well tolerated in animals after intranasal administration, and, even when administered twice daily for 4 weeks, no toxicity was found in beagle dogs (data not shown). These nanoparticles were also evaluated as a vaccine delivery system after nasal administration in humans and were found to be well tolerated (data not shown).

As morphine, due to its low lipophilicity (16), has a low brain bioavaibility, we chose it to evaluate the potential effect of nanoparticles in mice using the tail flick test. Comparison was made with a known permeation enhancer, sodium deoxycholate, and a potential mechanism of action of Biovector™ nanoparticles is discussed.

MATERIALS AND METHODS

Chemicals

Francopia (France) provided morphine hydrochloride and naloxone, and NaDOC (Sodium deoxycholate) were provided by Sigma. [^{3}H]morphine, specific activity 63 Ci/mmol, was supplied by New England Nuclear. Glucidex (US pharmacopeia maltodextrin (grade USP 23 NF 18 p 2263) was provided by Roquette, Lille, France).

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0724-8741/00/0600-0743$18.00/0

Preparation of Biovectors™

Polysaccharidic particles were prepared from maltodextrin as described previously (13–14). Briefly, 100 g of maltodextrin was dissolved in 2N sodium hydroxide under magnetic stirring at room temperature. Epichlorydrin (4.72 ml) and glycidyltrimethylammonium chloride (hydroxycholine) were then added. After 20 h reaction, the gel was neutralized with acetic acid and finally sheared under high pressure in a Minilab homogenizer (Rannie, APV Baker, Evreux, France). The 60 nm neutral, cationic polysaccharidic nanoparticles obtained were ultrafiltrated on an SGI Hi-flow system. (30UFIB/1 S.6/ 40 kD) (Setric Genie Industriel, Toulouse, France) in order to remove low molecular weight reagents and salts.

Biovectors™ nanoparticles were prepared in a Minilab homogenizer by the mixing of polysaccharidic nanoparticles, dipalmitoyl phosphatidyl choline (DPPC), and cholesterol at a temperature above the gel-to-liquid phase transition temperature of the phospholipid (17). Polysaccharide and phospholipid concentrations were 1.0 mg/ml and 0.3 mg/ml, respectively. Phospholipid concentration was determined using Bartlett's method (18). Cholesterol was analyzed using an enzymatic assay. Polysaccharide concentration was determined using Dubois' method (19). The mean diameter of Biovectors™ was determined by laser light scattering with the N4MD Coulter nanoparticle analyzer (Coultronics, Margency, France). Using this technique, the mean diameter obtained was 60 ± 15 nm.

Association Between Morphine and Biovector™ Nanoparticles

Morphine (50 mg/ml) and [^{3}H] morphine (1 μCi/ml) were mixed in 15 mM PBS (Phosphate Buffer Saline, pH 7.4) with increasing amounts of nanoparticles (1–130 μg/10 μl). The solutions (200 μl) were ultrafiltered on microcon 100 kD (Amicon) by centrifugation at 3000 g for 15 min. Aliquots (50 μl) taken before and after filtration were analyzed by liquid scintillation counting in a β-counter Packard Tri-Carb 2100 TR (Packard Instrument SA, France) for 2 min. (56% efficiency for tritium).

Animals

Male Swiss mice (25–30 g, C. E. Depré, France) were used in all experiments. Animal housing, care, and experimental procedures were in accordance with the recommendations of the International Association for the Study of Pain. Animals were maintained at 22°C on a normal dark/light cycle with access to food and water *ad libitum*.

Groups of 10 mice were used for each dose unless otherwise indicated.

Drug Administrations

For nasal administrations, 10 μl (5 μl/nostril) were instilled with a P20 Pipetman in mice maintained in a supine position for about 15 s.

For subcutaneous injections, drugs were injected at a constant volume (200 μl).

Analgesia

Nociceptive responses were assessed using the radiant heat tail-flick test (20). The intensity of heat stimulus was adjusted so that a tail-flick was observed after 2–3 sec. Each mouse was tested twice prior to drug administration. The tail-flick responses were measured 15, 30, 60, 90, 120, 240, and 360 min after drug administration. The cut-off time was set to 8 s to prevent tissue damage. Changes in latency responses were converted to maximum percentage effect (%MPE) for each animal, calculated from the equation:

$$[(T - T_0)/(8 - T_0)]*100,$$

where T_0 and T are the latencies before and after administration respectively.

