Original Article

Comparison of pharmacological action of Neurotropin® and nonsteroidal anti-inflammatory drugs on chronic inflammatory pain in adjuvant-induced arthritic rat

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Abstract

Neurotropin®, a non-protein extract from the inflamed skin of rabbits inoculated with vaccinia virus, is clinically used for treatment of chronic pain. In this study, we compared the pharmacological effects of repeated oral administration of Neurotropin® and non-steroidal anti-inflammatory drugs (NSAIDs) loxoprofen sodium, etodolac and celecoxib, in the adjuvant-induced arthritic rats. Neurotropin® produced analgesic effect but not anti-inflammatory effect and gastric ulcer. All NSAIDs tested produced analgesic and anti-inflammatory effects but loxoprofen and etodolac produced gastric ulcer as well. Neurotropin® may be an analgesic without anti-inflammatory action. Without causing stomach problems, Neurotropin® may be good for the treatment of chronic pain.

Key words: Neurotropin; Non-steroidal anti-inflammatory drug (NSAID); Chronic pain; Adjuvant-induced arthritis

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INTRODUCTION

Neurotropin® is a non-proteinaceous extract containing biologically active small molecules (molecular weights of less than 2,000) isolated from the inflamed skin of rabbits inoculated with vaccinia virus. This Neurotropin is clinically used as an analgesic drug for treatment of chronic pain such as lumbago, osteoarthritis,

shoulder-neck-arm syndrome, symptomatic neuralgia ¹⁾ and postherpetic neuralgia. The efficacy of Neurotropin in the treatment of reflex sympathetic dystrophy has also been reported ^{2,3)}.

The analgesic effects of Neurotropin have been shown in some animal models, such as specific alternation of rhythm in environmental temperature (SART) stress model with hyper-

algnesia^{4,5,6,7}), chronic nerve constriction injury model⁸⁾ and spinal nerve ligation (SNL) model⁹⁾ with painful peripheral neuropathy. Recently, we reported that Neurotropin exerted anti-nociceptive effect by activating of the descending pain inhibitory systems in rats with adjuvant-induced arthritis¹⁰⁾, an animal model of chronic inflammatory pain¹¹⁾. On the other hand, nonsteroidal anti-inflammatory drugs (NSAIDs) have been clinically used as first choice analgesics for pain managements in conditions such as lumbago, osteoarthritis and shoulder-neck-arm syndrome, which are similar to the indications of Neurotropin^{12,13)}.

In this study, to investigate the difference in modes of action of Neurotropin and NSAIDs, we compared anti-nociceptive and anti-inflammatory effects of Neurotropin and NSAIDs in a rat model of adjuvant-induced arthritis. In addition, we investigated the gastrointestinal ulcer, a major adverse effect of NSAIDs, after chronic administration of these drugs.

MATERIAL AND METHODS

Animals

Male Wistar rats (Japan SLC, Hamamatsu) were used; they were nine weeks of age at the start of experiments. The animals were housed at $22 \pm 2^\circ\text{C}$ under 12-h light and dark cycle (light turned on from 8:00 to 20:00). Laboratory chow and tap water were available ad libitum. The experiment was done in accordance with Guiding Principles for the Care and Use of Laboratory Animals and the Guidelines for Animal Experimentation approved by The Japan Pharmacological Society and The Japanese Association for Laboratory Animal Science.

Drugs

Neurotropin was prepared by Nippon Zoki Pharmaceutical Co. (Osaka). The analgesic potency of Neurotropin was expressed as Neurotropin unit (NU), and was evaluated by analgesic bioassay using SART stressed mice, which the mice

were kept at 24°C and 4°C for alternate 1 hour periods from 10:00 to 17:00 and then at 4°C from 17:00 to 10:00 the next morning for 5 days. Loxoprofen sodium, etodolac and celecoxib were extracted from Loxonin[®] (Sankyo, Tokyo), Hypen[®] (Nippon Shinyaku, Kyoto) and CelebrexTM (Pfizer, NY, USA), respectively.

