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Ethyl pyruvate and ethyl lactate down-regulate the production of pro-inflammatory cytokines and modulate expression of immune receptors

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ABSTRACT

Esters of α-oxo-carbonic acids such as ethyl pyruvate (EP) have been demonstrated to exert inhibitory effects on the production of anti-inflammatory cytokines. So far, there is no information about effects, if any, of ethyl lactate (EL), an obviously inactive analogue of EP, on inflammatory immune responses. In the present study, we provide evidence that the anti-inflammatory action of α-oxo-carbonic acid esters is mediated by inhibition of glyoxalases (Glo), cytosolic enzymes that catalyse the conversion of α-oxo-aldehydes such as methylglyoxal (MGO) into the corresponding α-hydroxy acids using glutathione as a cofactor. *In vitro* enzyme activity measurements revealed the inhibition of human Glo1 by α-oxo-carbonic acid esters, whilst α-hydroxy-carbonic acid esters such as EL were not inhibitory. In contrast, both EP and EL were shown to suppress the Lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin (IL)-1β, IL-6 and IL-8 from human immunocompetent cells, and modulated the expression of the immune receptors HLA-DR, CD14 and CD91 on human monocytes. Here, we show a crossing link between glyoxalases and the immune system. The results described herein introduce glyoxalases as a possible target for therapeutic approaches of immune suppression.

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Abbreviations: BGCD, *p*-bromobenzylglutathione cyclopentyl diester; EL, ethyl lactate; EP, ethyl pyruvate; HAGH, hydroxyacyl glutathione hydrolase (glyoxalase 2); IFN, interferon; Glo1, glyoxalase 1; GSH, L-glutathione; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; mAb, monoclonal antibody; MFI, mean fluorescence intensity; MGO, methylglyoxal; NAC, *N*-acetyl cysteine; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; PMSF, phenylmethylsulfonyl fluoride; TBS, Tris-buffered saline.

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1. Introduction

Recently, esters of pyruvate such as EP have been demonstrated to provide protection from ischemia/reperfusion-induced tissue injury and to rescue mice from endotoxin-induced lethality and from polymicrobially induced severe peritonitis [1,2]. The rationale behind pointed toward attenuation of release of early and late pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and high-mobility group box 1 (HMGB1). The molecular basis of EP action was suggested to be its interference with NF-κB signal transduction pathway, however, the detailed mechanism is not fully elucidated yet [3]. In the present study, we provide evidence that the anti-inflammatory action of EP is mediated by inhibition of glyoxalases. Glyoxalase 1 (Glo1, E.C. 4.4.1.5) and Glo 2 (E.C. 3.1.2.6) are cytosolic enzymes that catalyse the conversion of α-oxo-aldehydes into the corresponding α-hydroxy acids using L-glutathione (GSH) as a cofactor [4]. MGO is a reactive α-oxo-aldehyde that is mainly formed from non-enzymatic fragmentation of triose phosphates along the glycolytic pathway. MGO, when present in high concentrations, reacts rapidly with both, proteins and nucleic acids, and this is the presumed basis of its cytotoxicity [5].

To protect cells against damage caused by MGO, the glyoxalase system provides an efficient ubiquitous detoxification pathway. In many prokaryotic and eukaryotic cells, an increased MGO burden is prevented by up-regulation of glyoxalases. This enables tumor cells to escape intoxification by MGO formed paracatalytically via increased aerobic glycolysis [6].

Here, we show a link between glyoxalases and the immune system. Our studies reveal a remarkable and unexpected regulation of different immune functions by Glo1. Balanced inhibition of Glo1 in immune cells were found to modulate LPS-mediated release of pro-inflammatory cytokines, surface expression of receptors involved in antigen uptake and processing as well as Th1/Th2 cell responses upon antigenic and mitogenic stimulation. The results reveal glyoxalases as new targets for immune suppression.

