

Anti-inflammatory activity of (*E*)-1-(3,4-dimethoxyphenyl) butadiene from *Zingiber cassumunar* Roxb.

Rattima Jeenapongsa^{a,*}, Krongtong Yoovathaworn^b,
Kittima M. Sriwatanakul^b, Ubonwan Pongprayoon^c,
Kampon Sriwatanakul^b

^a Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences,
Naresuan University, Muang, Phitsanulok 65000, Thailand

^b Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand

^c Thailand Institute of Scientific and Technological Research, Bangkok, Thailand

Received 3 September 2002; received in revised form 22 February 2003; accepted 13 March 2003

Abstract

This study aimed to investigate the anti-inflammatory activity of (*E*)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD), isolated from *Zingiber cassumunar* Roxb., using in vivo and in vitro models. The results show that DMPBD dose-dependently inhibited the rat ear edema induced by ethyl phenylpropionate (EPP), arachidonic acid (AA) and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and it was more potent than any other standard drugs being used. In EPP-induced edema IC₅₀ of DMPBD and oxyphenbutazone were 21 and 136 nmol per ear, respectively. The IC₅₀ of DMPBD and phenidone were 60 and 2520 nmol per ear, respectively, in AA-induced edema whereas DMPBD was 11 times more potent than diclofenac in TPA-induced edema (IC₅₀ = 660 and 7200 pmol per ear, respectively). DMPBD and diclofenac inhibited the rat paw edema induced by carrageenan but not by platelet activating factor (PAF). In in vitro study DMPBD, aspirin and phenidone inhibited collagen-induced platelet aggregation with IC₅₀ of 0.35, 0.43 and 0.03 mM, respectively. Whereas IC₅₀ of these agents in ADP, AA and PAF inductions were 4.85, 3.98 and 1.30 mM; 0.94, 0.13 and 0.04 mM; and 1.14, 6.96 and 2.40 mM, respectively. These results indicate that DMPBD possesses a potent anti-inflammatory activity through the inhibition of CO and LO pathways and seems to have more prominent effects on the LO pathway.

© 2003 Published by Elsevier Science Ireland Ltd.

Keywords: *Zingiber cassumunar*; DMPBD; Anti-inflammatory activity; Platelet aggregation; Ear edema; Paw edema

1. Introduction

In many Asian countries, *Zingiber cassumunar* Roxb. (Zingiberaceae) is widely used in folklore remedies as a single plant or as a component of herbal recipes. Compounds with known chemical structures have been isolated from *Zingiber cassumunar* Roxb. Among these the hexane extract seemed to possess a potent anti-inflammatory activity. Compound D, isolated from the hexane extract, exhibited a strong inhibitory activity on the edema formation in carrageenan-induced rat paw edema. In rat pleurisy model, it exerted markedly inhibitory activity on the exudates formation, the accumulation of leukocytes and the

prostaglandin-like activity of the exudates (Panthong et al., 1990).

(*E*)-1-(3,4-Dimethoxyphenyl) butadiene (DMPBD) was isolated from the hexane extract of *Zingiber cassumunar* Roxb. by Thailand Institute of Scientific and Technological Research (TISTR). Its chemical structure is shown in Fig. 1. Preliminary studies suggested that DMPBD is an active ingredient of the essential oil derived from *Zingiber cassumunar* Roxb. Our in vivo preliminary study revealed the effect of DMPBD on both cyclooxygenase (CO) and lipoxygenase (LO) pathways. Therefore, this study aimed to characterize the anti-inflammatory activity of DMPBD using in vivo and in vitro models. Several inducers and standard drugs were employed so that possible mechanisms of action of DMPBD on arachidonic acid (AA) metabolic pathway could be postulated.

* Corresponding author. Tel.: +66-55-261000-4 Ext 3620;
fax: +66-55-261057.

E-mail address: rattimaj@yahoo.com (R. Jeenapongsa).

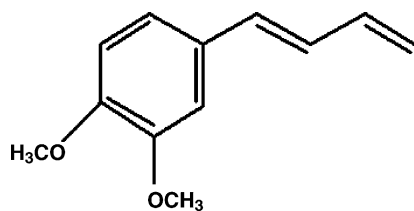


Fig. 1. Chemical structure of (*E*)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD).