The areas under the curve (AUC) were calculated by trapezoidal approximation up to 240 min.

Blood and Brain Profiles of Nasal [^{3}H]morphine

For each time at 0, 2, 5, 15, 30, 60, 90, 120, 240, and 360 min, 9 mice were killed by decapitation. The brains were extracted immediately and homogenized in 10 ml of Tris HCl 50 mM, pH 7.4, per g of tissue. Blood was collected in heparinized tubes and immediately centrifuged at 9500 g for 10 min. Aliquots of 100 μl of plasma or of brain homogenates were analyzed by liquid scintillation counting as described above.

Statistical Analysis

Values were compared with control data in a one-way analysis of variance (ANOVA) followed, if significant differences occurred, by a Student's *t-test* (Statview™). P-values of less than 0.05 were considered to indicate statistical significance. The data are reported as means ± S.E.M.

RESULTS

Effect of Biovector™ Nanoparticles on the Analgesic Efficacy of Nasal Morphine

Nanoparticles alone, at doses up to 130 μg, had no antinociceptive effect in mice (data not shown). Morphine (50–600 μg) instilled nasally induced a dose-dependent antinociception in the tail-flick test (Fig. 1). The ED_{50} value of morphine was calculated to be 33.6 ± 15.6 mg/kg. The morphine dose-response curve was shifted to the left when the opiate was co-administered with 2 μg of nanoparticles. The ED_{50} value (14.4 ± 7.6 mg/kg) was half than that of morphine alone, indicating this dose of nanoparticles improved the analgesic efficacy of morphine. The morphine maximal effect remained unchanged, but administration of nanoparticles significantly enhanced its duration (30 to 120 min compared to 30 to 60 min for morphine alone, Fig. 2). Co-administration of NaDOC 1% had no effect on morphine antinociceptive activity.

The effect of different doses of nanoparticles on the efficacy of 500 μg of morphine was examined. Morphine-induced analgesia increased in the presence of nanoparticles only at doses ranging from 1.5 to 2.5 μg (Fig. 3).

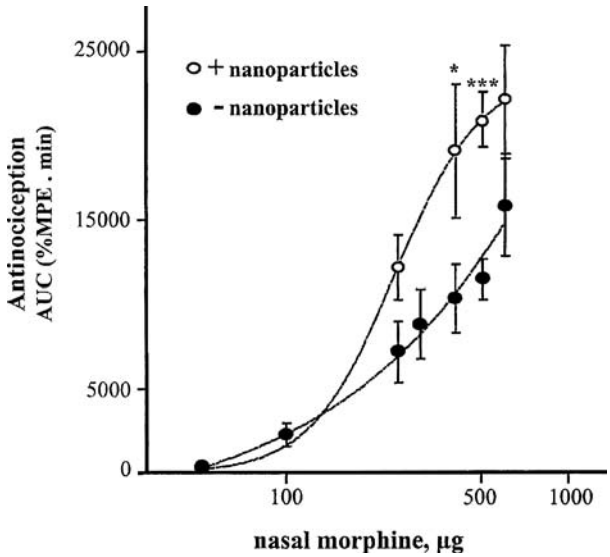

Fig. 1. Dose-dependent antinociceptive activity of nasal morphine in mice. Mice received 50 to 600 µg morphine in 10 µl (5 µl/nostril) with or without 2 µg of nanoparticles. The antinociceptive activity was evaluated in the tail-flick test. AUC were calculated on 240 min after nasal instillation of morphine. * $p < 0.05$; *** $p < 0.001$ *vs.* morphine alone (Student's *t-test*).

