Knockdown of Liver-Derived C1 Inhibitor Results in Brain Pathology in Mice

Running title: C1 Inhibitor, the Gatekeeper of the CNS

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Abstract

Activation of the innate immune system, mediated by the complement and the contact systems, induces inflammation. The inhibition of these pathways by C1 inhibitor (C1INH) has been shown to decrease pro-inflammatory response and mediate vascular permeability in the periphery. However, the role of peripheral C1INH has not been associated with brain function. Using an antisense oligonucleotide (ASO) to deplete circulating liver-derived C1INH in wildtype (WT) mice, we induced the activation of the kallikrein-kinin system (KKS), which produced bradykinin in the plasma, resulting in hypotension. Interestingly, the complement system was quiescent. Depletion of liver-derived C1INH increased blood-brain barrier (BBB) permeability, increased expression of bradykinin 2 receptor, activated resident glial cells to secrete pro-inflammatory mediators such as Il-1beta, Il-6, TNF-alpha and iNOS, and induced cognitive decline. The results of this study emphasize the important role of circulating C1INH in mediating brain function through the activation of the KKS. Thus, manipulation of C1INH in neuro-vascular disorders might be therapeutically beneficial.

Keywords: C1 inhibitor, Kallikrein-kinin system, bradykinin, vascular permeability, neuroinflammation, cognition.

Significant statement

By using Antisenseoligonucleotide (ASO) techniques we knocked-down liver-derived C1INH for 12 weeks. This elimination led to the activation of the kallikrein-kinin system in the plasma to produce elevated levels of bradykinin leading to increases bradykinin 2 receptor expressions in the brain, blood-brain barrier permeability, activation of astrocytes and microglial cells towards a pro-inflammatory response, and cognitive decline. These results suggest that normal levels of liver-derived C1INH is important for maintaining brain functions.

Introduction

The involvement of the peripheral immune system in brain pathology is now supported by mounting evidence. C1 inhibitor (C1INH) belongs to the super-family of serine protease inhibitors (serpins). C1INH inhibits the initiating proteases of the complement system(1) and the kallikrein system(2). The complement system and KKS share important features(3), and both involve in proteolytic cascades resulting in a pro-inflammatory response(4).

Activation of the KKS is induced by plasma kallikrein which cleaves high molecular weight kininogen (HK) to liberate bradykinin, a 9-amino acid peptide, known to cause vasodilation, reduce blood pressure, and induce a pro-inflammatory response through activation of bradykinin receptors 1 and 2 (B1R and B2R)(5).

The distribution of C1IHN is very similar in mouse and human tissues(6). Hepatocytes are thought to be the main source for C1INH biosynthesis(7) (Colten, 1972). In the CNS, C1INH is highly expressed in the cortex and the external capsule of the forebrain(6). The synthesis and/or secretion of C1INH have been detected in astrocyte-derived tumor cell lines(8), brain tissue, neuroblastoma cell lines, microglia, monocytes(9, 10), and neurons(11).

In this study, we show the important role of normal liver-derived C1INH in maintaining brain integrity and function. By using ASO-mediated knockdown, we eliminated liver-derived C1INH in mouse plasma, which resulted in kallikrein-kinin system (KKS) activation. KKS activation led to the production of bradykinin, which in turn led to hypotension and increased the expression of bradykinin 2 receptor (B2R) in the brain. Impaired blood-brain barrier (BBB) integrity, activated astrocytes and microglia cells to secrete pro-inflammatory mediators, and impaired cognition were the pathological result of this knockdown. These results suggest that it is crucial to maintain normal levels of liver-derived C1INH which mediates brain function.

Results

Subcutaneous administration of C1INH ASO eliminates C1INH in the plasma and activates the KKS. In order to prevent C1INH production in the liver, we subcutaneously administered antisense oligonucleotide (ASO)

targeted towards liver-derived C1 inhibitor protein (C1INH ASO) or control ASO (CTRL ASO) to ten-week-old WT mice for three months. Plasma levels of C1INH were significantly downregulated in mice treated with C1INH ASO compared to CTRL ASO (Fig 1A and B and Supplementary figure 1A for C57/C3H). Similar results were obtained in non-reduced conditions (Supp Fig.1B-C). In addition, C1INH ASO treatment, did not eliminate the protein expression in splenocytes and macrophages measures by FACS (Supp. Fig 1D-F). These findings suggest that the reduction of C1INH in the circulation is due to the targeted treatment of C1INH in the liver which results in decreased expression of the secreted form of C1INH but does not target other peripheral immune cells.

Since C1INH can also inhibit the KKS through plasma kallikrein, we analyzed the cleavage of high molecular weight kininogen (HK) after C1INH ASO administration. Using Western blot analysis, we found a 50% reduction in levels of intact HK (iHK; mHK+ΔD5) in plasma from C1INH ASO-treated mice compared to CTRL ASO-treated mice (Fig 1C and D and Supplementary figure 2A), indicative of increased HK cleavage as previously suggested(12). Non-reduced HK levels were also significantly reduced in C1INH ASO mice (Supplementary figure 2B, C). Furthermore, levels of plasma kallikrein, which activates HK cleavage, were significantly increased in plasma from C1INH ASO-treated mice compared to controls (Fig 1E, F). However, when we evaluated the plasma after eight weeks of treatment, we found no significant differences in iHK and plasma kallikrein levels in C1INH targeted by ASO (Supplementary figure 2D), suggesting that only long term C1INH reduction results in KKS activation. Therefore, we continued our evaluations using the chronic treatment time point (12 weeks of treatment).

We measured kallikrein activity in plasma of C1INH ASO-treated mice compared to CTRL ASO, as previously described(12). Kallikrein activity levels were significantly increased by 48% ($p=0.0244$) compared to plasma from control treated mice (Fig 1G and H), supporting the results of KKS activation seen in western blots.

The end product of HK cleavage is a 9-amino acid peptide called bradykinin. Bradykinin is unstable in the plasma and rapidly eliminated by degradation.

Using a sensitive ELISA kit for detecting bradykinin, we found that levels of bradykinin were significantly increased in C1INH ASO-treated mice compared to CTRL ASO-treated mice (Fig 1I). These results suggest that chronic knockdown of liver-derived C1INH leads to activation of the KKS and to the generation of bradykinin.

Studies have shown that elevated levels of bradykinin correlate with low blood pressure(13-15). Since plasma from our C1INH ASO-treated mice showed a significant increase in bradykinin, we examined the blood pressure of these mice. We found a significant decrease in systolic, diastolic, and mean blood pressure in C1INH ASO-treated mice compared to CTRL ASO-treated mice (Fig 1J), strengthening the results of elevated bradykinin in the plasma.

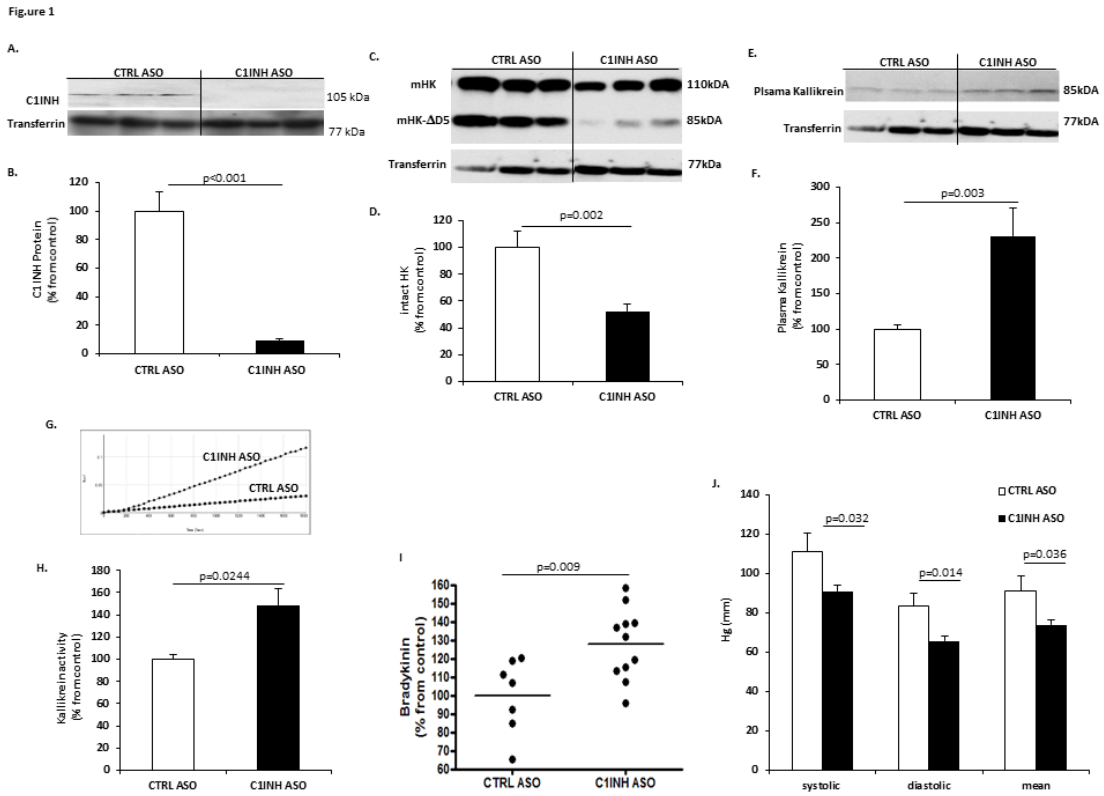

Figure 1. Elimination of C1INH in plasma of C1INH ASO treated mice and activation of the KKS. (A) Representative Western blot of plasma from mice treated with CTRL ASO vs C1INH ASO, probed with an anti-C1INH (A), HK comprised of two bands (110 kDa and 85 kDa) (C) and plasma kallikrein (E) antibodies under reduced conditions. Quantification of C1INH (B) levels show a significant reduction in C1INH ASO treated mice compared to controls (p<0.001, n=12/group). Quantification of Western blots showed a significant decrease in the total level of intact HK (110 kDa + 85 kDa; D; p=0.002, n=9-10/group) and a significant increase in the level of plasma kallikerin (F; p=0.003, n=9-10/group) between plasma from C1INH ASO treated mice compared to CTRL ASO treated mice. Samples were normalized to transferrin (TF). (G, H) Representative kallikrein activity plot and quantification of only males treated with CTRL ASO or C1INH ASO. Plasma from C1INH ASO treated mice show a significant increase in KKS activity (p=0.0244, n=5-7/group) compared to CTRL ASO. (I) ELISA analysis indicates there was significantly more bradykinin in the plasma of C1INH ASO treated mice compared to the

