The persistent mitochondrial oxidative stress in cerebral arteries due to chronic arterial hypertension can be reversed by SS-31 in mild traumatic brain injury rat model


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Introduction

• Traumatic brain injury (TBI) induces cerebrovascular oxidative stress, which is associated with neurovascular uncoupling, autoregulatory dysfunction and persisting cognitive decline in both preclinical models and patients. However, single mild TBI (mTBI), the most frequent form of brain trauma increases cerebral generation of reactive oxygen species (ROS) only transiently.

• The present study was designed to test the hypothesis that pre-existing arterial hypertension exacerbates TBI-induced cerebrovascular oxidative stress, by up-regulating TBI-induced mitochondrial ROS (mtROS) production in vascular cells.
Method(s)

• mTBI induction in normotensive and spontaneously hypertensive rats (SHR)
• Cytoplasmic and mitochondrial superoxide (O2-) production was assessed by confocal microscopy in isolated middle cerebral arteries (MCA) two weeks after mTBI using dihydroethidine (DHE) and the mitochondria-targeted redox sensitive fluorescent indicator dye MitoSox
• The protective effects of in vivo treatment with the potent mitochondrial-targeted antioxidative Szeto-Schiller (SS) peptide SS–31 was assessed
• Data were analyzed by analysis of variance (ANOVA). A p value less than 0.05 was considered statistically significant. Data are expressed as mean±S.E.M.
Results

- We found that mTBI induced a significant increase in long term cytoplasmic and mitochondrial $O_2^-$ production in MCAs of SHRs, which was reversed to the normal level by treating the animals with the cell-permeable, mitochondria-targeted antioxidant peptide SS-31.
Figure 1. Mild TBI induces long term oxidative stress in cerebral arteries of hypertensive rats, which is reversed by treatment with SS-31. A: Bar graphs depict summary data of dihydroethidine (DHE) fluorescence indicating production of reactive oxygen species (ROS) in the smooth muscle layer of middle cerebral arteries (MCA) isolated from wistar rats, wistar rats two weeks after mild traumatic brain injury (mTBI), spontaneously hypertensive rats (SHR), and SHRs after two weeks of mild traumatic brain injury treated with vehicle or the mitochondria-targeted antioxidant peptide SS-31. The treatment started after TBI was applied and last for two weeks (5.7 mg Kg-1day-1 i.p.). Data are means±S.E.M. (n=5 in each group) *P<0.05 vs. Wistar, #P<0.05 vs. Wistar+mTBI &P<0.05 vs. SHR, $P<0.05 vs. SHR+mTBI. Lower panel shows representative confocal images of red DHE fluorescence in the smooth muscle layer (identified in the outer layer of arteries by the spindle-shaped nuclei that are perpendicular to the long axis of the artery) of MCAs in each group. Panel B shows summary data of endothelial DHE fluorescence in MCAs from each group after the same treatment protocols. Data are means±S.E.M. (n=5 in each group) *P<0.05 vs. Wistar, #P<0.05 vs. Wistar+mTBI &P<0.05 vs. SHR, $P<0.05 vs. SHR+mTBI. Lower panel demonstrates confocal images of red DHE fluorescence indicating ROS production in the endothelium (identified as the inner layer of arteries by elongated nuclei that are mostly parallel to the long axis of the artery) of MCAs in each group. Scale bar is 50 µm.
Figure 2. Mild TBI induces long term mitochondrial oxidative stress in cerebral arteries of hypertensive rats, which is reversed by SS-31. A: Bar graphs show summary data of MitoSox staining (representing mitochondrial production of reactive oxygen species (ROS)) in the smooth muscle layer of middle cerebral arteries (MCA) isolated from wistar rats, wistar rats two weeks after mild traumatic brain injury (mTBI), spontaneously hypertensive rats (SHR), and SHRs after two weeks of mild traumatic brain injury treated with vehicle or the mitochondria-targeted antioxidant peptide SS-31. The treatment started after TBI was applied and last for two weeks (5.7 mg Kg$^{-1}$day$^{-1}$ i.p.). Data are means±S.E.M. (n=5 in each group) *$P<0.05$ vs. Wistar, &$P<0.05$ vs. SHR, $P<0.05$ vs. SHR+mTBI. Lower panel shows representative confocal images of red MitoSox fluorescence in the smooth muscle layer (identified in the outer layer of arteries by the spindle-shaped nuclei that are perpendicular to the long axis of the artery) of MCAs in each group. Panel B shows summary data of endothelial MitoSox fluorescence in MCAs from each group after the same treatment protocols. Data are means±S.E.M. (n=5 in each group) *$P<0.05$ vs. Wistar, &$P<0.05$ vs. SHR, $P<0.05$ vs. SHR+mTBI. Lower panel demonstrates confocal images of red MitoSox indicating mitochondrial ROS production in the endothelium (identified as the inner layer of arteries by elongated nuclei that are mostly parallel to the long axis of the artery) of MCAs in each group. Scale bar is 50 µm.
Conclusions

We propose, that hypertension- and mTBI-induced cerebrovascular oxidative stress likely lead to dysregulation of CBF and cognitive dysfunction, which might be reversed by SS-31 treatment.