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Inhibition of plasma extravasation by abruquinone A, a natural isoflavanquinone isolated from *Abrus precatorius*

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Abstract

Polymyxin B-induced hind-paw edema was suppressed by abruquinone A, an isoflavanquinone isolated from *Abrus precatorius*, in normal as well in adrenalectomized mice. Unlike dexamethasone, abruquinone A did not increase the liver glycogen content in fasting adrenalectomized mice. The volume of exuded plasma was significantly reduced by abruquinone A in neurogenic inflammation, passive cutaneous anaphylactic reaction and compound 48/80-induced ear edema. Histamine-, serotonin-, bradykinin- and substance P-induced plasma extravasation in ear edema was also suppressed by abruquinone A. Abruquinone A, like isoproterenol, significantly reduced the bradykinin- and substance P-induced plasma extravasation in normal as well as in compound 48/80-pretreated mice. In addition, abruquinone A suppressed the bradykinin- and substance P-induced ear edema to a significantly greater extent than diphenhydramine/methysergide did. In the in vitro experiments, abruquinone A suppressed the compound 48/80-induced histamine and β-glucuronidase released from isolated rat peritoneal mast cell preparations. These results suggest that the anti-inflammatory effect of abruquinone A is mediated partly via the suppression of the release of chemical mediators from mast cells and partly via the prevention of vascular permeability changes caused by mediators. The glucocorticoid activity and the release of glucocorticoid hormones from the adrenal gland are probably not involved.

Keywords: Abruquinone A; Paw edema; Neurogenic inflammation; Passive cutaneous anaphylaxis; Vascular permeability; Compound 48/80; Mast cell degranulation

1. Introduction

Vascular plasma leakage in response to locally generated mediators is one of the characteristic features of acute local inflammation. Chemical mediators, such as histamine, serotonin, arachidonate metabolites, bradykinin, substance P and platelet-activating factor etc., contribute to the development of plasma leakage by reacting with specific receptors on the vasculature, which in turn increase or enhance vascular permeability (Owen et al., 1980; Lembeck et al., 1982; Marceau et al., 1983; Page et al., 1983; Saria et al., 1983; Hwang et al., 1986; Andrews et al., 1989). The mast cell is an important inflammatory cell that participates in acute inflammation, including passive cutaneous anaphylactic reaction and neurogenic inflammation (Austen, 1979; Saria et al., 1983; Foreman and Jordan, 1984). A variety of chemical mediators, preformed and newly formed, are released from mast cells through cell activation with immunologic and non-immunologic secretagogues (Ishizaka et al., 1972; Austen, 1979).

Abruquinone A, a natural isoflavanquinone, was originally isolated from the roots of a leguminous plant, *Abrus precatorius* L. (Alessandro et al., 1979). The roots of *Abrus precatorius* have been used as a folk medicine for diuresis and the relief of fever, sore throat, bronchitis and hepatitis in the Far East. The purpose of this study was to evaluate the effect on plasma extravasation of abruquinone A against the acute inflammation evoked by a local increase in chemical mediators. In order to assess the possible mechanism of action of abruquinone A, the effects of

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abruquinone A on mast cell degranulation in vitro, and on phlogist-induced local acute inflammation in normal, in adrenalectomized as well as in compound 48/80-pretreated mice were also studied in vivo.

2. Materials and methods

2.1. Materials

Abruquinone A (mol. wt. 360) was isolated and purified from *Abrus precatorius* as previously described (Alessandro et al., 1979). Sodium pentobarbital, bovine serum albumin, polymyxin B, diphenhydramine, indomethacin, compound 48/80, dinitrophenyl-albumin, monoclonal anti-dinitrophenyl antibody (clone no. SPE-7; mouse IgE), Evans blue, isoproterenol, histamine, serotonin, bradykinin, [Thi^{5,8},D-Phe^{7}]bradykinin, substance P and [D-Pro^{2},D-Trp^{7,9}]substance P were purchased from Sigma Chem. Co., St. Louis, USA. Dimethylsulfoxide (DMSO) was obtained from Merck Taiwan, Taiwan. Methysergide was supplied by Sandoz Pharmaceutical, Basle, Switzerland.

