Pharmacological Profile of Alminoprofen among Four Writhing Models of Mice Caused by Kaolin, Zymosan, Acetylcholine and Phenylquinone

Toshio FUJIYOSHI,* Hiroyuki IIDA, Miho KUWASHIMA, Masaharu DOZEN, Nobuhiro TANIGUCHI, and Kenro IKEDA

Pharmaceuticals Research Laboratories, Fujirebio Inc., 51 Komiya-cho, Hachioji, Tokyo 192, Japan

(Received July 4, 1989)

The effects of alminoprofen and other non-steroidal antiinflammatory drugs (NSAIDs) on the writhing reaction caused by kaolin, acetylcholine, phenylquinone and zymosan were studied. Aspirin, indomethacin, ibuprofen and diclofenac-Na, as cyclooxygenase inhibitors, showed similar potency ratios on four writhing tests, although, alminoprofen exhibited a somewhat rather higher potency ratio on kaolin- and zymosan-induced writhing models than on acetylcholine- and phenylquinone-induced writhing models. All NSAIDs, cyclooxygenase inhibitors except alminoprofen showed similar shapes in illustrations of potency ratio when the potency of aspirin was expressed as 1.0. The potency of alminoprofen produced a figure unlike those of other cyclooxygenase inhibitors. These results suggest that alminoprofen has a different pharmacological profile from other general NSAIDs in terms of analgesic action. This combination method with potency ratios for writhing reactions caused by the above four inducers could be a simple method for classification of pharmacological profiles of the analgesic actions of NSAIDs.

Keywords — alminoprofen; analgesic effect; writhing; kaolin; zymosan

Introduction

(±)-2-[*p*-[(2-Methylallyl)amino]phenyl]-propionic acid (alminoprofen), a new non-steroidal antiinflammatory drug (NSAID) was selected from a series of phenylpropionic acid derivatives in which an amino group is introduced into the structure of ibuprofen on the basis of its potent analgesic and antiinflammatory action within a wide safety margin.¹⁾ In previous reports, authors have described that the analgesic and antiinflammatory activities of alminoprofen are more potent than those of ibuprofen.^{2,3)} In addition, it was found that several of the pharmacological properties of alminoprofen differed from those of other acidic NSAIDs such as aspirin, indomethacin and ibuprofen.^{2–5)} In this present study, we examined the potency of NSAIDs, including alminoprofen, on the writhing reaction evoked by four different inducers, and demonstrated the classification of pharmacological profiles comparing alminoprofen with other NSAIDs.

Materials and Methods

1. Animals — Male ICR mice (aged 5 weeks and weighing about 20 g) were purchased from Shizuoka Laboratory Center (Hamamatsu).

2. Reagents — The reagents used were kaolin (Wako), zymosan (Sigma), phenylquinone (PQ; Sigma), and acetylcholine hydrochloride (ACh; Dainippon Pharmaceuticals). The drugs used were alminoprofen (Bouchara), ibuprofen (Sigma), diclofenac-Na (Ciba Geigy), aspirin (Sigma) and indomethacin (Sigma). All other reagents used were of the highest quality commercially available. All test drugs were suspended in 5% gum arabic solution before administration.

3. Procedures — All drugs were administered orally 1 h before the intraperitoneal injection of ACh (1.4 mg/0.2 ml/mouse), PQ (60 µg/0.2 ml/mouse), kaolin (2.5 mg/0.5 ml/mouse) or zymosan (1 mg/0.5 ml/mouse) into mice (*N* = 10–15). ACh-, PQ- and zymosan-induced writhing models were produced by the methods described by Amanuma,⁶⁾ Siegmund⁷⁾ and Doherty,⁸⁾ respectively. The kaolin-induced

* To whom correspondence should be addressed.

