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CD163 Expression in Neurons after Experimental Intracerebral Hemorrhage

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Abstract

Background and Purpose—CD163, a receptor for hemoglobin, is involved in hemoglobin clearance following intracerebral hemorrhage (ICH). In contrast to microglial/macrophage CD163, neuronal CD163 hemoglobin has not been well studied. The current study examined the expression of neuronal CD163 in a pig model of ICH as well as *in vitro* rat cortical neurons, and the impact of deferoxamine on that expression.

Methods—There were 2 parts to this study. In the *in vivo* part, piglets had injection of autologous blood into the right frontal lobe. The time course of CD163 expression and the effect of deferoxamine on the expression of CD163 after ICH were determined in the grey matter. In the *in vitro* part, the levels of CD163 and neuronal death, and the effect of deferoxamine were examined in rat cortical neurons culture treated with hemoglobin.

Results—CD163 positive cells were found and the CD163 protein levels were upregulated in the ipsilateral grey matter after ICH. The CD163 levels peaked at days 1 and 3. The CD163 positive cells were co-located with NeuN, HO-2 and TUNEL positive cells. Deferoxamine treatment attenuated ICH-induced CD163 upregulation, and significantly reduced both brain CD163 and hemoglobin levels at day 3. Treating neuronal cultures with hemoglobin for 24 hours resulted in CD163 upregulation and increased cell death. Deferoxamine significantly attenuated the hemoglobin-induced neuronal death and CD163 upregulation.

Conclusions—CD163 is expressed in neurons and upregulated after ICH. Deferoxamine reduced ICH-induced CD163 upregulation and brain cell death *in vivo*, and hemoglobin-induced CD163 upregulation and neuronal death *in vitro*.

Keywords

Cerebral hemorrhage; CD163 antigen; Deferoxamine; Swine; Hemoglobin

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Subject Terms

Intracranial Hemorrhage

INTRODUCTION

Hemoglobin release from an intracerebral hemorrhage (ICH) has deleterious effects on perihematomal brain tissues, resulting in brain edema, neuronal death, and blood-brain barrier disruption¹. The haptoglobin-CD163-HO-1 pathway exerts as a first defense system in hemoglobin induced toxicity^{2, 3}, and CD163 acts as a main hemoglobin receptor mediating the intracellular uptake of hemoglobin into macrophages⁴.

While macrophages play a central role in hemoglobin clearance, evidence indicates that other cell types may also be involved⁵. Hemoglobin mostly remains encapsulated within erythrocytes until they are phagocytosed and degraded by microglia and infiltrating macrophages⁶. However, hemoglobin can also be taken up by neural cells and metabolized locally^{7, 8}. **Neuronal exposure to hemoglobin for one day induces a concentration-dependent cell death**⁹. While astrocytes and hippocampal neurons can take up hemoglobin¹⁰, neurons are more vulnerable to hemoglobin toxicity^{11, 12}.

We and others have demonstrated that neurons can express the hemoglobin receptor CD163^{13, 14}, but the trafficking of CD163 in neural cells has not been intensively investigated. The present study examined the expression of neuronal CD163 *in vivo* and *in vitro*. The effect of an iron chelator, deferoxamine, on CD163 expression was also examined.

METHODS

Animal preparation and intracerebral blood injection

Animal use protocols were approved by the University of Michigan Committee on the Use and Care of Animals and were conducted in accordance with United State Public Health Service's Policy on Humane Care and Use of Laboratory Animals. A total of 78 male piglets (8–10 kg; Michigan State University, East Lansing) were used in this study. One piglet was excluded from this study because of brain infection at the second day after ICH. The ICH model was performed as previously described^{15–17}. Piglets were sedated with ketamine (25 mg/kg, IM) and anesthetized with 2% isoflurane via nose cone. The isoflurane concentration was maintained at 1.0 to 1.5% during the surgical procedures. Body temperature was maintained at 37.5 ± 0.5 °C by a feedback-controlled heating pad (Gaymar, Orchard Park, NY). The right femoral artery was inserted with a polyethylene catheter (PE-160) to obtain blood for injection and to monitor arterial blood pressure, blood gases and glucose concentrations.

A cranial burr hole (1.5 mm) was then drilled 11 mm to the right of the sagittal and 11 mm anterior to the coronal suture. An 18-mm-long 20-gauge sterile plastic catheter was placed stereotaxically into the center of the right frontal cerebral white matter and cemented in place. Using an infusion pump, first 1 mL of autologous arterial blood (**without anticoagulants**) was infused over 15 minutes and another 1.5 mL of blood was injected over

15 minutes after a 5 minutes break. After injection, the needle was removed, the burr hole was filled with bone wax, and the skin incision closed with sutures. Sham animals underwent the same procedure without blood infusion.

Experimental groups

The *in vivo* study was divided into two parts. In the first part, piglets had 2.5 ml blood injected into the right frontal lobe. Brains were harvested at 4 hours (n=7), day 1 (n=7), day 3 (n=7) and day 7 (n=7). Sham piglets were euthanized at day 3 (n=7). Brains were used for histology and immunoblots. In the second part, piglets had blood injected into the frontal lobe and were treated with vehicle (saline) or deferoxamine (50 mg/kg, i.m. at 2 hours after ICH and then every 12 hours for 7 days). Brains were harvested at days 1, 3 and 7 (n=7 each group) for brain histological examines and Western blotting. The investigators were blinded for the treatment. YH performed surgeries and RL and SC did all other measurements.

Brain *in situ* freezing and brain histology

Brains were frozen *in situ* by decanting liquid nitrogen into a 12 oz. bottomless foam cup adhered to the head with Dow-Corning high vacuum grease (Dow Corning, Midland, MI), as described previously¹⁸. The frozen head was then cut with a band saw into 5 mm thick coronal sections. Tissues were sampled from the regions of interest.

For brain histology, piglets were reanesthetized and the brains perfused *in situ* with 10% formalin. Brains were sectioned coronally. Paraffin embedded brain was cut into 10 μ m thick sections.

