# Pharmacological Studies on EB-382, a New Non-steroidal Antiinflammatory Agent: Mode of Action of the Antiinflammatory Effects

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**The mode of action of (±)-2-[*p*-[(2-methylallyl)amino]phenyl]propionic acid (EB-382) was studied in terms of its antiinflammatory effect. EB-382 displayed a more potent inhibitory effect than that of ibuprofen on carrageenin-induced paw edema in rats, and its inhibitory action was unaffected by adrenalectomy. EB-382 had a less potent inhibitory effect than that of ibuprofen on the prostaglandin $E_2$ biosynthesis of 3T6 mouse fibroblast cells. EB-382 displayed a much more potent inhibitory effect than that of ibuprofen on carrageenin-induced pleurisy in rats. EB-382 exhibited a dose-dependent and significant inhibitory effect on the bradykinin and prostaglandin contents released into the pleural cavity in rat pleurisy induced by carrageenin, and its potency was much higher than that of ibuprofen. EB-382 strongly inhibited the leukocyte emigration into the pleural cavity, and its potency was almost equal to that of indomethacin. EB-382 did not affect the kinin-forming enzyme and kininase activities of rat plasma *in vitro*. The above results suggest that the antiinflammatory effect of EB-382 was probably due to inhibition of the production of bradykinin and prostaglandins derived from emigrating leukocytes in the local inflamed tissue besides a weak inhibitory effect on prostaglandin biosynthesis.**

**Keywords** EB-382; non-steroidal antiinflammatory agent; antiinflammatory effect; bradykinin; prostaglandin

## Introduction

(±)-2-[*p*-[(2-Methylallyl)amino]phenyl]propionic acid (EB-382) was selected as a new non-steroidal antiinflammatory compound from a series of phenylpropionic acids which has been synthesized by Bouchara Research Laboratories in France.[1] In previous papers, we reported that the antiinflammatory and analgesic activities of EB-382 were more potent than those of ibuprofen in acute and chronic inflammation, while less gastric damage was observed following treatment with EB-382.[2,3] In addition, it was found that several of the parmacological properties of EB-382 differed from those of other acidic antiinflammatory drugs such as aspirin, ibuprofen and indomethacin.[2-4]

The present study was conducted to clarify the precise mode of action of EB-382.

## Experimental

**Animals** Male Wistar rats were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Shizuoka).

**Drugs and Reagents** EB-382 was supplied by Bouchara Research Laboratories. The other test compounds were indomethacin (Sigma) and ibuprofen (Sigma). The reagents used included arachidonic acid (Sigma), soybean trypsin inhibitor (Sigma), 1,10-*o*-phenanthroline (Seikagaku Kogyo), bradykinin (Osaka Peptide Institute), λ-carrageenin (Zushi Chemicals), aprotinin (Bayer), actin (Kokusai Shiyaku), z-Phe-Arg-MCA (Sigma), Dulbecco's modified Eagle's salts (Flow Laboratories), fetal calf serum (Gibco), trypsin–EDTA (Flow Laboratories), octadodecyl silica (ODS; Wako Pure Chemical Industries), prostaglandin $E_2$, 6-keto-prostaglandin $F_{1\alpha}$, prostaglandin $F_{2\alpha}$, and thromboxane $B_2$ RIA kit (NEN).

All other reagents were of the highest quality commercially available. All test compounds were suspended in 5% arabic gum solution before administration or dissolved in dimethyl sulfoxide (DMSO) *in vitro*.

**Carrageenin-Induced Paw Edema in Adrenalectomized Rats** Male Wistar rats weighing about 200 g were adrenalectomized bilaterally from the dorsal region under anesthesia with pentobarbital-Na. Sham-operated rats were also prepared simultaneously. The adrenalectomized animals were maintained thereafter by supplying saline solution instead of drinking water. On the 4th postoperative day, these animals were divided into groups of 8 rats, and the effects of the test drugs on carrageenin-induced hind paw edema were examined. One-tenth milliliter of 1% carrageenin solution was injected subcutaneously (s.c.) into the plantar region of the rat hind paw according to the method of Winter *et al.*[5] The paw volume was measured every 1 h for 5 h after the s.c. injection of carrageenin. The test drugs were administered orally 1 h before the s.c. injection of carrageenin. The percentage inhibition was calculated from the difference in mean swelling value between the treated animals and the control group.

