Biochemical Pharmacology 132 (2017) 48–62

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Mechanism of ascaridole activation in *Leishmania*

Gerald Geroldinger [a], Matthias Tonner [a], Hubert Hettegger [b], Markus Bacher [b], Lianet Monzote [a,c], Martin Walter [d], Katrin Staniek [a], Thomas Rosenau [b], Lars Gille [a,*]

[a] *Institute of Pharmacology and Toxicology, Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria*
[b] *Department of Chemistry, University of Natural Resources and Life Sciences, Tulln, Austria*
[c] *Parasitology Department, Institute of Tropical Medicine "Pedro Kouri", Havana, Cuba*
[d] *Department of Environmental Geosciences, University of Vienna, Vienna, Austria*

A R T I C L E I N F O

Article history:
Received 5 January 2017
Accepted 28 February 2017
Available online 2 March 2017

Chemical compounds:
Ascaridole (Pubchem CID: 10545)
Artemisinin (Pubchem CID: 68827)

Keywords:
Ascaridole
Endoperoxides
Leishmania
Electron spin resonance
Iron

A B S T R A C T

Endoperoxides (EP) are an emerging class of drugs which have potential in antiparasitic therapy, but also in other fields. For malaria therapy the EP artemisinin (Art) and its derivatives are successfully used. We have shown in the past that the EP ascaridole (Asc) is useful for the treatment of cutaneous leishmaniasis in a mouse model. Biomimetic experiments suggested that these drugs need activation in the respective target pathogens to exert their function. In spite of this idea, direct activation of EP to radicals inside cells has never been demonstrated. Therefore, this study was initiated to explore the activation of Asc in biomimetic systems and inside *Leishmania* in comparison to Art. Using electron paramagnetic resonance spectroscopy (EPR) in combination with spin-trapping we identified the secondary alkyl radical intermediates arising from reduction by Fe^{2+} in cell-free systems. Combined GC/NMR analysis confirmed the loss of isopropyl residues from Asc during this process as intermediates. This activation of Asc was stimulated by low molecular Fe^{2+} complexes or alternatively by hemin in conjunction with thiol reductants, such as cysteine (Cys). In *Leishmania tarentolae* promastigotes (LtP) as model for pathogenic forms of *Leishmania* carbon-centered radicals were identified in the presence of Asc by EPR spin-trapping. Both Asc and Art inhibited the viability in LtP with IC_{50} values in the low micromolar range while IC_{50} values for J774 macrophages were considerably higher. A similar structure without EP bridge (1,4-cineole) resulted in no detectable radicals and possessed much less cytotoxicity in LtP and no selectivity for LtP compared to J774 cells. The Asc-derived radical formation in LtP was inhibited by the iron chelator deferoxamine (DFO), and stimulated by Cys (a suitable reductant for hemin). The IC_{50} values for LtP viability in the presence of Asc or Art were increased significantly by the spin trap DMPO, while Cys and DFO increased only IC_{50} values for Art. In a heme association assay Asc demonstrated a lower binding affinity to heme than Art. ICP-OES measurements revealed that in LtP the total iron concentrations were twice as high as values in J774 macrophages. Since low molecular iron was important in Asc activation we studied the influence of Asc on the labile iron pool (LIP) in LtP. Low temperature EPR experiments demonstrated that Asc shifts the redox balance of iron in the LIP to its oxidized state. These data demonstrate that univalent cleavage of Asc/Art in LtP is an essential part of their pharmacological mechanism. The structure of the EP determines whether activation by low molecular iron or heme is favored and the availability of these intracellular activators modulates their cytotoxicity. These findings may be helpful for synthesis of new Asc derivatives and understanding the action of EP in other cell types.

© 2017 Elsevier Inc. All rights reserved.

