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□ Experimental Research Article □

Effect of intrathecal oxcarbazepine on rat tail flick test-determined morphine tolerance

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Background: Repeated administration of morphine leads to characteristic tolerance. We tested the effects of intrathecal oxcarbazepine (OXC) on spinal morphine tolerance in rats using the tail flick test.

Methods: Sprague-Dawley rats received intrathecal injections of 10 μl saline alone, or 10 μl of solutions containing 100 μg OXC, 15 μg morphine, or OXC + morphine for 7 days. Different groups of rats received OXC on days 1-7, 1-3, or 5-7. The tail-flick assay was used to measure acute and chronic nociception. The nociceptive stimulus consisted of dipping the distal 5 cm of the tail into warm water before and 30 min after drug injection. On day 8, an antinociceptive dose-response curve was plotted, and the 50% effective dose for morphine (given alone) was determined for all groups.

Results: Morphine or OXC both produced acute antinociception; OXC + morphine resulted in a significantly larger response than obtained with morphine alone. Morphine tolerance was produced by intrathecal injection of morphine over 7 days. Co-administration of morphine and OXC completely blocked morphine tolerance, but tolerance developed when OXC injection was stopped, and morphine potency was partially restored by co-administration of OXC in tolerant rats.

Conclusions: The antinociceptive effect of both acute and chronic morphine therapy is increased with intrathecal OXC, and antinociceptive morphine tolerance is attenuated in rats. **(Korean J Anesthesiol 2009; 57: 337~41)**

Key Words: Intrathecal, Morphine, Oxcarbazepine, Tail flick test, Tolerance.

INTRODUCTION

Morphine is one of the most effective drugs currently used for pain management. However, its prolonged administration for chronic pain produces tolerance [1] to the analgesic effects, and thus limiting their therapeutic potential. These effects depend on the interaction between opiate and non-opiate receptor systems including gamma-aminobutyric acid [2], N-methyl-D-aspartate [3], adenosine [4], and α-adrenergic receptors [5] in the CNS. In addition, mechanisms of opioid tolerance include spinal changes involving translocation and activation of protein kinase C [3], dynorphin activity [6], calcitonin gene-related peptide activity [7], and cyclooxygenase activity [8].

Oxcarbazepine (OXC) is a newer generation anti-epileptic drug that is structurally related to carbamazepine, but with improved pharmacokinetic, safety, and tolerability profiles [9]. OXC has also been used in the treatment of neuropathic pain [10]. And there is some evidence that OXC could be used against inflammatory pain as well, since they exerted antinociception in certain animal models of inflammatory pain [11]. However, the mechanism of analgesic action of OXC is not yet fully understood and the effect of intrathecal OXC on opioid tolerance has not been studied. Thus, in this study, we investigate the hypothesis that intrathecal OXC could prevent and reverse chronic opioid tolerance.

MATERIALS AND METHODS

All studies were performed in accordance with a protocol approved by the Animal Use and Care Committee. The experiments were carried out on male Sprague-Dawley rats (weight

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200－250 g), which were housed in group cages on a 12-hour night/day cycle with access to food and water at all times in rooms that were temperature and humidity controlled.

For intrathecal drug administration, a polyethylene (PE-10) tubing was implanted through an incision in the atlanto-occipital membrane to the lumbar enlargement [12]. The catheter was externalized on the top of the skull and secured with a piece of steel wire. Rats showing postoperative neurological motor deficits were sacrificed immediately. Proper location was confirmed by a temporary motor block of both hindlimbs after injection of 2% lidocaine 10 μℓ. Drugs were intrathecally administered in a volume of 10 μℓ, followed by an additional 10 μℓ normal saline by a microinjection syringe (Microliter™ #702, Hamilton Co., USA) over a 60-second interval. All persons performing experiments were blinded to drugs and doses administered.

Tail-flick assay was used for the measurement of acute nociceptive sensitivity. The nociceptive stimulus consisted in dipping the distal 5 cm of the tail into a 50°C water bath [13] as this temperature produced an average baseline of withdrawal latency of 3.5 ± 0.12 s in naïve rats. Baseline was measured with three trials of tail-flick latency at 3-min intertrial intervals. The latency to tail-flick or withdrawal of the tail from the water was taken as the endpoint with a cutoff of 10 s in order to avoid tissue damage. The stimulus was applied prior (e.g., baseline latency) to and after (e.g., test latency) drugs administration. Animals with a baseline latency of over 5 s were discarded. Results are reported at peak effect time for each compound. The antinociceptive activity was expressed as a percentage of maximal possible effect (%MPE) and calculated as follows; %MPE = [(post-drug latency − baseline latency) / (cut-off time − baseline latency)] × 100%.