Primary cortical neuronal cultures

Primary cortical neurons were obtained from embryonic day 17 Sprague-Dawley rats (Charles River Laboratories, MI). Cultures were prepared according to a previously described procedure with some modifications^{19, 20}. Briefly, cortices were dissected, stripped of meninges, dissociated by a combination of 0.5% trypsin digestion and mechanical trituration, and plated in neurobasal medium with 2% B27, 0.5mM glutamine and 1% Antibiotic-Antimycotic in 6-well plates with a density of $2 \times 10^6/\text{cm}^2$ and cultured in a humidified incubator at 37 °C with 5% CO₂. Half of the culture media was changed every 3–4 days. The neurons were used at day-7.

Treatment of the neurons

The *in vitro* studies were divided into two parts. In the first part, primary cultured neurons were treated with either vehicle or hemoglobin at concentrations of 5 or 20 μ M (Cat No. 151234, MP Biomedicals, LLC). **The hemoglobin concentrations used in this study were based on a prior study by Regan and Panter⁹.** In the second part, neurons were treated with hemoglobin (5 or 20 μ M) with or without deferoxamine (5 μ M). Deferoxamine was applied 2 hours prior to hemoglobin administration. Cells were collected at 24 hours later and used for Western Blot and immunocytochemistry.

Neurotoxicity assay

Culture medium (50 μ L) was taken after 24 hours treatment and neuronal death was quantified by lactate dehydrogenase (LDH) release assay (G1780, Promega Corporation, WI, USA) as previously described²¹. The mean basal LDH activity in sister cultures subjected to medium exchange (sham wash) alone was subtracted from all values to quantify the signal specific to the cytotoxic exposure, following the protocol of Koh and Choi²².

Western blot analysis

Western blot analysis was performed as previously described^{15, 17}. Ipsi- and contralateral grey matter tissues were sampled. Protein concentration was determined by Bio-Rad protein assay kit (Hercules, CA). An equal amount of protein (50 μ g) was suspended in loading buffer, denatured at 95°C for 5 minutes and loaded on an SDS-PAGE gel. After being electrophoresed and transferred onto Hybond-C pure nitrocellulose membrane (Amersham), the membrane was blocked with nonfat milk buffer for 1 hour and then incubated with the primary antibodies. The primary antibodies were polyclonal rabbit anti CD163 (Abcam, Ab87099, 1:1000), rabbit monoclonal anti hemoglobin subunit alpha (Abcam, Ab92492, 1:2000), monoclonal mouse anti-GAPDH (Fitzgerald, 10R-G109A, 1:1000000) and monoclonal mouse anti- β actin (Sigma-Aldrich, A3854, 1:100000). The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hour at room temperature. The second antibodies were HRP-conjugated goat anti-mouse IgG (1:2000, Bio-Rad) and HRP-conjugated goat anti-rabbit IgG (1:2000, Bio-Rad). Protein band densities were detected by Kodak X-OMAT film and quantified by NIH Image J. CD163 levels were expressed as the ratio of CD163/GAPDH or CD163/ β actin. Hemoglobin level was expressed as the ratio of hemoglobin/GAPDH.

Immunohistochemistry and immunofluorescence staining

Immunohistochemical staining was performed using the avidin-biotin complex method as described previously^{15, 17}. Paraffin-embedded brains were cut into 10- μ m-thick sections. The sections were deparaffinized in xylene and rehydrated in a graded series of alcohol dilutions. Antigen retrieval was performed by the microwave method using citrate buffer (10mM, pH 6.4). Sections were permeabilized in 0.3% Triton X-100 (v/v) for 15 min and washed three times in PBS. Non-specific binding was blocked by 30 min treatment in 10% normal goat serum/PBS. The sections were incubated with primary antibody at 4 °C overnight. The primary antibody was polyclonal rabbit anti CD163 (Abcam, 87099, 1:500). After washing three-time in PBS, sections were incubated with biotinylated goat anti-rabbit IgG (1:500, Vector Laboratories) for 2 hours at room temperature, followed by 2 hours incubation in avidin-biotinylated peroxidase complex (1:500, Vectorstain Elite Kit) at room temperature. Diaminobenzidine (DAB; Invitrogen) was used as chromogen, with reactions sustained for 2–5 min at room temperature. For negative controls, the primary antibody was replaced with normal goat serum and no specific positive staining was detected. Stained sections were examined on an Olympus BX51 microscope. Images used for analysis were captured at the same contrast setting and exposure times.

For double labeling immunofluorescence staining, paraffin-embedded sections were treated with the same protocol as described above. The primary antibodies were monoclonal mouse

anti-pig CD163 (AbD serotec, MCA2311, 1:500), monoclonal mouse anti-rat CD163 (AbD serotec, MCA342, 1:500), polyclonal rabbit anti CD163 (Abcam, ab87099, 1:500), polyclonal mouse anti-FOX3/NeuN (AbD serotec, MAB377, 1:1000), polyclonal rabbit anti HO-2 (Enzo Life Sciences, ADI-OSA-200, 1:500) and monoclonal mouse anti porcine GFAP (Novus Biologicals, NBP 1-22545, 1:500). Secondary antibodies were Alexa Fluor 488 donkey anti-mouse IgG (1:500, Invitrogen), and Alexa Fluor 594 donkey anti-rabbit IgG (1:500, Invitrogen). Fluoroshield™ with DAPI (F6057) was used for nuclear labeling.

Immunocytochemistry of isolated cells was performed by the indirect immunofluorescence method. The cultures were washed twice with PBS and fixed with 4% paraformaldehyde in PBS for 15 min, then washed twice more with PBS at room temperature. The staining protocol and the primary and second antibodies were the same as described above.

Terminal dUDP nick end labeling (TUNEL) staining and cell counting

TUNEL staining was performed to assess DNA fragmentation using an Apop Tag® Peroxidase In Situ Apoptosis Detection Kit (S7100, Millipore, Billerica, MA, USA) and Apop Tag® Fluorescein In Situ Apoptosis Detection Kit (S7110, Millipore, Billerica, MA, USA). Counting of TUNEL positive cells was performed on high-power images ($\times 40$ magnification) taken using a digital camera. Counts were performed on 3 areas in each brain section.

