Abstract: 2287

Endothelial SIRT6 exerts a beneficial role in cerebral ischemia/reperfusion injury by preserving blood-brain barrier integrity

Authors:
L Liberale¹, A Akhmedov¹, N Bonetti¹, V Nageswaran¹, S Costantino¹, J Pahla¹, CM Matter¹, F Montecucco², JH Beer¹, F Paneni¹, TF Luescher¹, GG Camici¹, ¹University of Zurich, Center for Molecular Cardiology - Schlieren - Switzerland, ²University of Genoa, Department of Internal Medicine - Genoa - Italy,

Topic(s):
Basic Science - Vascular Diseases

Citation:
Swiss National Science Foundation

Introduction: Stroke is a major cause of mortality and morbidity worldwide. Yet, therapeutic strategies are limited to the early reperfusion which can, on the other hand, worsen the brain damage trough ischemia/reperfusion (I/R) injury. Post-stroke blood-brain barrier (BBB) impairment is associated with worsened outcome. Aging is a major risk factor for stroke and genes regulating lifespan also contribute to the determination of cerebral damage during I/R injury. Purpose: Given the pivotal role of endothelial cells in BBB, we hypothesized that the endothelial-specific expression of the longevity gene SIRT6 may protect the BBB from ischemia/reperfusion damage thus having a beneficial role on stroke outcome. Methods: Endothelial-specific SIRT6 knockout (eSIRT6-/-) mice and control littermates (CTRL) underwent transient middle cerebral artery occlusion (tMCAO) for 45 min followed by 48 hours of reperfusion. Immunohistochemistry (IHC) was used to investigate BBB permeability by IgG extravasation and molecular mechanisms. Primary human brain microvascular endothelial cells (HBMVECs) transfected with either SIRT6 (siSIRT6) or scrambled (siSCR) small interfering RNA were subjected to hypoxia/reoxygenation (H/R). An in vitro BBB model consisting of a monolayer of siRNA-treated HBMVECs was established and barrier function was assessed by 48 h-lasting transendothelial electrical resistance measurement. SIRT6 expression in monocytes from stroke patients was correlated with the short-term neurological outcome \( \text{?NIHSS%} = \frac{(\text{NIHSS discharge}-\text{NIHSS admission})}{\text{NIHSS admission}*100} \). Results: eSIRT6-/- displayed higher infarct volumes and lower survival rate compared to WT mice 48 h after tMCAO. The increased infarct volume was functionally relevant as eSIRT6-/- also showed worse post-stroke neurological impairment. Analysis of brain sections revealed increased BBB damage and increased endothelial expression of cleaved caspase-3 in eSIRT6-/- as compared to control littermates. In vitro, H/R reduced SIRT6 expression in HBMVECs. Mirroring the animal results, SIRT6 silencing impaired the barrier function of HBMVECs 48 h after exposure to H/R. In line with this, SIRT6-silenced HBMVECs showed reduced viability, increased cleaved caspase-3 expression and reduced activation of the anti-apoptotic survival pathway Akt as compared to control cells after H/R. The direct interaction between SIRT6 and Akt was confirmed by co-immunoprecipitation. In ischemic stroke patients, SIRT6 expression was higher in those with short-term neurological improvement \( ?\text{NIHSS%} > 0 \) and negatively correlated with ? NIHSS%. Conclusion: Endothelial SIRT6 exerts a beneficial role in ischemic stroke by blunting I/R-mediated BBB damage. Specifically, SIRT6 reduces endothelial I/R-induced apoptotic death through activation of the protective Akt pathway. The longevity gene SIRT6 may represent a novel therapeutic target for the treatment of ischemic stroke.
Abstract: Endothelial SIRT6 exerts a beneficial role in cerebral ischemia/reperfusion injury by preserving blood-brain barrier integrity.

Authors: L Liberale 1, A Akhmedov 1, N Bonetti 1, V Nageswaran 1, S Costantino 1, J Pahla 1, CM Matter 1, F Montecucco 2, JH Beer 1, F Paneni 1, TF Luescher 1, GG Camici 1

1 University of Zurich, Center for Molecular Cardiology – Schlieren – Switzerland, 2 University of Genoa, Department of Internal Medicine – Genoa – Italy

Introduction: Stroke is a major cause of mortality and morbidity worldwide. Yet, therapeutic strategies are limited to the early reperfusion which can, on the other hand, worsen the brain damage through ischemia/reperfusion (I/R) injury. Post-stroke blood-brain barrier (BBB) impairment is associated with worsened outcome. Aging is a major risk factor for stroke and genes regulating lifespan also contribute to the determination of cerebral damage during I/R injury.

Purpose: Given the pivotal role of endothelial cells in BBB, we hypothesized that the endothelial-specific expression of the longevity gene SIRT6 may protect the BBB from ischemia/reperfusion damage thus having a beneficial role on stroke outcome.

Methods: Endothelial-specific SIRT6 knockout (eSIRT6−/−) mice and control littermates (CTRL) underwent transient middle cerebral artery occlusion (tMCAO) for 45 min followed by 48 hours of reperfusion. Immunohistochemistry (IHC) was used to investigate BBB permeability by IgG extravasation and molecular mechanisms. Primary human brain microvascular endothelial cells (HBMVECs) transfected with either SIRT6 (siSIRT6) or scrambled (siSCR) small interfering RNA were subjected to hypoxia/reoxygenation (H/R). An in vitro BBB model consisting of a monolayer of siRNA-treated HBMVECs was established and barrier function was assessed by 48 h-lasting transendothelial electrical resistance measurement. SIRT6 expression in monocytes from stroke patients was correlated with the short-term neurological outcome [ΔNIHSS% = (NIHSS discharge - NIHSS admission)/NIHSS admission*100].

Results: eSIRT6−/− displayed higher infarct volumes and lower survival rate compared to WT mice 48 h after tMCAO. The increased infarct volume was functionally relevant as eSIRT6−/− also showed worse post-stroke neurological impairment. Analysis of brain sections revealed increased BBB damage and increased endothelial expression of cleaved caspase-3 in eSIRT6−/− as compared to control littermates. In vitro, H/R reduced SIRT6 expression in HBMVECs. Mirroring the animal results, SIRT6 silencing impaired the barrier function of HBMVECs 48 h after exposure to H/R. In line with this, SIRT6-silenced HBMVECs showed reduced viability, increased cleaved caspase-3 expression and reduced activation of the anti-apoptotic survival pathway Akt as compared to control cells after H/R.

Conclusion: Endothelial SIRT6 exerts a beneficial role in ischemic stroke by blunting I/R-mediated BBB damage. Specifically, SIRT6 reduces endothelial I/R-induced apoptotic death through activation of the protective Akt pathway. The longevity gene SIRT6 may represent a novel therapeutic target for the treatment of ischemic stroke.