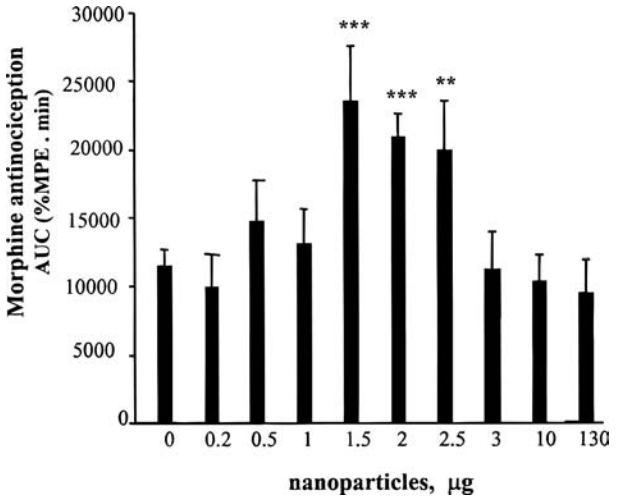

Fig. 3. Dose-effect of nanoparticles on the antinociceptive activity of 500 µg of nasal morphine in mice. The antinociceptive activity was evaluated in the tail-flick test. AUC were calculated up to 240 min after nasal co-administration with 500 µg of morphine and increasing amounts of nanoparticles (0.2–130 µg). ** $p < 0.01$; *** $p < 0.001$ *vs.* morphine alone (Student's *t-test*).

Reversion of Nasal Morphine Antinociception by Naloxone

The antinociceptive effects produced by 500 µg of morphine in the presence or absence of nanoparticles were both inhibited by the subcutaneous administration of 1 mg/kg of the opioid antagonist, naloxone (Fig. 4), clearly showing morphine effects are still reversible in the presence of nanoparticles.

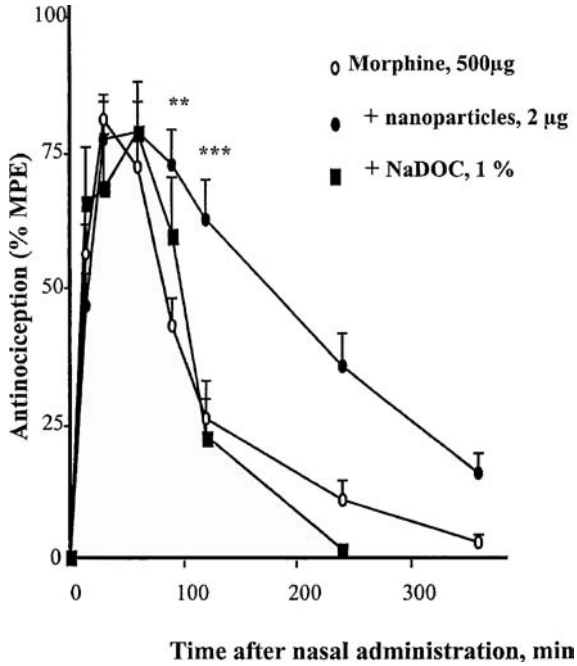

Fig. 2. Time course of nasal morphine antinociception in the tail-flick test in the presence or absence of 2 µg of nanoparticles or NaDOC. Mice were nasally administered with 500 µg morphine alone and in the presence of 1% NaDOC or 2 µg Biovector™ nanoparticles. ** $p < 0.01$; *** $p < 0.001$ *vs.* morphine alone (Student's *t-test*).

Effect of Biovector™ Nanoparticles on S.C. Morphine Antinociception

Nanoparticles had no effect on antinociception induced by the subcutaneous injection of morphine (Fig. 5). The same result was observed after oral administration (data not shown).

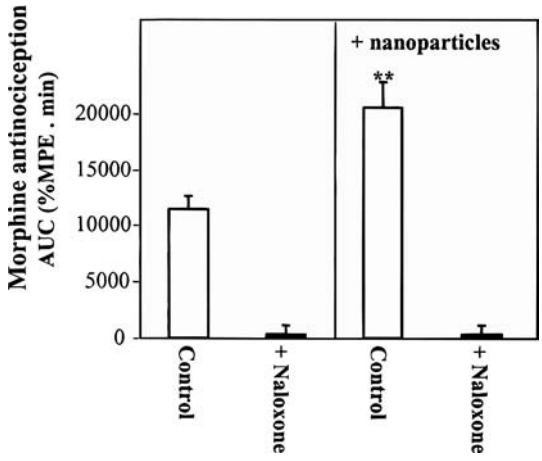

Fig. 4. Reversion by naloxone (1 mg/kg, s. c.) of the antinociceptive activity of nasal morphine administered or not in presence of nanoparticles. The antinociceptive activity was evaluated in the tail-flick test. AUC were calculated up to 240 min after nasal administration of 500 µg of morphine with or without 2 µg nanoparticles. ** $p < 0.01$ *vs.* morphine alone (Student's *t-test*).

