EFFECT OF THE PAF-RECEPTOR ANTAGONIST SM-12502 ON HUMAN PLATELETS

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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 human platelet aggregation as well as mediator release. After incubation of human platelet with different concentrations of SM-12502 the cells were subsequently stimulated with either the Ca ionophore A23187, with human thrombin, or with an activator of heterotrimeric G-proteins, sodium fluoride (NaF, in the presence of Al³⁺). Preincubation of platelets with the PAF receptor antagonist led to an inhibition of 12-lipoxygenase derived 12(S)-HETE and cyclooxygenase derived 12(S)-HHT. Pretreatment of platelets with the PAF receptor antagonist SM-12502 prior to activation with the Ca ionophore A23187 or PAF also inhibited platelet aggregation.

Our data clearly indicate an inhibitory effect of the new PAF receptor antagonist SM-12502 on the formation of platelet derived inflammatory mediators of the lipoxygenase pathway as well as of the cyclooxygenase pathway, and furtherone, treatment with the PAF receptor antagonist diminished platelet aggregation after subsequent specific and unspecific activation.

INTRODUCTION

Inflammatory reactions are induced and maintained by the interaction of different cells. In addition to their participation in hemeostatic reactions platelets are involved in inflammatory reactions by various mechanisms e.g. release of the precursor molecules (CTAP-3, connective tissue-activating protein; β-TG, β-thromboglobulin), which are further converted by intercellular metabolism to the potent chemokine NAP-2 (1).

Activated platelets release cyclooxygenase as well as lipoxygenase products. The monohydroxyeicosatetraenoic acid 12(S)-HETE is the major platelet lipoxygenase product. Besides chemotactic effects (e.g. neutrophils) 12(S)-HETE plays a crucial role on various enzymatic pathways of the lipoxygenases, cell movement, cell attachment intracellular calcium homeostasis and cell growth.
We could demonstrate that 12(S)-HETE is rapidly formed after interaction of the platelet with bacteria (E. coli, S. aureus) or bacterial toxins (E. coli α-hemolysin) (3–6). The pathophysiologic response of 12(S)-HETE in inflammatory skin diseases, e.g. in psoriasis, has been described (7). Recently we have shown that 12(S)-HETE formation was significantly elevated in patients with atopic diseases compared to healthy donors (6,8).

Communication between platelets and other cell populations occurs via cell-cell interaction by adhesion molecules (e.g. P selectin/CD15) or soluble mediators. 12(S)-HETE is rapidly incorporated into cellular membrane of bystander cells (e.g. endothelial cells, neutrophils) and metabolized e.g. to dioxygenated eicosatetraenoic acids. In this regard 12(S)-HETE is converted to 5(S), 12(S)-DiHETE (9) and 12(S),20(S)-DiHETE (10) by activated or resting neutrophils, respectively.

Human platelet thromboxane synthase catalyses the equimolar formation of thromboxane A2 (TxA2) and 12(S)-HHT plus malondialdehyde in a common enzymatic pathway (11). 12(S)-HHT is a metabolite of prostaglandin H2, which exerts as an unstable endoperoxide potent proaggregatory activity (12). The endoperoxides possess a greater platelet aggregatory potency as compared with TxA2 (13). In contrast to the potent proaggregatory and vasoactive TxA2 (14), 12(S)-HHT was not reported to possess similar biological potency.

The platelet activating factor (PAF) is one of the most potent platelet agonists. It activates the platelets at concentrations of 1–10 nM (15). High affinity receptors specific for PAF have been demonstrated on human platelets and account for the PAF induced platelet responses (e.g. aggregation) (16).

Since platelets express multiple functions including inflammatory responses we studied the efficacy of SM-12502 on defined parameters, e.g. 12(S)-HHT (cyclooxygenase product), 12(S)-HETE (lipoxygenase product) formation and platelet aggregation. As stimuli were used the Ca-ionophore A23187, human thrombin, the G-protein activator sodium fluoride as well as PAF.

**MATERIALS AND METHODS**

**Materials.** 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 eicosanoids 12(S)-HHT, 12(S)-HETE were generous gifts from Merck Frost (Pointe Claire/Dorval, Quebec, Canada). Ca-ionophore A23187 and sodium fluoride (NaF) were obtained from Sigma (Munich, FRG). The PAF-receptor antagonist SM-12502 was a generous gift from Sumitomo Pharmaceuticals Co., Ltd., Osaka (Japan). All other fine chemicals were purchased from Sigma (Munich, FRG).

**Buffers.** Unless stated otherwise, for the experiments a modified Dulbecco PBS (referred to as PBS) buffer consisting of 137 mM NaCl, 8 mM Na2HPO4, 2.7 mM KH2PO4 and 2.7 mM KCl (pH 7.4) was used.