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Gabapentin enhances the analgesic response to morphine in acute model of pain in male rats

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Abstract

Whenever opioids as drug of choice result in inadequate analgesia, the combinational therapy would be the solution. In this study the co-administration of gabapentin with morphine is evaluated in acute model of pain. Therefore the antinociceptive effect of gabapentin (30 or 90 mg/kg, s.c.) and morphine (0.5, 1 or 3 mg/kg, s.c.) alone or in combination were measured by tail-flick test in intact adult male rats. Control rats received normal saline.

Tail-flick latency time and Area Under Curve (AUC), as antinociception index were calculated for each groups. There was not any significant difference between the antinociceptive response of 0.5 mg/kg morphine and 30 mg/kg gabapentin as compared to controls, but co-administration of these subanalgesic doses increased significantly AUC as compared to morphine alone. The co-administration of gabapentin with analgesic doses of 1 and 3 mg/kg morphine had also increased significantly AUC. Therefore gabapentin enhanced the antinociceptive effect of both analgesic and subanalgesic doses of morphine in a dose dependent manner. In conclusion co-administration of gabapentin with low doses of morphine produced therapeutic analgesia which could have important clinical application.

Keywords: Gabapentin; Morphine; Acute pain; Antinociception

1. Introduction

Opioids are still the drug of choice in severe pain treatment (Way et al., 2001). Its single dose in clinical use is limited by side effects such as sedation, nausea, constipation and respiratory depression (Dickenson, 1994). In persistent pain opioid dose should be increased steadily due to irresponsiveness to increased pain and the development of tolerance. On the other hand repeated somnistration of opioids leads to dependency, however certain types of pain may not respond to opioids (Portenoy, 1994). Dependency, tolerance and irresponsiveness which limit opioid use and result in inadequate analgesia can be overcome by combination therapy. Therefore non opioid analgesics are proposed to enhance opioid analgesic effect and also to attenuate side effects (Way et al., 2001).

Gabapentin, an alkylated amino butyric acid analogue, is a safe and well-tolerated anticonvulsant drug (Haig et al., 2001; Rosa and Kam, 2002) which have demonstrated analgesic efficacy across a wide spectrum of pain states (Mao and Chen, 2000). It has been shown to be effective in animal model of neuropathic pain (Abdi et al., 1998), diabetic neuropathy (Cesena and Calcutt, 1999), trigeminal neuropathic disorders (Christensen et al., 2001) and herpetic pain induced by virus infection in mice (Takasaki et al., 2001). Also the antinociceptive effect of gabapentin was investigated in formalin induced inflammatory pain in rats (Dixit et al., 1999; Patel et al., 2001) and postoperative pain (Dirks et al., 2002; Turan et al., 2004).

Through these studies, it was demonstrated that gabapentin reduces pain transmission (Gilren, 2002), so it was proposed that gabapentin might enhance morphine analgesia whenever a nerve injury was occurred. For example in rat model of neuropathy, gabapentin increased morphine analgesia (Smiley et al.,

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2004). Also some clinical studies showed that gabapentin can reduce morphine consumption after mastectomy (Dirks et al., 2002) and after spinal surgery (Turan et al., 2004).

However, its analgesic effect in acute pain model has not been understood well. So this study was performed to evaluate the analgesic effect of gabapentin alone and its co-administration with morphine in acute model of pain, using tail-flick test in rats.

2. Experimental procedures

2.1. Animals

Male Wistar rats (200–300 g) were housed three or four per cage at a controlled temperature (23±1 °C) and a 12-h light/dark cycle. Food and water were available continuously. Experiments were performed on light cycle at the same time in all groups. Each animal was used only once and received drugs subcutaneously. The protocol has been approved by ethical committee of Kerman Azad University, Kerman, Iran (grant no A12/2004).

2.2. Drugs

The following drugs were used: Gabapentin (Park Davis company, Italy), Morphine sulfate (Temad Co., Iran). The drugs were dissolved in saline and were given subcutaneously. The control animals received normal saline.

2.3. Antinociception measurement

The tail-flick test was used to assess the antinociceptive effect of the study drugs. Radiant heat was applied to the tail at 5–8 cm from the tip using a tail-flick apparatus (PANLAB 7160, Spain). Tail-flick latency time (TFL) was measured as the time from the onset of the heat exposure to the withdrawal time of the tail. The intensity of the radiant heat was adjusted to establish the baseline latencies of 3–5 s. The heat stimulus was discontinued after 20 s to avoid tissue damage. (Cut off point=20 s).

