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Adenosine A_2 receptor-induced inhibition of leukotriene B_4 synthesis in whole blood *ex vivo*

E. Krump, G. Lemay & [1]P. Borgeat

Centre de Recherche en Rhumatologie et Immunologie, Centre de Recherche du CHUL and Université Laval, Québec, Canada, G1V 4G2

1 Engagement of adenosine A_2 receptors suppresses several leukocyte functions. In the present study, we examined the effect of adenosine on the inhibition of leukotriene B_4 (LTB_4) synthesis in heparinized human whole blood, pretreated with lipopolysaccharide (LPS) and tumour necrosis factor α (TNF-α) and stimulated with the chemotactic peptide, N-formyl-Met-Leu-Phe (FMLP).

2 The FMLP-induced synthesis of LTB_4 in whole blood pretreated with LPS and TNF-α was dose-dependently inhibited by adenosine analogues in the following order of potency; 5'(N-ethyl)carboxamidoadenosine (NECA) ≅ CGS 21680 > 2-Cl-adenosine > N^6-cyclopentyladenosine (CPA), indicating the involvement of the adenosine A_2 receptor subtype. The IC_{50} values for NECA, CGS 21680, 2-Cl-adenosine, and CPA were 6 nM, 9 nM, 180 nM, and 990 nM, respectively.

3 Dipyridamole, an agent that blocks the cellular uptake of adenosine by red cells and causes its accumulation in plasma, also inhibited the synthesis of LTB_4 in LPS and TNF-α-treated whole blood stimulated by FMLP; moreover, this inhibition was reversed upon addition of adenosine deaminase.

4 A highly selective antagonist of the adenosine A_2 receptor, 8-(3-chlorostyryl)caffeine (CSC), reversed the inhibition of LTB_4 synthesis by 2-Cl-adenosine and dipyridamole in LPS and TNF-α-treated whole blood, stimulated by FMLP.

5 LTB_4 synthesis in whole blood originates predominantly from neutrophils and to a lesser extent from monocytes. 2-Cl-adenosine also inhibited the synthesis of LTB_4 induced by FMLP in these isolated LPS and TNF-α-treated cells; however, 2-Cl-adenosine was a more potent inhibitor of LTB_4 synthesis in neutrophils than monocytes.

6 The present data demonstrate that adenosine, acting through A_2 receptors, exerts a potent inhibitory effect on the synthesis of LTB_4 and thus contribute to the understanding of its anti-inflammatory properties.

Keywords: Adenosine; adenosine A_2 receptor; dipyridamole; leukotriene B_4; whole blood

Introduction

Adenosine, via occupancy of A_2 receptors of neutrophils, inhibits their adherence to endothelial cells, the generation of superoxide anions, and phagocytosis (reviewed by Cronstein, 1994). Moreover, adenosine was shown to inhibit the synthesis of pro-inflammatory cytokines by lipopolysaccharide (LPS)-treated monocytes (Le Vraux *et al.*, 1993; Bouma *et al.*, 1994) and macrophages (Parmely *et al.*, 1993). In lymphocytes, adenosine inhibits the synthesis of immunoglobulins (Moroz & Stevens, 1980) and lymphocyte-mediated cytolysis (Wolberg *et al.*, 1975). Recent *in vivo* studies have demonstrated a protective role of adenosine and its structural analogues in models of acute inflammation such as experimental adjuvant arthritis (Green *et al.*, 1991), ischaemia-reperfusion (Grisham *et al.*, 1989; Kaminski & Proctor, 1989; Forman *et al.*, 1993; Marts *et al.*, 1993) and carregeenin-induced pleural inflammation (Schrier *et al.*, 1990). Furthermore, methotrexate, an antifolate commonly used in the treatment of rheumatoid arthritis patients, causes accumulation of adenosine and inhibition of leukocyte migration in inflammatory exudates in mice (Cronstein *et al.*, 1993). For these reasons, adenosine is increasingly viewed as a potent anti-inflammatory agent.