2.2. Hind-paw edema

Mice (ICR, 20–25 g) were used in this study. Hind-paw edema was induced as previously described (Wang et al., 1992). Briefly, a single subplantar injection of 5 μl of 0.2% polymyxin B or sterile saline was given in the right and left hind-paws, respectively, of normal or adrenalectomized mouse. The volumes of both hind-paws of each mouse were measured with a plethysmometer at the beginning and at 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h after induction of edema. Hind-paw swelling was calculated as following: paw swelling (%) = {[(right paw volume − initial volume)/(right paw initial volume)] − [(left paw volume − initial volume)/(left paw initial volume)]} × 100. The data were also analyzed to compare the area under the time (h)-paw swelling (%) curve (AUC) based on the Trapezoidal rule (Tallarida and Murray, 1987).

2.3. Adrenalectomized mice

Adrenalectomized mice were prepared as described (Waynforth, 1980). Briefly, pentobarbital (60 mg/kg i.p.)-anesthetized mice were carefully adrenalectomized bilaterally through the shaved dorsal region. Adrenalectomized mice were given normal physiological saline to drink ad libitum. On the fourth day after the operation, the animals were used for experiments.

For determination of the glucocorticoid activity of test compounds, adrenalectomized mice were deprived of food and saline for 18 h before intraperitoneal administration of the test drugs, and then 8 h after drug treatment the mice were killed (Schiatti et al., 1986). Liver glycogen was isolated and determined (Good et al., 1933; Fong et al., 1953)

2.4. Neurogenic inflammation

The saphenous nerve on one side of pentobarbital-anesthetized mice was carefully exposed, cut at the thigh, and the distal end was placed on bipolar platinum electrodes (Wang et al., 1993). The nerve was covered with paraffin oil. Evans blue (25 mg/kg i.v.) in saline was given and 5 min afterwards the nerve was stimulated for 10 min (1 V/10 Hz per 20 ms). The animals were killed 30 min after the stimulation, and the dorsal skin of the hind-paw was removed. Exuded blue dye in the skin was extracted as described (Katayama et al., 1978). Briefly, the tissue sample was soaked in 1 N NaOH at 37°C overnight and then dye was extracted with a mixture of 0.6 N H_3PO_4 and acetone (5:13, v/v). After centrifugation, blue dye in the supernatant was measured by spectrophotometry at 620 nm. In order to evaluate the volume of exuded plasma, an absorbance-plasma volume standard curve was prepared by measuring the absorbance of different volumes of plasma isolated from mice pretreated with Evans blue. Therefore, the volume of plasma leakage of each tissue sample was calculated by interpolation on this standard curve. The intensity of the inflammatory response was monitored by measuring the difference in the volume of exuded plasma between the two tissue samples of each animal.

2.5. Passive cutaneous anaphylactic reaction

Monoclonal anti-dinitrophenyl antibody 0.1 μg or sterile saline was injected into the right and left ears, respectively, of pentobarbital-anesthetized mice (Wang et al., 1994a). After 48 h, 20 mg/kg of Evans blue with 60 mg/kg of sodium pentobarbital in saline was injected through the caudal vein, followed 5 min later by injection of dinitrophenyl-albumin (10 mg/kg i.v.). The animals were killed 45 min after the induction of edema. A sample of tissue (9 mm diameter) was punched out from both the right and left ears. Exuded blue dye in the tissue sample was extracted as previously described

2.6. Non-immunologic phlogist-induced ear edema

A single injection of a phlogist (3 μg histamine, 1 μg compound 48/80, 0.3 μg bradykinin, 0.1 μg serotonin or 0.1 μg substance P) or sterile saline into the right and left ears, respectively, was given 5 min after the intravenous injection of 0.5% Evans blue and 1.5%

sodium pentobarbital in saline (4 ml/kg) (Wang et al., 1994a). The animals were killed 45 min after the induction of edema. Exuded blue dye in the tissue sample was extracted as previously described.

2.7. Depletion of histamine and serotonin

The mice were injected with compound 48/80 or sterile saline into the right and left ears, respectively, twice a day for six doses (Wang et al., 1993). Briefly, the dose of compound 48/80 was 1 μg for the first three injections, and 3 μg for the last three injections. After this treatment, the histamine content of the ear, measured as described by Shore et al. (1959), was reduced to less than 15% of the control value.

