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Anti-inflammatory Effect of Magnolol, Isolated from *Magnolia officinalis*, on A23187-induced Pleurisy in Mice

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Abstract

In the present study, A23187-induced pleurisy in mice was used to investigate the anti-inflammatory effect of magnolol, a phenolic compound isolated from Chinese medicine Hou p'u (cortex of *Magnolia officinalis*).

A23187-induced protein leakage was reduced by magnolol (10 mg kg^{-1}, i.p.), indomethacin (10 mg kg^{-1}, i.p.) and BW755C (30 mg kg^{-1}, i.p.). A23187-induced polymorphonuclear (PMN) leucocyte infiltration in the pleural cavity was suppressed by magnolol and BW755C, while enhanced by indomethacin. Like BW755C, magnolol reduced both prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) levels in the pleural fluid of A23187-induced pleurisy, while indomethacin reduced PGE_2 but increased LTB_4 formation. In the rat isolated peripheral neutrophil suspension, magnolol (3·7 μM) and BW755C (10 μM) also suppressed the A23187-induced thromboxane B_2 (TXB_2) and LTB_4 formation.

These results suggest that magnolol, like BW755C, might be a dual cyclo-oxygenase and lipoxygenase inhibitor. The inhibitory effect of magnolol on the A23187-induced pleurisy is proposed to be, at least partly, dependent on the reduction of the formation of eicosanoids mediators in the inflammatory site.

Magnolol is a phenolic compound isolated from Hou p'u, cortex of *Magnolia officinalis* Rehd. et Wils., *M. biloba* Rehd. et Wils., or *M. obovata* Thunb. (Magnoliaceae) (Fujita et al 1972). Hou p'u has for centuries been used in Chinese medicine for the relief of fever, headache, anxiety, diarrhoea and stroke. Magnolol has been found to be a novel antiplatelet-aggregating agent by diminishing thromboxane formation and calcium mobilization (Teng et al 1988), to relax rat aorta by releasing endothelium-derived relaxing factor (EDRF) and by inhibiting calcium influx into vascular smooth muscle (Teng et al 1990), and to prolong tail bleeding time in mice (Teng et al 1991). Recently magnolol was shown to exert anti-inflammatory effects on mouse hindpaw oedema, and on cutaneous plasma extravasation in anaphylactic reaction and in neurogenic stimulation (Wang et al 1992, 1993).

Pleurisy induced by the calcium ionophore A23187 in rat has been proposed as a convenient model for studying drug effect on arachidonate metabolism in-vivo (Hagiwara et al 1991). We have demonstrated that A23187-induced pleurisy in mice is also a useful model for the evaluation of anti-inflammatory drugs (Wang et al 1994). Since magnolol has been shown to suppress prostaglandin formation (Teng et al 1988; Wang et al 1992), the antiinflammatory effect of magnolol on A23187-induced pleurisy in mice was, therefore, investigated in the present study.

Materials and Methods

Materials

Magnolol was isolated and purified from the cortex of *Magnolia officinalis* Rehd. et Wils. (Fujita et al 1972). The purity of magnolol was more than 98% as checked by HPLC. A23187, sodium pentobarbitone, indomethacin, Trypan blue, sodium diatrizoate, Ficoll-400, HEPES, and EDTA were purchased from Sigma Chem. Co. (St Louis, MO, USA). Dextran T500 was purchased from Pharmacia Taiwan Branch Office (Taipei, Taiwan, ROC). Coomassie brilliant blue G 250 dye was obtained from Bio-Rad Lab.(-Hercules, CA, USA). Isoton-II was obtained from Coulter Electronic Ltd (Hong Kong). Hematall LAS reagent was obtained from Fisher Scientific (Singapore). Hemacolor and dimethylsulphoxide (DMSO) were obtained from Merck, Taiwan Ltd (Taipei, Taiwan, ROC). BW755C (3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline hydrochloride) was supplied from Wellcome Research Laboratories (Beckenham, UK). [^{3}H]Bicyclic PGE_2 and [^{3}H]TXB_2 RIA kits were purchased from Amersham International plc (Buckinghamshire, UK). [^{3}H]LTB_4 RIA kit was purchased from Du Pont NEN Research Products (Boston, MA, USA).

