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Imatinib reduces cholesterol uptake and matrix metalloproteinase activity in human THP-1 macrophages

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ABSTRACT

Background: Imatinib mesylate (Glivec®, formerly STI-571) is a selective tyrosine kinase inhibitor used for the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors. However, there are reports suggesting that imatinib could be atheroprotective by lowering plasma low-density lipoprotein (LDL).

Aim: To investigate the potential inhibitory effect of imatinib on cholesterol uptake in human macrophages as well as its effect on matrix metalloproteinase (MMP) activity.

Methods and results: Uptake of fluorescence-labeled LDL was analyzed using flow cytometry. Macrophages treated with imatinib showed a 23.5%, 27%, and 15% decrease in uptake of native LDL ($p < 0.05$), acetylated LDL ($p < 0.01$), and copper-modified oxidized LDL ($p < 0.01$), respectively. Gel-based zymography showed that secretion and activity of MMP-2 and MMP-9 were inhibited by imatinib. Using GeneChip Whole Transcript Expression array analysis, no obvious gene candidates involved in the mechanisms of cholesterol metabolism or MMP regulation were found to be affected by imatinib. Instead, we found that imatinib up-regulated microRNA 155 (*miR155*) by 43.8% and down-regulated ADAM metallopeptidase domain 28 (*ADAM28*) by 41.4%. Both genes could potentially play an atheroprotective role and would be interesting targets in future studies.

Conclusion: Our results indicate that imatinib causes post-translational inhibition with respect to cholesterol uptake and regulation of MMP-2 and MMP-9. More research is needed to further evaluate the role of imatinib in the regulation of other genes and processes.

Introduction

Cardiovascular disease is the main reason for premature death worldwide. One of the underlying causes of cardiovascular disease is atherosclerosis, which leads to the formation of unstable plaques followed by thrombus formation, occlusion of coronary arteries, heart failure, and death [1,2]. The earliest change in the development of atherosclerosis is endothelial dysfunction in the vascular wall that leads to lipid accumulation in the arterial intima. This dysfunction is initiated by several factors including elevated levels of low-density lipoprotein (LDL) that result in the attraction of monocytes [3]. Once the monocytes have passed through the endothelium and have differentiated into macrophages, they start ingesting oxidized LDL and become foam cells. This leads to the development of fatty streaks and, eventually, a lesion in the vascular wall [1,3]. The macrophage foam cell formation is thought to be caused by a dysfunction in the cellular mechanism controlling cholesterol uptake and efflux [1]. Continued progression of atherosclerosis results in the formation of a fibrous cap over the lesion, which might rupture due to protease degradation by matrix metalloproteinases (MMPs) and result in a myocardial infarction or stroke [1,3].

Imatinib mesylate (Glivec®) is a selective tyrosine kinase inhibitor that is currently Food and Drug Administration (FDA) approved for the treatment of chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST) because it inhibits the growth of malignant cells [4]. Imatinib is also pre-registered for acute lymphoblastic leukemia and is in phase II clinical trials for

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sarcoma and melanoma [5]. The mechanism of imatinib might be useful not only against tumors but also against non-malignant proliferative disorders such as rheumatoid disorders, pulmonary hypertension, and atherosclerosis. Imatinib functions by occupying the ATP-binding sites and inhibiting the phosphorylation of tyrosine residues of platelet-derived growth factor receptor (PDGFR)-α, PDGFR-β, c-kit, and breakpoint cluster region-Abelson (Bcr-Abl) [2,4]. The binding of a ligand to a tyrosine kinase receptor leads to homodimerization and auto-phosphorylation. The phosphorylation of tyrosine residues induces a signaling cascade and activates downstream pathways that promote cell proliferation and survival. Because imatinib binds to the ATP-binding site of tyrosine kinase receptors, substrate phosphorylation does not occur and the subsequent downstream signaling is inhibited.

In addition to the anti-malignant effects of imatinib, there are reports that the drug also decreases and normalizes plasma lipid levels within one month after therapy suggesting that imatinib could be used in the prevention of diseases such as atherosclerosis [6–8]. Furthermore, it has been shown that patients with CML who are treated with imatinib demonstrate increased levels of adiponectin, a protein that plays an atheroprotective role [9].

The aim of this study was to investigate the potential effects of the tyrosine kinase inhibitor imatinib on cholesterol uptake and MMP activity, two key targets in the prevention and treatment of atherosclerosis.

Materials and methods

Cell culture

Monocytic THP-1 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in RPMI 1640 medium with GlutaMAX™ (Invitrogen, Gibco®, Paisley, UK) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (PAA Laboratories GmbH, Pasching, Austria), 2 mM L-glutamine (PAA Laboratories GmbH), and 50 U/mL penicillin/streptomycin (Invitrogen, Gibco®). Cells were cultured in suspension at 37 °C in a humidified 5% CO_2 atmosphere. Macrophages were grown in RPMI 1640 medium supplemented with 50 μM β-mercaptoethanol (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and 1 mM sodium pyruvate (Invitrogen, Gibco®). To differentiate monocytes into macrophages, 1×10^6 cells/mL were treated with 50 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma–Aldrich, St Louis, MO, USA) for 24 h. To remove excessive PMA, the cells were washed with Dulbecco's phosphate-buffered saline (DPBS).

