

IMMUNOPHARMACOLOGICAL STUDIES ON PICRORHIZA KURROA ROYLE-EX-BENTH PART IV : CELLULAR MECHANISMS OF ANTI-INFLAMMATORY ACTION

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Summary : In this work abrogation of anti-inflammatory effect of *Picrorhiza kurroa* extract (PK) by β -adrenergic blockade was confirmed, which suggests alteration in cell-surface biology by PK treatment. Blockade of protein synthesis by cycloheximide pretreatment reduced PK effect, suggesting protein mediation. Metabolic inhibitor dinitrophenol inhibited inflammatory oedema equally in control and PK treated animals, and masking of PK effect was concluded. Discriminations of anti-inflammatory mechanism (s) of PK and the latter two cytotoxic agents was inferred from these observations and from existing knowledge. Selective PK influence on membrane linked activation events in inflammatory effector cells could be the basis of anti-inflammatory and perhaps other biological activities reported with the herb.

Key words : picrorhiza kurroa anti-inflammatory activity anti-inflammatory drug inflammation

INTRODUCTION

Anti-inflammatory effect mediated through increased sensitivity of β -adrenergic receptors and functional impairment of proinflammatory cells, as well as stabilization of mast cells against anaphylaxis were reported with *P. kurroa* treatment (1-3). An alteration of structure/function of cell membrane and cell-biochemistry was forwarded as basis of biological activities (2, 3). Activation and secretion processes of specialized effector cells in inflammation and allergy are understood to depend on metabolic activity of cells, which is under influence of many membrane linked and cytoplasmic events (4-6). The present study attempts to elucidate the

role of these aspects in anti-inflammatory activity of *P. kurroa*.

MATERIAL AND METHODS

Water soluble fraction of the alcoholic rhizome extract (PK), was prepared as reported earlier (7). Dose of PK represents the dry weight of parent alcoholic extract. Experiments were conducted in male Wistar rats (100-150 g), acclimatized to the laboratory conditions and diet for at least one week. Oral PK-treatment once at 9 a. m. daily for three days in 100 mg/kg dose was adopted as in earlier studies. Controls received 2 ml/100 g of distilled (DW) similarly.

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Evaluation of role of β -adrenoceptor function : 1 Hr after the last dose of 3 day oral PK (or DW) treatment, different groups were given either propranolol-HCl 25 mg/kg, iv, or timolol maleate 100 mg/kg, iv, through the tail vein. An hour later the animals were subjected to carrageenin induced pedal oedema test as described by Winter et al. (8). Inflammatory oedema developed at 3 hr after subplanter injection of 0.1 ml of 1% carrageenin solution was determined with help of mercury displacement in a manometric assembly.

Evaluation of protein/peptide dependent mechanisms : Different groups of rats were administered cycloheximide (100 mg/kg, ip). PK (or DW) treatment was started after 6 hr of this treatment and given daily for 3 days. Two hr after last PK dose carrageenin pedal oedema test was performed in such animals.

Evaluation of role of alterations in metabolic energy : One hr after the last dose of 3 day oral PK treatment, the animals were given 2-4 dinitrophenol (15 mg/kg, ip). One hr later they were subjected to carrageenin induced pedal oedema test.

RESULTS

Role of altered β -adrenoceptor function : Pretreatment with either of the β -blockers, propranolol and timolol *per se* resulted in insignificant proinflammatory outcome. Both the agents antagonised the anti-inflammatory effect of PK, significantly. Conversely, these results indicate enhancement of proinflammatory effect of propranolol and timolol by PK treatment (Table I : groups I-III).

TABLE I : Effect of drug pretreatments on carrageenin induced pedal oedema in control and PK-treated rats (n=number of animals).

Pretreatment	% increase in paw volume 3 hr postinsult (Mean \pm S.E.M.)			
	Control (DW)	n	Treated (PK)	n
1. None	33.4 \pm 3.2	5	17.3 \pm 3.8	8
2. Propranolol	42.0 \pm 4.3	7	32.6 \pm 4.3**	7
3. Timolol	36.8 \pm 2.8	6	31.1 \pm 3.8*	7
4. Cycloheximide	22.0 \pm 3.1*	6	15.7 \pm 1.8	6
5. Dinitrophenol	12.6 \pm 4.1***	5	13.4 \pm 2.7	5

P values (with respective group I) :
* < 0.05, ** < 0.02 and *** < 0.01

Role of peptide/protein mechanisms : Cycloheximide pretreatment significantly inhibited carrageenin oedema in control animals. After such pretreatment, PK treatment led to some further reduction pedal oedema, but no synergism was evident between anti-inflammatory actions of two agents (Table I : groups I and IV).

Role of changes in metabolic energy : Pretreatment with dinitrophenol (DNP) markedly reduced the carrageenin inflammation. Magnitudes of inflammation in both control and PK-treated animals were similar, following pretreatment of DNP. Compared to cycloheximide (group IV), DNP influence was much drastic and contribution of PK-treatment to anti-inflammatory effect was not detectable in the combined regimen (Table I : groups I and V).

DISCUSSION

Proinflammatory tendency of β -adrenoceptor antagonists seen in these experiments is in accord with previous report (9). Possibility of β -adrenoceptor upregulation by PK treatment raised by earlier study (2), could not be substantiated in these experiments. Enhancements in proinflammatory effect of timolol, a highly polar β -blocker (10), as well as propranolol by PK treatment however, favours a change in biology of cell surface. The observations are thus, consistent with earlier reports (2, 11).

No significant turnover of proteins with bearing on inflammation is documented to take place within 3 hr of carrageenin insult. Suppression of inflammation by cycloheximide treatment, thus, may be a reflection of reduced status of proinflammatory proteins/peptides, viz. kininogens, complement, cytokines etc. Cycloheximide pretreatment did not complement, but in fact partly reduced the anti-inflammatory effect of PK. Inhibition of protein synthesis by cycloheximide contrasts with the reported enhancement of recovery of liver functions and enzyme activities by P. kurroa treatment in rats subjected to carbon tetrachloride insult (12), and

would favour involvement of dissimilar mechanisms in anti-inflammatory actions of PK and cycloheximide.

Inhibition of carrageenin inflammation by the metabolic inhibitor DNP, may result from disorder of metabolic burst, necessary to proinflammatory function of effector cells (5). In contrast to findings with cycloheximide, almost equal magnitudes of inflammation were seen in the control and PK treated groups following DNP pretreatment. These observations reveal the profound anti-inflammatory effect of DNP in the study-dose. As per Wilder's law of initial values (13), powerful anti-inflammatory influence of DNP, may have masked the additional effect of PK, if any. Hence, any comment on the DNP-PK interaction in inflammation will be naive. In view of high toxicity and understood singular metabolic uncoupling mode of DNP action (14), which contrasts with virtual innocuity (7) and variegated activity (1, 7, 11, 12) of PK, we think that

dissimilar mechanisms are operative in the anti-inflammatory effects of the two agents.

Results of the present study, apparently negate any toxic influence of the given dose of PK on vital cytochemical processes. Compatible with lack of major pharmacological effects of PK in normal animals, but prominent activity against inflammation and allergy (1-3, 7), the present observations suggest that membrane events with enough modulatory reserve, which are critical in triggering of effector cell activation/function, following inflammagen/allergen insult, need to be examined as likely targets of PK action.

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