Leukotriene B_4 (LTB_4), a 5-lipoxygenase metabolite of arachidonic acid, stimulates several functions of leukocytes, such as chemotaxis, adherence to vascular endothelial cells, superoxide anion generation, and the release of lysozomal enzymes (Samuelsson *et al.*, 1987; Ford-Hutchison, 1990). Furthermore, LTB_4 was found to modulate the production of cytokines by monocytes (Rola-Pleszczinski & Lemaire, 1985; Horiguchi *et al.*, 1989; Rola-Pleszczinski & Stankova, 1992) and the proliferation of lymphocytes (Payan *et al.*, 1984; Gualde *et al.*, 1985; Atluru & Goodwin, 1986; Yamaoka *et al.*, 1989). The recent development of LTB_4 antagonists and inhibitors of 5-lipoxygenase have outlined a role for this lipid mediator in acute inflammatory states such as in endotoxic shock (Matera *et al.*, 1988; Fujimoto & Kobayashi, 1988; Coggeshall *et al.*, 1988; Yoshikawa & Goto, 1992). Moreover, by targeted disruption of the 5-lipoxygenase gene in mice, Chen *et al.* (1994) have demonstrated the involvement of leukotrienes in defined inflammatory states. LTB_4 is thus believed to play a significant role in several inflammatory conditions.

In view of the facts that adenosine inhibits several leukocyte functions and exerts anti-inflammatory effects *in vivo*, studies were undertaken to investigate the effects of adenosine on the synthesis of pro-inflammatory lipid mediators. In the present study, using a previously described experimental model where LTB_4 synthesis is induced by the chemoattractant peptide, N-formyl-Met-Leu-Phe (FMLP) in LPS- and tumour necrosis factor α (TNF-α)-primed whole blood (Surette *et al.*, 1993), we demonstrate that adenosine is a potent inhibitor of LTB_4 synthesis.

Methods

Whole blood collection and preparation of isolated leukocytes

Human venous peripheral blood was collected from healthy donors by venepuncture into heparinized tubes. For experiments with whole blood, 1 ml aliquots were dispensed into

[1] Author for correspondence.

polypropylene tubes and incubated as described in the figure legends. For experiments with isolated neutrophils and peripheral blood mononuclear cells, the cells were separated as previously described (Boyum, 1968). Briefly, whole blood was centrifuged at 180 *g* for 15 min and the resulting platelet-rich plasma was collected and depleted of its platelets by centrifugation at 2000 *g* for 30 min. Leukocytes were obtained following erythrocyte sedimentation at 1 *g* in 2% dextran T500. Neutrophils and mononuclear cells were separated by centrifugation on Ficoll-Paque cushions at 900 *g* for 20 min. Neutrophils and mononuclear cells were resuspended in HEPES-buffered HBSS (pH 7.4) containing 1.6 mM Ca^{2+} at 5×10^6 cells ml^{-1}. The viability of the final cell suspensions were >98%, as assessed by trypan blue exclusion, and the purity was >95% neutrophils. All cell isolation procedures were carried out at room temperature and cell incubations were carried out as described in the figure legends.

Measurement of LTB_4

Whole blood incubations were stopped by placing the tubes in an ice-water bath. After centrifugation at 180 *g* for 15 min at 4°C, a 200-μl aliquot of plasma was removed and denatured with 2 ml of acetonitrile containing 12.5 ng each of 19-hydroxy-prostaglandin (PG) B_2 and PGB_2 as internal standards. The denatured plasma was centrifuged at 2000 *g* for 15 min to remove the precipitated material and the supernatants were analyzed for 5-lipoxygenase products by reverse-phase high-performance liquid chromatography (r.p.-h.p.l.c.) as previously described (Surette *et al.*, 1994). Isolated neutrophils and mononuclear cells were treated as described in the figure legends; incubations were stopped by adding 1 volume of ice-cold methanol/acetonitrile (50/50; v/v) containing 12.5 ng each of 19-hydroxy-PGB_2 and PGB_2 as internal standards and processed for LTB_4 measurement by r.p.-h.p.l.c. as previously described (Borgeat *et al.*, 1990).