2.8. Mast cell release reaction

Rat peritoneal mast cells were prepared as previously described (Wang et al., 1989). Briefly, heparinized Tyrode solution was injected into the peritoneal cavity of exsanguinated rats (Sprague-Dawley, 250–300 g). After abdominal massage, the cells in the peritoneal fluid were harvested and separated in 38% bovine serum albumin in glucose-free Tyrode solution. The cell pellet was washed and suspended in Tyrode solution of the following composition (mM): NaCl 137, KCl 2.7, $NaHCO_3$ 12, NaH_2PO_4 0.3, $MgCl_2$ 1.0, $CaCl_2$ 1.0, dextrose 5.6 and 0.1% bovine serum albumin, to a final concentration of $1–1.5 \times 10^6$ cells/ml.

The cell suspension was preincubated at 37°C with DMSO or abruquinone A for 3 min, and then the release reaction was triggered by the addition of compound 48/80 (10 μg/ml). The final DMSO concentration was less than 0.5%. The reaction was terminated 15 min later by the addition of ice-cold Tyrode solution and the mixture was then centrifuged for 10 min at $1000 \times g$. The contents of histamine and β-glucuronidase in the supernatant were determined by fluorescence spectrophotometry at 350/450 nm (Håkanson and Rönnberg, 1974) and by spectrophotometry at 550 nm (Barrett, 1972), respectively, and expressed as % release of the total content. The total content of histamine and β-glucuronidase was measured after treatment of the cell suspension with Triton X-100.

2.9. Statistical analysis

Data are presented as the means ± S.E.M. The statistical significance of changes was analyzed with one-way analysis of variance followed by Newman-Keuls test. P-values < 0.05 were considered to be significant.

3. Results

3.1. Hind-paw edema and glucocorticoid activity

Pretreatment with indomethacin 3 mg/kg i.p. and diphenhydramine 10 mg/kg i.p., caused a significant reduction of the polymyxin B-induced hind-paw swelling beginning at 2 and 0.5 h, respectively, up to 6 h after induction of the edematous response in normal mice (Fig. 1A) and beginning at 1 h and 4 h, respectively, up to 6 h after induction of paw edema in adrenalectomized mice (Fig. 1B). Inhibition by abruquinone A (10 and 30 mg/kg i.p.) of paw swelling was observed in normal mice (61.6 ± 3.5% and 70.6 ± 3.4% inhibition for 10 and 30 mg/kg, respectively, in the 6-h period AUC, $P < 0.01$). The inhibitory effect on hind-paw edema was also demonstrated in adrenalectomized mice pretreated with abruquinone A

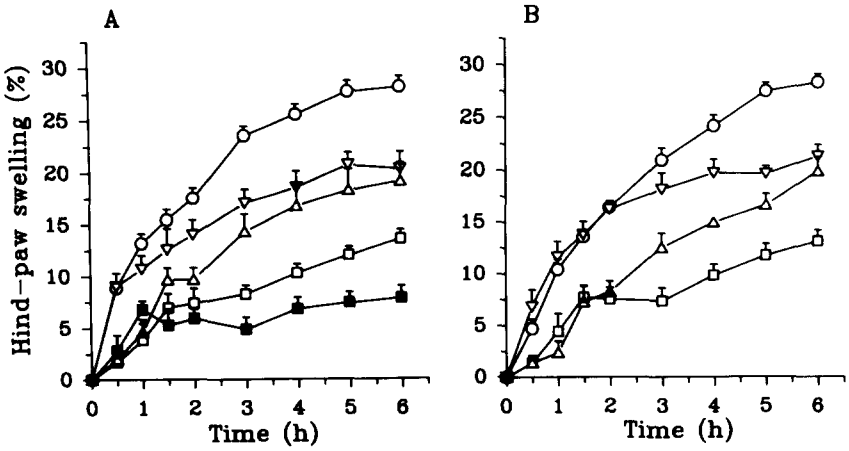

Fig. 1. Effects of abruquinone A, indomethacin and diphenhydramine on polymyxin B-induced hind-paw edema in mice. (○) Control, no pretreatment with anti-inflammatory drugs; abruquinone A (□) 10 and (■) 30 mg/kg; (▽) indomethacin 3 mg/kg; (△) diphenhydramine 10 mg/kg were injected intraperitoneally 30 min before subplantar injection of 10 μg polymyxin B into the right hind-paw of (A) normal or (B) adrenalectomized mice. Values are expressed as the means ± S.E.M. for seven to nine animals.

