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IMMUNOPHARMACOLOGICAL STUDIES ON PICRORHIZA KURROA ROYLE EX BENTH PART VI : EFFECT ON ANAPHYLACTIC ACTIVATION EVENTS IN RAT PERITONEAL MAST CELLS

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Summary : Mechanism of inhibion of mast cell anaphylaxis by P. kurroa-extract (PK) treatment in rats was investigated. Mast cell-IgE binding, assessed from induction of passive sensitization, was not affected. Calcium-independent early activation events in mast cell anaphylaxis indicated on inhibitory influence of PK-treatment. Inhibition of membrane-protease release by PK-treatment was suggested by study of gastric secretion and exhibition of saturable synergism with Diisopropyl fluoro phosphate on inhibition of anaphylactic degranulation. pH-independance of mast cell stabilizing effect negates any PK-influence on phospholipid transmethylation. The results complement findings of earlier studies on indirect effects of PK through alteration of membrane structure/-function.

Key words: antiallergic drug mast cell-activation mast cell-stabilizing action Picrorhiza kurroa.

INTRODUCTION

Antiallergic mast cell stabilizing effect of P. kurroa rhizome (Kutki) extract was reported earlier (1). Mast cell stabilization appeared gradually on repeated administration in vivo only (1). Antihepatotoxic and choleretic activities of the herb have been ascribed to the presence of iridoid glycosides (2, 3), which probably exert nonspecific biological effects (4). This report elaborates on mechanisms of mast cell stabilization, during early events of anaphylactic activation by treatment with water soluble fraction of the alcoholic rhizome extract rich in the above glycosides.

MATERIALS AND METHODS

Procedural details of preparation of the extract have been reported earlier (1, 5). All experiments were conducted in male Wistar rats (100-150 g), acclimatised to laboratory diet and conditions for at least one week. Quantities of water soluble fraction of the alcoholic extract (PK) represent dry weight of the parent alcoholic rhizome extract. PK treatment was oral (100 mg/kg, daily for 3 days). Controls received equivalent volumes of distilled water (DW). Two hrs after the last dose of PK or DW, animals were used as described below.

Effect on induction of passive sensitization of mesenteric mast cells : Procedures of sensitization of donor rats with horse serum and microscopic morphometry of degranulated mast cells have been reported elsewhere (1). 15 Days after sensitization of donor rats with horse serum, IgE-rich antisera were harvested. Mesenteric tissue was obtained from control (DW) and PK-treated animals for in vitro induction of passive sensitization with donor sera. Briefly, sensitised donor blood was collected in

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sterile vials and kept at 4°C for 4 hr and sera were pooled after centinuous oxygenation. Mesenteric pieces from control or PK-treated rats were added to separate weighed cuvets containing Ringer-Locke solution at 37°C under continuous oxygenation. Cuvets were weighed again to give weight of the added tissue. Mesenteric tissue was then incubated in aliquotes of donor sera (5 ml/g) for 30 min at 4°C. Mesenteric tissue from fresh rats was obtained and incubated separately with the above postincubation serum samples from control and PK-treated tissues. Fresh mesenteric tissue after similar 30 min incubation at 4°C was removed and challenged with horse serum (0.5 mg/ml) for 5 min. Challenged tissue was then stained and microscopically examinned for percentage of degranulated mast calls.

Effect on antigenic activation of sensitised mast cells: Passively sensitised mesenteric pieces from control and PK-treated rats were suspended in Ca^{++}-free solution, otherwise having same composition, at 0°C under continuous oxygenation. They were challenged with horse serum (antigen) and subsequently were transferred to fresh Ca^{++}-free solution at 0°C. After 3 washes with antigen-free solution, some of the pieces were removed and processed for microscopic examination. Half of the remaining sample was incubated at 0°C and the rest at 37°C in Ca^{++}-free solution for 5 min. Following such incubation different sets of challenged pieces were suspended in normal Ringer Locke solution at 37°C for 5 min, and were finally processed for microscopic examination.

Effect on mast cell anaphylasis in DFP-treated mesenteries: Mesenteric tissue was obtained from control (DW) and PK-treated rats 2 hr after completion of the treatments. The pieces were separately subjected to passive sensitization as before. Such pieces were then taken in Ringer Locke solution at 37°C under oxygenation. In both the sets of mesenteric tissue, Di-isopropyl fluoro phosphate (DFP) was added to give final concentration either of 10^{-3} M or 10^{-5} M. After 5 min of such a treatment different sets were challenged with horse serum and % of mast cell degranulation was assesed microscopically.

