DIFFERENCE IN THE ANTI-INFLAMMATORY EFFECT OF INDOMETHACIN BETWEEN ACUTE AND CHRONIC STAGES OF CARRAGEENIN-INDUCED INFLAMMATION

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A phlogistic agent, carrageenin, was injected into preformed subcutaneous air pouch on the back of rats to induce carrageenin-air-pouch inflammation. After 1.0, 2.0, 3.0, 4.0, 8.0, 10, 12, 16, 24, 48 and 72 hours and 5, 7 and 8 days, vascular permeability of the inflammatory tissue was measured with the aid of radioiodinated human serum albumin. The time course study of the vascular permeability indicated the existence of two separate periods in the course of the vascular permeability change, *i. e.* acute and chronic stages. The acute stage elevation exhibited a sharp peak over the period of 2—8 hours after the carrageenin injection. After exhibiting the sharp peak of the increased vascular permeability, the vascular leakage declined rapidly coming to a level of 1/5 as low as the peak value during the period of 16—24 hours. This low level of the permeability was maintained for several hours, and then it turned upwards once again increasing slowly along with the formation of granulation tissues. The peak of the permeability in this chronic, proliferative, stage of the inflammation was attained around day 7.

Between the acute stage and the chronic, proliferative, stage there was a marked difference in the sensitivity of leaking blood vessels to a non-steroid anti-inflammatory drug indomethacin. In the acute stage the vascular permeability was inhibited considerably in agreement with the data reported in most literatures of indomethacin, whereas in the chronic, proliferative, stage indomethacin was shown to be almost inert. Underlying mechanisms are discussed with special reference to the role of E type prostaglandins considered in connection with the data of our previous reports.

Keywords —acute inflammation; chronic inflammation; proliferative inflammation; indomethacin; vascular permeability; prostaglandin E

Elevation of the permeability to macromolecules in local vascular networks is recognized as one of the most prominent features in the inflammatory process. Clinical symptoms such as tissue edema and fluid accumulation in body cavities result principally from the elevation of the vascular permeability in the local tissues. Therefore, anti-inflammatory activity of drugs have been commonly evaluated in the experimental pharmacology on the basis of the suppressive action against elevated vascular permeability.

Among a number of methods which have been developed up to date, carrageenin-induced paw

edema of the rat is one of the most common models of the inflammation and this is extensively used in the evaluation of anti-exudative activity of drugs.¹⁻⁵⁾ Injection of carrageenin into a hind paw of a rat provokes elevation of vascular permeability and produces profound swelling of the paw in a few hours. The edema reaches the maximum level within 6—12 hours after the injection of carrageenin and then it declines. This type of inflammation, even though it is an excellent model for the acute inflammation, may not be comparable to the inflammation of synovia in rheumatoid arthritis, since the synovitis is an specialized type of

chronic granulomatous nature.

On the basis of above mentioned considerations we have developed carrageenin-air-pouch method as an experimental model which covers not only acute but also chronic stages of the inflammatory processes.⁶⁾ Using this type of inflammation model, we have already done a variety of experimental studies on the mode of action of steroid and non-steroid anti-inflammatory substances.⁶⁻⁸⁾ In the present experiment we have followed change with time of the vascular permeability in the carrageenin-air-pouch inflammation over the entire course through acute exudative and chronic proliferative stages. In addition, the response to a typical non-steroid anti-inflammatory agent indomethacin, which is a potent inhibitor of prostaglandin synthetase,⁹⁾ has been also investigated in the attempt to compare the nature of the responses between acute and chronic stages of the inflammation.

MATERIALS AND METHODS

Chemicals—Seakem # 202 carrageenin was purchased from Marine Colloid Inc., Springfield, N. J., U.S.A., ¹³¹I-human serum albumin (specific activity: 5 μ Ci/mg) from Dainabot Co., Tokyo, and ¹²⁵I-human serum albumin (specific activity: 2.5 μ Ci/mg) from Kaken Kagaku Co., Tokyo. Indomethacin was a gift from Nippon Merk Ban-yu Co., Tokyo. As the commercial preparations of ¹³¹I- and ¹²⁵I-human serum albumins contain a significant quantity of small molecular radiochemical impurity, purification through Sephadex G-100 was performed as reported in a previous paper.¹⁰⁾

