J Anesth (2014) 28:702–707
DOI 10.1007/s00540-014-1795-9

ORIGINAL ARTICLE

Evaluation of the peripheral analgesic effect of sufentanil lipid nanoparticles

Hongjun Li · Hui Qiao · Hongjie Lu · Changqi Liu

Received: 21 October 2013 / Accepted: 19 January 2014 / Published online: 7 March 2014

Abstract
Objective We wished to evaluate the effect of sufentanil lipid nanoparticles on peripheral analgesia of inflammatory pain model rats.
Methods Ninety SD rats were randomly divided into an inflammatory model group (group A, $n = 54$) and a blank control group (group B, $n = 36$). Group A was further divided into the sufentanil lipid nanoparticles group (group A1, $n = 18$), the sufentanil group (group A2, $n = 18$), and the inflammatory pain model group (group A3, $n = 18$); group B was divided into the sufentanil lipid nanoparticles group (group B1, $n = 18$) and the sufentanil group (group B2, $n = 18$). Rats of group A were given a formalin injection in the foot to produce the inflammatory pain model. Group B rats were given a normal saline foot injection of the same dosage. Then, groups A1 and B1 were given sufentanil lipid nanoparticles (0.82 μg/kg) treatment. Groups A2 and B2 were given sufentanil of the same dosage, and group A3 were given normal saline. Pain scores of Group A rats were recorded and analyzed. The ELISA method was adopted to determine drug concentration in rat brain, plasma, and the inflammatory pain/subcutaneous area.
Results Pain scores of rats in group A3 were always higher than those in groups A1 and A2, and the pain scores of group A2 were higher than in group A1 0–30 min after administration ($P < 0.05$). The brain drug concentration in groups A2 and B1 fluctuated over time; the brain drug concentrations of groups A2 and B2 were respectively higher than those of groups A1 and B1 ($P < 0.05$). There was no significant difference between the plasma drug concentrations of different groups at the same time point ($P > 0.05$); however, there was a notable difference within each group at different time points ($P < 0.05$), and the drug concentration of the inflammatory tissues in group A1 changed significantly over time ($P < 0.05$). Thirty minutes after administration, drug concentration in the inflammatory site of group A1 was higher than that of groups A2, B1, and B2 ($P < 0.05$).
Conclusion Sufentanil lipid nanoparticles had a comparatively weak effect on the central nervous system because of their features such as large particle size and targeted and controlled release. They have shown a remarkable analgesic effect in the peripheral inflammatory pain areas.

Keywords Sufentanil · Lipid nanoparticle · Inflammatory pain model · Rat · Drug concentration

Introduction

Since morphine was isolated from opium, opioids have been widely applied in clinical situations. They function through combining with opioid receptors located in the central or peripheral nervous system. Opioid analgesics also have a specific anti-injury effect on peripheral opioid receptors. Sufentanil is the highly selective agonist of μ-opioid receptors, which can have an effective peripheral analgesic effect by acting on the peripheral opioid receptor. However, the side effects that are generated such as respiratory depression are also connected with its effect on the central nervous system [1].

Nanotechnology is an evolving technology that has seen many improvements as the result of its implications in medicine [2]. One of the important issues that is considered in this field is the use of nano-carriers or nano-machines, which

H. Li (✉) · H. Qiao · H. Lu · C. Liu
Department of Anesthesiology, People's Hospital of Zhengzhou, Zhengzhou 450000, China
e-mail: lihongjunli1111@163.com

constitute a nanoscale delivery system. These delivery systems help prevent the unwanted exposure of tissues to a drug [3]. In particular, advances in nanotechnology have led to the development of nanoparticle drug-delivery vehicles. Nanoparticles are particularly well suited for cancer or trauma applications as they passively accumulate in tumors or lesions through enhanced permeability and retention effect [4].

To increase the bioavailability of sufentanil and its analgesic effect and drug distribution in inflammatory tissues [5], we wrapped sufentanil into solid lipid for the preparation of sufentanil lipid nanoparticles. We explored the drug distribution of sufentanil lipid nanoparticles in the brain, plasma, and inflammatory damage area, analyzed the pain response score changes of inflammatory pain model animals, and observed the features of this nano-drug, including targeted distribution and pharmacokinetics, to provide an experimental basis for clinical administration of anesthetics and analgesic drugs.