Drug administration

All drugs suspended with 0.5% carboxymethyl cellulose sodium solution were administered orally once a day for 7 or 14 days after adjuvant treatment. The dosages of Neurotropin (200 NU/kg per day, p.o.) and NSAIDs (10 mg/kg per day, p.o.) used in this study were set from the previous observation¹⁰⁾ and preliminary experiments (data not shown), respectively.

Induction of adjuvant-induced arthritis

Rats were given a subcutaneous injection of 0.1 mL of adjuvant (0.5% killed Mycobacterium butyricum suspended in liquid paraffin) into the hind paw^{14,15)}.

Measurements of nociceptive thresholds, paw edema and gastrointestinal ulceration

Nociceptive threshold was determined using an analgesy meter (Ugo basile, Milan, Italy). A pressure stimulus was applied on the center of the hind paw and was increased at a rate of 32 g/sec. The pressure intensity (g) that caused escape reaction was defined as the nociceptive threshold. The anti-inflammatory effect was evaluated by increased percentage of paw edema. The percentage of edema was calculated by the next equation.

$$\text{Edema (\%)} = [(\text{post paw volume} - \text{pre paw volume}) / \text{pre paw volume}] \times 100$$

Paw volume was measured using a plethysmometer (Ugo basile, Milan, Italy). The paw edema was measured before drug treatment, on day 7 and 14 after adjuvant treatment.

Gastrointestinal ulceration was observed as follows: the stomach and duodenum were isolated on day 7 and 14 after adjuvant treatment.

Fig. 1 Anti-nociceptive effects of Neurotropin, loxoprofen sodium, etodolac and celecoxib after 7-day administration in rats with adjuvant-induced inflammation.

Neurotropin (NTP, 200 NU/kg per day), loxoprofen sodium (LXP, 10 mg/kg per day) etodolac (ETD, 10 mg/kg per day) and celecoxib (CLC, 10 mg/kg per day) and vehicle (VH) were administered orally once a day for 7 days, and nociceptive threshold was determined immediately before (24 hours after 6th administration; open columns) and 60 min after (closed columns) the last administration. Values represent the means and S.E.M. of 10 animals. * $P < 0.05$ when compared with pre-administration (paired t -test).

Fig. 2 Anti-nociceptive effects of Neurotropin, loxoprofen sodium, etodolac and celecoxib after 14-day administration in rats with adjuvant-induced inflammation.

Neurotropin (NTP, 200 NU/kg per day), loxoprofen sodium (LXP, 10 mg/kg per day) etodolac (ETD, 10 mg/kg per day) celecoxib (CLC, 10 mg/kg per day) and vehicle (VH) were administered orally once a day for 14 days, and nociceptive threshold was determined immediately before (24 hours after 13th administration; open columns) and 60 min after (closed columns) the last administration. Values represent the means and S.E.M. of 10 animals. * $P < 0.05$ when compared with pre-administration (paired t -test). # $P < 0.05$ when compared with pre-VH of adjuvant group (Dunnett's multiple comparison test).

The isolated tissues were fixed with 1% formalin solution. After fixation, we evaluated the ulceration of gastric and duodenal mucosa by total length and number of ulcers under a stereoscopic microscope.

Statistical analysis

All data were expressed as means $\pm$ S.E.M. Statistical significance between two groups was determined by paired t -test. Statistical significance more than two groups was analyzed using Dunnett's multiple comparison test. P values less than 0.05 were considered statistically significant.

RESULTS

Anti-nociceptive effects of Neurotropin and NSAIDs

Adjuvant inoculation markedly decreased the nociceptive threshold of the affected hind

paw; changes in the nociceptive threshold 7 and 14 days after inoculation were shown in **Figs. 1 and 2**, respectively. Since the effect of Neurotropin peaked 60 min after oral administration⁹, the nociceptive threshold was measured before and 60 min after administration. Repeated oral administration of Neurotropin (200 NU/kg per day) and the NSAIDs, loxoprofen sodium, etodolac and celecoxib (10 mg/kg per day) for six days did not affect the nociceptive threshold about 24 hours after the last administration (**Fig. 1**). The slight but significant increase and an increased tendency ($P = 0.0604$) of the nociceptive threshold were obtained 60 min after the seventh administration of celecoxib (10 mg/kg) and Neurotropin (200 NU/kg), respectively, but loxoprofen sodium and etodolac at a dose of 10 mg/kg were without acute effects (**Fig. 1**).