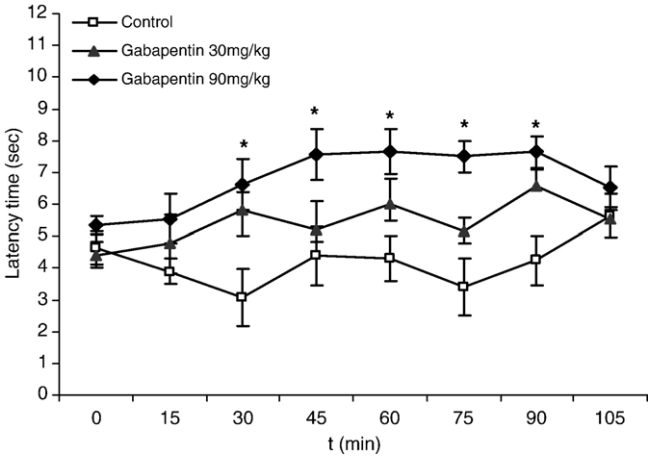

Fig. 1. The antinociceptive effect of various doses of gabapentin on tail-flick test. The animals received subcutaneously normal saline (control; $n=10$) and gabapentin (30 and 90 mg/kg). The latency time was increased significantly 30 min after injection of 90 mg/kg of gabapentin as compared to controls. Data are expressed as the mean+SEM of at least 6 rats. *$p<0.05$ compared with control group.

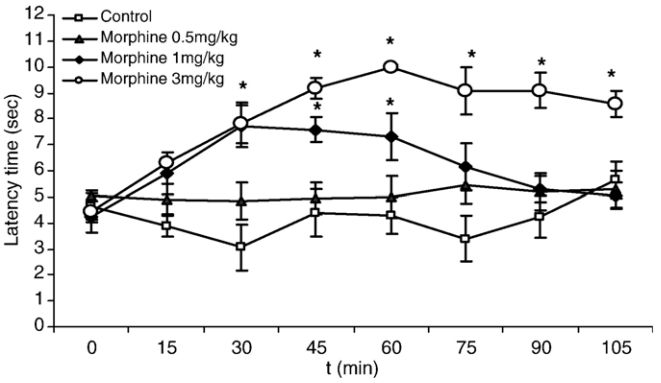

Fig. 2. The antinociceptive effect of various doses of morphine on tail-flick test. The animals received subcutaneously normal saline (control; $n=10$) and morphine (0.5, 1 and 3 mg/kg). The latency time was increased significantly 30 min after injection of 1 and 3 mg/kg of morphine as compared to controls. Data are expressed as the mean+SEM of at least 6 rats. *$p<0.05$ compared with control group.

For each animal, baseline latency was obtained as the mean of three measurements before administration of any drug and latency times were determined at every 15 minutes intervals from immediately up to 105 min after the administration of drug (s) or saline. Antinociception was quantified as either tail-flick or the area under curve of responses from 15 to 105 min after drug administration. The following formula based on Trapezoid rule was used to calculate the AUC:

$$\mathrm{AUC} = 15 \times \mathrm{TLF}[(\mathrm{min15}) + (\mathrm{min30}) + (\mathrm{min45}) + (\mathrm{min60}) \ldots + (\mathrm{min105})/2].$$

2.4. Experiments

To determine the effect of subanalgesic and analgesic doses of gabapentin and morphine, different doses of gabapentin (30 and 90 mg/kg s.c.) and morphine (0.5, 1 and 3 mg/kg s.c.) were injected to different groups of rats. Latency response time was measured every 15 min up to 105 min using tail-flick test after drug or saline administration. Control rats received only saline.

Also the analgesic effect of co-administration of above mentioned drugs was evaluated by tail-flick test. Animals received gabapentin 10 min before morphine administration (Meimandi et al., 2005).

2.5. Statistical analysis

One way and repeated measure ANOVA models were used to asses the effects of time and drug. In these models, the dependent variable was latency time, latency time just before injections was considered as covariant. As post-hoc, Dunnet test was used for a pairwise comparison between control and each drug-treated group. Data were expressed as Mean±SEM of at least six rats. To deal with multiple comparison effect, the sample size of

