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Effect of mammalian lignans on fMLP-induced oxidative bursts in human polymorphonuclear leucocytes

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Abstract—We examined the effects of mammalian lignans, enterolactone, prestegane B and 2,3-dibenzylbutane-1,4-diol (DBB) on superoxide production and luminol-dependent chemiluminescence (LCL) response in human polymorphonuclear leucocytes (PMNs). The three lignans had no direct effect on the responses of human PMNs. DBB and prestegane B enhanced the superoxide production and LCL response induced by formylmethionyl-leucyl-phenylalanine (fMLP), but enterolactone inhibited fMLP-induced effects. The effects of DBB were stronger than those of prestegane B and the effects of DBB were inhibited by bromophenacyl bromide, mepacrine, *N*-(6-aminophenyl)-5-chloro-1-naphthalene sulphonamide and trifluoroperazine, but not by gossypol, nordihydroguaretic acid, indomethacin, staurosporine, 1-(5-isoquinolinesulphonyl)-2-methylpiperazine dihydrochloride or (*R,S*)-2-methoxy-3-(octadecyl-carbamoyloxy)-propyl-2-(2-thiazolio)-ethylphosphate. These results suggest that DBB primes the responses of human PMNs, and the priming effect is caused by the activation of phospholipase A_2—and Ca^{2+}-calmodulin-pathways, but not by the activation of lipoxygenase, cyclo-oxygenase and protein kinase C or by the release of platelet activating factor.

Some lignans, enterolactone, enterodiol, prestegane B and 2,3-dibenzylbutane-1,4-diol (DBB) are present in the urine of man and other animals (Setchel et al 1980; Stitch et al 1980; Hirano et al 1989). These lignans have digitalis-like actions (Fagoo et al 1986; Hirano et al 1989), anti-PAF actions (enterolactone and prestegane B (Plante et al 1986)) or diuretic actions (prestegane B (Plante et al 1986)). The oxidative burst (superoxide and hypohalous acid) is a key function of polymorphonuclear leucocytes (PMNs) required for the killing of bacteria. Lipopolysaccharide, interleukin-1, tumour necrosis factor alpha (Sample & Czuprynski 1991) and substance P (Wozniak et al 1989) enhance several activities of PMNs, including adherence, secretion of protein and formyl-methionyl-leucyl-phenylalanine (fMLP)-induced superoxide generation. In experiments in-vitro, such enhancement of PMN functions is called priming. Priming might be related to the phenomenon that administration of a small amount of stimulus results in increased resistance to bacterial infection (Aida & Pabst 1991). These observations suggested that priming had an important role in the modulation of PMN functions, but the mechanism of priming is unclear. We have reported that DBB did not stimulate the superoxide production by itself, but enhanced fMLP- or A23187-induced superoxide production of human PMNs (Morikawa et al 1990). In the present study to examine the mechanism of DBB-induced priming, we compared the effects of enterolactone, prestegane B and DBB on superoxide production and luminol-dependent chemiluminescence (LCL) response of human PMNs stimulated by fMLP. Also, we have investigated the effects of inhibitors of phospholipase A_2 (PLA_2), lipoxygenase, cyclo-oxygenase, calmodulin and protein kinase C, and platelet activating factor (PAF)-antagonism on the priming effect of DBB.

Materials and methods

Chemicals. The following chemicals were used: DBB, enterolactone and prestegane B (synthesized in our laboratory); fMLP, gossypol, luminol, trifluoroperazine and mepacrine (Sigma Chemical Co.); indomethacin (Japan Merck Co.); nordihydroguaretic acid (NDGA) and staurosporine (Biomol. Res. Lab.); 3-(*N-R*-octadecylcarbamoyloxy)-2-methoxypropyl 2-thiazolium ethylphosphate (CV-3988) (Molecular Prove); *N*-(6-aminophenyl)-5-chloro-1-naphthalene sulphonamide dihydrochloride (W-7) and 1-5-isoquinolinesulphonyl-2-methylpiperazine (H-7) (Seikagaku Kougyo); bromophenacyl bromide (BPB) (Wako Chemicals); dextran (Dextran T-500) and Ficoll (Pharmacia); and Hank's balanced salt solution (HBSS, MA Bioproducts).

Preparation of polymorphonuclear leucocytes (PMNs). PMNs were obtained from healthy donors and were separated by the dextran sedimentation described by Persidsky & Olson (1978). PMNs were resuspended in HBSS (pH 7·4) at a final concentration of 4×10^5 cells mL^{-1}.

Determination of superoxide production. Superoxide production was measured as superoxide dismutase-inhibitable reduction of cytochrome C following the method described by Taniguchi & Takanaka (1984).

Luminol-enhanced chemiluminescence (LCL) response. The oxidative response of human PMNs was assayed by LCL response following the method of Anderson et al (1977). PMNs (2×10^5 cells) were incubated with 10 nM fMLP in a cuvette of a Lumiphotometer (Model 1000, Nition, Tokyo, Japan). After 30 s, 10 μM luminol was added.