The *in vivo* studies were carried using rat ear edema and rat paw edema models whereas platelet aggregation model was employed as an *in vitro* study.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, 50–70 and 120–130 g, were obtained from the National Laboratory Animal Center of Mahidol University. Before conducting any studies animals were acclimatized for 1 week at 24–26 °C with food and water *ad libitum*.

Adult male and female albino rabbits weighing 3–5 kg were supplied by the Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.

2.2. Materials

DMPBD was supplied by TISTR in the form of white crystal. Ethyl phenylpropionate (EPP), AA, 12-*O*-tetradecanoylphorbol 13-acetate (TPA), carrageenan, platelet activating factor (PAF), oxyphenbutazone, diclofenac, phenidone, salbutamol, collagen and adenosine diphosphate (ADP) were obtained from Sigma (St. Louis, MO). All other solvents and chemicals employed were of analytical grade. Compounds were prepared as solutions in acetone (reagent grade) just prior to application.

2.3. *In vivo* study

2.3.1. Rat ear edema

Ear edema was induced in male Sprague–Dawley rats weighing 50–70 g, according to the modified method of Brattsand et al. (1982) and Pongprayoon et al. (1991) with some modifications. The edema was induced by an application of a solution of EPP, AA or TPA in acetone at concentrations of 5, 10 and 0.02%, respectively. Each solution in volume of 0.01 ml was applied to each of inner and outer surfaces of both ears by means of an automatic micropipette.

For ear thickness determination, a dial caliper (no. 7039, Mitutoyo) was applied to the tip of the ear. In order to minimize technical variations among measurements, the same investigator performed the measurements throughout

the experiment. Prior to any treatment, ear thickness was determined as a basal value. In the EPP- and AA-induced ear edema, ear thickness was determined at 0.5, 1 and 2 h and 0.5, 1, 1.5, 2, 3 and 4 h after the treatment, respectively. In the TPA treated group, the thickness was determined at 2, 4, 6, 8, 10 and 12 h after the TPA application.

Drug was applied simultaneously with EPP or AA. Oxyphenbutazone or DMPBD was dissolved in the 5% EPP solution to obtain the final drug concentrations of 0.005, 0.05, 0.5 and 5%. In the AA treatment group, phenidone or DMPBD was dissolved in 10% AA solution to obtain the drug concentration of 5%.

In the TPA-induced edema, diclofenac was prepared in a mixture of ethanol and acetone (4:6) and DMPBD was prepared in acetone. The solution of diclofenac or DMPBD was applied on the ear at 1 h after the TPA application.

2.3.2. Rat paw edema

Paw edema was induced in male Sprague–Dawley rats weighing 120–130 g by subplantar injection of 0.1 ml carrageenan or PAF by a modified method described by Winter et al. (1962) with some modifications.

Carrageenan (1%, w/v) in 0.9% normal saline solution was injected into the left hind paw. Paw volume was measured before and at 1, 2, 3, 4, 5 and 6 h after the injection of carrageenan. Diclofenac or DMPBD was dissolved in acetone and 0.06 ml of the drug was applied over the upper and lower surfaces of the injected paw at 1 h after the carrageenan injection. Edema volume was determined plethysmographically at 1, 2, 3, 4, 5 and 6 h after the treatment.

PAF was dissolved in 0.9% normal saline to obtain a concentration of 8 µg/ml. Diclofenac and DMPBD were prepared at the same concentration as those used in the study induced by carrageenan. A solution of salbutamol (5%) was obtained by dissolving salbutamol in a mixture of methanol and acetone (1:1). Then 5% salbutamol was diluted with acetone to give the concentrations of 1, 0.5 and 0.05%. Drug solutions were applied over the upper and lower surfaces of the paw 30 min before the injection of PAF. Edema volume was determined at 0.5, 1, 1.5, 2 and 3 h after the PAF injection.

2.3.3. Platelet aggregation

2.3.3.1. Preparation of platelet suspension. Blood was taken from the central ear artery of the rabbits. An aliquot (9 ml) of blood sample was placed into a polystyrene tube containing 1 ml of 3.2% (w/v) sodium citrate as an anti-coagulant. Citrated blood was centrifuged at 160 × *g* for 10 min and the supernatant was removed as platelet rich plasma (PRP). The remaining blood was further centrifuged at 4000 × *g* for 10 min to obtain the supernatant as platelet-poor plasma (PPP). Platelet concentration of the PRP was adjusted to 3 × 10⁵ to 5 × 10⁵/ml by diluting with the PPP as required. Platelet aggregation tests were performed during 30 min and 3 h after the blood had been drawn.

