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Inhibiting the Na+/H+ exchanger reduces reperfusion injury: a small animal MRI study

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1. ABSTRACT

We used magnetic resonance imaging (MRI) to assess the efficacy of Na^+/H^+ exchanger isoform 1 (NHE-1) inhibition following cerebral ischemia. Transient focal cerebral ischemia was induced in wild-type controls (NHE-$1^{+/+}$), NHE-1 genetic knockdown mice (NHE-$1^{+/-}$), and NHE-$1^{+/+}$ mice treated with the selective NHE-1 inhibitor HOE642. Diffusion weighted imaging (DWI) revealed a brain lesion as early as 1 hour following reperfusion and illustrated significant protection in NHE-$1^{+/-}$ mice (16.2 +/- 7.9 mm^3 in NHE-$1^{+/-}$ mice vs. 47.5 +/- 16.6 mm^3 in NHE-$1^{+/+}$ mice). Knockdown of NHE-1 showed significantly smaller infarct at 72 hours on T2 imaging (21.2 +/- 12.6 mm^3 in NHE-$1^{+/-}$ mice vs. 64.6 +/- 2.5 mm^3 in NHE-$1^{+/+}$ mice). Administration of HOE642 prior to reperfusion or during early reperfusion reduced ischemic damage. Thus, high resolution T2 images can be used for consistent and precise calculation of lesion volumes, while changes of DWI are a sensitive early marker of ischemic injury. The results of this study demonstrate the therapeutic potential for inhibition of NHE-1 in treating cerebral ischemia.

2. INTRODUCTION

Loss of intracellular ionic homeostasis plays an important role in neuronal injury after cerebral ischemia (1). The Na^+/H^+ exchangers (NHEs) are a family of secondary active ion transport proteins responsible for maintaining normal intracellular pH and cell volume (2, 3). The NHEs catalyze the electroneutral exchange of Na^+ and H^+ ions across the cell membrane down their electrochemical gradients (3). Overstimulation of NHE following ischemia and reperfusion leads to a rise in intracellular Na^+, which subsequently triggers reverse mode operation of the Na^+/Ca^{2+} exchanger and Ca^{2+}-dependent cell death (4). Pretreatment of gerbils with the amiloride derivative ethylisopropylamiloride (EIPA), a non-selective NHE inhibitor, significantly reduces the extent of CA1 pyramidal neuron loss following global ischemia (5). Inhibition of NHEs with a non-specific NHE inhibitor SM-20220(N-aminoiminomethyl-1-methyl-1-indole-2-carboxamide methanesulfonate) significantly attenuates brain Na^+ and water content following 2 hours of

transient focal ischemia and 4 hours of reperfusion in rats (6).

NHE isoform 1 (NHE-1) activation has been suggested to play a role in cerebral ischemic cell damage (7, 8). Genetic ablation of NHE-1 attenuates intracellular Na^+ and Ca^{2+} accumulation in neuronal cultures after oxygen and glucose deprivation (OGD) and results in less cell death (9). Pharmacologic inhibition of NHE-1 by administration of its potent inhibitor HOE642 prior to ischemic induction is neuroprotective in the mouse model of transient focal ischemia (9, 10). Interestingly, inhibition of NHE-1 blocks intracellular Na^+ overload during the post-OGD period (reoxygenation phase) (11), suggesting a role for NHE-1 activation in reperfusion injury.

In this study, we used magnetic resonance imaging (MRI) to assess development of brain lesion during reperfusion and the efficacy of NHE-1 inhibition in neuroprotection. We chose to use the inhibitor HOE642 because it is a potent and specific inhibitor of the NHE-1 isoform (12). T2 and diffusion weighted MRI (DWI) revealed that either genetic knockdown or pharmacologic inhibition of NHE-1 was neuroprotective as early as 1 hour reperfusion, illustrating the therapeutic potential for targeting NHE-1 following cerebral ischemia.

3. MATERIALS AND METHODS

3.1. Animal preparation

$NHE\text{-}1^{+/+}$ and $NHE\text{-}1^{+/-}$ were obtained by breeding $NHE\text{-}1^{+/-}$ heterozygous mice as described before (9). The NHE-1 transgenic mouse line (SV129/Black Swiss) was established previously (13). The genotype of each mouse was determined by a polymerase chain reaction (PCR) of DNA from tail biopsies as described before (14). A total of 44 adult mice were used in this study. Animals that died after surgery (n = 3) or developed cerebral hemorrhage (n = 5) were excluded from the study. All animal procedures used in this study were conducted in strict compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Wisconsin Center for Health Sciences Research Animal Care Committee.

