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High Mobility Group Box1, Receptor for Advanced Glycation End products and IkappaB kinase are potential pharmacological targets in cerebral ischemia

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Stroke is the major cause of severe disability in the elderly and it is the third leading cause of death after cardiovascular disease and cancer in industrialized countries. Thrombolysis is the only option of therapy if patients reach to hospital in 3 hours after the onset of a stroke. Unfortunately, due to many contraindications only a minority of patients are treated with thrombolysis. Therefore, stroke research has been focused on the sub acute phase of cerebral ischemia that occurs hours after stroke and where the key players are inflammation and delayed apoptotic cell death. Severe cerebral ischemia triggers excitotoxicity induced necrotic neuronal death in the ischemic core. In the surrounding area (penumbra) where ischemia is less severe, tissue damage is due to apoptosis and inflammation. High mobility group box 1 (HMGB1) has been shown to link necrotic cell death to inflammation in several other forms of tissue damage. HMGB1 is a DNA binding protein that is widely expressed in various tissues including the brain. Moreover, HMGB1 is a cytokine-like mediator of delayed endotoxin lethality and acute lung injury. HMGB1 binds to the receptor for advanced glycation end products (RAGE) and to TOLL-like receptors (TLR) 2 and TLR4. In this study we show that HMGB1 is released by primary cortical neurons exposed to oxygen and glucose deprivation. In stroke patients we found an increase of serum HMGB1 concentrations 24 hours after admission to hospital and the animals after middle cerebral artery occlusion (MCAO) showed elevated serum HMGB1 levels after 4 hours. Furthermore, the an intraperitoneal application of neutralizing HMGB1 antibody and HMGB1 box A 15 minutes before MCAO significantly reduced the infarct volume. Animals lacking RAGE showed significantly reduced infarct volumes after permanent middle cerebral artery occlusion. However, opening of the blood-brain barrier in cerebral ischemia was not affected by RAGE

deficiency nor did physiologic parameters such as blood pressure, cerebral blood flow, and blood gases differ between $RAGE^{-/-}$ and control animals. Intraperitoneal administration of soluble RAGE 15 minutes before and 90 min after middle cerebral artery occlusion significantly reduced the infarct volume as well as the microglial activation. This data suggests that RAGE mediates ischemic brain damage in part due to binding of HMGB1 which is released from ischemic brain tissue. RAGE is known to activate NF-κB in neurons by a still unknown mechanism. IκB kinase complex (IKK) is a central component of the signalling cascade that controls NF-κB-dependent gene transcription. So far, the functional role of IKK in the brain was largely unknown. This data demonstrates that IKK is activated in penumbra after 30 minutes of MCA occlusion and remains active over 5 hours in a mouse model of stroke. To explore the function of IKK in brain ischemia we investigated mice that contain a targeted deletion of the IKK2 gene in neurons or that express a dominant inhibitor of IKK in neurons in our MCA occlusion model. In both lines, inhibition of IKK activity markedly reduced infarct size. In contrast, constitutive activation of IKK2 enlarged the infarct size. A selective small-molecule (BMS 345441) inhibitor of IKK mimicked the effect of genetic IKK inhibition in neurons reducing the infarct volume and cell death in a therapeutic time window of 4.5 hours.

These data suggests therapeutic potential of HMGB1 and RAGE inhibitors in ischemic insult and reveal a key function of IKK in ischemic brain damage and suggest a potential role for IKK inhibitors in stroke therapy.