Materials

2-Cl-adenosine, adenosine deaminase (EC 3.5.4.4) (calf intestinal type VIII), LPS (*Escherichia coli* 0111:B4), dipyridamole, and HEPES were purchased from Sigma (St. Louis, MO, U.S.A.). 5'(N-ethyl)carboxamidoadenosine (NECA) and N^6-cyclopentyladenosine (CPA), were from ICN Biomedicals Canada Ltd. (Mississauga, Ont., Canada). 2-*p*-(2-carboxyethyl)phenetylamino-5'-N-ethylcarboxamidoadenosine HCl (CGS 21680), 3,7-dimethyl-1-propargylxanthine (DMPX) and 8-(3-chlorostyryl)caffeine (CSC) were from Research Biochemicals International (Natick, MA, U.S.A.). TNF-α was a generous gift from Knoll Pharmaceuticals (Whippany, NJ, U.S.A.). Dextran T500 and Ficoll-Paque were purchased from Pharmacia (Dorval, Qué., Canada), Hanks' balanced salt solution (HBSS) was from GIBCO (Burlington, Ont. Canada), and solvents (all h.p.l.c. grade) were purchased from Anachemia (Montréal, Qué., Canada).

Statistical analysis

Statistical analysis was performed using Student's one-tailed paired *t* test.

Results

Inhibition of LTB_4 synthesis by adenosine and adenosine analogues in whole blood

In the present study, we investigated the effect of adenosine analogues on LTB_4 synthesis. Toward this end, we used a previously characterized model for studies of LTB_4 synthesis consisting of whole blood primed with 1 $\mu g\,ml^{-1}$ LPS and 500 u ml^{-1} TNF-α and stimulated with 1 μM FMLP. While TNF-α, LPS or FMLP, alone, do not induce h.p.l.c.-detectable LTB_4 synthesis, whole blood pretreated with LPS + TNF-α and stimulated with FMLP generates substantial amounts of LTB_4 (Surette *et al.*, 1993). In such experimental settings, the addition of adenosine analogues in blood inhibited the synthesis of LTB_4 in a dose-dependent fashion with the following order of potency: NECA ≅ CGS 2180 > 2-Cl-adenosine > CPA (Figure 1). The IC_{50}s for NECA, CGS 21680, 2-Cl-adenosine, and CPA were 6 nM ($n=3$), 9 nM ($n=3$), 180 nM ($n=3$), and 990 nM ($n=3$), respectively.

In these experiments, adenosine could not be tested because of its short half-life in blood (< 1 s), a consequence of the rapid uptake of adenosine by erythrocytes (Möser *et al.*, 1989). However, in order to assess specifically the inhibitory activity of adenosine (as opposed to adenosine analogues) on LTB_4 synthesis, whole blood was pretreated with dipyridamole, an agent that blocks the cellular uptake of adenosine (IC_{50} of 2–3 μM in blood), thereby increasing the plasma concentration of adenosine (Möser *et al.*, 1989). Whole blood was pretreated with 1 $\mu g\,ml^{-1}$ LPS + 500 u ml^{-1} TNF-α and increasing concentrations of dipyridamole for 30 min, then challenged with 1 μM FMLP. Figure 2 shows that dipyridamole dose-dependently inhibited the synthesis of LTB_4, with a maximum inhibition occurring at 30 μM dipyridamole.

Since the profile of inhibition of LTB_4 synthesis by the adenosine analogues is suggestive of the involvement of the adenosine A_2 receptor, we next assessed the reversibility of this inhibition with CSC, a highly selective A_2 receptor antagonist (Jacobson *et al.*, 1993). As shown in Figure 3, CSC (30 μM) efficiently reversed the inhibition of LTB_4 synthesis by 2-Cl-adenosine (1 μM) in blood pretreated with LPS + TNF-α and stimulated with FMLP (73.4 ± 18.9% of control, mean ± s.e.mean, $n=4$, $P=0.03$ *vs* no CSC). Similarly, CSC (30 μM) also substantially reversed the inhibition of LTB_4 by dipyridamole (10 μM) in blood (76.2 ± 11.8% of control, mean ± s.e.mean, $n=4$, $P=0.03$ *vs* no CSC). Moreover, the suppression of LTB_4 synthesis by dipyridamole was also reversed by adenosine deaminase (which transforms adenosine into its biologically inactive metabolite, inosine), in further support of a role of enhanced plasma adenosine level in the inhibitory effect of dipyridamole. Indeed, the addition of 10 u ml^{-1} of adenosine deaminase to whole blood pretreated with dipyridamole and LPS + TNF-α substantially reversed the inhibition of LTB_4 synthesis (87.4 ± 9.4% of control, mean ± s.e.mean, $n=4$, $P=0.01$ *vs* no ADA) induced by

