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PRIMING EFFECT OF 2,3-DIBENZYLBUTANE-1,4-DIOL (MAMMALIAN LIGNAN) ON SUPEROXIDE PRODUCTION IN HUMAN NEUTROPHILS

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We investigated the effect of 2,3-dibenzylbutane-1,4-diol(DBB), a mammalian lignan, on superoxide production and $[Ca^{2+}]i$ mobilization in human neutrophils. DBB did not generate superoxide production by itself, but enhanced the FMLP or A23187-induced superoxide production in a dose dependent manner. DBB did not influence the OAG-induced superoxide production. The priming effect of DBB was inhibited by W-7 or trifluoroperazine, but not by H-7 or staurosporine. And the priming effect of DBB was observed in the presence or absence of extracellular Ca^{2+}. DBB enhanced the low dose FMLP-induced $[Ca^{2+}]i$ mobilization. These results suggest that the priming effect of DBB in human neutrophils may be caused by the activation of the calcium-calmodulin pathway but not the protein kinase pathway.

It has been reported that the lignans derived from mammalian animals have several pharmacological actions; a diuretic action (prestegane B)(1), a digitalis-like action (enterolactone)(2) and an antagonistic action of platelet-activating factor (PAF) receptor (3). Recently, 2,3-dibenzylbutane-1,4-diol (DBB) has been found in human urine (4) but its physiological significance is unknown.

Neutrophils have some important roles in the mammalian host defense system against microbial infection. During phagocytosis of bacteria, a plasma-membrane-bound enzyme-complex (the NADPH oxidase) in neutrophils is stimulated, leading to the production of superoxide radicals(5).

Some lignans isolated from plants, have effects on cancer, bacteria (6) and virus (7).

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In this study, we investigated the effect of DBB on superoxide production and $[Ca^{2+}]i$ mobilization in human neutrophils.

MATERIALS AND METHODS

Materials : DBB was provided from Tsumura. FMLP, Cytochrome C (type VI), OAG, superoxide dismutase, chlorotetracycline (CTC) and trifluoroperazine (TFP) were purchased from Sigma. Dextran and Ficoll-Paque were products of Pharmacia. A23187 was purchased from Hoechst. Fura 2-AM and HEPES were obtained from Dojin Laboratories. Hanks' balanced salt solution (HBSS) was purchased from MA Bioproducts. H-7 and W-7 were purchased from Seikagaku Kogyo. Staurosporine was obtained from Biomol Research Laboratories Inc.

Fractionation of neutrophils : Neutrophils from healthy donors were isolated by the Dextrane-sedimentation, the centrifugation through Ficoll and the haemolysis of residual erythrocytes as described (8). The separated neutrophils were suspended ($1x10^5$ cells/ml)in HBSS (pH 7.4).

Determination of superoxide production: NADPH-dependent superoxide production was determined by the superoxide dismutase-sensitive rate of cytochrome c reduction as described by Cross et al.(9).

Measurement of calcium mobilization : Neutrophils were loaded Fura 2-AM (3 μM)(10) or CTC (100 μM)(11) for 40 min at 37 C. The change of fluorescence was measured using a Ca^{2+} analyser (model CAF-100, Jasco). Intracelular free calcium concentration ($[Ca^{2+}]i$) was calculated as described previously (10).

RESULTS AND DISCUSSION

Effect of DBB on superoxide production

By preincubating with some agonists at low concentration that do not evoke any observed cell-functions by themselves, the functions of human neutrophils are augmented relative to untreated cells. This phenomenon is referred to as priming and has been studied using a broad variety of stimuli (12,13).

We investigated the effect of DBB on superoxide production in human neutrophils. Fig.1 shows the effect of DBB on FMLP- or A23187-induced superoxide production. DBB did not generate the superoxide production by itself, but enhanced the FMLP- or A23187-induced superoxide production in dose dependent manner(1 μM-100 μM). However, DBB did not enhance the OAG-induced superoxide production. These results indicate that DBB has a

