Journal of Molecular and Cellular Cardiology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Original article

Red blood cells serve as intravascular carriers of myeloperoxidase ☆

Matti Adam [a,b,*], Silvie Gajdova [c], Hana Kolarova [c], Lukas Kubala [c,d], Denise Lau [e], Anne Geisler [e], Thorben Ravekes [f], Volker Rudolph [f], Philip S. Tsao [a,b], Stefan Blankenberg [e], Stephan Baldus [f], Anna Klinke [f]

[a] Stanford University, Division of Cardiovascular Medicine, Stanford, CA, USA
[b] Stanford Cardiovascular Institute, Stanford, CA, USA
[c] Academy of Sciences of the Czech Republic, Institute of Biophysics, Brno, Czech Republic
[d] International Clinical Research Center—CBCE, St. Anne's University Hospital Brno, Brno, Czech Republic
[e] University of Hamburg, Heart Center, Department of Cardiovascular Medicine, Hamburg, Germany
[f] Heart Center, Department of Cardiology and Cologne Cardiovascular Research Center, University of Cologne, Cologne, Germany

ARTICLE INFO

Article history:
Received 12 September 2013
Received in revised form 23 May 2014
Accepted 18 June 2014
Available online xxxx

Keywords:
Myeloperoxidase
Erythrocyte
Cell membranes
Vascular endothelium-dependent relaxation
Systemic vascular resistance

ABSTRACT

Myeloperoxidase (MPO) is a heme enzyme abundantly expressed in polymorphonuclear neutrophils. MPO is enzymatically capable of catalyzing the generation of reactive oxygen species (ROS) and the consumption of nitric oxide (NO). Thus MPO has both potent microbicidal and, upon binding to the vessel wall, pro-inflammatory properties. Interestingly, MPO – a highly cationic protein – has been shown to bind to both endothelial cells and leukocyte membranes. Given the anionic surface charge of red blood cells, we investigated binding of MPO to erythrocytes. Red blood cells (RBCs) derived from patients with elevated MPO plasma levels showed significantly higher amounts of MPO by flow cytometry and ELISA than healthy controls. Heparin-induced MPO-release from patient-derived RBCs was significantly increased compared to controls. *Ex vivo* experiments revealed dose and time dependency for MPO-RBC binding, and immunofluorescence staining as well as confocal microscopy localized MPO–RBC interaction to the erythrocyte plasma membrane. NO-consumption by RBC-membrane fragments (erythrocyte "ghosts") increased with incrementally greater concentrations of MPO during incubation, indicating preserved catalytic MPO activity. *In vivo* infusion of MPO-loaded RBCs into C57BL/6J mice increased local MPO tissue concentrations in liver, spleen, lung, and heart tissue as well as within the cardiac vasculature. Further, NO-dependent relaxation of aortic rings was altered by RBC bound-MPO and systemic vascular resistance significantly increased after infusion of MPO-loaded RBCs into mice. In summary, we find that MPO binds to RBC membranes *in vitro* and *in vivo*, is transported by RBCs to remote sites in mice, and affects endothelial function as well as systemic vascular resistance. RBCs may avidly bind circulating MPO, and act as carriers of this leukocyte-derived enzyme.

© 2014 Published by Elsevier Ltd.

1. Introduction

Myeloperoxidase (MPO) is a heme-containing enzyme abundantly expressed in immune cells, including polymorphonuclear neutrophils (PMN), monocytes and macrophages. MPO is stored in large amounts in the azurophilic granules of neutrophils and is secreted into the phagosome and the extracellular space upon activation by inflammatory stimuli [1]. Enzymatically capable of generating reactive oxygen species (ROS), in particular hypochlorous acid (HOCl), it is considered one of the major bactericidal proteins, taking part in the first line of host defense [2].

Besides its bactericidal properties, MPO has been found to potently affect vascular homeostasis. MPO catalytically consumes nitric oxide (NO), thereby directly impairing endothelial function. Also, MPO catalyzes the oxidation of thiols by HOCl, such as thioester and tyrosyl residues, thereby structurally impairing vascular integrity [3]. Moreover, MPO exhibits leukocyte-attracting properties irrespective of its catalytic function. Electrostatic interactions between the highly-cationic MPO and the anionic glycocalyx residues of PMNs and endothelial cells lead to attraction of PMNs to the endothelial surface [4]. Given the potent pro-inflammatory properties of MPO, its distribution in the microcirculation is crucial for its pathobiological significance.

