Analgesic Action of Amfenac Na, a Non-steroidal Anti-inflammatory Agent

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(Received March 14, 1988)

Amfenac Na is a new non-steroidal analgesic anti-inflammatory drug which is clinically used for ailments such as rheumatoid arthritis and pain and/or inflammation after surgery.

In this paper, amfenac Na is studied on the bradykinin induced-flexor reflex and the simultaneous recording of the cortical somatosensory-evoked response (SER) and the electromyogram of digastric muscle (d-EMG) evoked by a tooth pulp stimulation. Amfenac Na at doses of 0.1–1 mg/kg p.o. suppressed hindlimb flexor reflexes induced by bradykinin infusion in the rat. This effect was the most potent among the drugs used; the order of potency was as follows: amfenac Na > flottefenine > loxoprofen > piroxicam > emorfazone > mfenamic acid. Similarly, the intravenous injection of amfenac Na completely suppressed the flexor reflex with a dose as low as 0.1 mg/kg; the potency was almost equal to that of morphine. On the SER and d-EMG evoked by tooth pulp stimulation, a high dose (100 mg/kg i.v.) of amfenac Na showed very weak inhibition, whereas morphine (10 mg/kg i.v.) suppressed those responses. These data suggest that amfenac Na showed a very potent analgesic effect comparable to morphine, and that the site of action is mainly the periphery.

Keywords — analgesic anti-inflammatory agent; flexor reflex; amfenac Na; tooth pulp stimulation; somatosensory evoked response; digastric muscle electromyogram

Materials and Methods

Hind-Limb Flexor Reflex by Bradykinin Infusion — Male Sprague Dawley rats (200–260 g) were anesthetized with ether. A polyethylene cannula (0.6 – 0.8 mm o.d.) was inserted retrogradely into the right femoral artery so that the tip reached the bifurcation of the abdominal aorta. A bradykinin solution (2 μg/0.2 ml each) was injected through the cannula flowing into the left femoral artery. The right femoral vein was catheterized for drug injection. The animal was fixed horizontally in a stainless steel cage with a slit through which only the left hind-limb was exposed. The left hind-limb flexor reflexes were recorded on a minipolygraph (Nihon Kohden RM-6100) through an isometric transducer (Nihon Kohden SB-1T).

For the study of the analgesic effects, animals were left in the cage for at least 2 h after the operation for recovery from the anesthesia. Then three values of bradykinin treatment were obtained for each rat 10 min apart before drug administration of p.o. (0.1 ml/100 g body weight) or i.v. (0.1 ml/100 g body weight). The flexor reflexes were determined at appropriate intervals.
after drug administration. The analgesic activities were then calculated by comparison of the mean of three control values.

The ED<sub>50</sub> value of each drug was determined from a linear regression analysis of the dose response curves.

**Recording of Action Potentials by Tooth Pulp Stimulation** — Recording of action potentials by the stimulation of tooth pulp was carried out according to the method of Toda and his coworkers.<sup>9,10</sup> Wistar male rats (Shizuoka Agr. Coop. Assn. Lab. Animal, Shizuoka) weighing 220—300 g were used. Each animal was anesthetized with thiamylal sodium (Isozol, Yoshitomi Pharm. Co.), given intraperitoneally in an initial dose of 60—80 mg/kg and cannulated in the trachea. A dose of 5 mg/kg thiamylal was given intraperitoneally as required to maintain the light anesthesia.

A bipolar stimulating electrode (interpolar distance, 2 mm) of stainless steel wires of 0.1 mm in diameter, insulated except for the tips, was inserted into the lower incisor pulp through a small hole which was made about 7 mm from the top of the incisor. The hole was covered with dental wax to prevent a short-circuiting by saliva. Rectangular pulses of 0.2 ms duration were delivered to the tooth pulp at 1 Hz via electric stimulator (Nihon Kohden SEN 3201). The intensity of the stimulation was adjusted so that the amplitude of the SER was about 50% of the maximal response. After the animals were placed in a stereotaxic frame, SERs were recorded from the surface of the skull contralaterally with silver ball electrodes 0.8 mm in diameter. An indifferent electrode was placed in the muscle of the neck. The d-EMG activities were recorded from the anterior belly of the digastric muscle, one of the jaw openers, using a bipolar stainless needle electrode (interpolar distance of 4 mm). The SER and d-EMG were amplified through preamplifiers (Nihon Kohden RM-5), displayed on a storage oscilloscope (Matsushita VP-5730) taking the average of 64 responses, and recorded with a Polaloid camera.

