SULODEXIDE AND EVEROLIMUS: TWO POTENTIAL PHARMACOLOGICAL INHIBITORS OF THE HYPOXIA-INDUCED ALLOGRAFT FIBROSIS

Gianluigi Zaza[1], Valentina Masola[1], Maria Francesca Secchi[2], Simona Granata[1], Luigino Boschiero[4], Giovanni Gambaro[3], Antonio Lupo[1].

[1]Renal Unit, Department of Medicine, University-Hospital of Verona, Italy; [2]Department of Biomedical sciences, University of Padova, Italy; [3] Division of Nephrology and Dialysis, Columbus-Gemelli Hospital, Catholic University, Rome, Italy

Background

Progressive interstitial fibrosis contributes to the progressive graft loss after kidney transplantation.

The biological machinery involved in this process are multiple and most of the time starting at early post-transplant phase. As largely described, epithelial to mesenchymal transition (EMT) of tubular cells seems to have an important role in the onset/development of the pro-fibrotic machinery.

EMT is a process by which differentiated epithelial cells, after specific stimuli (including hypoxia), acquire the expression of mesenchymal markers (e.g., a-SMA, vimentin and fibronectin).

Additionally, as recently published by our group, EMT can be blocked by inhibiting heparanase (HPSE) with low molecular weight heparins (LMWH) such as Sulodexide.

Moreover, EMT can be regulated by several pharmacologic agents. In particular, low-therapeutic doses of everolimus (EVR), an mTOR-inhibitor, recently introduced in renal transplant medicine, may have anti-fibrotic properties.

Aim of the study

Therefore, the aim of our study has been to investigate whether a pharmacological therapy using LMWH alone (Sulodexide) or in combination with EVR was able to regulate EMT in renal tubular cell lines undergoing hypoxia.

Methods

Several biomolecular strategies (including RT-PCR, immunofluorescence, western-blotting) have been used to assess the capability of Sulodexide (50 µg/ml) and/or EVR (10 and 100 nM) to regulate the expression of several EMT markers [(alpha-smooth muscle actin (alpha-SMA), Vimentin (VIM), Fibronectin (FN)] in human renal epithelial proximal tubular cells (HK-2) in hypoxic condition (24 hours).

Results

Biomolecular experiments demonstrated that 24 hours hypoxia were able to induce a significant up-regulation of alpha-SMA, VIM and FN at both gene-expression and protein levels (Fig. 1-4).

However, the addition of both Sulodexide and EVR alone to the culture medium was able to significantly reduce the expression of the above EMT markers (Fig. 1-4).

Interestingly, the anti-EMT effect was higher when cells were co-treated with the two drugs.