Red blood cells (RBCs) are the most common cells in peripheral blood, with a concentration of 4–6 million cells per microliter. High amounts of sialic acid, bound to glycophorins in the erythrocyte membrane, are responsible for a halo of negative charge that surrounds RBCs [5].

☆ This work has been supported by the Stanford University Dean (M.A.), the Deutsche Forschungsgemeinschaft (KL 2516/1-1 to A.K., BA 1870/7-1, BA 1870/9-1 and BA 1870/10-1 to S.B.; RU 1876/1-1 to V.R), the Czech Science Foundation (P305/12/J038 to L.K.) and the European Regional Development Fund—Project FNUSA-ICRC (No. CZ.1.05/1.1.00/02.0123) (L.K.).

* Corresponding author at: Stanford University School of Medicine, Division of Cardiovascular Medicine, 300 Pasteur Drive, Falk CVRB, Stanford, CA 94305, USA. Tel.: +1 650 498 6353.
E-mail address: matti.adam@stanford.edu (M. Adam).

http://dx.doi.org/10.1016/j.yjmcc.2014.06.009
0022-2828/© 2014 Published by Elsevier Ltd.

Please cite this article as: Adam M, et al, Red blood cells serve as intravascular carriers of myeloperoxidase, J Mol Cell Cardiol (2014), http://dx.doi.org/10.1016/j.yjmcc.2014.06.009

UNCORRECTED PROOF

ARTICLE IN PRESS

We postulated that cationic MPO would be attracted to erythrocyte membrane. Due to the large number of RBCs and their close interaction with endothelial cells and other blood cells or even with RBCs themselves, depending on the region of vasculature, we investigated RBC capability to bind to, transport, and deliver MPO, with potential relevance for changing endothelial function and vascular tone.

2. Methods

Human studies were performed after informed consent, in accordance with the Declaration of Helsinki and approved by the Board of Physicians Ethics Committee Hamburg. All animal experiments were approved by the local ethics committee an in accordance with the US National Research Council's Guide for the Care and Use of Laboratory Animals.

2.1. Isolation of RBCs

Blood for erythrocyte isolation was collected either from healthy volunteers or from patients in intermediate or intensive care unit. Patients had either been diagnosed with acute coronary syndrome within the last week or had proof of severe heart failure, and were excluded for fever (body temperature >38 °C) or for c-reactive protein (CRP) level >50 mg/dl. Investigators were blinded to anticoagulation regime or additional clinical data.

Venous blood was mixed with EDTA (0.5 M pH 8.0) 100:1 and centrifuged for 10 min, supernatant and buffy-coat with leukocytes were removed, and 2 subsequent washing steps with phosphate buffered saline (PBS-Invitrogen) were performed. The pellet was diluted to 5 million RBC/μl stock solution and further diluted with PBS as needed. Isolation of RBCs from C57BL/6J mice (Jackson Laboratory) was performed using the same protocol.

2.2. Isolation of RBC-ghosts

RBCs were lysed in a 10-fold volume of 5 mM Tris–HCl, pH 7.0, 1 mM EDTA and mixed for 15 min at room temperature. Afterwards, samples were centrifuged at 21,000 ×*g* for 30 min at 4 °C. The pellet was resuspended in 5 mM Tris–HCl, pH 7.0, 1 mM EDTA and washed again. High salt wash was performed with 0.05 M Tris–HCl, pH 7.0, 1 mM EDTA, 0.5 M NaCl, and then twice resuspended and washed with 5 mM Tris–HCl, pH 7.0, 1 mM EDTA, while centrifuging at 21,000 ×g for 12 min at 4 °C. RBC ghosts were washed until they were white in color. Storage of RBC ghosts was performed in a small volume of 0.05 M Tris–HCl, pH 7.0 (500 μl per pellet) for a maximum of 1 day. Before use, membrane fragments were transferred into PBS and diluted to a stock solution using spectrometric OD 280 nm absorbance to create arbitrary units.

2.3. Flow cytometry

The presence of MPO on the surface of RBC was determined using isolated RBCs from healthy volunteers and patients (described above). After washing steps, RBCs were incubated with R-Phycoerythrin (PE)-conjugated anti-MPO antibody or isotype control (Acris, USA) for 10 min at RT. RBCs were analyzed using flowcytometer FACSCanto (BD Biosciences, USA). RBCs were defined by forward- and side-scatter characteristics, and 10,000 individual events were collected within the RBC gate. Cells stained with appropriate isotype controls were used to determine non-specific background signal.