3.2. Focal ischemic model

Focal cerebral ischemia was induced by occlusion of the left middle cerebral artery (MCA), as described previously (15). Mice were anesthetized with 3% isoflurane vaporized in N_2O and O_2 (3:2) for induction and 1.5% isoflurane for maintenance. The left common carotid artery was exposed and the occipital artery branches of the external carotid artery were isolated and coagulated. The internal carotid artery was isolated and the extracranial branch was dissected and ligated. A polyamide resin glue-coated suture (6–0 monofilament nylon) was used to block the MCA blood flow. Regional cerebral blood flow (rCBF) was measured with a laser Doppler probe. Changes in rCBF at the surface of the left cortex were recorded using a blood perfusion monitor (Laserflo BPM^2, Vasamedics, Eden Prairie, MN, USA). For reperfusion, the suture was withdrawn after 30 – 60 min MCAO. The incision was closed and the mice recovered under a heating lamp to ensure that the core temperature (36.0 – 37.0 °C) was maintained during recovery. Animals were randomly assigned into the control and drug-treated groups. In the HOE642 treated animals, 0.5 mg/kg of HOE642 in saline containing 1% DMSO was initially administered by intraperitoneal (IP) injection 30 min prior to the onset of reperfusion or at 1 hour after reperfusion. To maintain the optimal plasma concentration (half-life of HOE642 is 40 min in rats (16)), the same dose of HOE642 was administered at 24 and 48 hours after reperfusion. In the control group, an equivalent volume of 0.9% NaCl was administered by IP injection at the same time points.

3.3. Magnetic Resonance Imaging

MRI was performed using a Varian 4.7T Small Animal MRI scanner. Mice were anesthetized with 1.5% isoflurane in an oxygen/air mixture administered through a nose cone and then secured in a cradle positioned within the center of the magnet bore. Respiratory rate and body temperature were monitored with a MR-compatible physiology monitoring unit, and temperature was maintained within physiologic limits (37.0 ± 0.2 °C) using a heated airflow unit. Serial imaging was performed over the 72 hours of reperfusion after MCAO. T2-weighted fast spin-echo images [repetition time (TR) = 4000 ms, effective echo time (TE) = 60 ms, echo train length = 8, matrix size 128 × 128, averages = 40] were acquired in twelve contiguous axial slices with a field of view (FOV) = 17 × 17 mm and a slice thickness of 1 mm. Maps of the apparent diffusion coefficient (ADC) were calculated from a set of diffusion-weighted spin-echo images (matrix size = 32 × 64, TR = 1600 ms, TE = 60 ms, averages = 3, b value = 0 and 1125 s/mm^2). The total acquisition time for the entire set of MR scans was approximately 27 minutes per animal at each experimental time point.

3.4. Calculation of infarct volume

After 72 hours of reperfusion, mice were anesthetized with 5% isoflurane vaporized in N_2O and O_2 (3:2) and then decapitated. Brains were removed and frozen at -80°C for 5 minutes. Two-millimeter coronal slices were made with a rodent brain matrix. The sections were stained for 20 min at 37°C with 2% 2, 3, 5-triphenyltetrazolium chloride monohydrate (TTC). In a blinded manner, MR images and TTC stained brain sections were analyzed using ImageJ software (NIH). The ischemic lesion was traced in each slice, and the total volume of infarction was calculated with correction for edema as described by Swanson (17). Briefly, the ischemic area for each slice was calculated by subtracting the non-infarct area in the ipsilateral hemisphere from the total area of the contralateral hemisphere. The infarct areas were summed across all slices, and multiplied by the slice thickness giving the total infarct volume (mm^3).

