CHANGES IN FUNCTIONAL CERULOPLASMIN CONCENTRATIONS OF PLASMA AND EXUDATE AND THE EFFECT OF EXOGENOUS CERULOPLASMIN ON THE CARRAGEENIN-INDUCED INFLAMMATION IN RATS

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Changes in functional ceruloplasmin (Cp) concentrations of plasma and exudate during acute and chronic inflammatory processes were studied in detail by measuring Cp oxidase activity in the carrageenin-induced inflammation in rats. In contrast with the plasma functional Cp level as an acute-phase reactant, the exudate functional Cp level was very low during the acute phase, increased in the chronic phase and reached a constant value which was only half value of the plasma functional Cp level. The locally injected Cp had no effect on the carrageenin-induced inflammation, suggesting that exogenous Cp has no anti-inflammatory effect on the inflamed rats eating normal diet.

Keywords — ceruloplasmin; inflammation; carrageenin-induced inflammation; steroidal anti-inflammatory drug

INTRODUCTION

Serum ceruloplasmin (Cp) has been reported as an acute-phase reactant which rapidly increases in the acute phase of experimental inflammation in animals.1-5 In man, the elevated serum Cp level has been found in inflammatory diseases including rheumatoid arthritis.6-8 Copper in serum is firmly bound to Cp, and a strong positive correlation between copper and Cp concentrations is found in the sera of both inflamed rats3-5 and rheumatoid patients.9 Cp has, at least, three physiological functions; oxidase activity which acts as a ferroxidase, copper transport and maintenance of copper homeostasis.10 Thus, Cp probably participates in important biochemical processes in inflammation such as free radical metabolism and biosyntheses of prostaglandin and collagen.11

Change with time in serum Cp level has been studied in acute phase of inflammation,2-5 but no precise study on the change in exudate Cp level which reflects the Cp level of inflammatory lesion has been reported. In the present paper, by measuring Cp oxidase activity we report the changes in functionally active Cp concentrations of both plasma and exudate not only in the acute phase but also in the chronic phase of the carrageenin-induced inflammation in rats. We also report here on the effect of locally injected Cp on the inflammation induced by carrageenin.

MATERIALS AND METHODS

Measurement of Cp Oxidase Activities of Plasma and Exudate — Male Sprague-Dawley rats (Specific-pathogen free, from Charles River Japan Inc.), weighing 150-180 g, were used. An inflammation in the form of carrageenin-air-pouch was induced by subcutaneous injection of 4 ml of a 2% (w/v) solution of carrageenin (Seakem 202, from Marine Colloid Inc.,

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U.S.A.) into a preformed air-pouch on the back of rats. At various times after carrageenin injection, tail artery was cut with a surgical blade and oozed blood (about 150 μl) was collected in heparinized capillary tube (0.2 × 11 cm). Blood was repeatedly collected from the same rat at 0, 7, 12, 18, 24, 35, 55 and 88 h (the first experiment) or on 5, 8, 11 and 16 d (the second experiment) after carrageenin injection. In each experiment, blood from normal rat was also repeatedly collected at the same time intervals. Each experiment included seven inflamed rats and six normal rats. Plasma (20 μl) was obtained by centrifugation of the capillary tube at 1100 × g for 20 min at 4°C and diluted with 0.78 ml of 0.1 M acetate buffer (pH 5). Cp oxidase activity of the diluted plasma was measured on that day to avoid the decrease of Cp oxidase activity during storage.

The pouch fluid was repeatedly collected from the same rat at 0, 10, 24 and 72 h, and on 5, 7, 10 and 16 d after carrageenin injection (the third experiment, 10 rats). The pouch fluid (exudate, 0.25 ml) was collected and mixed with 2 ml of 0.1 M acetate buffer (pH 5). After centrifugation of the pouch fluid mixed with the buffer at 70000 × g for 60 min at 2°C, the supernatant (0.8 ml) was used for Cp assay on that day.

Functionally active Cp concentrations of plasma and exudate were determined by measuring Cp oxidase activity using o-dianisidine as a substrate. The enzymatic activity of Cp is expressed in International Units, in terms of substrate consumed; Cp oxidase activity (U/ml) = (A15 - A5) × 6.25 × 1.0 U/ml. A15 and A5 are the measured absorbances of the solutions incubated for 15 and 5 min, respectively.

