**Introduction**

Stroke ranks as the third leading cause of death in the U.S. Acute ischemic stroke, the most common form, is caused by clotting in the cerebral arteries leading to brain oxygen deprivation and cerebral infarction. Matrix metalloproteinases (MMPs), MMP-9 in particular, are known to be involved in neuronal cell death, blood-brain barrier (BBB) breakdown and hemorrhage. Recently, we reported a novel, "pathologically-activated therapeutic (PAT)" (Scheme 1) thriiane MMP inhibitor SB-3CT (Scheme 2) for neuroprotection in the ischemic mice. SB-3CT is the first mechanism-based MMP inhibitor selectively for MMP-9/-2 due to the ability of gelatinase to facilitate the requisite rate-limiting deprotonation event leading to thriiane-ring opening, giving tight-binding inhibition by the thiolate generated within the active site. This "suicide type" of inhibition is unique among MMP inhibitors developed to date.

**Scheme 1. Activation of a pathologically activated therapeutic (PAT) drug by its target.** The drug is not active until it interacts with its target, for example, an MMP enzyme. The MMP inhibitor shown here is then activated upon binding to the catalytic site of the enzyme. Hence, increased or pathologic activity of the enzyme results in further activation of the drug that in turn inhibits enzyme activity, resulting in blockade of MMP action in a feedback-type manner.

**Hypothesis:** MMP-9 causes proteolytic changes resulting in neurovascular damage after focal cerebral ischemia in mice. Inhibition of MMP proteolysis with SB-3CT should result in decreased apoptosis of neurons and improved behavioral outcomes.

**Methods**

- **Focal cerebral ischemia models.** C57BL/6J mice were subjected to the right middle cerebral artery (MCA) occlusion induced by either a 6-0 filament or a 10-mm embolus under isoflurane anesthesia.

- **Administration of SB-3CT**
  The mice were injected with SB-3CT or vehicle in a double-blind manner at 2 and 4 hrs, and then evaluated 24 hours after cerebral ischemia in mice.

- **Regional cerebral blood flow (rCBF).** rCBF was monitored with a Laser-Doppler Perfusion Monitor to ensure ischemia in the MCA. The mice were checked at 2 and four hours after ischemia to check for dissolution or movement of the clot. A 70% reduction was required for inclusion in the data.

- **Neurobehavioral test.** At 24 hours following the ischemic stroke, the mice were observed and graded based on a 14-point scales of the neurologic severity score system (NSSs) for mouse neurobehavioral tests (Li, Chopp et al. 2000). There are several motor and sensory deficits like circling and bending at the thorax which indicate ischemic damage.

- **Quantification of infarct volumes and cellular damage.** Following the behavioral test, we sectioned the mouse brains into 100-μm thickness, stained with 2,3,5-triphenyl-tetrazolium chloride (TTC) and calculate the infarct volumes after cerebral ischemia in mice. Neuronal cell damage was determined by cresyl violet staining and quantified with scale 0 representing no cellular damage, 1 or 2 for less than 1/3 or 2/3 of cellular damage, and 3 for more than 2/3 of cellular damage.

- **Analysis of MMP-9/-2 levels and activities.** Following the behavioral test, we harvested fresh brain tissues and dissected into sub-regions of cortex, striatum and hippocampus, MMP-9/-2 levels and activities in brain homogenates were determined by gelatin zymography.

**Results**

**Figure 1. SB-3CT inhibits increased MMP-9 activity and protects against neuronal damage after embolus-induced MCA occlusion in mice.**

**Figure 2. SB-3CT attenuates brain infarct volumes and ameliorates neuro-behavioral outcome after embolic MCA occlusion in mice.**

**Conclusions**

- The highly specific inhibitor SB-3CT inhibits increased MMP-9 activity;

- SB-3CT protects against neuronal damage

- SB-3CT attenuates brain infarct volumes

- SB-3CT ameliorates neuro-behavioral outcome.

We conclude that MMP-9 is a highly promising drug target in stroke patients and that demonstrate that the SB-3CT class of MMP inhibitors manifesting as a novel, "pathologically-activated therapeutic (PAT)" lead compound deserves further study.

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