Statistical Analysis

Measurements were performed by personnel blinded to treatment groups. Data were expressed as mean \pm standard deviation, and comparisons between groups were performed by Student *t* tests or one-way ANOVA as appropriate. Differences were considered significant at $p < 0.05$.

RESULTS

There were some CD163 positive cells in the ipsilateral grey matter, with fewer in contralateral grey matter at 4 hours after ICH. CD163 positive cells were located mostly in the perihematoma area and numbers increased at day 1, became abundant at day 3, and decreased at day 7 in the ipsilateral grey matter (Fig. 1A). CD163 protein levels were upregulated after ICH, it was peaked at day 3 (CD163/GAPDH: 0.57 ± 0.34 vs. 0.03 ± 0.03 at 4 hours, $P > 0.05$, 0.79 ± 0.43 at day 1, $P < 0.05$ and 0.23 ± 0.13 at day 7, $p > 0.05$) in grey matter (Fig. 1B). Compared to sham-operated animals and contralateral hemisphere, ICH induced significantly higher CD163 protein levels in the ipsilateral grey matter at day 3 after ICH (CD163/GAPDH: 0.78 ± 0.84 vs. 0.11 ± 0.04 in sham, $p < 0.05$, 0.01 ± 0.01 in contralateral grey matter, $p < 0.01$, Fig. 1C).

In order to determine whether CD163 expressed in neurons, double immunofluorescence staining was performed 24 hours after ICH. CD163 positive stained cells were co-localized with NeuN (a neuronal marker, Fig. 2), HO-2 (neuronal isoform of heme oxygenase, Fig. 2) and TUNEL (marker for double DNA strains damaged cells, Fig. 2) positive cells in the perihematoma area. **Many neurons were also TUNEL positive.** This indicated that CD163

expressed in the injured neurons after ICH. However, astrocytes did not express CD163 in this piglet ICH model (Fig. 2).

Systemic deferoxamine treatment significantly reduced CD163 immunoactivity and protein levels in the ipsilateral grey matter at day 1 (CD163/GAPDH: 0.04 ± 0.03 vs. 0.34 ± 0.14 in vehicle, $p<0.01$), day 3 (CD163/GAPDH: 0.09 ± 0.03 vs. 0.70 ± 0.48 in vehicle, $p<0.05$) and day 7 (CD163/GAPDH: 0.10 ± 0.05 vs. 0.58 ± 0.36 in vehicle, $p<0.05$) after ICH (Fig. 3A). Deferoxamine treatment reduced the numbers of the TUNEL positive cells in the perihemotoma region at day-1 (TUNEL positive cells 495.30 ± 22.82 vs. 258.50 ± 63.39 in vehicle, $p<0.01$, Fig. 3B). And there was no TUNEL positive cell in the sham operation group. ICH induced significantly upregulation of hemoglobin in ipsilateral grey matter at day 3 (hemoglobin/GAPDH: 1.46 ± 0.70 vs. 0.39 ± 0.54 in sham, $p<0.05$, Fig. 3C), and that was blocked by deferoxamine treatment (hemoglobin/GAPDH: 0.77 ± 0.27 vs. 1.46 ± 0.70 in vehicle, $p<0.05$, Fig. 3C).

To further confirm neuronal expression of CD163, primary cultured neurons were treated with hemoglobin (0, 5 or $20\mu\text{M}$) for 24 hours. Immunofluorescence double labeling revealed co-localization of CD163 with NeuN, a neuronal marker (Fig. 4A). Treatment with $5\mu\text{M}$ hemoglobin caused a slight decrease in neuronal number and mild morphological changes. In contrast, treatment with $20\mu\text{M}$ hemoglobin caused marked decreases in neuronal numbers, cell body shrinkage and axonal disruption. Also, most degenerating neurons expressed weaker NeuN immunoreactivity (Fig. 4A). Neuronal CD163 levels were upregulated significantly after treatment with hemoglobin for 24 hours (0.69 ± 0.03 in $5\mu\text{M}$, $p<0.05$ and 0.75 ± 0.12 in $20\mu\text{M}$, $p<0.01$ vs. 0.39 ± 0.11 in control, Fig. 4B).

To investigate the effect of deferoxamine on neuronal CD163 expression, cultured neurons were pretreated with deferoxamine ($5\mu\text{M}$) for 2 hours before hemoglobin ($20\mu\text{M}$) was administered. Immunocytochemistry revealed that axons were partially preserved in neurons that were pretreated with deferoxamine (Fig. 5A). Western blot analysis showed that CD163 levels were less in the deferoxamine-treated cells than in the vehicle-treated controls (0.39 ± 0.19 vs. 1.18 ± 0.64 , $p<0.05$; Fig. 5A). In addition, media LDH levels were significantly increased after treating neurons with $20\mu\text{M}$ of hemoglobin compared to controls (107 ± 37 vs. 18 ± 4 , $p<0.01$, Fig. 5B). This was attenuated by the pretreatment of deferoxamine (33 ± 7 vs. 107 ± 37 in vehicle, $p<0.01$, Fig. 5B).

DISCUSSION

The major findings of this study are as follows: (1) CD163 is expressed in neurons in grey matter; (2) in the ipsilateral grey matter, CD163 protein levels increased at day 1 after ICH, peaked at day 3 and decreased at day 7; (3) hemoglobin induced CD163 upregulation in cultured neurons; and (4) deferoxamine reduced ICH-induced CD163 expression *in vivo* and hemoglobin-induced neuronal CD163 expression *in vitro*.