Abbreviations: ACN, acetonitrile; Art, artemisinin; Asc, ascaridole; BHI, brain heart infusion; $CDCl_3$, deuterated chloroform; Cin, 1,4-cineole; Cys, cysteine; DFO, deferoxamine; DMEM, Dulbecco's Modified Eagle Medium; DMPO, 5,5-dimethyl-1-pyrroline N-oxide; DMSO, dimethyl sulfoxide; EP, endoperoxide; EPR, electron paramagnetic resonance; FCS, fetal calf serum; FID, flame ionization detector; GC, gas chromatography; IC_{50}, half maximal inhibitory concentration; ICP-OES, inductively coupled plasma-optical emission spectrometry; J774, J774 macrophage/monocyte cell line; $KCrOx_3$, potassium chromium(III) oxalate; LFR1, ferric iron reductase; LHR1, *Leishmania* heme response-1 transporter; LIP, labile iron pool; LIT1, ferrous iron transporter; LtP, *Leishmania tarentolae* promastigotes; MNP, 2-methyl-2-nitrosopropane; NMR, nuclear magnetic resonance; OD, optical density; PBS, phosphate buffered saline; Pen, pentamidine; ROS, reactive oxygen species; XO, xylenol orange; YEM, yeast extract medium.

* Corresponding author at: Institute of Pharmacology and Toxicology, Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria.
E-mail address: Lars.Gille@vetmeduni.ac.at (L. Gille).

http://dx.doi.org/10.1016/j.bcp.2017.02.023
0006-2952/© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Oxidative stress is a common threat for all aerobic organisms. The evolutionary adaptation enabled organisms to cope with normal levels of reactive oxygen species (ROS) which partially arise from small amounts of free iron as building block for various enzymes in the cell [1]. Only excessive amounts of ROS in mammalian organisms cause pathologies. The mechanisms of oxidative defense include chemical, enzymatic and genetic processes in cells to prevent excessive oxygen radical formation. In analogy, also eukaryotic microorganisms have to defend themselves against these species. However, their defense mechanisms slightly differ from mammalian cells although following similar principles [2]. These facts on the first view do not favor the use of peroxides/ROS as pharmacological weapons against these microorganisms since host cells usually can detoxify them and microorganisms have their own defense system. Nevertheless, specific types of peroxides/ROS have been shown to be effective pharmacological agents against intracellular protozoal pathogens in mammalian host cells [3]. The prototype of this drug class is artemisinin (Art) which is effectively used against plasmodia parasites residing inside erythrocytes [4]. This is astonishing since erythrocytes possess a highly efficient antioxidant defense with millimolar glutathione concentrations [5].

Leishmaniasis is another protozoal disease, which threatens millions of people in tropical and subtropical regions [6–8]. *Leishmania* parasites are transmitted as promastigotes via sandflies to mammalian hosts. Upon infection these parasites are phagocytized by macrophages and transformed into amastigotes residing inside the phagolysosome. In this location, parasites survive and multiply since they are hidden from the host immune system. Depending on the *Leishmania* species parasites can spread in the mammalian organism leading to different clinical manifestations, such as cutaneous, mucocutaneous or visceral leishmaniasis [6]. Although there are many differences between plasmodia and *Leishmania,* there are some common features, such as the strong dependence on iron/heme supply by the mammalian host cell [2]. Free iron, specifically located in the labile iron pool (LIP), is a major trigger of oxidative stress in all aerobic organisms what makes these pathogens specifically susceptible to peroxides [9]. On the other hand, iron may play a role in the differentiation of *Leishmania* [2].

Organic peroxides represent a major group of ROS and arise from the reaction of carbon-centered radicals with oxygen. However, resulting hydroperoxides against intracellular protozoal pathogens are efficiently detoxified in mammalian host cells and also partially in protozoal pathogens [2]. In addition, pharmaceutical application of hydroperoxides is limited because to reach the protozoal pathogen they would have to cross the mammalian host cell. Therefore, another class of peroxides, the endoperoxides (EPs) appear to be more promising since they partially can escape from the detoxification system of the host cell. So far, preferably glutathione-S-transferase has been shown to cleave the EP Art [10]. In previous works it was shown in a mouse model that the EP ascaridole (Asc) is effective against cutaneous leishmaniasis caused by *Leishmania amazonensis* [11,12]. Furthermore, Asc was effective against cell culture models of *Leishmania* promastigotes and amastigotes from different *Leishmania* species [11,13,14] and against specific cancer cells [15]. Because only few EPs exist temporarily in the mammalian host cells, such as during prostaglandin synthesis, obviously these compounds are less targeted by the mammalian antioxidative defense.

