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LEUKOTRIENE B_4: BIOLOGICAL ACTIVITIES AND THE CYTOSKELETON

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1 The aggregation and chemokinesis of either rat or human polymorphonuclear leucocytes (PMNs) induced by leukotriene B_4 isomer III (LTB_4) are inhibited significantly by colchicine (10^{-6}–10^{-3} M) and vinblastine (10^{-7}–10^{-4} M). Random migration of the leucocytes is inhibited by colchicine 10^{-3} M and vinblastine 10^{-4} M.

2 Cytochalasin B (4×10^{-7}–4×10^{-6} M) caused the aggregation of rat PMNs but inhibited their random migration. The aggregation of the PMNs induced by LTB_4 was enhanced by cytochalasin B but the chemokinesis was inhibited.

3 It is suggested that both microtubules and microfilaments may be involved in the aggregatory and chemokinetic responses of PMNs to LTB_4.

Introduction

The synthetic chemotactic peptide, N formylmethionylleucyl-phenylalanine (F-Met-Leu-Phe), the complement derived peptide C5a and leukotriene B_4 isomer III have been shown to stimulate the aggregation and chemokinesis of polymorphonuclear leucocytes (PMNs), and the release of lysosomal enzymes from cytochalasin B pretreated human PMNs (Wilkinson & Lackie, 1979; Hoffstein, 1980; Smith, 1981). Microtubules and microfilaments could be involved in the above phenomena since C5a and F-Met-Leu-Phe have been shown to promote microtubular assembly in PMNs (Hoffstein, 1980). In the present work the involvement of microtubules, in LTB_4-stimulated chemokinesis and aggregation, has been studied, using the microtubular disruptive agents colchicine and vinblastine, and that of microfilaments using cytochalasin B.

Methods

Female King's Wistar rats (200–250 g) were used.

Preparation of LTB_4

Purified LTB_4 isomer III was prepared from rat peritoneal PMNs exposed to the ionophore A23187 as described previously (Ford-Hutchinson, Bray, Doig, Shipley & Smith, 1980).

Aggregation assay

Rat peritoneal PMNs were obtained 24 h after the intraperitoneal injection of sodium caseinate as described previously (Cunningham, Ford-Hutchinson, Smith & Walker, 1979). The cells were washed and resuspended at a concentration of 1×10^7/ml in Eagle's Minimum Essential Medium (MEM) buffered to pH 7.4 with 30 mM HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid). PMN aggregation assays were carried out using a Payton aggregometer (Cunningham, Shipley & Smith, 1980). PMNs were preincubated for 30 min at 37°C in a shaking water bath with either colchicine or vinblastine. After 30 min the cells were washed and resuspended in fresh MEM; 500 µl aliquots were placed in aggregometer cuvettes and the aggregation responses to an ED_{50} concentration of LTB_4 (750 pg/ml) compared to those of control cells preincubated with dimethylsulphoxide (DMSO) alone.

Cytochalasin B-stimulated aggregation was measured after addition of the cytochalasin to 500 µl aliquots of stirred PMNs. The effect of pretreatment with cytochalasin B (Cyt B) upon LTB_4-stimulated aggregation was examined by adding Cyt B to the cells, stirring in an aggregometer cuvette, 5–10 min before addition of LTB_4, after which time the cells had aggregated maximally to Cyt B. The responses were measured as mm on the pen recorder and drug effects have also been expressed as a percentage of the control.

Chemokinesis assay

Human PMNs were obtained from heparinized venous blood by dextran sedimentation and Ficoll-Hypaque separation and assayed by the agarose microdroplet technique (Smith & Walker, 1980). At

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least six replicates of each sample were randomly distributed in the microtitre plate and migration assessed after 1.5 h at 37°C in a humid atmosphere. The area (mm^2) moved by the PMNs in the presence of MEM or LTB_4 500 pg/ml, with or without colchicine, vinblastine or cytochalasin B, was measured by planimetry.