The present study using a piglet model indicated that neurons can express CD163. **It is well known that CD163 is a hemoglobin receptor, predominantly expressed in microglia/macrophages². Our recent study showed that CD163 positive cells infiltrated into the**

hematoma from edge to center after ICH. A significant increase of CD163 positive cells in the hematoma was found at day 3 in the piglet model of ICH¹⁷. Recently, neuronal CD163 has been detected and may be involved in hemoglobin clearance after brain hemorrhage^{10, 13, 14}. Neuronal expression of high levels of HO-2 and heme can be metabolized by HO-2 inside neurons²³. Co-localization of HO-2 and CD163 revealed that both CD163 and HO-2 participate in hemoglobin degradation. Besides CD163-macrophage-HO-1 pathway, CD163-neuron-HO-2 pathway may also be responsible for the degradation of hemoglobin (Fig. 6). A difference in CD163 expression may explain why neurons are more vulnerable to hemoglobin toxicity than astrocytes¹¹.

Our results indicate that hemoglobin upregulates CD163 expression in neurons. In the pig model, there was little CD163 expression in the brain of sham animals and in the contralateral hemisphere after ICH. CD163 upregulation after ICH was time dependent. It was significantly upregulated at 24 hours and remained elevated at 3 days and then downregulated at 7 days. **Neuron-like CD163 positive cells were also found in the contralateral grey matter, which may result from hemoglobin release from the clot.** In the *in vitro* study, CD163 expression in cultured neurons was upregulated by hemoglobin in a dose-dependent manner. Therefore, ICH-induced increase of neuronal CD163 may result, at least in part, from hemoglobin release after ICH into perihematomal tissue as red blood cells lyse. DFX reduced the hemoglobin-induced increase of neuronal CD163 suggesting that iron may be involved in CD163 upregulation in neurons. **In should be noted, however, that microglia also expressed CD163 in grey matter.**

Hemoglobin is pivotal in the pathogenesis of ICH-induced brain damage²⁴. Hemoglobin uptake into neurons is through CD163. In addition to uptake of the hemoglobin-haptoglobin complex, CD163 can also facilitate the uptake of free hemoglobin². **A recent study found that haptoglobin increases hemoglobin-induced neuronal death *in vitro*¹⁴.** Some evidence also indicates that the neurotoxic effects of hemoglobin are HO-2 dependent. HO-2 is constitutively expressed by neurons and it accounts for most brain HO activity under normal conditions⁵. While HO-2 activity is not markedly increased over constitutive levels during exposure to hemoglobin²⁵, deletion of HO-2 isoform not only reduced oxidative stress but also conferred resistance of neurons to hemoglobin^{12, 26, 27}. Our current data showed many CD163 positive cells in the grey matter could be dying cells (TUNEL positive). **In the current study, however, we did not establish a causal relationship between neuronal CD163 and neuronal death. Whether CD163 is involved in ICH-induced brain cell death should be determined in the future studies.**

Deferoxamine is approved by the FDA for the treatment of acute iron intoxication and chronic iron overload in transfusion-dependent anemia. In the piglet ICH model, DFX treatment resulted in less hemoglobin accumulation, less ICH-induced upregulation of CD163 and less brain cell death in the grey matter. **Our previous study demonstrated that deferoxamine can reduce ICH-induced neuronal death²⁸.** There is evidence that deferoxamine can inhibit hemin-induced erythrocyte lysis²⁹ and lessen the rate of hematoma clearance in rats³⁰. Therefore, reduced CD163 upregulation in the deferoxamine-treated group may be due to less erythrocyte lysis and less hemoglobin release.

The effect of hemoglobin on neuronal CD163 was also examined in neuronal culture. **The nontoxic concentrations of deferoxamine are 0.6 to 5 μ M³¹, and the maximal nontoxic concentration of deferoxamine (5 μ M) was used in these experiments.** The current *in vitro* study indicated that deferoxamine suppressed the expression of CD163 induced by hemoglobin in neurons and decreased the hemoglobin-induced neuronal death. There may be a role of iron in CD163 modulation.

A number of preclinical studies have shown the neuroprotective effects of deferoxamine after ICH^{28, 30, 32}. Previous studies revealed that deferoxamine attenuates brain edema and reduces neuronal death after ICH³², but the underlying mechanisms are still not well understood. While the actions of deferoxamine may involve chelation of iron and reduced free radical production, it may also prevent neuronal death by stabilizing hypoxia-inducible factor-1 α (HIF-1 α) protein in neurons³³. In addition, in cultured neurons, some of the protective effects of deferoxamine were mediated by enhancing release of heme from neurons rather than an effect on heme intake³⁴. The present study that shows deferoxamine can also modulate brain CD163 levels. Thus, it appears that deferoxamine has multiple and diverse neuroprotective properties in relation to hemorrhagic insults.

The involvement of neurons in the immediate response to hemoglobin might be responsible for some of ICH symptoms. For example, seizures may occur when the hemoglobin degradation is impaired¹⁰. Therapies targeting at neuronal CD163 may protect those cells by limiting hemoglobin uptake reducing the oxidative stress induced by hemoglobin/iron. This merits further study. Salvaging neurons by targeting hemoglobin-induced injury may also bring clinical benefits.

In summary, we found that neurons express CD163 and that brain levels are increased after ICH. Neuronal CD163 can be upregulated by hemoglobin and may be associated with hemoglobin-induced neuronal death (Fig. 6). Deferoxamine can modulate CD163 levels and cell death *in vivo* and *in vitro*.

