Agents and Actions, vol. 32, 1/2 (1991)

0065-4299/91/020073-02 $ 1.50 + 0.20/0
© 1991 Birkhäuser Verlag, Basel

Leukotriene B_4 stimulation of macrophage cyclooxygenase metabolite synthesis

G. R. Elliott [1], A. P. M. Lauwen [2] and I. L. Bonta [2]

[1] Pharmacology Dept., M.B.L., P.O.B. 45, 2280 AA, Rijswijk, The Netherlands (present address)
[2] Pharmacology Dept., Erasmus University Rotterdam, P.O.B. 1738, 3000 DR, Rotterdam, The Netherlands

Introduction

The essential poly-unsaturated fatty acid, arachidonic acid (AA), is metabolised by macrophages via the cyclooxygenase (CO) and lipoxygenase (LO) pathways. In general CO metabolites, in particular prostaglandin (PG) E_2, are considered to inhibit macrophage functions while LO products, in particular leukotrienes (LTs), stimulate them. Thus, the CO : LO metabolite ratio is important in regulating macrophage activities. For example, LTC_4 stimulates the secretion of the lysosomal enzyme β-glucuronidase (β-GLU) by macrophages. This response is enhanced by indomethacin, a CO inhibitor, and inhibited by PGE_2. LTC_4 also stimulates macrophage PGE_2 synthesis indicating that LTC_4-stimulated β-GLU secretion is self-limiting [1]. We have recently shown that calcium-ionophore (A23187)-stimulated macrophage LTB_4 synthesis is enhanced by indomethacin and inhibited by PGE_2 [2]. It is possible, therefore, that LTB_4-stimulation of macrophage functions is also modified by an associated increase in endogenous PGE_2 formation. We now report the effect of LTB_4 on the release of macrophage CO metabolites [PGE_2, thromboxane (TX) A2 (assayed as TXB_2) and prostacyclin (assayed as 6-keto$PGF_{1\alpha}$)] and β-GLU.

Materials and methods

The materials and methods used have been published previously [1]. Briefly, 4 day carrageenin-elicited rat peritoneal macrophages were isolated by density gradient centrifugation over Ficoll-Isopaque and suspended at 2×10^6 cells ml^{-1} in Dulbecco's modified Eagle's minimum essential medium (DMEM). Aliquots of the cell suspension (1 ml) were incubated for 1 h or 3 h at 37 °C in polypropylene reaction vials. The tubes were then centrifuged and the supernatant fractions stored at −70 °C for later analysis of PGE_2, 6-keto$PGF_{1\alpha}$ and TXB_2 by radioimmunoassay and for β-GLU activity.

Results

The effect of LTB_4 on macrophage PGE_2, 6-keto$PGF_{1\alpha}$ and TXB_2 synthesis

LTB_4 (10^{-12} to 10^{-7} *M*) had a biphasic, stimulatory effect on the synthesis and release of PGE_2 and TXB_2 during a 1 h incubation period. A maximum stimulation of both CO metabolites was observed at 10^{-9} *M* LTB_4. The basal release of all three CO metabolites was greater during the 3 h incubation period. However, LTB_4 now had no effect on PGE_2 formation whereas TXB_2 synthesis was stimulated, again with a maximum effect at 10^{-9} *M* LTB_4. No effect on 6-keto$PGF_{1\alpha}$ production was observed during either the 1 h or 3 h incubation periods (Table 1). LTB_4 (10^{-9} *M* and 10^{-8} *M*) and a slight, but not significant, stimulatory effect on β-GLU secretion during the 3 h incubation (control = 100%, 10^{-9} *M* = 129%, 10^{-8} *M* = 135%).

Table 1
The effect of leukotriene (LT) B_4 on macrophage prostaglandin (PG) E_2, thromboxane (TX) B_2 and 6-keto prostaglandin $F_{1\alpha}$ (6-keto) release.