2. Materials and methods

2.1. Reagents and cells

Ficoll-Paque Plus® was purchased from GE Healthcare Life Science (Freiburg, Germany). RPMI-1640 medium and heat-inactivated fetal calf serum (FCS) were purchased from Invitrogen (Karlruhe, Germany). Magnetic cell separation system (MACS®) was supplied by Miltenyi Biotec (Bergish Gladbach, Germany). GSH was purchased from Roth (Karlsruhe, Germany). Cell proliferation reagent WST-1 was obtained from Roche (Mannheim, Germany). BD™ Cytometric Bead Array (CBA Human Inflammation kit), BD FACS™ Lysing Solution and isotype control antibodies were obtained from BD Biosciences (Heidelberg, Germany). EP, EL, MGO, N-acetyl cysteine (NAC), Phytohemagglutinin (PHA), BCIP/NBT-Blue Liquid substrate, HEPES, $MgCl_2$, KCl, EDTA, glycerol, Triton X-100, phenylmethylsulfonylfluoride (PMSF), dithiothreitol (DTT), diamino benzidine (DAB), H_2O_2, protease inhibitors and LPS (*E. coli* Serotype O111:B4) were purchased from Sigma (Steinheim, Germany). Sodium dodecyl sulfate (SDS) was purchased from Serva (Heidelberg, Germany). Cellulose nitrate membranes were obtained from Whatman Schleicher & Schuell (Dassel, Germany). Trypan blue, phosphate-buffered saline (PBS) and Tris-buffered saline (TBS) were purchased from Seromed (Berlin, Germany). RNeasy Mini Kit was purchased from Qiagen (Hilden, Germany), AMV Reverse Transcriptase und Oligo (dT) Primer were from Promega (Heidelberg, Germany) and Ready-To-Go™ PCR beads from GE Healthcare (Munich, Germany). Goat serum was purchased from Dako (Glostrup, Denmark). APC-labeled anti-HLA-DR and PE-labeled anti-CD14 mAbs were obtained from Immunotech (Beckman Coulter, Krefeld, Germany), FITC-labeled anti-human CD91, anti-human Glo1 (clone 4 C10) and anti-mouse Glo1 (clone 4C7) mAbs from BioMac (Leipzig, Germany), biotin-labeled anti-human IFN-γ mAb (1-D1K) and streptavidin-labeled-horse radish peroxidase (HRP) from Mabtech (Hamburg, Germany). Rabbit anti-human β-actin Ab was purchased from Abcam (Cambridge, UK), goat anti-mouse HRP-Ab from Dako (Glostrup, Denmark), and Alexa 488-labeled goat anti-mouse Ig from Invitrogen (Karlsruhe, Germany). ELISPOT-plates (ELISPOT Multiscreen-IP, 45 μm) were delivered by Millipore (Beford, U.S.A). Cell culture plates were obtained from Greiner Bio-one (Frickenhausen, Germany).

Blood samples used in this study were drawn from healthy donors at the Blood Bank of Leipzig University Clinic, Germany. The experiments were approved by the local authorities and the informed consent of all participating subjects was obtained. Mouse macrophages (RAW 264.7, ATCC, No. TIB-71) were supplied by LGC Promochem (Wesel, Germany).

2.2. Glyoxalase assay

The determination of Glo1 activity has been adapted according to Mannervik et al. [7]. To evaluate the effect of EP and EL on Glo1 enzymatic reaction, variable concentrations of these compounds were pre-incubated with 2 mM GSH for 4 min and the reaction was started by addition of 2 mM MGO and the enzyme. IC_{50} values were calculated from the inhibition curves. In another set of experiments, Glo1 activity was evaluated at fixed concentrations of EP/MGO and variable concentrations of GSH. Human Glo1 was purified as formerly described [8].

2.3. Whole blood assay

LPS (10 ng/ml), test substances and 200 μl heparinized human blood were co-incubated with RPMI-1640 medium (1 ml final volume) in 24-well culture plates for 6 h at 37 °C with 5% CO_2. Following incubation, plates were centrifuged at 2000 × *g* for 10 min and supernatants were stored at −20 °C for later evaluation for pro-inflammatory cytokines.

2.4. Isolation of human blood monocytes

Peripheral blood mononuclear cells (PBMC) were separated from heparinized human blood by Ficoll-Paque Plus® separation and CD14+ monocytes were purified further by magnetic