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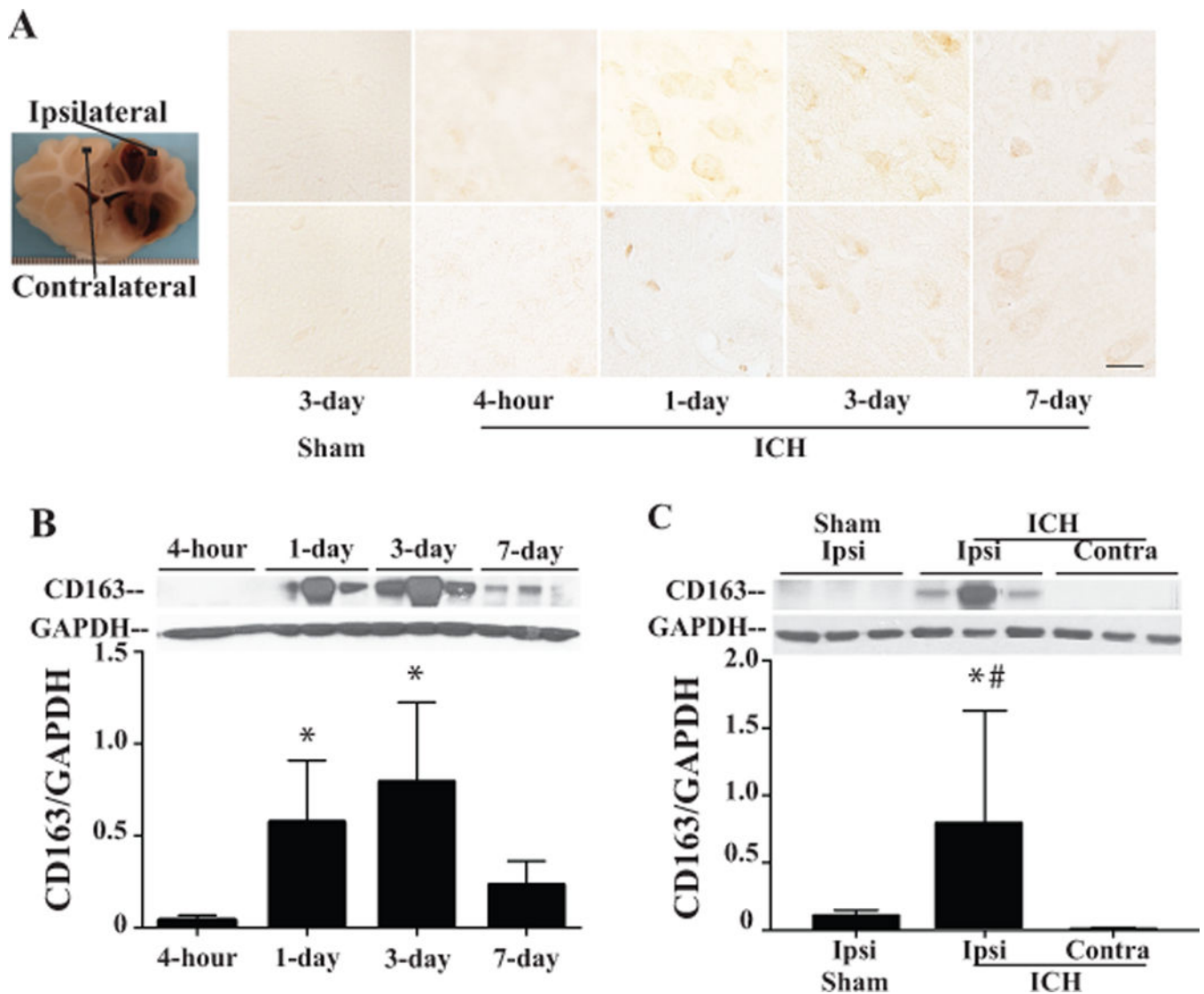


Figure 1.

Time course of CD163 immunoreactivity (A) and protein levels (B) in the ipsilateral and contralateral grey matter after 2.5ml of autologous blood injected into the right frontal lobe. Scale bar=50 μ m. Values are mean \pm SD, * p <0.05 vs. 4 hours. (C) Protein levels of CD163 in the ipsi- and contralateral grey matter after ICH, and in ipsilateral grey matter after sham operation at day 3. Values are mean \pm SD, * p <0.05, vs. the ipsilateral side of sham, # p <0.01 vs. the contralateral side of ICH.