**Compounds Used** — Amfenac Na (Lot No. FZP-2) was prepared in our laboratory. Mefenamic acid (SIMS, Firenze, Lot No. 1722) and piroxicam (Japan Pharm. Co., Tokyo, Lot No. 2698) were purchased. Floctafenine (Eizai Pharm. Co., Tokyo), loxoprofen Na (Sankyo Pharm. Co., Tokyo) and emorphazone (Morishita Pharm. Co., Tokyo) were extracted from tablets in our laboratory. Morphine HCl was purchased from Sankyo Pharm. Co. (Tokyo).

**Statistics** — Data were expressed as the mean ± the standard error of the mean. The statistical analysis was the unpaired Student's t-test, and a p value of less than 0.05 was accepted as being statistically significant.

**Results**

Effect on Hind-Limb Flexor Reflex Induced by the Bradykinin Infusion

A time course study of analgesic effects induced by oral administration of amfenac Na is shown in Figs. 1 and 2. Although the smallest dose 0.1 mg/kg amfenac Na did not show analgesia, the two higher doses, 0.3 and 1.0 mg/kg, showed significant analgesia. The analgesic effect in the highest dose of 1.0 mg/kg amfenac Na was evident 20 min after administration, and the maximal effect was 30 min, lasting over a period of 90 min.

When the time course of the analgesic effect of amfenac Na by oral administration was com-

![Graph](image-url)

Fig. 1. Time Course Change of the Hind-Limb Flexor Reflex Induced by Bradykinin after Amfenac Na Administration (p.o.)

The points indicate means ± S.E. of five experiments. ---, 0.1 mg/kg p.o.; ----, 0.3 mg/kg p.o.; ---, 1.0 mg/kg p.o.
pared with floctafenin, loxoprofen, piroxicam, emorphazone and mefenamic acid, the initiation of analgesic action of amfenac Na was fast and the duration of the action was the shortest among these. Dose-dependent analgesic action of all these drugs was clearly detectable (Fig 3). The ED$_{50}$ values are shown in Table I. The order of the analgesic effect was amfenac Na > floctafenin > loxoprofen >> piroxicam = emorphazone > mefenamic acid.

**Table I.** ED$_{50}$ Values of Amfenac Na and Reference Drugs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>ED$_{50}$ value (mg/kg p.o.)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amfenac Na</td>
<td>0.29</td>
<td>1.0</td>
</tr>
<tr>
<td>Floctafenine</td>
<td>0.43</td>
<td>1.5</td>
</tr>
<tr>
<td>Loxoprofen</td>
<td>0.56</td>
<td>1.9</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>2.34</td>
<td>8.1</td>
</tr>
<tr>
<td>Emorphazone</td>
<td>2.90</td>
<td>10.0</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>5.12</td>
<td>17.7</td>
</tr>
</tbody>
</table>
Fig. 4. Time Course Changes of the Hind-Limb Flexor Reflex Induced by Bradykinin after Intravenous Injection of Amfenac Na and Morphine
A) amfenac Na, 0.1 mg/kg i.v., B) morphine HCl, ---, 0.01 mg/kg i.v.; ---, 0.03 mg/kg i.v.; ---, 0.1 mg/kg i.v. The points indicate means ± S.E. of five experiments.

When amfenac Na and morphine were given intravenously, the bradykinin-induced flexor reflex was immediately suppressed after administration. The analgesic effect of 0.1 mg/kg amfenac Na was nearly equal to the effect of the same dose of morphine HCl (Fig. 4).

