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Vascular permeability responses and the role of prostaglandin E_2 in an experimental allergic inflammation of air pouch type in rats

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1 Rats were sensitized with azobenzene arsonate-conjugated acetyl bovine serum albumin. An allergic inflammation was induced in the preformed air pouch in the dorsum of the sensitized rats by injecting the antigen dissolved in a 2% sodium carboxymethyl cellulose solution into the air pouch.

2 Time course changes of vascular permeability, accumulated pouch fluid volume and prostaglandin E_2 (PGE_2) levels in the pouch fluid were compared in sensitized and non-sensitized rats to characterize the allergic inflammatory reaction.

3 Effects of three cyclo-oxygenase inhibitors (indomethacin, diclofenac sodium and tiaprofenic acid) on vascular permeability and accumulated pouch fluid volume 4 and 24 h after the immunological challenge injection were examined to elucidate a possible role of PGE_2 in the inflammatory response.

4 Four h after initiating the allergic reaction, although the level of PGE_2 in the pouch fluid reached a high level, the vascular permeability response, measured over the period 3.5–4 h, was not suppressed by treatment with the three cyclo-oxygenase inhibitors and neither was the pouch fluid volume measured over the period 0–4 h. However, vascular permeability and accumulated pouch fluid volume at 24 h were suppressed by the cyclo-oxygenase inhibitors in a dose-dependent manner.

5 These observations suggest that in this model, endogenous PGE_2 does not affect oedema formation measured at 4 h. However, oedema formation measured at 24 h may be dependent on PGE_2 generation.

Introduction

The allergic air pouch inflammation established in our laboratory (Tsurufuji *et al.*, 1982; Ohuchi *et al.*, 1982) is a novel experimental model that is suitable for analysis of allergic inflammatory processes (Ohuchi *et al.*, 1983; 1984; Kurihara *et al.*, 1983; 1984; Tsurufuji *et al.*, 1984). In a previous paper (Ohuchi *et al.*, 1982) we suggested the possible participation of prostaglandin E_2 (PGE_2) in this experimental allergic inflammation, based on the finding that the pouch fluid accumulation and migration of polymorphonuclear leukocytes into the pouch fluid at 24 h were inhibited by treatment with indomethacin with a concomitant decrease in PGE_2 levels. This finding prompted us to examine how vascular permeability and the level of PGE_2 in the pouch fluid change with time after the immunological challenge. This paper is aimed firstly at characterizing the time course of the allergic inflammatory reaction in respect of vascular permeability and PGE_2 levels in the pouch fluid and secondly, at investigating the effects of several cyclo-oxygenase inhibitors, in an attempt to clarify the role of PGE_2 in the vascular permeability responses.

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Methods

Immunization and induction of allergic air pouch inflammation was carried out as described previously (Tsurufuji *et al.*, 1982; Ohuchi *et al.*, 1982). Male rats of the Sprague-Dawley strain, specific pathogen free, 39–41 days old and weighing 150–170 g (Charles River Japan, Inc., Kanagawa, Japan) were used. The antigen used was azobenzene arsonate-conjugated acetyl bovine serum albumin (ABA-AcBSA), which was prepared in our laboratory according to the procedure described by Tabachnick & Sobotka (1959).

This antigen has been shown to induce type IV hypersensitivity as revealed through footpad reactions in mice (Ohuchi *et al.*, 1981). Five mg of the antigen in 250 μl saline was emulsified with an equal volume of Freund's complete adjuvant (FCA, Difco Laboratories, Detroit, Mich., U.S.A.); 500 μl of the emulsion was divided into five portions and injected intradermally on two nuchal and three lumbar sites of each rat. Nine days after the immunization, 8 ml of air was injected subcutaneously on the dorsum under light ether anaesthesia to form an air pouch in the shape of an ellipsoid. Twenty four h after the injection of air, 2 mg of the antigen, ABA-AcBSA, dissolved in 4 ml of sterilized 2% (w/v) solution of sodium carboxymethyl cellulose (CMC-Na, Cellogen F3H, Dai-ichi Kogyo Seiyaku, Niigata, Japan) in saline supplemented with antibiotics (0.1 mg penicillin G potassium and 0.1 mg dihydrostreptomycin sulphate per ml of the solution) were injected into the air pouch as a challenge injection. One group of rats, which had not been sensitized, was prepared as 'non-sensitized rats'. Thirty min before the designated times, i.e., 1, 4, 8, 15, 24 and 48 h after the challenge, 1 μCi aliquot of ^{131}I-human serum albumin (^{131}I-HSA, Dai-ichi Kagaku Yakuhin, Tokyo, Japan), which had been purified by gel filtration to remove low molecular impurity of ^{131}I (Tsurufuji *et al.*, 1977), in 0.2 ml of 0.9% NaCl solution was injected intravenously into the femoral vein as a tracer for the vascular permeability measurement. The rats were then killed by cutting the carotid artery at the designated time and the entire volume of the pouch fluid was collected and measured. In addition, one ml of the pouch fluid was counted in a well-type scintillation spectrometer (Aloka JDC-751, Aloka, Co., Tokyo, Japan). The radioactivity was then calculated for the entire volume of the pouch fluid for each rat. Vascular permeability was expressed as a percentage of the total radioactivity injected (Ohuchi *et al.*, 1977).