Drug effects on random migration have been expressed as a percentage of the response to medium alone and the effect on chemokinesis expressed as a percentage inhibition of the area moved by the cells in response to LTB_4 minus that area moved by the cells in the presence of medium alone.

Materials

Colchicine, vinblastine and cytochalasin B (Cyt B) (Sigma Chemical Co.) were dissolved in dimethylsulphoxide (DMSO), and the final concentration of DMSO used in experiments was never more than 0.1%. No effects of DMSO alone were apparent at this concentration.

Results

Effects of colchicine and vinblastine

Preincubation of rat PMNs for 30 min with colchicine (10^{-6}–10^{-3} M) and vinblastine (10^{-7}–10^{-4} M) significantly inhibited LTB_4-stimulated aggregation. The amount of inhibition seen with each drug did not increase with increasing concentration (Table 1).

Inhibition of the chemokinetic response to LTB_4 (500 pg/ml) was also obtained in the presence of colchicine (10^{-7}–10^{-3} M) and vinblastine (10^{-8}–10^{-4} M). Random migration was only inhibited significantly at high concentrations of the two drugs (10^{-3} M colchicine and 10^{-4} M vinblastine) (Table 2).

Effects of cytochalasin B

The addition of cytochalasin B to rat PMNs (4×10^{-7}–4×10^{-6} M) caused a dose-related aggregation. Higher concentrations of the drug could not be used because the concentration of DMSO would have exceeded 0.1% (Figure 1). Aggregation occurred 5–10 s after addition of the drug and was maximal by 5 min. In contrast to the response obtained with LTB_4, the aggregation of PMNs in response to cytochalasin B was irreversible. When leukotriene B_4 (250 pg/ml–3 ng/ml) was added to the aggregometer cuvette 5 min after a fixed dose of

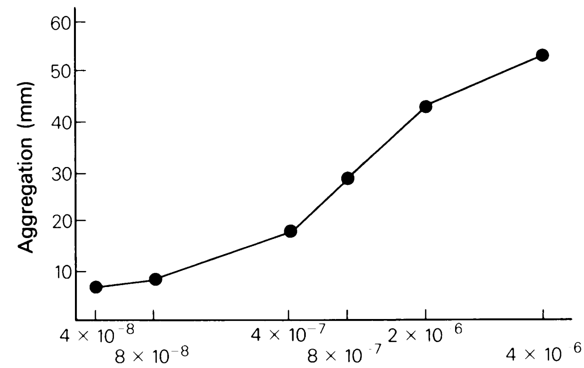

Figure 1 Cytochalasin B-stimulated aggregation of rat polymorphonuclear leucocytes; $n = 4$.

Table 1 The effect of colchicine and vinblastine (30 min preincubation) on leukotriene B_4 (LTB_4)-stimulated aggregation of rat polymorphonuclear leucocytes (PMNs)

Treatment	*Conc* (M)	n	*Height of response* (mm)	*% inhibition*
Control		4	37.5 ± 3.7	
Colchicine	10^{-7}	5	35.8 ± 1.9	5
	10^{-6}	5	20.6 ± 1.4	45*
	10^{-5}	5	20.2 ± 1.0	46*
	10^{-4}	5	23.0 ± 1.4	39*
	10^{-3}	5	19.6 ± 1.3	48*
Control		6	29.3 ± 1.8	
Vinblastine	10^{-8}	6	26.2 ± 1.4	11
	10^{-7}	5	14.4 ± 1.2	51*
	10^{-6}	6	14.0 ± 1.3	52*
	10^{-5}	4	13.8 ± 1.7	53*
	10^{-4}	6	13.1 ± 0.9	55*

Results are expressed as mean ± s.e.mean.
*$P < 0.01$ when compared to the corresponding control.