control group (p=0.009, n=7-11/group). (J) Blood pressure measurement was determined by tail cuff plethysmography. Systolic, diastolic, and mean blood pressure readings indicate that C1INH ASO treated mice are hypotensive in comparison to the control group (p=0.032, p=0.014, p=0.036; n=9-11/group).

C1INH was first characterized as the major inhibitor of the complement system initiation cascade, therefore we explored if the downregulation of peripheral C1INH induced complement system protein activation. Surprisingly, we found no changes in the levels of C1qA and C1r by Western blot (Supp Fig 3A-D and Supplementary figure 3H). Furthermore, levels of peripheral C3a were not changed when depleting peripheral C1INH, examined by Western blot and ELISA (Supp Fig 3E-G). These results suggest that chronic knockdown of liver-derived C1INH does not induce the activation of the complement system in the periphery.

ASO treatments target mRNA in the liver which may cause liver dysfunction resulting in pro-inflammatory responses(16-18), neurological deficits, brain edema, and brain inflammation(19-21). We ruled out liver dysfunction as a possible outcome of ASO treatment effects by using alanine aminotransferase (ALT) assay and ELISA for pro-inflammatory cytokines in the plasma (Supp Fig 4A-D) which showed no significant changes between the treated groups. Furthermore, we measured the spleen weight as another indication of liver dysfunction and found no differences in weight between the groups (Supplementary figure 4E), suggesting no toxic liver dysfunction apparent due to the ASO treatment. We also ruled out stress as a possible mechanism of KKS activation(22), measured by corticosterone (CORT) levels (Supplementary figure 4F) in the plasma and compared to immobilized induced-stress experiments. These low levels of CORT indicate that the chronic injections did not induce a stress response which might result in activation of the KKS.

Chronic knockdown of liver-derived C1INH increased bradykinin 2 receptor (B2R) expression in the brain and BBB dysfunction. The bradykinin system is known to be involved in BBB integrity(23), and B2R antagonists can prevent BBB permeability induced by elevated levels of bradykinin(24). Bradykinin was suggested to be an important pro-inflammatory inducer in the nervous system(25). Since we detected elevated levels of plasma bradykinin in C1INH ASO-treated mice, we measured the levels of

B2R in the brain. Both gene and protein expression levels of B2R were significantly increased by two-fold in brains of C1INH ASO mice compared to controls (Fig 2A-C), suggesting an activation of the bradykinin pathway in the brain.

Next, we examined BBB integrity by immunofluorescence staining of coronal brain sections for IgG which is usually not detected in healthy brain. There was increased staining of IgG in the parenchyma, outside laminin-positive vessels, specifically in borders of the lateral ventricle and the fimbria (Fig 2D). Using Evans blue, we evaluated the BBB leakage in the brains of C1INH ASO-treated mice compared to CTRL ASO-treated mice. We found an increase in blue staining in the freshly frozen brains of C1INH ASO mice (Fig 2E-F). Moreover, Evans blue dye was more readily detected by fluorescence microscopy in the brain tissue of C1INH ASO mice, specifically in the margins of the ventricles and parenchyma (Fig 2G). When we analyzed the brains for differences in water content, to test for brain edema, we did not find any changes between groups (Supp Fig 5). These results suggest a possible breakdown of the BBB specifically in the lateral ventricle region, with increased expression of B2R.

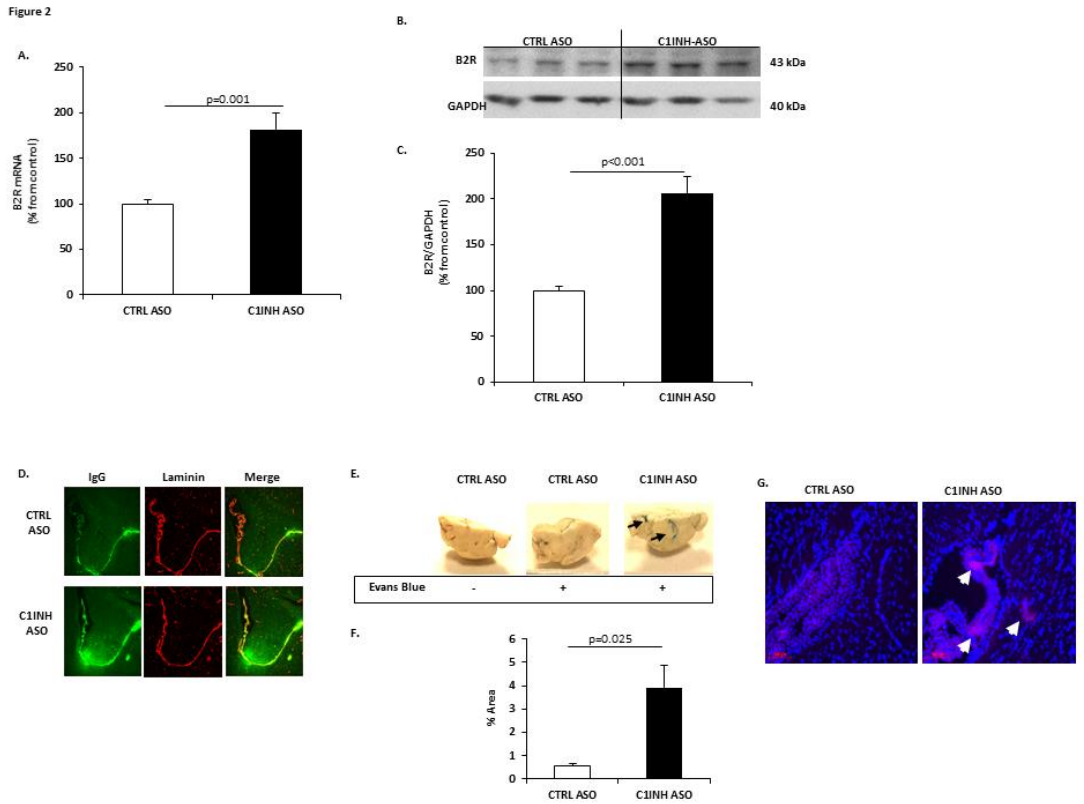

Figure 2. Increased B2R expression and BBB permeability is caused by depletion of peripheral C1INH. RTPCR of coronal brain samples showed elevated B2R gene expression in mouse brains from C1INH-ASO treated mice compared to controls (A; p=0.001, n=6-7/group). (B, C) Western blot of coronal brain homogenates were evaluated for B2R protein expression between groups. C1INH ASO group showed significantly higher levels of B2R in the brain than did the CTRL ASO group (p<0.001, n=5/group). GAPDH was used for normalization. (D) IgG immunofluorescence staining (green) at the fimbria of the hippocampus outside the vessels of C1INH ASO treated mice compared to that of CTRL ASO indicates a disruption of BBB integrity (arrows). Laminin staining (red) was used to outline blood vessels in the region (100μm scale). (E) Representative images of brain hemispheres from mice after Evans blue injection and perfusion. C1INH ASO mice administered Evans blue show a much greater amount of blue coloring in their brains than Evans blue-injected CTRL ASO mice and untreated mice. (F) Quantification of blue area from brain sectioned area in percentage using ImageJ (p=0.025, n=3) (G) Representative staining of Evans blue (red) in the brain parenchyma and the lateral ventricle. Blue signal is DAPI staining (100μm scale).

Chronic depletion of liver-derived C1INH induced activation of resident glial cell towards a pro-inflammatory response and cognitive decline. Emerging evidence suggests that peripheral inflammation can induce brain pathology leading to neuroinflammation and cognitive decline(26-28). We therefore examined this phenomenon in our studies. We found increased expression of GFAP and CD11b, markers of astrocyte and microglial cells activation, respectively, in the cortex of C1INH ASO-treated mice compared to CTRL ASO-treated mice (Fig 3). Expression levels of both genes and proteins were significantly increased in the cortex as shown by mRNA analysis (Fig 3A for GFAP; 3E for CD11b) and western blot analysis (Fig 3B&C for astrocytes; Fig 3F&G for microglia/macrophages). We have also confirmed the results using immunofluorescence staining for astrocytes and microglia (Fig 3D for astrocytes; Fig 3H for microglia/macrophages). These results suggest that the activation of resident immune cells in the brain is influenced by reduced levels of liver-derived C1INH in the periphery.