Radioimmunoassay of PGE_2 in the pouch fluid was performed after extraction according to the procedure described previously (Ohuchi *et al.*, 1982). Serological specificity of the anti-PGE_2 antiserum has been described elsewhere (Alam *et al.*, 1979).

The drugs used were the non-steroidal anti-inflammatory agents, indomethacin (Nihon Merck Banyu, Tokyo, Japan), diclofenac sodium and tiaprofenic acid (Roussel Medica, K.K., Tokyo, Japan), each of which was dissolved in ethanol and added to the antigen solution in order to administer them directly into the inflammatory site. The final ethanol concentration in the antigen solution was adjusted to 0.1%. The doses of the drug used were 1 and 10 μg per ml of the antigen solution, 4 μg and 40 μg per rat, respectively. Effects of the drugs on vascular permeability were evaluated at 4 and 24 h after the immunological challenge injection. A portion of the pouch fluid was diluted and the numbers of cells were counted in a haemocytometer.

Results were analyzed for statistical significance by Student's *t* test for paired observations.

Results

Table 1 shows the time course of pouch fluid volume collected from the non-sensitized and sensitized rats following antigen challenge. In the sensitized group, exudate production was prominent. Even 1 h after the injection of the antigen solution, the difference in the volume of the pouch fluid between sensitized and non-sensitized rats was statistically significant ($P < 0.005$). Pouch fluid volume of the sensitized rats increased during the course of the first 24 h, while in the non-sensitized rats, the pouch fluid volume increased slightly during the first 4 h and then levelled off. At 24 h, the volume of the pouch fluid from the sensitized rats was on average 2.3 times as much as that from the non-sensitized rats.

Time changes in vascular permeability and the level of PGE_2 in the pouch fluid from the sensitized rats are shown in Figure 1. The vascular permeability response developed rapidly to a peak observed at 1 h, then declined to a low level observed at 15 h. Thereafter it was again elevated to a plateau over the period from 24 to 48 h. The level of PGE_2 in the pouch fluid increased with time to reach a peak at 8 h and then changed in a parallel way with the time course of the vascular

Table 1 Time changes in the pouch fluid volume after injection of antigen solution into the preformed air pouch of non-sensitized and sensitized rats

Time after injection (h)	*Pouch fluid volume* (ml) *Non-sensitized rats*	*Sensitized rats*
1	4.72 ± 0.09	5.38 ± 0.09**
4	5.58 ± 0.30	6.50 ± 0.13*
8	5.60 ± 0.16	7.09 ± 0.16***
15	5.11 ± 0.42	7.67 ± 0.36***
24	3.73 ± 0.40	8.65 ± 0.33***
48	2.25 ± 0.16	10.52 ± 0.48***

Values are the mean ± s.e.mean from 5 rats. Four ml of antigen solution was injected into the preformed air pouch of non-sensitized and sensitized rats. Pouch fluid volume was measured at indicated times after the injection of the antigen solution.
Statistical significance: *$P < 0.05$; **$P < 0.005$; ***$P < 0.001$, compared to the corresponding non-sensitized rats.

