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Inhibition of human neutrophil locomotion by the polyamine oxidase-polyamine system

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Summary. The polyamines, spermine and spermidine, in the presence of either bovine serum [containing polyamine oxidase (PAO) activity] or partially purified PAO, inhibited human neutrophil locomotion. This effect could not be produced by either bovine serum, PAO, or the polyamines alone. The results suggested that at least two of the products generated during the oxidation of polyamines by PAO, namely H_2O_2 and ammonia, are not responsible for the effects on neutrophils. Acrolein, a breakdown product of other products (aminoaldehydes), was found to inhibit the neutrophil functions. Since polyamines, and possibly PAOs, accumulate at inflammatory sites, products of the PAO-polyamine reaction could function as regulators of the inflammatory response.

INTRODUCTION

Polyamines are present in all living tissues. Their levels may dramatically increase in tissue fluids as a consequence of tissue damage and regeneration (Gaugas, 1980b). The enzymes which catalyse the oxidation of the polyamines (polyamine oxidases) are present in most mammalian tissues (Seiler *et al.*, 1980). Polyamine oxidases (PAOs) are found naturally in significant levels in ruminant blood (Morgan, Ferluga & Allison, 1980; Morgan & Christensen, 1983). The enzyme activity may also markedly increase during pregnancy, and this has been postulated to play a role in the generation of immuno-suppressive factors which may contribute to fetal protection against maternal immune rejection (Byrd, Jacobs & Amoss, 1977; Gaugas, 1980a; Morgan, 1981, 1983). Of further potential importance is the finding that macrophages not only possess PAO activity, but can also release PAO in culture by incubation with bacterial lipopolysaccharide (Morgan *et al.*, 1980). Thus, macrophages could deliver PAOs in close proximity to other inflammatory cells, and in this manner regulate the functions of cells at inflammatory sites. In the present study, the effect of the PAO-polyamine system on the locomotion of one population of inflammatory cells, the neutrophil, was examined.

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MATERIALS AND METHODS

Preparation of neutrophils

Neutrophils were prepared from 10–20 ml of (heparinized) blood from healthy volunteers by a rapid one-step method (Ferrante & Thong, 1978, 1980, 1982). Briefly, 5 ml of blood was layered onto 3 ml of Ficoll-Hypaque medium (density = 1·114 g/ml) and centrifuged at 400 *g* for 20–30 min. During centrifugation, the leucocytes resolved into two distinct bands. The second band, containing neutrophils was harvested. The preparations consisted of >96% neutrophils with >99% viability. Viability was assessed by the trypan blue dye exclusion technique under previously described conditions (Ferrante, Rowan-Kelly & Thong, 1979).

Neutrophil chemotaxis and random mobility

Neutrophil chemotaxis and random movement were measured by the agarose technique (Nelson, Quie & Simmons, 1975) with some modification (Ferrante, Beard & Thong, 1980). For chemotaxis, sets of two wells (2·5 mm diameter) were cut into 1% agarose in medium 199. Wells in each set were cut 3·0 mm apart. To one well of each set was added 5 μl of neutrophils ($2{\cdot}5 \times 10^5$) and to the other, 5 μl of the chemoattractant. In order to assay for random movement, single wells were cut in agarose and each well received $2{\cdot}5 \times 10^5$ neutrophils. Chemotactic migration or random movement was measured after 2 hr of incubation at 37°.

The chemotactic factors used were either human serum activated with the opportunistic fungus *Torulopsis glabrata* (1×10^9 fungi/ml of serum) or N-formyl-L-methionyl-L-phenylalanine ($2{\cdot}5 \times 10^{-7}$ M). The former was used to mimic the clinical situation of an organism activating the complement system and generating chemotactic factors. This system has previously been used in this laboratory for this purpose (Ferrante *et al.*, 1980), and the fungus has been shown to activate human complement by the alternative pathway (Ferrante & Thong, 1979). A volume of 5 μl of either these was added to the wells. Distance of random migration, or migration in a chemotactic gradient, was measured with an inverted microscope (under phase).

The various agents to be tested for their effects on chemotaxis were mixed to the specific concentrations directly with the agarose.

Each experiment was conducted in triplicate plates and each plate contained eight wells or eight sets of two wells.

Enzymes

PAO (beef plasma, EC 1.4.3.6.) was obtained from Miles Laboratories (Goodwood, South Africa) and was that used previously (Ferrante, Hirumi & Allison, 1982; Ferrante, Rzepczyk & Saul, 1984; Rzepczyk, Saul & Ferrante, 1984). Specific activity of the enzyme was 28·0 units/g material (one unit being that amount of enzyme required to form 1 μmole benzylaldehyde per min from benzylamine at 25°). In this laboratory, using a radiochemical method, it was found that this enzyme preparation contained 32·2 U/g material, where the International Unit of enzyme activity is μmol product formed/min (Ferrante, Rzepczyk & Saul, 1984). The bovine serum used contained 34·3 U/l (Ferrante, *et al.*, 1984).

Catalase (bovine liver, EC 1.11.1.6.) was obtained from Sigma Chemical Company (St Louis, MO).

