Gp91ds-tat, a selective Nox2 peptide inhibitor, protects in vitro blood-brain barrier from ischaemic injury

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Background: Oxidative stress, associated with excessive availability of reactive oxygen species (ROS), plays a major role in blood-brain barrier (BBB) damage during ischaemic stroke. Nox2 constitutes the main source of vascular oxidative stress. This study investigates whether selective inhibition of Nox2 by gp91ds-tat can prevent ischaemic injury-induced BBB damage.

Methods: An in vitro model of human BBB was established by co-culture of human brain microvascular endothelial cells (HBMEC), astrocytes (HA) and pericytes (HP) before their exposure to 4 h of oxygen-glucose deprivation (OGD), OGD followed by 20 h of reperfusion (OGD+R), or to OGD±R with/out gp91ds-tat (50 μM). The integrity and function of the BBB were studied by measurements of trans-endothelial electrical resistance (TEER) and paracellular flux of low (sodium fluorescein; NaF, 376Da) and high (Evan's blue-labelled albumin; EBA, 67kDa) molecular weight permeability markers, respectively. NADPH oxidase activity and superoxide anion levels were assessed by (modified) lucigenin chemiluminescence assay. The cytoskeletal organisation of all three cell lines was examined by actin filament staining.

Results:

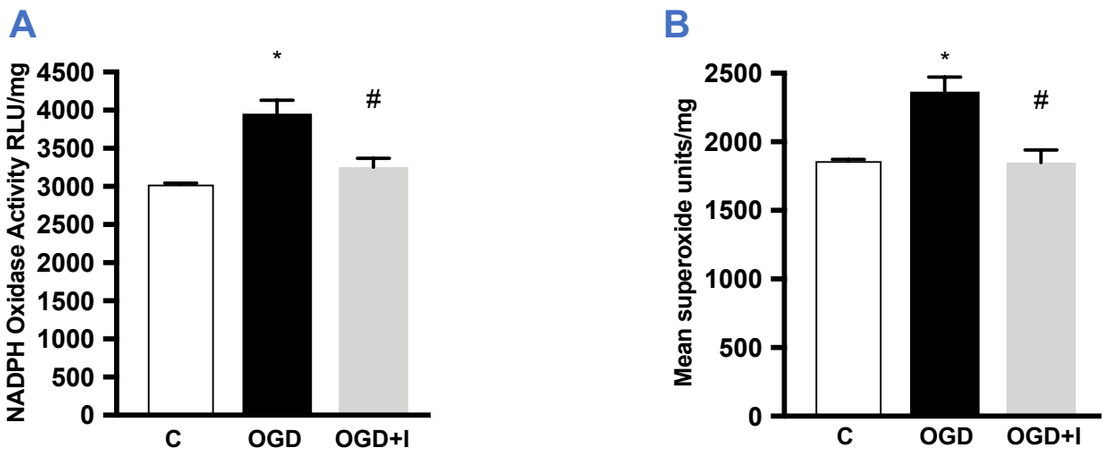

Figure.1 shows Nox2 activity (A) and superoxide anion levels (B) in human brain microvascular endothelial cells (HBMEC). *p<0.05 compared to control, #P<0.05 compared to OGD. Data expressed as a mean ± SEM from n≥3.
C= control , I = inhibitor, gp91ds-tat.