Table 1
Effects of abruquinone A and dexamethasone on liver glycogen content in adrenalectomized mice

Drugs [a]	Dose (mg/kg)	Glycogen content [b] (mg/g liver)
Control		0.75 ± 0.14
Abruquinone A	3	1.22 ± 0.05
	10	0.84 ± 0.18
Dexamethasone	0.5	26.39 ± 5.25 [c]

[a] Mice were deprived of food and water for 18 h before intraperitoneal administration of DMSO, abruquinone A or dexamethasone, and 8 h later the animals were killed, and then liver glycogen content was determined. [b] Values are expressed as the means ± S.E.M. for five to six animals. Statistically significant difference from the control value is noted as [c] $P < 0.01$.

10 mg/kg i.p. (57.6 ± 4.2% inhibition in 6-h period AUC, $P < 0.01$). Table 1 shows that dexamethasone 0.5 mg/kg i.p. markedly increased ($P < 0.01$) the liver glycogen content in fasting adrenalectomized mice. However, abruquinone A, at a dose which exerted a significant anti-inflammatory effect, had no effect on liver glycogen.

3.2. Plasma exudation in neurogenic inflammation

Antidromic stimulation of the saphenous nerve (1 V/10 Hz per 20 ms) evoked plasma extravasation in the dorsal skin of the hind-paw. Pretreatment with diphenhydramine (10 mg/kg i.p.), methysergide (3 mg/kg i.p.) and isoproterenol (1 mg/kg i.p.) significantly reduced ($P < 0.01$) the volume of exuded plasma in neurogenic inflammation (Fig. 2). However, the edematous response was not modified by indomethacin (3 mg/kg i.p.). Abruquinone A exerted a dose-dependent inhibitory effect on plasma exudation of 45.6 ± 3.5% and 72.0 ± 7.2% inhibition at 1 and 3 mg/kg i.p., respectively.

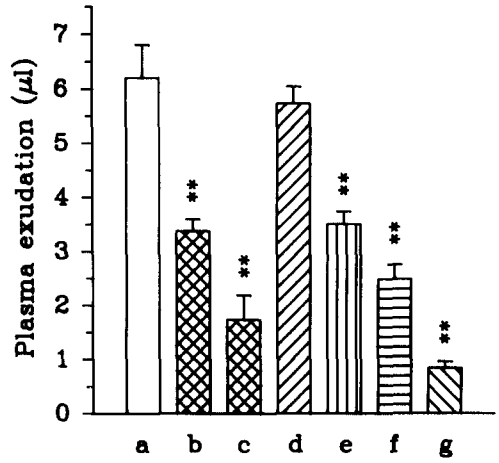

Fig. 2. Effects of abruquinone A, indomethacin, diphenhydramine, methysergide and isoproterenol on neurogenic inflammation in mice. (a) Control, no pretreatment with anti-inflammatory drugs; abruquinone A (b) 1 and (c) 3 mg/kg; (d) indomethacin 3 mg/kg; (e) diphenhydramine 10 mg/kg; (f) methysergide 3 mg/kg; (g) isoproterenol 1 mg/kg were injected intraperitoneally 30 min before antidromic stimulation of the saphenous nerve (1 V/10 Hz per 20 ms). Values for plasma exudation are expressed as the means ± S.E.M. for five to six animals. Statistically significant difference from the control value (a) is noted as ** $P < 0.01$.

3.3. Plasma exudation in passive cutaneous anaphylactic reaction

Ear edema was induced after intravenous injection of dinitrophenyl-albumin into the mice which had been sensitized by injection of anti-dinitrophenyl antibody into the ear. Fig. 3 shows that the immunologic ear edema was suppressed by diphenhydramine (10 mg/kg i.p.), methysergide (3 mg/kg i.p.) and isoproterenol (3 mg/kg i.p.), whereas indomethacin (3 mg/kg i.p.) was devoid of an inhibitory effect in this respect. Abruquinone A in a dose of 10 mg/kg i.p. showed a

Table 2
Effects of abruquinone A, indomethacin, diphenhydramine, methysergide and isoproterenol on histamine-, serotonin- and compound 48/80-induced ear edema

