Intracerebral Hematomas Disappear on T2*-Weighted Images During Normobaric Oxygen Therapy

Thomas Gaberel, MD, MSc; Clement Gakuba, MD, MSc; Marie Hebert, MSc; Axel Montagne, PhD; Veronique Agin, PhD; Marina Rubio, PhD; Evelyne Emery, MD, PhD; Denis Vivien, PhD; Maxime Gauberti, PhD

Background and Purpose—The aim of the present study was to investigate the effects of normobaric oxygen (NBO) therapy on T2*-weighted images of intracranial hemorrhages (ICHs).

Methods—Two common models of ICH were performed in mice, and longitudinal T2*-weighted images of the hematomas were acquired under normoxia or NBO. The effects of NBO were also investigated on perfusion-weighted imaging, susceptibility-weighted imaging, and molecular imaging of vascular cell adhesion molecule-1 after ICH. Last, we performed neurological testing, including neuroscore, actimetry, and gait analysis (Catwalk), to study the influence of NBO on neurological outcome of mice presenting ICH.

Results—Our results demonstrated that NBO, even during a short period of time, dramatically reduces the sensitivity of T2*-weighted imaging to detect ICH. Moreover, we provide evidence that the disappearance of ICH on T2*-weighted imaging could be used to improve accuracy of perfusion-weighted imaging and to allow molecular imaging after ICH. Importantly, a 30-minute NBO preparation 24 hours after ICH onset does not influence neurological outcome.

Conclusions—We provide an experimental demonstration that NBO significantly affects T2*-weighted imaging in ICH. Although this phenomenon could lead to inaccurate assessment of ICH volume, it could also be safely used to allow perfusion-weighted imaging and molecular imaging. (Stroke. 2013;44:00-00.)

Key Words: behavior therapy ■ cerebral hemorrhage ■ magnetic resonance imaging ■ molecular imaging

Magnetic resonance imaging (MRI) is currently one of the recommended modalities to evaluate patients with acute stroke.1 Distinction between ischemic and hemorrhagic strokes is usually performed by exclusion of hemorrhage on computed tomography in the emergency setting and in rarer cases in which stroke MRI is performed acutely by demonstrating presence of diffusion-weighted imaging lesion and absence of hypointense signal on T2*-weighted imaging. Although normobaric oxygen (NBO) therapy is frequently used in stroke,1 the potential effects of NBO on T2*-weighted imaging of intracranial hemorrhages (ICHs) remain unknown.

In the present study, we investigated the effects of NBO in 2 mouse models of ICH, with a particular focus on standard T2*-weighted imaging, perfusion-weighted imaging (PWI), and molecular imaging (Figure 1). Moreover, we investigated whether NBO affects neurological outcome in mice with ICH.

Methods

Experimental Models of Intracerebral Hemorrhage Experiments complied with the European Directives and the French Legislation on Animal Experimentation. Male Swiss mice (35–40 g; CURB, Caen, France) were anesthetized with isoflurane (2%) in 70%/30% NO2/O2. Using a stereotactic frame, a 30-gauge needle was inserted into the right striatum at bregma: 0.5 mm; mediodlateral: 2.5 mm; dorsoventral: 3 mm. For the collagenase model, 0.5 μL of saline containing 0.04 IU of collagenase VII-S (Sigma, France) was injected. For the direct intrastriatal blood injection model, 15 μL of whole blood was obtained from a first mouse. Immediately thereafter, in another mouse, 5 μL of this blood was injected in the striatum over 3 minutes, followed 7 minutes later by 10 μL injected over 5 minutes, as previously described.1 All the animals were included in the final analyses.

Oxygen Blood Level Measurement Mice were anesthetized using isoflurane and exposed to normoxia (fraction of inspired oxygen [FiO2]=20%) or NBO (FiO2=100%) during 30 minutes. Arterial blood was obtained by carotid puncture. Oxygen blood levels were measured using an automatic gas analyzer (Rapidlab; Siemens).