Materials and methods

All procedures were approved by and supervised by the Experimental Animal Center of Henan Province, and this study was approved by the Experimental Animal Ethics Committee in Experimental Animal Center of Zhengzhou University.

Experimental animals and materials

Ninety healthy male adult SD rats, each weighing 255.8 ± 11.4 g, were provided by the Experimental Animal Center of Henan Province.

Animal groupings

The rats were randomly divided into two groups: the formalin inflammatory model group (group A, $n = 54$) and blank control group (group B, $n = 36$). Group A was divided into the sufentanil lipid nanoparticles group (group A1, $n = 18$), sufentanil group (group A2, $n = 18$), and inflammatory model group (group A3, $n = 18$); group B was also divided into a sufentanil lipid nanoparticles group (group B1, $n = 18$) and a sufentanil group (group B2, $n = 18$).

Establishment of rat inflammatory model group and dosage regimen

To prepare the sufentanil lipid nanoparticles, 1 mg standard product of sufentanil, 10 mg stearic acid, and 2 mg lecithin were added to 10 ml anhydrous ethanol. After about 30 min ultrasound, the components were fully dissolved, thus constituting the oil phase. The surfactant F-68, accurately weighed, was added to 30 ml ddH_2O. After about 10 min ultrasound, this was fully dissolved, thus constituting the aqueous phase. Both the oil phase and the aqueous phase were heated to 75 ° ± 1 °C. By syringe the oil phase was then slowly injected into the mixing aqueous phase at the same temperature until the organic solvent had completely evaporated and the system had been concentrated to 5 ml, thus forming the nano-emulsion. After the nano-emulsion was rapidly mixed into 5 ml ice water with a 30 min ice bath ultrasound, the sufentanil lipid nanoparticles suspension was acquired. Under electron microscopy, it could be seen that the sufentanil lipid nanoparticles showed a round or quasi-circular shape, well distributed, with a diameter of 100–125 nm (Fig. 1).

All rats were under the state of abrosia at the time of 12 h before the experiment and dehydrated 4 h before experiment. After pelma disinfection of the left rear foot, rats of group A were given a hypodermic injection of 100 μl formalin with the volume fraction of 2.5 %; rats of group B were given a hypodermic injection of 100 μl normal saline. Analgesic drug delivery was conducted in rats of every group immediately after the formalin injection. Rats of groups A1 and B1 were given a quick intravenous injection of 0.5 ml normal saline containing 0.82 μg/kg sufentanil solid lipid nanoparticles; groups A2 and B2 were given a quick intravenous injection of 0.5 ml normal saline containing 0.82 μg/kg sufentanil; and group A3 was given a quick intravenous injection of 0.5 ml normal saline.

Sample processing and determination of sufentanil concentration

With rats fixed, blood (3 ml) was extracted from the hearts of rats in every group at 15, 30, and 60 min, respectively,