Special reagents

Spermine tetrahydrochloride, spermidine trihydrochloride and 2,3-dihydroxybenzoic acid were obtained from Sigma. Acrolein was purchased from Merck (Frankfurt, Germany).

RESULTS

The effect of polyamines and bovine serum

The polyamines, spermine and spermidine, inhibited migration of neutrophil in a chemotactic gradient and neutrophil random migration in the presence of bovine serum (Tables 1 and 2). Marked inhibition of migration was observed with concentrations of 40 μM for spermine or spermidine. If human serum albumin was used instead of bovine serum, the polyamines failed to inhibit leucocyte locomotion.

The effect of polyamines and PAO

Neutrophil migration in a chemotactic gradient and neutrophil random migration were inhibited in the presence of non-toxic concentrations of PAO and either spermine or spermidine (Table 3). The enzyme alone had no effect on neutrophil locomotion.

The role of acrolein, H_2O_2 or ammonia

Some of the known products of the PAO-polyamine reaction were examined for effects on neutrophil locomotion. These were acrolein, H_2O_2 and ammonia. The results showed that direct addition of acrolein, not H_2O_2 or ammonia, inhibited migration of neutrophils in a chemotactic gradient and neutrophil random movement. Acrolein at concentrations of 100 μM caused approximately 50% inhibition of neutrophil migration (Table 4) while, in three experimental runs, both H_2O_2 and ammonia at a concentration range of 10–200 μM showed no effect (data not presented).

The H_2O_2 scavengers, catalase and 2,3-dihydroxybenzoic acid (Graziano *et al.*, 1976) failed to prevent the PAO-mediated inhibition of neutrophil locomotion (Table 5).

Effects of neutrophil viability

In order to show that the PAO-mediated inhibitory

Table 1. Effect of spermine and spermidine on chemotaxis

Polyamine concentration (μM)	% inhibition (M ± SD)* Spermine AHS† (5 exp.)	Spermine Tripeptide† (3 exp.)	Spermidine AHS† (3 exp.)	Spermidine Tripeptide† (3 exp.)
10	18·1 ± 10·7	13·0 ± 1·6	12·3 ± 8·9	7·5 ± 1·4
40	71·1 ± 7·1	60·1 ± 10·2	53·9 ± 4·6	32·0 ± 6·2
160	84·8 ± 5·8	85·8 ± 3·7	89·1 ± 2·9	79·1 ± 2·1

* Chemotaxis was carried out in the presence of 5% bovine serum (heated 56°, 30 min). Similar concentrations of polyamine alone (i.e. in presence of 5% human serum albumin) had no effect on chemotaxis. Percentage inhibition of migration in a chemotactic gradient was calculated by the following:

$$\% \text{ inhibition} = \frac{(\text{migration in control} - \text{migration in test})}{\text{migration in control}}$$

† The chemotactic agents used were fungi-activated human serum (AHS) or the tripeptide (N-formyl-L-methionyl-phenylalanine).

Table 2. Effect of spermine and spermidine on random migration

Polyamine concentration (μM)	% inhibition* Spermine	Spermidine
10	33·1 ± 3·6	3·8 ± 3·7
40	62·1 ± 3·9	29·7 ± 3·5
160	83·8 ± 8·8	68·7 ± 6·1

* Random migration was carried out in the presence of 5% bovine serum (heated 56°, 30 min). Results are expressed as mean ± SD of three experiments. Similar concentrations of polyamines alone (i.e. in the presence of 5% human serum albumin) had no effect on random migration. Percentage inhibition of random migration was calculated as for Table 1.

effects on neutrophils were not due to a loss of cell viability, the effect of PAO-polyamines on cell viability was examined. In this study, neutrophils were incubated with 100 μg/ml of PAO + 100 μM of polyamines or 100 μM of acrolein for 2 hr. After incubation, viability was assessed by the trypan blue dye exclusion method. It was consistently found that these concentrations of enzyme + polyamines or acrolein had no effect on cell viability.

DISCUSSION

Products generated during the oxidation of polyamines by PAOs have adverse effects on normal function of mammalian cells such as lymphocytes (Gaugas & Curzen, 1978; Byrd *et al.*, 1977; Swanson & Gibbs, 1980; Hussain, Smith & Allen, 1983), tumour cells (Bachrach, Abzug & Bekierkunst, 1967), and also

Table 3. Effect of polyamines and polyamine oxidase on chemotaxis and random movement

Additions	% inhibition* Chemotaxis AHS	Chemotaxis Tripeptide	Random migration
100 μM spermine + 100 μg PAO	85·0 ± 7·5	68·7 ± 2·3	75·0 ± 17·8
100 μM spermidine + 100 μg PAO	49·1 ± 11·2	47·6 ± 6·4	62·5 ± 27·0

* Results are expressed as the mean ± SD of three experiments. PAO alone produced no significant effect on chemotaxis or random migration.