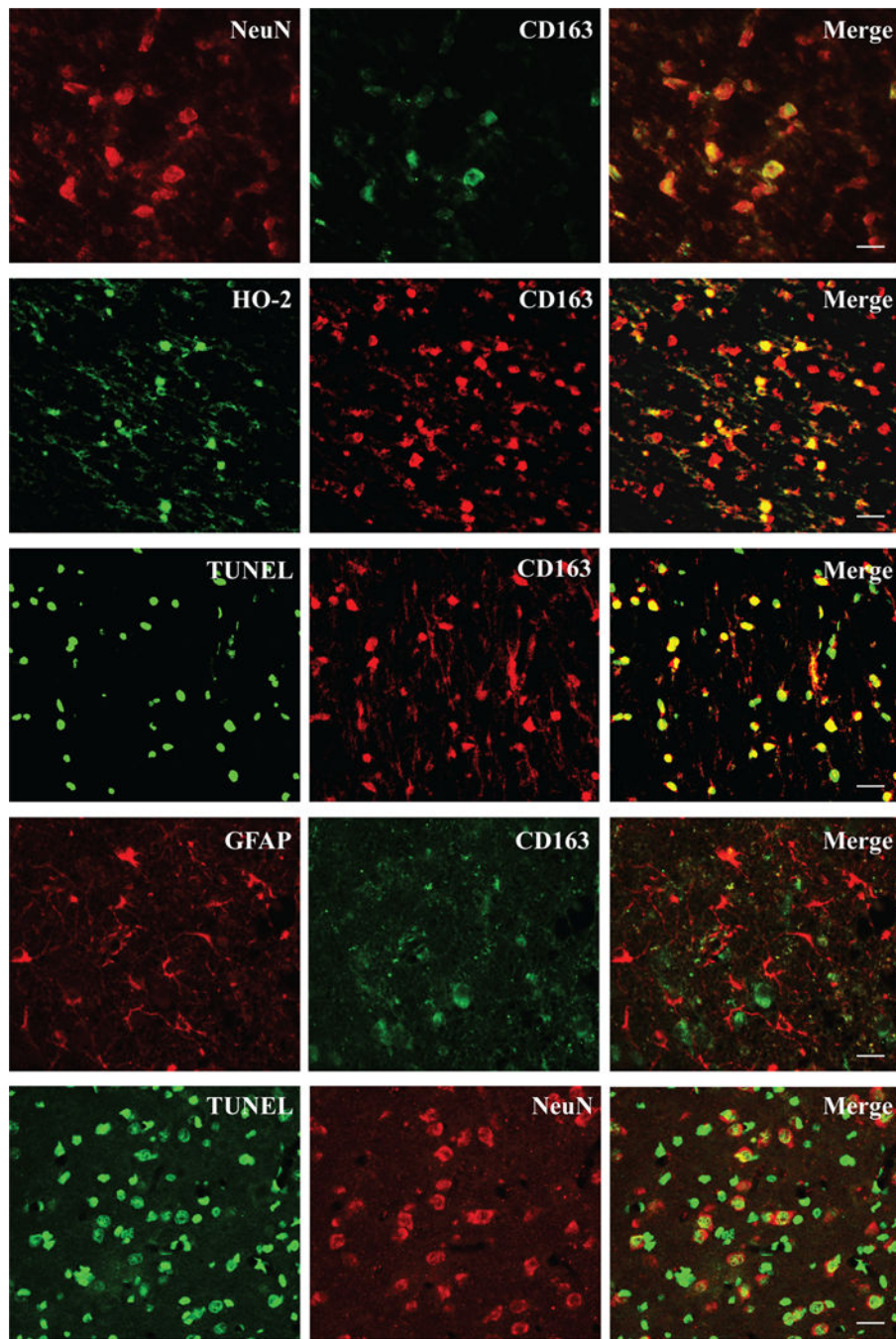


Figure 2. Double labeling of CD163 immunoreactivity with NeuN, HO-2, TUNEL, and GFAP positive cells in the perihematoma area at 24 hours after blood injection. **Double labeling was also performed for TUNEL and NeuN.** Scale bar= 50 μ m.

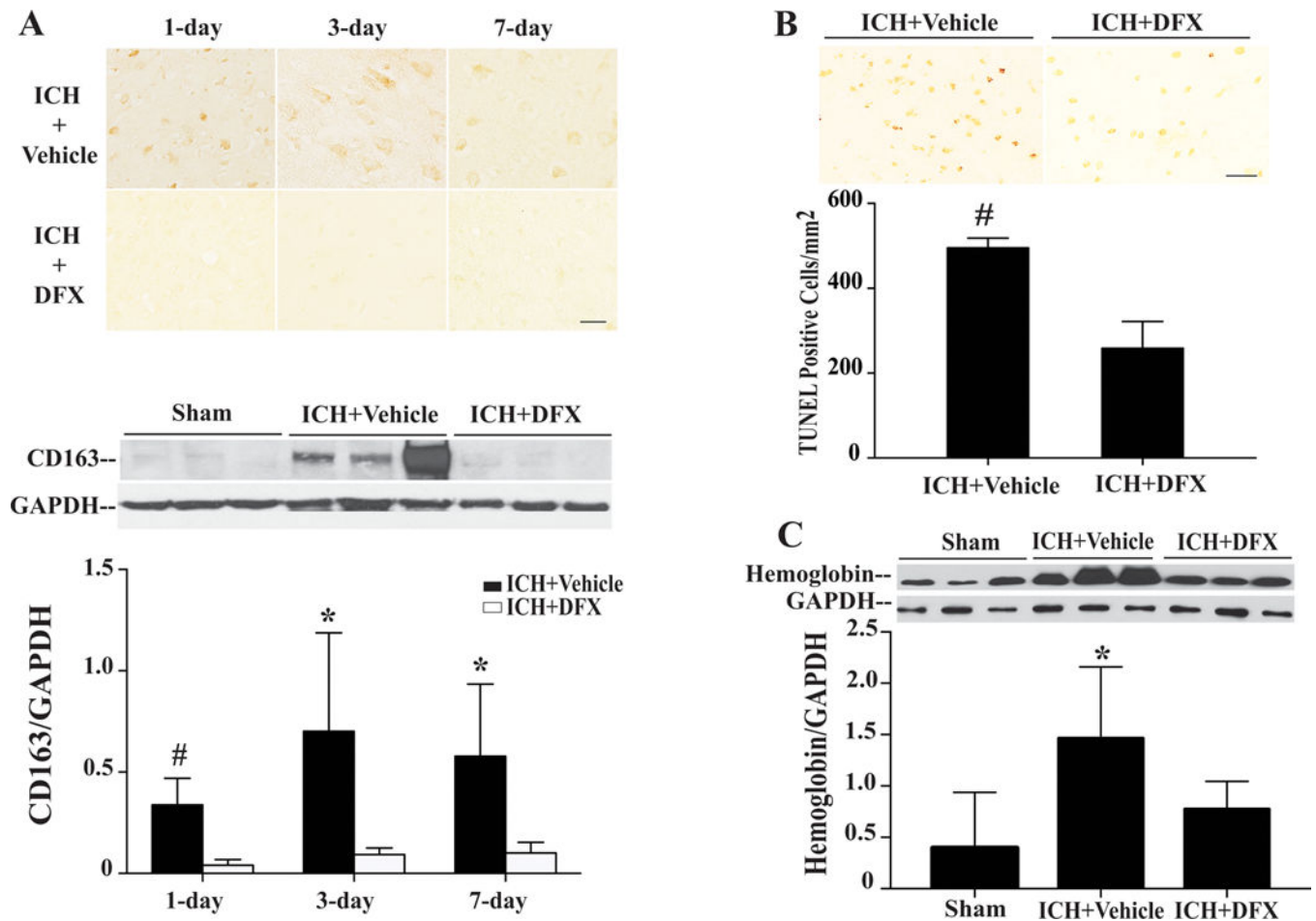


Figure 3. CD163 immunoreactivity and protein levels in the ipsilateral grey matter of piglets treated with deferoxamine or vehicle after ICH (A); numbers of TUNEL positive cells in the perihematoma cortex of piglets treated with deferoxamine or vehicle at day 1 after ICH (B); levels of hemoglobin in the ipsilateral grey matter of piglets at day 3 after ICH or sham operation treated with deferoxamine or vehicle (C). Scale bar= 50 μ m, values are mean \pm SD, # p <0.01, * p <0.05 vs. the DFX-treated group.