Effect on proteolytic activity of rat gastric secretion: Rats were treated with DW or PK for 3 days. Between the 2nd and 3rd ration, they were fasted overnight. 4 hr after the last dose, rats were anaesthesised by pentobarbitone sodium (40 mg/kg, ip) and were subjected to surgical pylorus-ligation (6). 4 hr later, they were sacrificed by cervical fracture and gastric juice was collected through a niche in fundus. After centrifugation and filtration, clear gastric juice was obtained for analysis of proteolytic activity using haemoglobin as substrate, by a modification of Anson's method (7) reported elsewhere (8). Proteolytic activity is expressed in terms of amount of tyrosine produced on digestion of haemoglobin over 20 min at 37°C.

Effect of in vitro PK-admixture on gastric juice proteolytic activity: Fresh rats fasted overnight were used to obtain gastric juice as above. Duplicate aliquots of equal volume were taken from gastric juice samples of each rat using dilutions with distilled water wherever necessary. One of the aliquots served as control while to the other, 5 mg/ml of PK was added. Both control and PK-mixed samples were incubated for 10 min at 37°C. After incubations, volume of the respective control and PK-mixed samples were matched by adding DW to the former. Subsequently proteolytic activity of the samples was determined as before.

Effect on mast cell anaphylaxis at varying pH of incubation medium: pH of Ringer Locke solutions was adjusted with 0.1 N NaNH or HCI at 6.5, 8.5 and neutral (7 4). Passively sensitised mesenteric tissues from PK-treated rats were challenged with horse serum in above solution separately and % of degranulated mast cells after challenges was evaluated.

RESULTS

Induction of passive sensitization : Mesenteric pieces from fresh rats when incubated with donor sera, through which mesenteric pieces from either the control (DW) or PK-treated rats had passed before, acquired lesser degree of passive sensitization in comparison to that acquired by similar pieces incubated with maiden samples of donor sera. This was evident from lower challenge degranulations in the former as compared to the latter mesenteric sets. Reductions in passive sensitizing efficacy of donor serum were similar, whethere mesenteric pieces passed were from the control or PK-treated rats (Table I).

TABLE I : Passive sensitization inducing capacities of Donar serum following passage of control or PK treated rat mesenteries (n=5 in each group).

Incubation Group	*Prior treatment of Mesentery Donar Rate*	*% of mast cells degranulating of passive anaphylaxis (mean±sem)*
I Primary	.....	66.5±5.7
II Secondary		
A	DW X 3 Days	50.6±4.6
B	PK X 3 Days	46.4±5.8*

* P<0.05 (with respect to I)

Antigenic activation in calcium-free environment : Challenge with antigen in Ca^{++}-free environment significantly reduced the anaphylactic degranulation of mast cells. After rinsing off the antigen, incubation in normal solution resulted in significant degranulation of mast cells, if maintained at 0°C but not at 37°C. Such degranulation was significantly inhibited in cells from PK-Treated animals (Table II).

TABLE II : Degranulation of passively sensitized rat mesenteric mast cells in Ca^{++} containg medium after anaphylactic activation in Ca^{-++} free medium (n=5 in each group).

Group	*Incubation Temperature*	*% of Mast Cells Degranulated (Mean±Sem)*	*P Value (Vs Group Indicated in Parentheses)*
I Control			
A	37°C	34.9±4.2	<0.01(I of TableI)
B	0°C	50.4±4.1	<0.05(I of TableI)
II PK-Treated			
A	37°C	38.2±4.6	
B	0°C	32.4±6.2	<0.05 (I-B)

Effect on mast cell anasphylaxis in presence of DFP : Antigen challenge in presence of DFP significantly reduced the anaphylactic degranulation of mast cells (Table III). Comparative inhibitions with PK-

TABLE III : Anaphylactic Degranulation of Passively sensitized Mesenteric Mast Cells of control and PK Treated Rats : Effects of DFP and Altered pH of Medium (n=5 in each group)

Group	*Changes in Inclubation Medium*	*% of Degranulated Mast Cells (Mean±Sem)*	
		A. Control Rats	*B.PK Treated Rats*
I	None	66.5±5.7	46.2±4.5
II. 1	DFP 10^{-5}M	58.2±4.2	36.7±4.3@@
II. 2	DFP 10^{-3}M	42.2±4.8*	38.2±4.6
III. 1	pH 6.5	78.4±6.0	50.6±4.0@@
III. 2	pH 8.5	58.5±4.4	38.8±4.2@

* P<0.02 (Vs. respective values in group I)

@ F<0.02 and @@ P<0 01 (Vs. respective control group A values)

treatment alone or PK+DFP treatment, did not differ significantly, though a superior effect of combined treatment was apparent in numerical figures. However, despite 100-fold differences in molar concentration of DFP, synergistic inhibition of degranulation in both the PK-treated groups were similar. Further, significant synergism of PK-treatment with DFP-treatment was evident only at low, i e. 10^{-5}M concentration.