Data analysis. Data were analysed using Dunnet's 2-sided analysis or Student's paired *t*-test with $P < 0.05$ considered significant. Results are expressed as the mean ± s.e. (n = number of experiments).

Results

Effects of lignans on superoxide production and LCL response in human PMNs. LCL response appears to correlate closely with the production of hypochlorous acid rather than the initial increase in superoxide production and is dependent on myeloperoxidase. DBB, prestegane B and enterolactone did not directly influence the superoxide production or LCL response of human PMNs. Fig. 1 shows the effects of DBB, prestegane B and enterolactone on fMLP-induced superoxide production and LCL response of human PMNs. DBB enhanced fMLP-induced superoxide production from 3·65 ± 0·21 to 6·42 ± 0·54 nmol/10^5 cells (a 98% increase), and LCL response from 4·40 ± 0·47 to 16·2 ± 2·22 mV/10^5 cells (a 260% increase). Prestegane B enhanced fMLP-induced superoxide production (by 28%) and LCL response (by 55%). Enterolactone inhibited fMLP-induced superoxide production (by 45%) and LCL response (by 55%). The priming effects of DBB were dose-dependent (1–100 μM) and were stronger than those of prestegane B.

Effects of inhibitors of arachidonate metabolism, calmodulin, protein kinase C and PAF-antagonist on the priming effect of DBB. Table 1 shows the effects of arachidonic acid (AA) metabolism inhibitors, calmodulin inhibitors, protein kinase C

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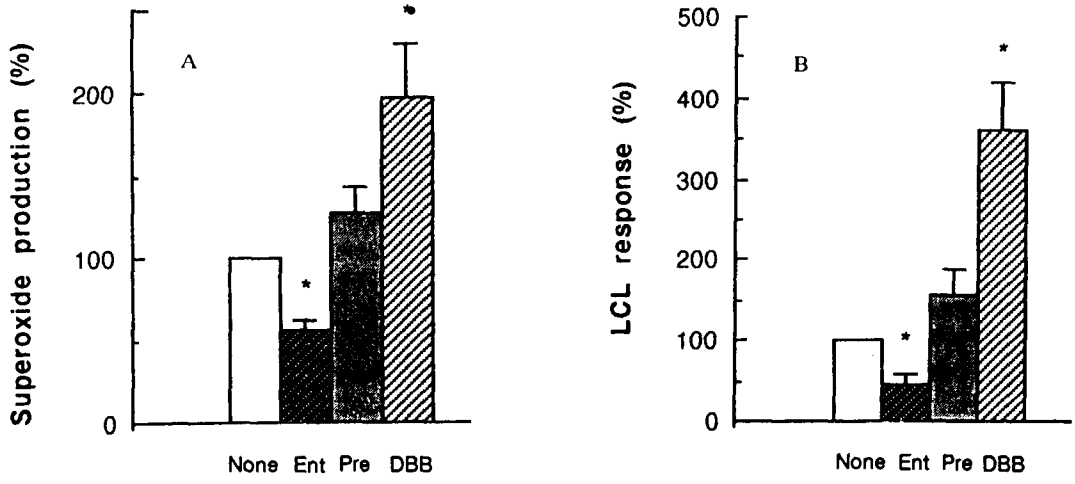

FIG. 1. Effect of lignans on fMLP-induced superoxide production or LCL response. A. Effect of lignans on superoxide production induced by fMLP (10 nM). B. Effect of lignans on LCL response induced by fMLP (10 nM). None, fMLP alone; Ent, enterolactone (100 μM); Pre, prestegane B (100 μM). Each point was expressed as the percentage response in the absence of lignan. Mean ± s.e. (n = 5), $*P < 0.05$.

Table 1. Effect of inhibitors on fMLP-induced LCL in human PMNs.

		LCL (mV)	
Inhibitor	μM	Control	DBB-treated
None		4·40 ± 0·47	16·28 ± 2·22
Bromophenacyl bromide	0·32	3·29 ± 0·30	3·57 ± 0·15
	1·0	1·12 ± 0·26*	1·31 ± 0·21
	3·2	0·37 ± 0·13*	0·41 ± 0·12
Mepacrine	10·0	1·42 ± 0·16*	2·20 ± 0·34
	32·0	0·36 ± 0·09*	0·47 ± 0·09
NDGA	0·5	0·80 ± 0·24*	4·82 ± 1·20†
	1·0	0·20 ± 0·07*	1·24 ± 0·40†
Gossypol	1·0	3·22 ± 0·49	13·20 ± 1·64†
	10·0	1·80 ± 0·23*	5·64 ± 0·68†
Indomethacin	20·0	2·20 ± 0·31*	8·78 ± 0·96†
	100	0·41 ± 0·09*	2·02 ± 0·38†
H-7	10	4·69 ± 0·21	18·84 ± 0·90†
	100	3·54 ± 0·43	14·80 ± 0·93†
Staurosporine	0·10	3·59 ± 0·23	14·88 ± 1·14†
	0·15	2·10 ± 0·32*	9·84 ± 0·97†
W-7	10·0	2·14 ± 0·68*	1·45 ± 0·37
	20·0	0·26 ± 0·26*	0·20 ± 0·13
Trifluoroperazine	3·2	3·25 ± 0·13*	1·94 ± 0·26
	10·0	0·37 ± 0·71*	0·24 ± 0·2
CV-3988	0·32	4·10 ± 0·54	16·03 ± 0·48†
	1·0	3·16 ± 0·22	12·04 ± 2·04†