Effect on the SER
Figure 5 shows the typical examples obtained
Fig. 6. Time Course Change of SER after Intravenous Injection of Amfenac Na (A) and Morphine (B)

A) amfenac Na i.v., ○, 10 mg/kg (n = 9); ●, 30 mg/kg (n = 7); □, 100 mg/kg (n = 4). B) morphine HCl i.v., ○, 1 mg/kg (n = 5); ●, 3 mg/kg (n = 7); □, 10 mg/kg (n = 5). The numbers in parentheses indicate numbers of experiments. a) p < 0.05, b) p < 0.01, compared with the values before drug administration.

Fig. 7. Typical Recording of the d-EMG after Intravenous Injection of 100 mg/kg Amfenac Na and 10 mg/kg Morphine

The d-EMG is composed of two action potentials, P1 and P2, as indicated in the figure. Calibrations are indicated in the figure.

with amfenac Na and morphine on SER, and Fig. 6 shows the time course of the effects of these drugs on SERs. As shown in Fig. 5, SERs were found to be composed of four main components, namely, a positive wave (P1), a negative wave (N1), a positive wave (P2), and a negative wave (N2). The latencies of the waves were about 10, 15, 28 and 45 ms, respectively. As the
Fig. 8. Time Course Changes of the d-EMG after Intravenous Injection of Amfenac Na (A) and Morphine (B)

A) amfenac Na i.v., ○, 10 mg/kg (n = 4); ●, 30 mg/kg (n = 5); □, 100 mg/kg (n = 3). B) morphine HCl i.v., ○, 1 mg/kg (n = 5); ●, 3 mg/kg (n = 5); □, 10 mg/kg (n = 4). The numbers in parentheses indicate numbers of experiments. a) p < 0.05, b) p < 0.01, compared with the values before drug administration.

### Table II. Effect of Amfenac Na and Morphine on d-EMG Latency

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg)</th>
<th>Control (mean ± S.E.)</th>
<th>After treatment (mean ± S.E.)</th>
<th>nba)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amfenac Na</td>
<td>10</td>
<td>5.9 ± 0.30</td>
<td>6.2 ± 0.28</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5.1 ± 0.13</td>
<td>5.7 ± 0.39</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6.1 ± 0.60</td>
<td>7.1 ± 0.68</td>
<td>3</td>
</tr>
<tr>
<td>Morphine</td>
<td>1</td>
<td>5.0 ± 0.36</td>
<td>5.8 ± 0.34</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.5 ± 0.40</td>
<td>6.4 ± 0.41</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.5 ± 0.54</td>
<td>7.6 ± 0.30 (c)</td>
<td>3</td>
</tr>
</tbody>
</table>

a) The values were obtained at 15—30 min after drug administration. b) Number of experiments. c) p < 0.05, compared with control values.

The intensity of the stimulation increased the peak-to-peak amplitude of the P1 and N1 waves was potentiated, this was taken as the magnitude of the SER. In Fig. 6, the amplitudes of the SERs are expressed relative to the value before the drug injection. Amfenac Na, at doses of 10 and 30 mg/kg i.v., had little effect on SER, whereas 100 mg/kg injection of this drug suppressed the SER by 13.6% (statistically not significant) 15 min after the injection.

On the other hand, morphine, a narcotic analgesic, reduced the SER by 13.6% (p < 0.05) 5 min after the injection of 3 mg/kg; however, 1 mg/kg of morphine had no appreciable effect. Ten mg/kg of morphine inhibited the SER to about 47% of control (p < 0.01) after 15 min, and then it was gradually restored.

**Effect on the d-EMG**

Figure 7 shows typical results obtained with amfenac Na and morphine on d-EMG activity; Fig. 8 shows the time course of the effects of the drugs on d-EMG activities. The average latency of the onset of d-EMG was 5.5 ± 0.16 ms (n = 24); as the measure of d-EMG, we took the peak height of the first positive peak (P1). Although 10 and 30 mg/kg amfenac Na i.v. did not show any effects on d-EMG activities, 100 mg/kg amfenac Na suppressed the d-EMG activities by about 21% (p < 0.01), at 15—30 min after the injection. Morphine at doses of 1 and 3
mg/kg i.v. did not show any significant effect on d-EMG. The mg/kg of morphine, however, suppressed the d-EMG activities over 50%. The maximal value of the suppression (62.1%, p < 0.05) was obtained 30 min after the injection. Morphine, unlike amfenac Na, delayed the latency of the d-EMG in a dose-dependent manner. In particular, 10 mg/kg morphine significantly (p < 0.05) delayed the latency at 15—30 min after the injection (Table II.)

