BASIC SCIENCE
Nanomedicine: Nanotechnology, Biology, and Medicine
11 (2015) 1163 – 1167

nanomedjournal.com

Short Communication

Effects of singlet oxygen generated by a broad-spectrum viral fusion inhibitor on membrane nanoarchitecture

Axel Hollmann, PhD[a], Sónia Gonçalves, PhD[a], Marcelo T. Augusto, MSc[a], Miguel A.R.B. Castanho, PhD[a], Benhur Lee, PhD[b], Nuno C. Santos, PhD[a,*]

[a]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal
[b]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Received 5 November 2014; accepted 19 February 2015

Abstract

Targeting membranes of enveloped viruses represents an exciting new paradigm to explore on the development of broad-spectrum antivirals. Recently, broad-spectrum small-molecule antiviral drugs were described, preventing enveloped virus entry at an intermediate step, after virus binding but before virus–cell fusion. Those compounds, including an oxazolidine-2,4-dithione named JL103 that presented the most promissing results, act deleteriously on the virus envelope but not at the cell membrane level. In this work, by using atomic force microscopy (AFM), we aimed at unraveling the effects that JL103 is able to induce in the lipid membrane architecture at the nanoscale. Our results indicate that singlet oxygen produced by JL103 decreases membrane thickness, with an expansion of the area per phospholipid, by attacking the double bonds of unsaturated phospholipids. This membrane reorganization prevents the fusion between enveloped virus and target cell membranes, resulting in viral entry inhibition.

From the Clinical Editor: The recent development of a family of innovative broad-spectrum small-molecule antiviral drugs that block virus cell entry has provided exciting armors against viruses. In this research paper, the authors utilize atomic force microscopy to investigate the mechanism of action of viral blockade. The findings have resulted in new understanding of cell membrane behavior, which may help in further drug design.
© 2015 Elsevier Inc. All rights reserved.

Key words: Broad-spectrum antiviral; Singlet oxygen; AFM; Membrane organization

Recently, a family of innovative broad-spectrum small-molecule antiviral drugs was described.[1–3] These molecules prevent enveloped viruses entry at an intermediate step, between virus binding and virus–cell fusion. These compounds act deleteriously on the virus envelope, but not at the cell membrane level. Later, we showed that LJ001[1,3] and its oxazolidine counterpart (JL103),[2,4] all belonging to this new class, act as membrane-targeted photosensitizers. They generate singlet oxygen ($^1O_2$) in the membrane, changing its biophysical properties and leading to the inhibition of the viral–cell membrane fusion necessary for target cell infection.[4,5] $^1O_2$-mediated lipid oxidation targets the C═C double bonds of unsaturated phospholipid acyl chains, introducing polar hydroperoxide groups in the hydrophobic region of the lipid bilayer.[6] The presence of these oxidized phospholipids results in several membrane properties changes, which negatively impact on its ability to undergo the extreme membrane curvature transitions necessary for virus–cell fusion. These compounds only affect the viral membrane, showing no significant cytotoxicity and not affecting cell–cell fusion.[1,2,4] This is related to the differences between the biogenic properties of cell plasma membranes and those of static viral membranes. Metabolically active host cells have intrinsic mechanisms to repair the lipid damage induced by reactive oxygen species, while viruses lack this ability to revert membrane alterations.

Methods

POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) and DPPC (1,2-palmitoyl-*sn*-glycero-3-phosphocholine) were

Sources of support for research: Fundação para a Ciência e Tecnologia, Ministério da Educação e Ciência (FCT-MEC, Portugal) project VIH/SAU/0047/2011, and fellowships SFRH/BPD/72037/2010 and SFRH/BD/95624/2013 to A.H. and M.T.A., respectively, as well as NIH grants U01 AI070495 and U01 AI082100. JL103 and LJ025 were generously provided by Michael E. Jung and Jihye Lee (University of California, Los Angeles). The authors have declared that no competing interests exist.

* Corresponding author. Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal.
E-mail address: nsantos@fm.ul.pt (N.C. Santos).

http://dx.doi.org/10.1016/j.nano.2015.02.014
1549-9634/© 2015 Elsevier Inc. All rights reserved.

Please cite this article as: Hollmann A, et al, Effects of singlet oxygen generated by a broad-spectrum viral fusion inhibitor on membrane nanoarchitecture. *Nanomedicine: NBM* 2015;11:1163-1167, http://dx.doi.org/10.1016/j.nano.2015.02.014

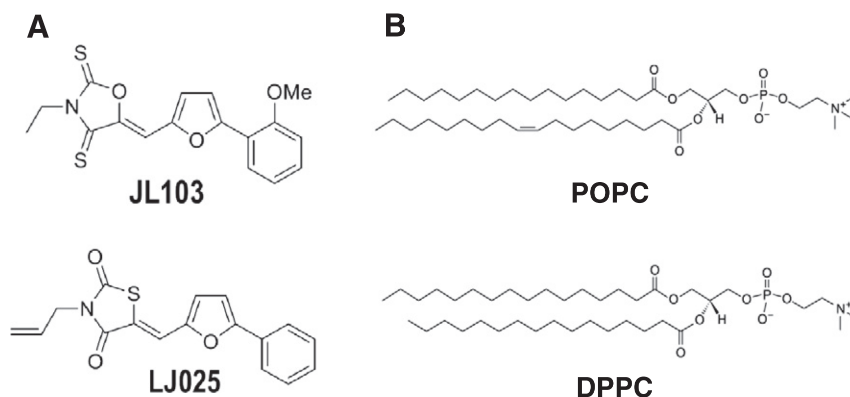

Figure 1. **(A)** Molecular structures of JL103 and its inactive analogue LJ025.[1,2] **(B)** Molecular structures of the phospholipids POPC and DPPC.