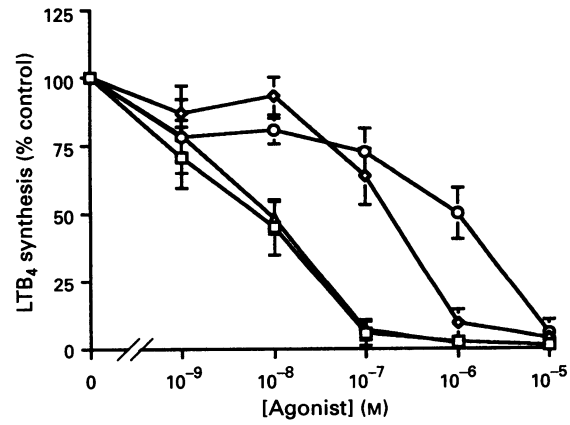

Figure 1 Effect of adenosine analogues on the synthesis of LTB_4 in LPS plus TNF-α-primed whole blood stimulated with FMLP. Blood aliquots (1 ml) were pretreated with 1 $\mu g\,ml^{-1}$ LPS plus 500 u ml^{-1} TNF-α for 30 min at 37°C in the presence of various concentrations of NECA (□), CGS 21680 (△), 2-Cl-adenosine (◇) or CPA (○), then stimulated with 1 μM FMLP for 15 min. Control level (100%) of LTB_4 synthesis was 11.8 ± 1.3 ng ml^{-1} of plasma. Data shown are the mean ± s.e. mean of 3 separate experiments, each performed in triplicate.

FMLP. The addition of dipyridamole, adenosine deaminase or CSC, alone, to whole blood or LPS + TNF-α-treated whole blood, failed to induce h.p.l.c.-detectable LTB_4 synthesis (not shown).

We next examined whether the synthesis of LTB_4 in response to stimulation of blood with the calcium ionophore A23187 was inhibited by 2-Cl-adenosine. Whereas 1 μM 2-Cl-adenosine accomplished near complete inhibition of LTB_4 synthesis (~95%) in LPS + TNF-α-primed whole blood stimulated with 1 μM FMLP, only a 20% (mean ± s.e.mean, $n=4$) inhibition of LTB_4 synthesis was noted with 1 μM 2-Cl-adenosine in whole blood challenged with 60 μM A23187 (Figure 4). With a lower concentration of A23187 (10 μM) resulting in levels of LTB_4 synthesis similar to those measured upon FMLP stimulation of LPS + TNF-α-primed blood, 1 μM 2-Cl-adenosine inhibited the synthesis of LTB_4 by only 30% (mean ± s.e.mean, $n=4$).

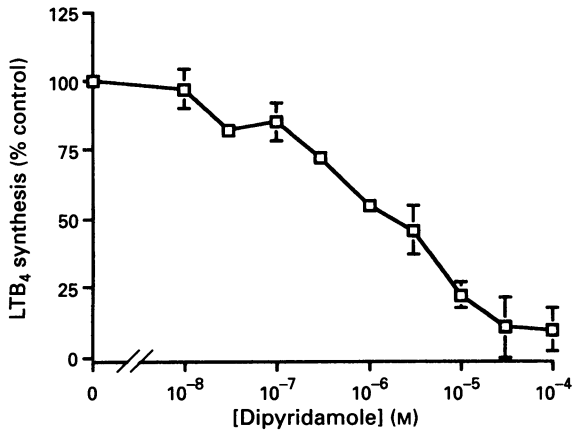

Figure 2 Effect of dipyridamole on the synthesis of LTB_4 in LPS plus TNF-α-primed whole blood stimulated with FMLP. Blood aliquots (1 ml) were pretreated with 1 μg ml^{-1} LPS plus 500 u ml^{-1} TNF-α for 30 min at 37°C in the presence of increasing concentrations of dipyridamole, then stimulated with 1 μM FMLP for 15 min. Control level (100%) of LTB_4 synthesis was 23.1 ± 1.4 ng ml^{-1} of plasma. Data shown are the mean ± s.e. mean of 4 separate experiments, each performed in triplicate.