Carrageenin-air-pouch Inflammation—Inflammation of carrageenin-air pouch was induced according to the method of Fukuhara and Tsurufuji⁶⁾ with a slight modification. Male rats of Sprague-Dawley strain, 6 weeks old and weighing 150–180 g, fed on laboratory solid food (Funabashi Farm, Chiba) and tap water, were injected under light ether anesthesia with 8.0 ml of air subcutaneously on the back to make an air pouch in the shape of oval or ellipsoid. After 24 hours 4.0 ml of 2 (w/v) % carrageenin solution in 0.9% NaCl was injected into the air pouch already

formed. The carrageenin solution was sterilized by autoclaving at 110° for 15 min and injected, after cooling, in the temperature at 40–45°. Immediately before the injection, penicillin and streptomycin were added each in the concentration of 0.1 mg/ml to the carrageenin solution. The day of carrageenin injection was designated as day 0.

Measurement of the Vascular Permeability

At an appropriate time interval after the carrageenin treatment 1 μ Ci of the purified radioiodinated human serum albumin in 0.2 ml of 0.9% NaCl was injected into the femoral vein of the animal. After 30 min, entire exudate was withdrawn from the carrageenin-air-pouch and served as a sample to measure the leakage of the tracer into the pouch fluid. The volume of the entire pouch fluid was also measured. Total radioactivity in the entire pouch fluid of each rat was assayed using a well type scintillation spectrometer as described in a previous paper¹⁰⁾ and expressed in the term of the percentage of the radioactivity injected and used as an index of the vascular permeability of the inflamed tissue.

RESULTS

Time Course of the Inflammation as Reflected in the Local Vascular Permeability

Rats were sacrificed in group 1.0, 2.0, 3.0, 4.0, 8.0, 10, 12, 16, 24, 48 and 72 hours and 5, 7 and 8 days after the injection of the phlogistic agent. Each rat had been injected intravenously 30 min prior to the sacrifice with 1 μ Ci of ¹³¹I-human serum albumin and then 1.0 ml of the carrageenin-air-pouch fluid was taken at the time of sacrifice and served as a sample for the measurement of the vascular permeability. Results are summarized in Fig. 1. The abscissa indicates the time of sacrifice and the ordinate shows the vascular permeability value as defined in the section of the methods. At 1 hour after the injection of the phlogistic agent, the vascular permeability was very low and it showed sudden elevation up to the almost maximum level at 2 hour and it persisted for a few hours. During the period beyond 8 hour until 24 hour the permeability had declined down to a very low level, and then it was again elevated slowly towards the

FIG. 1. Change with Time of the Vascular Permeability and Exudate Volume in Carrageenin-air-pouch Inflammation in Rats ●—●: vascular permeability as measured in the time of the quantity of leaked radioiodinated serum albumin and expressed as % of the injected radioactivity. ○—○: exudate volume. Vertical bars in the figures indicate standard error of the mean. Number of rats used for the assay was 11, 18, 24, 14, 17, 6, 7, 7, 12, 6, 7, 6, 167 and 39, respectively, expressed in order of the time of the assay.

second highest level which was attained around day 7. The overall vascular permeability response indicates a characteristic profile having two prominent stages. The elevation in the early stage corresponds to the acute phase of inflammation and the second elevation in the late stage occurs along with proliferation of granuloma pouch tissues.⁶⁻⁸⁾

In agreement with the profile of time change of the vascular permeability, elevation in the volume of the pouch fluid occurred also biphasically as shown in Fig. 1. The increase in the acute stage occurred during the first 24 hours ($p < 0.025$), followed by intermission persisting for about 2 days and then the increase in the proliferative stage started and persisted over the period of several days.

Anti-exudative Effect of Indomethacin

Anti-exudative effect of a potent anti-inflammatory agent indomethacin was tested in both of the acute and the proliferative, chronic, stages of the carrageenin-air-pouch inflammation. Indomethacin was given orally through a stomach tube as a suspension in 0.5% carboxymethyl cellulose solution. In the experiment for the proliferative,

TABLE I. Effect of Indomethacin on the Vascular Permeability in the Chronic, Proliferative, Stage of Carrageenin-air-pouch Inflammation

