ROLE OF HUMAN PLACENTAL EXTRACT ON SUCCINIC DEHYDROGENASE ACTIVITY IN CARRAGEENIN-INDUCED EDEMA IN RATS IN VIVO AND ITS EFFECT ON ERYTHROCYTE LYSIS, PLATELET AGGREGATION AND TRYPsin ACTIVITY IN VITRO

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Abstract: Significant increase of liver succinic dehydrogenase (SDH, EC 1.3.99.1) activity was produced by carrageenin-induced edema in rats. Pretreatment with human placental extract inhibited the increased liver SDH activity in a dose-dependent manner. Placental extract was found to have little or no effect on the liver SDH activity in normal rats. Furthermore, heat-induced erythrocyte lysis was inhibited to a substantial extent by the extract and was found to be dose-responsive. However, adenosine diphosphate (ADP)-induced platelet aggregation and trypsin activity were not changed by the placental extract in vitro.

The study indicates that the membrane stabilization and depletion of adenosine triphosphate (ATP) synthesis may contribute to antiinflammatory effect of the extract.

Key words: human placental extract, succinic dehydrogenase, erythrocyte lysis, antiinflammatory

INTRODUCTION

Human placental extract has been claimed to have actions in diseases of diverse aetiology (1). The application of placental extracts in many chronic inflammatory conditions commonly encountered in gynaecology, rheumatic affections, psoriasis, corneal ulcer, vitiligo etc. was evidenced world-wide (2-6) and these preparations are known to contain human chorionic gonadotrophin (HCG), human placental lactogen (HPL), corticotropin-releasing like factor (CRF), lactoferrin (LF) etc. (7-9).

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in vitro for studying the adherence of platelet to subendothelial structures in injured blood vessels (12,13).

We have reported the significant suppression of carrageenin-induced inflammation by the administration of human placental extract (14). In the present study, we present the effects of human placental extract on liver SDH activity in carrageenin-induced edema in vivo, heat-induced erythrocyte lysis, ADP-induced platelet aggregation and trypsin activity in vitro in order to have the insight into the possible mechanism by which the human placental extract could exert its antiinflammatory effect.

METHODS

Carrageenin-induced edema in rats: Male Wistar rats (100-125 g) having free access to a standard laboratory food and water ad libitum. Rats were divided into groups of 8 in each. Group 1 received normal saline (0.1 ml/animal) into the planter region of the hind paw. In Group 2, edema was produced acutely by subcutaneous injection of 0.1 ml of 1% w/v carrageen in into the planter region of the hind paw of the rat (15). Group 3 to 7 received placental extract ip in different doses (1, 2, 3, 4 and 5 ml/kg) 30 min before carrageenin administration. Vehicle (benzyl alcohol, 1.5% v/v) in different doses (1 to 5 ml/kg) were injected ip in the rats of another set of group 3 to 7 instead of placental extract 30 min before carrageenin treatment. Four h later the injection of carrageenin or normal saline, the animals were sacrificed, livers were removed and kept at 0-4°C for the enzymatic analysis.

Liver succinic dehydrogenase (SDH) activity: A portion of the liver was homogenized in 0.1 M phosphate buffer (pH 7.4) in ice-cold Potter-Elvehjem homogenizer to give a 10% w/v homogenate which was centrifuged at 15000 x g for 30 min in a Sorvall refrigerated centrifuge. The supernatant was used as enzyme preparation. SDH activity was assayed spectrophotometrically (16) and was expressed as μmol of succinate oxidized/h.

Heat-induced erythrocyte lysis: Blood from rat was collected in a heparinized vial and plasma was separated. The blood cells were washed 3 times with normal saline and finally suspended as 2 x 10^6 cells/μl. 0.25 ml of this suspension was taken in 50 test tubes containing 2.5 ml of 0.15 M phosphate buffer (pH 7.4) in each. Placental extract was added to each experimental tube. A set of tubes with 1.5% benzyl alcohol in each served as control. Volume in each tube was made up to 5.0 ml with distilled water. The tubes were inverted for thorough mixing, kept at 53°C for 20 min, cooled and centrifuged. The absorbance of the supernatant was measured at 540 nm (17).

