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LY 255283 [(1-(5-ethyl-2-hydroxy-4-(6-methyl-6-)1H-tetrazol-5-yl)-heptyloxy) phenyl)ethanone], a specific leukotriene B_4 (LTB_4) receptor antagonist, inhibited the production of LTB_4 in human peripheral blood polymorphonuclear leukocytes (PMNL) and in monocytes activated by calcium ionophore A23187. In human monocytes activated by ionophore it inhibited also the production of thromboxane B_2 (TXB_2). The effect of LY 255283 on 5-lipoxygenase (5-LO) and LTA_4 hydrolase activities which catalyse the production of LTB_4 and LTA_4 has not been studied yet. It is thought that LY 255283 may inhibit the production of LTB_4 and TXA_2 by antagonising the effect of ionophore-induced LTB_4 on 5-lipoxygenase and cyclooxygenase in human peripheral blood PMNL and monocytes.

Key words: Human monocytes, Human polymorphonuclear leukocytes (PMNL), Leukotriene B_4 (LTB_4), LY 255283, Thromboxane B_2 (TXB_2)

Inhibitory effect of LY 255283 on the synthesis of leukotriene B_4 and thromboxane A_2 in human peripheral blood polymorphonuclear leukocytes and monocytes

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Introduction

LTB_4, a 5-lipoxygenase metabolite of arachidonic acid (AA), is an inflammatory mediator released from immunoinflammatory cells, especially from neutrophils and monocytes. A variety of stimuli, including calcium ionophores, particulate (zymosan) and soluble stimuli (N-formyl-methionyl-leucyl-phenylalanine) and complement component C5a[1,2] can elicit the synthesis of LTB_4 from neutrophil and monocytes.

It has been shown that LTB_4 stimulates various neutrophil functions such as chemotaxis, adherence, aggregation and degranulation.[3] LTB_4 also has some effects on the function of monocytes.[4]

Increased levels of LTB_4 have been shown during various immunoinflammatory conditions.[5,6] Since LTB_4 is accepted as an inflammatory mediator and the level of LTB_4 is increased during various immunoinflammatory conditions, several 5-lipoxygenase inhibitors and LTB_4 receptor antagonists are developed for the purpose of obtaining an effective antiinflammatory drug.[7]

Recently, McDonald *et al.* have shown that exogenously applied LTB_4 activates the human neutrophil 5-lipoxygenase and causes the overproduction of LTB_4.[8] During our ongoing studies with LTB_4 receptor antagonists, we have observed that LY 2455283, a specific LTB_4 antagonist,[9] can inhibit calcium ionophore A23187-induced LTB_4 synthesis in human neutrophils, monocytes and TXB_2 synthesis in monocytes.

Materials and Methods

Cell separation: Citrated blood was collected by venipuncture from healthy volunteers who had abstained from any drugs for at least 1 week before the sampling. A method described by Dewar was used for the isolation of human PMNL and monocytes.[10] Briefly, platelet rich plasma was discarded after the centrifugation of whole blood. Mononuclear cells and PMNL were separated by centrifugation on Ficoll–Paque cushions. The PMNL were separated from erythrocytes by dextran sedimentation and NH_4Cl lysis. After the isolation procedure, the purity of PMNL was $>98\%$ and viability determined by Trypan blue exclusion $>95\%$. Monocytes were further purified by adherence. 1.5 ml portions of the mononuclear cell suspension (2×10^6 cells/ml) were layered onto 30 mm plastic tissue culture dishes (Greiner) and were incubated at 37°C under an atmosphere of 5% CO_2 and 95% air for 1 h. The nonadherent cells were removed and additional 1.5 ml portions of mononuclear cell suspension (2×10^6 cells/ml) were added to each plate and the same incubation protocol was repeated. By quantitation of the adherent cells, it was determined that 85 to 90% of the adherent cells were monocytes.