The doses of morphine (0.3, 1, 3, and 10 μg) were administered to intrathecal space (n = 6 per subgroup) for the measurement of the time to peak effect and the 50% effective dose (ED_{50}) estimated to produce 50% maximal possible effect (%MPE).

In the first experiment, to evaluate the acute effects of OXC on morphine antinociception, we intrathecally administered single doses of 5 μg morphine, 100 μg OXC, and a combination of 5 μg morphine plus 100 μg OXC. Measurements were taken before and 15, 30, 45, 60, 90, 120, and 180 min after an intrathecal dose of the drug(s).

In the second set of experiments, to evaluate the effects of OXC on the development of morphine tolerance, OXC 100 μg was intrathecally coadministered with morphine 15 μg once daily for 7 days. For induction of tolerance to morphine, we intrathecally injected 15 μg of morphine once daily for 7 days according to the previously reported procedure [14]. Behavioral testing was performed before and 30 min after drug administration. To characterize the offset of the effect of OXC on morphine tolerance, we intrathecally coinjected OXC with morphine for days 1－3 followed by daily morphine alone on days 4－7.

In the final experiment, to evaluate the effects of OXC on established morphine tolerance, intrathecal morphine 15 μg was administered once daily for 4 days to induce tolerance. On the following 3 days, intrathecal OXC 100 μg was injected in combination with morphine.

After second and third experiment, we plotted cumulative dose-response curves and estimated the ED_{50} values of morphine on day 8. To construct these curves, animals received increasing doses of morphine (3, 9, 21, 45, and 93 μg) for morphine alone group and morphine plus OXC (5－7 days) group every 30 min, and behavioral testing was performed 30 min after each drug administration. For saline group, morphine plus OXC (1－3 days) group, and morphine plus OXC (1－7 days) group, animals received increasing doses of morphine (0.3, 0.9, 2.1, 4.5, 9.3 and 18.9 μg) and behavioral testing was also performed. This protocol continued until maximal antinociception was obtained.

Morphine sulfate (MW = 668.76; Sigma, USA) was dissolved in 0.9% saline. OXC (MW = 252.27; Sigma, USA) was dissolved in dimethyl sulfoxide (DMSO, minimum 99.5%; Sigma) and diluted with 0.9% saline.

All data are expressed as mean maximum percentage effect (±SEM). The ED_{50} values were determined using nonlinear regression analysis. Statistical significance ($P < 0.05$) was determined using one-way ANOVA followed by a Dunnett's test for multiple comparisons between groups.

RESULTS

Intrathecally administered morphine produced dose-dependent antinociceptive effect (Fig. 1) without any signs of severe motor impairment. The ED_{50} values and slopes (95% confidence intervals) are 3.4 (2.2－5.3) μg and 45.2 (31.6－58.8) for morphine.

In the first experiment, intrathecally injected morphine 5 μg or OXC 100 μg resulted in peak antinociceptve effect (49.8 ±

5.2 %MPE or 51.3 ± 4.0 %MPE respectively) 30 minutes after injection (Fig. 2). With combination of both drugs, these doses of morphine and OXC produced maximal, and supra-additive antinociception (98.9 ± 0.5 %MPE) (Fig. 2). The peak effect was observed between 30 and 45 min after administration. In both tests, antinociceptive effects gradually decreased to baseline by 180 minutes after injection.

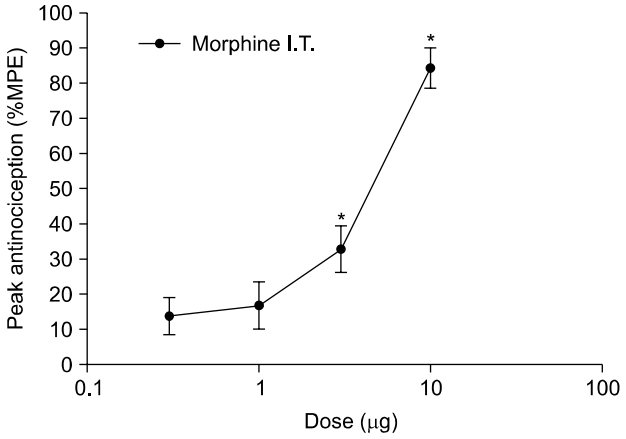

Fig. 1. Dose-response curves from the peak effects of percent maximal possible effect (%MPE) for antinociception in the morphine groups (n = 6 per subgroup). These curves show a dose-dependent antiallodynic effect. Data are expressed as mean ± SEM. Doses (μg) are represented logarithmically on the *x* axis and peak antinociception (%MPE) is represented on the *y* axis. Asterisks indicate that the mean %MPE of each group is significant compared with the smallest dose. *P < 0.05. I.T.: intrathecal.

