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Erdosteine inhibits viral infection and modulates innate immunity and stress oxidative state in RSV-infected A549 cells

Sergio Strizzi[1], Fiammetta Danzo[2], Claudia Vanetti[1], Mara Biasin[1], Dejan Radovanovic[2], , Pierachille Santus[2], Daria Trabattoni[1]

[1]Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Via G.B. Grassi, Milan, Italy

[2]Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Division of Respiratory Diseses, University Hospital "L. Sacco", Milan, Italy

Introduction/Summary

- The decrease in the antioxidant glutathione (GSH) content characterizes several viral infections, suggesting that maintenance or restoration of GSH levels may be a novel potential therapeutic approach for these diseases[1].
- Unfortunately, a limit to GSH use as a therapeutic agent is given by its biochemical and pharmacokinetic properties. Thus, other molecules have been proposed to restore or increase GSH levels[2].
- Among these, erdosteine active metabolite MET-1 seem to have a rationale in the treatment of patients affected by respiratory viruses as Respiratory Syncytial Virus (RSV).

Aims

The main objectives of this study were:

- To evaluate effects of erdosteine active metabolite (MET-1) in RSV infection in order to detect a potential anti-viral effect
- To evaluate whether erdosteine active metabolite can modulate the inflammatory state of human cells playing a role in the innate immunity pathways

Methods

- Viral infection assay on A549 cell line has been set up adding Erdosteine active metabolite MET-1 at different doses (100µg/mL and 1000µg/mL) before and after infection with human Respiratory Syncytial Virus (hRSV - A2001/3-12) at concentration of 1.26 TCID50/µL.
- Viral replication in culture supernatant was assessed by qPCR method at 48h and 72h post-infection (hpi) by means of two specific primers targeting RSV sequences (N, NS1). To quantify viral copies in supernatant, the last obtained timepoint was processed trough Digital Droplet PCR.
- At 72hpi innate immune response signaling and oxidative stress pathway were analyzed using RT-PCR custom-array with a set of 60 optimized primers.

Results

- Post-treatment with erdosteine active metabolite MET-1 1000µg/mL resulted in a statistically significant antiviral effect (p= 0.002) against hRSV
- Infected cells treated with erdosteine active metabolite MET-1 1000µg/mL displayed an overexpression of innate immunity (IFITM1, IFITM3) and oxidative stress (CAT, GPX2) components. Interestingly, a decrease in expression level of CASP1, CASP4, CCL2, CXCL2, DDX58, HLA-A, ICAM1, IFNA1, IFNAR1, IL1B, IL6, IL8, ISG20, NFKB1, AHR, GPX3, and SOD2 was observed.

Figure 1

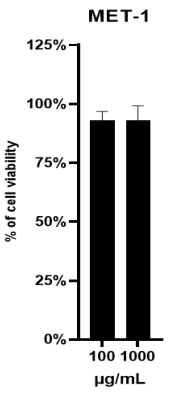

Figure 1: MTT assay

MTT viability assay performed on cells cultured in the presence of 100/1000 µg/mL concentrations of MET-1 over 72-hour period. 100% viability value has been set based on untreated cells as control (data not shown).

