A novel controlled release drug delivery system for naltrexone administration combined with intermittent morphine to induce antinociception

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Co-administration of ultra-low dose opioid antagonists and opioid agonists has been shown to have some advantages in pain management. Objectives: We tried to design a controlled release drug delivery system (CRDDS) to supply controlled release of ultra-low dose naltrexone (ULDN) which was accompanied with intermittent subcutaneous morphine injections. Injectable implants were prepared by dissolving PLGA (Resomer RG504H) in N-methyl-2-pyrrolidone(NMP) containing ethyl heptanoate. This CRDDS provided controlled release of ULDN in mice bodies and was followed by subcutaneous morphine injection (3 mg) at days 2, 5, 10, 15, 20, 25 and 30. Pain response was determined by Tail Flick test. Daily ULDN of 300 ng/kg led to the most effective antinociception rate and tolerance inhibition in mice after a short delayed time interval than those mice which received 3000 ng/kg/day naltrexone (P: 0.01) and those that received no naltrexone (P: 0.02). The CRDDS which was designed in this study shows acceptable outcomes in providing daily ULDN to increase morphine efficacy. The highest antinociception efficacy along with tolerance inhibition was observed when 300 ng/kg ULDN was administered daily followed by intermittent morphine subcutaneous injections.

Key words: Drug resistance– Morphine – Naltrexone – Pain – Polymeric drug delivery systems.

Controlled release drug delivery system (CRDDS) is a sophisticated method that is designed to release specific amount of drug within a predetermined time period. Many formulations for designing such a system exist and specific materials are cautiously selected to fit the background purpose. Route of drug delivery, which is mostly oral, dermal or injections, plays an important part in material selection. Previously several formulations for various aims are proposed but the specific characteristics of each medication mostly require a unique design. In between some previously tested materials are mucoadhesive beads of glipizide [1], hydrochloride-loaded niosomes to provide a CRDDS for oral metformin administration [2], specific drug delivery systems to administer antibiotics [3] and even the development of electrohydrodynamic devices are proposed in this field [4]. These formulations can be designed to be used orally [5] or through injections. Along with various formulations that are shown to be suitable in providing a CRDDS, such as microencapsulation [6], it seems that CRDDS for every single drug needs unique designing to be fitted according to purposes.

Here we have tried to design a controlled released drug delivery system for morphine administration in inducing analgesia. More efficient drug delivery methods such as CRDDS along with evolution of the existing drug molecules from a conventional form to a polymeric novel delivery system can significantly improve medications performance in terms of patient's compliance, safety and efficacy [7].CRDDS not only provide lowest doses of drug which effect sufficiently but also reduce undesired drug level fluctuations and thus diminish side effects.

Pain management is one of the major concerns in clinical practice and opioids are one of the major medications used in this field. Many efforts have been done to reduce or inhibit the pathway of tolerance development by opioid administration. Crain and Shen [8] suggested that alternations in opioid receptors coupling to G-protein linked receptors have the key role in tolerance development. The stimulation of these receptors can exert excitatory effect in low doses and inhibitory receptor-mediated effects in high doses. Excitatory effect after opioid receptors stimulations can be inhibited by ultra-low dose of opioid antagonists such as naloxone and naltrexone. The fact that naloxone and naltrexone inhibit the excitatory effect of opioid receptor stimulation by prolonging the action potential duration was first described by Crain and Shen [9]. Many combination variations of an opioid agonist and ultra-low dose antagonists were reported to have the similar outcomes [10, 11].

The aim of this study was to design a CRDDS to provide ultra-low dose naltrexone which was combined by subcutaneous morphine administration on specific day intervals to induce antinociception in mice.

Recently, biodegradable polymer based injectable solutions, named "*in situ* forming implants" (ISFI), have received increasing attention due to several advantages: ease of application, prolonged delivery periods, decreased drug dosage, improved compliance and reduced invasion [12, 13]. We looked forward to see if this CRDDS design can exert the enhancement of opioid efficacy while controlling the tolerance development in mice.

I. MATERIALS AND METHODS

1. Materials

PLGA (50:50, Resomer RG 504H) was purchased from Boehringer Ingelheim (Ingelheim, Germany).). N-methyl 2-pyrolidone was supplied by Merck (Darmstadt, Germany) and naltroxone was purchased from Sun pharmaceutical Co. (India).Morphine sulfate kindly supplied by Temad Co. (Tehran, Iran). Other reagent was supplied by Merck (Darmstadt, Germany) and used as received.

2. Study population

This study was performed on swiss Webster mice with weight of 25-30 g. All mice were male and were provided by Iran Pastur Institute. Previously Terner *et al*. [14] showed that various races of mice respond differently to morphine and ultra-low dose naltrexone.

Their result showed that naltrexone enhanced morphine antinociception and attenuated the development of morphine tolerance in male and female Sprague-Dawley and Long-Evans rats while no such an effect could be detected in Lewis rats. Along with race, rat gender was also reported as a factor influencing opioid efficacy including kappa opioid-induced antinociception [15] and tolerance development through mu-opioid receptor [16]. By considering the effect of race and sex in opioid administration response along with opioid antagonists we designed this study using only male Swiss Webster mice.