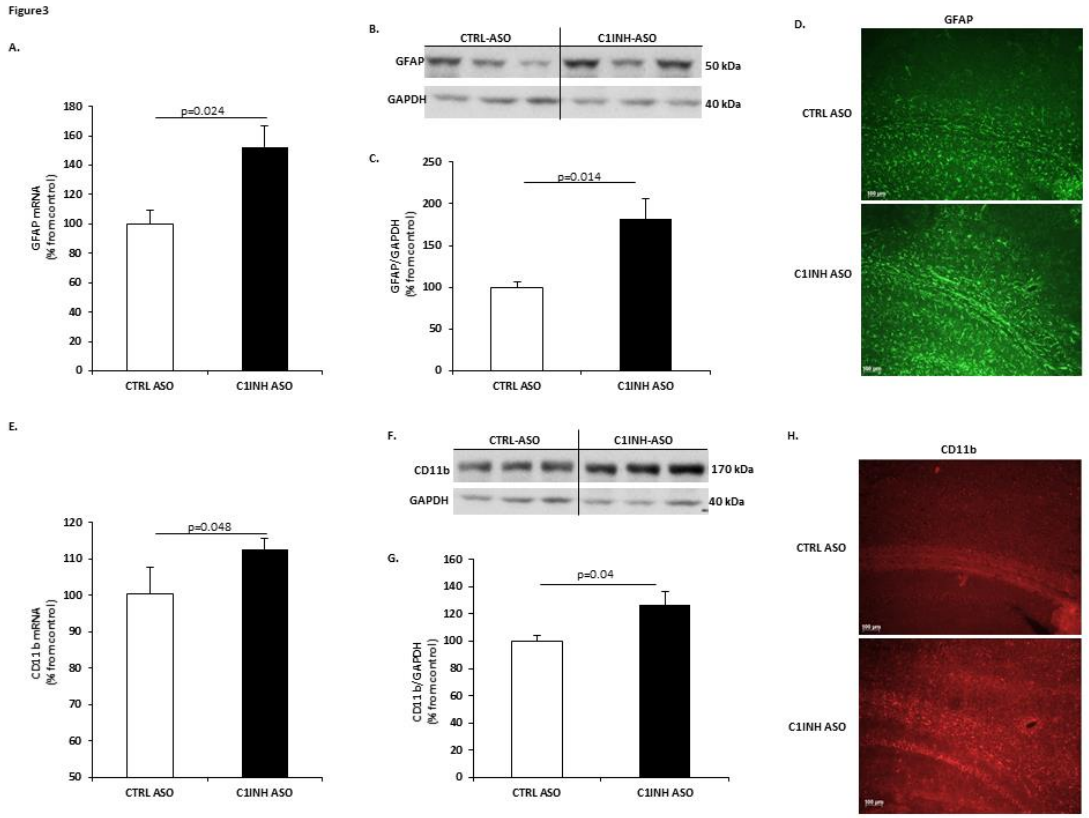

Figure 3. Glial cell activation in the cortex is induced by depletion of peripheral C1INH. (A) Gene expression of GFAP is increased (p=0.024; n=4-10/group) in the cortex of mice treated with C1INH ASO compared to control treatment. (B) Representative Western blot of GFAP in the cortex of C1INH ASO treated mice vs. CTRL ASO treated mice. Samples were normalized to GAPDH. (C) GFAP protein expression is significantly increased (p=0.014, n=5) in the cortex of C1INH ASO treated mice compared to CTRL ASO treated mice. (D) Representative immunofluorescence staining of GFAP in the cortex of C1INH ASO vs. CTRL ASO treated mice (100μm scale). (E) Gene expression of CD11b is increased (p=0.048, n=4-10/group) in cortex of C1INH ASO vs. CTRL ASO treated mice. (F) Representative Western blot of CD11b in the cortex of C1INH ASO vs. CTRL ASO treated mice. Samples were normalized to GAPDH. (G) CD11b protein expression is increased (p=0.04, n=5) in the cortex of C1INH ASO treated mice compared to CTRL ASO treated mice. (H) Representative immunofluorescence staining of CD11b in the cortex of C1INH ASO vs CTRL ASO treated mice (100μm scale).

Activation of glial cells can trigger inflammatory pathways(29). In order to understand the response of resident glial cell activation, we measured levels of pro-inflammatory cytokines in the brain. We first measured gene expression of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α, since they are the major secreted cytokines from activated glial cells. Comparing brain samples from C1INH ASO- and CTRL ASO-treated mice, RT-PCR analysis confirmed the elevation in pro-inflammatory cytokine gene expression (Fig 4A-C) and ELISA confirmed an increase in their proteins (Fig 6D-F). Furthermore, since nitric oxide is known to be secreted from activated resident immune cells during pro-inflammatory response(29), we used immunofluorescence and

Western blotting to examine changes in inducible nitric oxide synthase (iNOS). We observed an increase in iNOS expression in brains of C1INH ASO mice compared to CTRL ASO-treated mice (Fig 4G-I).

At the end of the chronic treatment, we performed behavioral tests to evaluate motor and cognitive function in mice. Using the fear conditioning paradigm, C1INH ASO-treated mice showed reduced percentage of total freezing time on the test day compared to the CTRL ASO group (Fig 4J), indicative of memory impairment. We have also examined cognitive function after 8 weeks of treatment, and found no significant changes (Supp Fig 6A), suggesting the chronic, rather than short term, depletion of liver-derived C1INH induced brain dysfunction. In order to evaluate motor abilities that might be affected by the ASO treatment, we quantified the average distance traveled on the first day of the fear conditioning test prior to foot shock. No motor dysfunction was observed between the groups (Supp Fig 6B). We also performed an open field test to compare the distance which mice traveled after treatment and found no differences (Supp Fig 6C). Since there was no difference observed in motor function between the C1INH ASO and CTRL ASO groups, the reduced freezing time during fear conditioning is most likely a result of cognitive impairment due to the chronic depletion of peripheral C1INH. Although cognitive impairment is often correlated with neurodegeneration, we did not observe neuronal degeneration measured apoptosis (Supplementary figure 6D). This result suggests that the cognitive decline observed in C1INH ASO-treated mice might be due to the loss of BBB integrity and the pro-inflammatory response in the brain, resulting from peripheral activation of the KKS. In conclusion, these results suggest that brain function is mediated by peripheral C1INH.

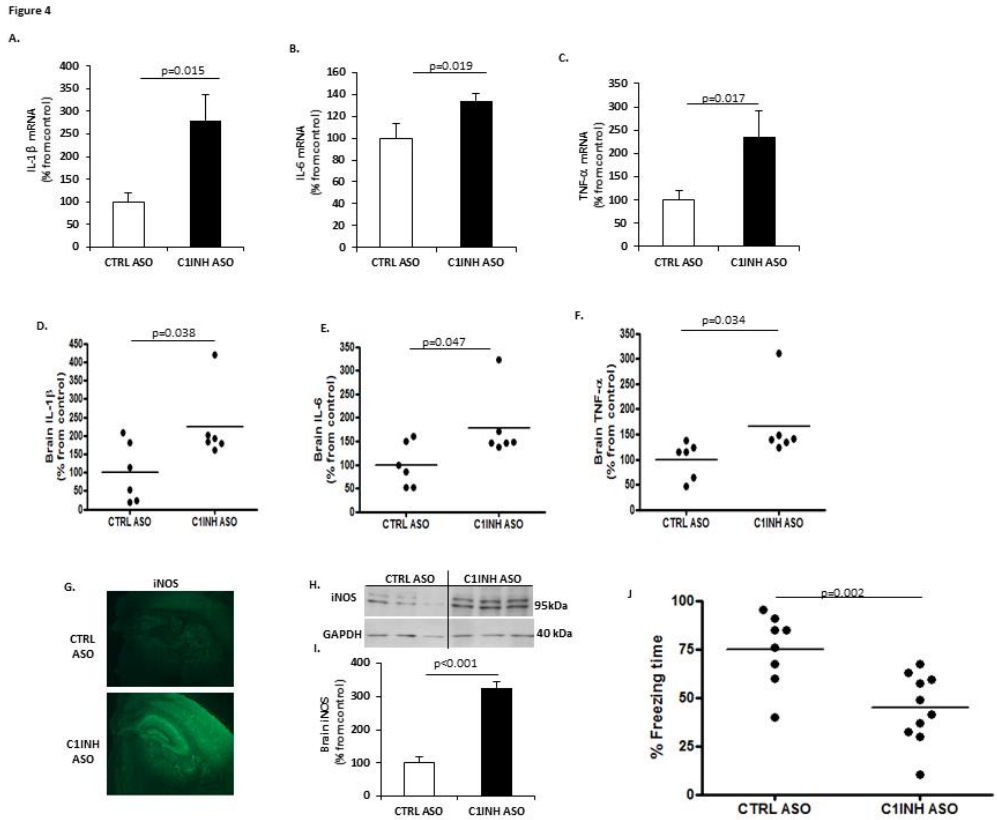

Figure 4. Increased pro-inflammatory response and cognitive decline in the CNS of mice treated with C1INH ASO. (A-C) Elevated gene expression of (A) IL-1β (p=0.015, n=5-7/group), (B) IL-6 (p=0.019, n=5-7/group), and (C) TNF-α (p=0.017, n=5-7/group) in brains of C1INH ASO treated mice compared to CTRL ASO treated mice. (D-F) Protein expression levels of pro-inflammatory cytokines are increased in brains of C1INH ASO treated mice compared to controls: (D) Il-1β (p=0.038, n=6), (E) IL-6 (p=0.047, n=6), and (F) TNF-α (p=0.034, n=6). (G) Representative immunofluorescence staining of iNOS in the brains of C1INH ASO treated mice compared to CTRL ASO treated mice. (H) Representative Western blot of iNOS in brain homogenates of C1INH ASO treated mice compared to controls. (I) iNOS protein expression is increased (p<0.001, n=6) in C1INH ASO treated mice compared to controls. See my handwritten suggestions and notes on this Figure. (J) Fear conditioning test (day 2) demonstrates decreased freezing time in C1INH ASO treated mice compared to controls (74.80 ± 6.521 vs. 74.80 ± 6.521 N=8, p=0.022, n=8-10/group).