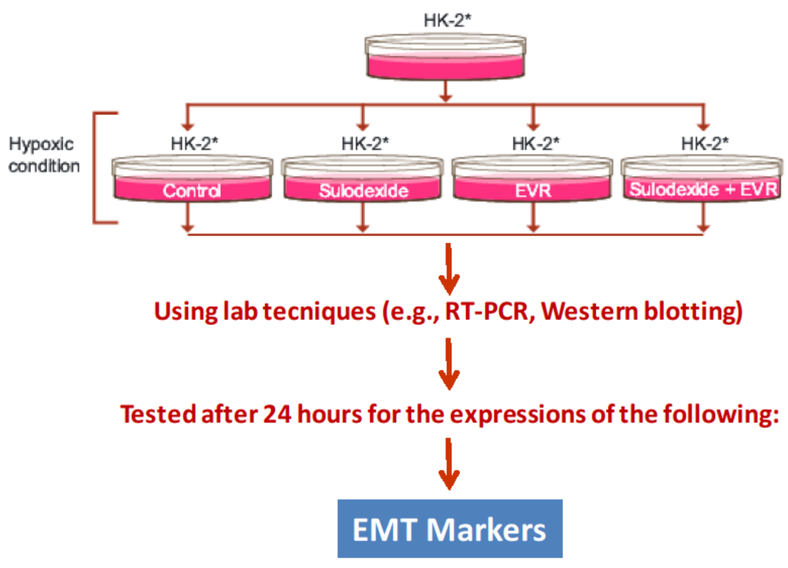


Fig.1

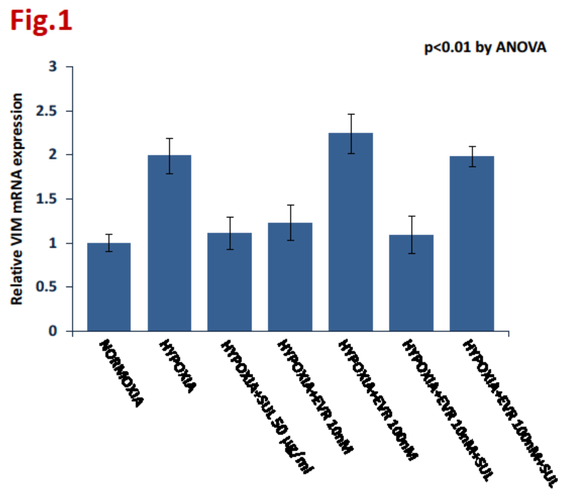


Fig.2

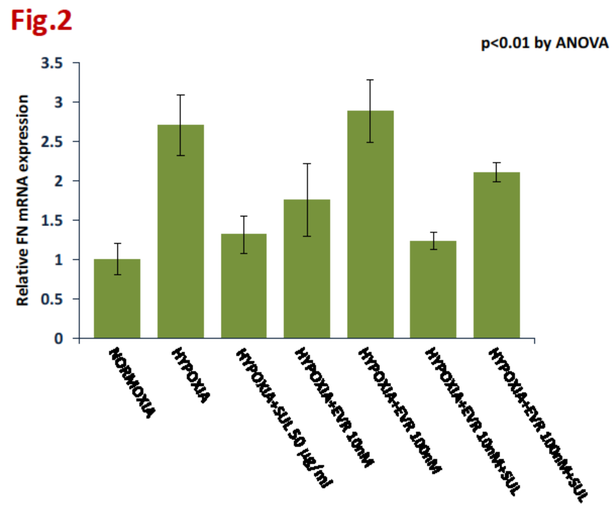


Fig.3

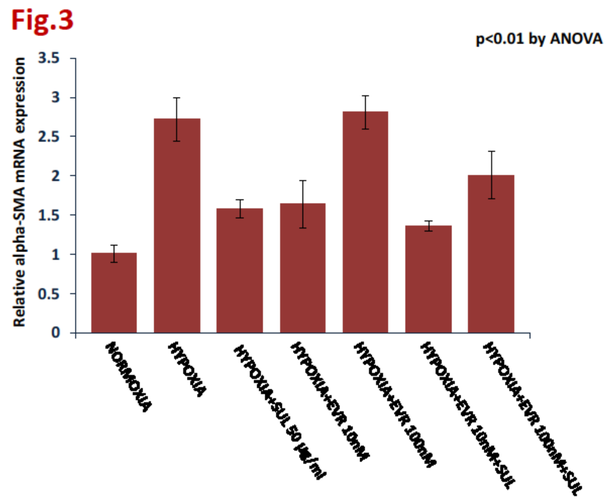
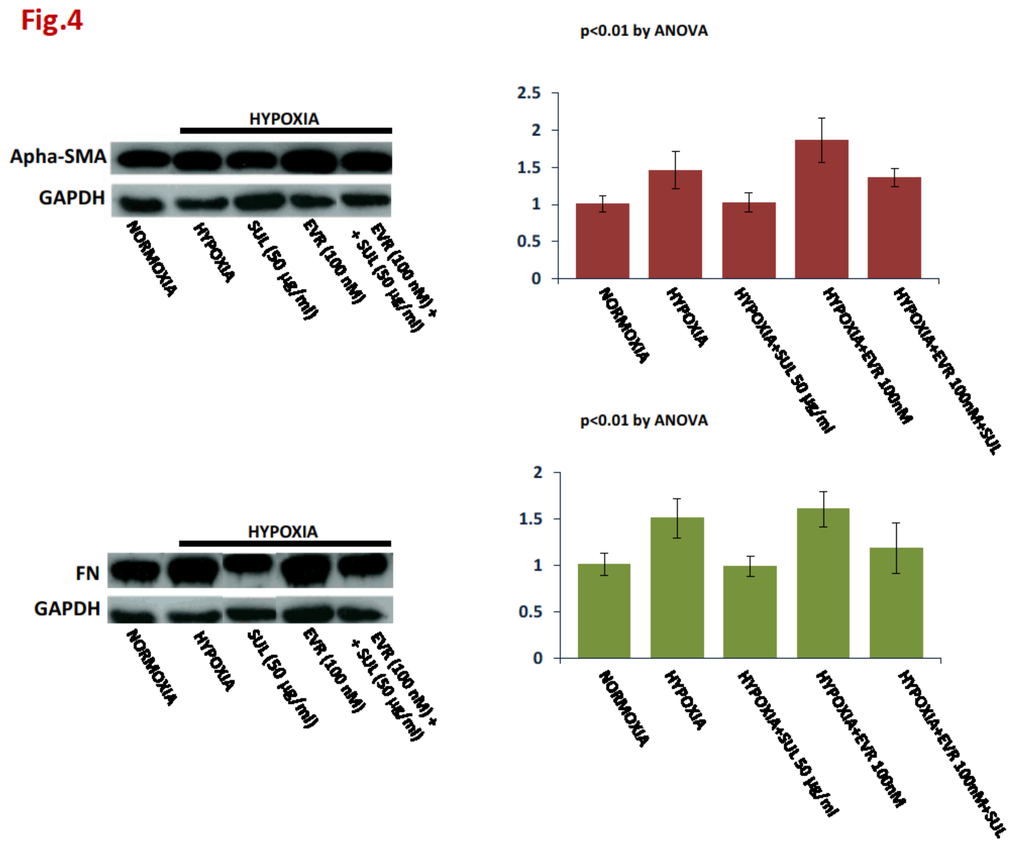


Fig.4



Conclusions

Therefore, our data all together suggest that Sulodexide and EVR alone or in combination may inhibit the hypoxia-induced EMT of renal tubular cells.

Moreover, the co-administration of these drugs may help to prevent fibrotic-related kidney damage occurring in several post-transplant hypoxic condition (e.g., delyed graft function).

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Gianluigi Zaza
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