Repeated administration of etodolac and

Fig. 3 The effects of Neurotropin, loxoprofen sodium, etodolac and celecoxib on adjuvant-induced edema, gastric ulcer and body weight.

Neurotropin (NTP, 200 NU/kg per day), loxoprofen sodium (LXP, 10 mg/kg per day), etodolac (ETD, 10 mg/kg per day), celecoxib (CLC, 10 mg/kg per day) and vehicle (VH) were administered orally once a day for 7 (open columns) or 14 days (closed columns). The edema (A), the number of gastric ulcers (B) and the total length of the gastric ulcer (C) were determined 60 min after the last administration. Body weight was determined before the adjuvant inoculation and the last administration. Values represent the means and S.E.M. of 6–10 animals. * $P < 0.05$ when compared with the corresponding VH + adjuvant group (Dunnett's multiple comparison test).

celecoxib at a dose of 10 mg/kg per day for 13 days increased the nociceptive threshold about one day after the last administration, but Neurotropin (200 NU/kg per day) and loxoprofen sodium (10 mg/kg per day) were without long-lasting effects (Fig. 2). The significant increase of the nociceptive threshold was obtained 60 min after the 14th administration of Neurotropin (200 NU/kg per day), but all NSAIDs tested (10 mg/kg per day) were without acute effects (Fig. 2).

The effects of Neurotropin and NSAIDs on adjuvant-induced paw edema, gastric ulcer and body weight

An inoculation of adjuvant produced long-lasting edema in the treated hind paw, which was marked and significant 7 and 14 days after inoculation (Fig. 3A). The edema was significantly inhibited by repeated administration of loxoprofen sodium, etodolac and celecoxib at daily dose of 10 mg/kg, and Neurotropin (200 NU/kg per day) was without effects (Fig. 3A).

There were no gastrointestinal ulcer observed 7 and 14 days after adjuvant inoculation (Fig. 3B, C). Repeated administration of loxoprofen sodium and etodolac at daily doses of 10 mg/kg caused gastric ulcers in all animals examined, shown as increases in the number and length of the ulcers (Fig. 3B, C). A few gastric ulcers were observed in 3 out of 6 animals examined after repeated administration of celecoxib (10 mg/kg per day). One gastric ulcer was observed in 1 out of 6 animals after repeated administration of Neurotropin (200 NU/kg per day) (Fig. 3B, C). No duodenum ulcers were observed after repeated administration of Neurotropin at a dose of 200 NU/kg per day and all NSAIDs tested at a dose of 10 mg/kg per day (data not shown).

Adjuvant inoculation produced a marked decrease in the body weight 7 days after inoculation, and the slight recovery was observed after 14 days (Fig. 3D). Repeated administration of Neurotropin (200 NU/kg per day) did not affect the decrease of body weight caused by adjuvant treatment (Fig. 3D). Loxoprofen sodium (10 mg/kg per day) showed a tendency to enhance the decrease of body weight during the initial seven days (Fig. 3D). Repeated administration of celecoxib and etodolac at a dose of 10 mg/kg per day significantly inhibited the decrease of the body weight (Fig. 3D).