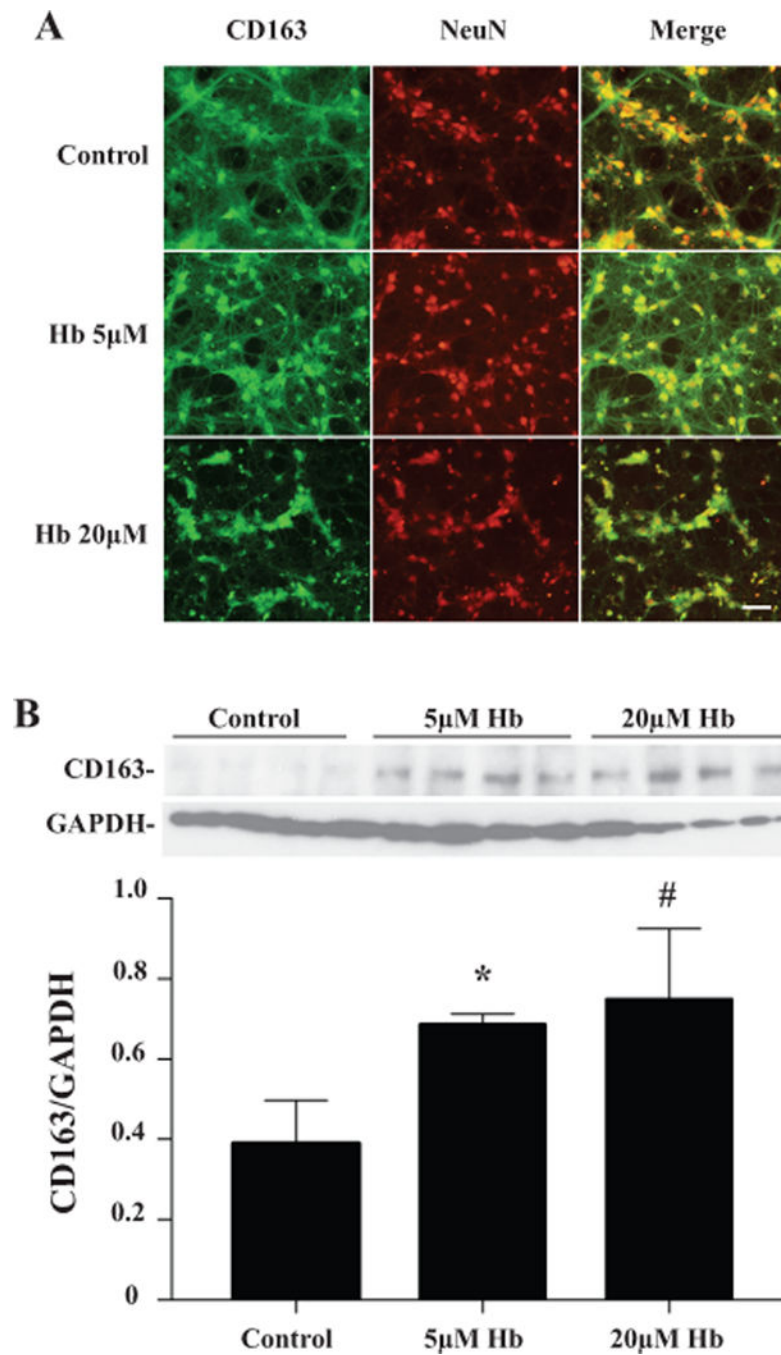


Figure 4. (A) Double labeling of CD163 and NeuN in primary cultured neuron treated with 0 (control), 5 or 20µM hemoglobin for 24 hours. Scale bar = 50µm. (B) Quantification of CD163 protein levels after hemoglobin exposure by Western blot. Values are mean ±SD, n=4, * p <0.05, # p <0.01 vs. the control.