Discussion

Emerging evidences are rising regarding the effect of the peripheral system and brain function. Blood proteins and activated immune cells are known to play a role in many brain pathologies. However, the exact mechanism and the routes involved are still under investigation.

In this study, we show that chronic depletion of liver-derived C1INH lead to BBB permeability, brain inflammation, and cognitive dysfunction. The mechanistic link underlying these results is through the activation of the KKS to produce bradykinin in the periphery but spares the activation of the

complement system.

By using an ASO technique that targets the production of C1INH in the liver, we reduced the protein expression in the periphery. Being described first as an inhibitor of the complement system, we tested whether C1INH ASO mice demonstrated changes in the initiation factors of the complement system and found no differences in the activation state of the complement system in the plasma or the brain. However, we observed significant increased activation of the KKS, through the cleavage of intact HK by plasma kallikrein, resulting in a production of bradykinin. This toxic protein was shown to induce peripheral vascular permeability and hypotension mediated by bradykinin 2 receptor (14, 30) similar to the results we present here. However, no information is presented regarding the influence of liver-derived C1INH and brain integrity and function.

ASOs that target the liver may produce liver disorders which can correlate with an enlarged spleen, elevated pro-inflammatory cytokines(31), and induce neurological deficits and brain inflammation(19-21). We analyzed liver dysfunction and secretion of pro-inflammatory mediators in the plasma and found no liver stress measured by ALT or pro-inflammatory response such as IL-6, IL-1β, and TNF-α. Spleen weight was also unchanged between the treated groups, suggesting no toxic liver dysfunction apparent due to the ASO treatment. Altogether, these results indicate that the activation of the KKS in the periphery is not associated with the subcutaneous chronic treatment of the ASO but rather by the reduction of liver-derived C1INH.

In the brain, we observed leakage of the BBB, pro-inflammatory response and cognitive decline which are not a result of liver dysfunction due to the chronic C1INH ASO treatment, but because of the reduced expression of C1INH levels in the plasma.

Our mouse model partially mimics the clinical pathology of Hereditary angioedema (HAE) type-1, a condition caused by mutations in the SERPING1 gene leading to depletion of C1INH production in humans. HAE type-1 and acquired angioedema (AAE) type-1 are diseases characterized by recurrent episodes of severe, localized inflammation and increased vascular permeability affecting soft tissues, including the gastrointestinal tract, upper

airways, and the mucosa(32, 33). The acute attacks in HAE patients are thought to be mediated by the activation of the KKS and the complement system(34). Plasma from C1INH-deficient patients contains decreased levels of intact HK(35). During inflammatory attacks, HK is cleaved(36) and bradykinin is released(37). Activation of the contact system was shown to mediate vascular permeability via B2R n in the C1INH-/- model(30). Today, HAE patients are treated during attacks with either recombinant C1INH or kallikrein inhibitors, which halt the production of bradykinin(38). Moreover, prophylactic treatment with C1INH was shown to reduce the frequency of acute attacks(39). Previously, the term angioneurotic edema was used to characterize angioedema because of the psychological stress involved with this disorder(40) suggesting an involvement of stress hormones. Physiological stress is considered a trigger for acute attacks in the cases of HAE (41). To understand if the chronic treatment induced a stress response and consequently contributed to the activation of the KKS, we tested corticosteroid levels in the plasma of ASO-treated mice compared to restrained mice, which demonstrate high levels of plasma corticosteroid. ASO-treated mice did not show elevated levels of plasma corticosteroid, suggesting that the cause of KKS activation and bradykinin overproduction was due to the depletion of the C1INH itself, indicating an important role of controlled, balanced levels of C1INH in the plasma. Depletion of peripheral C1INH led to low blood pressure likely due to the high levels of bradykinin detected in the plasma, which causes blood vessels to dilate. Thus, we concluded KKS activation upon C1INH ASO treatment was not driven by stress or inflammation in the periphery and was specific to C1INH depletion.

C1INH was first suggested to be involved in brain injury in 1983(42). Twelve years later, C1INH was shown to be synthesized in the brain and was correlated with Alzheimer's disease (AD) pathology(11). Our group has also shown a decreased expression of C1INH in AD plasma compared to non-demented controls(43).

The therapeutic roles of C1INH have been observed in several animal models of peripheral inflammatory disease including sepsis(44-47), xenograft transplant rejection(48, 49), myocardium ischemia-reperfusion injury(50, 51), and hemorrhagic shock (52). Beyond the common clinical treatment with

recombinant C1INH for treating HAE, C1INH was shown to be therapeutically beneficial in humans in coronary and myocardial infarction(53, 54), septic shock and vascular leak syndrome (55), and other acute inflammatory diseases(56, 57). Moreover, mice infected with *Streptococcus pneumonia* and treated with C1INH showed an improvement in clinical signs of bacterial clearance in the cerebrospinal fluid (CSF) and blood, decreased leukocyte infiltration to the CSF, meningitis, and reduced Il-6 levels(58). Administration of C1INH was shown to have neuroprotective roles in neurodegenerative diseases such as stroke and traumatic brain injury (59-61) by reducing the infarct volume and the neuronal damage in the temporal cortex, striatum, hippocampus, and thalamus.

Our data suggest that chronic depletion of C1INH can cause serious brain pathologies via the activation of the KKS alone, and treatment with recombinant C1INH, KKS inhibitors, or B2R antagonists might be considered a necessary prophylactic treatment for down-regulated C1INH brain derived-disorders. Furthermore, manipulation of C1INH levels in the periphery might be used to intentionally open the BBB for drug administration. It will also be interesting to determine the role of peripheral C1INH protein in acute and chronic neurodegenerative diseases such as stroke and Alzheimer's disease and evaluate the therapeutic potential of recombinant C1INH, KKS inhibitors, or B2R antagonists in neuro-vascular disorders.

Materials and Methods

Animals. All animal experiments were conducted in accordance with the guidelines of the US NIH Guide for the Care and Use of Laboratory Animals and with approval from the Animal Care and Use Committee of The Rockefeller University. C57/Bl6J mice (males and females) were used for all experiments. A second WT model, C57/C3H, was also used. C1INH ASO (murine sequence)(62) and control ASO (CTRL ASO, no homologies to the mouse genome) were provided by Ionis Pharmaceuticals.

ASO preparation and treatment. ASOs were dissolved in saline and injected subcutaneously at 150 mg/kg/week for two weeks and then 50 mg/kg/week for 10 weeks. Ten-week-old WT mice were treated with C1INH ASO or CTRL ASO for 12 weeks (n=6-14 mice/group).

Plasma processing. Plasma was collected the day before the treatment started, after two weeks of treatment with 150mg/kg/week (sub-mandibularly), and the day of sacrifice (12 weeks, cardiac puncture). Animals were anesthetized at the end of the treatment using regulated CO_2. Mice were then transcardially perfused with saline. Upon collection, blood was immediately processed in EDTA-containing tubes (BD Microtainer). Heparin was avoided as it has been shown to bind to C1INH(63). Blood was centrifuged at 1300 rpm for 15 min at room temperature (RT). The upper phase of the supernatant was transferred to a second tube containing 0.5 M EDTA PH8 and centrifuged again. The plasma was aliquoted and immediately frozen and stored at -80^{0}C until analysis. During the lifespan of the mice, blood was taken submandibularly. At the end of the experiment, blood was taken from the heart.

Splenocytes and peritoneal macrophages. Spleens and peritoneal macrophages were taken from CTRL ASO and C1INH ASO-treated mice, and single-cell suspensions were prepared (64, 65). CD4+ cells and CD8+ cells were positively stained (BD Bioscience) along with C1INH-conjugated FITC (biorbyt) for FACS analysis.

Chronic restraint stress. Chronic restraint stress (CRS) was induced (66) with slight modifications. Mice were immobilized for 6 hours using a disposable netting. Blood was collected following the restraint period, and plasma was prepared. Levels of plasma corticosterone were determined using Corticosterone Enzyme Immunoassay Kit (K014-H1, Arbor Assays).

LPS treatment. Mice were injected intraperitoneally (i.p.) with 100 μl of 1 mg/ml lipopolysaccharide (LPS; L-2630 Sigma-Aldrich). Blood was collected and plasma prepared 12 hours after LPS injection. This source was used as a positive control during all ELISA examinations (see below).

Alanine aminotransferase (ALT) activity assay. Liver enzyme function was examined using the ALT Activity Assay (MAK052, Sigma-Aldrich). As a positive control group for this assay, we used lipopolysaccharide (LPS) to induce high levels of secreted ALT levels in the plasma(67).

Blood pressure. Blood pressure was measured at the end of ASO treatment using tail-cuff plethysmography (Kent Scientific). An average of 3 readings was obtained for each animal during measurement.