2.3.3.2. Preparation of chemical reagents. Collagen and ADP were dissolved in distilled water at the concentration of 10 g/ml and 0.2 mM, respectively. AA was dissolved in a small volume of absolute ethanol (not higher than 5% in final concentration) and then adjusted with 0.9% normal saline to obtain the final concentration of 12.38 mM. PAF was prepared by evaporating the solution of PAF in chloroform under nitrogen gas and reconstitute with normal saline to achieve a final concentration of 10 mg/ml.

Aspirin was dissolved in distilled water at the concentrations of 1 and 5 mg/ml. Phenidone (1%, w/v) was prepared by dissolving the drug in propylene glycol (60% in final concentration) and then the final volume was adjusted with normal saline. DMPBD was dissolved in propylene glycol (40% in final concentration) and adjusted the volume with normal saline.

2.3.3.3. Platelet aggregation study. PRP (450 μ l) was placed into siliconized glass cuvettes. The aggregating agent was added into the stirred platelet suspension to induce platelet aggregation. When studying the inhibitory effects of aspirin, phenidone and DMPBD on platelet aggregation induced by these four agents, the studied compound was added into the stirred PRP 2 min before the addition of the aggregating agent. The extent of platelet aggregation was shown as a percentage of change in light transmission through the cuvette comparing with that of PPP suspension. The inhibitory effect was observed as the decrease in light transmission comparing with that observed in the aggregation without an inhibitor. Absence of platelet aggregation was defined by having no changes in light transmission after the period of 5 min.

3. Results

3.1. DMPBD inhibits ear edema induced by EPP, AA and TPA

Topical application of 5% EPP produced ear swelling within 30 min. Erythema was also observed. The maximum effect of EPP was at 1 h, thereafter the ear swelling decreased

gradually. DMPBD and oxyphenbutazone when applied concomitantly with 5% EPP concentration-dependently inhibited the EPP-induced edema formation with IC_{50} of 21 and 136 nmol per ear, respectively (Table 1). DMPBD was about six times more potent than oxyphenbutazone.

AA produced a rapid edema formation with a maximum effect at 30–60 min and the edema then gradually decreased. DMPBD and phenidone exhibited concentration-related inhibitory profile with the maximal activity at 30 min after the topical administration. DMPBD (IC_{50} of 60 nmol per ear) was much more potent than phenidone (IC_{50} 2520 nmol per ear) in inhibiting AA-induced ear edema in rats.

TPA-induced edema formation was clearly observed at 2 h after the topical application and reached its maximum effect at 8 h after the application. DMPBD and diclofenac applied 1 h after TPA application inhibited ear edema with the maximum activity at 2 h. DMPBD (IC_{50} of 660 pmol per ear) was approximately 11 times more potent than diclofenac (IC_{50} of 7200 pmol per ear) in inhibiting TPA-induced ear edema.

3.2. DMPBD inhibits paw edema induced by carrageenan but not by PAF

The injection of carrageenan resulted in paw edema within 1 h and the maximum effect was reached at 3 h. This reaction was inhibited by topical application of diclofenac in a concentration-dependent fashion with an IC_{50} at 3 h of 14 μ mol per paw. The topical application of DMPBD also showed a concentration-dependent effect with an IC_{50} at 3 h of 22 μ mol per paw.

The subplantar injection of PAF into a rat hind paw rapidly induced edema formation. The topical application of diclofenac or DMPBD showed no significant inhibition on PAF-induced paw edema. However, salbutamol significantly inhibited the PAF-induced paw edema in a dose-related manner with IC_{50} of about 1.9%.

3.3. DMPBD inhibits platelet aggregation induced by collagen, ADP, AA and PAF

Collagen-induced platelet aggregation in a concentration-dependent manner with a lag period of 2–3 min.

