I. Supplemental Material

*Animals and Drugs*

Pregnant Sprague–Dawley rats were purchased from Harlan Laboratories, (Indianapolis, IN). 92 P7 male and female rat pups were then randomly divided into the sham-operated (n=20), collagenase-injected vehicle-treated (n=44), collagenase-injected SD-208 treated (N=28) animals. For GMH induction, animals were anesthetized with 3% isoflurane and placed onto a stereotaxic frame. Isoflurane concentration was then reduced to 2%. The scalp area was sterilized and bregma was exposed. Using bregma as a reference point, the following stereotactic coordinates were measured: 1.8 mm (rostral), 1.5 mm (lateral). A burr hole (1mm) was drilled. A 27 gauge needle was inserted at a rate of 1 mm/min at the depth of 2.8 mm from the dura. Using a microinfusion pump (Harvard Apparatus, Holliston, MA) 0.3 units of clostridial collagenase VII-S (Sigma, St Louis, MO) in 0.5µl was infused through the Hamilton syringe. The needle remained in place for an additional 10 min after injection to prevent “back-leakage”. After the needle was removed, the burr hole was sealed with bone wax and the incision suture closed, and the animals were allowed to recover on a 37 °C heated blanket. Upon recovering from anesthesia, the animals were returned to their dams. Sham operation consisted of needle insertion alone without collagenase infusion.

*Neurological Examination*

The developmental profile was assessed for seven days after GMH induction by negative geotropism and righting reflex tests. For negative geotropism, the time needed to turn 180° after being placed head down on a 20° angle slope was recorded. For righting reflex, the time required for the rat pups to completely rollover onto all four limbs after being placed on their backs was measured. The maximum allotted time was 60 s per trial (3 trials/day) for these tests. The average value of all three trials was used for further evaluation.

*Cognitive function*

The animals were assessed using the water maze and T-maze tests.
• The water maze was performed as described previously\textsuperscript{2, 3}. The apparatus consisted of a metal pool (110 cm in diameter), filled with water to within 15 cm of the upper edge, containing a small platform (11 cm in diameter) for the animal to climb onto, and swim path length was digitally analyzed by Noldus Ethovision tracking software (with a maximum of 60 s/trial). The cued trials, with the escape platform obviously visible from above the water's surface, measured general associative learning, sensorimotor abilities, and motivation to escape the water. The platform's location changed every other trial. The spatial trials measured spatial learning, with the platform submerged just below the water’s surface, hidden but discoverable. The probe trials measured spatial memory by recording time spent in the target quadrant once the platform was removed.

• The T-maze assessed short-term (working) memory ability\textsuperscript{4}. For each trial, the rat was placed into the stem (40 cm×10 cm) of the T-maze and allowed to explore until either the left or right arm was chosen. Following a sequence of 10 trials, the rate of spontaneous alternation (0\%=none and 100\%=complete; alternations/trial) was calculated. Test was performed on day 21.

\textbf{Motor function}

For motor function evaluation, neurodeficit scales and foot fault tests were performed on day 24.

• Neurodeficit scales quantifies the neurodeficit using a series of six tests measuring functional deficits: 1) proprioceptive limb placing, 2) lateral limb placement, 3) forelimb placement, 4) postural reflex, 5) back pressure towards edge, and 6) lateral pressure towards edge. Score 10 is considered as severe, 5 as moderate and 0 as no neurological deficits\textsuperscript{3}.

• Foot-fault testing. The number of complete limb missteps through wire-grid openings was documented per limb over 120 s while the animal explored an elevated wire (3 mm) suspension (20 cm×40 cm) grid\textsuperscript{5}.