	LTB_4 (M)					
	0	10^{-12}	10^{-10}	10^{-9}	10^{-8}	10^{-7}
1 h incubation						
PGE_2	0.35	0.48*	0.65*	1.32*	0.71*	0.52*
TXB_2	2.33	2.78	2.97	3.27*	3.21	2.61
6-keto	1.48	1.63	1.62	1.75	1.79	1.49
3 h incubation						
PGE_2	1.18	1.08	1.05	1.13	1.22	1.13
TXB_2	5.75	7.74*	11.28*	12.54*	11.50*	8.60*
6-keto	3.40	3.07	3.20	3.49	3.46	3.18

Results (ng/2×10^6 cells) are mean values, $n = 12$. *$p < 0.05$ vs control (Mann-Whitney U-test).

Discussion

LTB_4 had a differential effect on the synthesis of PGE_2, TXB_2 and 6-keto$PGF_{1\alpha}$ by carrageenin-elicited peritoneal macrophages. Whereas TXB_2 release was stimulated by LTB_4 during 1 h and 3 h incubation, PGE_2 formation was only stimulated during the 1 h incubation period. PGE_2 synthesis appeared to reach a maximum (1 h incubation with LTB_4 or a 3 h incubation without LTB_4) after which its synthesis stopped. Release of 6-keto $PGF_{1\alpha}$ was not modified by LTB_4 (1 h and 3 h incubations) even though its production was not maximal. LTB_4 had little effect on macrophage lysosomal enzyme release, under the conditions used an it has not proved possible to relate changes in CO metabolite synthesis with this marker of macrophage activation. It is possible that CO inhibitors, such as indomethacin, could enhance LTB_4-stimulated enzyme release as reported for LTC_4-induced lysosomal enzyme secretion [1].

LTB_4 has been reported to stimulate macrophage phosphatidylinositol turnover and to act as a calcium ionophore, which could result in stimulation of PLA_2 [3]. Both activities could stimulate the release of AA from phospholipid pools resulting in an increase in CO metabolite formation. We have previously shown that synthesis of 6-keto$PGF_{1\alpha}$ by granuloma macrophages is saturated at lower AA concentrations than the formation of PGE_2 or TXB_2 [4]. Thus is could be that the basal release of AA is sufficient to maximally stimulate 6-keto $PGF_{1\alpha}$ formation. PGE_2 inhibits A23187-stimulated macrophage TXB_2 formation and LTC_4-stimulated macrophage PGE_2 synthesis is associated with a reduction in TXB_2 formation [1, 2]. Thus, it could be that the apparent increase in LTB_4-stimulated TXB_2 production observed during the 3 h incubation, compared to the 1 h data, was due to the relative reduction in PGE_2 synthesis. Further experiments are necessary before we can suggest a reason for the saturation of PGE_2 synthesis.

References

[1] E. J. Schenkelaars and I. L. Bonta, *Cyclooxygenase inhibitors promote the leukotriene C_4 induced release of β-glucuronidase from rat peritoneal macrophages: prostaglandin E_2 suppresses.* International Journal of Immunopharmacology *8,* 305–311 (1986).

[2] G. R. Elliott, A. P. M. Lauwen and I. L. Bonta, *Prostaglandin E_2 inhibits, and indomethacin and aspirin enhance, A23187-stimulated leukotriene B_4 synthesis by rat peritoneal macrophages.* British Journal of Pharmacology *96,* 265–270 (1989).

[3] A. Holian, *Leukotriene B_4 stimulation of phosphatidylinositol turnover in macrophages and inhibition by pertussis toxin.* FEBS Letters *201,* 15–19 (1986).

[4] G. R. Elliott, M. J. P. Adolfs and I. L. Bonta, *Arachidonic acid an in vivo inhibitor of carrageenin-induced granulomas in the rat.* European Journal of Pharmacology *110,* 367–371 (1985).