Table 1
Inhibitory effects of DMPBD and standard drugs on rat ear edema and rat paw edema. Data are expressed as IC_{50} values

	DMPBD	IC_{50}			
		Oxyphenbutazone	Phenidone	Diclofenac	Salbutamol
Ear edema					
EPP	21 nmol/ear	136 nmol/ear	–	–	–
AA	60 nmol/ear	–	2520 nmol/ear	–	–
TPA	660 pmol/ear	–	–	7200 pmol/ear	–
Paw edema					
Carrageenan	22 μ mol/paw	–	–	14 μ mol/paw	–
PAF	No effect	–	–	No effect	1.9%

– is non-applicable.

Table 2
Inhibitory effects of DMPBD, aspirin and phenidone on platelet aggregation induced by collagen, ADP, AA and PAF

Aggregating agents	DMPBD	Standard drugs	
		Aspirin	Phenidone
Collagen	0.35	0.43	0.03
ADP	4.85	3.98	1.30
AA	0.94	0.13	0.04
PAF	1.14	6.96	2.40

Data are shown as IC₅₀ (mM) values.

Preincubation of platelets with aspirin, phenidone and DMPBD inhibited collagen-induced platelet aggregation with IC₅₀ of 0.43, 0.03 and 0.35 mM, respectively (Table 2). The lag phases were prolonged by these agents. The inhibition was concentration-related and among these phenidone was the most potent inhibitor.

ADP-induced platelet aggregation differently from that of collagen, in that the lag period was not observed with ADP. The aggregation was observed immediately after the addition of ADP solution into the platelet suspension. The response to ADP increased as ADP concentration was increased. Higher concentrations of aspirin, phenidone and DMPBD were needed to inhibit platelet aggregation induced by ADP than those required in the collagen induction. The IC₅₀ of aspirin, phenidone and DMPBD were 3.98, 1.30 and 4.85 mM, respectively.

Platelet aggregation profile induced by AA was similar to that induced by ADP. The platelets aggregated immediately after the addition of the aggregating agents. Aggregation profiles in the presence of the inhibitors were similar but it was slightly delayed when DMPBD was used. Aspirin, phenidone and DMPBD concentration-dependently inhibited AA-induced platelet aggregation with IC₅₀ of 0.13, 0.04 and 0.94 mM, respectively.

Platelet response occurred immediately after the addition of PAF solution into the stirred PRP suspension. Aspirin, phenidone and DMPBD inhibited platelet aggregation with IC₅₀ of 6.96, 2.40 and 1.14 mM, respectively.

4. Discussion

Murine models of inflammation provide a relative system for studying the physiological and biochemical mechanisms of anti-inflammatory agents (Jacobson and Jacobs, 1992). In this study, DMPBD was clearly shown to exert its effects on all models tested, except in PAF-induced rat paw edema.

DMPBD showed a marked inhibitory activity on EPP-induced ear edema. This action is more potent than oxyphenbutazone. Immediately after the application of EPP, the vascular permeability was increased (Patrick et al., 1987). The combined actions of kinins and PGs on vascular permeability seemed to be involved in the early response to EPP while increased in inflammatory cells in the tissues

provided a new source of mediators that contributed to the modulation of the blood flow. The response to EPP during the first hour was partially suppressed by pretreatment with indomethacin, aprotinin and mechlorethamine (Patrick et al., 1987).

The topical application of TPA onto a rat ear induced a long-lasting edema formation. The majority of TPA actions appear to involve or be dependent on AA release and metabolism (Patrick et al., 1987). At the biochemical level, cAMP, PGI₂, PGE₂ and PGF₂ are elevated within minutes to hours after TPA application (Ashendel and Boutwell, 1979; Furstenberger and Marks, 1980). Phospholipase inhibitor, CO inhibitors and LO inhibitors as well as corticosteroids are effective at edema suppression after topical application of high dose of TPA (Young et al., 1983; Carlson et al., 1985), confirming a role of AA release and metabolism. DMPBD, if possessing the same pharmacological effect as diclofenac, should possess inhibitory activity on the CO pathway. However, when comparing the IC₅₀ of both compounds in inhibiting TPA-induced ear edema, DMPBD with IC₅₀ of 660 pmol per ear is more potent than diclofenac.