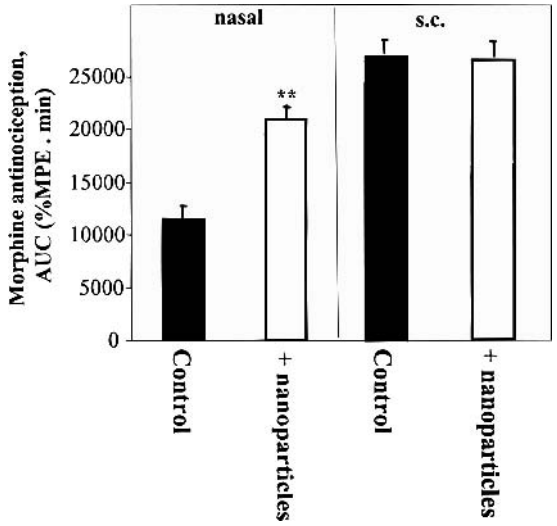

Fig. 5. Comparison of nasal and subcutaneous (s.c.) routes of morphine administration in the presence or absence of 2 μg of nanoparticles. The antinociceptive activity was evaluated in the tail-flick test. AUC were calculated up to 240 min after administration of 500 μg of morphine. *** $p < 0.01$ *vs.* morphine alone (Student's *t-test*).

Characterization of the Mechanism of Action of Biovector™ Nanoparticles

The ability of morphine to bind to nanoparticles at various concentrations (1 to 130 μg) was evaluated by centrifuge ultrafiltration. No direct interaction could be detected between nanoparticles and morphine since the opiate, in contrast to nanoparticles (60 nm), was not retained by 100 kD filters (Table 1). Moreover, the nasal administration of morphine, followed two minutes later by nanoparticles or in reverse order, had no effect on the antinociceptive effects of morphine (data not shown). The effects of nanoparticles disappeared if the two administrations were separated by a time interval of at least 2 min, indicating that co-administration is required to obtain an increase in morphine antinociceptive effects.

The antinociceptive activity of nasal morphine in the presence of a permeation enhancer, NaDOC was also tested. This bile acid is known to increase the rate of transport from the nasal mucosa to the blood (21). As shown in the Fig. 2, the analgesic potency of nasal morphine was unaffected by co-administration of NaDOC. Moreover, no improvement of [^{3}H]morphine plasma profiles was observed in presence of Biovector™ nanoparticles (Fig. 6). Brain homogenates were analyzed after instillation of tritiated morphine and we observed no significant difference after nasal administration in presence and absence of Biovector™ (data not shown). These results suggest the increased antinociceptive activity observed in presence of nanoparticles was not the consequence of an increased delivery of morphine to the blood, but of a direct delivery to the brain.

Table 1. Lack of Association Between [^{3}H]morphine and Biovector™ Nanoparticles

	(a) Before ultrafitration Radioactivity (nCi/ml)	(b) After ultrafitration Radioactivity in the ultrafiltrate (nCi/ ml)	% association
[^{3}H]morphine + nanoparticles			
0 μg	103	106	0
1 μg	103	107	0
2 μg	101	107	0
10 μg	104	108	0
130 μg	148	160	0

Note: Association between [^{3}H] morphine and Biovector™ nanoparticles was evaluated by centrifuge ultrafiltration, nanoparticles are retained on the filter (cut-off: 100 kD), analysis of the ultrafiltrate.

DISCUSSION

The administration of morphine via the nasal route represents an interesting therapeutic alternative to the parenteral or oral route.

In the present study, morphine, a highly hydrophilic drug with low brain bioavaibility (22) was selected in order to differentiate between the contributions of direct and systemic pathways to uptake by the brain after administration by nasal route.