3.5. Pure cortical neuron cultures

Pure cortical neurons from embryonic day 14-16 mouse fetuses (SV129/Black swiss) were prepared as described previously (18). The cortices were removed from E14-16 fetuses and treated with 0.5 mg/ml trypsin at 37ºC for 25 min. The cells were centrifuged at 300 g for 4

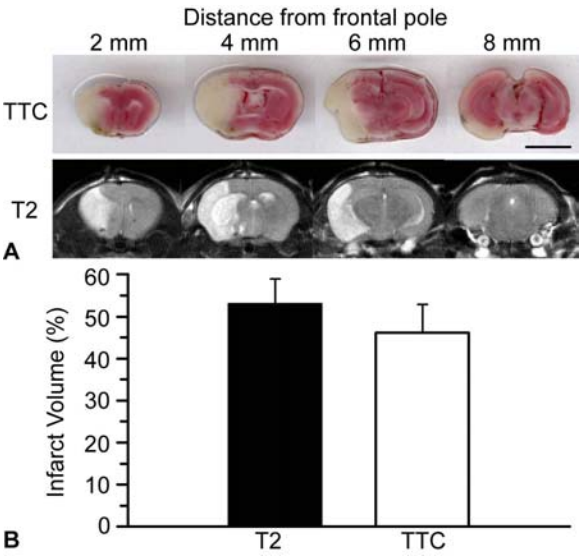

Figure 1. Correlation of T2 images with TTC staining. A. Representative TTC stained sections and T2 weighted images from NHE-$1^{+/+}$ brains at 72 hours of reperfusion after 30 minute MCAO. Images shown are coronal sections 2 mm, 4 mm, 6 mm and 8 mm from the frontal pole. Scale bar = 5 mm. B. Summarized data comparing infarct volume calculated from T2 images and TTC stained sections. Infarct volumes are expressed as percentage of contralateral hemispheres (mean ± S.D.), n = 4.

minutes. The cell pellet was diluted in B-27 supplemented neurobasal medium (2%) containing 0.5 mM L-glutamine and penicillin/streptomycin (100 units/ml and 0.1 mg/ml, respectively). The cells were seeded at a density of 1 x 10^5 cells/ cm^2 on glass coverslips in 6-well plastic plates coated with poly-D-lysine. The cultures were maintained in an incubator (model 3130, Thermo Forma, Waltham, MA) with 5% CO_2 and atmospheric air at 37ºC. Half of the medium was replaced twice a week. DIV 10-15 cultures (days in culture) were used in the study.

3.6. Oxygen and glucose deprivation (OGD) treatment

DIV 10-15 neuronal cultures grown on coverslips in 6-well plates were rinsed with an isotonic OGD solution (pH 7.4) containing (in mM): 0 glucose, 26 $NaHCO_3$, 120 NaCl, 5.36 KCl, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 1.27 $CaCl_2$, and 0.81 $MgSO_4$. This solution has a K^+ concentration (~ 5.8 mM) which is similar to the neurobasal medium (5.6 mM) used for cell cultures. The cells were incubated in 1 ml of OGD solution for 2 hours in a hypoxic incubator (model 3130, Thermo Forma) containing 94% N_2, 1% O_2, and 5% CO_2. Normoxic control cells were incubated for 2 hours in 5% CO_2 and atmospheric air in a buffer identical to the OGD solution except for the addition of 5.5 mM glucose. Reoxygenation (REOX) was achieved by addition of glucose (5.5 mM) and incubation at 37ºC in 5% CO_2 and atmospheric air.

3.7. Neuronal mortality measurement

Cell viability was assessed by propidium iodide (PI) uptake and retention of calcein as described previously (9). Briefly, cultured neurons were rinsed and incubated with 1 µmol/L calcein-AM and 10 µg/ml PI in HEPES-MEM at 37ºC for 30 minutes. Cells were then rinsed and visualized using a Nikon TE 300 inverted epifluorescence (Tokyo, Japan) and 20X objective lens. Calcein and PI fluorescence signals were obtained using FITC filters and Texas Red filters, respectively. Images were collected using a Princeton Instruments (Trenton, NJ) MicroMax CCD camera. In a blind manner, a total of 1000 cells/condition were counted using MetaMorph image-processing software (Universal Imaging Corp., Downingtown, PA). Cell mortality was expressed as the ratio of PI-positive cells to the sum of calcein-positive and PI-positive cells.

3.8. Statistics

Values are expressed as the mean ± S.D. Statistical analysis was performed using Mann-Whitney rank sum test, or ANOVA (the Bonferroni post test) in the case of multiple comparisons (SigmaStat, Systat Software, Point Richmond, CA, USA). A p-value smaller than 0.05 was considered statistically significant.