Cell incubation: Incubations were performed at 37°C under an atmosphere of 5% CO_2 and 95% air. PMNL (4×10^6 cells/ml) were suspended in Dulbecco's phosphate buffered saline containing

Ca^{2+}/Mg^{2+} ($DPBS^{++}$) (composition in g/l NaCl 8, KCl 0.2, Na_2HPO_4 1.15, KH_2PO_4 0.2, $MgCl_2.6H_2O$ 0.1 and $CaCl_2$ 0.1) in tissue culture tubes (Greiner). After 10 min preincubation with LY 255283, PMNL were stimulated with 1 μM A23187. After an incubation period of 5 min, the cell suspension was centrifuged and the supernatant was stored at −70°C until the quantitation of LTB_4 and TXB_2.

The monocyte monolayers were washed and fresh Dulbecco's modified eagle medium (DMEM) containing 5% foetal calf serum was added. After 10 min preincubation with LY 255283 (10^{-6} M), monolayers were stimulated with 1 μM A23187. The supernatants were decanted, centrifuged and stored at −70°C.

Enzyme immunoassay experiments: LTB_4 and TXB_2 were measured by enzyme immunoassay (EIA).[11] The assay was performed in a total volume of 150 μl with each of the following components being added in a 50 μl volume: standards or biological samples, enzymatic tracer and specific antiserum. After overnight incubation at room temperature, the plates were washed and 200 μl Ellman's reagent was dispensed into each well. After 1–2 h, the absorbance at 405 nm of each well was measured. A standard curve from 15 pg/ml to 1 ng/ml was used in order to evaluate the concentrations of LTB_4 and TXB_2. Results are calculated in terms of per cent of B/Bo.

Chemicals: Calcium ionophore A23187 was purchased from Sigma Chemical Company (St Louis, MO). Monoclonal anti-rabbit IgG, standards and enzymatic tracers of LTB_4 and TXB_2 and antibody to LTB_4 were purchased from Cayman Chemical Company (Ann Arbor, MI). DMEM was purchased from Gibco BRL (Middlesex, UK). LY 255283 and antibody to TXB_2 were kindly provided by Thomas L. Jeatran (Lilly Research Laboratories, Indianapolis), and Professor GianCarlo Folco (Institute of Pharmacological Sciences, University of Milan) respectively. A23187 and LY 255283 were dissolved in absolute ethanol and dimethylsulfoxide respectively and were diluted with $DPBS^{++}$.

Statistics: The results are expressed in ng/10^6 cells and ng/dish for PMNL and monocytes respectively. The statistical assessment of groups was made by using a nonparametric method, Mann–Whitney–Wilcoxon test. The minimal level of significance was considered as $p < 0.05$.

Results

Calcium ionophore A23187 (10^{-6} M) elicited an increase in the synthesis of LTB_4 and TXB_2 in human PMNL and monocytes. This increase was more prominent on the synthesis of LTB_4, especially in monocytes (Figs 1 and 2).

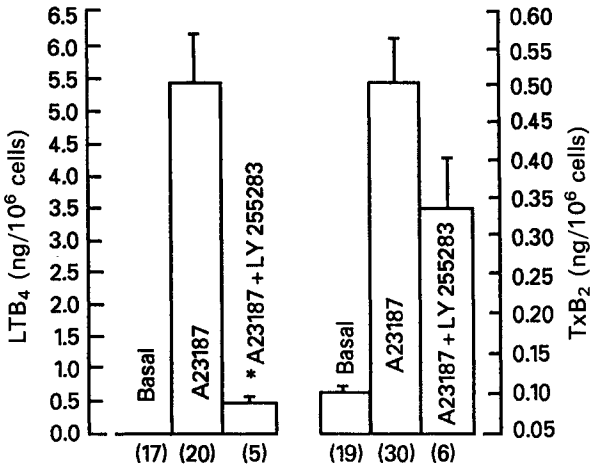

FIG. 1. Inhibition by LY 255283 (10^{-6} M) of the A23187-induced (10^{-6} M) increase of LTB_4 (*$p < 0.0007$) in human PMNL. ( ) number of experiments.

LTB_4 receptor antagonist LY 255283, at the concentration of 10^{-6} M, significantly inhibited the ionophore-induced LTB_4 synthesis in both cell types (Figs 1 and 2). In preliminary experiments, LY 255283 (ranging from 10^{-9} to 10^{-6} M) caused a dose-dependent inhibitory effect on LTB_4 release (data not shown). LY 255283 also inhibited the synthesis of TXB_2, the stable metabolite of TXA_2 in monocytes (Fig. 2). However, LY 255283 did not significantly inhibit the synthesis of TXB_2 in PMNL (Fig. 1).