Behavioral Assessment ICH was induced by intrastriatal collagenase injection in 16 mice. One mouse died during the surgery and was therefore excluded from the analysis. Three behavioral tests were performed before the ICH and at +24 hours to assess the functional deficits induced by the ICH.
Stroke December 2013

in mice. The general state of the mice was assessed using a 5-point neuroscore scale (0=no apparent deficit; 1=slight deficit; 2=circling; 3=heavy circling or no movement at all; or 4=death). The spontaneous global locomotor activity was quantified during 10 minutes using an actimeter (Imetronic, Pessac, France) as described previously. Briefly, global locomotor activity was quantified using activity cages equipped with horizontal infrared beams located across the long axis of the cage (IMETRONIC, France). Mice were placed in individual acrylic chambers (30×20×20 cm) for 10 minutes. The number of horizontal movements (horizontal crossings) was determined by breaks in movement-sensitive photobeams that were then converted into locomotor activity counts. A gait analysis was also performed using the Catwalk XT system (Noldus Information Technology, Wageningen, The Netherlands). The apparatus consists of an enclosed walkway (130×68×152 cm) on a glass plate that is traversed by the mice from one side to the other. Green light enters at the long edge of the plate and is completely internally reflected. Light is able to escape only at those areas where the animal’s paws make contact with the glass plate; as a result, the light is scattered. Real footprints are captured by a high-speed video camera that is positioned underneath the walkway. The video camera transforms each scene into a digital image that is transferred to a computer. The video capture is then processed by the Catwalk XT software (version 9). After training (3 explorations of the walkway before the baseline), the mice were subjected to 3 consecutive compliant runs (<60% of speed variation) at each end point. Three mice were unable to cross the walkway because of heavy circling and were therefore excluded from the study of gait analysis.

Thereafter, to assess the potential effects of NBO on the acquired functional deficits, the same mice were randomized to receive either NBO preparation or normoxia for 30 minutes. Then, the 3 behavioral tests were performed 1 more time. To compare data before and after NBO/normoxia between the groups for the gait analysis experiment, a delta was calculated for each parameter and each mouse as:

\[ \text{Delta}_{\text{parameter x}} = \text{value after gas}_{\text{parameter x}} - \text{value before gas}_{\text{parameter x}} \]

All behavioral tests were conducted by experimenters blinded to the treatment group.

MRI Experiments

MRI was performed on a 7T Pharmascan MRI system (Bruker, Germany) at 24 hours after collagenase or blood injection. Mice were anesthetized with 2% isoflurane in 80%/20% NO2/O2 (normoxia) or 100% O2 (NBO). T2-weighted images were acquired using a rapid acquisition with relaxation enhancement sequence, with repetition time (TR)/echo time (TE) 2500/60 ms. T2*-weighted images were acquired using a fast low-angle short sequence, with TR/TE=500/8 ms. Hemorrhagic volumes were measured using ImageJ 1.45. Quantitative analyses were conducted blinded to oxygen level. PWI was performed using a T2*-weighted fast imaging with steady-state precession sequence (TR/TE=10/5 ms; temporal resolution=911 ms; spatial resolution=100×100 µm; slice thickness=0.75 mm). A gadolinium bolus (1 mL/kg, Dotarem; Guerbet, France) was injected intravenously when appropriate. Parametric mapping of area under the curve, negative index, and time to peak was performed using a homemade macro written in ImageJ. Four regions of interest were manually defined as follows: (1) in the center of the hematoma=hematoma center; (2) at the periphery of the hematoma=hematoma periphery; (3) in the area surrounding the hematoma=perihematoma; and (4) in the contralateral striatum=contralateral striatum. Susceptibility mapping was performed using a gradient echo sequence (TR/TE=3000/8 ms; resolution=70×70×500 µm) and the integrated susceptibility-weighted imaging macro of the MRI console (Paravision 5.1).

Molecular MRI

Molecular imaging experiments were performed as previously described. Briefly, mice underwent NBO preparation during 30 minutes. Thereafter, mice received intravenous microparticles of iron oxide targeting vascular cell adhesion molecule-1 (MPIO-αVCAM-1) (1 mg/kg) 24 hours after ICH onset (collagenase model). Then, T2*-weighted imaging was performed using a 3-dimensional gradient echo with flow compensation sequence with TR/TE=200/12 ms.

Statistics

Results are expressed as mean±SD. Statistical analyses were performed with the JMP software package (v10.00). An α level of P<0.05 was used for determination of significance in all statistical
tests. All $P$ values are 2-tailed. Wilcoxon signed-rank tests were performed to compare 2 matched samples (ie, baseline versus +24 hours for neurological tests). Mann–Whitney tests were performed to compare 2 independent samples (eg, normoxia versus NBO). Quantitative analyses were conducted blinded to oxygen level.