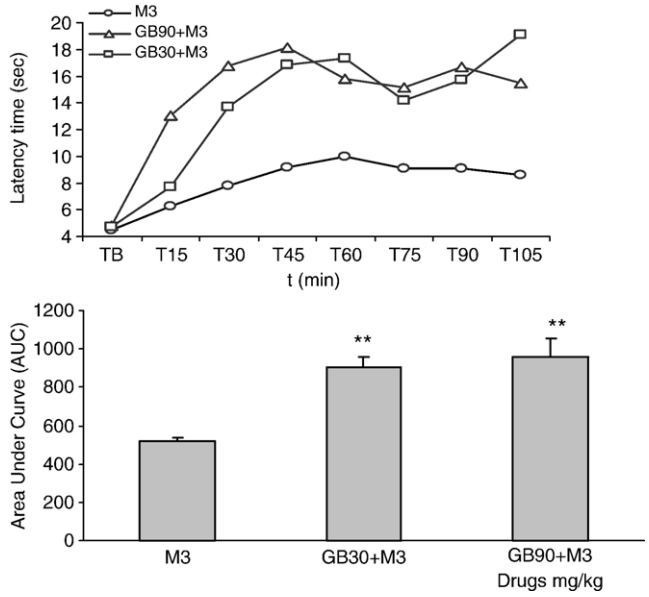

Fig. 3. The antinociceptive effect of different doses of gabapentin on 3 mg/kg of morphine in tail-flick tests. The animals received subcutaneously morphine 3 mg/kg (M3), gabapentin 30 mg/kg plus morphine (GB30+M3) and gabapentin 90 mg/kg plus morphine (GB90+M3). The time–effect curve and respective Area Under Curve (AUC) represents the antinociception in each group. Data are expressed as the mean+SEM of at least 6 rats. $^{**}p<0.001$ compared with morphine treated group.

control group ($n=10$) was 40% greater than other drug treated groups. The value of $P<0.05$ was considered as statistically significant.

3. Results

3.1. Antinociceptive effect of morphine and gabapentin alone

To determine the antinociceptive effect of morphine and gabapentin alone, different doses of morphine (0.5, 1 and 3 mg/kg) and gabapentin (30 and 90 mg/kg) were injected subcutaneously to at least six-adult male rats in each group.

Based on the result of repeated ANOVA model, the temporal variations and their pattern in different doses of gabapentin were not significant (temporal variation: $p=0.84$, interaction between time and dose: $p=0.55$). However a significant difference was observed between control and gabapentin at dose of 90 mg/kg. ($p=0.001$, $F=10.16$). Injection of gabapentin at dose of 90 mg/kg (but not at dose of 30 mg/kg) increased significantly the latency time from 30 to 90 min compared to control group (Fig. 1). The temporal variations of latency time among different doses of morphine were not significant ($p=0.54$); however the interaction between dose and time was observed ($p=0.01$). It means that the doses of 3 and 1 mg/kg of morphine (not 0.5 mg/kg) increased latency time compared to control group ($p=0.000$, $p=0.007$, $F=19.84$). The latency time of 3 mg/kg of morphine was increased 30 min after morphine injection and lasted up to 105 min, while that of 1 mg/kg of morphine was increased after 30 min and lasted up to 60 min as compared to control group (Fig. 2).

Based on the above finding, subanalgesic doses of gabapentin and morphine were 30 and 0.5 mg/kg respectively. The

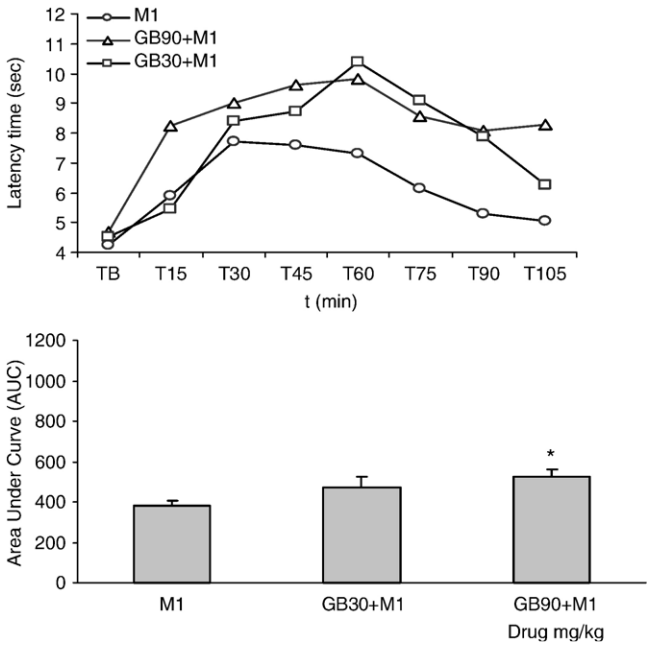

Fig. 4. The antinociceptive effect of different doses of gabapentin on 1 mg/kg of morphine in tail-flick tests. The animals received subcutaneously morphine 1 mg/kg (M1), gabapentin 30 mg/kg plus morphine (GB30+M1) and gabapentin 90 mg/kg plus morphine (GB90+M1). The time–effect curve and respective Area Under Curve (AUC) represents the antinociception in each group. Data are expressed as the mean+SEM of at least 6 rats. $^{*}p<0.05$ compared with morphine treated group.