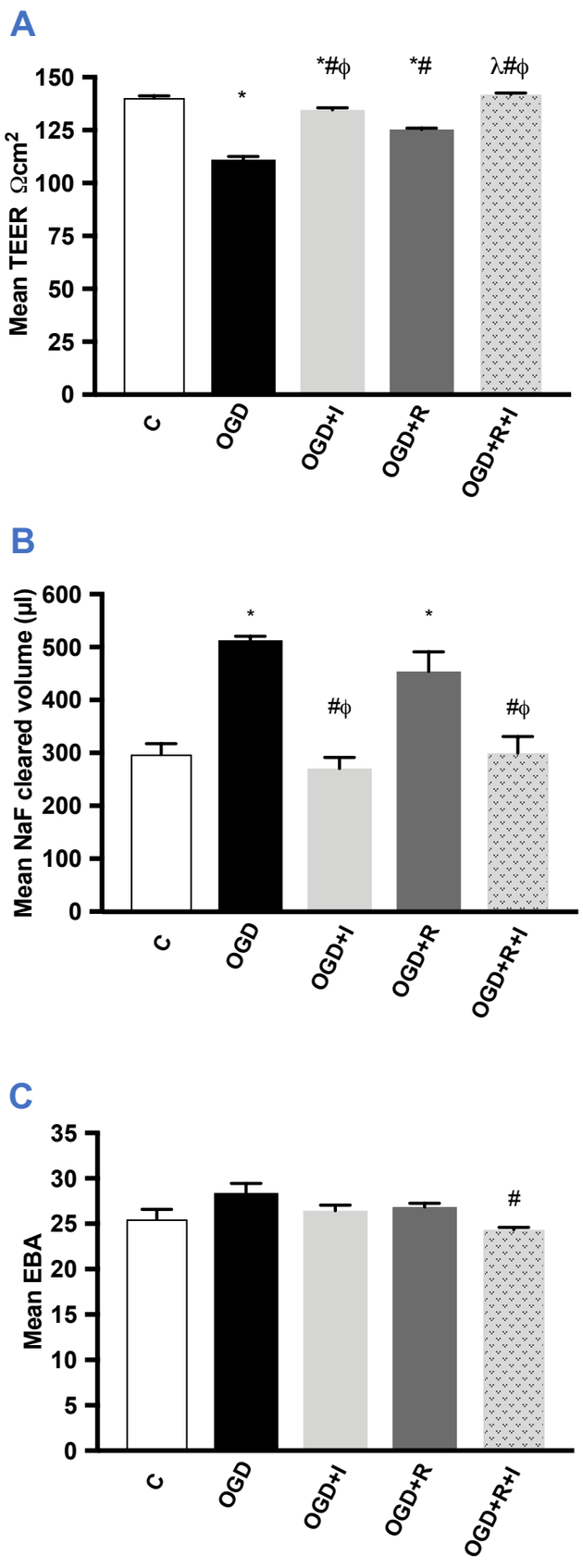

Figure.2 shows trans-endothelial electrical resistance (TEER) (A), and NaF (B) and EBA (C) flux across an in vitro models of human BBB.
*P<0.05 compared to control.
#P<0.05 compared to OGD.
ϕP<0.05 vs OGD+R.
λP<0.05 compared to OGD+I.
Data expressed as a mean ± SEM from n≥3.
C= control, I= inhibitor, gp91ds-tat.

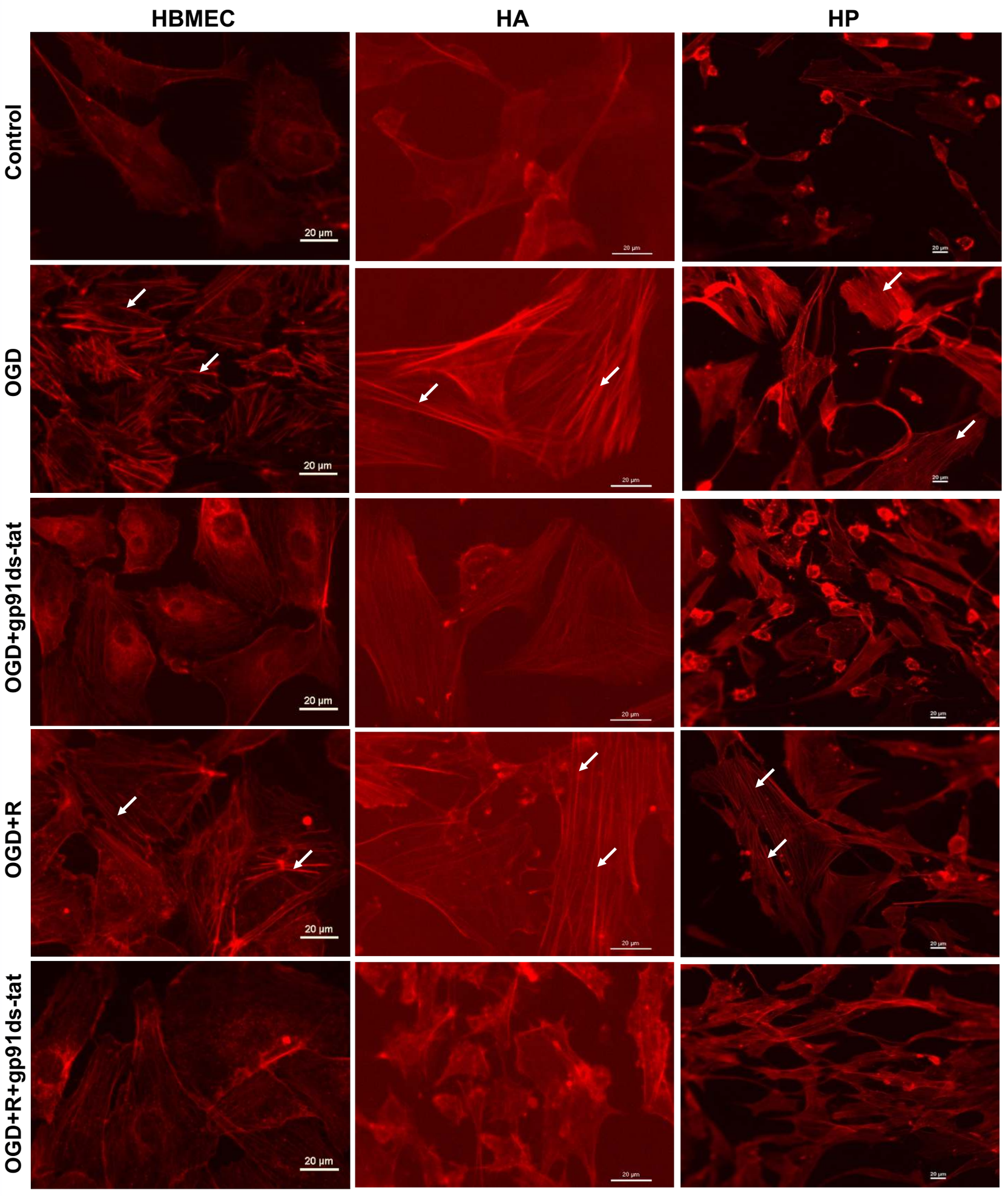

Figure.3 F-actin staining OGD±R induced stress fibres formation in HBMEC,HA, and HP (indicated by white arrows). Inhibition of Nox2 by gp91ds-tat significantly reduced actin filament formation. Scale bars = 20μm

Conclusion: Selective inhibition of Nox2 appears to protect human BBB integrity and function during or after an ischaemic injury by attenuating oxidative stress and maintaining cellular architecture.