ADP-induced platelet aggregation: Rat blood was withdrawn into a plastic syringe containing 3.8% trisodium citrate (9:1 v/v). Platelet rich plasma (PRP) was obtained by the centrifugation of the dilute plasma at 200 x g for 10 min at 0°C. Platelet count in PRP was adjusted to 200000/μl. Placental extract (0.1 to 0.3 ml) was added to 0.5 ml of PRP of rat after 2 min preincubation and then 1 min later 0.1 ml of the ADP solution was added to induce aggregation. A set of tubes with 1.5% benzyl alcohol in each served as control. Aggregation was estimated by the measurement of light transmission at 600 nm (12).

Trypsin activity in presence of absence of placental extract: The incubation mixture consisted of the following reagents in 0.4 ml : 5 μg of trypsin, 4 mg of azocasein and different volumes of placental extract (0.05 - 0.3 ml). A set of tubes with 1.5% benzyl alcohol in each served as control. Trypsin activity in presence or absence of placental extract was assayed by the method of Barret et al (18).

Commercial preparation of human placental extract was used in the study. Each ml of this extract was derived from 0.1 g of fresh human placenta and benzyl alcohol (1.5% v/v) was used as preservative (14). Carrageenin, ADP, trypsin, azocasein were from Sigma, St. Louis, USA. All other chemicals were of analytical grade from E. Merck (India) Limited.

The statistical analysis was performed using student’s t-test.

RESULTS AND DISCUSSION

The results of study are summarized in Table I. SDH activity in liver was increased markedly in rats with carrageenin-induced edema. 30 min pretreatment with placental extract inhibited the increase of liver
TABLE 1: Effect of human placental extract on liver SDH in carrageenan induced edema in rats and its in vitro effect on heat-induced erythrocyte lysis.

<table>
<thead>
<tr>
<th>Group</th>
<th>SDH activity (µmol of succinate oxidized/h)</th>
<th>Placental extract (ml)</th>
<th>% inhibition of erythrocyte lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>35.1 ± 7.7</td>
<td>0.5</td>
<td>20 ± 6.2</td>
</tr>
<tr>
<td>Carrageenan-induced edema</td>
<td>76.5 ± 5.2***</td>
<td>1.0</td>
<td>35 ± 4.1</td>
</tr>
<tr>
<td>Extract 1 ml/kg</td>
<td>56.7 ± 4.1*</td>
<td>1.5</td>
<td>48 ± 2.0*</td>
</tr>
<tr>
<td>Extract 2 ml/kg</td>
<td>51.8 ± 4.3*</td>
<td>2.0</td>
<td>56 ± 5.3*</td>
</tr>
<tr>
<td>Extract 3 ml/kg</td>
<td>47.4 ± 3.2**</td>
<td>2.5</td>
<td>51 ± 4.0*</td>
</tr>
<tr>
<td>Extract 4 ml/kg</td>
<td>42.4 ± 3.0**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract 5 ml/kg</td>
<td>38.8 ± 4.0**</td>
<td></td>
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</table>

Results are expressed as Mean ± SEM (n=8 or 5). ***P < 0.001 as compared to normal saline. *P < 0.01 and **P < 0.001 as compared to carrageenan-induced edema. *P< 0.05 and **P< 0.01 as compared to corresponding control.

SDH activity in a dose-dependent manner. In normal rats liver SDH activity was not affected by the extract. Placental extract inhibited the heat-induced erythrocyte lysis in vitro in a dose dependent manner upto 2.0 ml (Table I). On the other hand, placental extract did not change the trypsin activity in vitro as well as aggregating effect of platelet induced by ADP.

Human placental extract prevents the increase in the liver SDH activity produced during inflammation with carrageenan. The inhibition of liver SDH activity, the key enzyme linked with energy (ATP) yielding citric acid cycle, by the placental extract during inflammation as seen in this study would result in the depletion of ATP supply to the liver tissue. This may be true in case of other body tissues including the inflamed tissue as well. However, usually many biochemical changes in the liver of the animals occur long before secondary physiological change that may be associated with the experimental conditions and the changes in the liver provide much more sensitive indication for the same.

Erythrocyte lysis was inhibited by the placental extract in vitro. Non-steroidal antiinflammatory drugs were reported to stabilize the erythrocyte. The process of inflammation could be visualized as wave of leaky membranes and antiinflammatory drugs could exert their effect by stabilization of the cell membrane (17). The protective effect of placental extract on heat-induced erythrocyte lysis in vitro partially confirm the idea.

Placental extract did not affect the ADP-induced platelet aggregation in vitro apparently suggesting antiinflammatory action of placental extract may not be mediated via prostaglandin inhibition.

Placental extract also did not change the trypsin activity in vitro which too is unlikely to be involved in the antiinflammatory effect.

REFERENCES

References