from Avanti Polar Lipids (Alabaster, AL, USA). The working buffer used throughout the studies was HEPES 10 mM, pH 7.4, in NaCl 150 mM. JL103 and LJ025 (10 mM) stock solutions were prepared in DMSO. Large and small unilamellar vesicles (LUVs and SUVs, respectively) were prepared by extrusion methods, as described elsewhere.[7,8] Planar supported lipid bilayers (SLBs) were prepared by SUVs deposition, as previously described.[9] SLBs were then allowed to equilibrate for at least 1 h before measurement.

Atomic force microscopy (AFM) measurements were performed on a JPK Instruments Nanowizard II (Berlin, Germany) mounted on a Zeiss Axiovert 200 inverted microscope (Jena, Germany). Imaging was performed in 10 mM HEPES buffer pH 7.4, in contact mode, using uncoated silicon cantilevers CSC38 from MikroMasch (Tallinn, Estonia), with a tip radius of 10 nm, resonant frequency of approximately 14 kHz and spring constant of 0.05 N/m. Images with a scan size of $5 \times 5\ \mu m^2$ and resolution 512×512 pixels^2 were obtained with scan rates between 0.6 and 1.0 Hz and set points close to 0.2 V. At least three independent images from three different preparations were obtained for each experimental condition. Height and error signal images were collected and analyzed. After 100 min of imaging, SLBs remained unaltered, without domain fusion or membrane degradation after successive scannings.

Results and discussion

In previous studies, the antiviral compound oxazolidine-2,4-dithione (JL103) revealed to be the most active against several enveloped viruses, in good agreement with its most efficient production of $^1O_2$.[2,5] Here, for the first time, we evaluated the effects of JL103 on SLBs, using AFM to assess the morphological changes induced in membrane structure and organization. For the sake of comparison, LJ025, an inactive analogue of JL103[1,2] was also included in this study (Figure 1, *A*).

AFM was used to evaluate the effects of JL103 after 20 min incubation at 100 μM final concentration on SLBs of POPC:DPPC 1:1 molar ratio. This lipid mixture was selected because it forms a stable and well defined liquid disordered-gel (l_d–s_o) membrane phase coexistence.[10,11] In this context, a binary mixture of phospholipid with a double bond at C9 in the oleoyl chain (POPC) and a completely saturated phospholipid (DPPC) (Figure 1, *B*) is a simple model to evaluate the effect of the $^1O_2$ produced by JL103 on membrane nanoarchitecture. As we previously demonstrated, photosensitization of viral membranes requires the presence of unsaturated acyl chains[2,4,5,12] (as the case of POPC), in contrast with DPPC, a saturated lipid, where there are no carbon-carbon double bonds available for $^1O_2$ attack. Moreover, JL103 has a low affinity for DPPC bilayers, as shown by fluorescence spectroscopy assays (*vd.*, Supporting Information). Phosphatidylcholines (such as POPC and DPPC) are the most common phospholipids in several biological membranes, being POPC frequently the most common phosphatidylcholine.[13,14] POPC–DPPC membranes are extensively used in the literature as the simplest model to represent the coexistence of two distinctive lipid phases, sensitive to domain alterations in the presence of interacting molecules or upon the change of any other experimental parameter. Furthermore, the presence of a carbon–carbon double bond in one of the POPC fatty acyl chains and its absence in DPPC make this the most suitable lipid mixture to study the oxidation effect in the presence of JL103.

By comparing the AFM topography images obtained prior and after incubation with JL103 (Figure 2 and Figure S2), we observed changes in the edge region of POPC:DPPC l_d–s_o phase limits, as well as the coalescence of smaller DPPC domains (Figure 2). These changes were not observed on control experiments using the negative control LJ025, despite of its insertion in membranes[1,2,5], or using buffer with an amount of DMSO equivalent to that added to the bilayers in the experiments with JL103 and LJ025, as solvent from their stock solutions (Figure 2).

In order to get an insight on JL103 effects at the level of the bilayer nanoarchitecture, we measured the fraction of the total area corresponding to each type of domain (l_d and s_o). In the control images, there was *ca.* 50% of each one, as expected, as well as for LJ025. Upon incubation with JL103, there was an 8.7% ± 1.5 % increase on POPC (l_d) area (Figure 3, *A*). In order to demonstrate that the changes on lipid bilayer architecture are induced by the $^1O_2$ produced by JL103, instead of by the simple insertion of this compound in the membrane, we observed that the increase of POPC area is reverted by the addition of 15 mM

Download English Version:

https://daneshyari.com/en/article/10435984

Download Persian Version:

https://daneshyari.com/article/10435984

Daneshyari.com