Each value represents the mean ± s.e. for 5 experiments. $*P < 0.05$ compared with experiment with no inhibitor. $\dagger P < 0.05$ compared with corresponding control.

inhibitors and a PAF-antagonist on LCL response induced by fMLP. fMLP-induced LCL response was inhibited by mepacrine, BPB, NDGA, gossypol, indomethacin, W-7, trifluoroperazine and staurosporine. On the other hand, the priming effect of DBB in fMLP-stimulated PMNs was inhibited by BPB, mepacrine, W-7 and trifluoroperazine; NDGA, gossypol, indomethacin and CV-3988 did not inhibit the priming effect of DBB.

Discussion

Mammalian lignans have several pharmacological actions. In previous reports, we showed that DBB enhances fMLP- or A23187-induced superoxide production of human PMNs (Morikawa et al 1990) and DBB inhibits contractile responses induced by high K^+ and noradrenaline in rabbit and rat aorta (Abe et al 1989, 1991).

The oxidative burst in PMNs is a major biochemical response that is associated with their interaction with a soluble stimulus (phorbol myristate acetate), a chemotactic agent (fMLP) or a particulate stimulus (opsonized zymosan). The oxidative burst in PMNs includes the generation of superoxide and hypohalous acid. LCL response is myeloperoxidase-dependent and reflects hypohalous acid production (Brestel & McClain 1983). It is possible then that the enhancement in LCL response is due to greater release of hypohalous acid rather than an initial increase in superoxide production in PMNs (Robert et al 1989).

The results from the present study show that DBB and prestegane B enhance not only superoxide production but also LCL response in PMNs stimulated by fMLP, but enterolactone, a lactone-type mammalian lignan, does not. Also the priming effects of DBB are stronger than those of prestegane B.

We have studied the effects of several drugs on the priming effect of DBB. The activation of Ca^{2+}-calmodulin- and protein kinase C-pathways and the release of PAF occur following the stimulation of PMNs (Rossi 1986). It has been reported that mepacrine and bromophenacyl bromide, inhibitors of PLA_2, inhibit the superoxide generation stimulated by a wide range of stimuli in PMNs (Duque et al 1986; Taniguchi et al 1988; Henderson et al 1989). The activation of the protein kinase C-pathway is involved in the activation of PMNs (Rossi 1986); however, protein kinase C inhibitors (including H-7) do not inhibit the activation of oxidative bursts. In this study, fMLP-induced oxidative burst in human PMNs was inhibited by inhibitors of PLA_2, cyclo-oxygenase, lipoxygenase, calmodulin and protein kinase C. On the other hand, the priming effect of DBB was inhibited by inhibitors of PLA_2 and calmodulin, but not by inhibitors of cyclo-oxygenase, lipoxygenase and protein kinase C. These results support the hypothesis that AA cascade, calmodulin- and protein kinase C-pathways have direct roles in fMLP-stimulation, but the mechanism of DBB-induced priming correlates with the activation of PLA_2- and Ca^{2+}-calmodulin-pathways, and is elicited by AA released by PLA_2 itself but not by its metabolites.

It has been reported that membrane-permeant fatty acids such as AA act as a second messenger in signal transduction. For

example, AA mediates signalling in neuronal cells and activates potassium-selective channels in both cardiac and smooth muscle cells (Piomelli et al 1987; Kim & Clapham 1989; Ordway et al 1989). AA also induces intact PMNs to aggregate, generate superoxide anions and release the contents of lysosomes (Curnutte et al 1984; Smith et al 1987). It is unclear what is the mechanism of AA-promoted cell activation and whether such effects are direct or via metabolites. Ligeti et al (1988) have reported that AA stimulates the superoxide anion generation in the cell-free system of PMNs. There is evidence that the effect of AA is independent of protein kinase C and is due to the capacity of AA to increase the binding of GTP to a cytosolic, pertussis toxin-insensitive GTP binding protein (Ligeti et al 1988; Gabig et al 1988). Abramson et al (1991) have reported that the activation induced by AA in human PMNs is consequent to the capacity of AA to increase the binding of GTP to the regulatory G protein. Our data suggest that the AA released via the activation of PLA_2 may play an important role in the mechanism of priming induced by DBB.

These results suggest that the priming effect of DBB on oxidative bursts induced by fMLP may be due to the release of AA initiated by PLA_2 or the activation of the Ca^{2+}-calmodulin-pathway, and DBB may enhance the host defence system against microbial infection.

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