Effect of Steroidal Anti-inflammatory Drugs on the Plasma Cp Level — Steroidal anti-inflammatory drugs or vehicle were injected into the carrageenin-air-pouch immediately after carrageenin injection, and plasma Cp oxidase activity was measured 20 h later. Effect of anti-inflammatory drugs on plasma functional Cp level was evaluated by comparing with the plasma functional Cp level of control group. The suppression by the drugs of the increase in plasma functional Cp level at 20 h after carrageenin injection was calculated as follows:

% inhibition = \[1 - \left( \frac{T_{20} - T_0}{C_{20} - C_0} \right) \times 100\]

where \(C_0\) and \(C_{20}\) are the measured plasma Cp oxidase activity of control group at 0 and 20 h, respectively, after carrageenin injection, while \(T_0\) and \(T_{20}\) are those of the drug-treated group.

Treatment in Vivo with Cp — Cp was partially purified from day 14-exudate according to the method of Deutsch, and the partially purified Cp solution (1.3 U/ml of 0.9% NaCl, 5.5 mg protein/ml) was obtained. One group was injected with 2 ml of the Cp solution into the carrageenin-air-pouch immediately after carrageenin injection, and then 1 ml of the Cp solution was repeatedly injected into the pouch every 12 h (at 12, 24, 36, 48 and 60 h after carrageenin injection), while another group (control group) was injected with 0.9% NaCl at the same time intervals. Exudate (0.25 ml) was collected at 1, 25, 49, 72 and 100 h and blood (150 μl) was collected at 0, 10, 30, 49, 72 and 100 h after carrageenin injection. Cp oxidase activities of exudate and plasma were measured as described above. Anti-inflammatory action of the locally injected Cp was estimated by measuring the wet weight of granulation tissue and weight of exudate at 100 h (day 4) after carrageenin injection.

RESULTS

Functional Cp Concentrations of Plasma and Exudate

Changes in functional Cp concentrations of plasma and exudate after carrageenin injection were summarized in Fig. 1. The plasma functional Cp concentration of normal rats remains constant throughout the experimental period, suggesting that repeated collections of blood from the same rat had no effect on the plasma functional Cp concentration. The plasma functional Cp concentration of inflamed rats increased after a lag period of about 7 h, reached a maximum about 30 h after carrageenin injection, and then gradually decreased (Fig. 1). On the other
hand, the exudate functional Cp concentration increased only after a lag period of about 20 h (Figs. 1 and 3), reached a maximum level on day 5 after carrageenin injection, and then maintained a constant level. In the chronic phase of the carrageenin-induced inflammation, the plasma functional Cp concentration was held constant at about 0.223 U/ml, which was significantly higher than functional Cp concentrations of exudate (about 0.122 U/ml) and normal rat plasma (about 0.149 U/ml). The origin of exudate Cp may be serum Cp, because the increase in exudate functional Cp corresponds to the decrease in plasma functional Cp (Fig. 1) and plasma functional Cp level increased by the injection of Cp into the carrageenin-air-pouch (Fig. 3).

Effect of Steroidal Anti-inflammatory Drugs on the Plasma Functional Cp Level

Plasma Cp oxidase activity markedly increases after carrageenin injection (Fig. 1), suggesting that plasma functional Cp level is an

FIG. 1. Changes with Time in Functional Cp Concentrations of Plasma and Exudate in the Carrageenin-Induced Inflammation in Rats

- ○ ○ the plasma functional Cp concentration of normal rats.
- ● ● the plasma functional Cp concentration of inflamed rats.
- ▲ ▲ the exudate functional Cp concentration of inflamed rats.

Each point represents the mean ± S.E.M. (vertical bars).
Experimental details are shown in the text.

FIG. 2. Inhibitory Effect of Steroidal Anti-inflammatory Drugs on the Plasma Functional Cp Level

Each point represents the mean ± S.E.M. of 5—7 determinations (rats).
Experimental details are shown in the text.

FIG. 3. Effect of Repeated Injections of Partially Purified Cp on the Functional Cp Concentrations of Exudate and Plasma in the Carrageenin-Induced Inflammation in Rats

- ○ ○ the plasma functional Cp concentration of control rats.
- ▲ ▲ the exudate functional Cp concentration of control rats.
- ● ● the plasma functional Cp concentration of Cp-treated rats.
- ▲ ▲ the exudate functional Cp concentration of Cp-treated rats.

Each point represents the mean ± S.E.M. (vertical bars).
* p < 0.01, ** p < 0.05 (Student’s t-test).
Experimental details are shown in the text.
index of the intensity of inflammation. To ascertain whether this possibility is true, we studied the effect of dexamethasone, prednisolone and hydrocortisone on the increase in the plasma functional Cp level. As shown in Fig. 2, the steroid anti-inflammatory drugs suppressed the rise of the plasma functional Cp level at 20 h after carrageenin injection in dose-response manner and the suppressive action was proportional to the anti-inflammatory potency of the steroids.