The topical application of AA provokes a rapid and intense inflammatory response of the ear. This response is not affected by CO inhibitors (Young et al., 1983) but is inhibited by agents that inhibit both CO and LO pathways of AA metabolism (Young et al., 1984). It was found that AA-induced ear edema is predominantly mediated by leukotriene C₄ and other LO products while PGE₂ (in the presence of LTs) acts to facilitate ear swelling (Inoue et al., 1988). In this study DMPBD and phenidone, a dual CO and LO inhibitor, inhibited AA-induced ear edema with IC₅₀ of 60 and 2520 nmol per ear, respectively, suggesting that DMPBD may also acts on the LO pathway.

In the rat paw edema model, carrageenan induces edema formation in three distinct phases according to the mediators involved (Di Rosa et al., 1971). The initial phase occurring during the first hour after subplantar injection of carrageenan into rat hind paw is mediated by histamine and 5-HT. After that the increased vascular permeability in the second phase is maintained by kinin release up to 2.5 h. Then the third phase, from 2.5 to 6 h, the mediators involved are PGs (Di Rosa et al., 1971; Hwang et al., 1986). The biosynthesis of LTs is suggested to be involved in the fourth phase of carrageenan-induced edema formation (Holsapple and Yim, 1984). All non-steroidal anti-inflammatory drugs (NSAIDs) are effective in inhibiting edema formation especially in the late phase in which PGs involves (Niemegeers et al., 1964). In this study, diclofenac showed the maximum inhibitory effect on edema formation at 5 h. This corresponds with the period of PG phase (Di Rosa and Willoughby, 1971). DMPBD also inhibited edema formation with maximum activity at the same period as diclofenac. These results support the suggestion from the rat ear edema model that DMPBD may interfere with the CO pathway of AA metabolism.

It has been observed that the injection of PAF into rat hind paw provokes an inflammatory response characterized by an

acute edema (Cordeiro et al., 1986). The subplantar injection of PAF to rat hind paw-induced dose-related edema formation with the maximum response at 1 h (data not shown). The results are in agreement with the study reported by Castro-Faria-Neto et al. (1990). In this study, PAF-induced paw edema was suppressed by β_2 -adrenergic agonist, salbutamol. It strongly inhibited paw edema while diclofenac and DMPBD did not, suggesting that DMPBD and diclofenac do not affect the pathways involved in PAF-induced paw edema.

The platelet aggregation study represents an attempt to explore the effects of DMPBD on AA metabolism in platelet, since AA metabolites, PGs and thromboxane A₂ (TBA₂) are involved in the platelet aggregation. The effects of the anti-platelet aggregating agents, aspirin and phenidone, on collagen-induced platelet aggregation produced characteristics similarly to that reported in the previous study (Karniguiian et al., 1990). Collagen-induced platelet aggregation in a concentration-related fashion and a lag phase was observed before the aggregation occurred. The earlier study suggested that the lag phase is the time that is needed for collagen to activate phosphatidylinositol 4,5-diphosphate-specific phospholipase C (Karniguiian et al., 1990).

The results are in agreement with the previous studies that aspirin could inhibit collagen-induced aggregation (O'Brien, 1968; Kuster and Frolich, 1986). Phenidone, a CO and LO inhibitor, was slightly more potent than aspirin. In this study, phenidone was the most potent inhibitor of collagen-induced platelet aggregation. Regarding the greater potent anti-aggregating effects of phenidone compared to aspirin, it may be postulated that LTs, which are synthesized via the LO pathway, may also be involved in the platelet aggregation. DMPBD may affect both CO and LO pathways but it is less potent than phenidone in the collagen-induced aggregation.

NSAIDs including aspirin are reported to be effective inhibitors of ADP-induced platelet aggregation (Zucker and Peterson, 1968; Chars et al., 1977). In this study, phenidone seemed to have a higher potency than aspirin and DMPBD. This observation suggests that both CO and LO pathways are involved in the activity of ADP and DMPBD may exert its inhibitory effect on these pathways.

In the AA-induced platelet aggregation, AA is converted to endoperoxides and thromboxanes by platelets and these compounds are further responsible for platelet aggregation (Kinlough-Rathbone et al., 1976). Aspirin, phenidone and DMPBD inhibited the effect of AA on platelets but with different potency. Phenidone and aspirin were more potent than DMPBD (Table 1). Based on the action of aspirin and phenidone, it may be postulated that DMPBD may affect the common pathways of anti-platelet aggregation with that of aspirin and phenidone, possibly CO and LO pathways.