Table 2 The effect of colchicine and vinblastine on the movement of human polymorphonuclear leucocytes (PMNs)

Treatment	Conc (M)	n	Random migration: Area of migration (mm^2)	Random migration: % inhibition/ enhancement of movement	n	Chemokinesis: Area of migration (mm^2)	Chemokinesis: % inhibition of movement
Control		11	3.9±0.1		7	9.0±0.3	
Colchicine	10^{-7}	9	4.0±0.2	4↑	10	6.1±0.4	59*
	10^{-6}	8	4.1±0.2	6↑	10	5.3±0.2	76*
	10^{-5}	10	3.7±0.2	5↓	8	5.4±0.4	67*
	10^{-4}	10	3.9±0.2	0.9↑	10	4.3±0.3	92*
	10^{-3}	9	1.3±0.1	68↓*	10	2.0±0.1	86*
Control		10	3.8±0.1		8	9.9±0.4	
Vinblastine	10^{-8}	10	4.0±0.2	4↑	9	7.1±0.3	49*
	10^{-7}	8	4.0±0.3	3↑	9	6.5±0.4	59*
	10^{-6}	9	3.5±0.2	8↓	10	5.3±0.3	70*
	10^{-5}	10	3.5±0.2	8↓	9	5.3±0.3	71*
	10^{-4}	9	2.5±0.3	34↓*	10	3.2±0.2	89*

Results are expressed as mean ± s.e.mean (pooled data from 2 subjects. Chemokinesis stimulated by LTB$_4$ 500 pg/ml.
*$P < 0.001$ when compared to corresponding control.

cytochalasin B (8×10^{-7} M), the response to LTB$_4$ was enhanced (Figure 2). A similar effect was observed after 10 min (results not shown). Both random migration and chemokinesis were inhibited in the presence of cytochalasin B. This effect was also apparent at concentrations that neither promoted aggregation, nor augmented the response to LTB$_4$ (10^{-8} and 10^{-7} M).

Discussion

Leukotriene B$_4$ isomer III (5(*S*), 12 (*R*)-dihydroxy-6, 14-*cis*-8, 10-*trans*-eicosatetraenoic acid, LTB$_4$), a product of the lipoxygenase pathway of arachidonic acid metabolism, is released from PMNs in response to a variety of stimuli, including the ionophore A23187 (Borgeat & Samuelsson, 1979). LTB$_4$, in common with other cytotaxins, such as C5a and the synthetic tripeptide formyl methionyl-leucylphenylalanine (F-Met-Leu-Phe), has been shown to be a potent chemokinetic and chemotactic agent, to cause the aggregation of PMNs and the release of lysosomal enzymes from PMNs pretreated with cytochalasin B (Smith, 1981).

The cellular responses that occur following combination of a cytotaxin with the surface membrane (O'Flaherty & Ward, 1979) may require cytoskeletal elements such as microtubules and microfilaments, since C5a and F-Met-Leu-Phe have been shown to promote microtubular assembly in PMNs (Hoffstein, 1980). Agents that inhibit microtubular assembly also inhibit cell movement and aggregation (Lackie, 1974; Malech, Root & Gallin, 1977). The intracellular events occurring after combination of LTB$_4$ with the PMN have not been fully investigated, although it has recently been reported that, in common with C5a and F-Met-Leu-Phe, LTB$_4$ enhances the influx of calcium ions in rabbit neutrophils (Naccache, Sha'afi, Borgeat & Goetzl, 1981). In the present work the role of microtubules and microfilaments in the aggregatory and chemokinetic responses of PMNs to LTB$_4$ has been studied using the microtubular disruptive drugs colchicine and vinblastine, and using cytochalasin B, an agent known to inhibit microfilament polymerization in phagocytes (Hartwig & Stossel, 1976).