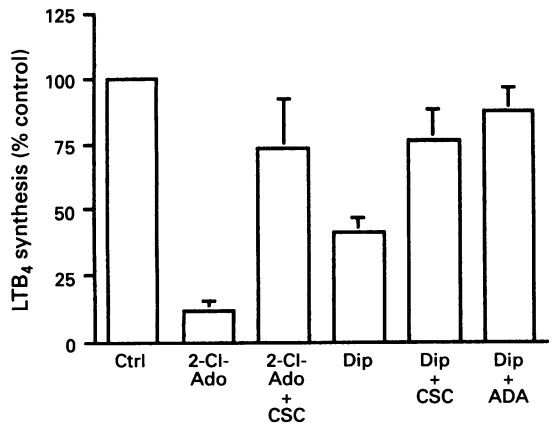

Figure 3 Reversibility of the 2-Cl-adenosine and dipyridamole inhibition of LTB_4 synthesis in LPS + TNF-α-primed whole blood stimulated with FMLP. Blood aliquots (1 ml) were pretreated with 1 μg ml^{-1} LPS plus 500 u ml^{-1} TNF-α for 30 min at 37°C, in the presence or absence of 10 μM dipyridamole or 1 μM 2-Cl-adenosine then stimulated with 1 μM FMLP for 15 min. The reversibility of the dipyridamole inhibition was assessed by adding either 10 u ml^{-1} ADA or 30 μM CSC and the reversibility of 2-Cl-adenosine inhibition was assessed by adding 30 μM CSC during the pretreatment. Control level (100%) of LTB_4 synthesis was 18.6 ± 4.3 ng ml^{-1} of plasma. Data shown are the mean ± s.e. mean of 4 separate experiments, each performed in triplicate. Dip, dipyridamole; 2-Cl-Ado, 2-Cl-adenosine.

Inhibition of LTB_4 synthesis by 2-Cl-adenosine in isolated leukocytes

The synthesis of LTB_4 in blood originates predominantly from neutrophils and from monocytes (Surette *et al.*, 1993; Palmantier *et al.*, 1994). We therefore assessed whether adenosine affects LTB_4 synthesis in isolated neutrophils and peripheral blood mononuclear cells, as observed in whole blood. The priming of neutrophils or mononuclear cells with LPS + TNF-α was performed in the presence of plasma which was shown to be required for the CD14 antigen-mediated LPS priming of LTB_4 synthesis in neutrophils (Surette *et al.*, 1993). Neutrophils and mononuclear cells were treated with 1 μg ml^{-1} LPS + 100 u ml^{-1} TNF-α for 30 min in the presence of 10% plasma, washed, then further incubated for 5 min with various concentrations of 2-Cl-adenosine, and stimulated with 1 μM FMLP. The addition of FMLP, 2-Cl-adenosine, LPS + TNF-α, alone, to neutrophils or mononuclear cells failed to induce r.p.-h.p.l.c.-detectable LTB_4 synthesis. Figure 5a shows that the addition of 2-Cl-adenosine to LPS + TNF-α-treated and FMLP-stimulated neutrophils resulted in a dose-dependent inhibition of LTB_4 synthesis, as observed in whole blood. However, 2-Cl-adenosine was a less potent inhibitor of LTB_4 synthesis by mononuclear cells.

The involvement of the adenosine A_2 receptor in the suppression of LTB_4 synthesis in isolated human neutrophils was next assessed by use of selective antagonists, namely CSC and DMPX. Figure 5b shows that the addition of 1 μM CSC reversed the suppression of LTB_4 synthesis by 1 μM 2-Cl-adenosine (91.5 ± 5.7% of control, mean ± s.e.mean, $n=4$, $P=0.001$) in neutrophils treated with LPS + TNF-α and stimulated with FMLP. Similarly, the addition of 30 μM DMPX substantially reversed the 2-Cl-adenosine inhibition of LTB_4 synthesis (69.0 ± 18.1% of control, mean ± s.e.mean, $n=3$, $P=0.03$) in human neutrophils.

