Amiodarone affects Ebola virus binding and entry into target cells

Cristiano Salata¹, Denis Munegato¹, Francesco Martelli¹, Cristina Parolin¹, Arianna Calistrì¹, Aldo Baritussio², Giorgio Palu¹

¹Department of Molecular Medicine, University of Padua, Italy; ²Clinica Medica I, Department of Medicine, University of Padua, Italy

SUMMARY

Ebola Virus Disease is one of the most lethal transmissible infections characterized by a high fatality rate. Several research studies have aimed to identify effective antiviral agents. Amiodarone, a drug used for the treatment of arrhythmias, has been shown to inhibit filovirus infection in vitro by acting at the early step of the viral replication cycle. Here we demonstrate that amiodarone reduces virus binding to target cells and slows down the progression of the viral particles along the endocytic pathway. Overall our data support the notion that amiodarone interferes with Ebola virus infection by affecting cellular pathways/targets involved in the viral entry process.

Received July 5, 2017
Accepted December 18, 2017

©2018 by EDIMES - Edizioni Internazionali Srl. All rights reserved
Amiodarone inhibits EBOV entry

and 0.08±0.09 vs 0.15±0.11, respectively). By contrast, after 2 hours the colocalization with LAMP1 was higher in amiodarone-treated than in control cells (0.19±0.11) (Figure 2). By analyzing the colocalization of VLPs with NPC-1, we found that:

1) VLPs colocalized with NPC-1 to a greater extent than with the other endocytic markers;
2) the colocalization increased over time;
3) there was no statistically significant difference between control and amiodarone-treated cells (Figure 2), indicating that amiodarone does not prevent the arrival of VLPs in a NPC-1 rich compartment.

Thus, amiodarone appears to interfere with the progression of EBOVLPs along the endocytic pathway at discrete steps, by decreasing binding to target cells and by slowing the acquisition of late endocytic markers to the vacuole containing the virus. Since amiodarone stimulates autophagy (Morissette et al., 2009; Piccoli et al., 2011; Salata et al., 2016), we also asked whether amiodarone could favor the autophagy of internalized virions trapped in late endosomes. To this end, after 5 hours of incubation in the presence of amiodarone, colocalization of VLPs with LC3B, a marker of autophagosomes, was investigated (Piccoli et al., 2011). As shown in Figure 2, in amiodarone-treat-
ed cells we found that the colocalization between VLPs and LC3B was modest and not statistically different from the control. Similar data were obtained after 2 hours of incubation (data not shown). Thus, increased autophagy does not appear to play a role in the antiviral activity of amiodarone.

We previously showed that amiodarone blocks EBOV entry into target cells at the level of the LE, by inhibiting the fusion of the viral envelope with the LEs membrane (Salata et al., 2015). Here, we show that amiodarone reduces the efficiency of viral binding to the cell surface and slows the progress along the endocytic pathway supporting the view that amiodarone, and likely other CADs with anti-EBOV activity, may act at multiple steps during virus entry.

**Competing interests**
The authors declare that there is no conflict of interest regarding the publication of this article.

**Funding**
This work was supported by University of Padova grants (ex 60% to CS, AC, CP, AB and Progetti di Ateneo 2008 to AB), and Regione Veneto grants to GP.

**Acknowledgements**
The authors are grateful to Vincenzo Ciminale, Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua, Italy, for the colocalization analysis of EBOVLPs, to Gary Nabel, Vaccine Research Center, NIH, Bethesda, USA, for providing the ZEBOV GP plasmid, to Christopher Basler, Mount Sinai School of Medicine, New York, USA, for donating the VP40-GFP plasmid, and to Michael Whitt, University of Tennessee, Memphis, USA, for providing the recombinant Vesicular Stomatitis Virus.

**References**