A23187-induced pleurisy

Pleurisy was induced in pentobarbitone-anaesthetized mice (ICR 25–30 g) by intrapleural injection of 7·5 nmol A23187 (A23187 was prepared as 10 mM stock solution in DMSO and diluted with sterile saline) as previously described (Wang et al 1994). Mice were killed at the appropriate times after induction. The pleural cavity was opened, and then washed once with 0·5 mL 0·2% (w/v) EDTA in phosphated-buffered saline. The pleural fluid was harvested and kept in an ice bath.

Protein assay

Protein in the pleural fluid was determined as previously described (Bradford 1976). Briefly, pleural fluid, harvested

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at 0·5 h after A23187 challenge, was centrifuged at 600 *g* at 4°C for 5 min. An aliquot of supernatant was mixed with Coomassie brilliant blue G 250 dye, and then detected by spectrophotometry at 595 nm. Protein content was calculated by interpolation on a standard curve.

White blood cell counts

For determination of total cell counts, an aliquot of pleural fluid, harvested 3 h after A23187 challenge, was suspended in Isoton-II. Red blood cells were lysed by the addition of Hematall LAS reagent and cell counts were determined using a Hemalaser 2 instrument (Sebia). For differential cell counts, the pleural fluid was centrifuged at 600 *g* at 4°C for 5 min. The cell pellet was resuspended in murine cell-free plasma and spread on glass slides, air-dried and then stained with Hemacolor. Polymorphonuclear (PMN) leucocytes were counted under the microscope.

Isolation of neutrophils

Rat peripheral neutrophils were isolated by a modification of the procedure described by Böyum (1968). Fresh blood was obtained from the abdominal aorta of pentobarbitone-anaesthetized rat (Sprague-Dawley 300–350 g) and mixed with 50 mM EDTA. Neutrophils were separated from other blood cells using dextran sedimentation, centrifugation on Ficoll-hypaque density gradient, and hypotonic lysis of contaminated erythrocytes. Cells were resuspended in Hank's balanced salt solution containing 4 mM $NaHCO_3$ and 10 mM HEPES pH 7·4 to a final concentration of 2×10^6 cells mL^{-1} (viability approx. 95% by Trypan blue exclusion).

Determination of arachidonate metabolites

For the neutrophil preparation, the cell suspension was preincubated at 37°C with DMSO or test compounds for 3 min, then challenged with A23187 (3 μM). Forty-five minutes later, the reaction was terminated by immersing the test-tube into an ice bath. After centrifugation at 2000 *g* at 4°C for 5 min, supernatant was stored at −70°C. The prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) levels in the pleural fluid, harvested at 0·5 and 1 h, respectively, and the thromboxane B_2 (TXB_2) and LTB_4 content in the neutrophil supernatant were determined by radioimmunoassay according to the procedure described by the manufacturers.

Statistical evaluations

The statistical significance of changes was determined by one-way analysis of variance followed by the Newman-Keuls test. $P < 0.05$ was considered to be significant.

Results

Protein leakage

We had previously shown that the level of protein in pleural fluid peaked at 0·5–2 h after intrapleural injection of 7·5 nmol A23187 into mice (Wang et al 1994); a significant increase of protein in the pleural fluid, harvested at 0·5 h after A23187 challenge, was also observed in the present study. Mice pretreated with indomethacin (10 mg kg^{-1}, i.p.), BW755C, a dual cyclo-oxygenase and lipoxygenase inhibitor (Higgs et al 1979) (30 mg kg^{-1}, i.p.), and magnolol (10 and 30 mg kg^{-1}, i.p.) showed significant reduction of protein levels in pleural fluid (Fig. 1).