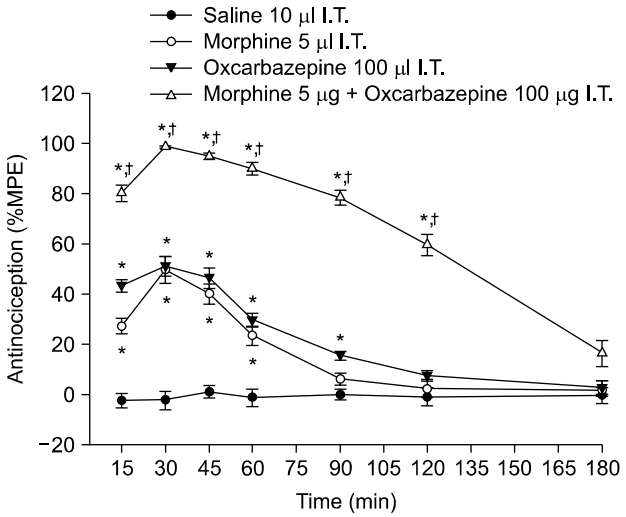

Fig. 2. Acute tail-flick responses (mean ± SEM) to saline, morphine, oxcarbazepine and morphine plus oxcarbazepine (n = 9 per subgroup). All doses of morphine and oxcarbazepine are 5 μg and 100 μg, respectively. *P < 0.05 versus saline, †P < 0.05 versus morphine. I.T.: intrathecal.

In the second experiment, intrathecally administered morphine 15 μg produced maximal antinociception on day 1 (99.4 ± 0.4 %MPE) and the antinociception was gradually decreased to baseline levels by day 7. With coadministration of intrathecal morphine 15 μg plus intrathecal OXC 100 μg, the decrease in morphine effect was completely blocked throughout the entire 7-day period (Fig. 3). When OXC was coadministered with morphine only for days 1−3, maximal antinociceptive effect with morphine was still maintained on day 4, but the effect was subsequently decreased from days 5−7 (Fig. 3). Administration of morphine for 7 days significantly increased the ED_{50} value five- to sixfold more than that observed in saline-treated animals (Table 1). With coadministration of OXC plus morphine for the entire 7-day period, the ED_{50}s were significantly lower than values for the morphine alone group (Table 1).

In the final experiment, although chronic administration of morphine alone on days 1−4 resulted in a decreased antinociception similar to that observed previously (Fig. 3), with coadministration of OXC on days 5−7, the morphine effect

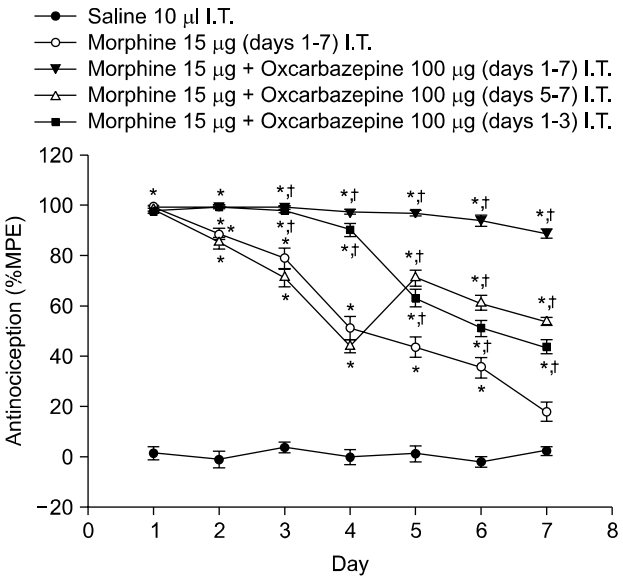

Fig. 3. Tail-flick responses (mean ± SEM) to chronic saline, morphine, and morphine plus oxcarbazepine (oxcarbazepine given on days 1−7, days 5−7, or days 1−3) (n = 9 per subgroup). All doses of morphine and oxcarbazepine are 15 μg, and 100 μg, respectively. *P < 0.05 versus saline, †P < 0.05 versus morphine. I.T.: intrathecal.