Discussion

It is well known that a variety of stimuli, including the calcium ionophore A23187, can elicit an increase in the synthesis of cyclooxygenase (prostaglandin E_2, prostacylin, TXA_2) and lipoxygenase (mainly LTB_4) metabolites of AA in human

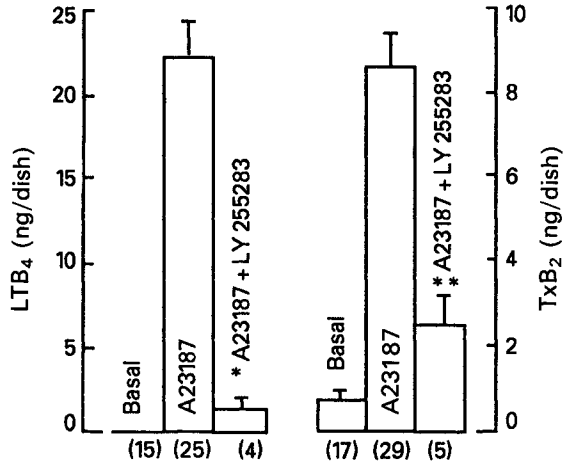

FIG. 2. Inhibition by LY 255283 (10^{-6} M) of the A23187-induced (10^{-6} M) increase of LTB_4 (*$p < 0.001$) and TXB_2 (**$p < 0.003$) in human monocytes. ( ) number of experiments.

PMNL and monocytes.[1,2] In the present study, calcium ionophore A23187, produced an increase in the synthesis of LTB_4 and TXB_2 in these cells. The ionophore-induced synthesis of LTB_4 was more prominent than that of TXB_2, a finding which supports previous observation of Moroney *et al.*[12]

LTB_4 is also known as one of the activators of human PMNL and monocytes. It is therefore expected that LTB_4 may activate its own synthesis in these cells. McDonald *et al.*[8] have described that 14,15-dideuterio-LTB_4 (D2-LTB_4) activates 5-lipoxygenase in PMNL and elicits an increase of LTB_4 synthesis. In this study, increased synthesis of LTB_4 by D2-LTB_4 in PMNL occurred when the cells were incubated with exogenous AA. It is well known that synthesis of LTB_4 from endogenous AA results from the activation of two independent calcium-dependent events. First, the release of AA from membrane phospholipids and second, the activation of the 5-LO.[13] In the present study, calcium ionophore A23187 increases Ca^{2+} influx as well as intracellular Ca^{2+} and therefore causes the release of AA from membrane phospholipids and activation of 5-LO.

Another interesting facet of the present study is that LY 255283 causes a decrease in the level of TXB_2 in monocytes. LTB_4 increases PGE_2 and TXA_2 synthesis in rat peritoneal macrophages elicited by carrageenin injection.[14] The results of the present study indicate that LY 255283 inhibited the synthesis of TXB_2 of human peripheral monocytes, one of another mononuclear cell population. A possible explanation of this result is that the increase of LTB_4 induced by ionophore may activate the cyclooxygenase in human peripheral monocytes; therefore, this may increase the synthesis of TXA_2. LY 255283 may decrease the level of TXB_2 in monocytes by inhibiting the synthesis of LTB_4.

One can assume that the LTB_4 receptor blocker, LY 255283, may also suppress the synthesis of LTB_4 by inhibiting 5-LO or LTA_4 hydrolase. Such an effect of the compound has not been described previously by other investigators yet and needs further studies using purified 5-LO or LTA_4 hydrolase.

In conclusion, besides LTB_4's activation of immunoinflammatory cells like human peripheral PMNL and monocytes, LTB_4 can also activate its own synthesis in these cells and activate the synthesis of TXB_2 in human monocytes. LTB_4 receptor antagonists may provide an additional useful effect by antagonising the self-stimulatory effect of LTB_4.

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