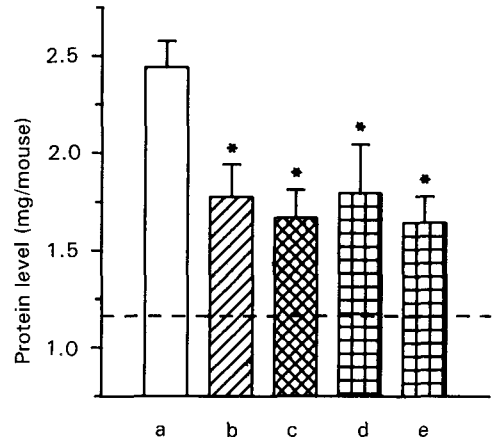

FIG. 1. Effects of indomethacin, BW755C and magnolol on protein leakage in A23187-induced pleurisy. Mice were intraperitoneally injected with a. DMSO; b. indomethacin (10 mg kg^{-1}); c. BW755C (30 mg kg^{-1}); d. magnolol (10 mg kg^{-1}); or e. magnolol (30 mg kg^{-1}), 30 min before intrapleural injection of 7·5 nmol A23187. Pleural fluid was harvested at 0·5 h after A23187 challenge, and then centrifuged for determination of protein in the supernatant. The dashed line represents the background value. Values are expressed as the means ± s.e.m. of 7–10 animals. $*P < 0.05$ compared with control values.

Leucocyte counts

The numbers of PMN leucocytes infiltrating the pleural cavity peaked at 3 h after intrapleural injection of A23187 (Wang et al 1994). As shown in Fig. 2, both total cell and PMN leucocyte counts in pleural fluid of A23187-induced pleurisy were increased in mice pretreated with indomethacin (10 mg kg^{-1}, i.p.). In contrast, BW755C (30 mg kg^{-1}, i.p.) abrogated the leucocyte accumulation. Magnolol (10

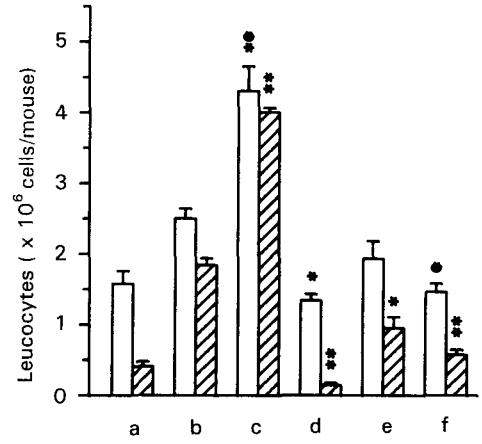

FIG. 2. Effects of indomethacin, BW755C and magnolol on leucocyte accumulation in A23187-induced pleurisy. Mice were intraperitoneally injected with b. DMSO; c. indomethacin (10 mg kg^{-1}); d. BW755C (30 mg kg^{-1}); e. magnolol (10 mg kg^{-1}); or f. magnolol (30 mg kg^{-1}), 30 min before intrapleural injection of 7·5 nmol A23187. Pleural fluid was harvested at 3 h after A23187 challenge for determination of total cell (blank column) and PMN leucocyte (hatch column) counts. The numbers of total cells and PMN leucocytes in the pleural cavity at 3 h after intrapleural injection of sterile saline is shown in column a. Values are expressed as the means ± s.e.m. of 8–10 animals. $*P < 0.05$, $**P < 0.01$ compared with the corresponding values from DMSO-injected controls.