Figure 2

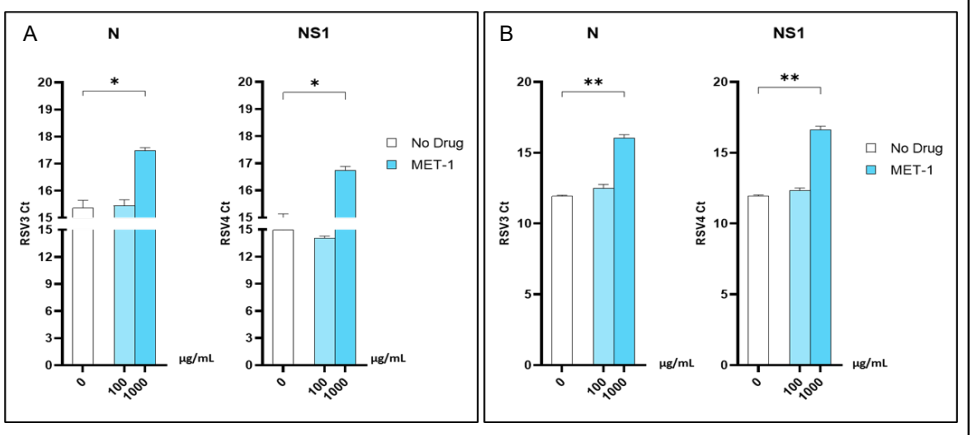

Figure 2: qPCR - MET-1 antiviral effects on hRSV infected A549 cells

hRSV infection on A549 cells has been assessed by means of two different viral targets (N, NS1) in qPCR at 48hpi (A) and 72hpi (B). RSV-infected cells treated with erdosteine active metabolite MET-1 at a concentration of 1000µg/mL (azure bar) show an enhance of Ct value which is statistically significant [A: p = 0,02; B: p = 0,002].

Figure 3

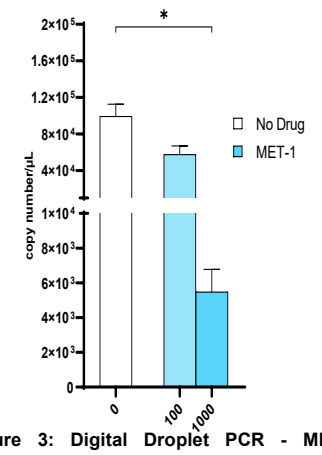

Figure 3: Digital Droplet PCR - MET-1 antiviral effects on hRSV infected A549 cells

Viral copies produced by A549 cells infected with hRSV have been assessed by DD-PCR at 72hpi. RSV-infected cells treated with 1000µg/mL MET-1 (azure bar) show a statistically significant decrease in viral copies number [p = 0.04].

Figure 4

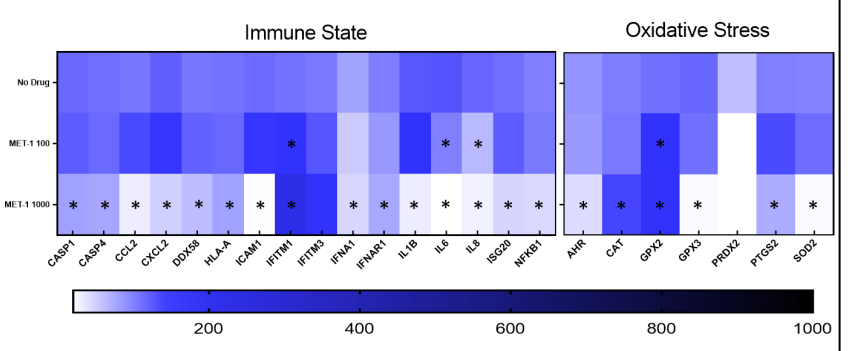

Figure 4: Transcriptome analysis display modulation of the immune state and oxidative stress by erdosteine active metabolite MET-1 in hRSV infected cells.

Transcriptome analysis of a set of 60 optimized RT-PCR primers were employed to study innate immune response signaling pathways and oxidative stress using RT-PCR custom-array. GAPDH and ACTB housekeeping genes were used for normalization of dataset for each condition. hRSV-infected cells treated with 1000 µg/mL of erdosteine active metabolite MET-1 showed an overexpression of innate immunity (IFITM1, IFITM3) and oxidative stress (CAT, GPX2) components. Interestingly, a decrease in expression level of CASP1, CASP4, CCL2, CXCL2, DDX58, HLA-A, ICAM1, IFNA1, IFNAR1, IL1B, IL6, IL8, ISG20, NFKB1, AHR, GPX3, and SOD2 was observed.

Conclusions

- Erdosteine modulates several molecular pathways involved in induction of anti-viral state. This is associated with upregulation of some ISGs and reduction of pro-inflammatory cytokines.
- **Erdosteine can be effectively used as adjunctive antioxidant treatment to improve the outcomes of antiviral therapies**

References

1. Santus P, Danzo F, Zuffi A, Pini S, Saad M, Visconti A, Radovanovic D. Oxidative stress and viral Infections: rationale, experiences, and perspectives on N-acetylcysteine. Eur Rev Med Pharmacol Sci. 2022 Nov;26(22):8582-8590
2. Mitrea M, Silvestro G, Savu S: Reduced (GSH) and oxidised (GSSG) levels in plasma and BAL of chronic bronchitic patients treated with erdosteine or N-acetylcysteine (NAC). 6th Int Conf Bronchoalveolar Lavage. Corfù, Greece, 24–27 June, 1998.