Table 4. Effect of acrolein on chemotaxis and random migration

Acrolein concentration (μM)	% inhibition* Chemotaxis AHS	Chemotaxis Tripeptide	Random migration
10	10·1±6·0	2·6±3·6	3·3±4·7
100	45·8±13·4	63·3±4·2	44·8±21·0
200	81·5±8·6	83·5±2·0	87·8±2·7

* Results are expressed as mean±SD of three experiments. Chemotaxis and random migration were carried out in the presence of 5% human serum albumin.

Table 5. Effect of catalase or 2,3-dihydroxybenzoic acid (DHB) on PAO-mediated inhibition of chemotaxis and random migration

Additions	Chemotaxis (tripeptide)*	Random migration*
No additions	2·6±0·3	0·7±0·2
PAO+spermine†	0·4±0·2	0·2±0·2
PAO+spermine+catalase†	0·4±0·3	0·2±0·2
PAO+spermine+DHB	0·3±0·2	0·1±0·1

* Results are expressed as mean±SD of three experiments (mm/2 hr). Assays were conducted in the presence of 5% human serum albumin.

† Concentrations of PAO, spermine, catalase, and DHB were 100 μg/ml, 100 μM, 200 U/ml and 1 mM, respectively.

parasitic protozoa (Morgan & Christensen, 1983; Ferrante *et al.*, 1982; Ferrante, Rzepczyk & Allison, 1983; Rzepczyk, *et al.*, 1984; Ferrante *et al.*, 1984). In the present study, it was demonstrated that the polyamines, spermine and spermidine, in either the presence of sera known to have PAO activity (bovine serum) or a partially purified PAO, caused marked inhibition of neutrophil migration in a gradient generated by serum complement factors or a chemotactic tripeptide. Since neutrophil random migration (locomotion) was also inhibited, this is the most likely reason for the reduced chemotactic migration. These effects were not associated with loss of leucocyte viability, and the inhibitory effects on the leucocyte function were not produced with either enzyme or polyamines alone. It is viewed with interest that these polyamines which are positively charged do not behave like most polycations in inhibiting cell movement. Perhaps the concentration of polyamines used is not adequate to achieve observable inhibition. Obviously, this question needs to be resolved in future studies together with their effects on cell adhesion which is an important requirement for cell locomotion. However, in preliminary studies, 100 μM concentration of spermine showed no inhibitory effect on neutrophil adherence (A. Ferrante, unpublished observations).

Oxidation of polyamines by PAOs results in the generation of reactive aminoaldehydes, H_2O_2 and ammonia (Morgan, 1980). The aminoaldehydes are highly unstable and may give rise to breakdown products such as acrolein (Alarcon, 1964, 1970; Ferrante *et al.*, 1984). Evidence was provided which suggests that H_2O_2 and ammonia are not the products responsible for inhibition of neutrophil random migration and migration in a chemotactic gradient. Acrolein at concentrations expected to be generated under these conditions (10–100 μM) Ferrante *et al.*, 1984) was found to be inhibitory on neutrophil locomotion. However, it is most unlikely that acrolein participates in the inhibition caused by the PAO-polyamine system since the aminoaldehydes are highly reactive, and these would react with neutrophils and probably not give rise to breakdown products. While it would be of interest to examine whether the aminoaldehydes are the responsible agents, such studies are

complicated by the highly unstable nature of these aldehydes.

The mechanism of accumulation of neutrophils in tissues during an inflammatory process, although remaining to be identified, probably involves either chemotaxis and/or a process of random migration and immobilization in tissues. Oxidized polyamines could limit an inflammatory reaction by inhibiting neutrophil locomotion. Oxidized polyamines may arise as a consequence of tissue damage (Gaugas, 1980b); these agents could serve to limit further neutrophil accumulation and concomitantly trap cells already present at inflammatory sites. Thus, the products of the PAO-polyamine reaction may function as regulators of inflammatory responses. This must be considered of particular importance since macrophages contain PAOs and the cells can secrete the enzyme following stimulation (Morgan *et al.* 1980). Possibly, this could be one means by which the monocytes/ macrophages outphase the neutrophils at inflammatory sites.

It is feasible that oxidized polyamines could be, in part, responsible for the anti-inflammatory activity found in inflammatory exudates, arthritic rat plasma, human pregnancy serum, human peritoneal fluids, extracts from regenerating liver following damage of this tissue, sponge-induced exudates in rats, and human rheumatoid synovial fluids (Rindani, 1956; Persellin, 1972; Lewis, Capskick & Best, 1976; Hempel, Fernandez, & Persellin, 1970; Billingham, Robinson & Robson, 1969; Robinson & Robson, 1964; Capstick, Lewis & Cosh, 1975). Of relevance to this view is the observation that polyamines are present in sponge exudates in rats and polyamines are markedly elevated in damaged rat liver compared to normal rat liver (Bird, Mohd-Hidir & Lewis, 1983). In the same study, it was shown that spermidine and putrescine were anti-inflammatory *in vivo* in the carrageenan-induced oedema rat model and also in the adjuvant arthritic rat model (Bird *et al.*, 1983). Obviously, further studies on anti-inflammatory properties of the PAO-polyamine system would be interesting, and identification of products responsible could lead to the synthesis of a new class of compounds for the treatment of inflammatory diseases.

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