

Naltrexone microspheres: *in vitro* release and effect on morphine analgesia in mice

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ABSTRACT

AIM: To study *in vitro* release and *in vivo* effect of four different types of sustained-release naltrexone microspheres on morphine analgesia. **METHODS:** Release of naltrexone from four types of biodegradable microspheres was investigated by HPLC. Their antagonist effects on morphine analgesia were observed using mouse hot-plate procedure. **RESULTS:** Poly lactide-coglycolide (PLGA) composition had a remarkable effect on naltrexone release from microspheres and its antagonism towards morphine analgesia. Two formulations of PLGA 50:50 formulation released more than 80 % of total naltrexone and lost their antagonism by 8 d. The PLGA 75:25 formulation with 20 % and 30 % drug loadings did not release 95 % of total drug and lose antagonism until 40 d and 30 d, respectively. Increasing the drug loading enhanced naltrexone release from microspheres and seemed to shorten the analgesic antagonistic effect of naltrexone. **CONCLUSION:** Antagonism by naltrexone microspheres towards morphine analgesia correlates well with the drug release *in vitro*.

INTRODUCTION

The narcotic antagonist naltrexone is by far the only drug approved by FDA to keep deaddicted patients from readdiction. A major problem with naltrexone is its poor compliance, which may be overcome by a sustained-

release delivery system providing for a 30-d period eliminating the need for daily drug administration⁽¹⁻³⁾.

We have been developing an injectable naltrexone microsphere system using biodegradable poly lactide-coglycolide (PLGA) excipient, which is quite biocompatible and has been allowed by FDA for clinical use. PLGA has been widely applied in sustained-release drug delivery system since the biodegradation rate of the copolymer can be easily changed by altering the composition. The molecular weight and copolymer ratio of lactic acid (LA) and glycolic acid (GA) may have obvious influences on *in vitro* and *in vivo* release kinetics of the microspheres⁽⁴⁾.

In the present study, we tested four types of naltrexone microspheres, differing in either naltrexone loading or molar ratio of LA/GA in PLGA copolymer. The purpose of this study was to investigate *in vitro* release of the microspheres and their antagonism to morphine analgesia *in vivo*.

MATERIALS AND METHODS

Materials Placebo microspheres and naltrexone microspheres of four different types (Tab 1) were provided by National Pharmaceutical Engineering Research Center, Shanghai Institute of Pharmaceutical Industry, sterilized by γ -irradiation before injection. The mean size of microspheres was approximately 100 μm . The PLGA used to prepare the microspheres was purchased from

Tab 1. Different types of naltrexone microspheres.

Microsphere	Naltrexone loading/%	LA/GA molar ratio in PLGA copolymer
2050	20	50:50
3050	30	50:50
2075	20	75:25
3075	30	75:25
Placebo	0	75:25

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Mitsui Toatsu Chemicals, Inc, Japan (Lot No DGLP-003). Morphine injections were purchased from Qinghai Pharmaceutical Plant. Acetonitrile was of chromatographic grade and other reagents of analytical grade. Membra-cei dialysis membranes (14000 MWCO Daltons) were from Shanghai Green Island Co, Shanghai.

Mice Female mice of Kunming strain, weighing $(20.0 \pm s 2.0)$ g, were supplied by Animal Center, Shanghai Institute of Pharmaceutical Industry (Grade II, Certificate No 107).

Apparatus HPLC system was from Shimadzu Co, Japan. Including LC-10A pump, SPD-10A UV-Vis detector, CR-6A recorder, and a Suplco RP-18 column (4.6 mm \times 250 mm, particle size 5 μ m) with a guard column of the same material. Hot-plate pain detector (Model GJ-8402) was obtained from Baishi Medical Electronics, and thermostatic oscillator (Model HTY-2) was from Xidian Bioengineering Facilities, Ninghai, Zhejiang Province.

In vitro release Microspheres containing 5 mg naltrexone were sealed with Membra-cei dialysis membranes and were put in the sealed glass vials which were filled with 20 mL of 0.01 mol/L phosphate buffered solution (pH 7.4) containing 0.02 % NaN_3 and 0.02 % polysorbate 80 (Tween 80). Three vials were set for each type of naltrexone microspheres. All the vials were incubated with horizontal oscillation (80 cycles/min) at 37 $^\circ\text{C}$. Solutions were substituted completely with fresh ones at predetermined intervals. Substituted solutions at each time point were filtered through a 0.45 μ m filter and sampled on the HPLC column. The mobile phase was a mixture of acetonitrile and 0.1 mol/L KH_2PO_4 solution (14:86, v/v) at a flow rate of 1 mL/min. The amount of naltrexone released was detected at 283 nm according to the peak area in the chromatogram.