Peripheral whole blood was drawn from four healthy blood donors into Na-heparin-treated tubes. The blood was diluted with RPMI 1640 medium, layered on top of a ficoll gradient (GE Healthcare, Uppsala, Sweden) and centrifuged at $400 \times g$ for 30 min. Thereafter, the interface containing peripheral blood mononuclear cells (PBMC) was collected and washed two times with RPMI 1640 containing 2% fetal bovine serum at $300 \times g$ before culturing. The PBMC pellet was suspended in 1640 RPMI medium (without FBS, penicillin/streptomycin or L-glutamine) transferred into a cell culture flask and incubated for 2 h at 37 °C with 5% CO_2 to allow monocyte adherence. Non-adherent cells were then washed away and ice-cold PBS was added to detach the monocytes. One million monocytes were transferred to each well in a 24-well plate and cells were incubated in 1640 RPMI medium (without FBS, penicillin/streptomycin or L-glutamine) containing 100 ng/mL GM-CSF (Invitrogen) for 24 h at 37 °C with 5% CO_2. After 24 h the medium was replaced with new 1640 RPMI medium containing 10% FBS, 50 U/mL penicillin/streptomycin, 2 mM L-glutamine and 100 ng/mL GM-CSF. At day 5 cells were treated with 1 μM imatinib and cholesterol uptake was analyzed.

Cholesterol uptake and efflux

To evaluate the effects of imatinib on cholesterol uptake in macrophages, cells were treated with or without 1.0 or 0.1 μM imatinib (Selleck Chemicals LLC, Houston, TX, USA) or 1.0 μM bosutinib (Sigma–Aldrich) for 48 h and incubated with 10 μg/mL of BODIPY-labeled LDL, 10 μg/mL of BODIPY-labeled acetylated LDL (Invitrogen, Carlsbad, CA, USA), or 10 μg/mL of copper-oxidized 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (Dil)-labeled oxidized LDL for 4 h. Copper-oxidized Dil-labeled oxidized LDL was prepared with 200 μg/mL native Dil-labeled LDL (Biogenesis, Poole, UK) and dialyzed against PBS and oxidized using 40 μM $CuSO_4$. The oxidation was halted after 4 h through gel filtration using a PD-10 column (Amersham Biosciences, Piscataway, NJ, USA).

After incubation with fluorescently-labeled LDL, the cells were washed with DPBS and detached from the plate using Versene solution (Invitrogen, Gibco®). Cells were transferred to 5 mL polystyrene round-bottom tubes (BD Falcon™, Franklin Lakes, NJ, USA), washed in DPBS, and analyzed immediately using a Gallios™ flow cytometer (Beckman Coulter, Brea, CA, USA). Kaluza® Analysis Software (Beckman Coulter) was used for data analysis.

For efflux analysis, cells incubated with BODIPY-labeled LDL were starved overnight in media containing 1% bovine serum albumin and acyl-coenzyme A:cholesterol acyltransferase inhibitor (ACAT, 2 μg/mL, Sigma–Aldrich), to inhibit spontaneous cholesterol efflux, before receiving the cholesterol acceptor HDL_3 (MyBioSource LLC, San Diego, CA, USA) for 4 h. Cyclic AMP (0.3 mM, Sigma–Aldrich) was used as a positive control for efflux. The wells were washed with cold DPBS and then analyzed by flow cytometry.

Affymetrix® geneChip® whole transcript expression array analysis

To evaluate the effects of imatinib on gene expression, PMA-differentiated macrophages were treated with or without 1 μM imatinib (Selleck Chemicals LLC) and incubated for 24 h. The cells were lysed in RLT buffer (Qiagen, Hilden, Germany). The quality and integrity of total RNA was checked with an Agilent Technologies 2100 Bioanalyzer (Agilent Technologies; Waldbronn, Germany). The Ambion® WT Expression Kit (Life technologies, Ambion®, Carlsbad, CA, USA) and the GeneChip® WT Terminal Labeling and Hybridization Kit (Affymetrix, Santa Clara, CA, USA) were used for biotin labeling with 250 ng of total RNA. As recommended by the manufacturer, 5.5 μg of biotinylated cDNA were fragmented and placed in a hybridization cocktail containing four biotinylated hybridization controls (BioB, BioC, BioD, and Cre). Samples were hybridized to an identical lot of Affymetrix Gene ST 1.0 arrays (169 Format) for 17 h at 45 °C.

Washing and staining steps were performed using the Fluidics Station 450 with the GeneChip® Hybridization, Wash, and Stain Kit (Affymetrix) following the FS450-0007 protocol (Affymetrix). Image analysis was performed with a GCOS 3000 Scanner and the GCOS1.2 software suite (Affymetrix). GeneSpring 11.5.1 (Agilent Technologies, CA, USA) was used to analyze microarray data. Signal intensities (raw data) were log2 transformed and normalized using robust multichip average (RMA). Gene expression values for each condition are reported as relative to the mean expression value calculated from the intensities of all conditions (mean centralization, normalized data) from three separate experiments.

Gel-based zymography

Media from the cells were mixed with native loading buffer and separated using a 10% zymography gel (Invitrogen) to document cleavage of gelatin by MMP-2 and MMP-9. After electrophoresis, the proteins in the gel were renatured and the gels were developed