Treatment	No. of rats	Vascular permeability (Leak of tracer)		Permeability change (¹²⁵ I/ ¹³¹ I)	Relative permeability (% of control)	<i>p</i> -value
		Pretreatment (¹²⁵ I-HSA)	Post-treatment (¹³¹ I-HSA)			
Experiment 1						
Vehicle (0.5% CMC)	7	0.40±0.06 ^{a)}	0.42±0.07 ^{a)}	1.03±0.05 ^{a)}	100	
Indomethacin						
3.0 mg/kg	7	0.31±0.04	0.31±0.03	1.01±0.04	98.1	N.S.
10.0 mg/kg	7	0.43±0.07	0.37±0.06	0.88±0.05	85.4	N.S.
30.0 mg/kg	8	0.40±0.08	0.38±0.07	0.97±0.05	94.2	N.S.
Experiment 2						
Vehicle (0.5% CMC)	8	0.57±0.06	0.57±0.07	0.99±0.04	100	
Indomethacin						
3.0 mg/kg	8	0.78±0.08	0.66±0.06	0.87±0.05	87.9	N.S.
10.0 mg/kg	7	0.52±0.10	0.42±0.08	0.81±0.02	81.8	<i>p</i> <0.025
30.0 mg/kg	7	0.56±0.06	0.44±0.05	0.79±0.07	79.8	<i>p</i> <0.01

a) Mean ± S.E.M.

chronic, stage the double tracer method¹⁰⁾ with the aid of ¹²⁵I- and ¹³¹I-human serum albumins was applied on day 7 in order to measure vascular permeability change which might be induced by indomethacin, as described in our previous paper,¹⁰⁾ since this method had been demonstrated to be efficient in eliminating experimental error arising from individual variation in rats. In this method vascular permeability of each carrageenin granuloma pouch was measured twice, firstly with ¹²⁵I-human serum albumin immediately before the oral administration of indomethacin and secondly with ¹³¹I-human serum albumin at 4 hour after the drug treatment. In the vehicle-treated animals the ratio of the permeability values, ¹³¹I/¹²⁵I, came near unity as already reported,¹⁰⁾ since the vehicle gave no significant influence to the vascular permeability. The time interval of 4 hours between indomethacin administration and the permeability measurement was selected on the basis of the data from the literature¹¹⁾ which dealt with blood concentration of the drug. The experiments were repeated twice in order to make the conclusion more certain, and the results are shown in Table I.

On the other hand, in the acute stage experiment vascular permeability was measured 3

hours after the injection of carrageenin. Indomethacin was given 1 hour before injecting the phlogistic agent. Thus the time interval of 4 hours between the drug treatment and the permeability measurement was set agreeing with the case of the proliferative, chronic, stage experiment. The double tracer method which was applied in the case of the chronic stage experiment was not used in the acute stage experiment because measurement of the permeability prior to the drug treatment was impossible to be done as the inflammation had not started yet. Individual variation of the vascular permeability measurement was comparatively small in the acute stage experiment. The results in the acute stage experiment are summarized in Table II.

Comparing the results between the acute stage and the chronic, proliferative, stage it is apparent that indomethacin is potently antiinflammatory only in the acute stage. In the acute stage, dose-dependent inhibition was obtained, approaching maximum 50% inhibition at 3 mg/kg, whereas in the chronic stage the ten-fold dose (30 mg/kg) produced only 20% inhibition and 3.0 mg/kg gave no significant inhibition. Even 1 mg/kg produced sufficiently significant (30%) inhibition in the acute stage.

TABLE II. *Effect of Indomethacin on the Vascular Permeability in the Acute Stage of Carrageenin-air-pouch Inflammation*

Treatment	No. of rats	Vascular permeability (Leak of tracer) Post-treatment (¹³¹ I-HSA)	Relative Permeability (% of control)	p-value
Experiment 1				
Vehicle (0.5% CMC)	9	0.66 ± 0.05 ^{a)}	100	
Indomethacin				
1.0 mg/kg	8	0.44 ± 0.05	66.7	<i>p</i> < 0.005
3.0 mg/kg	9	0.32 ± 0.05	48.5	<i>p</i> < 0.005
10.0 mg/kg	9	0.32 ± 0.04	48.5	<i>p</i> < 0.005
Experiment 2				
Vehicle (0.5% CMC)	8	0.90 ± 0.09	100	
Indomethacin				
10.0 mg/kg	9	0.46 ± 0.09	51.1	<i>p</i> < 0.005

a) Mean ± S.E.M.