For mice nasal administration, according to Gizurarson (27), as the volume of the nasal cavity is about 30 μl, the volume to administer should not be over 3 μl per nostril. We found that increasing the total volume of administration, from 5 to 20 μl, had no effect on the antinociceptive activity (data not shown). This result suggests the effect observed is mainly due to nasal absorption of morphine. Some studies have shown

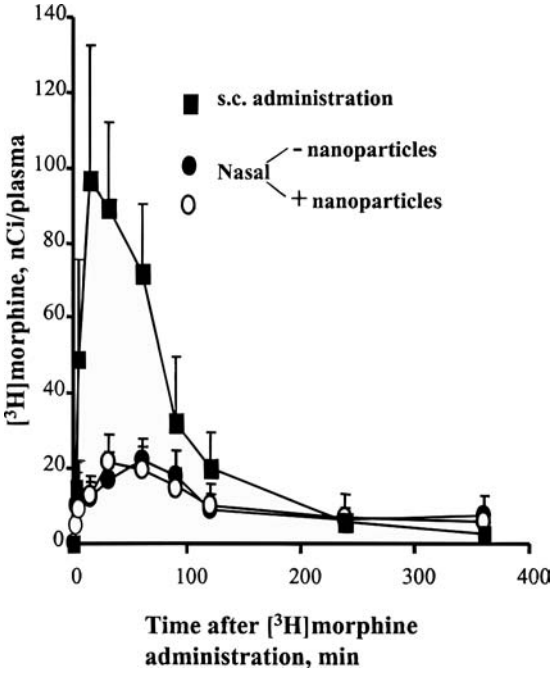

Fig. 6. Plasma kinetics of [^{3}H]morphine administered via nasal and subcutaneous (s.c.) routes of administration. Mice ($n = 9$) were administered with [^{3}H]morphine (2.4 μCi) + 500 μg morphine with and without nanoparticles (2 μg) via the nasal or subcutaneous route. * $p < 0.05$ *vs.* nasal route of administration (Student's *t-test*).

that, after intranasal administration, highly lipid-soluble molecules such as progesterone are transported directly to the cerebrospinal fluid (CSF) without first entering the peripheral blood compartment (23). As a consequence, the bioavailability of progesterone to the CSF is greater following nasal administration than after intravenous injection. However, although highly hydrophobic compounds such as progesterone have a high CSF bioavailability, this is not the case for highly hydrophilic compounds. Indeed, after nasal administration, a direct correlation has been reported between the lipophilicity of a drug and its CSF bioavailability (10,16,24). Since the brain microvessel endothelial cells are joined by tight junctions which limit the paracellular diffusion of hydrophilic compounds (25,26), a variety of strategies have been proposed and investigated to overcome the blood-brain barrier (24).

In our study, the dose-related antinociceptive effect of nasally administered morphine was significantly increased by the presence of nanoparticles. These nanoparticles were effective only at doses of 1.5 to 2.5 μg and led to an increase in the duration of the effects of morphine. This could not be attributed to the viscosity of the nanoparticles since even at high concentrations (15 g/l) the viscosity is only 7 mPa.s.

The reversal of the antinociceptive effects by naloxone demonstrates opioid receptors are involved and these nanoparticles do not modify the mechanisms of morphine action.

We attempted to characterize the mechanism by which these nanoparticles improved the antinociceptive efficacy of nasal morphine. Our results suggest this increase was not due to a specific carrier delivery of the drug to the brain since morphine did not bind to these nanoparticles, and it appears the nanoparticles do not cross the nasal epithelial barrier (28). Moreover, it is unlikely that a specific carrier system would account for the effects of nanoparticles only at a very specific dose.

Furthermore, after nasal administration an increase in blood delivery of morphine-6-glucuronide in the presence of chitosan has been shown (29). This result is not consistent with the lack of increase of radiolabeled morphine concentration in the blood found in our experiment. Similarly, by using NaDOC, a nasal permeation enhancer which is able to increase nose-blood delivery (21), no modification of morphine activity was observed.

In contrast, an increase in the direct nasal-brain transport of morphine in the presence of nanoparticles could more easily explain the results obtained. A number of previous studies support the possibility of a direct nose-CSF passage (8,9,11,23,26), but no data are presently available to show that this mechanism could be involved for hydrophilic compounds. In our studies, no increase of tritiated morphine was obtained in brain homogenates. However, measuring the whole brain does not exclude an increase in concentration in specific brain regions involved in analgesia. Furthermore, the presence of vessels in brain extracts could also explain the lack of increased of labeled morphine after nasal administration.

In conclusion, the co-administration of Biovector™ nanoparticles, only at specific doses, increases the antinociceptive activity of nasal morphine in mice, without modification in blood delivery of the drug. Since these nanoparticles, unlike permeation enhancers, are well tolerated in animals and humans after nasal administration (28,31), their potential for increasing antinociceptive activity in humans would merit investigation.

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