Results

NBO Increases $O_2$ Blood Level

We first wanted to determine the changes in arterial oxygen tension ($PaO_2$) induced by NBO therapy. To this aim, arterial blood from the common carotid artery was obtained after 30 minutes of NBO or normoxia and the $PaO_2$ was measured. After 30 minutes of NBO ($FiO_2=100\%$), the $PaO_2$ was 5-times higher than in mice under normoxia (493.0±87.0 versus 90.5±3.6 mm Hg; $P<0.05$; Figure I in the online-only Data Supplement).

NBO Induces Apparent Disappearance of Intracerebral Hematoma After ICH

Then, we investigated the effect of NBO in a collagenase model of ICH. We performed longitudinal T2*-weighted imaging of the hematoma under NBO or normoxia 24 hours after collagenase administration. Our results demonstrated that the apparent size of the hematoma rapidly decreases with time after NBO induction (Figure 2A). Thus, as soon as 30 minutes after the start of NBO, the apparent volume of the hematoma represented only $9.9±2.7\%$ of its initial volume (Figure 2B). In contrast, hypersignals in T2-weighted images were not affected (Figure 2C and 2D). Notably, there was no further reduction of the apparent hematoma size between 30 and 45 minutes, suggesting that this effect is saturable.

We confirmed these findings in another common mouse model of ICH induced by direct intrastratal injection of blood. Again, NBO induced a rapid and progressive reduction of the apparent hematoma size, which represented $18±5.6\%$ of its initial size after 30 minutes (Figure 3A and 3B in the online-only Data Supplement). Importantly, 1 hour of normoxia after the 30-minute period of NBO completely normalized the hematoma size on T2*-weighted imaging. Both disappearance and reappearance were from the periphery to the center of the hematoma (Figure 3C).

Altogether, these results demonstrated that NBO, even during a short period of time, dramatically reduces the sensitivity of T2*-weighted imaging to detect ICH. A similar effect was observed in susceptibility-weighted images (Figure II in the online-only Data Supplement).

NBO Preparation Allows Accurate Magnetic Resonance PWI After ICH

Subsequently, we looked for potential applications of NBO for T2*-weighted imaging of ICH. After ICH, some studies suggest that ischemia occurs in the perihematoma area. Monitoring of regional cerebral blood flow in patients with ICH may allow
optimizing the blood pressure to choose the best compromise between risk of hematoma enlargement and risk of ischemia. Unfortunately, magnetic resonance PWI is highly sensitive to the magnetic susceptibility effects of deoxyhemoglobin, precluding accurate imaging of the perihematoma area. We hypothesized that NBO preparation could be used to suppress the magnetic susceptibility effects of the hematoma, therefore allowing bolus tracking experiments in mice after collagenase injection.

To test this hypothesis, we performed bolus tracking experiments in mice after 30 minutes of NBO, 24 hours after ICH. As expected, 30 minutes of NBO preparation suppressed the susceptibility effects of deoxyhemoglobin and allowed tracking of a gadolinium bolus and subsequent parametric mapping of regional perfusion both outside and inside the hematoma (Figure 4A). In this proof-of-concept experiment, our results demonstrated that perilesional perfusion is not significantly affected by the hematoma in this model (Figure 4B). Whereas cerebral blood volume (area under the curve), cerebral blood flow (negative index), and time to peak were reduced inside the hematoma (ie, in both the center and periphery of the hematoma), those parameters were not significantly different from the contralateral side in the perihematoma area.

NBO Preparation Improves Molecular Imaging of Inflammation After ICH

Another potential application of NBO preparation is molecular imaging. Susceptibility effects of deoxyhemoglobin prevent accurate molecular imaging when negative contrast agents are used. We hypothesized that NBO preparation would suppress the signal voids induced by the hemorrhage without affecting the signal voids induced by negative contrast agents, therefore allowing more accurate molecular imaging. To test this hypothesis, mice underwent molecular MRI brain imaging 24 hours after ICH onset (collagenase injection). A 30-minute NBO preparation was performed immediately before intravenous injection of MPIO-αVCAM-1. This NBO preparation suppressed the hemorrhage-related signal voids and allowed tracking of MPIO-αVCAM-1–induced signal voids. In this model, ICH induced a strong vascular inflammation in the whole brain, which was particularly significant in the perihematoma area (Figure 5).