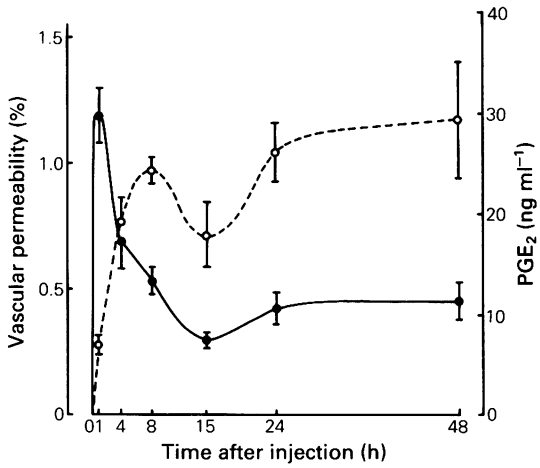

Figure 1 Time course of vascular permeability (left-hand scale) and the level of prostaglandin E_2 (PGE_2, right hand scale) in the pouch fluid of the allergic air pouch model of inflammation. Four ml of antigen solution was injected at time zero into the air pouch of the sensitized rat. Thirty min before the indicated times, 1 μCi aliquot of ^{131}I-HSA was injected intravenously. The rats were killed 30 min after the injection, and the entire volume of the pouch fluid was collected. Vascular permeability is expressed in terms of the leakage of the radioactivity into the pouch fluid during the 30 min period, as a percentage of the total radioactivity injected (●). PGE_2 (○) in the pouch fluid was radioimmunoassayed. Values are the mean (with s.e.mean shown by vertical lines) from 5 rats.

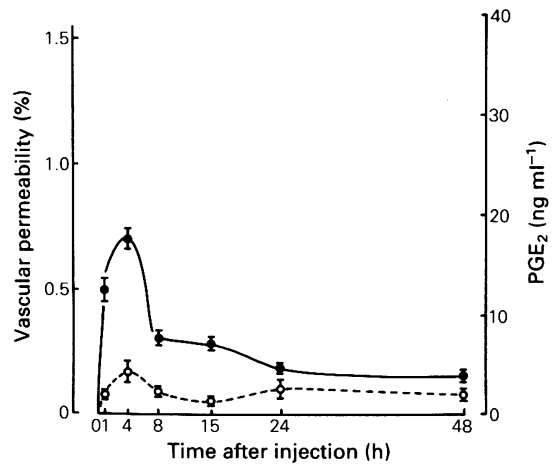

Figure 2 Time course of the vascular permeability (●) and the level of prostaglandin E_2 (PGE_2, ○) in the pouch fluid in non-sensitized rats. For details of the experiment, see Figure 1 legend. Values are the mean (with s.e.mean shown by vertical lines) from 5 rats.

permeability response towards the end of the experimental period. At the end of the 48 h period, the level of PGE_2 was approximately 30 ng per ml of pouch fluid.

Figure 2 shows the time course of vascular permeability and the PGE_2 level in pouch fluid in the non-sensitized group. The vascular permeability observed 1 h after the challenge injection was considerably lower compared with that of the sensitized rats ($P < 0.005$). The maximum peak of the vascular permeability was observed 4 h after the challenge injection. Vascular permeability in the non-sensitized group, determined 1, 8, 24 and 48 h after the injection, was significantly lower than that in the sensitized rats ($P < 0.001$). However, no significant difference was observed at 4 and 15 h. The level of PGE_2 in the pouch fluid from the non-sensitized rats reached a maximum at 4 h in agreement with the time of the maximum for the vascular permeability response, but it was significantly lower than the corresponding level in the sensitized rats ($P < 0.001$). The levels of PGE_2 in the pouch fluid of the sensitized rats were significantly higher than those in the non-sensitized rats throughout the experimental period ($P < 0.001$).

Table 2 shows the effects of various doses of indomethacin, diclofenac sodium and tiaprofenic acid on the pouch fluid volume, vascular permeability and the level of PGE_2 in the pouch fluid 4 h after the immunological challenge. All of the cyclo-oxygenase inhibitors examined failed to suppress the exudate accumulation or vascular permeability to any extent, whereas these drugs induced a dose-dependent reduction of the PGE_2 level in the pouch fluid. Tiaprofenic acid was less effective than indomethacin or diclofenac sodium. Polymorphonuclear leukocyte migration into the pouch fluid during the 4 h period was not affected by the indomethacin treatment (data not shown).

The effects of the cyclo-oxygenase inhibitors on the pouch fluid volume, total number of cells (more than 95% of the cells were polymorphonuclear leukocytes) in the pouch fluid and the vascular permeability response measured 24 h after the immunological challenge are shown in Table 3. The vascular permeability response was suppressed significantly and dose-dependently by all the cyclo-oxygenase inhibitors examined. The pouch fluid volume was also inhibited significantly at a dose of 10 μg ml^{-1}. The infiltration of polymorphonuclear leukocytes into the pouch fluid was similarly inhibited in a dose-dependent manner. When compared at a dose of 1 μg ml^{-1}, diclofenac sodium was most potent among the three drugs on the vascular permeability response. Only diclofenac sodium exerted significant inhibition of the pouch fluid volume at a dose of 1 μg ml^{-1}.

