Captopril Uncovers Kinin-Dependent Release of Arachidonic Acid Metabolites in Carrageenin-Induced Rat Pleurisy

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Abstract—As previously reported, pretreatment with captopril significantly enhanced pleural exudation of rat carrageenin-induced pleurisy. However, in high molecular weight kininogen-deficient rats (B/N-Katholiek), the pleural exudate volume was significantly less than that of the normal strain (B/N-Kitasato), and captopril pretreatment did not enhance exudation. In the present study, the following additional evidences were demonstrated: 1) Captopril did not increase 6-keto-PGF$_{1\alpha}$ level in the deficient strain, but it was significantly increased in the normal strain after captopril treatment; 2) simultaneous administration of soybean trypsin inhibitor with carrageenin markedly suppressed the exudate volume and levels of 6-keto-PGF$_{1\alpha}$ in the normal strain; and 3) indomethacin also suppressed pleural fluid accumulation and the production of arachidonate metabolites. These data suggest that carrageenin causes intrinsic kinin-release through the activation of plasma kallikrein and then in turn, the kinin stimulates the production of arachidonic acid metabolites. Thus these products and kinin may interact to induce more plasma exudation in carrageenin inflammation. The results also indicate that captopril uncovers the effects of bradykinin on exudation and stimulation of arachidonate metabolite production; otherwise, the biological effect of kinin is too slight to produce a clear effect at the initial phase of the inflammation.

In carrageenin induced edema in rats, an involvement of the kallikrein-kinin system has previously been reported (1–3). Our previous work also suggested that kinin involvement in rat carrageenin induced pleurisy, since a high molecular weight (HMW)-kininogen deficient rat strain showed significantly lower reactivity in this model (3, 4). It is well-known that bradykinin stimulates various tissues to release arachidonate metabolites since an early study reported that renal tissue produced a PGE-like substance by the infusion of bradykinin (5). Although there have been numerous in vitro studies demonstrating that bradykinin stimulates phospholipase(s) to release arachidonic acid and prostaglandins (6, 7), there is little in vivo data clearly demonstrating kinin-stimulation of prostaglandin production at the inflammatory site.

This study demonstrates that arachidonate metabolite production could be induced through the intrinsic kinin release in carrageenin-induced pleurisy by the use of captopril and the kininogen deficient rat strain (8).

Materials and Methods

Animals: Brown Norway Kitasato (B/N-Ki, normal) and Brown Norway Katholiek (B/N-Ka, HMW- and LMW-kininogens-deficient) rats were bred and kept in the animal laboratory of Kitasato University as previously reported (2). Sprague-Dawley (SD) rats, 7–9 week-old, were purchased from Shizuoka Experimental Animal Center (Hamamatsu).

Agents: Carrageenin (lambda, Sigma), indomethacin (Sigma), soybean trypsin inhibitor (SBTI, Worthington), pontamine sky blue (Tokyo Kasei) and anti-PGE$_2$ serum (Institut Pasteur Prod.) were purchased.

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Captopril (Sankyo Pharm. Co.), prostaglandins and antiserum to 6-keto-PGF1α (Ono Pharm. Co.) and thromboxane B2 (TXB2) were kind gifts.

Rat pleurisy: Production of rat pleurisy and sample collection were previously described (2). Captopril, 10 mg/kg, was injected intraperitoneally 30 min before the carrageenin injection. SBTI, 0.2 mg/rat, was injected simultaneously with 0.1 ml of 2% carrageenin in saline solution intrapleurally. Indomethacin, 5 mg/kg, suspended in saline solution containing 2% ethanol, was intraperitoneally injected into rats 30 min before the carrageenin injection.

Radioimmunoassay of arachidonate metabolites: Arachidonate metabolites in the pleural exudate was extracted through Sep-Pak C18 (Waters) and fractionated by HPLC and measured by radioimmunoassay as described previously (9).

Statistical analysis: Statistical analysis of data were carried out by Student's t-test at P<0.01 or P<0.05.

Results

Figure 1 illustrates the effect of captopril on carrageenin-induced pleurisy at 0.5 to 3 hr in Sprague-Dawley rats. Pleural fluid volume (panel A) and exudation rate (panel B) at 0.5, 1 and 2 hr, but not leukocytes (panel C) were significantly enhanced by pretreatment with captopril. Since enhancement at 1 hr was the most marked, effects of inhibitors were examined on 1 hr-pleurisy with or without pretreatment of captopril.

Figure 2 illustrates the effects of SBTI, an inhibitor of plasma kallikrein and Factor XII, and indomethacin on carrageenin induced-pleurisy at 1 hr. Increased pleural fluid volume (panel A) and exudation rate (panel B) by captopril were significantly suppressed (P<0.05) by simultaneous injection of SBTI with carrageenin and also suppressed by indomethacin.