Altogether, these data demonstrate the usefulness of NBO preparation to allow molecular imaging in mice with ICHs.

NBO Does Not Worsen Post-ICH Neurological Outcome

Because our data suggest that NBO preparation could be used to improve MRI performance in several settings, we wanted to make sure that NBO preparation does not affect the neurological outcome in mice with ICH. To this aim, we first performed a battery of neurological tests (neuroscore, actimetry, and gait analysis) before and 24 hours after ICH onset to determine which parameters are significantly modified by ICH alone (ie, in the absence of NBO). The neuroscore increased from 0 before ICH to 1.8±0.6 at +24 hours after ICH (P<0.0001; Figure 6A). Actimetry experiments revealed a reduced spontaneous locomotor activity in mice with ICH (P<0.0001;
For the gait analysis using the Catwalk system, we isolated a set of 76 parameters (of a total of 165) that were significantly affected by collagenase injection. Three of them presenting low P values are presented in Figure 4C, the others are presented as online-only Data Supplement.

Then, mice were randomized to receive either NBO or normoxia and underwent the same functional test battery to investigate whether NBO affects neurological outcome. Interestingly, NBO therapy did not significantly modify the neurobehavioral experiments in ICH mice. Neuroscores were 1.8±0.4 in the control group (n=7) and 1.5±0.7 in the NBO group (P=0.29; Figure 6A). Actimetry (Figure 6B) and Catwalk (Figure 6C) experiments also failed to reveal any significant differences between NBO-treated and normoxia-treated mice, with the exception of the parameter LF_MaxContactMeanIntensity that appeared improved in NBO-treated mice (P=0.0284). This parameter did not remain significantly improved after applying Bonferroni correction to counteract the problem of multiple comparisons. Altogether, these results suggest that 30 minutes of NBO preparation is safe in ICH animals.

Discussion

Our study provides evidence in 2 mouse models that ICH signal on T2*-weighted imaging is significantly affected by NBO. One likely explanation is the transformation of deoxyhemoglobin (paramagnetic) to oxyhemoglobin (diamagnetic) by the increased tissular concentration of O2. This leads to normalization of the tissular magnetic susceptibility and therefore to an increase in magnetic resonance signal on T2*-weighted images in hemorrhagic areas. Therefore, hematomas disappear on T2*-weighted images during NBO. Alternatively, vascular oxyhemoglobin could permeate through the disrupted blood–brain barrier. Therefore, the turnover of intraparenchymal hemoglobin will progressively reduce the concentration of deoxyhemoglobin and blunt its susceptibility effect. Another explanation that could involve mechanisms of increased
should be considered, such as mitochondrial oxygen as a delivery gas for anesthetic agents, especially if T2*-weighted imaging is performed to detect hemorrhages. During MRI in experimental studies should avoid the use of 100% oxygen therapy because the signal of these small hemorrhages would rapidly vanish on T2*-weighted images. Similarly, anesthesia during MRI in experimental studies should avoid the use of 100% oxygen as a delivery gas for anesthetic agents, especially if T2*-weighted imaging is performed to detect hemorrhages. In addition, NBO preparation could dramatically increase the sensitivity and specificity of molecular imaging in the presence of extravasated blood. Thus, NBO preparation could be used for high-resolution molecular imaging of the perihematoma area in ICH models. This also would be particularly interesting for molecular imaging of intracranial tumors by allowing accurate detection of contrast media-induced signal voids without hemorrhage-related artifacts. The signal void originating from extravasated blood remains one of the main limitations of imaging in cancer.

Using NBO preparation and PWI, we were able to measure cerebral blood perfusion both inside and outside the hematoma. We demonstrated that intrahematoma perfusion is reduced (in the core and periphery of the hematoma), whereas perihematoma perfusion is not significantly different from the contralateral side (Figure 4). Our data argue against the presence of ischemia in the perihematoma area, as previously suggested. Thus, other mechanisms for acute neural injury should be considered, such as mitochondrial dysfunction.