Drugs [a]	Dose (mg/kg)	Plasma exudation (μl) [b]		
		Histamine	Serotonin	Compound 48/80
Control		4.3 ± 0.4	7.2 ± 0.4	5.5 ± 0.3
Abruquinone A	3	3.2 ± 0.6	4.2 ± 0.5 [d]	2.4 ± 0.3 [d]
	10	1.6 ± 0.4 [d]	3.5 ± 0.8 [d]	1.9 ± 0.4 [d]
Indomethacin	3	3.9 ± 0.5	6.8 ± 0.2	4.2 ± 0.5
Diphenhydramine	10	1.9 ± 0.3 [d]	n.d.	3.7 ± 0.5 [c]
Methysergide	3	n.d.	1.8 ± 0.3 [d]	2.0 ± 0.2 [d]
Isoproterenol	1	1.2 ± 0.1 [d]	2.4 ± 0.1 [d]	3.5 ± 0.3 [c]

[a] Drugs were injected intraperitoneally 0.5 h prior to histamine (3 μg), serotonin (0.1 μg) or compound 48/80 (1 μg) injection into the ear. [b] Values are expressed as the means ± S.E.M. for six to eight animals; n.d., not determined. Statistically significant differences from the corresponding control values are noted as [c] $P < 0.05$, [d] $P < 0.01$.

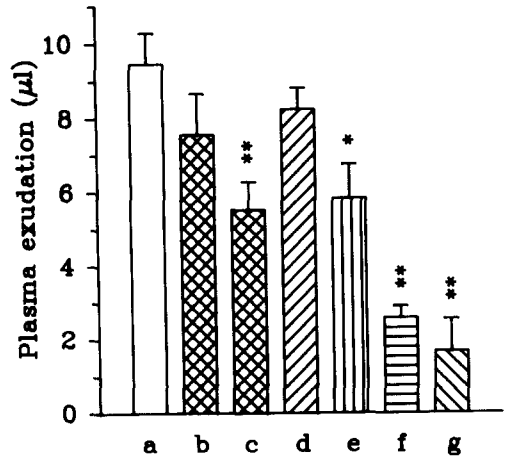

Fig. 3. Effects of abruquinone A, indomethacin, diphenhydramine, methysergide and isoproterenol on ear edema in the passive cutaneous anaphylactic reaction. (a) Control, no pretreatment with anti-inflammatory drugs; abruquinone A (b) 3 and (c) 10 mg/kg; (d) indomethacin 3 mg/kg; (e) diphenhydramine 10 mg/kg; (f) methysergide 3 mg/kg; (g) isoproterenol 3 mg/kg were injected intraperitoneally 30 min before dinitrophenyl-albumin 10 mg/kg intravenous injection. Values for plasma exudation are expressed as the means ± S.E.M. for seven to eight animals. Statistically significant differences from the control values (a) are noted as * $P < 0.05$, ** $P < 0.01$.

significant inhibitory effect against the immunologic edematous response (41.6 ± 8.0% inhibition, $P < 0.01$), but abruquinone A 3 mg/kg i.p. failed to alter it significantly.

3.4. Plasma exudation in histamine-, serotonin- and compound 48/80-induced ear edema

As shown in Table 2, diphenhydramine (10 mg/kg i.p.) and methysergide (3 mg/kg i.p.) suppressed the histamine- and serotonin-induced ear edema, respectively. In addition, compound 48/80-induced plasma extravasation was also reduced by diphenhydramine and methysergide. All three phlogist-induced edematous responses were inhibited by isoproterenol (1 mg/kg i.p.), but not affected by indomethacin (3 mg/kg i.p.). In the experiments with abruquinone A, a dose-dependent inhibitory effect was observed on the ear edema induced by three phlogists. The volumes of exuded plasma in mice pretreated with abruquinone A 3 and 10 mg/kg i.p. were reduced to 75.1 ± 14.1% and 37.1 ± 10.2%, respectively, of the histamine control value, to 58.3 ± 8.1% and 48.1 ± 11.1%, respectively, of the serotonin control value, and to 43.4 ± 5.5% and 35.5 ± 8.4%, respectively, of the compound 48/80 control value.