Evans blue and brain edema. Twelve hours before the sacrifice, 2% Evans blue in saline was injected into mice intraperitoneally. After perfusion with saline, one hemisphere was taken to assess percent of H_2O volume using the wet/dry procedure (68). Hemispheres were immediately weighed to obtain wet weight (WW) and heated to 100 ^{0}C for 24h. Then samples were weighed again to obtain the dry weight (DW). Brain water content was calculated as %H_2O = (WW-DW) X 100/WW. The other brain hemisphere was collected and sectioned. Loss of BBB integrity was revealed by visualizing Evans blue under the fluorescence microscope.

Immunohistochemistry. The primary antibodies used were: anti-GFAP (DAKO Z0334), anti-CD11b (Abcam ab-8878), IgG (Thermo scientific), anti-laminin (Fisher scientific RT-795-PO), and anti-iNOS (Abcam ab-129372). For secondary antibodies, we used IgG anti- mouse, rat, or rabbit, depending on the host of the first antibody (Thermo Scientific). Vectasheild-DAPI (Vector Labs) was used to seal the slides. Brain sections were visualized using Zeiss Axiovert200. Images were analyzed using ImageJ software.

Tunnel assay. Cell death by apoptosis was examined using the In-Situ Cell Death Detection Kit, TMR red (Roche).

Western blot analysis. Antibodies used for plasma samples: anti-HK light chain (R&D MAB22061); anti-FXII (HTI PAHFFXII-s); anti-plasma kallikrein (R&D AF2498); anti-transferrin (Abcam ab82411). Antibodies used for brain samples: anti-GFAP (DAKO Z0334); anti-CD11b (Abcam ab-133357); anti-B2R (LSBio LS-C405461); anti-GAPDH (Abcam ab9484 or Proteintech 60004-1-Ig). Protein levels were quantified using NIH Image J densitometry. Western blot results for plasma samples were normalized to transferrin, and brain protein extracts were normalized to GAPDH or Actin.

ELISA. ELISA was used to determine level of C3a (Molecular Innovations) and bradykinin (ENZO) in plasma and brain homogenate. The samples were normalized to 100% from CTRL-ASO to pool results from three different tests. For detection of pro-inflammatory cytokines, we used Mouse DuoSet ELISA kits for IL-1b, IL-6, and TNF-α (R&D). For a positive control in these assays, we used plasma from an LPS-injected mouse (69).

Gene expression. RNA was extracted from fresh cortex or frozen whole brain using RNeasy Lipid Tissue mini kit (74804, Qiagen). RNA was

converted to cDNA using High Capacity cDNA Reverse Transcription Kit (4368814, Life technologies). Gene expression levels were amplified using Taqman enzyme (Applied Biosystems, 4370048) and primers (IL1-β Mm00434228_m1, IL-6 Mm00446190_m1, TNF-α Mm00443258_m1, GFAP Mm01253033_m1, CD11b Mm00434455_m1) and normalized to endogenous mouse GAPDH (Mm99999915_g1). ΔΔCT was quantified and compared between samples.

Kallikrein activity assay. Plasma was evaluated for KKS activity using the chromogenic substrate S-2303 (Diapharma) or Pefachrome PK8092 (Pentapharm).

Behavioral analysis. All behavioral experiments were performed and analyzed with a researcher blinded to genotype and treatment. Fear conditioning was performed as previously described (70) with slight changes. Two foot shocks were given (0.7 milliamp, 0.5 sec), one after the first three minutes and the second at the end of the five minutes of the first day. Open field test was done as previously described (Gould, 2009).

Statistical analysis. All statistical analyses as indicated in the figure legends were determined by two-tailed t-test. One-tailed t-test was performed when the results confirmed the hypothesis. All numerical values presented in graphs are mean ±SEM.

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Authorship:

Contributions: D.F. designed the study, performed experiments, analyzed data, and wrote the manuscript; E.F. and A.R. performed experiments and provided technical assistance; E.H.N and S.S participated in data analysis and manuscript preparation. Conflict-of-interest disclosure: Alexey S. Revenko and A. Robert MacLeod are employees and stockholders of Ionis Pharmaceuticals.

The authors declare no additional competing financial interests.

References

1. Ziccardi RJ & Cooper NR (1979) Active disassembly of the first complement component, C-1, by C-1 inactivator. (Translated from Eng) J Immunol 123(2):788-792 (in Eng).
2. Schapira M, Scott CF, & Colman RW (1982) Contribution of plasma protease inhibitors to the inactivation of kallikrein in plasma. (Translated from eng) J Clin Invest 69(2):462-468 (in eng).
3. Bossi F, Peerschke EI, Ghebrehiwet B, & Tedesco F (2011) Cross-talk between the complement and the kinin system in vascular permeability. (Translated from eng) Immunol Lett 140(1-2):7-13 (in eng).
4. Ghebrehiwet B, Kaplan AP, Joseph K, & Peerschke EI (2016) The complement and contact activation systems: partnership in pathogenesis beyond angioedema. (Translated from Eng) Immunol Rev 274(1):281-289 (in Eng).
5. Schmaier AH & McCrae KR (2007) The plasma kallikrein-kinin system: its evolution from contact activation. (Translated from Eng) J Thromb Haemost 5(12):2323-2329 (in Eng).
6. Vinci G, et al. (2002) In vivo biosynthesis of endogenous and of human C1 inhibitor in transgenic mice: tissue distribution and colocalization of their expression. (Translated from Eng) J Immunol 169(10):5948-5954 (in Eng).
7. Johnson AM, Alper CA, Rosen FS, & Craig JM (1971) C1 inhibitor: evidence for decreased hepatic synthesis in hereditary angioneurotic edema. (Translated from eng) Science 173(3996):553-554 (in eng).
8. Gasque P, et al. (1993) Expression of the complement classical pathway by human glioma in culture. A model for complement expression by nerve cells. (Translated from Eng) J Biol Chem 268(33):25068-25074 (in Eng).
9. Gasque P, Fontaine M, & Morgan BP (1995) Complement expression in human brain. Biosynthesis of terminal pathway components and regulators in human glial cells and cell lines. (Translated from Eng) J Immunol 154(9):4726-4733 (in Eng).
10. Gasque P, Thomas A, Fontaine M, & Morgan BP (1996) Complement activation on human neuroblastoma cell lines in vitro: route of activation and expression of functional complement regulatory proteins. (Translated from Eng) J Neuroimmunol 66(1-2):29-40 (in Eng).
11. Walker DG, Yasuhara O, Patston PA, McGeer EG, & McGeer PL (1995) Complement C1 inhibitor is produced by brain tissue and is cleaved in Alzheimer disease. (Translated from Eng) Brain Res 675(1-2):75-82 (in Eng).
12. Zamolodchikov D, Chen ZL, Conti BA, Renne T, & Strickland S (2015) Activation of the factor XII-driven contact system in Alzheimer's disease patient and mouse model plasma. (Translated from Eng) Proc Natl Acad Sci U S A 112(13):4068-4073 (in Eng).
13. Guilarte M, Sala-Cunill A, Luengo O, Labrador-Horrillo M, & Cardona V (2017) The Mast Cell, Contact, and Coagulation System Connection in