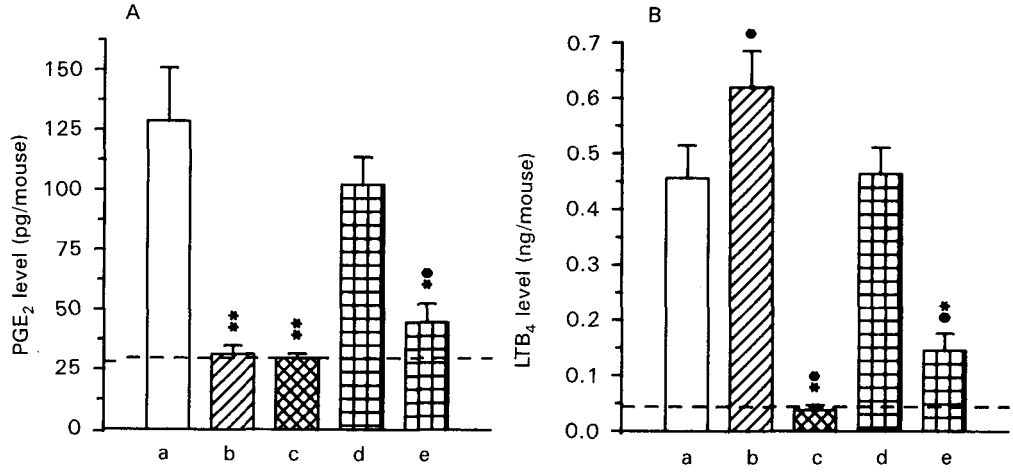

FIG. 3. Effects of indomethacin, BW755C and magnolol on PGE_2 and LTB_4 formation in A23187-induced pleurisy. Mice were intraperitoneally injected with a. DMSO; b. indomethacin (10 mg kg^{-1}); c. BW755C (30 mg kg^{-1}); d. magnolol (10 mg kg^{-1}); or e. magnolol (30 mg kg^{-1}), 30 min before intrapleural injection of 7·5 nmol A23187. The PGE_2 (A) and LTB_4 (B) levels in the pleural fluid, harvested at 0·5 and 1 h, respectively, after A23187 challenge, were then determined. The dashed line represents the background value. Values are expressed as the means ± s.e.m. of 6–8 animals. $^*P < 0.05$, $^{**}P < 0.01$ compared with corresponding values.

and 30 mg kg^{-1}) significantly reduced the PMN leucocyte counts to 37·9 ± 8·5 and 11·4 ± 3·8%, respectively, of the control value.

Arachidonate metabolite formation

After intrapleural injection of A23187, the PGE_2 and LTB_4 levels in the pleural fluid peaked at 0·5–1 h (Wang et al 1994). Fig. 3 demonstrates that PGE_2 levels in the pleural fluid of A23187induced pleurisy was abolished in mice pretreated with indomethacin (10 mg kg^{-1}, i.p.) or BW755C (30 mg kg^{-1}, i.p.). The elevated LTB_4 level in the pleural fluid was also abolished by BW755C but not by indomethacin, which significantly further increased the LTB_4 levels by about 40% of the control value. Magnolol at 10 mg kg^{-1} had no effect on, but at 30 mg kg^{-1} greatly attenuated both PGE_2 and LTB_4 levels. In rat isolated peripheral neutrophil suspension, A23187 (3 μM) induced both TXB_2 and LTB_4 formation. Magnolol (1·1 μM) as well as indomethacin (0·01 μM) reduced ($P < 0.01$) TXB_2 levels, but left the LTB_4 level unaffected (Table 1). However, magnolol (3·7 μM) and BW755C (10 μM) significantly reduced both TXB_2 and LTB_4 formation in neutrophil suspensions in response to A23187 challenge.

Table 1. Effect of indomethacin, BW755C and magnolol on A23187-induced TXB_2 and LTB_4 formation in neutrophil suspension.

Drugs (μM)	Arachidonate metabolites (ng/2 × 10^6 cells)	
	TXB_2	LTB_4
Control	8·03 ± 0·59	21·55 ± 2·33
Indomethacin (0·01)	3·59 ± 0·51**	21·33 ± 1·24
BW755C (10)	2·72 ± 0·40**	14·56 ± 1·31*
Magnolol (1·1)	2·54 ± 0·39**	25·80 ± 2·88
(3·7)	0·92 ± 0·21**	5·81 ± 1·77**

An aliquot of the neutrophil suspension was incubated with DMSO, indomethacin, BW755C or magnolol at 37°C for 3 min, then challenged with A23187 (3 μM). TXB_2 and LTB_4 in the supernatant of the reaction mixture were determined by radioimmunoassay. Values are expressed as the means ± s.e.m. of 6–8 separate experiments. $^*P < 0.05$, $^{**}P < 0.01$ compared with control values.