TABLE I. Effects of Alminoprofen and Other Non-steroidal Antiinflammatory Drugs on the Kaolin-, Acetylcholine-, Phenylquinone- and Zymosan-Induced Writhing Reaction in Mice

Compounds	Kaolin		Acetylcholine		Phenylquinone		Zymosan	
	ED ₅₀ (mg/kg) (95% C.L. ^{a)})	Potency ratio	ED ₅₀ (mg/kg) (95% C.L. ^{a)})	Potency ratio	ED ₅₀ (mg/kg) (95% C.L. ^{a)})	Potency ratio	ED ₅₀ (mg/kg) (95% C.L. ^{a)})	Potency ratio
Alminoprofen	0.76 (0.37–1.56)	61.1	2.76 (1.31–5.80)	8.8	12.1 (7.28–20.2)	3.9	0.22 (0.10–0.47)	22.3
Indomethacin	0.41 (0.22–0.75)	113.2	0.17 (0.09–0.31)	142.3	0.59 (0.28–1.22)	80.5	0.11 (0.07–0.16)	44.5
Ibuprofen	4.09 (2.24–7.47)	11.3	2.15 (1.33–3.50)	11.3	16.7 (9.33–30.0)	2.8	3.37 (1.90–5.97)	1.5
Aspirin	46.4 (24.2–89.1)	1.0	24.2 (13.5–43.3)	1.0	47.5 (24.0–93.9)	1.0	4.90 (2.60–9.30)	1.0
Diclofenac sodium	0.77 (0.29–2.07)	60.3	0.29 (0.17–0.50)	83.4	0.86 (0.40–1.83)	55.2	0.38 (0.20–0.72)	12.9

All drugs were administered orally 1 h before intraperitoneal injection of 0.5 ml of kaolin (5 mg/ml saline), 0.2 ml of acetylcholine (0.7% in saline), phenylquinone (0.03% in 5% ethanol) or zymosan (2 mg/ml saline) into mice ($N = 10-15$). Writhes were counted for 10 min after kaolin or acetylcholine injection. Writhes were counted for 30 min after zymosan injection. Writhes caused by phenylquinone were counted in the 5–20 min period following injection. The ED₅₀ value and ^{a)} 95% confidence limits were calculated by the method of Litchfield and Wilcoxon. Potency ratio of each drug is calculated as aspirin = 1.0.

writhing model was produced by the method described in previous reports.^{9,10)} Writhes were counted for 10 min after ACh or kaolin injection, and writhes caused by zymosan were counted for 30 min after injection. Writhes caused by PQ were counted in the 5–20 min period following injection. When the number of writhes were 50% or less than that of the vehicle control group, the dose was considered to be effective. ED₅₀ values of the drugs and their 95% confidence limits were calculated from the effective ratio (%) according to the method of Litchfield and Wilcoxon.¹¹⁾

Results and Discussion

The effect of NSAIDs on the writhing reaction caused by ACh, PQ, kaolin and zymosan were studied. All inducers used caused clear and reproducible writhing reactions to mice, and the mean number of writhes in vehicle control groups were 7.4, 8.0, 7.9 and 26.5 on kaolin, zymosan, ACh and PQ models, respectively. All NSAIDs tested exhibited dose-dependent inhibitory effects on writhing reactions induced by four different inducers. Although, in comparison with potency ratios of NSAIDs in each

TABLE II. Potency Index of Alminoprofen and Other Non-steroidal Antiinflammatory Drugs on the Kaolin-, Zymosan- and Phenylquinone-Induced Writhing Reaction in Comparison with Those on the Acetylcholine-Induced Writhing Reaction

Compounds	Potency index ^{a)}		
	Kaolin	Zymosan	Phenylquinone
Alminoprofen	3.63	12.6	0.23
Indomethacin	0.41	1.55	0.29
Ibuprofen	0.53	0.64	0.13
Aspirin	0.52	4.94	0.51
Diclofenac-Na	0.38	0.76	0.34

Each value shows the potency index of each drug on the writhing models caused by kaolin, zymosan and phenylquinone.
^{a)} Potency index: ED₅₀ value on the acetylcholine model/ED₅₀ value on the each other model.

model when the potency of aspirin was expressed as 1.0, alminoprofen appeared to have a higher potency ratio in the kaolin and zymosan models in comparison with those on the other models. On the other hand, ibuprofen appeared to have a somewhat higher potency in kaolin and ACh models than in the PQ and zymosan models, and diclofenac-Na appeared to have a somewhat higher potency on the kaolin, ACh

and PQ models than on the zymosan model, in comparisons of the potency on each model (Table I).