4. RESULTS

4.1. Correlation of T2 images with TTC staining

Brain infarct volume was determined in NHE-$1^{+/+}$ brains at 72 hours reperfusion by T2-weighted MRI and TTC staining following 30 minutes of MCAO. Qualitatively, the mature infarct seen on T2 images correlated closely in size and extent of lesion with TTC staining (Figure 1 A). In comparing lesion volumes determined by TTC and T2 MRI, lesion volumes were corrected for edema and normalized to the contralateral hemisphere by expressing lesion volume as a percentage of the contralateral hemispheric volume. There was no significant difference in lesion volumes calculated from TTC staining or T2 MRI (53.0 ± 5.8% vs. 46.2 ± 6.7%, n = 4, p = 0.18, Figure 1 B).

4.2. NHE-$1^{+/-}$ mice demonstrate smaller infarct on MRI

Diffusion weighted and T2 weighted MRI were performed at 1, 6, 24, 48, and 72 hours of reperfusion after 30 minutes of MCAO in NHE-$1^{+/+}$ and NHE-$1^{+/-}$ mice. A decrease in ADC on diffusion images and an increase in T2 signal intensity on T2 images reflects ischemic brain lesion. Images in Figure 2 were obtained from a brain section in the center of the ischemic core [3.8 mm rostral and 5.8 mm dorsal to the interaural line (19)]. On T2 images, a lesion was first visible at 6 hours of reperfusion in both groups, with increasing signal intensity over the following 72 hours and little increase in size of the lesion. The NHE-$1^{+/+}$ mice demonstrated an extensive injury throughout the affected hemisphere. In contrast, in the NHE-$1^{+/-}$ mice, the infarct was confined to the striatum and thalamus with sparing of the cortex **(**Figure 2 A**)**. The final infarct volume at 72 hours measured by T2 imaging was significantly smaller in the heterozygous animals (21.2 ± 12.6 mm^3 in NHE-$1^{+/-}$ mice vs. 64.6 ± 2.5 mm^3 in NHE-$1^{+/+}$ mice, n = 4, p < 0.005, Figure 2 C).

An ADC lesion was apparent in both groups at 1 hour of reperfusion (Figure 2 B). The ischemic volume at 1

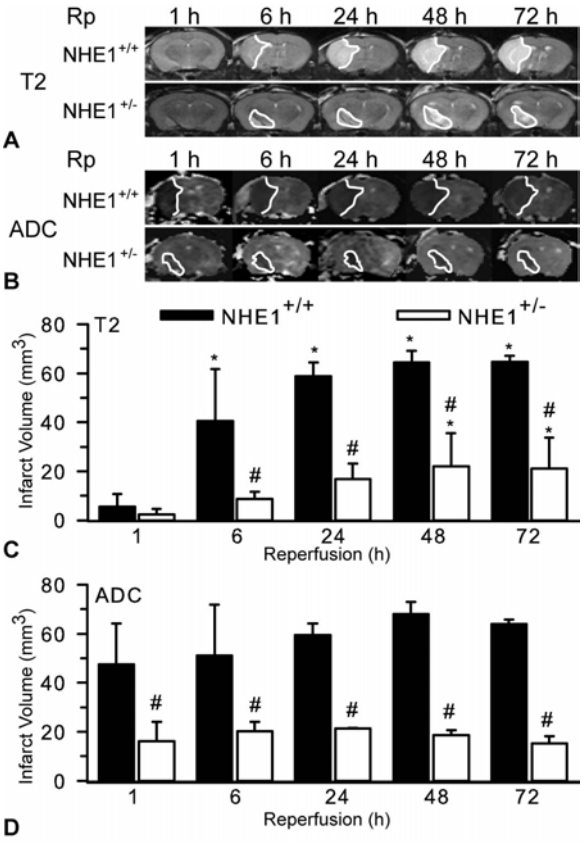

Figure 2. Time-course of ischemic damage 1-72 hours of reperfusion in NHE-$1^{+/+}$ and NHE-$1^{+/-}$ mice. A representative T2 (A) and ADC (B) image from the center of the ischemic lesion at 1, 6, 24, 48, and 72 hours of reperfusion following 30 minutes MCAO. Summarized data for the total infarct volume are shown for T2 (C) and ADC images (**D**). A lesion on the ADC images was consistently visible beginning at 1 hour of reperfusion, while the first apparent change on T2 images was at 6 hours of reperfusion. NHE-$1^{+/-}$ mice exhibited significantly smaller lesion volumes on ADC and T2 images. Values are expressed as the mean ± S.D., n = 4, * $p < 0.05$ vs. 1 hour time-point, # $p < 0.05$ vs. NHE-$1^{+/+}$ controls.