Potential Implications for Clinical Practice
In the present study, we also unveil potential pitfalls of NBO therapy for T2*-weighted imaging in patients presenting ICH. In addition to the well-known overestimation of the hematoma size in patients with ICH, our results suggest that T2*-weighted imaging could underestimate the hematoma size in patients treated by NBO. Therefore, our study suggests that information about PaO2 of each patient should be provided to neuroradiologists for reliable interpretation of T2*-weighted images. This would be particularly important for patients requiring respiratory support who are frequently ventilated with 100% O2 during transport to the MRI facilities. In addition, studies of cerebral microbleeds should avoid the use of oxygen therapy because the signal of these small hemorrhages would rapidly vanish on T2*-weighted images.

On the contrary, the described phenomenon of hematoma disappearance could be used to improve accuracy of MRI, especially because we (and others) demonstrated that NBO appears safe after ICH (Figure 6). For instance, we demonstrated here that perihematoma magnetic resonance PWI becomes feasible after a 30-minute preparation period of NBO. Similarly, identification of the underlying cause of ICH (eg, cerebral tumor or angiomatous cavernoma), which remains a challenging radiological problem, could benefit from NBO preparation by revealing the brain parenchyma inside the hematoma.

Limitations
We must, however, expose some limits of the present data. First, the absolute sizes of the hematomas in the present study were comparable with human microbleeds, but much smaller than large ICH. Second, we investigated the effects of NBO therapy at 24 hours post-ICH onset. Hence, whether the efficiency of NBO preparation to induce disappearance of ICHs on T2*-weighted imaging is time-dependent and volume-dependent remains to be investigated. Concerning the data regarding PWI, the different regions of interest were segmented manually and no algorithm of motion correction was applied. These limitations could have lowered the sensitivity of the experiment performed to detect subtle variations in cerebral blood flow. Moreover, the clinical implications of our findings remain elusive and deserve to be adequately confirmed in human studies.

Conclusions
In conclusion, we provide an experimental proof-of-concept that NBO affects T2*-weighted imaging in ICH. Although
this phenomenon could lead to inaccurate assessment of ICH volume, it could also be used to improve MRI performance by unmasking the brain parenchyma affected by the magnetic susceptibility effects of deoxyhemoglobin, thus allowing PWI and molecular imaging in and around the hemorrhage.

**Sources of Funding**

This work was supported by the Institut National de la Santé Et de la Recherche Médicale, the French Ministry of Research and Technology, and the Eurostroke-Arise Program (FP7/2007-2013-201024).

**Disclosures**

None.

**References**


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Stroke. published online October 8, 2013; Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231. Copyright © 2013 American Heart Association, Inc. All rights reserved. Print ISSN: 0039-2499. Online ISSN: 1524-4628

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SUPPLEMENTAL MATERIAL

Intracerebral hematomas disappear on T2*-weighted images during normobaric oxygen therapy

Thomas GABEREL, MD, MSc; Clement GAKUBA, MD, MSc; Marie HEBERT, MSc; Axel MONTAGNE, PhD; Veronique AGIN, PhD; Marina RUBIO, PhD; Evelyne EMERY, MD, PhD; Denis VIVIEN, PhD; Maxime GAUBERTI, PhD
Supplementary video legend

Supplementary Video I. NBO induces subtotal disappearance of intracerebral hematoma in a direct blood injection ICH model. 24 hours after autologous blood injection within the striatum, mice were placed in a 7T MRI for dynamic T2*-weighted brain imaging under NBO. One image was acquired every two seconds. In 30 minutes, the hyposignal induced by the hematoma was close to completely disappear. Disappearance was from the periphery to the center of the hematoma. Time is MM:SS.
Supplementary Figure I. NBO significantly increased arterial oxygen level

Bar graphs representing the relationship between fraction of inspired oxygen (FiO₂) and the arterial oxygen blood level (PaO₂). After 30 minutes of NBO (FiO₂=100%), the PaO₂ was five time higher than under normoxia. Induction of anesthesia was performed under FiO₂=33%: the resulting PaO₂ at the end of the anesthesia induction is given here for information (n=4).
Supplementary Figure II. NBO significantly affects phase images.

(A) Representative magnitude and phase images of a control animal generated from gradient echo data. (B) Representative longitudinal T2*-weighted magnitude and (C) phase images under normoxia or NBO 24 hours after collagenase-induced ICH. (D) Corresponding quantification of the absolute value of the phase (n=3). As already demonstrated for magnitude images, phase images of ICH are significantly affected by NBO. In contrast, the contralateral phase was not modified. Ipsi = Ipsilateral (hemorrhagic side). Contra = Contralateral.