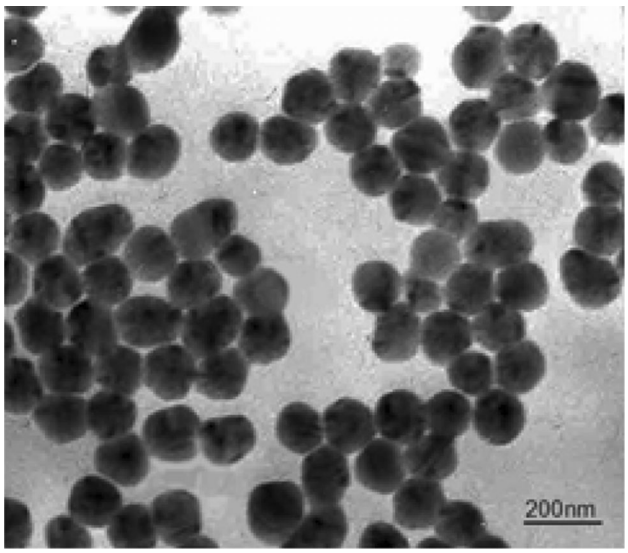

Fig. 1 Shape of sufentanil lipid nanoparticles under electron microscopy

after drug administration. To minimize the pain of the experimental rats, euthanasia (cervical vertebra dislocation method) was immediately carried out after the extraction of blood. Rat brain tissues (the hippocampus tissues), inflammatory tissues, and subcutaneous tissues were taken, each sample weighing 0.3 g. Then, each kind of tissue was homogenized by a homogenizer for the tissue homogenate. The homogenate was centrifuged at 3000 rpm for 5 min, and the supernate was reserved. The blood sample was centrifuged at 800 rpm for 8 min and the supernate was reserved. An enzyme-linked immunosorbent assay (ELISA) kit was used to determine the concentration of sufentanil in the tissues and plasma.

Record of pain score of the inflammatory pain rat group

According to the Dubuisson method and the actual site of injection, the behavior reaction of the wounded rats should be successively recorded, followed by determining the spontaneous pain score values during the observation period of 60 min at 5-min time intervals. Quantitative formalin-induced pain was measured by the total time spent in different behavioral states. Different behavioral states indicated all kinds of behaviors, such as animals licking and biting the injected foot or reducing weight-bearing on the injection foot (the load theory): 0 point, animal normal walking; 1 point, injected foot touching the floor slightly, not overweighted or slightly overweighted, limping when walking; 2 points, the position of the injection foot too high, not contacting any surface; 3 points, licking, biting, or intensely shaking the injected foot [6].

Statistical analysis

Data are shown in the form of ($x \pm s$). SPSS 17.0 software was used for statistical analysis. When the variance was homogeneous, one-way analysis of variance (ANOVA) was adopted for comparisons among groups, and the statistical method was least squares difference (LSD); when the variance was not homogeneous, the method of Welch was adopted to calibrate the F test and Dunnett's $T3$ test was used to do pairwise comparison. The chi-square test was used to analyze enumeration data; at $P < 0.05$, the difference was statistically significant.

Results

Pain scores of inflammatory pain model rats

After administration of analgesia, 5 min was taken to observe and evaluate the pain scores of rats in groups A1, A2, and A3. Statistical analysis showed that the pain score of rats in group A3 was higher than those in group A1 and group A2 at all times. However, during the period of time (0–30 min) after administration ($P < 0.05$), the pain score of group A2 was also higher than that of group A1 ($P < 0.05$). After 30 min, the pain scores of group A1 and group A2 did not differ ($P > 0.05$) (Fig. 2).

Comparison of sufentanil concentration of brain tissue homogenate (hippocampus tissue) in the different groups

The results of sufentanil concentration in brain tissue homogenate (hippocampus tissue) showed that after administration the drug concentrations of group A2 and group B1 had obvious differences at different time points ($P < 0.05$) (Table 1). Brain tissue homogenate concentration of group A1 and group B2 did not vary with time. The results of one-way ANOVA multiple comparisons showed that the drug level in group A2 was significantly higher than that in group A1 ($P < 0.05$); the drug concentration in group B2 was also higher than that in group B1 ($P < 0.05$). These results indicated that, compared with sufentanil, the sufentanil solid nanoparticles could not easily pass through the blood–brain barrier because of their large particle size, which led to the concentration distribution within the brain being lower than that of sufentanil.

Comparison of plasma concentrations of sufentanil in different groups

One-way ANOVA analysis showed that, among different groups and at different time points, the plasma concentration of sufentanil in the experimental animals had no significant difference at the same time point ($P > 0.05$) (Table 2). Within the same group, however, the difference was evident at different time points ($P < 0.05$). Concentrations of both drugs reached the highest level at 15 min