DISCUSSION

Time course studies in vascular permeability response in the acute inflammation have been repeatedly done by several workers^{2, 3, 12, 13)} and generally indicate the existence of two phases in the elevation of the permeability. The first phase temporary elevation, which occurs immediately after irritation and disappears within one hour, is likely mediated by histamine and serotonin^{2, 12)} and makes only a minor contribution in the entire process of the acute inflammation.¹²⁾ The second phase, which is commonly called delayed response in the acute inflammation and is suggested to be mediated by a combination of the E type prostaglandin and bradykinin,^{3, 4)} forms main part in overall exudative response in the acute inflammation.¹²⁾ On the other hand, however, details of the vascular permeability response in the chronic, proliferative, inflammation remains to be investigated. The present experiment presents complete data for the time course of the vascular permeability response over the full term of inflammatory process throughout from the stage of acute inflammation up to the stage of chronic, proliferative, inflammation.

There is a prominent resting period in the vascular permeability response between the acute and the chronic stages as shown in Fig. 1. This resting period coincides, as shown in Fig. 2, with the period of tentative fall in prostaglandin E level of which the data have been quoted from our papers reported previously.^{14, 15)} The early peak of the permeability corresponds to the early peak of the prostaglandin. As the level of the prostaglandin falls, the permeability declines. Thereafter, the permeability again turns upwards around 72 hour together with increase of the prostaglandin. And then, however, in the period later than 72 hour the prostaglandin level and the vascular permeability proceed in different ways respectively. Agreeing with this phenomena, indomethacin suppresses vascular permeability response only in the acute stage where prostaglandin E and the vascular permeability indicate changes of a similar way. These phenomena indicate the participation of the E type prostaglandin in the increased vascular per-

FIG. 2. *Correlation of the Vascular Permeability and Prostaglandin E Level in the Exudate in Carrageenin-air-pouch Inflammation in Rats* ●—●: vascular permeability as measured in the term of the quantity of leaked radioiodinated serum albumin and expressed as % of the injected radioactivity. ○—○: prostaglandin E level in the exudate. Vertical bars in the figures indicate standard error of the mean.

meability in the acute stage of carrageenin-air-pouch inflammation as well as in other forms of the acute inflammation.^{9, 16)} In contrast, prostaglandin E and vascular permeability take different attitude each other in the chronic, proliferative, stage and indomethacin is almost ineffective in this stage. Therefore, it seems rational to conclude that the E type prostaglandin mediates vascular permeability response only in the acute stage of the inflammation and consequently prostaglandin synthetase inhibitors can elaborate inhibitory influence only in the acute stage.

If we accept the concept implying that the E type prostaglandins do not substantially take part in the vascular permeability response in the proliferative inflammation, it becomes quite important to elucidate what mechanism other than prostaglandin E underlies in the process of long lasting elevation of vascular permeability in the proliferative inflammation. In order to consider this question, the findings reported by Schoeffl¹⁷⁾ and Cater and Wallington¹⁸⁾ seems to be informative. They disclosed that newly arising capillary vessels in the granulation tissues of proliferative inflammation was structurally different from the normal capillary, *i.e.* so immature as to be permeable

to colloidal particles, whereas the normal capillary was impermeable. In addition, it has been repeatedly demonstrated in the literature that postcapillary venules are almost exclusive site of the vascular leakage for colloidal particles in the cases that various probable chemical mediators of the acute inflammation involving prostaglandin E are applied to the microvasculature of local tissues.^{13, 19-21)} These findings reported in the literature and the results of the present experiment are consistent with the concept that there are some differences between acute stage and chronic, proliferative, stage of the inflammation not only in the site and the mode of vascular leakage but also in the sensitivity to some anti-inflammatory drugs. Namely, in the acute inflammation the E type prostaglandin collaborates with other mediators⁴⁾ such as bradykinin, histamine and serotonin to make postcapillary venules highly permeable for macromolecules, and to result in profound swelling of the local tissues and/or accumulation of fluids in body cavities. This phase can be strongly inhibited by an inhibitor of prostaglandin synthetase. On the other hand, in the chronic, proliferative, inflammation release of the E type prostaglandin is not sufficient to induce exudative reaction and the vascular leakage is likely attributable in most part to leaking structure of proliferating premature capillary blood vessels in the proliferating granulation tissues. Therefore, prostaglandin synthetase inhibitors elaborate hardly inhibitory effect to the exudative process in the proliferative, chronic, inflammation.

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