**Prostaglandin $E_2$ Biosynthesis in 3T6 Mouse Fibroblast Cells** Employing the method of Carty *et al.*,[6] 3T6 fibroblast cell (Flow Laboratories) monolayers were trypsinized with 0.3% trypsin–EDTA, and thereafter the trypsinized 3T6 cells ($5 \times 10$ cells/ml/dish) were seeded and exponentially grown for 24 h in Dulbecco's modified Eagle's salts (DMEM) containing 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 μg/ml streptomycin (final concentration). The cells were then washed three times with 1 ml of FCS-free DMEM, and 1 ml of fresh 10% FCS–DMEM was added with and without each test drug which was dissolved in 0.1% DMSO (final concentration). One-tenth percent DMSO did not affect the prostaglandin $E_2$ formation by the cells. After the monolayers had been incubated for 2 h, the medium was subjected to radioimmunoassay for prostaglandin $E_2$.

**Carrageenin-Induced Pleurisy in Rats** The method was based on that described by Katori *et al.*[7] Male Wistar rats weighing 200 to 270 g were lightly anesthetized with ether and 0.1 ml of 2% carrageenin in sterile saline was injected into the right pleural cavity through the chest skin sterilized with 70% ethanol 1 h after oral administration of each test drug. Three hours after the carrageenin injection, the ether-anesthetized rats were exsanguinated and the volume of pleural exudate was measured.

The leukocyte count in the exudate was determined by using an auto cell counter (Toa, CC-108). Classification of the leukocytes was performed on the basis of Papanicolaou and Wright–Giemsa staining.

**Bradykinin Assay in Pleural Exudate** After exsanguination, the pleural cavity was rapidly washed with 2 ml of ice-cold saline and the pleural exudate was collected into a polyethylene tube which contained a 2-fold volume of ice-cold methanol and immediately centrifuged at 3000 rpm for 10 min at 0 °C. The supernatant was dried under $N_2$ gas and served as the sample. The content of bradykinin was finally measured by radioimmunoassay according to the method of Takanashi *et al.*[8]

**Prostaglandin Assay in Pleural Exudate** After addition of indomethacin to a final concentration of $10^{-4}$ M, the pleural exudate was chilled immediately with 2 ml of washing medium (Tris-buffered saline, pH 7.4) as described previously, and centrifuged at 3000 rpm for 10 min at 0 °C. The supernatant obtained by the above procedure was treated according to the following method. One milliliter of sample was adjusted to pH 3.0 by the addition of 0.1 N HCl. Then after the addition of 80 mg of ODS and mixing for 10 min, the sample was allowed to stand. The resultant supernatant was decanted off, and the precipitate was washed twice with 1 ml of a 0.5 N HCl–ethanol (80 : 15) mixture and further with 1 ml of ether. This washed precipitate was extracted with 1 ml of ethyl acetate. The extract obtained was dried under $N_2$ gas, and served as the crude prostaglandin product. This product was applied to a silica gel column, washed with acetonitrile–chloroform–acetic acid (20 : 80 : 20), and then eluted with a mixture of acetonitrile–chloroform–acetic acid (80 : 20 : 0.5). After drying under $N_2$ gas, and dissolving in 50 mM phosphate-buffered saline (pH 6.8) containing 0.3% bovine γ-globulin, 0.005% Triton-X-100 and 0.05% sodium azide,

the contents of prostaglandin $E_2$, 6-keto-prostaglandin $F_{1\alpha}$, proataglandin $F_{2\alpha}$, and thromboxane $B_2$ were each measured by radioimmunoassay.

**Assay of Kinin-Forming Enzyme and Kininase Activity of Rat Plasma *in Vitro*** Kinin-Forming Enzyme: Seven-week-old male Wistar rats weighing about 200 g were used. The blood sample was collected into a polyethylene syringe containing 3.8% sodium citrate (1/10 volume) and centrifuged at 3000 rpm for 15 min at 0 °C. The plasma sample was diluted with ice-cold saline to a concentration of 1/50 as the prekallikrein preparation. The prekallikrein activity was assayed according to the modified method of Oh-ishi and Katori.[9] Twenty-five microliters of the prekallikrein preparation was incubated with 140 $\mu$l of 10% actin dissolved in 15 mM Tris–HCl (pH 7.4) and test compounds dissolved in 0.5% DMSO for 5 min, and further with 40 $\mu$l of z-Phe-Arg-MCA (0.5 mM) for 5 min. The enzyme activity was then measured with an autoanalyzer (COBAS BIO, Roche). The kinin-forming enzyme activity was expressed in arbitrary units (%) with the standard human plasma enzyme activity taken as 100.