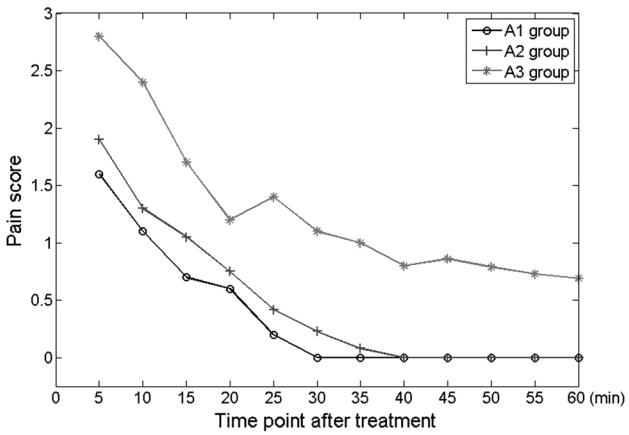

Fig. 2 Pain scores of inflammatory pain model rats after analgesic administration at different time points

Table 1 Analysis of drug concentration of hippocampus tissue homogenate among groups at different time points

Group	Time point			*F* value	*P* value
	15 min	30 min	60 min		
Group A1	0. 203 ± 0.012	0. 236 ± 0.019	0.221 ± 0.016	3.357	0.059
Group A2	0.245 ± 0.019	0.278 ± 0.014	0.232 ± 0.025	4.553	0.031
Group B1	0.204 ± 0.016	0.240 ± 0.021	0.212 ± 0.010	4.408	0.042
Group B2	0.242 ± 0.021	0.244 ± 0.032	0.243 ± 0.013	1.347	0.565
F value	6.172	4.124	3.146		
P value	0.004	0.036	0.061		

Table 2 Analysis of plasma drug concentration among groups at different time points

Group	Time point			*F* value	*P* value
	15 min	30 min	60 min		
Group A1	0.344 ± 0.018	0.308 ± 0.012	0.272 ± 0.022	25.638	0.001
Group A2	0.348 ± 0.016	0.312 ± 0.015	0.269 ± 0.018	48.662	0.000
Group B1	0.362 ± 0.015	0.313 ± 0.009	0.261 ± 0.010	67.404	0.000
Group B2	0.358 ± 0.021	0.320 ± 0.010	0.268 ± 0.011	69.747	0.000
F value	1.652	1.049	0.453		
P value	0.215	0.403	0.726		

after administration. The drug levels then showed a slowly decreasing trend with time. These results indicated that the metabolic rates of sufentanil lipid nanoparticles and sufentanil were close in vivo and that they had similar pharmacokinetic features.

Analysis of sufentanil concentrations in inflammatory or subcutaneous tissue homogenates among the groups at different time points

One-way ANOVA analysis showed that, among the groups and at different time points, sufentanil concentration in experimental animal inflammatory tissue and subcutaneous tissue homogenate had no significant differences ($P > 0.05$) (Table 3), except in group A1: at 30 min after administration, the drug concentration at the inflammatory site in group A1 was higher than that in groups A2, B1, and B2 ($P < 0.05$), and drug concentrations at the same time point in the other groups were not different ($P > 0.05$). These results indicated that, compared with sufentanil, the drug concentration of sufentanil lipid nanoparticles was higher at the inflammatory injury site, and the peripheral analgesic effects were more prominent.

Discussion

The exogenous opioid analgesics morphine, fentanyl, and sufentanil mainly act on the μ-receptor to bring about their clinical effect [7]. The receptor is widely expressed in the central nervous system and the peripheral nervous system. A study showed that opioids can produce the analgesia effect through a peripheral mechanism. In particular, the peripheral opioid activity is evident in inflammatory tissues. The interaction between peripheral opioid receptors and opioid peptides released by immune cells can produce effective peripheral analgesia [8].

Solid lipid nanoparticles, a novel kind of nano-colloid drug-loading system, were produced at a size of about 50–1000 nm with lipid as the carrier, forming the colloidal drug delivery system. The drug was entrapped in nanoparticles or adsorbed on the nanoparticle surface. Being solid at room temperature, the nanoparticles have advantages, including good physiological compatibility, and are easily degradable within the body with natural lipid material as the carrier, which readily allows release of drugs wrapped in the particles. Because of the features, such as targeted and controlled release, of the solid polymer nanoparticles [9], at the same time it was applied clinically to the development and utilization of anesthetic drugs to reduce their side effects. Pathak and Nargarsenker investigated the production process of a lipid carrier of solid lipid nanoparticles and nanostructures so as to improve the dermal delivery of a local anesthetic agent lidocaine (LID), finally obtaining a solid lipid nanoparticle gel coated with lidocaine and a gel with nanostructured lipid carriers containing lidocaine. The duration of anesthesia of the two gels was increased by fivefold and sixfold,