3.5. Bradykinin- and substance P-induced plasma exudation in normal and compound 48/80-pretreated mice

Pretreatment with diphenhydramine (10 mg/kg i.p.) and methysergide (3 mg/kg i.p.) significantly inhibited ($P < 0.01$) the bradykinin- and substance P-induced ear

Table 3
Effects of abruquinone A, indomethacin, isoproterenol, diphenhydramine, methysergide, [Thi^{5,8},D-Phe^{7}]bradykinin and [D-Pro^{2},D-Trp^{7,9}]substance P on bradykinin- and substance P-induced ear edema in normal or compound 48/80-pretreated mice

Drugs [a]	Dose (mg/kg)	Plasma exudation (μl) [b]	
		Normal	Compound 48/80-pretreated
(A) Bradykinin (BK)			
Control		8.3 ± 0.5	5.8 ± 0.2
Abruquinone A	3	6.0 ± 0.6 [d]	4.3 ± 0.9 [c]
	10	2.0 ± 0.3 [d]	2.0 ± 0.1 [d,e]
Indomethacin	3	7.7 ± 0.6	6.6 ± 0.3
Isoproterenol	3	1.4 ± 0.3 [d]	0.7 ± 0.2 [d,e]
[Thi^{5,8},D-Phe^{7}]BK		3.4 ± 0.3 [d]	n.d.
Diphenhydramine/	10		
methysergide	3	6.4 ± 0.4 [d]	5.2 ± 0.4
+[Thi^{5,8},D-Phe^{7}]BK		n.d.	0.4 ± 0.1 [d,e]
(B) Substance P (SP)			
Control		7.1 ± 0.3	5.0 ± 0.2
Abruquinone A	3	5.6 ± 0.4 [c]	2.2 ± 0.5 [d]
	10	0.4 ± 0.2 [d]	0.8 ± 0.2 [d,e]
Indomethacin	3	6.8 ± 0.2	4.3 ± 0.3
Isoproterenol	3	0.6 ± 0.1 [d]	0.4 ± 0.1 [d,e]
[D-Pro^{2},D-Trp^{7,9}]SP		4.2 ± 0.3 [d]	n.d.
Diphenhydramine/	10		
methysergide	3	4.0 ± 0.4 [d]	4.0 ± 0.3 [c]
+[D-Pro^{2},D-Trp^{7,9}]SP		n.d.	0.6 ± 0.1 [d,e]

[a] [Thi^{5,8},D-Phe^{7}]bradykinin (1 μg) and [D-Pro^{2},D-Trp^{7,9}]substance P (1 μg) were coinjected with bradykinin and substance P, respectively, whereas the other drugs were injected intraperitoneally 0.5 h prior to (A) bradykinin (0.3 μg) or (B) substance P (0.1 μg) injection into the ear.
[b] Values are expressed as the means ± S.E.M. for six to nine animals; n.d., not determined. Statistically significant differences from the corresponding control values are noted as [c] $P < 0.05$, [d] $P < 0.01$, and from the diphenhydramine/methysergide-treated values are noted as [e] $P < 0.01$.

edema in normal mice. In compound 48/80-pretreated mice, the bradykinin-induced edematous response was not affected, whereas the substance P-induced response was slightly, but significantly, reduced by diphenhydramine/methysergide. Both bradykinin- and substance P-induced edematous responses were markedly attenuated by isoproterenol (3 mg/kg i.p.), but not modified by indomethacin (3 mg/kg i.p.) (Table 3). [Thi^{5,8},D-Phe^{7}]bradykinin 1 μg and [D-Pro^{2},D-Trp^{7,9}]substance P 1 μg exerted a significant inhibitory effect on bradykinin- and substance P-induced plasma extravasation, respectively, in normal mice (59.0 $\pm$ 3.6% and 40.8 $\pm$ 4.2% inhibition, respectively, $P < 0.01$), and this inhibition was even more pronounced in the presence of diphenhydramine/methysergide in compound 48/80-pretreated mice (93.1 $\pm$ 1.7% and 88.0 $\pm$ 2.0% inhibition, respectively, $P < 0.01$). A dose-dependent inhibitory effect of abruquinone A on bradykinin- and substance P-induced ear edema was also observed in normal as well as in compound 48/80-pretreated mice. Abruquinone A 3 and 10 mg/kg i.p. reduced bradykinin- and substance P-induced plasma extravasation to 72.3 $\pm$ 7.2% and 24.1 $\pm$ 3.6%, respectively, of the bradykinin control value and to 78.9 $\pm$ 5.6% and 5.7 $\pm$ 2.8%, respectively, of the substance P control value in normal mice, and to 74.2 $\pm$ 15.5% and 34.5 $\pm$ 1.7%, respectively, of the bradykinin control value and to 44.0 $\pm$ 10.0% and 16.0 $\pm$ 4.0%, respectively, of the substance P control value in compound 48/80-pretreated mice (Table 3). Like isoproterenol, [Thi^{5,8},D-Phe^{7}]bradykinin and [D-Pro^{2},D-Trp^{7,9}]substance P, abruquinone A suppressed the bradykinin- and substance P-induced edematous response to a significantly greater extent ($P < 0.01$) than diphenhydramine/methysergide did in compound 48/80-pretreated mice.