Discussion

In the present study, A23187-induced pleurisy in mice was used to evaluate the anti-inflammatory effect of magnolol. The levels of protein leakage, the numbers of leucocytes infiltrating, and the levels of arachidonate metabolites (PGE_2 and LTB_4) in pleural fluid of A23187-induced pleurisy were investigated in mice pre-treated with or without anti-inflammatory drugs.

Arachidonate metabolites participate in the pathogenesis of inflammation (Insel 1990). PGE_2 does not have a direct effect on vascular permeability, but rather it may enhance the increase of vascular permeability caused by other inflammatory mediators (Williams 1979). LTB_4, a potent chemoattractant (Bray 1983), mediates plasma exudation indirectly via the circulating PMN leucocytes (Wedmore & Williams 1981). LTC_4 and LTD_4 are vasoconstrictors and cause plasma leakage (Ueno et al 1981). As expected, indomethacin, a cyclo-oxygenase inhibitor (Insel 1990), interupted the cyclo-oxygenase pathway, and resulted in a marked reduction of PGE_2 formation, whereas the increase of LTB_4 formation was probably due to elevation of the concentrations of arachidonate in the lipoxygenase pathway. Both PGE_2 and LTB_4 levels in the pleural fluid of A23187-induced pleurisy were reduced by BW755C, a dual cyclo-oxygenase and lipoxygenase inhibitor (Higgs et al 1979), as well as by magnolol. We also observed that magnolol suppressed both cyclo-oxygenase and lipoxygenase product formation in neutrophil suspensions challenged with A23187. However, the concentrations of magnolol needed for producing an inhibitory effect on arachidonate metabolism tended to be higher in LTB_4 than in TXB_2 formation. From the data at hand, it is assumed that magnolol, like BW755C, acted as a dual cyclo-oxygenase and lipoxygenase

inhibitor on arachidonate metabolism. Magnolol, with its phenolic structure, is proposed to possess antioxidant properties, contributing to this inhibitory effect. However, to assess the precise action the mechanism needs further study.

Indomethacin, BW755C and magnolol, all reduced the protein leakage in A23187-induced pleurisy. Vascular permeability can be increased by chemical mediators released from mast cells close to the vasculature, resulting in the plasma protein leakage. However, the inhibitory effect of indomethacin and BW755C on protein leakage is not through the suppression of preformed mediators from mast cells, since selective inhibition of prostaglandins and leukotriene generation did not alter mast cell degranulation (Sullivan & Parker 1979; Razin et al 1984). Magnolol had no effect on mast cell degranulation either (Wang et al 1993). Since indomethacin did not suppress leukotriene formation but did inhibit the protein leakage in A23187-induced pleurisy, one may expect that reduction of PGE_2 at the inflammatory site can contribute to the attenuation of the vascular permeability change. In addition, magnolol may also decrease the vascular permeability through nonselective inhibition on vascular tissue to prevent the permeability change caused by various mediators (Wang et al 1993).

Mice pretreated with indomethacin significantly increased the PMN leucocytes infiltration in the pleural cavity at 3 h after A23187 intrapleural injection. This result is consistent with our previous report (Wang et al 1994). It seems likely that the increase of LTB_4 levels in the pleural fluid of indomethacin-treated animals can be attributed to exaggerated PMN leucocyte accumulation. The decrease of LTB_4 levels in the pleural fluid of BW755C- and magnolol-treated animals is, therefore, accounted for by the reduction of PMN leucocyte counts in the pleural cavity.

The present results indicate that magnolol has an anti-inflammatory effect on A23187-induced pleurisy in mice. Inhibition of both cyclo-oxygenase and lipoxygenase pathway could cause the reduction of the protein leakage and decrease the PMN leucocyte infiltration.

Acknowledgements

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