Kininase Activity: Seven-week-old male Wistar rats weighing about 220 g were used. The blood sample was collected into a polyethylene syringe containing 3.8% sodium citrate (1/10 volume) and immediately centrifuged at 3000 rpm for 15 min at 0 °C. The plasma sample was diluted with ice-cold phosphate buffer (0.1 M, pH 7.4) to a concentration of 1/20, as the kininase preparation. This preparation was incubated with 0.1 ml of soybean trypsin inhibitor (2 mg/ml), 0.4 ml of test compound dissolved in 0.5% DMSO (as final concentration) and 1 ml of bradykinin ($10^{-6}$ g/ml) for 20 min at 37 °C. After the incubation, a 2-fold volume of ice-cold methanol was added and the residual bradykinin was determined by radioimmunoassay as described previously. The kininase activity was taken as 100% when the bradykinin was consumed completely by this enzyme.

**Statistical Analysis** Student's *t* test was employed statistical analysis of the results.

## Results

**Effect on Carrageenin-Induced Paw Edema in Adrenalectomized Rats** EB-382 (2—50 mg/kg, *p.o.*) showed a dose-dependent inhibitory effect on the carrageenin-induced edema in bilaterally adrenalectomized rats (Table I). EB-382 had almost the same potency in both adrenalectomized and sham-operated rats.

**Effect on the Prostaglandin $E_2$ Biosynthesis of 3T6 Fibroblast Cells *in Vitro*** EB-382 (1—10 $\mu$M) exhibited a dose-dependent inhibitory effect on the prostaglandin $E_2$ biosynthesis from arachidonic acid in 3T6 fibroblast cells *in vitro* (Table II). The potency of EB-382 was lower than that of ibuprofen.

**Effect on Carrageenin-Induced Pleurisy in Rats** EB-382 (1—30 mg/kg, *p.o.*) exhibited a dose-dependent inhibitory effect on the carrageenin-induced pleural exudate volume in rats (Table III). The potency of EB-382 was about 4 times higher than that of ibuprofen.

**Effect on Bradykinin Content of Pleural Exudate** EB-382 (0.3—3mg/kg, *p.o.*) exhibited a dose-dependent and significant inhibitory effect on the bradykinin content released into the pleural exudate induced by carrageenin. The potency of EB-382 was higher than that of ibuprofen and indomethacin (Table IV).

**Effect on Prostaglandin Content of Pleural Exudate** EB-382 (1—30 mg/kg, *p.o.*) exhibited a dose-dependent and significant inhibitory effect on the production of prostaglandin $E_2$, 6-keto-prostaglandin $F_{1\alpha}$, prostaglandin $F_{2\alpha}$ and thromboxane $B_2$ into the pleural exudate induced by carrageenin. Its potency was higher than that of ibuprofen (Table V).

**Effect on Leukocyte Count in Pleural Exudate** EB-382 (1—30 mg/kg, *p.o.*) exhibited a dose-dependent and significant inhibitory effect on the counts of leukocytes emigrating into the pleural exudate. Its potency was much higher

TABLE I. Effect of EB-382 on Carrageenin-Induced Hind Paw Edema in Rats

| Models | Compounds | Dose (mg/kg, *p.o.*) | *N* | Inhibition (%) |
|---|---|---|---|---|
| Normal | EB-382 | 2 | 8 | 14.6 |
| | | 10 | 8 | 33.0 |
| | | 50 | 8 | 50.1 |
| | Ibuprofen | 50 | 8 | 43.9 |
| Sham-operated | EB-382 | 2 | 8 | 12.8 |
| | | 10 | 8 | 31.5 |
| | | 50 | 8 | 48.7 |
| | Ibuprofen | 50 | 8 | 45.8 |
| Adrenalectomized | EB-382 | 2 | 8 | 16.5 |
| | | 10 | 8 | 33.8 |
| | | 50 | 8 | 48.7 |
| | Ibuprofen | 50 | 8 | 40.9 |

TABLE II. Effect of EB-382 on the Prostaglandin Biosynthesis of 3T6 Mouse Fibroblast Cells *in Vitro*

| Compounds | *N* | $IC_{50}$ ($\mu$M) |
|---|---|---|
| EB-382 | 8 | 3.06±0.79 |
| Ibuprofen | 8 | 1.39±0.30 |
| Indomethacin | 8 | 0.05±0.07 |

Each value represents the mean±S.E. The $IC_{50}$ value was calculated graphically from the dose–response curve of inhibition.