Proteolytic activity of gastric juice : PK-treatment for 3 days reduced secretion of gastric protease as seen from estimates of proteolytic activity of gastric juice. In terms of μM of tyrosine produced : the values in control and PK-treatment groups were 218.32 ± 17.42 and 179.45 ± 18.23 ($n=5$, $P<0.01$). Admixture of PK with gastric juice, in vitro (5mg/ml), had no major effect on proteolytic activity.

Influence of varying pH on PK-effect : Anaphylactic degranulation of mast cells seen at pH 6.5 was slightly higher and that occurring at pH 8.5 was slightly lower as compared to the degranulations at pH 7.4. A similar trend was observed in PK-treated groups as well. Thus change of pH did not interfere with the anti-anaphylactic action of PK (Table III).

DISCUSSION

Plasma membranes of mast cells and basophils are the loci for antigen IgE antibody reaction, the forerunner event of anaphylaxis. Broadly, the events are subdivided into four phases : antibody binding at specific membrane receptors; activation of biochemical processes following above complex-formation; phenomenon of mediator release; and finally autoinhibition of release process (see 9 for review).

Rat mast cells bear proteases on their surface which can reduce binding and persistence of IgE over the membrane receptors (10). Similar reductions in passive sensitization inducing capacity of donor serum following passage of control and PK-treated rat mesenteries, negates any interference by PK-treatment with surface binding of IgE, because binding of the latter is in direct proportion to serum titers (11).

Brindging of two adjacent IgE molecules by an antigen molecule sets perturbance in structure of cell membrane which initiates biochemical reactions preparatory to mediator release (12). An initial event is activation of serine esterase which is associated with the IgE receptors in the membrane (13). This is followed by activation of phospholipid transmethylating enzymes which pave the way for Ca^{++} influx (12). The relationship of protease activation to transmethylation however, remains ill defined. Phospholipid transmethylation in necessary for Ca^{++} influx and provides substrate for action of membrane phospholipase A_2 (14), that is activated by influxing calcium ion (14). Protease and transmethylase activations precede the Ca^{++} influx and hence are independent of extracellular Ca^{++} requirement (12, 13). They thus provide the activated state of the membrane in preparation of mediator release. The activated state of mast cells so attained, on challenge, quickly withers off at 37°C, if no influx of extracellular calcium takes place; but may persist for several minutes at 0°C (15). Validity of the technique adopted in the present study to detect effect of PK-treatment on anaphylactic mast cell activation is substantiated by observations complementing a temperature dependent maintenance of activation phase (Table II). Reduced degranulation of mesenteric mast cells, on subsequent provision of Ca^{++} in environment, in the PK-treated group, reveals a suppression of activation phase events by PK-treatment (Table II).

Observed synergism of PK-treatment with anti-anaphylactic effect of esterase inhibitor DFP, which did increase with increasing concentration of the latter, favours actions of the two treatments through a common mechanism both saturable and rate limiting on the release process. DFP is known to

inhibit anaphylactic degranulation of mast cells in a dose-dependent and saturable way (16). Inhibition of serine esterase activation following antigen challenge is thus of significance in antianaphylactic effect exibited by PK-treatment.

Lack of direct in vitro inhibition of gastric juice protease but reduction of gastric juice proteolytic activity in PK-treated animals, may support reported clinical benefit to pepticulcer patients with P. kurroa therapy (17). The findings are suggestive of an inhibition of enzyme release in the gastric secretion and not inactivation of the same. The observations help to suggest relevance of antiprotease mechanism of PK-treatment to antianaphylactic effect, substantiated by experiments involving co–administration of DFP. PK-treatment, thus, may be inhibiting release of protease active sites from membrane millieu, in consequence to IgE bridging by antigen molecule. As PK does not bind to interact with protease in vitro, the in vivo inhibitory action has to be viewed as indirect, involving alterations in membrane structure and/or function in the vicinity of protease molecules.

Another important biochemical event of activation phase is a sequential transmethylation of phospholipids through activation of two transmethylating enzymes, with opposite i.e. acidic and alkaline pH requirements for optimal activity (18). Membrane alterations initiating release process depend on the final product of phospholipid transmethylation, and hence activity of one of the above refered transmethylases with slower rate at body pH will be rate limiting. pH-Alterations towards or apart from the pH-optima of the rate limiting enzyme should enhance or inhibit the ultimate degranulation of mast cells. Further, any direct interaction of PK with the transmethylases can only be viewed as specific for one of the two enzymes, because substrate specificities and pH optimae of the two are different. Depending on the low or high functional state of the target enzyme at the concerned pH, inhibitory effect of PK-treatment, if any, must also become less or more prominent. Thus, similar trend of changes observed in magnitudes of mast cell degranulation at various pH conditions in both control and PK-treated groups indicates no influence of PK-treatment on transmethylases (Table III).