PAF-induced platelet aggregation accompanies with the increase in inositol phosphate metabolism (Dhar et al., 1990). When binding to its receptors, PAF activates phos-

pholipase C and phosphorylation of several proteins occurs (Dhar et al., 1990). TXA₂, an AA metabolic product, is not found to be important in the mechanism of PAF-induced aggregation (McCulloch and Vandongen, 1990). Aspirin and other NSAIDs do not affect this response (Kuster and Frolich, 1986). In this study, DMPBD strongly inhibited platelet response to PAF with IC₅₀ of 1.14 mM. Aspirin and phenidone also inhibited aggregation with lower potency than DMPBD (Table 1). Aspirin was effective only at much higher concentration than those of DMPBD and phenidone. This may suggest that the effects of aspirin on PAF action may occur via pathways other than CO inhibitor and DMPBD may also affect pathways other than the CO and LO pathways in PAF-induced aggregation.

The results obtained from the in vivo models of inflammation suggest that DMPBD exerts a clear anti-inflammatory effect. The differences in the potency of DMPBD in inhibiting platelet aggregation induced by each inducer point out the specificity of DMPBD on the pathways involved in platelet aggregation. The in vivo data suggest that DMPBD may inhibit CO and LO activities and seems to have more prominent effects on the LO pathway. However, further studies should be performed to explore the activities of DMPBD on the enzymatic levels of the inflammation before any final conclusion can be made.

This study is the first to demonstrate that DMPBD is a unique topically active anti-inflammatory agent. Possibly it is a modulator of AA metabolism having activities on both CO and LO enzymes. It has a potential for local therapeutic applications in inflammatory diseases. A drug development program should be systematically initiated to determine the possibility of making this compound a new pharmacological agent for the treatment of inflammatory disorders.

Acknowledgements

This work is supported by the University Developing Commissions Grant, Ministry of University Affairs, and the Faculty of Graduate Studies, Mahidol University, Thailand. The authors would like to thank all the staff at TISTR for their technical assistance and valuable suggestion.

References

- Ashendel, C.L., Boutwell, R.K., 1979. Prostaglandin E and F levels in mouse epidermis are increased by tumor-promoting phorbol esters. *Biochemical and Biophysical Research Communications* 90, 623–627.
- Brattsand, R., Thalen, A., Roempke, K., Kallatrom, L., Gruvstad, E., 1982. Influence of 16a,17a-acetal substitution and steroid nucleus fluorination on the topical to systemic activity ratio of glucocorticoids. *Journal of Steroid Biochemistry* 16, 779–786.
- Carlson, R.P., O'Neil-Davis, L., Chang, J., Lewis, A.S., 1985. Modulation of mouse ear edema by cyclooxygenase and lipoxygenase inhibitors and other pharmacologic agents. *Agents & Actions* 17, 197–204.