hour of reperfusion was significantly smaller in the NHE-$1^{+/-}$ mice (16.2 ± 7.9 mm^3 in NHE-$1^{+/-}$ mice vs. 47.5 ± 16.6 mm^3 in NHE-$1^{+/+}$ mice, $p < 0.05$, Figure 2 D). This degree of neuroprotection in NHE-$1^{+/-}$ mice was similar to that detected at 72 hours of reperfusion using T2 imaging (66% for ADC and 67% for T2).

4.3. Inhibition of NHE-1 with HOE642 is protective when administered prior to reperfusion

HOE642 in saline was administered in NHE-$1^{+/+}$ mice at 30 minutes prior to reperfusion, and again at 24 and 48 hours of reperfusion. T2 imaging was performed at 72 hours of reperfusion following 60 minutes of MCAO. In these studies, to determine the neuroprotective effects after a more severe insult, MCAO duration was increased to 60 min. A large infarct was again detected in NHE-$1^{+/+}$ mice treated with saline, affecting cortex, striatum, and thalamus. In contrast, HOE642-treated animals demonstrated a smaller lesion which was confined to thalamus and striatum (Figure 3 A). NHE-1 inhibition with HOE642 resulted in a 78% reduction in lesion volume, compared to saline treated control animals at 72 hours of reperfusion as measured by both T2 MRI and TTC staining (n = 5, $p < 0.05$, Figure 3 B and 3 C).

4.4. Inhibition of NHE-1 with HOE642 remains protective when administered during early reperfusion

In the next study, we investigated the HOE642 efficacy in post ischemia injury. First, inhibition of NHE-1 activity was tested in the *in vitro* model of ischemic neuronal damage (OGD/REOX). Pure cortical neuron culture exhibited ~ 70% cell death at 24 hours REOX following 2 hours OGD (Figure 4 A and B**)**. In contrast, addition of HOE642 (1 μM) at 0-2 hours REOX reduced cell death (at 24 hour REOX) by 40-50% ($p < 0.05$). This implies that post-ischemic application of NHE-1 inhibitors may remain effective following cerebral ischemia. Therefore, additional experiments were performed in NHE-$1^{+/+}$ mice with HOE642 administered at 1 hour reperfusion. Consistent with the *in vitro* studies, MRI analysis performed at 48 hours after injury revealed significant neuroprotection in the mice treated with HOE642 at 1 hour post-ischemia (Figure 4 C and D**,** n = 3, $p < 0.05$). At 72 hour reperfusion, TTC staining confirmed a ~ 60% reduction in infarct volume (Figure 4 C and D, $p = 0.057$**)**. The 48 hour reperfusion time-point was chosen for these imaging experiments based on the observation that brain lesion size detected with T2 imaging remained unchanged from 24-72 hours reperfusion (Figure 2). Taken together, these data indicate that administration of HOE642 at early reperfusion reduced ischemic brain damage. This study suggests the therapeutic potential for inhibition of NHE-1 in the treatment of cerebral ischemia during early reperfusion.

5. DISCUSSION

5.1. Quantification of ischemic brain lesion with MRI

In the current study, both T2 and DWI were used to assess ischemia-reperfusion injury in NHE-$1^{+/+}$ and NHE-$1^{+/-}$ mice. NHE-1 is the most abundant isoform of NHE in the CNS (20). The use of NHE-1 genetic knockout mice in these studies is not possible, as NHE-$1^{-/-}$ mice develop intractable seizures and die 1-2 weeks after birth (13). Consequently, we used NHE-$1^{+/-}$ mice, which exhibit a 70% reduction in NHE-1 protein expression (9).