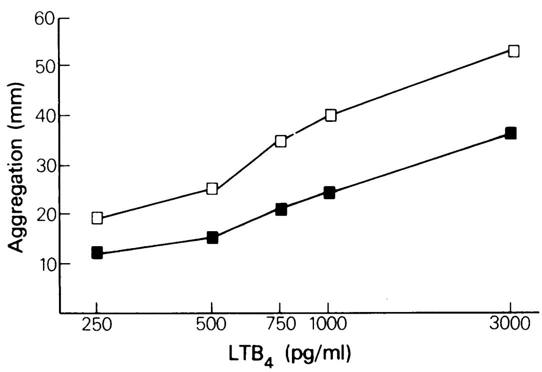

Figure 2 Leukotriene B$_4$ (LTB$_4$)-stimulated aggregation of rat polymorphonuclear leucocytes: effect of cytochalasin B: (■)LTB$_4$; (□)cytochalasin B (8×10^{-7} M) plus LTB$_4$. $n = 7–10$.

The aggregation of rat PMNs in response to LTB$_4$

Table 3 Effect of cytochalasin B (Cyt B) on leukotriene B_4 (LTB_4)-stimulated chemokinesis and random migration of human polymorphonuclear leucocytes (PMNs)

		Random migration			*Chemokinesis*	
Conc Cyt B (M)	n	*Area of migration* (mm^2)	*% inhibition of movement*	n	*Area of migration* (mm^2)	*% inhibition of movement*
Control	10	3.5 ± 0.4		10	6.1 ± 0.2	
10^{-9}	10	3.1 ± 0.2	11	8	5.7 ± 0.3	< 1
10^{-8}	10	3.1 ± 0.1	12*	8	5.4 ± 0.2	10*
10^{-7}	10	2.9 ± 0.1	16*	8	4.8 ± 0.2	28*
10^{-6}	10	0.9 ± 0.1	76*	8	1.6 ± 0.1	71*
10^{-5}	10	0.5 ± 0.1	87*	8	0.4 ± 0.1	100*

Results expressed as mean ± s.e.mean.
* $P < 0.05$ when compared to corresponding control.

was reduced by pretreatment of the cells with colchicine and vinblastine, suggesting that microtubules are involved in the response. Cytochalasin B alone caused rat peritoneal PMNs to aggregate, an effect not observed with human peripheral and guinea-pig and rabbit peritoneal PMNs (Oseas, Boxer, Butterick & Baehner, 1980). The mechanisms underlying PMN aggregation are unclear, but may involve exposure of previously concealed plasma membrane components which interact and cause intercellular adhesion. Cyt B may increase the deformability of the rat PMN plasma membrane by disruption of submembranous microfilaments, thus leading to exposure of components necessary for aggregation.

In addition, cytochalasin B augmented LTB_4-stimulated aggregation, an effect that was abolished by washing and resuspending the PMNs in fresh medium. Cytochalasin B has been shown to bind rapidly and reversibly to the surface of PMNs (Parker, Green & MacDonald, 1976), and the enhancing effect of Cyt B may be explained by interaction of the agent with a superficial site on the cell membrane, resulting in an increase in cation transport stimulated by LTB_4 (O'Flaherty, Kreutzer & Ward, 1979), and not by an effect on microfilaments. It is of interest that addition of a second aggregating agent to cells that have previously responsed to a different agent, results in an augmented response (O'Flaherty, Kreutzer, Showell, Becker & Ward, 1978).

The chemokinetic response of human PMNs to LTB_4 was inhibited by colchicine, vinblastine and cytochalasin B, present throughout the assay. Random migration was also reduced by cytochalasin B and by high concentrations of the microtubular disruptive drugs (Table 2). An increase in the random migration of human PMNs occurs after preincubation with colchicine (10^{-7}–10^{-5} M) and it has been suggested that microtubules exert a constraining effect on cell movement (Rich & Hoffstein, 1981). However, the results obtained in the present work suggest that microtubules are required for chemokinesis but not random migration. The inhibition of cell movement caused by Cyt B may be due to irreversible aggregation of the cells.

The responses of PMNs to LTB_4 appear to require an intact microtubular system. Microfilaments may also be involved in aggregation, but in a suppressive fashion.

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