Table 2 Effects of indomethacin, diclofenac sodium and tiaprofenic acid on the pouch fluid volume, vascular permeability and the level of prostaglandin E_2 (PGE_2) in the pouch fluid 4 h after the immunological challenge injection

Treatment	*No. of rats*	*Pouch fluid volume* (ml)	*Vascular permeability* (% of ^{131}I-HSA injected)	*Prostaglandin* E_2 (ng ml^{-1})
Control	6	5.87 ± 0.06	0.62 ± 0.03	16.44 ± 1.21
Indomethacin				
1 μg ml^{-1}	5	5.67 ± 0.08	0.56 ± 0.05	0.42 ± 0.03*
10 μg ml^{-1}	5	5.63 ± 0.12	0.63 ± 0.05	0.19 ± 0.01*
Diclofenac sodium				
1 μg ml^{-1}	6	5.67 ± 0.07	0.63 ± 0.05	0.40 ± 0.04*
10 μg ml^{-1}	5	5.78 ± 0.07	0.68 ± 0.03	0.17 ± 0.03*
Tiaprofenic acid				
1 μg ml^{-1}	6	5.96 ± 0.15	0.60 ± 0.06	5.99 ± 0.83*
10 μg ml^{-1}	6	5.60 ± 0.07	0.66 ± 0.05	1.07 ± 0.17*

Values are the mean ± s.e.mean.
Statistical significance: $*P < 0.001$ compared to the control group.

Table 3 Effects of indomethacin, diclofenac sodium and tiaprofenic acid on the pouch fluid volume, total numbers of cells and vascular permeability 24 h after the immunological challenge injection

Treatment	*No. of rats*	*Pouch fluid volume* (ml)	*No. of cells* ($\times 10^8$)	*Vascular permeability* (% of ^{131}I-HSA injected)
Control	7	8.13 ± 0.18	5.88 ± 0.25	0.31 ± 0.02
Indomethacin				
1 μg ml^{-1}	6	8.05 ± 0.24	4.62 ± 0.27	0.21 ± 0.02***
10 μg ml^{-1}	6	6.43 ± 0.27***	3.62 ± 0.13***	0.09 ± 0.02***
Diclofenac sodium				
1 μg ml^{-1}	6	6.93 ± 0.10***	4.37 ± 0.14**	0.14 ± 0.01***
10 μg ml^{-1}	6	6.65 ± 0.40**	3.78 ± 0.05***	0.09 ± 0.02***
Tiaprofenic acid				
1 μg ml^{-1}	6	7.79 ± 0.21	4.50 ± 0.32*	0.21 ± 0.01***
10 μg ml^{-1}	5	7.28 ± 0.22*	3.80 ± 0.30***	0.13 ± 0.01***

Values are the mean ± s.e.mean. Statistical significance: $*P < 0.025$; $**P < 0.005$; $***P < 0.001$.

Discussion

Some of the fundamental features of the allergic air pouch inflammation used in the present experiments have been described previously (Tsurufuji *et al.*, 1982; Ohuchi *et al.*, 1982). In the sensitized rats, a profound increase in vascular permeability was observed within 1 h following antigen challenge. In contrast, the vascular permeability increase was not observed in the non-sensitized rats. Consequently, this is considered to be the anaphylactic phase of the reaction. Analysis of this anaphylactic phase of the vascular permeability response, the details of which have been described elsewhere (Ohuchi *et al.*, 1985), revealed that histamine and 5-hydroxytryptamine (5-HT) are responsible for the elevation of vascular permeability and that leukotrienes of the slow reacting substance (SRS) type play only a minor role, if any, in spite of their apparent generation in the inflammatory locus (Ohuchi *et al.*, 1983; 1984). Subsequent to the sharp initial rise, vascular permeability in the allergic group declined quickly approaching a level comparable to the maximum peak in the non-sensitized group which was attained at 4 h (Figures 1 and 2). In other words, both the sensitized and non-sensitized groups reached almost equal states 4 h after the challenge injection into the air pouch in respect of the apparent level of their vascular permeability responses. However, as shown in Table 1 it should be noted that the 4 h accumulated pouch fluid volume in the sensitized group was significantly higher than that in the non-sensitized group. When a challenge injection with a

2% solution of CMC-Na was made in the absence of the antigen to the non-sensitized group, time changes of vascular permeability took substantially the same course (data not shown) as that indicated in Figure 2, in which the challenge injection was made to the non-sensitized rats with the CMC-Na solution containing the antigen. Therefore, the antigen, ABA-AcBSA, is considered to be inert with respect to the induction of the vascular permeability response, although the vehicle, a 2% solution of CMC-Na, irritates tissue so as to cause a mild non-specific inflammatory reaction.