Discussion

In the present study we have shown that adenosine is a potent inhibitor of LTB_4 synthesis in activated whole blood, as well as in isolated leukocytes. Adenosine analogues of different specificities for adenosine receptors, inhibited the synthesis of

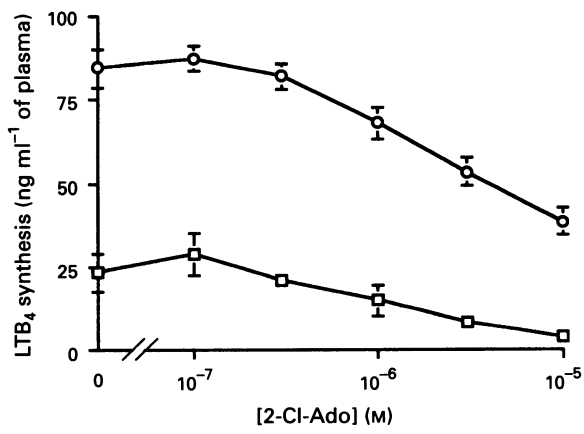

Figure 4 Effect of 2-Cl-adenosine on the synthesis of LTB_4 in whole blood stimulated with A23187. Blood aliquots (1 ml) were pretreated with an increasing concentration of 2-Cl-adenosine for 30 min at 37°C then stimulated with 10 μM (□) or 60 μM (○) A23187 for 15 min. Data shown are the mean ± s.d. of triplicate incubations from one experiment representative of four.

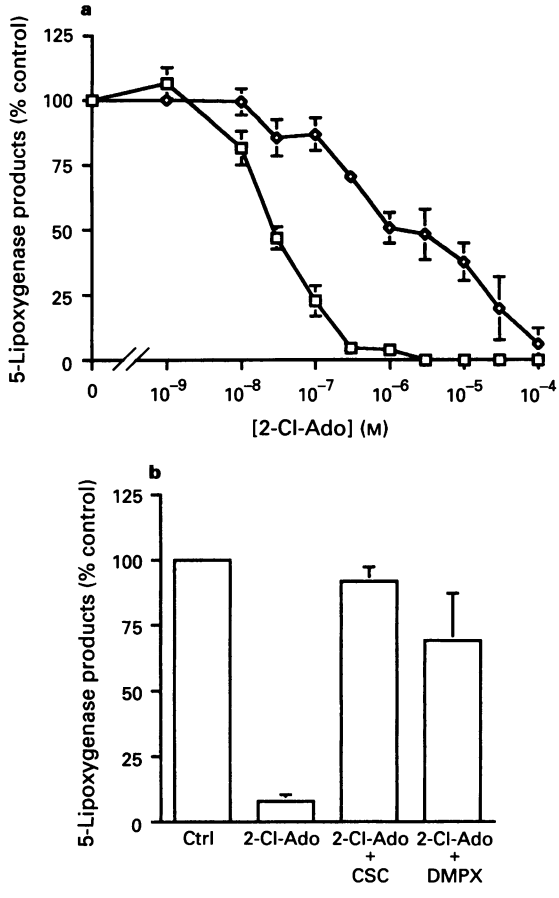

Figure 5 Effect of 2-Cl-adenosine on the synthesis of LTB_4 in isolated neutrophils and mononuclear leukocytes treated with LPS + TNF-α and stimulated with FMLP. (a) Neutrophils ($5 \times 10^6 ml^{-1}$) (□) and mononuclear cells ($5 \times 10^6 ml^{-1}$) (◇) were pretreated with 1 $\mu g ml^{-1}$ LPS plus 100 u ml^{-1} TNF-α in the presence of 10% autologous plasma for 30 min at 37°C. Plasma was then removed by washing, and cells were further incubated with increasing concentrations of 2-Cl-adenosine for 5 min, then stimulated with 1 μM FMLP for 15 min. Control level (100%) of 5-lipoxygenase products was 4.3 ± 1.0 ng per 5×10^6 neutrophils and 1.9 ± 0.8 ng per 5×10^6 mononuclear cells. (b) Neutrophils ($5 \times 10^6 ml^{-1}$) were pretreated and stimulated as described above except that 1 μM CSC or 30 μM DMPX (or their diluent) were added along with 1 μM 2-Cl-adenosine, as indicated on the figure. 5-Lipoxygenase products represent the sum of products detected in stimulated cells; 20-OH-LTB_4, 20-COOH-LTB_4, and LTB_4 for neutrophils and LTB_4 for mononuclear cells. Data shown are the mean ± s.e. mean of 3 (DMPX) and 4 (CSC) separate experiments, each performed in triplicate.