Table 3 Analysis of drug concentrations of inflammatory or subcutaneous tissues among groups at different time points

Group	Time point			*F* value	*P* value
	15 min	30 min	60 min		
Group A1	0.252 ± 0.019	0.283 ± 0.023	0.236 ± 0.016	4.656	0.034
Group A2	0.220 ± 0.018	0.219 ± 0.014	0.220 ± 0.018	0.112	0.895
Group B1	0.224 ± 0.023	0.220 ± 0.019	0.222 ± 0.011	0.416	0.673
Group B2	0.223 ± 0.016	0.218 ± 0.011	0.214 ± 0.015	1.205	0.338
F value	3.309	3.948	0.873		
P value	0.062	0.027	0.472		

respectively, compared to that of anesthesia of the usual lidocaine administration [10]. Leng et al. [11] employed monostearin (MS), glyceryl palmitostearate (GP), and stearic acid(SA) as lipids for the preparation of the lidocaine solid lipid nanoparticle (SLN). Results showed that in vitro release within 48 h of lidocaine from SLNs was 80 % with MS SLNs, 69 % with GP SLNs, and 89 % with SA SLNs.

Sufentanil lipid nanoparticles are made with a natural or synthetic biodegradable lipoid as the carrier, and the common lipoid is stearic acid. Stearic acid is a kind of endogenous physical substance, whose melting range is 50–60 °C. It has good biocompatibility and a fixed path of degradation [12, 13]. Research conducted by Guan et al. [14] found that release of the manufactured drug lipid nanoparticle in vitro showed a faster trend at the first stage and was slower at the second stage. After 12 h, the accumulative release of drugs reached 50 %. On the other hand, drugs entrapped in the matrix of degradable material were slowly released through matrix erosion, conforming to the Higuchi equation. Jigisha et al. [15] studied the in vitro release of cyclosporine lipid nanoparticles (using stearic acid as the solid lipid) and found that after 1 h release of the cyclosporine lipid nanoparticle reached 14 % and after 20 h reached 41.12 %. Zhang et al. [16] studied the in vitro release of 10-hydroxycamptothecine, modified by polyethylene glycol (PEG), and found that after 48 h the accumulative release of drugs reached 80 %. It is thus can be seen that the lipid nanoparticle is a good choice for the release of entrapped drugs.

This study used formalin to produce the rat inflammatory pain model to analyze the safety and efficacy of sufentanil nanoparticles from the aspect of peripheral analgesia through pain scores and drug distribution within animal tissues after administration. According to the pain score, it could be seen that within 30 min after administration, the analgesic effect in group A1 (sufentanil lipid nanoparticles group) was better than that in group A2 ($P < 0.05$). After 30 min, the analgesic effects of the two groups did not differ ($P > 0.05$). Drug concentration at the inflammatory injury site also confirmed this. In this experiment, the drug concentration in group A1 was higher than that of groups A2, B1, and B2 at three time points, indicating that compared with the usual sufentanil, drug concentration at the inflammation injury site is higher and sufentanil lipid nanoparticles have better targeting properties, which was confirmed in numerous studies [17]. Concerning the influence on the central nervous system, in the sufentanil groups (group A2 and group B2) the drug concentration in the brain tissue homogenate was significantly higher than that of the sufentanil lipid nanoparticles groups (group A1 and group B1). This finding shows that the blood–brain barrier permeability rate of sufentanil lipid nanoparticles is relatively low, so their distribution level in the brain tissue is also relatively low and the influence on the central nervous system is weak. In addition, the process of the targeted and controlled release of the nano-drug delivery system also needs a certain amount of time, which limits drug concentration distribution in the central nervous system. The plasma drug concentration analysis indicated that the animal drug plasma concentration of each group changes over time, whereas drug concentration at the same time point has no difference, S ($P > 0.05$). Analysis indicated that the pharmacokinetics of sufentanil lipid nanoparticles is similar to that of sufentanil, and their metabolic efficiency vivo is similar.