It has been reported that antiwrithing effects of NSAIDs on the ACh-induced writhing reaction is highly correlated with the inhibitory effect on cyclooxygenase, and the PQ-induced writhing reaction is similar to that of the ACh model.⁶⁾ When the ED₅₀ values of each NSAID

Fig. 1. Pharmacological Profiles of Alminoprofen and Other Drugs among Four Writhing Models Caused by Kaolin, Zymosan, Acetylcholine and Phenylquinone

Each point of figure indicates the potency ratio when the potency of aspirin was expressed as 1.0.

on the kaolin-, zymosan- and PQ-induced writhing models were compared with those on the ACh-induced writhing model, alminoprofen exhibited characteristically higher potency on the kaolin and zymosan models compared with that on the PQ model, and aspirin and indomethacin exhibited somewhat higher potency on the zymosan model than that in the other models. Ibuprofen and diclofenac-Na did not exhibit any characteristic potencies in the zymosan model (Table II). This result seems to show that the mode of analgesic action of alminoprofen is different from those of the others (Table II).

The kaolin-induced writhing reaction is highly related with bradykinin produced by activation of the kallikrein-kinin system, as a trigger of pain reaction.^{8,9,12)} Further, the zymosan-induced writhing reaction could indicate that activation of the complement system and the subsequent release of prostaglandin I₂ caused by zymosan play a major role in the writhing reaction.⁸⁾ Therefore, based on the above results, it was suggested that alminoprofen had characteristic effects on the inflammatory pain involved in the activation of kallikrein-kinin and complement systems. This result was in good agreement with previous reports,²⁻⁵⁾ in that alminoprofen exhibited a potent inhibitory effect on experimental models of inflammation involving activation of the kallikrein-kinin and complement systems.

Further, a comparison of pharmacological profiles of NSAIDs including alminoprofen was attempted by using illustrations of each potency ratio when the potency of aspirin as a typical cyclooxygenase inhibitor was expressed as 1.0. It is well known that acidic NSAIDs such as aspirin, indomethacin, ibuprofen and diclofenac-Na have a potent inhibitory effect on cyclooxygenase as the main mechanism of their antiinflammatory and analgesic actions.¹³⁻¹⁵⁾ On the other hand, we recently reported that alminoprofen was a weak inhibitor of cyclooxygenase with some other different mechanism of action.^{4,5)} In comparison with the illustrations of potency ratios on four writhing tests, all NSAIDs as potent cyclooxygenase inhibitors except for alminoprofen showed similar illustrations of potency ratios. Even ibuprofen and

diclofenac-Na, which expressed a somewhat lower potency ratio on the zymosan model, also showed similar illustrations. On the other hand, the representation of alminoprofen was shown to have a shape apparently different from those of other potent cyclooxygenase inhibitors (Fig. 1).

These results suggest that the pharmacological profile and mode of analgesic action of alminoprofen are apparently different from other acidic NSAIDs. The above results suggest that the combination method of the four writhing models in mice caused by different inducers, kaolin, zymosan, ACh and PQ, might be a simple method of classifying the pharmacological properties of NSAIDs in terms of their analgesic action.

It is well known that even kallikrein-kinin and complement systems play an important role in inflammatory reactions, besides the system of prostaglandin biosynthesis.^{16,17)} Accordingly, the mechanism of inflammatory pain is complicated. Therefore, antiinflammatory drugs possessing multiple modes of action in addition to the inhibition of cyclooxygenase seemed to be useful for more effective treatment of inflammatory disease. Actually, alminoprofen expressed more efficacy than that of ibuprofen, as cyclooxygenase inhibitor, in the clinical treatment of inflammatory disease.¹⁸⁾

As stated above, this combination method may be a clue to a simple method for the classification of pharmacological profiles of NSAIDs and the subsequent presumption of their efficacy in the treatment of inflammatory disease, through assessment of their various mode of analgesic action in four different models. However, it is necessary that the pharmacological profiles of many types of NSAIDs be fully compared in further studies using this combination method in order to establish more reliability and value. Comparative studies of various NSAIDs with pharmacological profiles using this experimental model and their efficacy in clinical treatment are needed for patient management in the future.

Acknowledgements We wish to thank prof. Yutaka Mizushima, Department of Inter-

nal Medicine, St. Marianna University, School of Medicine, and Prof. Sachiko Oh-ishi, Department of Pharmacology, Kitasato University, School of pharmaceutical Sciences, for their helpful discussions and advice.

