Modulation of aortic perivascular adipose tissue function by peroxynitrite

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Introduction: Peroxynitrite ($ONOO^-$) is formed by the rapid reaction of nitric oxide and superoxide and is an endothelium-independent vasodilator (1). Both superoxide and nitric oxide are produced by the perivascular adipose tissue (PVAT) surrounding blood vessels and the resulting $ONOO^-$ formed could modulate the function of the PVAT. In a previous study we demonstrated that treatment of isolated PVAT with $ONOO^-$ enhanced the ability of the PVAT to augment vessel relaxation (2). In this study we aimed to further investigate the effect of exogenous $ONOO^-$ on PVAT function and release profile.

Methods: Thoracic aortae from male C57BL/6 mice (20-25g) were removed and placed in ice-cold Krebs' solution. All experiments were in accordance with the Animals (Scientific Procedures) Act 1986 / ASPA Amendment Regulations 2012. The PVAT was removed carefully and treated with either vehicle or $ONOO^-$ (10^{-4}M for 1 hour at 37^{o}C in Krebs' solution). Following this, PVAT was incubated with dihydroethidium (DHE; 10^{-5}M for 30 minutes at 37^{o}C in the dark) to measure the effect of $ONOO^-$ on superoxide formation. In some experiments PVAT was homogenized in liquid nitrogen, protein concentration determined and adiponectin content measured using an ELISA kit (Quantikine® ELISA mouse adiponectin kit; R&D Systems). Respirometry (OROBROS Oxygraph-2k) was used to measure the effect of $ONOO^-$ on mitochondrial function in the PVAT.

Results: Analysis of the confocal projections of PVAT stained with DHE showed that $ONOO^-$ treatment increased superoxide formation ($p<0.01$, n=6). Incubation with superoxide dismutase (SOD) significantly reduced superoxide formation in PVAT ($p<0.01$, n=6) (Fig.1). In addition, $ONOO^-$-treated PVAT had higher adiponectin content compared to control (72.4 ± 5.5 ng/mg protein vs. 39.7 ± 7.6 ng/mg protein, $p<0.01$, n=11).

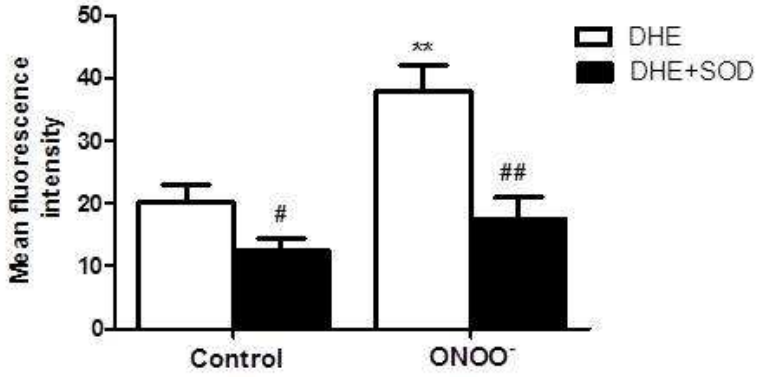

Fig.1 Effect of $ONOO^-$ on superoxide formation in PVAT (# p<0.05 vs Control DHE, ## p<0.01 vs $ONOO^-$ DHE, ** p<0.01 vs Control DHE, n=6)

Spirometry measurements revealed that $ONOO^-$ increased mitochondrial respiration which may be linked to the increased superoxide formation and adiponectin content.

Conclusion: The ability of PVAT to augment relaxation is increased following treatment with $ONOO^-$

and may be related to increased adiponectin content caused by increased mitochondrial activity.

References:

1. Ewart MA *et al*. (2014). *Atherosclerosis* **234(1)**: 154-161.

2. Ugusman A *et al.* (2016). In: EPHAR 2016 Congress Book: Turkey, p295.