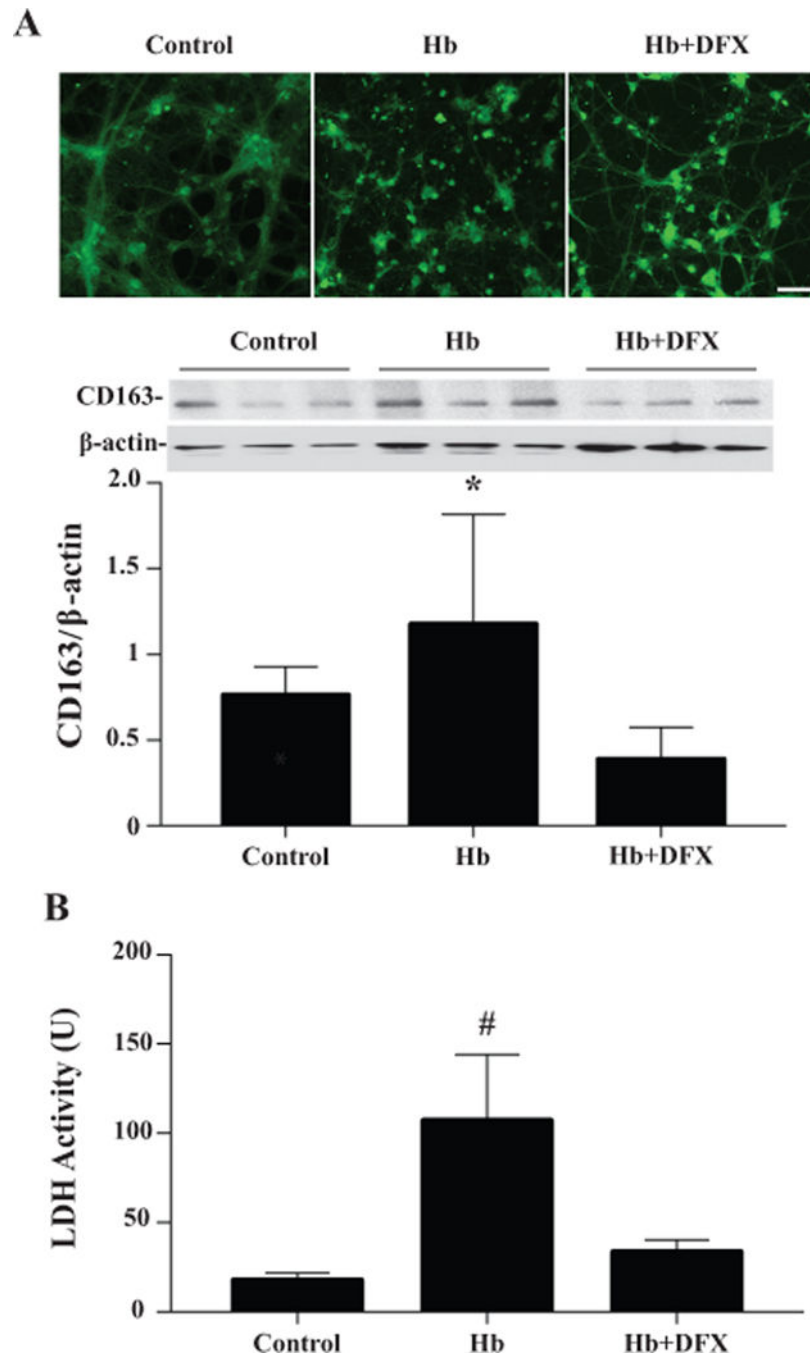


Figure 5. (A) CD163 immunoreactivity and protein levels in primary cultured neurons treated with hemoglobin (0 or 20 μ M) with or without deferoxamine (5 μ M) pretreatment. Scale bar= 50 μ m. (B) Media LDH levels 24 hours after the treatment of hemoglobin (0 or 20 μ M) with or without deferoxamine (5 μ M). Values are mean \pm SD, * p <0.05, # p <0.01 vs. the other groups.

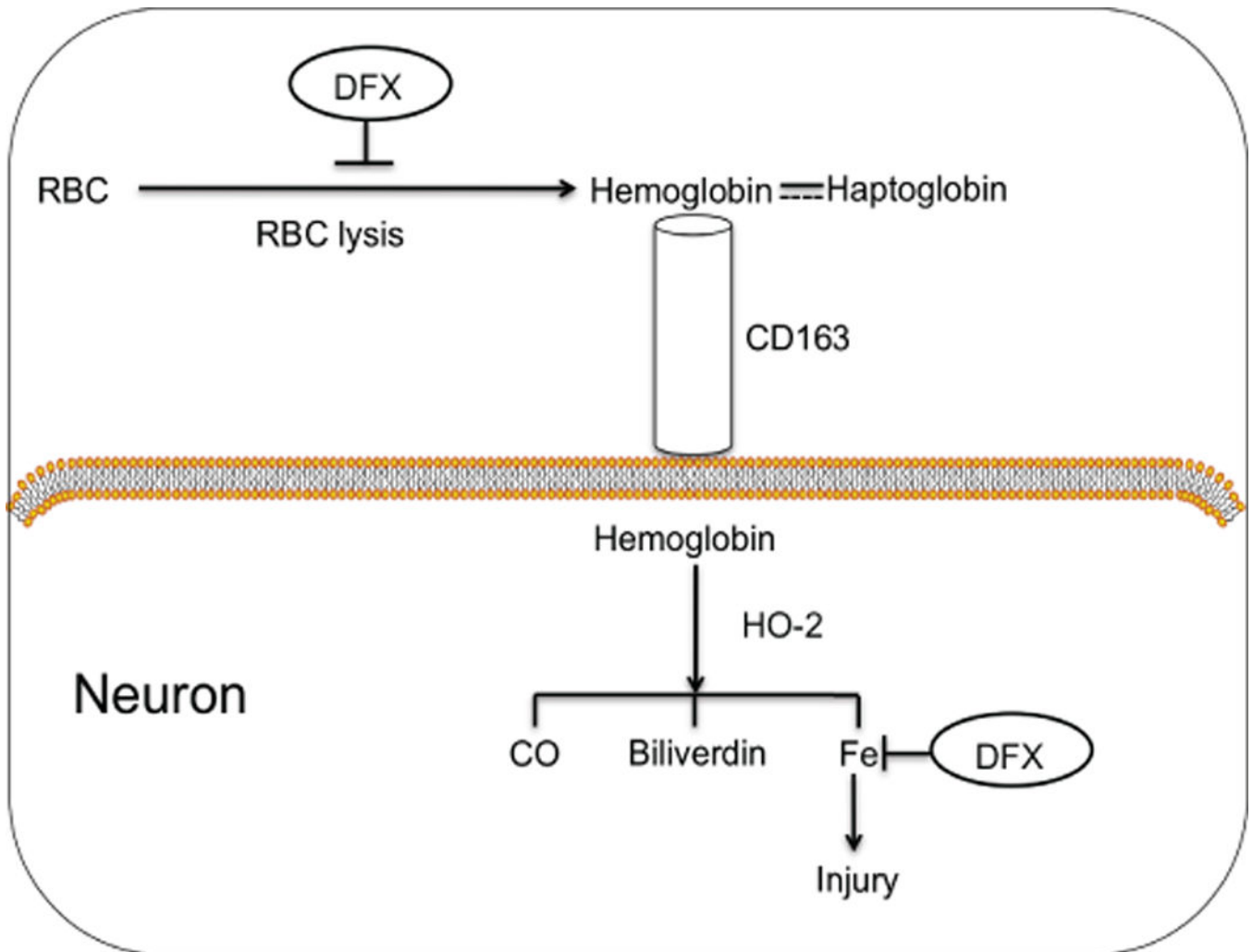


Figure 6. Model of the role of neuronal CD163 in relation to ICH and the effects of deferoxamine (DFX). Lysis of red blood cells (RBCs) in the clot leads to hemoglobin release into the extracellular space, which may be taken up into neurons via CD163 either alone or as part of a haptoglobin complex. Intracellularly, hemoglobin is degraded by heme oxygenase-2 (HO-2), producing iron, carbon monoxide and biliverdin. Deferoxamine may exert the neuroprotective effect by attenuating red blood cell lysis and iron chelation.