It has been established that DWI is a sensitive early measure of neuronal ischemia (21). The reduction in ADC is considered to represent cytotoxic edema formation resulting from the loss of ionic homeostasis after ischemia (22). Clinically, DWI has become an integral part of diagnosis and management of acute stroke (23). We report here that DWI detected a brain lesion as early as 1 hour of reperfusion. Moreover, NHE-$1^{+/-}$ mice exhibited a 70% reduction in ADC lesion size at 1 hour of reperfusion, which remained unchanged by 72 hours reperfusion. Thus, the changes in ADC during early reperfusion can accurately predict the degree of damage at 72 hours reperfusion. Our

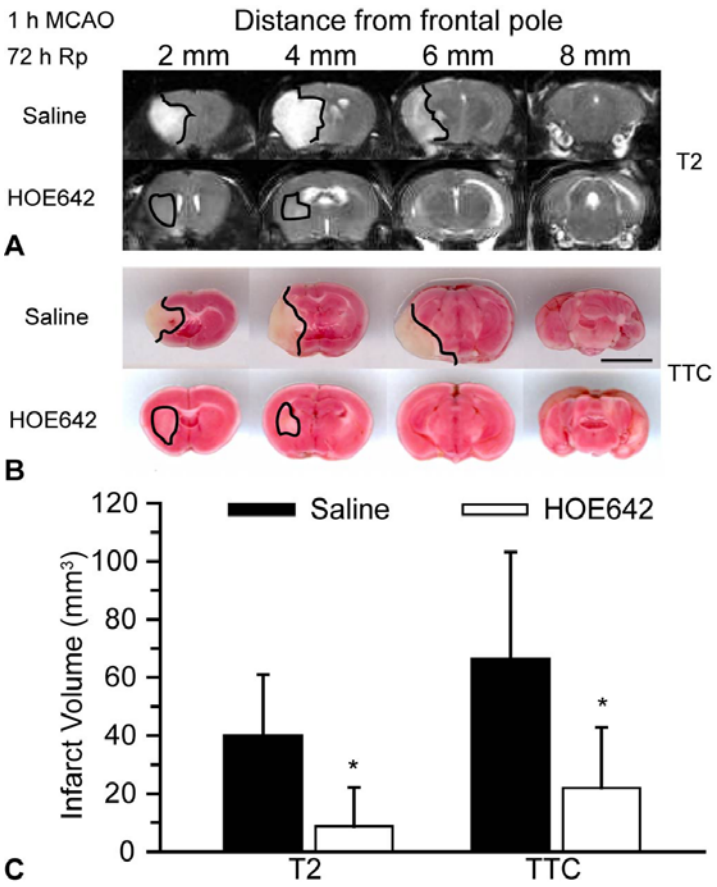

Figure 3. Reduced ischemic injury in NHE-$1^{+/+}$ mice treated with HOE642 prior to reperfusion. Representative T2-weighted images (A) and TTC stained sections (B) of NHE-$1^{+/+}$ saline control and NHE-$1^{+/+}$ mice treated with HOE642. Coronal sections (2 mm, 4 mm, 6 mm and 8 mm from the frontal pole) are shown in TTC staining at 72 hours of reperfusion after 1 hour MCAO. Scale bar = 5 mm. **C**. Summarized data. Values are expressed as the mean ± S.D., n = 5. * $p < 0.05$ vs. saline control.

DWI findings identified a reduction of cytotoxic edema formation after NHE-1 inhibition, and suggest that DWI is an ideal non-invasive measure of early ischemic injury.

Moreover, our results show that an ischemic lesion was first visible on T2 images at 6 hours of reperfusion. NHE-$1^{+/-}$ mice consistently demonstrated a significant reduction in lesion size at all tested time-points between 6 and 72 hours of reperfusion. Both qualitatively and quantitatively, the T2 images of stroke in these animals closely correlated with the infarct volume determined by TTC staining, but we are aware that the presence of microglia/macrophage in the lesioned brain tissue may result in underestimation of infarct volume by TTC. Recently, Na^+ magnetic resonance imaging has demonstrated a time-dependent increase in Na^+ signal within the ischemic lesion in acute stroke patients and in preclinical animal studies (24,22). Our study implies that excessive stimulation of NHE-1 may contribute to the Na^+ overload after cerebral ischemia.

5.2. Neuroprotection mediated by HOE642

It has been established in experimental animal models that inhibition of NHE-1 attenuates the detrimental consequences of myocardial ischemia and reperfusion, such as arrhythmias, contractile dysfunction, and tissue necrosis (12, 25, 26). The principal mechanism underlying the cardioprotective actions of NHE-1 inhibition is the attenuation of intracellular Na^+ accumulation, which in turn may reduce the rise of intracellular Ca^{2+} that occurs during both ischemia and subsequent reperfusion (4).