Discussion

Amfenac Na inhibited hind-limb flexor reflex caused by bradykinin and showed an analgesic effect by oral administration. The effect was most potent among the compounds used. When the analgesic effect of amfenac Na by intravenous injection (i.v.) was compared with morphine, the effect was almost comparable to morphine in doses of 0.1 mg/kg i.v. The flexor reflex was induced by bradykinin infusion in the present experiment. Bradykinin releases PGs, and the effect of bradykinin is potentiated by the PGE. Amfenac Na is reported to decrease PGs synthesis by inhibiting cyclooxygenase. Therefore, it is believed that amfenac Na reduced the activity of bradykinin sensitized by PGE at the peripheral free nerve ending by the inhibition of PGs synthesis and then suppressed the hind-limb flexor reflex. However, amfenac Na showed very weak inhibition on the SER and d-EMG caused by the tooth pulp stimulation at a dose as high as 100 mg/kg i.v. On the other hand, morphine inhibited the potentials at a dose of 10 mg/kg i.v. The results are in agreement with several reports that aspirin-like analgesic antiinflammatory agents have no or only a weak effect on SER evoked by tooth pulp stimulation. Inoki and Kudo proposed that stimulation of the surface of the dental pulp releases bradykinin which then stimulates the pain-conducting nerve endings of the tooth pulp. Although recently it has been reported that amfenac Na inhibits the bradykinin release at the hind-limb caused by heat stimulation (personal communication from Dr. Inoki), the activity did not seem to play a role in this study of tooth pulp stimulation. In our study, Aδ or C fibers are thought to be activated directly without the mediation of chemical transmitters such as bradykinin, for electrodes were inserted as deep as near the dental root and electric stimulation was delivered with enough intensity. Dubas and Parker reported that the hypothalamus is the main site of generation of analgesia because it is thought to be the site of generation of antipyretic action according to its abundance of prostaglandins and because it is part of the pain-conducting pathway. Thus, our results suggest that the central effect of amfenac Na is very weak and that the peripheral mechanisms were not included in this experiment because of the direct stimulation of the pulpal nerve.

In contrast, morphine strongly depressed the SER and d-EMGs evoked by tooth pulp stimulation. The effects of morphine were well antagonized by 1 mg/kg naloxone (data not shown). Furthermore, it prolonged the latency of d-EMG, which is the reflex potential, though the latency of the SER was not affected. A similar prolongation of the latency of d-EMG in rabbits was reported by Chan and Fung. Morphine is reported to have depressive effects, especially on sensory afferent pathways, the thalamocortico system, the afferent excitation system of reticular formation of the brain stem, and the thalamo-cortico tract. It is also reported that morphine has an analgesic effect by activating the inhibitory descending pathway through the brain stem structures, thus depressing the noxious impulses in the dorsal horn of the spinal cord or the medulla. From these reports, morphine seems to suppress SER and d-EMG by depressing or activating these varieties of ascending and descending pathways, respectively. Furthermore, it is interesting that morphine has different effects on latencies of SERs and d-EMG activities.

In conclusion, in contrast to narcotic analgesics such as morphine which acts on the central nervous system, amfenac Na slightly suppressed SER and d-EMG evoked by tooth pulp stimulation only in high dose, it is suggested that the main site of action of amfenac Na is the periphery. Furthermore, it is interesting that the analgesic effect of amfenac Na on peripherally
induced pain is comparable to that of morphine.

**Acknowledgement** We are thankful to Dr. Kazuo Toda, Dept. of Physiology, Tokyo Medical and Dental University, for his kindness in teaching us the experimental technique for SER and the d-EMG recording.

**References**