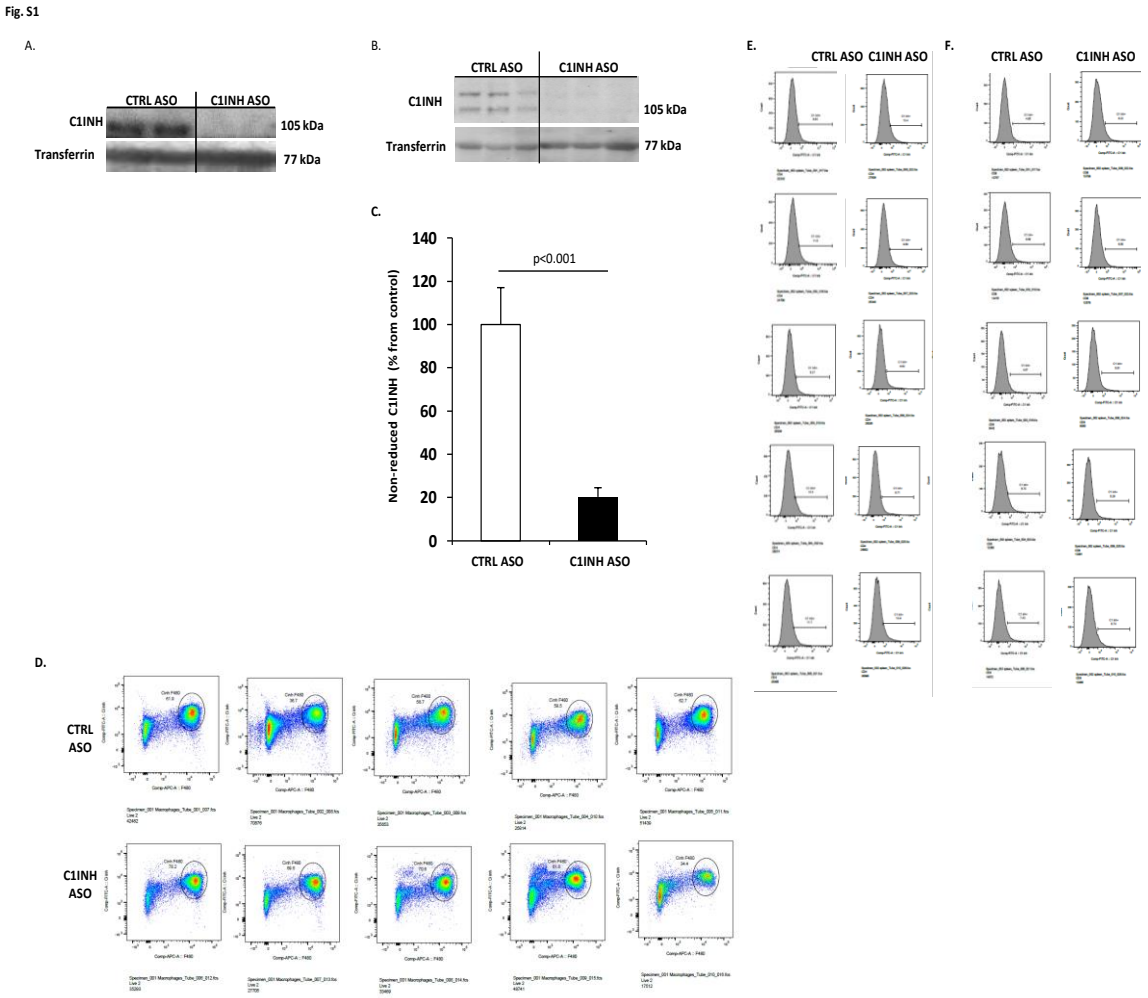
Anaphylaxis. (Translated from eng) Front Immunol 8:846 (in eng).
14. Sala-Cunill A, et al. (2015) Plasma contact system activation drives anaphylaxis in severe mast cell-mediated allergic reactions. (Translated from eng) J Allergy Clin Immunol 135(4):1031-1043 e1036 (in eng).
15. Waeber B, Nussberger J, Brunner HR, de Agostini A, & Schapira M (1984) [Role of bradykinin in hypotension induced by the active factor derived from factor XII]. (Translated from fre) Schweiz Med Wochenschr 114(48):1749-1751 (in fre).
16. Lapinski TW (2001) The levels of IL-1beta, IL-4 and IL-6 in the serum and the liver tissue of chronic HCV-infected patients. (Translated from eng) Arch Immunol Ther Exp (Warsz) 49(4):311-316 (in eng).
17. Mehta G, Mookerjee RP, Sharma V, & Jalan R (2015) Systemic inflammation is associated with increased intrahepatic resistance and mortality in alcohol-related acute-on-chronic liver failure. (Translated from eng) Liver Int 35(3):724-734 (in eng).
18. Liu Q (2009) Role of cytokines in the pathophysiology of acute-on-chronic liver failure. (Translated from eng) Blood Purif 28(4):331-341 (in eng).
19. Jiang W, Desjardins P, & Butterworth RF (2009) Cerebral inflammation contributes to encephalopathy and brain edema in acute liver failure: protective effect of minocycline. (Translated from eng) J Neurochem 109(2):485-493 (in eng).
20. O'Beirne JP, Chouhan M, & Hughes RD (2006) The role of infection and inflammation in the pathogenesis of hepatic encephalopathy and cerebral edema in acute liver failure. (Translated from eng) Nat Clin Pract Gastroenterol Hepatol 3(3):118-119 (in eng).
21. Sawhney R, et al. (2016) Role of ammonia, inflammation, and cerebral oxygenation in brain dysfunction of acute-on-chronic liver failure patients. (Translated from eng) Liver Transpl 22(6):732-742 (in eng).
22. Gomazkov OA, Komissarova NV, & Gorizontova MP (1981) [Participation of the lungs in regulating the activity of the kallikrein-kinin system during immobilization stress]. (Translated from rus) Biull Eksp Biol Med 91(6):652-654 (in rus).
23. Bartus RT, et al. (1996) Controlled modulation of BBB permeability using the bradykinin agonist, RMP-7. (Translated from eng) Exp Neurol 142(1):14-28 (in eng).
24. Marcos-Contreras OA, et al. (2016) Hyperfibrinolysis increases blood-brain barrier permeability by a plasmin- and bradykinin-dependent mechanism. (Translated from eng) Blood 128(20):2423-2434 (in eng).
25. Wahl M, et al. (1996) Vasomotor and permeability effects of bradykinin in the cerebral microcirculation. (Translated from eng) Immunopharmacology 33(1-3):257-263 (in eng).
26. Hu Z, Ou Y, Duan K, & Jiang X (2010) Inflammation: a bridge between postoperative cognitive dysfunction and Alzheimer's disease. (Translated from eng) Med Hypotheses 74(4):722-724 (in eng).
27. Obasi CN, et al. (2012) Association of Biomarkers for Inflammation, Endothelial Dysfunction and Oxidative Stress with Cognitive Impairment. The Epidemiology of Hearing Loss Study (EHLS). (Translated from eng) Oxid Antioxid Med Sci 1(3):169-173 (in eng).

28. Simen AA, Bordner KA, Martin MP, Moy LA, & Barry LC (2011) Cognitive dysfunction with aging and the role of inflammation. (Translated from eng) Ther Adv Chronic Dis 2(3):175-195 (in eng).
29. Farfara D, Lifshitz V, & Frenkel D (2008) Neuroprotective and neurotoxic properties of glial cells in the pathogenesis of Alzheimer's disease. (Translated from eng) J Cell Mol Med 12(3):762-780 (in eng).
30. Han ED, MacFarlane RC, Mulligan AN, Scafidi J, & Davis AE, 3rd (2002) Increased vascular permeability in C1 inhibitor-deficient mice mediated by the bradykinin type 2 receptor. (Translated from Eng) J Clin Invest 109(8):1057-1063 (in Eng).
31. Reid KB (1986) Activation and control of the complement system. (Translated from eng) Essays Biochem 22:27-68 (in eng).
32. Agostoni A & Cicardi M (1992) Hereditary and acquired C1-inhibitor deficiency: biological and clinical characteristics in 235 patients. (Translated from Eng) Medicine (Baltimore) 71(4):206-215 (in Eng).
33. Carugati A, Pappalardo E, Zingale LC, & Cicardi M (2001) C1-inhibitor deficiency and angioedema. (Translated from eng) Mol Immunol 38(2-3):161-173 (in eng).
34. Nielsen EW, et al. (1996) Activation of the complement, coagulation, fibrinolytic and kallikrein-kinin systems during attacks of hereditary angioedema. (Translated from eng) Immunopharmacology 33(1-3):359-360 (in eng).
35. Schapira M, et al. (1983) Prekallikrein activation and high-molecular-weight kininogen consumption in hereditary angioedema. (Translated from Eng) N Engl J Med 308(18):1050-1053 (in Eng).
36. Berrettini M, et al. (1986) Detection of in vitro and in vivo cleavage of high molecular weight kininogen in human plasma by immunoblotting with monoclonal antibodies. (Translated from Eng) Blood 68(2):455-462 (in Eng).
37. Nussberger J, et al. (1998) Plasma bradykinin in angio-oedema. (Translated from Eng) Lancet 351(9117):1693-1697 (in Eng).
38. Cugno M, Zanichelli A, Foieni F, Caccia S, & Cicardi M (2009) C1-inhibitor deficiency and angioedema: molecular mechanisms and clinical progress. (Translated from Eng) Trends Mol Med 15(2):69-78 (in Eng).
39. Sabharwal G & Craig T (2015) Recombinant human C1 esterase inhibitor for the treatment of hereditary angioedema due to C1 inhibitor deficiency (C1-INH-HAE). (Translated from eng) Expert Rev Clin Immunol 11(3):319-327 (in eng).
40. C.H Douglas JRB (1957) Familial paroxismal dysfunction of the autonomic nervous system (a periodic disease), often precipitated by emotional stress. Pediatrics, 18 (1957), pp. 782–793.
41. Nielsen EW, et al. (1995) C3 is activated in hereditary angioedema, and C1/C1-inhibitor complexes rise during physical stress in untreated patients. (Translated from eng) Scand J Immunol 42(6):679-685 (in eng).
42. Spiteri D, Viola L, Allegretti De Lista G, Galloro F, & Schisano G (1983) Changes of C1 esterase inhibitor, alpha2 globulin and sedimentation rate after severe brain injury. (Translated from Eng) Acta Neurol (Napoli) 5(6):420-425 (in Eng).
43. Zamolodchikov D, Renne T, & Strickland S (2016) The Alzheimer's disease

