INHIBITION OF LEUKOTRIENE FORMATION AND IL-8 RELEASE BY THE PAF-RECEPTOR ANTAGONIST SM-12502

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Abstract—We analyzed the effect of the PAF receptor antagonist (+)-cis-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride (SM-12502) on the release of leukotriene B4 and IL-8 from human leukocytes. Peripheral blood from healthy donors was separated in two different fractions: polymorphonuclear leukocytes (PMN) and a lymphocyte, monocyte and basophil granulocyte cell fraction (LMB). After incubation of the cell population with different concentrations of SM-12502 the cells were subsequently stimulated with either the Ca ionophore A23187, the bacterial derived peptide fMLP, or with an activator of heterotrimeric G-proteins, the sodium fluoride (NaF, in the presence of Al3+). The PAF receptor antagonist led to a concentration and time dependent inhibition of LTB4 formation and IL-8 release from PMN and LMB. Our data clearly indicate an inhibitory effect of the PAF receptor antagonist SM-12502 on the formation of mediators of the lipoxygenase pathway and on the release of IL-8.

INTRODUCTION

Inflammation, allergy, anaphylaxis and shock are induced and maintained by the interaction of various cell populations and soluble proinflammatory mediators such as lipid mediators or cytokines. Among them the stereospecific 5-lipoxygenase (5-LOX) metabolite leukotriene B4 (LTB4) and the platelet activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) are potent activators of various phagocyte responses, such as degranulation, oxygen radical generation, aggregation, adherence and chemotaxis (1-4). PAF is synthesized from a phospholipid precursor after cleavage of the fatty acid in position C2 by a phospholipase A2 (PLA2) and transfer of an acetyl residue by an acetyltran-
ferase. LTB₄ is formed via transformation of arachidonic acid which is liberated from membraneous phospholipids by PLA₂ or PLC. Further oxydation of the free acid by a 5-LOX leads to the metabolites of the leukotriene cascade. Currently much effort is directed to determine the mechanisms by which lipidmediator synthesis is regulated.

Leukotrienes and PAF are both synthesized upon activation of leukocytes by calcium ionophores such as the A23187, by receptor dependent soluble stimuli such as N-formyl-methionyl-leucyl-phenylalanine (fMLP), by C5a and IL-8, or by direct G-protein activation via fluoride ions (5). LTB₄ and PAF themselves also have the potency to induce PAF synthesis, an event which is enhanced following PMN priming with GM-CSF (granulocyte-macrophage colony-stimulating factor) or IL-8 (6-9).

Recent reports provide evidence that an autocrine enhancement of LT synthesis by endogenous PAF and LTB₄ represents an important amplification mechanism of cellular mediator release (10, 11). It is well established that distinct cytokines, such as IL-1, IL-8, TNF-α and GM-CSF, can prime leukocytes during a pretreatment period for a subsequent stimulus, e.g. fMLP or the Ca ionophore A23187 (10, 12-15). After priming the cells respond more vigorously towards a subsequent stimulus, which is reflected by an enhanced formation of cell specific lipid mediators and/or cytokines.

In this regard the relative concentrations of various proinflammatory lipid mediators and cytokines determined the final cellular reactivity. Thus, it was the purpose of this study to investigate the role of the new PAF receptor antagonist (+)-cis-3,5,-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride, SM-12502 (16-19) on the autocrine and paracrine cell activation of peripheral leukocytes by the use of distinct cell stimuli, which either bypass membraneous signaltransduction (e.g. Ca ionophore A23187), or activate cellular responses via ligand-receptor coupling (fMLP), or which directly stimulate heterotrimeric G-proteins (sodium fluoride, NaF).

**MATERIALS AND METHODS**

*Materials.* Ficoll 400 was obtained from Pharmacia (Uppsala, Sweden); Macrodex (6%, wt/vol) was from Knoll (Ludwigshafen FRG); sodium metrizoate solution (75%, wt/vol) from Nyegaard (Oslo, Norway); heparin was obtained from Sigma (Munich, FRG); acetonitrile (HPLC grade) was purchased from Baker Chemicals (Gross-Gerau, FRG); methanol, EDTA, dipotassiumhydrogen-phosphate, and phosphoric acid were from Riedel de Haen (Seelze, FRG).

Synthetic leukotrienes LTB₄, LTC₄, LTD₄ and LTE₄ as well as the omega-oxidated products 20-OH-LTB₄ and 20-COOH-LTB₄ were generous gifts from Merck Frost (Pointe Claire/Dorval, Quebec, Canada). Ca-ionophore A23187 and sodium fluoride (NaF) were obtained from Sigma.