2.4. Fluorescent staining of RBCs and confocal microscopy

RBC suspension was streaked on microscope slides and dried overnight. In the first set of experiments, RBC suspension was fixed in 100% ethanol for 30 min. Subsequently, samples were washed with PBS, blocked and permeabilized with 10% goat serum and 0.1% Triton X-100 in PBS for 30 min at RT. Non-permeabilized samples were blocked with 10% goat serum in PBS at RT for 1 h. Samples were incubated with antibodies against MPO (rabbit, Calbiochem EMD Millipore 1:250; rabbit polyclonal, Thermo Scientific, USA 1:125) and hemoglobin (goat, Thermo Scientific, 1:250) at 4 °C overnight, and finally labeled with secondary antibodies (Alexa 488 IgG anti-rabbit, DyLight 488 Thermo Scientific, Alexa 594 IgG anti-goat). Images were acquired with a Leica microscope (DMLB) and IVision software or a confocal microscope (TCS SP5, Leica, Germany) using a 63 × 1.4 oil immersion objective. A three-dimensional projection was reconstructed from a two-dimensional z-stack series using LAS AF Lite software (Leica).

2.5. Immunofluorescence staining of 3-chlorotyrosine in murine tissue

Harvested organs (as described in 2.6) were embedded and frozen in optimal cutting temperature compound (OCT) and cut to 4 μm sections. Sections were thawed, fixed in 3.7% formaldehyde solution, incubated with 0.1% Triton X-100 and blocked with 10% goat serum. Sections were treated with first antibody rabbit IgG to 3-chlorotyrosine (1:100, Hycult Biotech) and secondary antibody goat IgG to rabbit labeled with Alexa Fluor 488 (Molecular Probes). Nuclei were counterstained with DAPI. Images were acquired with a Keyence BZII Analyzer Software (Keyence).

2.6. NO-consumption by RBC-ghosts

RBC-ghosts of different concentrations (0, 10, 100 arbitrary units) after incubation with different MPO concentrations (0, 10, 50 μg/ml) were placed into glass vials. Using an NO electrode (AmiNO 700, Innovative Instruments, USA), connected to the ISO-NO MARK II potentiostat (WPI, USA) while stirring at 37 °C, NO concentration was measured as previously described [6] after administration of 15 μM H_2O_2 with 10 μM PAPANO as a NO-donor. Values were calculated from the maximal rate of NO consumption per second and are expressed as pA/s.

2.7. Animal experiments

Male C57BL/6J (Jackson Laboratory) aged 10–15 weeks were treated with an infusion of MPO-loaded RBCs. Briefly, murine blood was harvested in EDTA 1:100. RBCs were isolated as described above, and diluted in standard fashion with NaCl (Fresenius) for incubation with MPO or NaCl. Two subsequent washing steps followed. Treated murine RBCs were then reinfused into animals of the same gender and age via the right carotid artery under anesthesia with isoflurane and buprenorphine. After circulation for 30 min, animals were euthanized and organs were harvested in standard fashion after perfusing the animal with 5 ml of PBS via the carotid catheter.

2.8. Langendorff-perfusion

Freshly isolated mouse hearts were mounted on a Langendorff-perfusion system and rinsed with 500 μl of isotonic NaCl-solution at 37 °C. Subsequently, hearts were perfused with 2 ml of PBS-heparin (50 IU/ml) at 37 °C and the rinse was collected. The resulting solution was concentrated to 200 μl using speedvac-centrifugation and MPO-concentration was quantified by ELISA. Unless otherwise stated, all ELISA analysis was performed using a CardioMPO kit (Cleveland Heart Lab) according to manufacturer's instructions.

2.9. Organ bath

Aortic segments of euthanasized mice were carefully dissected, and endothelium-dependent and -independent relaxation were determined in response to increasing doses of acetylcholine.

(Ach, 10^{-9}–5×10^{-6} mol/l) and nitroglycerine (NTG) as previously described [6,7].

UNCORRECTED PROOF

Please cite this article as: Adam M, et al, Red blood cells serve as intravascular carriers of myeloperoxidase, J Mol Cell Cardiol (2014), http://dx.doi.org/10.1016/j.yjmcc.2014.06.009

Download English Version:

https://daneshyari.com/en/article/8474779

Download Persian Version:

https://daneshyari.com/article/8474779

Daneshyari.com