DISCUSSION

A rat model of adjuvant-induced arthritis is

a chronic inflammatory pain model and suitable to test the analgesic and anti-inflammatory effects of agents^{16,17}. Repeated administration of Neurotropin produced an inhibitory tendency and significant inhibition of pain after 7 and 14 days, respectively. With regard to NSAIDs, repeated administration of celecoxib produced a slight but significant inhibition of pain after 7 days and the inhibition of the continuous pain state after 14 days. Repeated administration of etodolac did not inhibit pain after 7 days but the inhibition of the continuous pain state after 14 days. Repeated administration of loxoprofen sodium was without effects after 7 and 14 days. These results suggested that the action mode of Neurotropin is different from those of NSAIDs, because Neurotropin showed a more significantly effect compared with NSAIDs after 14 days in chronic pain state.

All NSAIDs tested produced marked suppression of the long-lasting edema caused by adjuvant inoculation, but Neurotropin was without effects. The anti-inflammatory action of NSAIDs is primarily due to the inhibition of prostaglandin biosynthesis in the inflamed tissue. Neurotropin did not show anti-inflammatory effect, a result consistent with findings that Neurotropin does not inhibit the noxious stimulation-induced release of prostaglandin E₂ in the skin¹⁸. Thus, Neurotropin may be an analgesic without anti-inflammatory action.

We have so far reported that antinociceptive action of Neurotropin has been shown to involve the activation of the noradrenergic and serotonergic descending pain inhibitory systems, because the analgesic action of Neurotropin was significantly inhibited by intrathecal injections of the α 2-adrenoceptor antagonist and selective 5-HT₃ serotonin receptor antagonist. In addition, adjuvant-induced arthritic rats may have the dysfunction of descending pain inhibitory systems¹⁰. Neurotropin produces antinociceptive effects in SART stress model with chronic hyperalgesia^{4,5,6,7} and SNL model with

chronic hyperalgesia and allodynia⁹) without any inflammations. In both models, it is suggested that the mechanism of antinociceptive effect of Neurotropin is via enhancement of the descending pain inhibitory systems^{7,9}. In case of adjuvant-induced arthritic rats, the antinociceptive effect of Neurotropin showed more clearly 14 days in chronic state than 7 days in acute state. It is suggested that the dysfunction of the descending pain inhibitory systems may be caused by chronic pain, regardless of the presence of inflammation.

Adjuvant inoculation itself did not produce gastric ulcers, but repeated administration of loxoprofen sodium and etodolac markedly caused gastric ulcers. Single administration of celecoxib, a selective cyclooxygenase-2 inhibitor, at a dose of 100 mg/kg causes marked gastric ulcers in arthritic rats but not in normal rats, which was claimed to be due to the up-regulation of cyclooxygenase-2 in the stomach¹⁹. In this study, repeated administration of celecoxib at a lower dose of 10 mg/kg caused a few gastric ulcers in one-half of arthritic rats examined. Therefore, one should be careful in chronic medication, even when a selective cyclooxygenase-2 inhibitor is used at low dose. Repeated administration of Neurotropin caused one ulcer in 1 of 6 arthritic rats. Thus, Neurotropin may be relatively free from gastric adverse action even after repeated administration.

Adjuvant inoculation markedly decreased the body weight, which was not affected by repeated administration of Neurotropin. With regard to NSAIDs, etodolac and celecoxib markedly restored the weight loss. Loxoprofen sodium did not affect the weight loss 14 days after inoculation but rather enhanced it after 7 days. The weight loss and decrease in locomotor activity of arthritic rats are restored by the disconnection of the afferent pain tract in the spinal cord, suggesting that pain itself affects the morbidity²⁰. In this respect, the continuous pain state was reduced by etodolac

and celecoxib but not by loxoprofen sodium and Neurotrophin. One of reasons is thought that this effect was induced by the continuous anti-inflammatory actions of etodolac and celecoxib. Therefore, the long-lasting analgesic action may be important to treat the morbidity of chronic arthritis.

In summary, repeated administration of Neurotrophin is suggested to be effective in the treatment of arthritic pain without gastrointestinal adverse effects. It does not have anti-inflammatory action, and a combination therapy with Neurotrophin and anti-inflammatory agents may be useful for the treatment of chronic painful arthritis. We will investigate the effects of the combination therapy on the chronic arthritis in the near future.

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