We have previously shown that in NHE-$1^{+/+}$ neurons, OGD causes a 2-fold increase in $[Na^+]_i$, and 60 min of REOX triggers a 7-fold increase in $[Na^+]_i$ (9). Genetic ablation of NHE-1 or HOE642 treatment reduces the REOX-mediated second phase of Na^+_i rise by ~40-50%, accompanied by a significant reduction in cell death (9). Moreover, NHE-1 activity in astrocytes is also stimulated and accompanied by a five-fold rise in $[Na^+]_i$ and 26% swelling during reoxygenation following 2 hours OGD (14). In addition, a rise in $[Na^+]_i$ subsequently leads to Ca^{2+} influx via the reversed Na^+/Ca^{2+} exchange (NCX_{rev}). Pharmacologic inhibition of NHE-1 activity or genetic ablation of NHE-1 significantly attenuates Na^+ and Ca^{2+} influx, astrocyte swelling, and mitochondrial damage following OGD/REOX (14, 27). The observed reduction in changes of ADC and T2 with NHE-1 inhibition in the

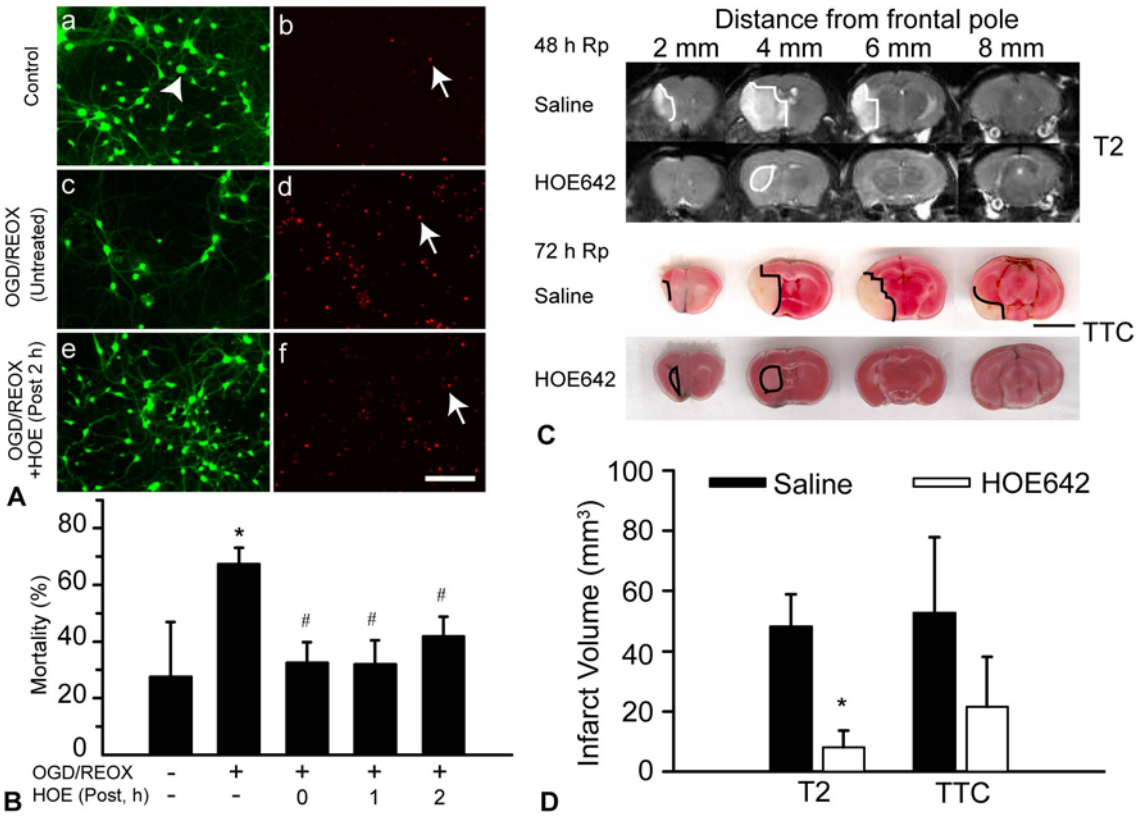

Figure 4. Neuroprotective effects of HOE642 administered post-ischemia. A. Cell mortality was assessed in NHE-$1^{+/+}$ neurons at 24 hours of reoxygenation (REOX) following 2 hours of OGD. For HOE642 treatment, 1 µM HOE642 was added in the medium at 0, 1, or 2 hours REOX. Sister NHE-$1^{+/+}$ cultures were incubated for 24 hours in normoxic control buffer. Live cells were stained with calcein-AM (a, c, e) and dead cells stained with propidium iodide (b, d, f), and cell images were acquired. Arrowhead: living cell. Arrow: dead cell. Scale bar = 100 µm. B. Summary data. n = 1000 cells from 3 cultures. * $p < 0.05$ vs. normoxic control, # $p < 0.05$ vs. untreated OGD/REOX. C. Representative T2-weighted images of coronal brain sections at 48 hours of reperfusion and TTC staining at 72 hours of reperfusion after 1 hour MCAO. HOE642 (0.5 mg/kg, IP) was administered at 1, 24, and 48 hours after reperfusion. Scale bar = 5 mm. D. Summary data. Values are expressed as mean ± S.D., n = 3. * $p < 0.05$ vs. saline control.

current study likely reflects the decrease of intracellular Na^+ and Ca^{2+} loading as well as cytotoxic edema in neurons and astrocytes during early reperfusion.

HOE642 (Cariporide) is safe for use in humans and has been studied extensively for its cardioprotective effects, including in two large clinical trials, the GUARDIAN study and the EXPEDITION trial (28, 29, 30). These studies demonstrated the cardioprotective efficacy of Cariporide in the setting of high-risk coronary artery bypass surgery. NHE-1 inhibition in these patients resulted in a 25% reduction in risk for myocardial infarction in the 6 months following surgery (30). However, while