peptide beta-amyloid promotes thrombin generation through activation of coagulation factor XII. (Translated from Eng) J Thromb Haemost 14(5):995-1007 (in Eng).
44. Croner RS, et al. (2004) C1-inhibitor reduces hepatic leukocyte-endothelial interaction and the expression of VCAM-1 in LPS-induced sepsis in the rat. (Translated from Eng) Microvasc Res 67(2):182-191 (in Eng).
45. Guerrero R, et al. (1993) Endotoxin-induced pulmonary dysfunction is prevented by C1-esterase inhibitor. (Translated from Eng) J Clin Invest 91(6):2754-2760 (in Eng).
46. Jansen PM, et al. (1998) Effect of C1 inhibitor on inflammatory and physiologic response patterns in primates suffering from lethal septic shock. (Translated from Eng) J Immunol 160(1):475-484 (in Eng).
47. Scherer RU, Giebler RM, Schmidt U, Paar D, & Kox WJ (1996) The influence of C1-esterase inhibitor substitution on coagulation and cardiorespiratory parameters in an endotoxin-induced rabbit model of hypercoagulability. (Translated from Eng) Semin Thromb Hemost 22(4):357-366 (in Eng).
48. Fiane AE, et al. (1999) C1-inhibitor attenuates hyperacute rejection and inhibits complement, leukocyte and platelet activation in an ex vivo pig-to-human perfusion model. (Translated from Eng) Immunopharmacology 42(1-3):231-243 (in Eng).
49. Hecker JM, Loss M, Klempnauer J, & Winkler M (2002) C1-inhibitor for therapy and prophylaxis of acute vascular rejection of porcine kidneys in cynomolgus monkeys. (Translated from Eng) Transplant Proc 34(6):2382 (in Eng).
50. Akita N, Nakase H, Kaido T, Kanemoto Y, & Sakaki T (2003) Protective effect of C1 esterase inhibitor on reperfusion injury in the rat middle cerebral artery occlusion model. (Translated from Eng) Neurosurgery 52(2):395-400; discussion 400-391 (in Eng).
51. Horstick G, et al. (1997) Intracoronary application of C1 esterase inhibitor improves cardiac function and reduces myocardial necrosis in an experimental model of ischemia and reperfusion. (Translated from Eng) Circulation 95(3):701-708 (in Eng).
52. Horstick G, et al. (2001) C1-esterase-inhibitor treatment at early reperfusion of hemorrhagic shock reduces mesentry leukocyte adhesion and rolling. (Translated from Eng) Microcirculation 8(6):427-433 (in Eng).
53. Bauernschmitt R, Bohrer H, & Hagl S (1998) Rescue therapy with C1-esterase inhibitor concentrate after emergency coronary surgery for failed PTCA. (Translated from Eng) Intensive Care Med 24(6):635-638 (in Eng).
54. de Zwaan C, et al. (2002) Continuous 48-h C1-inhibitor treatment, following reperfusion therapy, in patients with acute myocardial infarction. (Translated from Eng) Eur Heart J 23(21):1670-1677 (in Eng).
55. Hack CE, et al. (1993) C1-inhibitor substitution therapy in septic shock and in the vascular leak syndrome induced by high doses of interleukin-2. (Translated from Eng) Intensive Care Med 19 Suppl 1:S19-28 (in Eng).
56. Niederau C, et al. (1995) Effects of C1-esterase inhibitor in three models of acute pancreatitis. (Translated from Eng) Int J Pancreatol 17(2):189-196 (in Eng).
57. Tassani P, et al. (2001) Effect of C1-esterase-inhibitor on capillary leak and

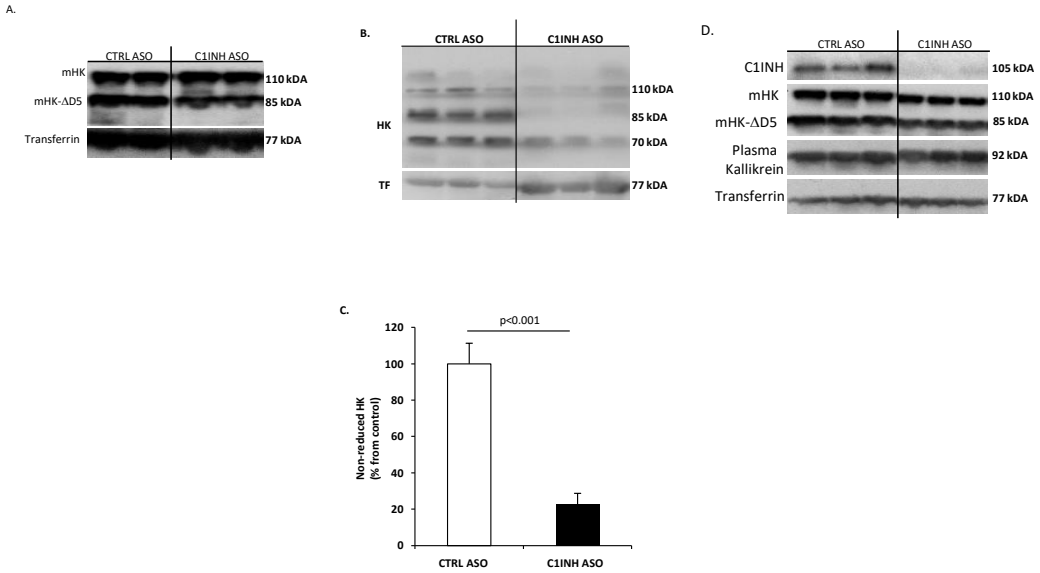
inflammatory response syndrome during arterial switch operations in neonates. (Translated from Eng) J Cardiothorac Vasc Anesth 15(4):469-473 (in Eng).
58. Zwijnenburg PJ, et al. (2007) C1 inhibitor treatment improves host defense in pneumococcal meningitis in rats and mice. (Translated from Eng) J Infect Dis 196(1):115-123 (in Eng).
59. Albert-Weissenberger C, et al. (2014) C1-Inhibitor protects from focal brain trauma in a cortical cryolesion mice model by reducing thrombo-inflammation. (Translated from Eng) Front Cell Neurosci 8:269 (in Eng).
60. De Simoni MG, et al. (2003) Neuroprotection by complement (C1) inhibitor in mouse transient brain ischemia. (Translated from Eng) J Cereb Blood Flow Metab 23(2):232-239 (in Eng).
61. Heydenreich N, et al. (2012) C1-inhibitor protects from brain ischemia-reperfusion injury by combined antiinflammatory and antithrombotic mechanisms. (Translated from Eng) Stroke 43(9):2457-2467 (in Eng).
62. Bhattacharjee G, et al. (2013) Inhibition of vascular permeability by antisense-mediated inhibition of plasma kallikrein and coagulation factor 12. (Translated from eng) Nucleic Acid Ther 23(3):175-187 (in eng).
63. Rossi V, et al. (2010) Functional characterization of the recombinant human C1 inhibitor serpin domain: insights into heparin binding. (Translated from Eng) J Immunol 184(9):4982-4989 (in Eng).
64. Farfara D, et al. (2011) gamma-Secretase component presenilin is important for microglia beta-amyloid clearance. (Translated from eng) Ann Neurol 69(1):170-180 (in eng).
65. Weiss R, Lifshitz V, & Frenkel D (2011) TGF-beta1 affects endothelial cell interaction with macrophages and T cells leading to the development of cerebrovascular amyloidosis. (Translated from eng) Brain Behav Immun 25(5):1017-1024 (in eng).
66. Wood GE, Norris EH, Waters E, Stoldt JT, & McEwen BS (2008) Chronic immobilization stress alters aspects of emotionality and associative learning in the rat. (Translated from eng) Behav Neurosci 122(2):282-292 (in eng).
67. Baveja R, et al. (2002) LPS-induced imbalanced expression of hepatic vascular stress genes in cirrhosis: possible mechanism of increased susceptibility to endotoxemia. (Translated from eng) Shock 17(4):316-321 (in eng).
68. Hellal F, et al. (2004) Pattern of cerebral edema and hemorrhage in a mice model of diffuse brain injury. (Translated from eng) Neurosci Lett 357(1):21-24 (in eng).
69. Qadri F, et al. (2016) Acute hypothalamo-pituitary-adrenal axis response to LPS-induced endotoxemia: expression pattern of kinin type B1 and B2 receptors. (Translated from eng) Biol Chem 397(2):97-109 (in eng).
70. Farfara D, et al. (2015) Low-level laser therapy ameliorates disease progression in a mouse model of Alzheimer's disease. (Translated from eng) J Mol Neurosci 55(2):430-436 (in eng).

Supplementary figures:



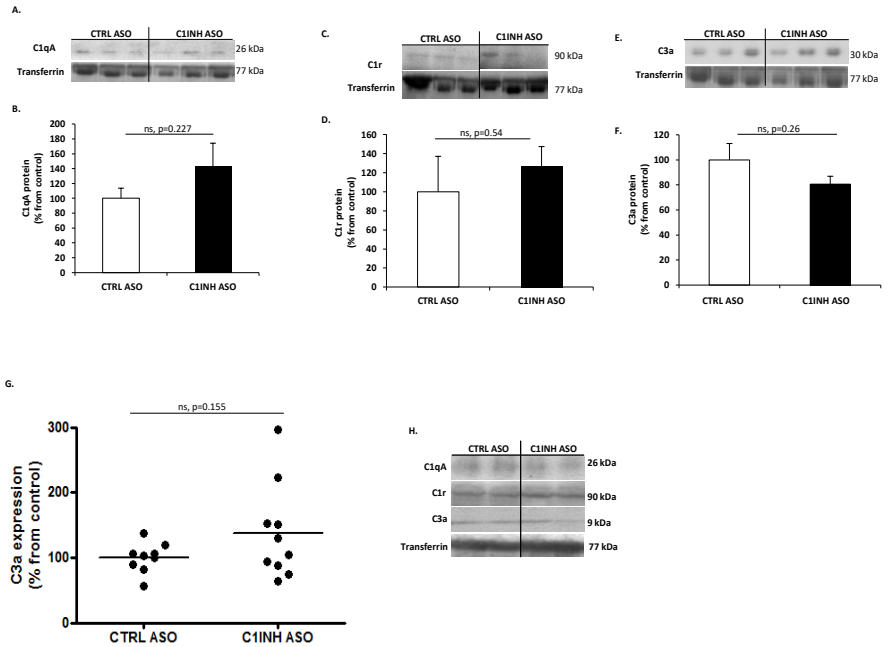
Supplementary Figure 1. Down regulation of liver-derived C1INH in C57/C3H and no observed changes in C1INH expression in macrophages or splenocytes nor activated the complement system. Plasma from C57/C3H mouse model was used to confirm the results seen in C57/Bl6J. (A) Representative Western blots of C1INH depletion from CTRL ASO compared to WT treated with C1INH ASO (B) Representative Western blots of non-reduced C1INH and (C) Quantification analysis of C1INH expression shows an 80% reduction ($p<0.001$, $n=6$). (D) FACS analysis of C1INH (FITC) from IP macrophages (F4/80+ APC) and (E) CD4+ cells and (F) CD8+ cells from spleen. (G) Representative Western blots of C1qA, C1r and C3a in C57/C3H mouse model. Transferrin was used as loading control.

