Analysis of Leukotriene-Inducing (Lipoxygenase, Glutathione-S-Transferase) and -Metabolizing (γ -Glutamyltranspeptidase, Dipeptidase) Enzymes from Human Polymorphonuclear Granulocytes

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Introduction

The outstanding role of polymorphonuclear granulocytes (PMN) to produce mediators of inflammation has been clearly established [2, 6–9]. After activation by extracellular stimuli the cells convert arachi-donic acid to three major groups of derivatives: the prostaglandins, the thromboxanes and the recently discovered leukotrienes are formed by oxygenation and further transformation. Among the lipoxygenase transformation products are factors with pronounced effect on leukocyte migration (leukotriene B₄ and iso-mers) and vascular permeability [11]. The biological activity referred to for 40 years as slow-reacting substance of anaphylaxis (SRS-A) consists of three related metabolites LTC₄, D₄ and E₄ [5, 10, 12]. The lipoxygenase pathway within human granulocytes is initiated by a 5-lipoxygenase which transforms arachi-donic acid to 5-HPETE. LTA₄ is converted via glutathione-S-transferase to LTC₄; LTC₄ is metabolized by a γ -glutamyltranspeptidase (γ -GT) to LTD₄ and LTD₄ by a dipeptidase to LTE₄. It was the purpose of the present investigation to determine the release of leukotriene-inducing (5-lipoxygenase, glutathione-S-transferase) as well as metabolizing (γ -GT, dipeptidase) enzymes [3, 10, 11].

Material and Methods

Human PMNs were obtained from heparinized blood of healthy donors and separated on a Ficoll Metrizoate gradient followed by dextran sedimentation [3, 4]. Homogenization was performed in a Potter-Elvehjem Teflon glass homogenizer; differential centrifugation was carried out at 400, 3,000 and 20,000 g for 15 min and at 200,000 g for 60 min leading to four pellets and a final supernatant fraction. Equilibrium gradient centrifugation was performed with a postnuclear supernatant on a 19–54% (w/w) continuous sucrose gradient and centrifuged for 11 h at 100,000 g. Leukotriene-inducing and -metabolizing enzymes were determined as follows: PMNs were stimulated with the calcium ionophore in the presence of ¹⁴C-arachidonic acid and indomethacin; the release of 5-HETE, 5-HPETE, LTB₄, 20-OH LTB₄ and phospholipids was detected by thin-layer chromatography of the labeled

compounds; 5-lipoxygenase activity in the cell-free supernatant was analyzed by their incubation with ^{14}C -arachidonic acid; alternatively LTB₄, C₄, D₄ and E₄ were determined by reversed-phase HPLC or radioimmunoassay for LTC₄ [1,3].

For analysis of γ -GT and dipeptidase activities cell supernatants and subcellular fractions were solubilized in Triton X 100 and incubated with either synthetic LTC₄ or LTD₄; metabolites were determined by reversed-phase HPLC. For LTC₄ generation fractions were incubated with LTA₄ or LTA₄-methyl-ester in the presence of 1 mM glutathione and 200 μM serine borate; LTC₄ generation was measured by radioimmunoassay; marker enzymes of granulocytes, further glutathione-S-transferase; L- γ -GT and dipeptidase were also studied photometrically [3].

Results

Stimulation of human PMNs with the calcium ionophore A 23187 led to a dose- and time-related release of LTB₄ and LTC₄. When the metabolism of labeled lipoxygenase products was analyzed six radioactive metabolites were detected, among them LTB₄, 20-OH LTB₄, 5-HETE, 5-HPETE and radioactively labeled phospholipids. The extraction procedure was not suited to detect the peptido-leukotrienes as well. Kinetic experiments demonstrated a rapid release of HPETE, HETE and LTB₄ with a rapid metabolism over time; the formation of 20-OH-LTB₄ steadily increased. The generation of lipoxygenase products can be inhibited with compounds affecting the various enzymatic pathways; for the 5-lipoxygenase are known esculetin, flavanoids and caffeic acid.

In order to analyze the leukotriene-inducing (5-lipoxygenase, glutathione-S-transferase) and -

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from granulocytes. Dipeptidase activity rapidly appeared in the supernatant and decreased in its activity over time; with prolonged incubation γ -GT transforming LTC₄ into LTD₄ became apparent. No soluble glutathione-S-transferase was detected. In view of the biological importance of these mediators in cell biological interactions as well as in immunopathological disease processes, extensive immunopharmacological efforts had been devoted to control leukotriene release from the various cells. A prerequisite, however, is the knowledge about the distribution of the leukotriene-specific enzymes on a subcellular level. Granulocytes were separated as to their nuclear, granular, micro-somal and cytosol fractions which were characterized by their appropriate marker enzymes. The 5-lipoxygenase resided in the granular and microsomal fractions, glutathione-S-transferase activity in the cytosol and to a lesser extent in the microsomal fractions, γ -GT in the microsomal fraction, and dipeptidase in the granular and microsomal fraction, which also contained the highest lysozyme content. By isopycnic centrifugation of a postnuclear supernatant, azurophilic granules were present at a density of 1.20–1.23 g/ml (β -glucuronidase, peroxidase), the microsomes and specific granules at a density of 1.12–1.18 g/ml (fig. 1). As to the leukotriene-specific enzymes, glutathione-S-transferase stayed on top of the gradient, 5-lipoxygenase at 1.17–1.18 g/ml, γ -GT at 1.20–1.23 g/ml and 1.12–1.18 g/ml and dipeptidase at 1.18–1.22 g/ml. These results demonstrate a compartmentalization of the various enzymes involved in leukotriene metabolism. The described system allows the evaluation of antiinflammatory compounds on leukotriene-inducing and -metabolizing enzymes.

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