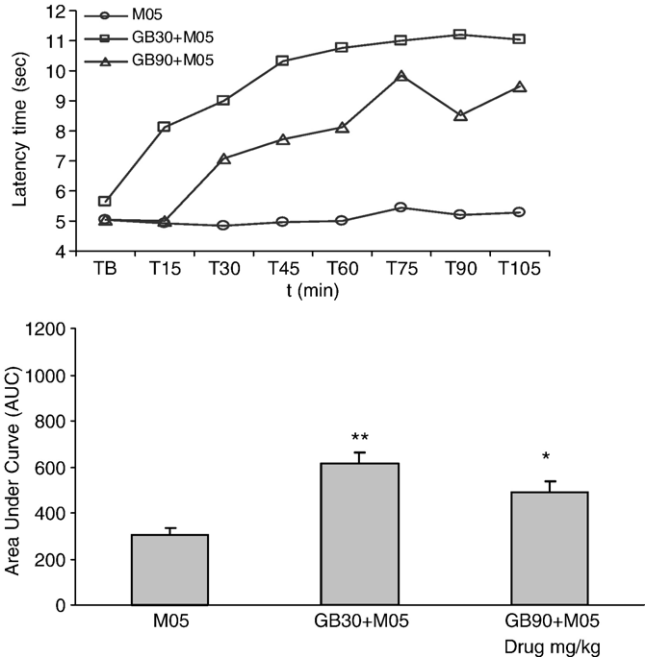

Fig. 5. The antinociceptive effect of different doses of gabapentin on subanalgesic dose of morphine in tail-flick tests. The animals received subcutaneously morphine 0.5 mg/kg (M05), gabapentin 30 mg/kg plus morphine (GB30+M05) and gabapentin 90 mg/kg plus morphine (GB90+M05). The time–effect curve and respective Area Under Curve (AUC) represents the antinociception in each group. Data are expressed as the mean+SEM of at least 6 rats. $^{*}p<0.05$, $^{**}p<0.001$ compared with morphine treated group.

analgesic effect of both morphine and gabapentin was also dose-dependent. (Figs. 1 and 2).

3.2. Effect of gabapentin on antinociceptive effect of morphine

Figs. 3, 4 and 5 showed that the co-administration of gabapentin increased both tail-flick latency time and duration of action compared to morphine alone in all cases. So Area Under Curve which includes both of these effects were reported. Analysis of variance showed that the AUC of 3 mg/kg of morphine (which had antinociceptive effect compared to control) in combination with gabapentin at dose of 30 mg/kg (which was not an analgesic dose) and also at analgesic dose of 90 mg/kg were increased significantly compared to morphine alone ($p=0.001$, $p<0.001$; $F=16.7$). (Fig. 3).

The co-administration of analgesic dose of gabapentin (and not subanalgesic dose of 30 mg/kg) increased the AUC of morphine at doses of 1 mg/kg ($p=0.049$, $F=2.9$). (Fig. 4). The AUC of subanalgesic doses of morphine (0.5 mg/kg) was increased by addition of gabapentin. ANOVA showed that gabapentin at doses of 90 mg/kg likewise at subanalgesic dose of 30 mg/kg were able to increase significantly the AUC compared to 0.5 mg/kg of morphine alone ($p=0.012$, $p=0.000$; $F=13.8$). (Fig. 5).

4. Discussion

The pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (IASP, 1994). Because of complexity and multidimensional aspects of pain, the management of severe pain relief, remains the subject of many experimental and clinical studies. Morphine and opioids as "gold standard" in treating severe pain may result in inadequate analgesia, tolerance and dependency (Way et al., 2001). So alternative drugs that produce therapeutic analgesia when co-administrated with low dose of morphine could have important clinical application. Gabapentin which is effective in different aspect of pain might be proposed as analgesic or coadjuvants (Mao and Chen, 2000).