\textit{Western Blot}
Animals were euthanized with isoflurane and perfused transcardially with 100 ml of cold PBS. Hemispheres were isolated and stored at -80°C until analysis. The whole-cell lysates were obtained by gently homogenizing in RIPA lysis buffer (Santa Cruz Biotechnology, Inc., sc-24948) and centrifuging (14,000 g at 4°C for 30 min). The supernatant was collected and the protein concentration was determined using a detergent compatible assay (Bio-Rad, Dc protein assay). Equal amounts of protein (30 µg) were loaded and subjected to electrophoresis on an SDS-PAGE gel. After being electrophoresed and transferred to a nitrocellulose membrane, the membrane was blocked and incubated with the primary antibody overnight at 4°C. The following primary antibodies were used:

- Rabbit polyclonal to TGFR1 1/500 (Millipore ABF17)
- Rabbit polyclonal to TGFβ1 1/500 (Abcam ab9758)
- Rabbit polyclonal to SMAD2+3 TGFβ1 1/200 (Abcam ab65847)
- Rabbit polyclonal to phosphorylated SMAD2+3 TGFβ1 1/1000 (Abcam ab63399)
- Rabbit monoclonal [EP873Y] to Vitronectin 1/20000 (Abcam, ab45139)
- Rabbit polyclonal to GFAP 1/50000 (Abcam, ab7260)

The same membrane was probed with an antibody against β-actin (Santa Cruz, 1:1000) for an internal control. Incubation with secondary antibodies (Santa Cruz Biotechnology) was done for 1 h at room temperature. Immunoblots were then probed with an ECL Plus chemiluminescence reagent kit (Amersham Biosciences, Arlington Heights, IL) and visualized with an imaging system (Bio-Rad, Versa Doc, model 4000). Data was analyzed using Image J software.

**Brain injury evaluation**

After neurological testing, animals were euthanized with isoflurane and perfused transcardially with 100 ml of cold PBS following 50 ml of 4% PBS. The brains were removed and the quantification of weight was performed using an analytical microbalance (model AE 100; Mettler Instrument Co., Columbus, OH) capable of 1.0 µg precision. Brains were post-fixed in 10% paraformaldehyde for 24 hours. Then brains were dehydrated in 30% sucrose for 3 days. Histopathological analyses used 10 µm thick coronal sections, caudally cut every 600 µm on a cryostat (Leica Microsystems LM3050S), then mounted and stained on poly-L-lysine-coated
slides. Morphometric analysis of cresyl violet slides involved computer-assisted (ImageJ 4.0, Media Cybernetics, Silver Spring, MD) hand delineation of the ventricle system and hemisphere (cortex, subcortex). The borders of these structures were based on criteria derived from stereologic studies using optical dissector principles\textsuperscript{3, 6}. The volumes were calculated: 

\[ \text{Volume} = \left( \frac{\text{Average Area of coronal section} \times \text{Interval} \times \text{Number of sections}}{7} \right) \]

Immunohistochemistry

Samples were collected and brains were cut as described above. 

Antigens were retrieved by 15 minutes incubation in 95\(^\circ\) C. After blocking, the following primary antibodies were applied overnight by 4\(^\circ\) C:

- Rabbit monoclonal [EP873Y] to Vitronectin 1/250 (Abcam, ab45139)
- Rabbit polyclonal to GFAP 1/500 (Abcam, ab7260)

Secondary antibody were applied for one hour by room temperature.

Imaging was performed in the LLUSM Advanced Imaging and Microscopy Core with support of NSF Grant No. MRI-DBI 0923559 (SM Wilson) and the Loma Linda University School of Medicine.
II. Supplemental Figure

![Bar chart showing body weight comparison across different groups.](image)

- **Sham (N=8)**
- **Acute Treatment (N=8)**
- **Vehicle (N=8)**
- **Delayed Treatment (N=8)**
III. Supplemental Figure Legend: SD 208 ameliorated GMH-induced body weight loss

24 days after GMH induction animals ( ) shown significant loss of body weight compared to sham-operated animals ( ). (* P<0.05 vs. sham) SD 208 acute treatment ( ) and delayed treatment ( ) ameliorated GMH-induced loss of body weight, however the difference in body weight between treated and untreated animals did not reach statistical significance.
IV. Supplemental References