Table 4
Effect of abruquinone A on the release of histamine and β-glucuronidase from isolated rat peritoneal mast cells challenged with compound 48/80

Drugs [a]	Dose (μg/ml)	Percent release (%) [b]	
		Histamine	β-Glucuronidase
Control		51.7 $\pm$ 0.6	35.9 $\pm$ 1.8
Abruquinone A	0.3	48.5 $\pm$ 6.2	31.2 $\pm$ 2.1
	1	24.3 $\pm$ 3.5 [c]	20.0 $\pm$ 2.3 [c]
	3	16.4 $\pm$ 1.6 [c]	14.7 $\pm$ 1.1 [c]

[a] Mast cell suspension was preincubated with DMSO or abruquinone A at 37°C for 3 min, then challenged with compound 48/80 (10 μg/ml). [b] Values are expressed as means $\pm$ S.E.M. for seven to eight experiments. Statistically significant difference from the corresponding control values is noted as [c] $P < 0.01$.

3.6. Mast cell release of histamine and β-glucuronidase

In the in vitro experiments, the compound 48/80-induced mast cell release reaction was inhibited by abruquinone A in a concentration-dependent manner, with IC_{50} values of 4.5 $\pm$ 0.7 μM in the histamine assay and 8.4 $\pm$ 2.2 μM in the β-glucuronidase assay (Table 4).

4. Discussion

Concerning the hind-paw swelling induced by subplantar injection of polymyxin B, this effect has previously been suggested to be mediated by prostaglandins, histamine and serotonin (Bertelli and Soldani, 1979; Wang et al., 1992). The observations that abruquinone A suppressed the polymyxin B-induced hind-paw edema in normal as well as in adrenalectomized mice, whereas abruquinone A had no effect on liver glycogen content, indicate that neither glucocorticoid activity nor release of glucocorticoid hormones from the adrenal gland is involved in the anti-inflammatory effect of abruquinone A.

Neurogenic inflammation evoked by the antidromic stimulation of sensory nerve causes vasodilation and plasma exudation (Jancsó et al., 1967). Substance P and related neurokinin released from sensory nerve endings during activation mediate this neurogenic inflammation (Lembeck and Holzer, 1979; Gamse et al., 1980). Substance P has been proposed to act directly on the vasculature and indirectly by activation of mast cells (Lembeck et al., 1982; Saria et al., 1983; Foreman and Jordan, 1984). Plasma extravasation evoked by antigen challenge of sensitized animals has been also proposed to occur partly via activation of sensory neurons, but mainly via the release of inflammatory mediators from mast cells (Perper et al., 1975; Saria et al., 1983). Compound 48/80 (Paton, 1951) is widely used as a mast cell degranulating agent, leading to the release of histamine and other mast cell constituents. The increase in vascular permeability caused by compound 48/80 is partly through the activation of capsaicin-sensitive neurons and partly mediated by mast cell constituents (Saria et al., 1984). Pretreatment of mice with abruquinone A significantly reduced the plasma exudation in neurogenic inflammation, passive cutaneous anaphylactic reaction and compound 48/80-induced ear edema. The inhibitory effects of diphenhydramine, a histamine receptor antagonist, and methysergide, a 5-HT receptor antagonist (Garrison, 1991), but not indomethacin, a cyclooxygenase inhibitor (Insel, 1990), on plasma extravasation in neurogenic inflammation, passive cutaneous anaphylactic reaction and compound 48/80-induced edema are con-

sistent with those of previous reports (Maling et al., 1974; Saria et al., 1983, Saria et al., 1984; Wang et al., 1994a, Wang et al., 1994b).