In conclusion, sufentanil lipid nanoparticles and sufentanil are very close in terms of pharmacokinetics, although the nanoparticles have a weak influence on the central nervous system because of their characteristics such as large particle size and targeted and controlled release. Sufentanil lipid nanoparticles also show a more remarkable analgesic effect on the peripheral inflammatory pain areas.

Acknowledgments The accomplishment of this dissertation is the joint efforts of all colleagues in the hospital. First, workmates in the department have offered great help in the construction of the dissertation. Second, the hospital offered the necessary experimental instruments. Thus, sincere gratitude is given to them.

References

1. Gebhart GF, Su X, Joshi S, Ozaki N, Sengupta JN. Peripheral opioid modulation of visceral pain. Ann N Y Acad Sci. 2000;909:41–50.
2. Dabbagh A, Rajaei S. Halothane: is there still any place for using the gas as an anesthetic? Hepat Mon. 2011;11(7):511–2.
3. Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. Mol Pharm. 2008;5(4):505–15.
4. Korotcov A, Shan L, Meng H, Wang T, Sridhar R, Zhao Y, Liang XJ, Wang PC. A nanocomplex system as targeted contrast agent delivery vehicle for magnetic resonance imaging dynamic contrast enhancement study. J Nanosci Nanotechnol. 2010;10(11): 7545–9.
5. de Araújo DR, da Silva DC, Barbosa RM, Franz-Montan M, Cereda CM, Padula C, Santi P, de Paula E. Strategies for delivering local anesthetics to the skin: focus on liposomes, solid lipid nanoparticles, hydrogels and patches. Expert Opin Drug Deliv. 2013;10:1551–63.
6. Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain. 1977;4:161–74.
7. Sehgal N, Smith HS, Manchikanti L. Peripherally acting opioids and clinical implications for pain control. Pain Physician. 2011;14(3):249–58.
8. Brack A, Rittner HL, Machelska H, Beschmann K, Sitte N, Schäfer M, Stein C. Mobilization of opioid containing polymorphonuclear cells by hematopoietic growth factors and influence on inflammatory pain. Anesthesiology. 2004;100:149–57.
9. Andreozzi E, Wang P, Valenzuela A, Tu C, Gorin F, Dhenain M, Louie A. Size-stable solid lipid nanoparticles loaded with Gd-DOTA for magnetic resonance imaging. Bioconjug Chem. 2013;24:1455–67.
10. Pathak P, Nagarsenker M. Formulation and evaluation of lidocaine lipid nanosystems for dermal delivery. AAPS PharmSciTech. 2009;10(3):985–92.
11. Leng F, Wan J, Liu W, Tao B, Chen X. Prolongation of epidural analgesia using solid lipid nanoparticles as drug carrier for lidocaine. Reg Anesth Pain Med. 2012;37(2):159–65.
12. Bocca C, Caputo O, Cavalli R, Gabriel L, Miglietta A, Gasco MR. Phagocytic uptake of fluorescent stealth and non-stealth solid lipid nanoparticles. Int J Pharm 1998;175(2):185–93.
13. Cavalli R, Bocca C, Miglietta A, Caputo O, Gasco MR. Albumin adsorption on stealth and non-stealth solid lipid nanoparticles. STP Pharm Sci 1999;9(2):183–9.
14. Guan QX, Zhu K, Lin TM, Guan QT, Guo J, Yin JY. Preparation, characterization and drug release of biodegradable solid lipid nanoparticles. Chem J Chin Univ. 2010;31(11):2298–302.
15. Jigisha KV, Shamsunder SD, Krutika KS. Cyclosporine A loaded solid lipid nanoparticles: optimization of formulation process variable and characterization. Curr Drug Deliv. 2008;5:64.
16. Zhang XX, Pan WS, Gan L, Zhu C, Gan Y, Nie S. Preparation of a dispersible PEGylate nanostructured lipid carrier (NLC) loaded with 10-hydroxycamptothecin by spray-drying. Chem Pharm Bull. 2008;56:1645–50.
17. Zhang L, Zhang N. How nanotechnology can enhance docetaxel therapy. Int J Nanomed. 2013;8:2927–41.