LTB_4 in whole blood in the following order of potency; NECA ≅ CGS 21680 > 2-Cl-adenosine > CPA. Such a profile of inhibition by the adenosine receptor agonists is characteristic of the involvement of the adenosine A_2 receptor (Collis & Hourani, 1993). This involvement of the A_2 receptor is further demonstrated by the ability of CSC to reverse the suppression of LTB_4 synthesis by 2-Cl-adenosine, an adenosine analogue which has an equal affinity for both the adenosine A_1 and A_2 receptor. Furthermore, our data strongly suggest the involvement of the adenosine A_{2a} receptor subtype since CGS 21680, an A_{2a} selective agonist, was amongst the most potent inhibitors of LTB_4 synthesis and that CSC, a selective A_{2a} antagonist, efficiently reversed the inhibition of LTB_4 synthesis by 2-Cl-adenosine. These results are consistent with several reported suppressive effects of adenosine on other leukocyte functions, such as the adherence to endothelial cells, the generation of superoxide anions, and phagocytosis, which were shown to involve adenosine A_2 receptors (Cronstein, 1994).

We also observed that 2-Cl-adenosine was a much more potent inhibitor of FMLP-stimulated LTB_4 synthesis than of A23187-stimulated LTB_4 synthesis; that the experiments with FMLP were performed following LPS + TNF-α priming, might have indicated that 2-Cl-adenosine inhibits the priming of PMN rather than their stimulation with FMLP or A23187. However, this hypothesis was ruled out since the addition of 2-Cl-adenosine, whether performed 0.5 min or 30 min prior to stimulation with FMLP (i.e. after or before the priming step), produced similar inhibitions of LTB_4 synthesis (data not shown). The reason for the difference in the inhibition by 2-Cl-adenosine of LTB_4 synthesis induced by FMLP and A23187 may be related to differences in the mechanisms by which ionophores and receptor-dependent agonists activate neutrophils. Indeed, it was suggested that the inhibitory effect of adenosine on superoxide anion synthesis through A_2 receptors involves an uncoupling of the FMLP receptor from its signal transduction elements (Cronstein *et al.*, 1992); accordingly, adenosine has been shown to inhibit the influx of Ca^{2+} induced by FMLP and PAF in human neutrophils (Tsuruta *et al.*, 1992), an effect which could impact on two Ca^{2+} dependent processes involved in LTB_4 synthesis, namely 5-lipoxygenase activation and arachidonic acid release. Thus, this putative mechanism of action of adenosine is compatible with our observation that adenosine does not efficiently inhibit the Ca^{2+} ionophore-induced LTB_4 synthesis.

While the inhibition of LTB_4 synthesis in whole blood was demonstrated with adenosine analogues, we confirmed the inhibitory effect of adenosine itself using dipyridamole. Indeed, the rapid uptake of adenosine by erythrocytes precludes the assessment of its biological activity in whole blood; however, dipyridamole blocks the cellular uptake of adenosine, which results in its extracellular accumulation (Möser *et al.*, 1989). Direct evidence for the involvement of adenosine in the inhibitory effect of dipyridamole was obtained in experiments where whole blood treatment with adenosine deaminase or CSC abrogated over 75% of the inhibitory effect of dipyridamole. These experiments demonstrated that endogenous adenosine can act as an efficient physiological inhibitor of LTB_4 synthesis in whole blood, and suggests that the modulation of adenosine action or concentration in blood or at inflammatory sites may represent a novel avenue for the pharmacological regulation of 5-lipoxygenase product synthesis *in vivo*. In this regard, recent studies have implicated an elevation of adenosine in the anti-inflammatory action of methotrexate. Indeed, Cronstein *et al.* (1993) showed a parallel accumulation of adenosine and inhibition of leukocyte migration at inflammatory sites of methotrexate-treated mice. Since LTB_4 is a potent chemotactic agent, it can be speculated that the inhibition of LTB_4 synthesis by adenosine may contribute to such anti-inflammatory effects of methotrexate. Previous studies have addressed the question of the effect of methotrexate therapy on LTB_4 synthesis by isolated blood neutrophils. The results of these studies were somewhat conflicting, some reporting a 30% decrease of LTB_4 synthesis (Sperling *et al.*, 1992; Leroux *et al.*, 1992) while in another study no effect of methotrexate treatment could be observed (Hawkes *et al.*, 1994). Such discrepancies may be due to the use of A23187 for inducing the synthesis of LTB_4 in the above studies, as we have shown in the present study that adenosine is a poor inhibitor of LTB_4 synthesis when stimulated by A23187. Studies are in progress to assess the effect of methotrexate therapy on LTB_4 synthesis by isolated neutrophils upon receptor-dependent cell activation.