TABLE III. Effect of EB-382 on Carrageenin-Induced Pleurisy in Rats

| Compounds | Dose (mg/kg, *p.o.*) | *N* | Pleural exudate (ml) | Inhibition (%) |
|---|---|---|---|---|
| Control | | 7 | 0.77±0.06 | |
| EB-382 | 1 | 7 | 0.55±0.04[a] | 28.2 |
| | 3 | 7 | 0.54±0.04[a] | 30.4 |
| | 10 | 7 | 0.37±0.05[a] | 50.5 |
| | 30 | 7 | 0.26±0.03[a] | 65.7 |
| Ibuprofen | 10 | 7 | 0.73±0.06 | 5.4 |
| | 30 | 7 | 0.50±0.06[a] | 35.1 |
| | 100 | 7 | 0.18±0.04[a] | 76.8 |
| Indomethacin | 1 | 7 | 0.75±0.05 | 2.6 |
| | 3 | 7 | 0.37±0.06[a] | 50.8 |
| | 10 | 7 | 0.19±0.03[a] | 72.1 |

Each value represents the mean±S.E. *a*) Significantly different from the control at $p<0.01$.

TABLE IV. Effect of EB-382 on Carrageenin-Induced Release of Bradykinin into the Rat Pleural Cavity

| Compounds | Dose (mg/kg, *p.o.*) | *N* | Bradykinin (pg/rat) | Inhibition (%) |
|---|---|---|---|---|
| Sham-operation | | 6 | 18.5± 4.1 | |
| Control | | 15 | 512.8± 41.4 | |
| EB-382 | 0.3 | 8 | 312.0± 49.9[a] | 39.2 |
| | 1 | 9 | 278.6± 25.9[a] | 45.6 |
| | 3 | 8 | 236.3± 56.9[a] | 54.0 |
| Ibuprofen | 1 | 8 | 694.5±111.8 | −18.3 |
| | 3 | 9 | 635.1±129.5 | −23.8 |
| | 10 | 8 | 418.7± 64.1 | 18.3 |
| Indomethacin | 0.3 | 8 | 589.7±150.3 | −15.0 |
| | 1 | 7 | 427.8± 54.5 | 16.6 |
| | 3 | 8 | 374.8± 50.9 | 26.9 |
| Bromelain[b] | 10 | 6 | 41.3± 10.4 | 91.9 |

Each value represents the mean±S.E. *a*) Significantly different from the control at $p<0.05$. *b*) Bromelain was injected intravenously.

TABLE V. Effects of EB-382 and Reference Drugs on Carrageenin-Induced Release of Prostaglandins into the Rat Pleural Cavity