the GUARDIAN Study reported no significant increase in cerebrovascular events (29), the cardioprotection afforded by HOE642 in the EXPEDITION trial was tempered by non-cardiac adverse effects including cerebrovascular events (30). It has been speculated that this adverse effect may result from either 1) severe acidosis due to NHE-1 inhibition or 2) an abrupt withdrawal of Cariporide leading to a rebound effect and resulting in rapid activation of the exchanger and platelet hyperactivity (30). The results of these clinical trials highlight the need for further studies to better understand the role of NHE-1 function in cerebral ischemia-reperfusion injury. In the current study, in contrast to the adverse effects of NHE-1 inhibition seen in the EXPEDITION trial (30), our findings clearly demonstrate that inhibition of NHE-1 activity is neuroprotective in cerebral ischemia. Our dosing strategy was similar to that used in the clinical Cariporide trials (29). Additional studies are warranted to further assess the therapeutic window of NHE-1 inhibition after cerebral ischemia, and to delineate the beneficial and adverse effects of NHE-1 inhibition in ischemic brains, especially regarding the possible effects on cerebral vascular function and platelet hyperactivity.

In summary, small animal MRI is useful in evaluating the evolution of brain injury following transient focal ischemia. High resolution T2 images can be used for consistent and precise calculation of lesion volumes, while DWI serves as a sensitive early marker of ischemic injury.

This study is the first to non-invasively characterize the neuroprotection mediated by inhibition of NHE-1 over the 72 hours following reperfusion. We report here that both genetic knockdown of NHE-1 and administration of HOE642 prior to reperfusion or at 1 hour of reperfusion are neuroprotective after cerebral ischemia. This study demonstrates the therapeutic potential for inhibition of NHE-1 in stroke treatment.

6. ACKNOWLEDGEMENTS

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Abbreviations: MRI: magnetic resonance imaging; NHE: Na+/H+ exchanger; DWI: diffusion weighted imaging; OGD: oxygen and glucose deprivation; PCR: polymerase chain reaction; MCA: middle cerebral artery; rCBF: regional cerebral blood flow; IP: intraperitoneal; TR: repetition time; TE: echo time; FOV: field of view; ADC: apparent diffusion coefficient; TTC: 2, 3, 5-triphenyltetrazolium chloride monohydrate; DIV: days in culture; REOX: reoxygenation; PI: propidium iodide; Rp: reperfusion. NCX: Na^+/Ca^{2+} exchanger

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