Our data showed that administration of high dose of 90 mg/kg s.c. of gabapentin in rats induced antinociception as well as morphine at doses of 1 and 3 mg/kg s.c., in tail-flick test. In accordance Dixit reported that gabapentin dose dependently increased hot-plate latency time at 90 mg/kg s.c., in rats. Its analgesic effect was comparable to morphine at dose of 3 mg/kg s.c. (Dixit et al., 2000). Also gabapentin caused antinociception especially in hot-plate test in mice (Pakulska and Czarnecka, 2004). Whereas the antinociceptive effect of 500 μg gabapentin was comparable to 300 μg of morphine at spinal level (Shimoyama et al., 1997).

In contrast, other authors sustained that gabapentin had no analgesic effect in tail-flick test (Taylor, 2001) and also in early phase of formalin test which are considered as physiologic acute model of pain (Field et al., 1997; Gilren, 2002) or at least gabapentin produced a negligible acute nociceptive effect at antiallodynic doses of 30–300 mg/kg i.p., (Hunter et al., 1997). This controversy may found explanation since gabapentin does not change pain transmission at nociceptor site nor does affect pain nociceptive threshold, but may have facilitator effect on nociceptive responses at spinal cord dorsal neurons (Mao and Chen, 2000).

The results of this study showed that gabapentin enhanced morphine antinociceptive effects in tail-flick test or acute model of pain. Gabapentin has been proposed as combinational therapy in pain relief management. In healthy volunteers gabapentin showed no analgesic effect alone, but enhanced the analgesic effect of morphine (Eckhardt et al., 2000). In another clinical study, it was also demonstrated that a single dose of gabapentin reduced morphine consumption after mastectomy (Dirks et al., 2002), probably acting like NMDA antagonists (Dirks et al., 2002; Eckhardt et al., 2000). In animal experimental models of pain, similar result was obtained. For example; the single dose of 10 mg/kg of gabapentin enhanced analgesic effect of 10 mg/kg of morphine in mice, in hot-plate but not in tail-flick test (Pakulska and Czarnecka, 2004). In an acute bradykinin pancreatic model of visceral pain in rats, spinal administration of subanalgesic doses of gabapentin with morphine reduced significantly pain behaviors (Smiley et al., 2004). Likewise intrathecal co-administration of gabapentin with morphine was effective in second phase of nociception after orofacial formalin test in rats (Grabow and Dougherty, 2002). Also spinal administration of gabapentin showed potentiation of morphine antinociception; i.e., the co-administration of subanalgesic doses of gabapentin increased significantly tail-flick latency time after subanalgesic dose of morphine (Shimoyama et al., 1997).

In accordance with some of the previous studies, the result of this study showed that gabapentin may potentiate the spinal antinociceptive effect, not only in sensitized nervous system, but also in acute model of pain in tail-flick in rats. Tail-flick test shows possible effect on normal sensory nociceptive function which could be mediated by spinal and supraspinal mechanisms of nociception (Hunter et al., 1997). So it is useful to determine the antinociceptive effect of gabapentin at spinal level and the effect of its co-administration with morphine in acute model of pain.

In this study the enhancement of analgesic effect of gabapentin on morphine is demonstrated when subanalgesic doses of gabapentin (30 mg/kg) were combined to subanalgesic dose of morphine (0.5 mg/kg) (Fig. 5). The AUC produced by this combination was significantly more than AUC of each drug alone and even more than AUC of analgesic dose of 3 mg/kg morphine. So gabapentin as a well tolerate safe drug (Rosa and Kam, 2002; Haig et al., 2001) could be considered as a co-adjuvant with morphine in acute severe pain treatment in a normal nervous system. Contrary to our results, other authors have widely limited the analgesic effect of gabapentin through neuroplastic changes in a sensitized nervous system or in the case of nerve injury (Abdi et al., 1998; Cesena and Calcutt, 1999; Christensen et al., 2001; Dirks et al., 2002; Dixit et al., 1999; Gilren, 2002; Patel et al., 2001; Takasaki et al., 2001; Turan et al., 2004). On the other hand the use of subanalgesic doses of gabapentin and morphine might overcome tolerance, dependency and irresponsiveness of each drug alone. It was

shown that in prolonged use of this combination in rats, gabapentin prevented morphine tolerance and decreased withdrawal signs (Andrews et al., 2001; Meimandi et al., 2005). For this reason it was proposed in treatment of opiate withdrawal (Martinez-Rage et al., 2004).

In conclusion the result of this study showed that gabapentin has analgesic effect in tail-flick test and also subanalgesic dose of gabapentin may enhance the antinociceptive effect of both analgesic and subanalgesic doses of morphine in acute model of pain. Future studies are needed to interpret additive or synergy statement of this combination.

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