| Compounds | Dose (mg/kg, *p.o.*) | *N* | $PGE_2$ | | $PGF_{2\alpha}$ | | 6-keto-$PGF_{1\alpha}$ | | $TXB_2$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | (ng/rat) | Inhibition (%) | (ng/rat) | Inhibition (%) | (ng/rat) | Inhibition (%) | (ng/rat) | Inhibition (%) |
| Control | | 7 | 11.2±3.4 | | 3.7±0.3 | | 55.1±10.9 | | 17.0±2.4 | |
| EB-382 | 1 | 7 | 7.3±0.9 | 34.8 | 3.4±0.2 | 8.1 | 47.2± 5.5 | 14.3 | 18.2±2.3 | −7.1 |
| | 3 | 7 | 4.4±0.7 | 60.7 | 2.0±0.3[b] | 45.9 | 18.2± 4.1[b] | 67.0 | 7.4±1.2[b] | 56.5 |
| | 10 | 7 | 4.3±0.5 | 61.6 | 2.4±0.2[b] | 35.1 | 19.1± 5.3[a] | 65.3 | 7.0±1.3[b] | 58.8 |
| | 30 | 7 | 1.6±0.2 | 85.7 | 1.5±0.1[b] | 59.5 | 5.9± 0.5[b] | 89.3 | 2.4±0.4[b] | 85.9 |
| Ibuprofen | 10 | 7 | 6.3±0.5 | 43.8 | 3.0±0.2 | 18.9 | 60.5±19.8 | −9.8 | 13.6±2.2 | 20.0 |
| | 30 | 7 | 1.9±0.3 | 83.0 | 1.3±0.1[b] | 64.9 | 6.6± 1.6[b] | 88.0 | 2.3±0.4[b] | 86.5 |
| | 100 | 7 | 1.2±0.2 | 89.3 | 1.3±0.1[b] | 64.9 | 4.7± 0.9[b] | 91.5 | 1.5±0.4[b] | 91.2 |
| Indomethacin | 1 | 7 | 3.0±0.5[a] | 73.2 | 2.0±0.2[b] | 45.9 | 18.7± 5.3[a] | 66.1 | 9.3±2.6 | 45.3 |
| | 3 | 7 | 0.8±0.1[b] | 92.9 | 1.3±0.1[b] | 64.9 | 4.8± 1.0[b] | 91.3 | 2.1±0.6[b] | 87.6 |
| | 10 | 7 | 0.6±0.1[b] | 94.6 | 1.1±0.1[b] | 70.3 | 2.5± 0.6[b] | 95.5 | 1.0±0.3[b] | 94.1 |

$PGE_2$, prostaglandin $E_2$; $PGF_{1\alpha}$, prostaglandin $F_{1\alpha}$; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; $TXB_2$, thromboxane $B_2$. Each value represents the mean±S.E. *a, b*) Significantly different from the control at $p<0.05$ and $p<0.01$, respectively.

TABLE VI. Effect of EB-382 on Leukocytes Emigration into the Rat Pleural Cavity Induced by Carrageenin

| Compounds | Dose (mg/kg, *p.o.*) | *N* | Leukocytes (counts × $10^8$/rat) | Inhibition (%) |
|---|---|---|---|---|
| Control | | 7 | 2.48±0.25 | |
| EB-382 | 1 | 6 | 2.34±0.18 | 5.6 |
| | 3 | 6 | 1.99±0.24 | 19.8 |
| | 10 | 6 | 1.84±0.20 | 25.8 |
| | 30 | 7 | 1.15±0.21[a] | 53.6 |
| Ibuprofen | 10 | 7 | 2.54±0.32 | −2.4 |
| | 30 | 5 | 2.51±0.50 | −1.2 |
| | 100 | 7 | 1.59±0.20 | 35.9 |
| Indomethacin | 1 | 7 | 2.58±0.38 | −4.0 |
| | 3 | 6 | 2.41±0.24 | 2.8 |
| | 10 | 7 | 1.30±0.22[a] | 47.6 |

Each value represents the mean±S.E. *a*) Significantly different from the control at $p<0.01$.

TABLE VII. Effect of EB-382 on Rat Plasma Kinin-Forming Enzyme and Kininase Activities *in Vitro*

| Compounds | Dose | Kinin-forming enzyme activity (%) | Kininase activity (%) |
|---|---|---|---|
| Control | | 65.4±0.93[a] | 52.7± 4.48[b] |
| EB-382 | $10^{-6}$ M | 64.8±1.16 | 50.6± 5.18 |
| | $10^{-5}$ M | 64.8±0.88 | 52.5±11.13 |
| | $10^{-4}$ M | 63.6±2.68 | 52.9± 4.64 |
| | $10^{-3}$ M | 66.3±1.94 | 39.3±10.70 |
| Ibuprofen | $10^{-3}$ M | 67.9±2.88 | 30.6± 6.00[d] |
| Indomethacin | $10^{-3}$ M | 59.4±1.98[c] | 56.0± 5.26 |
| Soybean trypsin inhibitor | $10^{-6}$ g/ml | 13.6±0.66[d] | — |
| 1,10-Phenanthroline | $10^{-4}$ M | — | 7.8± 4.91[d] |

Each value represents the mean±S.E. of 3—5 experiments. *a*) Arbitrary units (%): the standard plasma enzyme activity was taken as 100. *b*) The kininase activity was taken as 100% when the bradykinin was consumed completely by this enzyme. *c, d*) Significantly different from the control at $p<0.05$ and $p<0.01$, respectively.

than that of ibuprofen, and was almost equal to that of indomethacin (Table VI).