_Treatment in Vivo with Cp_

Effect of Cp on the carrageenin-induced inflammation was studied by the repeated injections of partially purified Cp. Cp was eluted as a single peak when applied to diethylaminoethyl (DEAE)-cellulose column at the final step of purification, suggesting that the Cp was fairly pure, though a minor band which had no oxidase activity was found in this Cp fraction on analytical polyacrylamide gel electrophoresis (data not shown).

Repeated injections of the partially purified Cp into the carrageenin-air-pouch markedly increased the exudate functional Cp concentration, which corresponded to a maximum level of the exudate functional Cp in the carrageenin-induced inflammation in rats (Figs. 1 and 3). On the other hand, a slight increase in the plasma functional Cp concentration was found in the Cp-treated group (Fig. 3). Although the local functional Cp level was high throughout the experimental period, the locally injected Cp had no significant effect on the weight of exudate and the formation of granulation tissue on day 4 after carrageenin injection (Table I). In addition, a single injection of Cp had no effect on the migration of polymorphonuclear leukocytes into the carrageenin-air-pouch at 2 and 6 h after carrageenin injection when the partially purified Cp (1.4 U in 1.5 ml of 0.9% NaCl) was injected into the carrageenin-air-pouch immediately after carrageenin injection (data not shown).

**DISCUSSION**

Functional Cp concentrations of plasma and exudate were determined not only in the acute phase but also in the chronic phase of the carrageenin-air-pouch inflammation in rats. Plasma functional Cp behaved as an acute-phase reactant in the acute phase and was maintained at a constant level in the chronic phase, which was significantly higher than the plasma functional Cp level of normal rats (Fig. 1). On the other hand, the exudate functional Cp (the local Cp) concentration remained at a very low level during the acute phase, increased in the chronic phase and reached a constant level which was only half value of the plasma functional Cp level in the chronic phase of the carrageenin-air-pouch inflammation (Fig. 1). This low level of exudate functional Cp may be accounted for by (1) the restricted passage of Cp (a high molecular weight glycoprotein) through blood vessel, because the exudate functional Cp level was significantly lower than the plasma functional Cp level (Fig. 1); (2) the utilization of Cp in inflammatory processes including free radical metabolism and biosyntheses of prostaglandin and collagen. The exudate functional Cp concentration increases with the development of granulation tissue; the formation of the tissue

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rats</th>
<th>Granulation tissue (g)</th>
<th>Exudate (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>3.78±0.17</td>
<td>6.97±0.85</td>
</tr>
<tr>
<td>Cp-treated</td>
<td>7</td>
<td>3.19±0.31</td>
<td>9.09±1.06</td>
</tr>
</tbody>
</table>

Weights of exudate and granulation tissue were measured on day 4 after carrageenin injection. Data are shown as means ± S.E.M. Experimental details are shown in the text.
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takes place 2—3 d after carrageenin injection and the wet weight of the tissue reaches a maximum on day 5 and then a constant wet weight remains until at least day 16 (data not shown), suggesting that Cp supplies copper involved in granulation tissue metabolism.

As shown in Fig. 2, the suppression of the increase in the plasma functional Cp level by the steroidal anti-inflammatory drugs depends on the anti-inflammatory potency of the steroids, suggesting that the plasma functional Cp level reflects the intensity of inflammation. Therefore, on the basis of the plasma functional Cp level (Fig. 1), it seems likely that a mild inflammation constantly progresses during the chronic phase of the carrageenin-air-pouch inflammation.

Milanino et al. demonstrated that the acute inflammatory process was enhanced in copper-deficient rat; the development of carrageenin-induced foot edema and pleurisy was enhanced in rats after 1 month of a 0.2 ppm copper-deficient diet. Denko also reported that experimental inflammation in copper-deficient rats was greater than that induced in control rats eating normal diet, and the injection of Cp resulted in reduction of the foot swelling with monosodium urate in copper-deficient rats. Based on these findings, it has been proposed that endogenous copper has a protective modulatory role in the inflammatory processes; copper-deficiency may induce a significant decrease in the activities of Cp and superoxide dismutase which protect tissues from free radical-induced damage. On the other hand, the data shown in Table I indicate that repeated injections of Cp have no inhibitory effect on the edudation, the formation of granulation tissue and the infiltration of polymorphonuclear leukocytes in the carrageenin-induced inflammation in rats eating normal diet. Our data suggest that exogenous Cp does not exert anti-inflammatory activity on the inflamed rats which are not copper-deficient, though Cp has anti-inflammatory effect on the inflamed rats under copper-deficient condition as reported by Denko.

REFERENCES
3) A. Conforti, L. Franco, R. Milanino and G. P. Velo: Copper and ceruloplasmin (Cp) concentrations during the acute inflammatory process in the rat, Agents Actions, 12, 303—307 (1982).