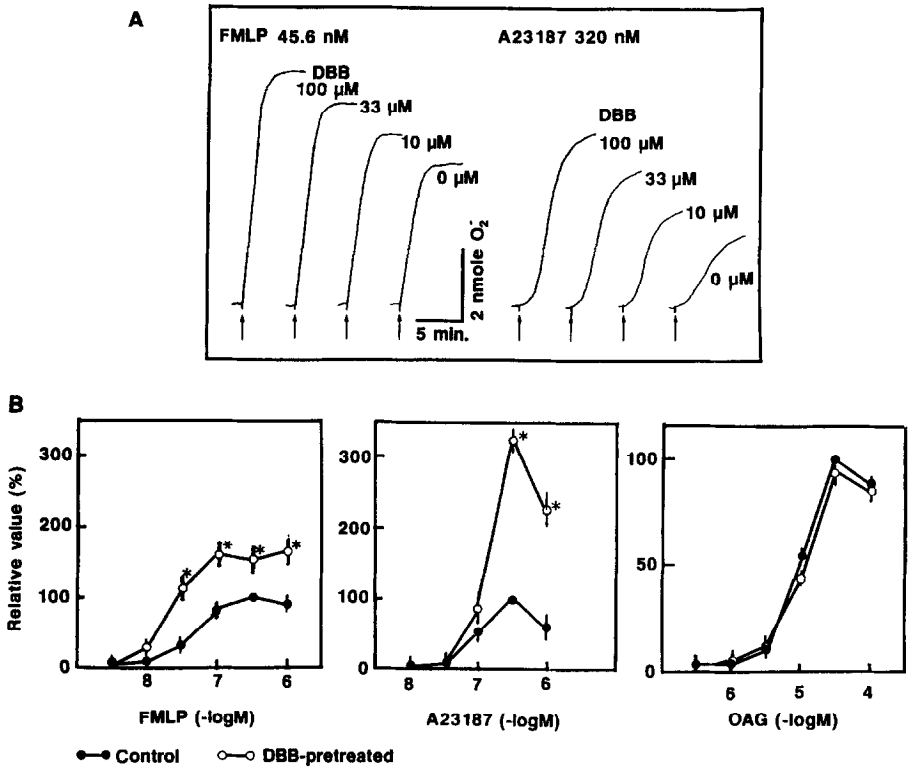

Fig. 1 Effect of DBB on FMLP-, A23187- or OAG-induced superoxide production in human neutrophils

A : Effect of DBB on FMLP-or A23187-induced superoxide production.

B : Effect of DBB (100 μM) on the dose-response curve of FMLP, A23187 or OAG for superoxide prodcution in human platelets. Relative value to the response induced by 320 nM FMLP, 320 nM A23187 or 32 nM OAG in control. Mean±S.E. (n=4), Significant difference compared with control. * $p<0.05$.

priming effect on the FMLP- or A23187-induced superoxide production in human neutrophils.

Table 1 shows the influence of extracellular calcium on the priming effect of DBB on the FMLP-induced superoxide production.

Table 1. Effect of DBB on FMLP-induced superoxide production in the presence or absence of extracellular calcium

	Superoxide production(nmol/4x10^{5}cells)	
	$+Ca^{2+}$	$-Ca^{2+}$
Control	3.50±0.51	0.87±0.14
DBB-pretreated	5.37±0.35*	1.66±0.31*

Mean±S.E.(n=5), Significant difference compared with each control value *$p<0.05$

Removing extracellular calcium, the FMLP-induced superoxide production decreased to about 25 %, but the priming effect of DBB did not change. It is suggested that extracelluar Ca^{2+} is not necessary to the priming effect of DBB.

Effect of DBB on calcium mobilization

It is reported that the increment of $[Ca^{2+}]i$ in neutrophils is induced by FMLP and triggers superoxide production. The increment of $[Ca^{2+}]i$ in human neutrophils stimulated with FMLP, is composed of two phases; the rapid Ca^{2+} mobilization from the intracellular Ca^{2+} store and the Ca^{2+} influx through the plasma membrane (14).