Based on the known reaction principles for ROS, it is expected that EPs in analogy to hydroperoxides have to be activated by single electron reduction to exert their pharmacological function via radical formation [16]. For Art this has been demonstrated in a broad range of publications using methods of biomimetic chemistry, i.e. not in cellular environments [17]. For Asc we demonstrated its activation by Fe^{2+} in organic solvents [18]. Although this mechanism might be of relevance for the pharmacological action of these compounds, it is surprising that, to our knowledge, so far no publication demonstrates directly (by spin-trapping) the activation of these EPs in the targeted pathogens. To study the activation of Asc in *Leishmania* we designed a study to identify its primary radical activation products in the chemical system and inside *Leishmania*. For comparison purposes Art as another EP and 1,4-cineole (Cin) with a structure similar to Asc but without peroxide bridge were used. To enable these mechanistic studies *Leishmania tarentolae* promastigotes (LtP) were chosen as a parasitic model organism and a J774 macrophage cell line was used as model for host cells. Our results elucidate the nature of the primary radical formed from Asc. For the first time, its activation inside *Leishmania* was demonstrated and also the effect of several influencing factors on its activation was observed.

2. Materials and methods

2.1. Chemicals

Glucose, acetonitrile, methanol, potassium chromium(III) oxalate ($KCrOx_3$), xylenol orange, H_2SO_4, HNO_3, glycerol, Tris, K_2HPO_4, KH_2PO_4, Na_2HPO_4, NaCl, KCl, HCl, $FeSO_4$, and $FeCl_3$ were obtained from Merck (Darmstadt, Germany). Hemin, 5,5-dimethyl-1-pyrroline N-oxide (DMPO), 2-methyl-2-nitrosopropane (MNP), penicillin-streptomycin solution, resazurin, pentamidine isethionate (Pen), artemisinin (Art), 1,4-cineole (Cin), brain heart infusion (BHI) medium, 4-oxo-2,2,6,6-tetramethylpiperidine-1-yl)oxyl (Oxo-Tempo), and $CHCl_3$ were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Butylated hydroxytoluene was from Roche (Basel, Switzerland). Dulbecco's Modified Eagle Medium (DMEM) was from Thermo Fisher Scientific (Waltham, MA, USA) and low-endotoxin fetal calf serum (FCS) from Bio&Sell (Nürnberg, Germany). Dimethyl sulfoxide (DMSO) was from Roth (Karlsruhe, Germany). D_2O, DMSO-d_6 and $CDCl_3$ (deuterated solvents) were from Euriso-top (Saint-Aubin, France).

Ascaridole (Asc) was synthesized according to a previously published procedure [18].

Yeast extract powder was supplied by Amresco (Solon, Ohio, USA). Deferoxamine (DFO) was obtained from Novartis (Basel, Switzerland). Yeast extract medium (YEM) consisted of 20.7 g/L yeast extract powder, 1.2 g/L K_2HPO_4, 0.2 g/L KH_2PO_4, 2.9 g/L glucose, pH 7.4. Phosphate buffered saline (PBS) contained 136 mM NaCl, 1.15 mM KH_2PO_4, 14 mM Na_2HPO_4, 2.7 mM KCl, pH 7.4.

2.2. Leishmania culture

Leishmania tarentolae promastigotes (LtP) strain P10 from Jena Bioscience (Germany) was used according to manufacturer instructions up to passage 50. Parasites were cultivated at 26 °C in brain heart infusion (BHI) medium (37 g/L) supplemented with 5 mg/L hemin and 50.000 U/L penicillin – 50 mg/L streptomycin in 50 mL Saarstedt tubes with gas-permeable caps and agitation in a tube shaker ($0.05\ s^{-1}$). LtP were always used one day after passage in the logarithmic growth phase.

Download English Version:

https://daneshyari.com/en/article/5552190

Download Persian Version:

https://daneshyari.com/article/5552190

Daneshyari.com