Histamine and serotonin are important mast cell constituents (Sjoerdsma et al., 1957), and can be released during cell activation with immunologic and non-immunologic secretagogues. Once released from mast cells, histamine and serotonin have been reported to increase vascular permeability by activation of specific receptors on the vasculature (Owen et al., 1980; Heltianu et al., 1982; Owen, 1987). Our results with abruquinone A, which reduced the plasma exudation caused by histamine and serotonin, indicate that abruquinone A may directly act on the vasculature to reduce the permeability changes caused by histamine and serotonin. This hypothesis could also explain the anti-inflammatory effects of abruquinone A on neurogenic inflammation, passive cutaneous anaphylactic reaction and compound 48/80-induced edematous response.

In order to assess whether or not the plasma extravasation caused by the direct action of other mediators, besides histamine and serotonin, on the vasculature is also blocked by abruquinone A, bradykinin and substance P were also used as phlogists. Bradykinin- and substance P-induced plasma extravasation was partially, but significantly, reduced by [Thi^{5,8},D-Phe^{7}]bradykinin, a bradykinin B_2 receptor antagonist (Regoli et al., 1986), and [D-Pro^{2},D-Trp^{7,9}]substance P, a tachykinin NK_1 receptor antagonist (Lembeck et al., 1981; Appell et al., 1992), respectively. The inhibitory effects on bradykinin- and substance P-induced responses were also observed in mice pretreated with abruquinone A. The results that indomethacin was ineffective against bradykinin- and substance P-induced responses are in line with our previous observations (Wang et al., 1994a, Wang et al., 1994b). However, mast cell constituents should be depleted before the direct action of bradykinin and substance P on the vasculature can be investigated, since the increase in vascular permeability elicited by bradykinin, like substance P, is proposed to be mediated indirectly through the release of mast cell-derived mediators and directly via the vasculature (Marceau et al., 1981; Wang et al., 1989). Pretreatment of mice with compound 48/80 for 3 consecutive days leads to a greatly reduced histamine content in tissues and organs (Saria et al., 1984; Wang et al., 1993). In this study, the histamine content of ear samples from compound 48/80-pretreated mice was reduced to less than 15% of the control value. In some experiments, diphenhydramine and methysergide were also introduced to eliminate the inflammatory effects of the remaining histamine and serotonin, which may be released during the challenge with bradykinin and substance P. Under these conditions, bradykinin- and substance P-induced plasma extravasation was greatly inhibited by [Thi^{5,8},D-Phe^{7}]bradykinin and [D-Pro^{2},D-Trp^{7,9}]substance P, respectively. Abruquinone A and isoproterenol, a β-adrenoceptor stimulant, also inhibited the bradykinin- and substance P-induced edematous responses in compound 48/80-pretreated mice. β-Adrenoceptor stimulants have been proposed to exert presynaptic inhibition of substance P release from sensory neurons, inhibit mast cell degranulation, and suppress the increase in vascular permeability, through an increase of the cellular cAMP level (Assem and Schild, 1969; Winslow and Austen, 1984; Kennedy et al., 1989; Advenier et al., 1992; Morikawa et al., 1993). The inhibitory effects of abruquinone A and isoproterenol against bradykinin- and substance P-induced plasma extravasation in compound 48/80-pretreated mice were significantly greater than those of diphenhydramine in combination with methysergide, which indicates that abruquinone A and isoproterenol may also suppress the direct action of bradykinin and substance P on the vasculature.

The finding that abruquinone A reduced the histamine and β-glucuronidase released from isolated mast cell preparations in a concentration-dependent manner indicates that abruquinone A may also suppress the vascular permeability change through the inhibition of the release of mast cell-derived mediators.

In conclusion, we have shown that the anti-inflammatory compound abruquinone A has no glucocorticoid activity and does not depend on secretion of glucocorticoid hormones from adrenal gland to exert its anti-inflammatory effect. Its anti-inflammatory activity is proposed partly to occur through the suppression of the release of chemical mediators from mast cells and partly through the prevention of vascular permeability changes caused by mediators.

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