LTB_4 is generated by the 5-lipoxygenase pathway of neutrophils, monocytes/macrophages, eosinophils, mast cells, basophils, and B cells in response to various stimuli (Borgeat & Naccache, 1990; Claesson *et al.*, 1992). While whole blood is composed of several cell types that have the potential to produce LTB_4, we have previously reported that approximately 80% of LTB_4 generated in whole blood originates from neutrophils, whereas the remaining 20% derives from monocytes (Surette *et al.*, 1993; Palmantier *et al.*, 1994). Our data showing that 2-Cl-adenosine suppressed the synthesis of LTB_4 in whole

blood and in isolated neutrophils with similar dose-inhibition curves are in agreement with a major contribution of neutrophils to the production of LTB_4 in blood. The difference in the potency of 2-Cl-adenosine to inhibit LTB_4 synthesis in neutrophils and mononuclear cells may be explained by differences in the level and/or function of A_2 receptors between the two cell types. In this regard, Eppell *et al.* (1989) reported that the expression and functionality of adenosine receptors increase during monocyte differentiation into macrophage. While our studies show a suppression of LTB_4 synthesis by adenosine in whole blood, a previous study by Peachell *et al.* (1991) reported that adenosine (and 2-Cl-adenosine) inhibited IgE-induced LTC_4 synthesis in isolated basophils, whereas stimulatory effects were observed in lung mast cells. The adenosine-mediated enhancement of LTC_4 synthesis in mast cells may be related to the novel A_3 receptor subtype recently identified in these cells, which have stimulatory effects on cellular functions (Ramkumar *et al.*, 1993).

While the current study has not investigated the mechanism whereby adenosine suppressed the synthesis of LTB_4, it is tempting to speculate that cyclic AMP may be involved in the signalling following the adenosine A_2 receptor engagement. Indeed, Iannone *et al.* (1989) demonstrated that the occupancy of adenosine A_2 receptors in combination with FMLP receptor stimulation resulted in a significant elevation of intracellular cyclic AMP in human neutrophils. This increase of intracellular cyclic AMP may suppress the synthesis of LTB_4 since cyclic AMP-elevating agents have been shown to inhibit the synthesis of LTB_4 in human neutrophils (Fonteh *et al.*, 1993). Further studies are required to assess the precise role of cyclic AMP in the suppressive effects of adenosine.

Finally, it should be pointed out that our demonstration that adenosine inhibits the synthesis of LTB_4 in whole blood primed with LPS and TNF-α has specific significance with regard to sepsis, a pathophysiological condition where both LPS and TNF-α are present (Beutler *et al.*, 1985; Martich *et al.*, 1991) and where leukotrienes have been implicated (Matera *et al.*, 1988; Fujimoto & Kobayashi, 1988; Coggeshall *et al.*, 1988; Yoshikawa & Goto, 1992). In addition to the previously reported inhibitory effects of adenosine on the synthesis of pro-inflammatory cytokines, such as TNF-α (Le Vraux *et al.*, 1993; Parmely *et al.*, 1993; Bouma *et al.*, 1994) and IL-8 (Bouma *et al.*, 1994), the adherence of PMN to endothelial cells (Cronstein *et al.*, 1986), and the generation of superoxide anions (Cronstein *et al.*, 1990), the ability of adenosine and adenosine-elevating agents to inhibit the synthesis of LTB_4 (in the current study) provides additional impetus towards the use of selective A_2 receptor agonists (or agents that affect the *in vivo* metabolism of adenosine) in the treatment of sepsis, a complex inflammatory condition which may only be controllable by a multi-target therapeutic approach.

In summary, the present study demonstrated the ability of adenosine and some structural analogues to inhibit the synthesis of LTB_4 in human whole blood through A_2 receptor engagement. In fact, with an IC_{50} under 10 nM, NECA and CGS 21680 are amongst the most potent inhibitors of LTB_4 synthesis in blood reported as yet. Our data not only add the synthesis of LTB_4 to the list of leukocyte functions that are suppressed upon stimulation of A_2 receptors, it also adds credence to the concept recently put forward that adenosine is a potent natural anti-inflammatory substance.

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