PK-treatment, thus interferes with anaphylactic activation events of mast cells by inhibiting protease activation indirectly through alterations in membrane structure/function as suggested earlier (1, 5, 19, 20). The latter mode of action has potential to influence consequent events related to release process, largely dependent on Ca^{++}-influx though it was not explored with PK; Antianaphylactic drugs may affect either the activation and release events or both.

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REFERENCES

1. Panday BL, Das PK. Immunopharmacological studies on picrorhiza kurroa Royle ex Benth Part II : Antiallergic activity. *Ind J Allergy appl Immunol* 1988; 2:21 34.
2. Ansari RA, Aswal BS, Chandar R. *et la.* Hepatoprotective activity of kutkin-the iridoid glycoside mixture of picrorhiza kurroa. *Ind J Med Res* 1988; 87:401-404.
3. Kloss P Schwabe W. Liver Protecting and choleretic picroside II from picrorhiza kurroa. *Chem Abstr* 1973; 79:108053r.
4. Bobbitt JM, Sagebarth KP. The iridoid glycosides and similar substances. In, Taylor, W.I.; Batters by, A.R. eds. Cyclopentanoid TerPene Derivatives. *New York, Marcel Decker Inc.* 1969; 1-146.
5. Pandey BL, Biswas M, Das PK. ImmunoPharmacological studies on picrorhiza kurroa Royleex Benth Part I : Anti-inflammatory activity. In Sadique, J. ed. *Proceedings of the Satellite symposium on traditional medicine, an*

adjunct to Asian Congress of Pharmacology. Thanjavur. Tamil University, 1986; 113-134.

6. Pandey BL, Goel RK, Das PK. A study of the effects of Tarmrabhasma, an indigenous preperation of copper on experimental gastric ulcers and secretion. *Ind J Exp Biol* 1983; 21 : 258 264.
7. Anson MC. Estimation of pepsin, trypsin, papain and cathepsin with haemoglobin *J Gen Physiol* 1938; 23 : 79-89.
8. Sanyal AK, Pandey BL, Goel RK. The effect of a traditional preparation of copper, Tamrabhasma on experimental ulcers and gastric secretion. *J Ethnophamac* 1982; 5:79-89.
9. Fadal RG. The immunobiology and immunoPharmacology of the allergic resPonse. *Otolaryngol Clin North Am* 1985; 18:649-674.
10. Bach MK, Brashler JR. On the nature of the presumed receptor of IgE on mast cells IV : Inhibition of passive cutaneous anaphylaxis blocking activity of cell-free particulate preperations and intact mast cells by inhibitors of prateases. *Ant Arch Allergy appl Immunol* 1977; 55:255-256.
11. Spencer E, James, T. Allergy problems : current therapy Miami Medical Publishers, 1981.
12. Ishizaka T, Conrad DH, Schulman ES, Sterk AR, Ishizaka K. Biochemical analysis of initial triggering events of IgE mediated histamine release from human lung mast cells. *J Immunol* 1983; 130:2357-2362.
13. Kaliner M, Austen KF. A sequence of biochemical events in the antigen-inauced release of chemical mediators from sensitized human lung tissues *J Exp Med* 1973; 138:1077 1094.
14. Sullivan TJ. Diacyl glycerol metabolism and the release of mediators from rat mast cells. In Becker, E.L., Simon P. and Austen, K.F. Eds. Biochemistry of acute allergic reactions. New York, Liss, 1981:229.
15. Lichtentein LM. The immidiate allergic response : in vitro separation of antigen activation, decay and histamine release. *J Immunol* 1977: 107:1122-1130.
16. Becker EL, Austen KF. Mechanism of immunologic injury of rat peritoneal mast cells I : The effect of phosphonate inhibitors on the homocytotropic antibody mediated histamine release and the first component of rat complement. *J Exp Med* 1966: 124:379-416.
17. Langer JG, Gupta OP, Atal CK. Clinical trials on Picrorhiza kurroa as immunomodulator (abstract). *Ind J Pharmac* 1981: 13:98-99.
18. Hirata T, Viveros OH, Diliberto EJ Jr, Axelrod JA. Identification and properties of two methyl tranferases in convertion of phosphatidyl ethanolamine to phosphatidyl choline. *Proc Natl Acad Sei USA* 1978: 75:1718-1721.
19. Pandey BL, Das PK. Immunopharmacological studies on Picrorhiza kurroa Royle ex Benth Part III : Adrenergic mechanisms of anti-inflammatory action. *Ind J Physiol Pharmac* 1988: 32:120-125.
20. Pandey BL, Das PK. Immunopharmacological studies on Picrorhiza kurroa Royle ex Benth Part V : Anti-inflammatory action relation with cell types involved in inflammation. *Ind J Physiol Pharmac* : 32:289-292.