References

- 1) B. Dumaitre, A. Fouquet, C. Perrin, P. Cornu, A. Boucherle, C. Portka, G. Domage and G. Streichenberger: Synthèse de quelques dérivés des acides amino-4 phénylacétique et (amino-4-phényl)-2 propionique possédant des activités analgésiques et anti-inflammatoires, *Eur. J. Med. Chem.*, **14**, 207 (1979).
- 2) T. Fujiyoshi, K. Ikeda, M. Saito, T. Yamaura, H. Iida, E. Maeda, N. Kase and T. Uematsu: The antiinflammatory effects of EB-382, *Nippon Yakurigaku Zasshi*, **87**, 379–395 (1986).
- 3) T. Fujiyoshi, H. Iida, M. Saito, K. Ikeda and T. Uematsu: The analgesic and antipyretic effects of non-steroidal antiinflammatory agent, EB-382, in experimental animals, *Nippon Yakurigaku Zasshi*, **87**, 527–536 (1986).
- 4) T. Fujiyoshi, H. Iida, M. Murakami, M. Kuwashima and T. Uematsu: Pharmacological studies of EB-382, a new non-steroidal antiinflammatory agent: Mode of action of antiinflammatory effects, *Chem. Pharm. Bull.*, **37**, 771–774 (1989).
- 5) T. Fujiyoshi, H. Iida, M. Murakami, M. Kuwashima and T. Uematsu: Pharmacological studies of EB-382, a new non-steroidal antiinflammatory agent: Mode of action of analgesic effects, *Chem. Pharm. Bull.*, **37**, 775–777 (1989).
- 6) F. Amanuma, C. Wakaumi, M. Tanaka, M. Muramatsu and H. Aihara: The analgesic effects of non-steroidal anti-inflammatory drugs on acetylcholine-induced writhing in mice, *Nippon Yakurigaku Zasshi*, **84**, 543–551 (1984).
- 7) E. A. Siegmund, R. A. Anderson, and E. J. DeBeer: A method for evaluating both narcotic and non-narcotic analgesics, *Proc. Soc. Exp. Biol. Med.*, **95**, 729–731 (1957).
- 8) N. S. Doherty, T. H. Beaver, K. Y. Chan, J. E. Coutant and G. L. Westrich: The role of prostaglandins in the nociceptive response induced by intraperitoneal injection of zymosan in mice, *Br. J. Pharmacol.*, **91**, 39–47 (1987).
- 9) T. Fujiyoshi, M. Kuwashima, H. Iida and T. Uematsu: A new writhing model of factor XII activator-induced pain for assessment of non-steroidal antiinflammatory agents. Kaolin-induced writhing in mice, *J. Pharmacobio-Dyn.*, **12**, 132–136 (1989).
- 10) T. Fujiyoshi, I. Hayashi, S. Oh-ishi, M. Kuwashima, H. Iida, N. Taniguchi, M. Dozen, K. Ikeda and H. Ohnishi: Kaolin-induced writhing in mice, A new model of possible bradykinin-induced pain for assessment of analgesic agents, *Agents Actions*, **27**, 332–334 (1989).
- 11) J. T. Litchfield and F. Wilcoxon: A simple method of evaluating dose-effect experiments, *J. Pharmacol. Exp. Ther.*, **96**, 99–113 (1949).
- 12) S. H. Ferreira and J. R. Vane: Aspirin and prostaglandins, "The prostaglandins," vol. 2, ed. by Ramwell, Plenum Press, New York and London, 1974, pp. 1–48.
- 13) R. J. Flower: Drugs which inhibit prostaglandin biosynthesis, *Pharmacol. Rev.*, **26**, 33–67 (1974).
- 14) S. H. Ferreira: Prostaglandins, "Handbook of inflammation," vol. 1, ed. by J. C. Houck, Elsevier, Amsterdam, 1979, pp. 113–151.
- 15) S. A. Mets: Antiinflammatory agents as inhibitors of prostaglandin synthesis in man, *Med. Clin. North. Amer.*, **65**, 713–757 (1981).
- 16) G. P. Lewis: Kinins in inflammation and tissue injury, *Handb. Exp. Pharmacol.*, **25**, 516–530 (1970).
- 17) M. Di Rosa, J. P. Giroud and D. A. Willoughby: Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenin and turpentine, *J. Pathol.*, **104**, 15–29 (1971).
- 18) H. Aritomi, T. Kageyama, S. Sugawara, W. Kawaji, M. Igarashi and T. Aoki: Clinical evaluation of EB-382 in the treatment of osteoarthritis of the knee. A double blind comparative study, *Rinsho Hyoka*, **14**, 723–756 (1986).