DEVELOPMENT OF TOLERANCE TO ANTIINFLAMMATORY EFFECT OF MORPHINE

Abolhassan Ahmadiani PhD*, Parichehr Hassanzadeh PharmD, Mahmood Aleboyeh PhD

Department of Pharmacology, Shaheed Beheshti University of Medical Sciences, Tehran, Iran

Opioid drugs, typified by morphine, are primarily used for their pain relieving properties, however, they produce a host of other effects due to the wide distribution of opioids and their receptors in both the brain and the periphery. The capacity of morphine in attenuation of inflammation and inflammatory pain has already been demonstrated. In addition, there has been evidence showing the systemic efficiency of morphine against some inflammatory diseases and reversal of such effects by naloxon. In the present study, after confirming the antiinflammatory effect of morphine against carrageenan-induced hind paw edema in mice (data not shown), we tried to assess the probable development of tolerance, a somehow troublesome phenomenon which extends to most effects of morphine, to its antiinflammatory effect in chronic administration.

Experiments were performed on adult male NMRI mice weighing 25 – 30 g which were housed at room temperature on 12/12 hours light/dark cycles and had free access to water and food at all times except during the experiments.

In the process of induction of inflammation, weights of the hind paws of mice were measured with a mercury plethysmometer prior to, and 1/2, 1, 2, 3, 4, 5, and 6 hours after intraplantar (IPL) injection of 0.05 mL of carrageenan (Fluka Co., Canada), 3% w/v, to the dorsal surface of the hind paw. The control group (n = 7) received equal volumes of saline. Finally, according to the density of the mercury, the weight of the hind paw of the animal was converted to volume, in order to assess the rate of the edema.

In the 2nd phase of the study, pretreatment with the intraperitoneal (IP) injection of morphine sulfate (Temad Co., Tehran, Iran), 7 mg/kg, 45 min before carrageenan injection (0.05 mL, 3% w/v, IPL) was performed to assess the effect of morphine on inflammation induced by carrageenan.

In the 3rd phase of the study, we tried to make the animals morphine-dependent; the weights of the hind paws of the control and treatment groups (n = 7) were measured; then morphine was given to the treatment group by IP injections three times a day at 8 am, 12 mid-day, and 4 pm for 3 days according to the dosage schedule. The first three doses were 50, 50, and 75 mg/kg; each of the doses was then increased by 25 mg/kg/day. The higher dose of the third day injection aimed to minimize any overnight withdrawal. On the 4th day, the treatment group received morphine (50mg/kg, IP) at 8 am; 2 hours later, a number of randomly chosen mice were tested to precipitate abstinence by an injection of naloxone HCl (Tolidarou, Tehran, Iran), 4 mg/kg. The animals were placed in a Perspex observation box (dimensions: 20 × 20 × 50 cm) and the number of jumps was recorded over a 30-min period. After making sure of morphine dependence, study continued on the rest of the animals. Weights of hind paws were measured and the treatment group received morphine (7 mg/kg, IP), 45 min prior to carrageenan (0.05 mL, 3% w/v, IPL), which was injected to both control and treatment groups. The rate of edema in each mouse was assessed 1/2, 1, 2, 3, 4, 5, and 6 hours after carrageenan injection. Data were expressed as the mean ± SEM. Unpaired Student t-test was used for analysis of data, with significance defined as p < 0.05.

According to the results, inflammation and swelling of the injected paw was apparent 30 min after carrageenan injection and the highest rate of

---

*Correspondence: A. Ahmadiani PhD, Department of Pharmacology, Shaheed Beheshti University of Medical Sciences, Tehran, Iran. P.O.Box: 19835-355. Fax: +98-21-2403150, E-mail: aahmadiani@yahoo.com.
Development of Tolerance to Antiinflammatory Effect of Morphine

inflammation was observed about 3 hours postinjection. Pretreatment with morphine (7 mg/kg, IP) resulted in a marked decrease in inflammation between hours 2 and 6 of carrageenan injection (data not shown). As shown in the Figure, morphine (7 mg/kg, IP) was not able to reduce inflammation in morphine-dependent animals.

Opioid receptors have been demonstrated on peripheral terminals of thinly myelinated and unmyelinated sensory nerves in animals and human.4 When these neuronal opioid receptors are occupied by an agonist, the excitability of nociceptive input terminal or the propagation of action potential is attenuated and the peripheral release of excitatory neuropeptides (e.g. substance P) is inhibited. These events may account not only for the antinociceptive but also for antiinflammatory actions of opioids in peripheral tissues.5 In the current study, the inflammation induced by carrageenan and the antiinflammatory effect of morphine were confirmed. We also showed that after chronic administration of increasing doses of morphine, its antiinflammatory effect against carrageenan was abolished. This finding suggests that tolerance develops to the antiinflammatory effect of morphine as to some other effects of this drug. Despite a great deal of research in the area, the mechanisms of opiate tolerance and dependence are not fully understood. Although the traditional mechanisms of receptor downregulation and desensitization seem to play a role, they cannot entirely explain the phenomena of tolerance and dependence; therefore, other mechanisms should be considered. Involvement of cAMP-mediated events and activation of the N-methyl-D-aspartate (NMDA) receptors have been implicated in the cellular mechanisms of opioid tolerance.6, 7 Mao et al have demonstrated that spinal neuronal apoptosis was induced in rats made tolerant to morphine and blockade of the spinal caspase-like activity, partially prevented morphine tolerance.8 Coupling of opiate receptors to alternative G-proteins has been suggested by Harrison et al and Tyr-W-MIF-1, as an antiope has been shown to act by inhibiting basal G-protein activation and may play a critical role in tolerance and dependence.9 Traditionally, opioids are considered to exert their analgesic effect within the central nervous system, however, recent evidence has begun to accumulate that potent analgesia can be elicited by activation of opioid receptors in peripheral tissues especially in inflamed tissues. All three opioid receptor types (mu, delta, and kappa) can be functionally active in peripheral tissues. In peripheral tissues such as joints, opioids act to reduce inflammation and morphine has been administered locally in relieving painful inflammatory disorders.10 Therefore, it can be suggested that antiinflammatory effect of morphine may follow a peripheral pathway. Confirmation of the current hypothesis, which is based on the development of tolerance to peripheral effect(s) of morphine in addition to its central nervous system (CNS) effects must await the application of more refined

Figure. Treatment group, after being morphine-dependent with different doses of morphine, received morphine (50 mg/kg, IP) on the fourth day. After confirming that the addiction was induced properly, the treatment received morphine (7 mg/kg, IP), 45 minutes prior to carrageenan injection (0.05mL, 3% w/v, IPL), which was injected to both control and treatment (n = 7 in each group). Each point represents mean ± SEM compared with corresponding time value.
techniques and encourages other studies in order to minimize the potentially serious long-term consequences of tolerance.

References