Table 1. The Effect of Oxcarbazepine on the Development and Reversal of Intrathecal Morphine Tolerance

Chronic treatment groups	ED_{50} (μg)
Saline 10 μl (days 1−7)	5.8 ± 0.8*
Morphine 15 μg (days 1−7)	27.2 ± 1.2
Morphine 15 μg + Oxcarbazepine 100 μg (days 1−7)	4.1 ± 1.0*
Morphine 15 μg + Oxcarbazepine 100 μg (days 1−3)	4.6 ± 0.9*
Morphine 15 μg + Oxcarbazepine 100 μg (days 5−7)	11.7 ± 1.1

Data shown as mean ± SEM. Following the end of the 7-day chronic treatment period, cumulative dose-response curves to acute morphine were generated on day 8. ED_{50} values were derived from these curves. *P < 0.05 compared to morphine alone (n = 9 per subgroup).

was partially restored (Fig. 3) and the antinociception was significantly greater than for morphine alone on days 5−7. The ED_{50} value on day 8 for this treatment group was significantly lower than values of morphine alone group (Table 1).

DISCUSSION

In this study, the development of antinociceptive tolerance to morphine was inhibited by intrathecally administered OXC. The antinociceptive responses to morphine was sustained in the presence of OXC for 7 days. The acute morphine dose-response curve was shifted leftward. The acute morphine ED_{50} value was decreased compared to those of morphine tolerant animals. However, within 48 h of discontinuing OXC, the tolerance to morphine became apparent, and this indicates that continued OXC is required to keep up opioid potency. Finally, we can suggest that morphine potency could be partially restored by OXC in tolerant rats. Summing up, these results support a role for OXC-morphine combinations or for the addition of OXC to morphine in the production of tolerance.

Several studies have suggested that opioid-induced antinociceptive tolerance may be a consequence of neuroplastic changes that result in enhanced release of excitatory neurotransmitters from primary afferent terminals in the spinal dorsal horn [15]. Meanwhile, the transmission of nociceptive stimuli depends crucially on several types of ion channels, such as persistent sodium channels, inwardly rectifying potassium channels and voltage-gated calcium channels, which regulate either cellular excitability or synaptic transmission [16]. Therefore, we can suppose that sodium- or calcium channels are also partially related to the mechanism of opioid-induced antinociceptive tolerance.

OXC and its active 10-hydroxy metabolite inhibit sustained, high-frequency, repetitive firing of voltage-gated sodium channels combined with the inhibition of the high-voltage P/Q and N-type calcium channels [17]. It is also reported that OXC inhibits, to a lesser extent, potassium channels [18]. There is increasing evidence that OXC is effective in different animal models of pain [11,19,20]. Primary sensory neurons (dorsal root ganglion neurons or trigeminal neurons) constitute the first link in the chain of neurons making up somatosensory pathways. They encode their messages in the form of a series of action potentials whose depolarizing upstroke is produced by sodium channels. Under normal circumstances, dorsal root ganglion and trigeminal neurons are relatively quiescent unless they are stimulated. However, following some injuries, primary sensory neurons can become hyperexcitable and can give rise to unprovoked spontaneous action potential activity or pathological bursting [21]. Based on their electrophysiological and pharmacological properties, the voltage-dependent calcium channels (VDCC) are classified into six subtypes (T, L, N, P, Q and R). N-type VDCCs are almost entirely restricted to neurons, where they play a major role in the release of synaptic mediators such as glutamate, acetylcholine, dopamine, norepinephrine, gamma-aminobutyric acid and calcitonin gene related peptide [22]. The function of N-type VDCCs has been studied in several neural structures including the spinal cord [23], as well as in DRG neurons [24]. P/Q-type VDCCs may be involved in the release of glutamate, aspartate, dopamine, serotonin, norepinephrine, GABA and probably glycine [23,25].

In other studies, OXC was just administered through oral (150−1,200 mg/day for human) [26]. or intraperitoneal (10−160 mg/kg for rat) [19,27] or intraplantar routes (500 μg/100 μl for rat) [28]. However, in this study, we administered OXC by intrathecal route, and the effective dose was relatively smaller (100 μg/10 μl for rat) than other studies. Therefore, we suggest that OXC could be effectively used by intrathecal administration with relatively smaller doses and reduced side effects.

In conclusion, therapeutic utility of opioid may be restricted by tolerance and an understanding of the underlying mechanisms may be helpful for treatment of pain in certain situations. In this study, it is suggested that the antinociceptive effect of both acute and chronic morphine therapy is increased with intrathecal OXC, and the antinociceptive morphine tolerance is also attenuated in rats. Further studies are needed to explain the exact sites and mechanisms of these actions.

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