Indian J Physiol Pharmacol 1994; 38(2) : 121-124

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*

KISHORE K. BANERJEE, ANUPAM BISHAYEE* AND MALAY CHATTERJEE*+

Department of Occupational Health,
All India Institute of Hygiene & Public Health,
110, Chittaranjan Avenue,
Calcutta - 700 073
and
**Division of Biochemistry,*
Department of Pharmaceutical Technology,
Jadavpur University,
Calcutta - 700 032

(Received on February 3, 1993)

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).

Inhibition of succinic dehydrogenase (SDH), a respiratory enzyme, was found to be an important criterion for antiinflammatory property (10). In inflammation, different proteases become activated and make the cells vulnerable to leakage to such an extent that it is possible to induce edema in rat by local administration of different proteases like trypsin and anti-inflammatory drugs could moderate its activity (11). It is also wellknown that inhibitor of endogenous prostaglandin synthesis usually interferes with platelet aggregation. The aggregation of platelet by adenosine diphosphate (ADP) is considered to be an useful system

+Corresponding Author

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 inection 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 × 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 2×10^6 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 upto 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 × 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 summerized 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 I : Effect of human placental extract on liver SDH in carrageenin induced edema in rats and its *in vitro* effect on heat-induced erythrocyte lysis.

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

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 carrageenin-induced edema. [a]P< 0.05 and [b]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 carrageenin. 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 inflammed 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

1. Windholz M, Merck Index. Rahway, Merck and Co., Inc. 1983 ; 1084.
2. Bertone C, Sgro LC. Clinical data on human placental extract's administration in gynaecological cases. *Int J Tiss Reac* 1982; 4 : 165-167.
3. Rosenthal M. The application of an extract of human placenta in the treatment of rheumatic affections. *Int J Tiss Reac* 1982; 4 : 147-151.
4. Lodi A. Local therapy of psoriasis with placentrex extract. *Ital J Dermatol Venerol* 1986 ; 121 : 15-17.

5. Girotis G, Malinverni W. Use of placental extract for the treatment of myopic and senile chorio-retinal dystrophies. *Int J Tiss Reac* 1982; 4: 169.

6. Sharma SK. Topical human placental extract for the treatment of vitiligo. *Indian J Dermatol Veneral Leprol* 1988; 54 : 199-201.

7. Chatterjee M, Munro HW. Structure and biosynthesis of human placental peptide hormones. *Vitam Horm* 1977 ; 35 : 149-208.

8. Klopper A. Biochemistry of placental proteins. Basel, Karger 1985 : 17-18.

9. Shibasaki T. Corticotropin-releasing-like activity in human placental extracts. *J Clin Endorcrinol Metabol* 1982 : 55 : 384 - 386.

10. Naik SR, Kalyanpur SG, Sheth UK. Effect of antiinfammatory drugs on glutathione levels and liver succinic dehyrogenase activity in carrageenin edema and cotton pellet granuloma in rats. *Biochem Pharmacol* 1972 ; 21: 511-516.

11. Bertelli A. Proteases and antiproteasic substance in the inflammatory responses. In Bernerd KF, ed. Chemical Biology of Inflammation. London, Pergamon Press 1968 : 229-240.

12. Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 162 ; 194 : 927 - 929.

13. Manabe S, Wada O, Matsui H, Takada M, Kobayshi N, Mackawa T. Triphenyltinfluride *in vitro* inhibition of rabbit platelet collagen-induced aggregation and ATP secretion and blockade of arachidonic acid metabolization from membrane phospholipids. *Biochem Pharmacol* 1983 ; 32 : 1627 - 1634.

14. Banerjee KK, Bharadwaj D, Ghosh DP, Hati RN. Effect of human placental extract 'Plaecentrex' on experimental inflammation. *Indian Med Gaz* 1990 ; 124 : 243-244.

15. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc Soc Exp Biol Med* 1962 ; 111 : 544-547.

16. Singer TP, Kearney EB. Determination of succinic dehydrogenase activity. In Glick D, ed. Methods of Biochemical Analysis. New York, Inter Science Publishers, Inc. 1957 : 307-333.

17. Gleen EM, Bowman JB, Koslowske TC. The systemetic response to inflammation. In Bernerd KF, ed. Chemical Biology of Inflammation. London, Pergamon Press 1968 : 27-49.

18. Barret AJ, Kirschke H, Cathepsin B, Cathepsin H, Cathepsin L. In Lorand L, ed. Methods in Enzymology, vol. 80. New York, Acdemic Press 1981 : 523-543.