Analgesic test In the hot-plate method^[5], the response of a mouse towards a noxious stimuli usually consists of licking of the paws or, less frequently, jumping out of the restraining cylinder. Pain threshold, the latency at which the mouse licks its hind paw, was determined on a 55 $^\circ\text{C}$ hot plate for each mouse before the test. Mice with threshold < 30 s were divided into six groups of 10 each. At the beginning, mice were injected sc with different types of microspheres suspended in 2.4 % (CMC-Na), placebo microspheres for group I and II, naltrexone microspheres 2050, 3050, 2075, and 3075 (40 mg/kg) for group III - VI, respectively. Mice were applied to hot-plate test 30 min before and af-

ter being injected sc with morphine (10 mg/kg, ED_{95}). Mice in group I were injected with normal saline instead of morphine. The response time (RT) before and after morphine injection was determined at 3, 8, 13, 18, 23, 28, 33, 38, and 43 d after the administration of microspheres for each group. Using the data of RT after morphine injection, the degree of antagonism of a narcotic antagonist (naltrexone) to morphine was calculated by: $\text{antagonism}/\% =$

$$\frac{(\text{Mean RT of morphine}) - (\text{Mean RT of morphine plus antagonist})}{(\text{Mean RT of morphine}) - (\text{Mean RT of vehicle control})}$$

Statistical analysis Data were expressed as $\bar{x} \pm s$ and compared with the unpaired *t*-test, a two-paired value of $P < 0.05$ was taken to indicate statistical significance.

RESULTS

The *in vitro* release profile (Fig 1) showed different release rates of naltrexone from the PLGA microspheres. For microspheres prepared from PLGA 50:50 (2050 and 3050), a burst of naltrexone release was observed, which increased with increasing naltrexone loading. The release rate of naltrexone elevated and then decreased quickly. Released naltrexone surpassed 95 % at d 18 for 2050 and d 4 for 3050, respectively. As for microspheres prepared from PLGA 75:25 (2075 and 3075), there was a "lag" time of nearly 6 d, after which naltrexone release rate increased and then decreased, giving a sigmoidal release profile. Over 95 % of naltrexone was released approximately at d 40 for 2075 and d 30 for 3075, respectively. The percentage naltrexone released at each sampling time enhanced with increasing loading of the microspheres.

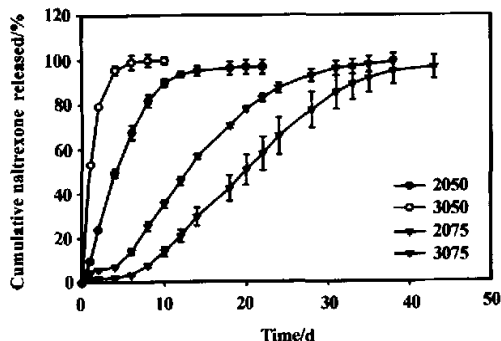


Fig 1. *In vitro* release-time profile for different types of naltrexone microspheres. $n = 6$ replicates. $\bar{x} \pm s$.

In mouse hot-plate experiment, there was no marked difference in response time for each group before morphine challenge ($P > 0.05$, Tab 2). Therefore, the initial status of all mice was regarded as homogenous.

The four types of naltrexone microspheres presented antagonism to morphine analgesia for various periods (Tab 3, Fig 2). Microspheres 2050 and 3050 antagonized morphine analgesia markedly ($P < 0.01$) with 87.0 % and 83.9 % of antagonism ratios, respectively, at d 3. But the effect diminished at d 8. By contrast, the analgesic antagonism of microspheres 2075 and 3075 maintained until d 38 and d 28 ($P < 0.01$) with 98.0 % and 98.9 % of antagonism ratios, respectively. Microspheres made from PLGA 75:25 (2075 and 3075) had much more enduring antagonism to morphine analgesia than those made from PLGA 50:50. For the former, the one having lower drug loading (2075) seemed to prolong the antagonistic effect, in comparison to that having a higher drug loading (3075).

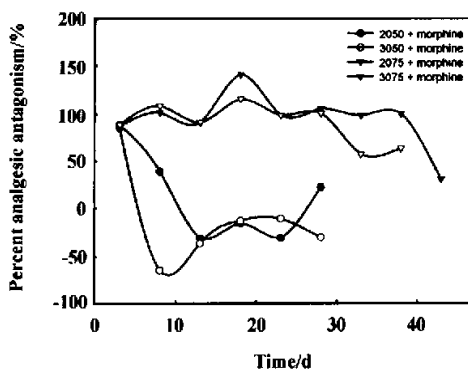


Fig 2. Percent analgesic antagonism-time profile of different types of naltrexone microspheres.

DISCUSSION

The data demonstrated a fine relationship between *in vitro* drug release and *in vivo* analgesic antagonism by

Tab 2. Response time assessed on the hot plate method before challenging with morphine (10 mg/kg, sc). n = 10 mice. $\bar{x} \pm s$.