Arachidonate metabolites in the exudate were measured by RIA and shown in panels D, E and F. Captopril significantly enhanced the levels of 6-keto-PGF1α (panel D) and TXB2 (panel E). This enhancement was also suppressed by SBTI, indicating that arachidonate metabolite production could be caused by the released plasma kinin. However, a small amount of PGE2 was found in the exudate, and no change was observed. Pretreatment with indomethacin also inhibited the pleural fluid accumulation, exudation rate and production of arachidonate metabolites by cap-

Fig. 1. Effect of captopril on carrageenin-induced rat pleurisy. (A) Pleural exudate volume at the indicated time after carrageenin injection. (B) Exudation rate: expressed by the leaked plasma volume during 20 min at the indicated time, and (C) Leukocyte number in the collected exudate. Closed circles show the data of the control group and open circles, those of the captopril-treated group. Captopril, 10 mg/kg, was injected intraperitoneally 30 min prior to the carrageenin injection. Each point expresses the mean of the indicated numbers of rats, with standard errors expressed by vertical bars. * and ** indicate values that are significantly different from those of the control group at P<0.05 and P<0.01, respectively. Figures by the circles in panel (A) indicate the numbers of rats used.
Fig. 2. Effect of SBTI and indomethacin on the carrageenin-induced pleurisy at 1 hr when rats were pretreated with captopril. Panels (A), (B) and (C) are the same as those in Fig. 1. Panels (D), (E) and (F) show the amounts of arachidonate metabolites, 6-oxo-PGF$_1\alpha$, TXB$_2$ and PGE$_2$, respectively, found in the exudate. Rats are divided into 4 groups: C, C-Ca, S-Ca and I-Ca, and numbers used are shown at the tops of the columns in panel (A). Group C is the control group that received saline intraperitoneally 30 min before the carrageenin injection; C-Ca is the group that received captopril, 10 mg/kg, intraperitoneally 30 min before the carrageenin injection; and S-Ca is the group that treated with captopril intraperitoneally as group C-Ca and also received SBTI, 0.2 mg/rat, simultaneously with carrageenin in the pleural cavity; and I-Ca is the group that received indomethacin, 5 mg/kg, intraperitoneally 30 min prior to the carrageenin injection. Other symbols are the same as those in Fig. 1. Each bracket indicates 2 groups that are compared, and when the difference between these two groups are statistically significant ($P<0.05$), this is indicated by an asterisk.

The same type of experiment was performed on a rat strain congenitally deficient in plasma HMW-kininogen and LMW-kininogen, B/N-Katholiek. As shown in Fig. 3, exudate volume at 1 hr of carrageenin pleurisy was less in the deficient strain than in the normal rat. Captopril treatment produced no enhancement in the former strain, whereas in the normal strain, B/N-Ki, it produced enhancement, which was mostly similar to that in SD rats (shown in Fig. 2). The amount of 6-keto-PGF$_1\alpha$ in the exudate of the normal strain increased markedly by captopril treatment; otherwise, the level was similar to that of B/N-Ka rats.

**Discussion**

As previously reported, the experimental inflammation in B/N-Katholiek rat was significantly less than that of the normal strain (2, 3). Captopril treatment markedly enhanced exudation of carrageenin pleurisy at the early phase in the normal strain, but not in the deficient strain, B/N-Ka, as previously reported (2). A similar result was reported for urate crystal edema in B/N-Katholiek rats in...
comparison with Wistar rats (10). In the present experiment, the above findings, especially the enhancement of inflammation by captopril in the normal strain, were confirmed.

Moreover, the production of 6-keto-PGF\textsubscript{1\alpha} in response to captopril was significantly different between the two strains, as shown in Fig. 3. The features of the normal strain B/N-Ki were similar to those of the SD rat except that the level of TXB\textsubscript{2} was less in B/N-Ki rats. The above fact indicates that PG\textsubscript{1\alpha} production in the exudate could be stimulated by intrinsic bradykinin whose biological life could be prolonged by captopril (otherwise, its half life is less than 10 sec in rat plasma (11, 12)) when the plasma kallikrein-kinin system is possibly activated by carrageenin to produce bradykinin from HMW-kinogen. Further evidence for the role of kinin was the observed suppression of pleural exudation by SBTI, a specific inhibitor of plasma kallikrein (13). The inhibitor also suppressed the production of 6-keto-PGF\textsubscript{1\alpha}, clearly indicating that the released kinin may stimulate the production of arachidonate metabolites.

Indomethacin mostly suppressed the production of arachidonate metabolites in the normal strain with or without pretreatment of captopril, but partially suppressed exudate volume as shown in Fig. 2. This remaining exudation may account for the direct action of bradykinin on vascular permeability, and the amount which was suppressed by indomethacin possibly accounts for the effect of PG\textsubscript{1\alpha}.

All these results indicate that arachidonate metabolites could be produced in response to stimulation by bradykinin, which could be produced by the activation of the kallikrein-kinin system by carrageenin. Therefore, the kinin could induce exudation by its intrinsic action and also does so by an indirect action through the production of arachidonate metabolites.

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