The fact that vascular permeability in the allergic group was so close to that in the non-sensitized group at 4 h (Figures 1 and 2) in spite of the marked difference in the PGE_2 level in the pouch fluid between these two groups (Figures 1 and 2), suggests that PGE_2 in the inflammatory sites of the sensitized group does not play any significant role in the vascular permeability response at this stage of the inflammation. In order to verify the above concept, a series of experiments was carried out with the aid of three nonsteroidal anti-inflammatory drugs which possess potent inhibitory activities against cyclo-oxygenase (Deraedt *et al.*, 1982). Results of the experiments summarized in Table 2 clearly indicate that none of the three cyclo-oxygenase inhibitors are capable of suppressing the vascular permeability response in the 4 h stage of the air pouch inflammation, whereas the PGE_2 level was significantly lowered in all the treated groups. Pouch fluid volume (measured over the period 0–4 h) was also not affected by treatment with the three cyclo-oxygenase inhibitors (Table 2).

In marked contrast with the results for the 4 h stage of the allergic air pouch inflammation, it was shown in our previous study (Ohuchi *et al.*, 1982) that indomethacin, when administered locally in a similar way to the present experiment, was effective in inhibiting exudate accumulation and lowering the level of PGE_2 in the pouch fluid when examined 24 h after the immunological challenge. In an attempt to reconfirm those previous findings, effects of the three cyclo-oxygenase inhibitors were tested on vascular permeability in the 24 h stage of the inflammation in a similar way to the 4 h experiment. In agreement with the results of the previous experiment for the 24 h stage (Ohuchi *et al.*, 1982) but in contrast to the results for the 4 h stage of the present experiment (Table 2), the three cyclo-oxygenase inhibitors exerted potent and dose-dependent inhibitory effects on the vascular permeability at 24 h (Table 3). Total numbers of cells in the pouch fluid were also decreased dose-dependently by treatment with the cyclo-oxygenase inhibitors. The percentage of polymorphonuclear leukocytes was not significantly changed by the cyclo-oxygenase inhibitors. The mechanism of the inhibitory effects of these drugs on leukocyte migration remains to be elucidated. However, as suggested by Brown & Collins (1977), it is possible that the cyclo-oxygenase inhibitors may affect chemotaxis by a mechanism that is independent of cyclo-oxygenase inhibition.

At present we cannot show what type of cells are responsible for PGE_2 production. However, a part of PGE_2 production may be due to invading cells especially polymorphonuclear leukocytes, since the numbers of polymorphonuclear leukocytes in the pouch fluid were elevated 4 h after the antigen challenge (Tsurufuji *et al.*, 1982) and histological analysis revealed that many polymorphonuclear leukocytes appeared in the subcutaneous tissue adjacent to the air pouch. The levels of 13,14-dihydro-15-keto-PGE_2, 6-keto-$PGF_{1\alpha}$ and PGD_2 in the pouch fluid collected 1, 8 and 24 h after the antigen challenge were described in a previous paper (Ohuchi *et al.*, 1984). The level of the PGE_2 metabolite was highest at 8 h. It may reflect the decreased level of PGE_2 at 15 h (Figure 1). E-type prostaglandins are known to act synergistically with a variety of chemical mediators of inflammation to induce oedema e.g. histamine (Williams & Morley, 1973; Movat *et al.*, 1978), 5-HT (Chahl, 1976), bradykinin (Williams & Morley, 1973), leukotrienes of SRS type (Williams & Piper, 1980), C5a (Jose *et al.*, 1981), leukotriene B_4 (Bray *et al.*, 1981) and platelet-activating factor (Wedmore & Williams, 1981). Consequently, the vascular permeability response at 24 h could be attributed to PGE_2 together with some of the factors quoted above. When 4 ml of the antigen solution containing 3 and 30 $\mu g\,ml^{-1}$ of pyrilamine maleate was injected into the preformed air pouch of the sensitized rats, the anaphylactic increase of vascular permeability was suppressed to 54.9% and 25.6% of control, respectively (Ohuchi *et al.*, 1985). However, at these doses of pyrilamine maleate, no significant inhibition of vascular permeability was observed at 4 h. The levels of histamine and 5-HT in the pouch fluid were decreased when measured 90 min after antigen challenge, although these levels were high at 30 min (Ohuchi *et al.*, 1985). These observations suggest that histamine and 5-HT do not participate in the vascular permeability response nor collaborate with PGE_2 at the 4 h stage but are involved in the anaphylactic phase of the reaction. The failure to suppress vascular permeability at 4 h by treatment with the cyclo-oxygenase inhibitors suggests that PGE_2 plays no significant role in provoking the vascular permeability response at this stage and that this response in this model should be ascribed to some unknown factors other than those quoted above.

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