Day	Group					
	I (placebo + vehicle)	II (placebo + morphine)	III (2050 + morphine)	IV (3050 + morphine)	V (2075 + morphine)	VI (3075 + morphine)
3	12 ± 4	11 ± 4	15 ± 5	12 ± 6	13 ± 7	12 ± 5
8	12 ± 6	9.8 ± 2.6	11 ± 5	16 ± 9	11 ± 7	11 ± 6
13	14 ± 6	12 ± 8	13 ± 6	16 ± 7	9 ± 4	12 ± 6
18	13 ± 4	12 ± 4	11 ± 6	14 ± 6	11 ± 5	12 ± 6
23	12 ± 4	12 ± 6	10 ± 3	11 ± 5	12 ± 7	11 ± 5
28	12 ± 5	10 ± 4	10 ± 3	9 ± 4	7.8 ± 2.2	10 ± 3
33	14 ± 5	14 ± 6	-	-	9.7 ± 2.9	12 ± 6
38	10 ± 4	9 ± 4	-	-	8 ± 3	11 ± 6
43	11 ± 6	9 ± 4	-	-	7.7 ± 2.7	-

Tab 3. Response time assessed on the hot plate method after challenging with morphine (10 mg/kg, sc). n = 10 mice. $\bar{x} \pm s$. ^a $P < 0.01$ vs Group I. ^b $P < 0.05$, ^c $P < 0.01$ vs Group II.

Day	Group					
	I (placebo + vehicle)	II (placebo + morphine)	III (2050 + morphine)	IV (3050 + morphine)	V (2075 + morphine)	VI (3075 + morphine)
3	11 ± 5	42 ± 17 ^a	15 ± 6 ^f	16 ± 4 ^f	16 ± 8 ^f	15 ± 7 ^f
8	13 ± 6	31 ± 18 ^a	24 ± 15	44 ± 21	13 ± 5 ^f	11 ± 7 ^f
13	12 ± 6	37 ± 21 ^a	45 ± 12	46 ± 17	15 ± 11 ^f	15 ± 13 ^e
18	14 ± 6	24 ± 7 ^c	26 ± 13	26 ± 12	10 ± 4 ^f	13 ± 3 ^f
23	11 ± 6	27 ± 18 ^a	32 ± 19	29 ± 15	12 ± 5 ^e	12 ± 4 ^e
28	12 ± 7	30 ± 19 ^a	26 ± 14	36 ± 14	11 ± 4 ^f	12 ± 5 ^f
33	11 ± 6	33 ± 19 ^a	-	-	12 ± 5 ^f	21 ± 10
38	13 ± 4	37 ± 20 ^a	-	-	13 ± 6 ^f	22 ± 9
43	10 ± 3	37 ± 22 ^a	-	-	29 ± 14	-

naltrexone microspheres. More than 80 % of total naltrexone had been released from microspheres 2050 and 3050 while their analgesic antagonism had been lost by 8 d. And also, the time when 95 % of naltrexone was released coincided with the time when the analgesic antagonism diminished for microspheres 2075 (about d 40) and 3075 (about d 30). PLGA composition had a remarkable effect on naltrexone release from microspheres and accordingly its antagonism towards morphine analgesia. The PLGA 50:50 formulation showed a faster release profile *in vitro* and a shorter antagonism profile *in vivo* than the PLGA 75:25 formulation. The faster drug release from the microspheres with PLGA 50:50 was due to the more rapid degradation of the copolymer. The influence of composition on erosion rate is generally attributed to the lower crystallizing ability of the 50:50 copolymer allowing greater absorption of water into the polymer and faster erosion and degradation^[6].

Naltrexone release from PLGA microspheres was also sensitive to the drug loading. The degradation-controlled kinetics of drug release might also be getting assisted by the diffusion pattern of the drug. The initial release of naltrexone was associated with drug present at or near the surface which diffused out with water penetration. As the drug loading of the microspheres is increased, a higher amount of drug is expected to be near the surface; thus resulting in an increase in the initial release. Consequently, more and larger holes will be left in the microspheres with higher drug loading, which will promote the release of the drug inside^[7].

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纳曲酮微球的体外释药和对小鼠吗啡镇痛的影响

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关键词 纳曲酮; 微球体; 疼痛测定; 镇痛

目的: 探讨四种纳曲酮缓释微球的体外释放及其对体内吗啡镇痛效果的影响. 方法: 用高效液相色谱法检测四种纳曲酮微球体外释放. 用小鼠热板法评价它们对吗啡镇痛作用的拮抗效果. 结果: 微球中 PLGA 的组成对纳曲酮微球的体外释药及其拮抗吗啡镇痛的作用有显著影响. 给药后第八天, 以 PLGA 50:50 为载体的两种微球体外释药已达 80 % 以上, 且失去了对吗啡的拮抗作用. 以 PLGA 75:25 为载体, 含药量分别为 20 % 和 30 % 的微球分别在给药后 40 天和 30 天, 其体外释药达 95 %, 这两种微球拮抗吗啡的作用也分别持续了约 40 天和 30 天. 提高药物含量加快了药物的体外释放, 也缩短了微球有效作用的时间. 结论: 微球中药物的体外释放与其拮抗吗啡镇痛作用的效果有良好的相关性.

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