Fig S2



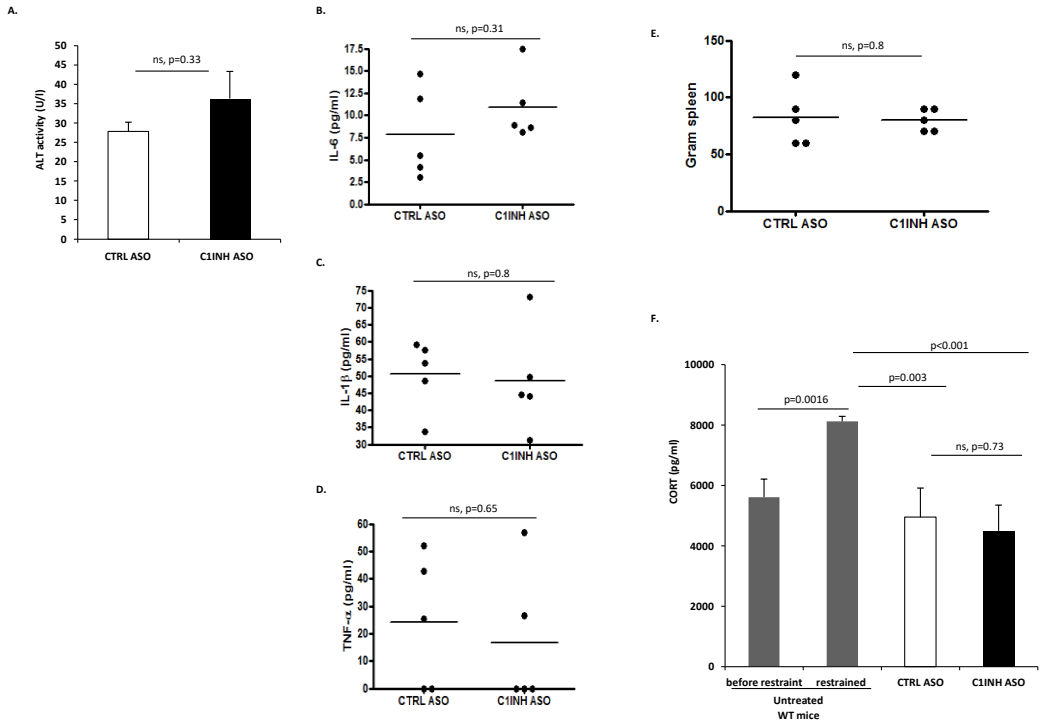
Supplementary Figure 2. Activation of the KKS in C57/C3H but no activation seen after eight weeks of treatment. (A) Representative Western blots of iHK (mHK+ΔD5) from C57/C3H mouse model treated with C1INH ASO compared to CTRL ASO shows decreased levels of iHK. (B) Non-reduced expression of iHK from plasma of CTLR ASO compared to C1INH ASO. (C) Quantification analysis of non-reduced iHK shows a decreased expression of 78% in treated C1INH ASO mice compared CTRL ASO ($p<0.001$, n=6). (D) Representative western blot of plasma from CTRL ASO compared to C1INH ASO treated for eight weeks show down regulation of C1INH expression but no differences in iHK and plasma kallikrein. Transferrin was used as loading control.

Fig S3



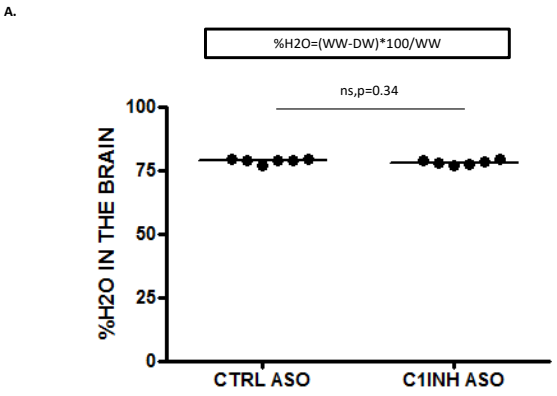
Supplementary Figure 3. Knockdown of C1INH does not induce complement activation. Representative Western blots of plasma from ASO-treated mice probed with antibodies against C1qA (A), C1r (C) and C3a (D). Samples were normalized to transferrin (TF). Quantification of Western blots shows that levels of C1qA (B), C1r (D) and C3a (E) were not significantly changed upon depletion of plasma C1INH ($p=0.227$, $p=0.54$ and $p=0.26$; n=6/group). (G) ELISA analysis of plasma demonstrates no significant change in C3a levels between C1INH ASO treated mice and CTRL ASO treated mice ($p=0.155$; n=9-10/group). (H) Representative Western blots of plasma from ASO-treated mice probed with antibodies against C1qA, C1r, and C3a from C57/C3H mice.

Fig. S4

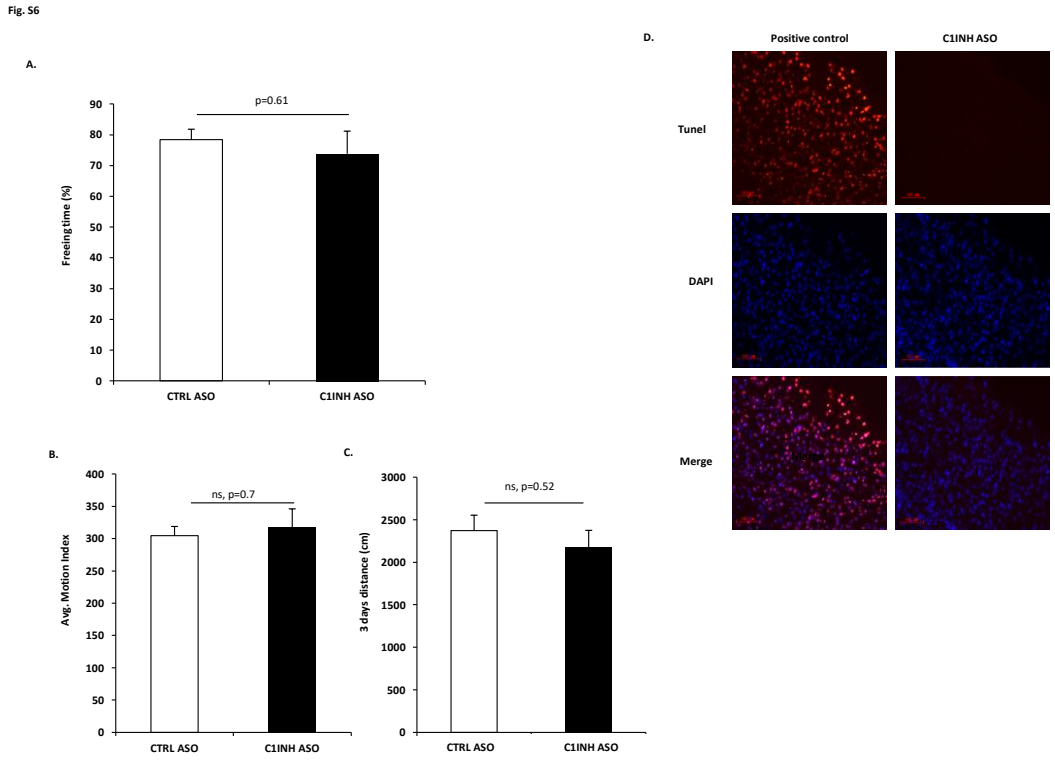


Supplementary Figure 4. No toxicity induced by C1INH ASO treatment. (A) Activity level of liver function measured by alanine transferase (ALT) assay was examined in mouse plasma. ALT activity did not change between C1INH ASO treated mice and CTRL ASO mice (p=0.33; n=4-5/group). (B-D) Levels of inflammatory cytokines did not change between C1INH ASO mice and CTRL ASO mice: IL-6 (p=0.31, n=5), IL-1β (p=0.8, n=5), and TNF-α (n=0.65, n=5). (E) Spleen weight after treatments show non differences between the groups. (F) No differences in spleen weight between the treated groups (82.00 ± 11.14, 80.00 ± 4.472, p=0.8718). (H) Corticosterone (CORT) levels did not change in plasma of C1INH ASO treated mice compared to CTRL ASO treated and mice that underwent acute restraint (p<0.001, n=5-7/group).

Fig. S5



Supplementary Figure 5. Measurement of brain edema. Brains of C1INH ASO treated mice were dried and measured for water content percentage. No differences were found between the treated groups in water content, indicating no brain edema (p=0.34, n=6/group).



Supplementary Figure 6. Cognitive dysfunction, motor function and apoptosis were not impaired after chronic elimination of liver-derived C1INH. (A) Percentage of freezing time on day 2 of the FCT showed no differences between treated groups after 8 weeks of treatment (P=0.61, n=6/group). (B) Calculation of the average motion index in the first day of the fear conditioning test prior to foot shock, which demonstrated no differences between the groups (p=0.7, n=6/group). (C) In an open field test, average of distance traveled was calculated over three days of testing. No differences were found between the groups (p=0.52, n=3-6/group). (D) Representative image of for neurodegeneration using TUNEL staining for apoptosis. No positive staining for apoptosis was determined.