**Effect on Kinin-Forming Enzyme and Kininase Activities of Rat Plasma *in Vitro*** EB-382 ($10^{-6}$—$10^{-3}$ M) did not exert any significant effect on the prekallikrein and kininase activities of rat plasma *in vitro* (Table VII). Among the test drugs, indomethacin exhibited a weak but significant inhibitory effect on the prekallikrein activity at a high dose ($10^{-3}$ M).

## Discussion

EB-382 revealed a dose-dependent inhibitory effect on carrageenin-induced paw edema in rats. The antiinflammatory effect of EB-382 on the paw edema induced by carrageenin was stronger than that of ibuprofen. It was concluded that the antiinflammatory effect of EB-382 was not due to an indirect effect *via* stimulation of the adrenals, since adrenalectomy did not influence the effect of the drug.

In our previous experiments,[2,3] EB-382 showed a weak inhibitory effect on the prostaglandin biosynthesis of sheep seminal vesicle microsomes *in vitro* in spite of its potent antiinflammatory and analgesic effects. Indeed, EB-382 also displayed a weak inhibitory effect on the prostaglandin biosynthesis of 3T6 mouse fibroblast cells *in vitro* in the present experiments. It is assumed therefore that EB-382 acts through different mechanisms from those of other antiinflammatory drugs such as aspirin, ibuprofen and indomethacin.

It has been clearly shown that carrageenin-induced pleurisy in rats is closely related to the kallikrein–kinin system at the early inflammatory stage.[7,10,11] The carrageenin-induced pleural exudation through an increase in vascular permeability has been attributed to the bradykinin released by activation of the Hageman factor followed by activation of kallikrein.[12,13] The bradykinin produced causes release of prostaglandins, and the released prostaglandins amplify the bradykinin-induced exudation in the pleural cavity.[7,12] EB-382 exerted a marked inhibitory effect on the pleural exudation induced by carrageenin in rats, and its potency was much higher than that of ibuprofen. Furthermore, EB-382 displayed a significant inhibitory effect on the release of bradykinin into the pleural cavity, and the potency of EB-382 appeared to be higher than that of ibuprofen and indomethacin. EB-382 did not affect the prekallikrein and kininase activities of rat plasma *in vitro*. Based on these results, it was inferred that EB-382 does not influence the synthesis and degradation process of bradykinin in the plasma, but affects some stage of the release process of the mediator from the plasma leaking into the inflammatory

site. It was assumed therefore that bradykinin was intimately involved in the antiinflammatory effect of EB-382.

EB-382 showed a much more potent inhibition of the release of prostaglandin $E_2$, 6-keto-prostaglandin $F_{1\alpha}$, prostaglandin $F_{2\alpha}$ and thromboxane $B_2$ into the pleural cavity than did ibuprofen. In addition, EB-382 exhibited a dose-dependent inhibitory effect on the emigration of leukocytes into the pleural cavity at a relatively low dose, although its potency was almost equal to that of indomethacin.

It is well known that emigrating leukocytes release several arachidonic acid metabolites into inflamed tissue.[13] EB-382 was assumed therefore to suppress effectively the production of prostaglandins, derived from the leukocytes emigrating into the inflammatory sites. It has been shown that in inflamed tissue, emigrating leukocytes can lead to aggravation of the prognosis at the chronic stage on inflammation due to the release of lysosomal enzymes. Accelerated emigration of leukocytes has been reported to play an important role especially in the chronic stage of inflammation,[14] and reduction of the lysosomal enzyme activity in adjuvant arthritis is therefore clearly related to the antirheumatoid activity of antiinflammatory drugs.[15] We found previously that EB-382 exhibited an excellent inhibitory effect on edema and lysozyme activity in an adjuvant arthritis model,[2] and its potency was higher than that of ibuprofen which revealed only a weak inhibitory activity on leucocyte emigration in the present study.

From the above results, it is suggested that the antiinflammatory effect of EB-382 was probably due to inhibition of the production of bradykinin and prostaglandins derived from emigrating leukocytes in the local inflamed tissues in addition to a weak inhibitory effect on prostaglandin biosynthesis. The above findings indicate that EB-382 has some characteristic pharmacological effects which differ from those of other acidic antiinflammatory drugs, especially substances such as ibuprofen.

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