3. Pain determination

To determine the pain response after analgesics administrations Tail Flick test was used. Tail Flick test which was first described by D'Amour and Smith [17] is a standard method which evaluates pain response in a similar way to hot plate test. In this procedure a light beam (ultra-red) is focused on mice's tail and meanwhile a timer starts. The duration since beginning of the test until animal flicks its tail is considered as a measure for pain threshold. This recorded time is expressed as a percentage of maximal possible effect (MPE) which is calculated according this formula: MPE% = ((post-injection latency - base line latency)/(cut-off time - base line latency)) * 100.

Before any opioid injection Tail Flick test was performed once (base line latency) and 30 min after injection it was performed again (post-injection latency) to provide a proper comparison. In cases in which pain sensation was impaired (due to drug administration) and mice did not flick its tail, the test was interrupted after 10 s to prevent tissue damage. Pain determination test was performed on days 0, 2, 5, 10, 15, 20, 25 and 30.

4. Preparation of controlled release naltrexone injectable formulation

In this system, PLGA-polymers are dissolved in water-miscible solvents, such as N-methyl-2-pyrrolidone (NMP) [18]. Here we used PLGA-polymers with NMP containing ethyl heptanoate with 68/31/1 (w/w) ratio. Prior to injection the drug was added and formed an injectable solution. After subcutaneous injection of the formulation into the body, the organic solvent dissipates into the surrounding tissue as aqueous body fluids penetrate into the implant. This leads to phase separation and precipitation of the polymer, forming a depot at the injection site (*Figure 1*). The active pharmaceutical ingredient (API) gets entrapped within the matrix as it solidifies and is released by diffusion processes or as the implant biodegrades in controlled manner [19].

Based on our previous studies the required amount of naltrexone to deliver 300 and 3000 ng/kg/day for a 25 g mice (40 % excess of naltrexone because of burst release and 100 mg excess of total formulation because of remained amount into the syringe) dissolved in polymeric solution. Then the solution totally injected to mice, thus there isn't any loss of API and we could claim we have a 100 % of encapsulation efficiency. If the mice weight was higher than 25 g the correction would be applied to total formulation weight. For adding naltrexone to the formulation the required amount of naltrexone was preformulated in a portion of solvent and then that portion would added to polymeric solution.

Injectable implants were prepared by dissolving PLGA (Resomer RG504H) in NMP containing ethyl heptanoate with 68/31/1(w/w) ratio. For this propose, PLGA was added slowly to NMP while it was mixed by mechanical stirring, and was kept in room temperature till achieve a perfect bubble free mixture. A quantity of naltrexone equivalent to 0.0002 and 0.002 % (w/w) were added to the polymer solution to achieve a homogeneous solution.

The actual formation of depot in mice body could be observed a day after injectection of ISFI. In two mice that were expired, we opened the tissues to see the formation of depot (*Figure 1*). The percent of

Figure 1 - *In vivo* administration of ISFI (*in situ* forming implants) plus naltrexone which is become solid after exposure to intracellular fluid of mice body.

API was calculated in preparation of formulations so we are sure that the complete amount of API was entrapped within the depot delivery systems.

5. Protocols of drug administration in mice

Mice were divided into 6 groups and each group contained 8 mice which were similar in race, weight. Medication administrations in these six groups are shown in *Table I*.Three formulations for controlled release of naltrexone were designed. The designs of these formulations which included specific dose of naltrexone are shown in *Table I*.

Groups A and B presented case groups and we tried to determine the appropriate dosage of naltrexone in CRDDS that optimized the efficacy of morphine by comparison of these two groups' outcomes. Groups C and D did not received morphine and were designed to understand if naltrexone in ultra-doses can mediate analgesic effect itself so that the bias effect of additive analgesic could be ruled out. Group E presents the control group in which no naltrexone was administered and mice received only subcutaneous morphine, the existence of this control group gave us the possibility to recognize whether morphine efficacy was enhanced by administration of ULDN by comparing the results of this group with groups A and B. Group F was a mere control group which received neither morphine nor naltrexone.

6. Analysis

All statistical analyses were performed using SPSS 18.0 (SPSS, Inc., Chicago, IL, United States). One-way analysis of variances (ANOVA) was used to compare means of MPE % in all 6 groups. P value < 0.05 was considered statistically significant.

Table I - Protocols of opioid administration in each group of mice.

Mice group	Device which was injected subcutaneously at the day 0	Morphine/saline which was received subcutaneously on days 2, 5, 10, 15, 20, 25 and 30
A	I	3 mg/kg morphine
B	II	3 mg/kg morphine
C	I	3 mg/kg saline
D	II	3 mg/kg saline
E	III	3 mg/kg morphine
F	III	3 mg/kg saline

I: *In situ* forming implants (ISFI) formulation provides about 300 ng/kg/day naltrexone. II: ISFI formulation provide about 3000 ng/kg/day naltrexone. III: Placebo (only ISFI formulation without naltrexone).