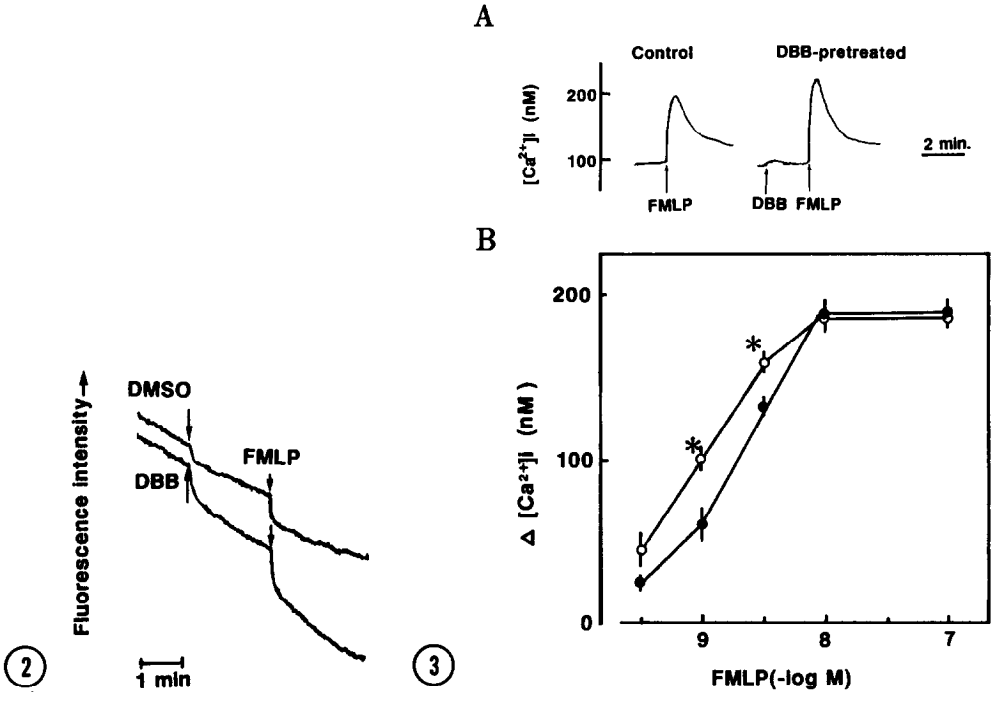

Fig. 2 Effect of DBB on fluorescence change induced by FMLP in CTC-loaded neutrophils
FMLP: 45.6 nM, DBB: 100 μM.

Fig. 3 Effect of DBB on $[Ca^{2+}]i$ mobilization induced by FMLP in human neutrophils
A. Typical pattern of $[Ca^{2+}]i$ mobolization in Fura 2-loaded human neutrophils.
B. Effect of DBB on the dose-response curve of FMLP for $[Ca^{2+}]i$ mobilization.
Mean±S.E. (n=6), Significant difference compared with control
* $p<0.05$.

Fig. 2 shows the effect of DBB on the fluorescence change induced by FMLP in CTC-loaded neutrophils. DBB induced the release of membrane bound calcium and enhanced the effect induced by FMLP. Fig.3 shows the effect of DBB on $[Ca^{2+}]i$ mobilization in human neutrophils. DBB induced a slight $[Ca^{2+}]i$ increment by itself, and enhanced the effect induced by low dose FMLP.

Effect of W-7, TFP, H-7 or staurosporine on FMLP-induced superoxide production

It is reported that the mechanism of FMLP for the activation of NADPH oxidase relates to the activation of protein kinase pathway and calcium/calmodulin pathway. But the mechanism of priming induced by any agonist is not clearly defined. The primed response to FMLP has been throught to depend on an upregulation of its receptor. The role of an upregulated protein kinase C induced by FMLP also has been invoked, but certain conditions did not exhibit either a translocation of protein kinase C to the membrane or an increase in its activity(15).

Table 2 shows the effect of W-7, TFP, H-7 or staurosporine on the priming effect of DBB on the FMLP-induced superoxide production in human neutrophils. The priming effect of DBB was inhibited by W-7 (10,20 μM) or TFP (10, 30 μM) ; as a calmodulin inhibitor. But H-7 (10, 100 μM) or staurosporine (100, 150 nM) ;

Table 2. Effect of W-7, TFP, H-7 or staurosporine on effect of DBB on FMLP-induced superoxide production

		Superoxide prodcution(nmol/4x10^5cells)	
		-DBB	+DBB
Control		3.50±0.51	5.37±0.35*
W-7	10 μM	2.05±0.60	1.21±0.32
	20 μM	0.15±0.07	0.13±0.04
TFP	3 μM	3.25±0.12	1.94±0.20
	10 μM	0.37±0.07	0.17±0.02
H-7	10 μM	4.45±0.21	6.47±0.36*
	100 μM	3.40±0.40	7.11±0.36*
Staurosporine	100 nM	2.97±0.23	5.93±0.51*
	150 nM	2.02±0.33	4.77±0.36*

TFP: Trifluoroperazine, Mean±S.E.(n=4), Significant difference compared with each value in the absence of DBB *$p<0.05$

as a protein kinase C inhibitor, did not inhibited the priming effect of DBB.

These results suggest that the priming effect of DBB in human neutrophils may be caused by the activation of the calcium-calmodulin pathway but not the protein kinase C pathway.

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