- Castro-Faria-Neto, H.C., Silva, P.M.R., Martins, M.A., Silva, P.S., Henriques, M.G., Cordeiro, R.S., Vargaftig, B.B., 1990. Pharmacological modulation of 2-methyl-carbamate-PAF-induced rat paw oedema. *Journal of Pharmacy & Pharmacology* 42, 203–204.
- Chars, I.F., Feinman, R.D., Dewiler, T.C., 1977. Interaction of platelet aggregation and secretion. *Journal of Clinical Investigation* 60, 866–873.
- Cordeiro, R.S.B., Martins, M.A., Silva, P.M.R., Castro-Faria-Neto, H.C., Castanheira, J.R.C., Vargaftig, B.B., 1986. Desensitization to PAF-induced rat paw edema by repeated intraplantar injections. *Life Sciences* 39, 1871–1878.
- Dhar, A., Paul, A.K., Shukla, S.D., 1990. Platelet-activating factor stimulation of tyrosine kinase and its relationship to phospholipase C in rabbit platelets studies with genistein and monoclonal antibody to phosphotyrosine. *Molecular Pharmacology* 37, 519–525.
- Di Rosa, M., Giroud, J.P., Willoughby, D.A., 1971. Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *Journal of Pathology* 104, 15–29.
- Di Rosa, M., Willoughby, D.A., 1971. Screens for anti-inflammatory drugs. *Journal of Pharmacy & Pharmacology* 23, 297–298.
- Furstenberger, G., Marks, F., 1980. Early prostaglandin E synthesis is an obligatory event in the induction of cell proliferation in mouse epidermis in vivo by the phorbol ester TPA. *Biochemical and Biophysical Research Communications* 92, 749–756.
- Holsapple, M.P., Yim, G.K.W., 1984. Therapeutic reduction of ongoing carrageenan-induced inflammation by lipoxygenase but not cyclooxygenase inhibitors. *Inflammation* 8, 223.
- Hwang, S.B., Lam, M.H., Li, C.L., Shen, T.Y., 1986. Release of platelet activating factor and its involvement in the first phase of carrageenan-induced rat foot edema. *European Journal of Pharmacology* 120, 33–41.
- Inoue, H., Mori, T., Koshihara, Y., 1988. Sulfidopeptide-leukotrienes are major mediators of arachidonic acid-induced mouse ear edema. *Prostaglandins* 36, 731–739.
- Jacobson, P.B., Jacobs, R.S., 1992. Fuscoidin: an anti-inflammatory marine natural product which selectively inhibits 5-lipoxygenase. Part I. Physiological and biochemical studies in murine inflammatory models. *Journal of Pharmacology & Experimental Therapeutics* 262, 874–882.
- Karniguian, A., Grelae, F., Levy-Toledano, S., Legrand, Y.J., Rendu, F., 1990. Collagen-induced platelet activation mainly involves the protein kinase C pathway. *Biochemical Journal* 268, 325–331.
- Kinlough-Rathbone, R.L., Reimers, H.J., Mustard, J.F., 1976. Sodium arachidonate can induce platelet shape change and aggregation which are independent of the release reaction. *Science* 192, 1011–1012.
- Kuster, L.J., Frolich, J.C., 1986. Platelet aggregation and thromboxane release induced by arachidonic acid, collagen, ADP, and platelet-activating factor following low dose acetylsalicylic acid in man. *Prostaglandins* 32, 415–423.
- McCulloch, R.K., Vandongen, R., 1990. Mechanisms of platelet activating factor-induced aggregation and secretion in human platelets. *Prostaglandins* 39, 13–21.
- Niemegeers, C.J.E., Verbruggen, F.J., Janssen, P.A.J., 1964. Effect of various drugs on carrageenan-induced oedema in the rat hind paw. *Journal of Pharmacology* 16, 810–816.
- O'Brien, J.R., 1968. Effects of salicylates on human platelets. *Lancet* 13, 779–783.
- Panthong, A., Kanjanapothi, D., Niwatananun, V., Tantiwachwuttikul, P., Reutrakul, V., 1990. Anti-inflammatory activity of compounds isolated from *Zingiber cassumunar*. *Planta Medica* 56, 655.
- Patrick, E., Kurkhalter, A., Maibach, H.I., 1987. Recent investigations of mechanisms of chemically-induced skin irritation in laboratory mice. *Journal of Investigative Dermatology* 88, 24s–31s.
- Pongprayoon, U., Bohlin, L., Soonthornsaratune, P., Wasuwat, S., 1991. Anti-inflammatory activity of *Ipomea pes-casprae* (L.) R. Br. *Phytotherapy Research* 5, 63–66.
- Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proceedings of the Society for Experimental Biology & Medicine* 111, 544–547.
- Young, J.M., Wagner, B.M., Spires, D.A., 1983. Tachyphylaxis in 12-O-tetradecanoylphorbol acetate and arachidonic acid-induced ear edema. *Journal of Investigative Dermatology* 80, 48–50.
- Young, J.M., Spires, D.A., Bedord, C.J., Wagner, B., Ballaron, S.J., De Young, L.M., 1984. The mouse ear inflammatory response to topical arachidonic acid. *Journal of Investigative Dermatology* 82, 367–371.
- Zucker, M.B., Peterson, J., 1968. Inhibition of adenosine diphosphate-induced secondary aggregation and other platelet functions by acetylsalicylic acid ingestion. *Proceedings of the Society for Experimental Biology & Medicine* 127, 547–551.