Role of magnesium in the reduction of ischemic depolarization and lesion volume after experimental subarachnoid hemorrhage

WALTER M. VAN DEN BERGH, M.D., J. KAREL ZUUR, M.D., NIELS A. KAMERLING, M.D., JAN THIES H. VAN ASSELDONK, M.D., GABRIËL J. E. RINKEL, PH.D., M.D., CORNELIS A. F. TULLEKEN, PROF., PH.D., M.D., AND KLAAS NICOLAY, PROF., PH.D.

Department of Neurosurgery, Experimental In Vivo Nuclear Magnetic Resonance and Neurology, University Medical Center Utrecht, The Netherlands

Object. Ischemia-induced tissue depolarizations probably play an important role in the pathophysiology of cerebral ischemia caused by parent vessel occlusion. Their role in ischemia caused by subarachnoid hemorrhage (SAH) remains to be investigated. The authors determined whether ischemic depolarizations (IDs) or cortical spreading depressions (CSDs) occur after SAH, and how these relate to the extent of tissue injury measured on magnetic resonance (MR) images. In addition, they assessed whether administration of MgSO₄ reduces depolarization time and lesion volume.

Methods. By means of the endovascular suture model, experimental SAH was induced in 52 rats, of which 37 were appropriate for analysis, including four animals that underwent sham operations. Before induction of SAH, serum Mg²⁺ levels were measured and 90 mg/kg intravascular MgSO₄ or saline was given. Extracellular direct current potentials were continuously recorded from six Ag/AgCl electrodes, before and up to 90 minutes following SAH, after which serum Mg²⁺ levels were again measured. Next, animals were transferred to the MR imaging magnet for diffusion-weighted (DW) MR imaging. Depolarization times per electrode were averaged to determine a mean depolarization time per animal.

No depolarizations occurred in sham-operated animals. Ischemic depolarizations occurred at all electrodes in all animals after SAH. Only two animals displayed a single spreading depression-like depolarization. The mean duration of the ID time was 41 ± 25 minutes in the saline-treated controls and 31 ± 30 minutes in the Mg²⁺-treated animals (difference 10 minutes; p = 0.31). Apparent diffusion coefficient (ADC) maps of tissue H₂O, obtained using DW images approximately 2.5 hours after SAH induction, demonstrated hypointensities in both hemispheres, but predominantly in the ipsilateral cortex. No ADC abnormalities were found in sham-operated animals. The mean lesion volume, as defined on the basis of a significant ADC reduction, was 0.32 ± 0.42 ml in saline-treated controls and 0.11 ± 0.06 ml in Mg²⁺-treated animals (difference 0.21 ml; p = 0.045). Serum Mg²⁺ levels were significantly elevated in the Mg²⁺-treated group.

Conclusions. On the basis of their data, the authors suggest that CSDs play a minor role, if any, in the acute pathophysiology of SAH. Administration of Mg²⁺ reduces the cerebral lesion volume that is present during the acute period after SAH. The neuroprotective value of Mg²⁺ after SAH may, in part, be explained by a reduction in the duration of the ID of brain cells.

Key Words • experimental subarachnoid hemorrhage • magnesium • diffusion-weighted magnetic resonance imaging • depolarization • rat

Much research has been devoted to the treatment of SAH and its complications, but these have only led to modest improvements in overall outcome. The lack of a major improvement is explained by the initial impact of the hemorrhage, which is responsible for 15% of cases of immediate death from SAH and is also a major cause of overall morbidity and mortality. The initial impact of aneurysm rupture is probably also associated with the occurrence of secondary ischemia after SAH, because parameters of the impact of the initial bleeding (that is, the amount of blood shown on the computerized tomography scan, duration of unconsciousness, and clinical condition at admission) are the most important predictors of secondary ischemia. The pathophysiology of acute cerebral damage after SAH remains largely unclear. Increased insight into this pathophysiology may help curb the effects of the initial ischemia and improve prevention and treatment of secondary ischemia.

Shortly after SAH there is a decrease in CBF. In experimental models of SAH the decreased CBF has been linked to a rapid and transient increase in ICP in the initial phase following hemorrhage but after this period CBF is decreased and thus, probably, the period of raised ICP is accompanied and followed by acute vasoconstriction, which
Magnesium reduces ischemic depolarization and SAH volume

...is independent of changes in ICP and perfusion pressure. In patients, this period of diminished CBF is reflected by a period of unconsciousness, and can lead to brain infarction.

A recent study in which a fast echoplanar MR imaging diffusion sequence was used found a sometimes transient decline in the ADC of H2O during the hyperacute phase of an endovascularly induced SAH in the rat, indicating that CSDs occur after SAH. In the case of focal cerebral ischemia, CSDs are known to represent undulating changes in extracellular K+ concentration, which occur in the border zone of evolving brain infarctions and are believed to play a role in the development of the infarction. Diffusion-weighted MR imaging is an important tool in stroke research, because of its ability to visualize ischemic tissue shortly after disease onset. Ischemia is accompanied by a reduction in the ADC of brain-tissue H2O, which results in regional hypointensities on quantitative H2O ADC maps.

The aim of this work was to measure the DC potential in the rat cortex to determine whether IDs or CSDs occur after SAH, and to assess lesion volume during the acute phase following SAH by MR imaging performed shortly after DC potential measurements. In view of the role of IDs in the pathophysiology of ischemic stroke, we considered it of interest to examine the possible correlation between depolarization time and the volume of the lesion as it is depicted on MR images. Moreover, we studied the effect of MgSO4 on CSDs, depolarization time, and lesion volume following SAH, because we have previously shown that MgSO4 reduces the frequency of CSDs in a rat model of artificially evoked CSD.

Materials and Methods

Animal Preparation

The experiments were performed in 52 male Wistar rats, each of which weighed between 300 and 380 g. Anesthesia was induced by administering a subcutaneous injection of a mixture of 0.55 ml/kg fentanyl citrate (0.315 mg/ml), fentanyl (0.1 mg/ml), and 0.55 ml/kg midazolam (5 mg/ml). After transoral intubation had been initiated, anesthesia was maintained by administration of 0.8% halothane in a 70:30 gas mixture of N2O/O2 and artificial ventilation was regulated at a rate of 30 breaths/minute. The tidal CO2 was continuously monitored and kept within physiological boundaries. Body temperature was maintained at 37 ± 0.5°C by means of a feedback-controlled heating pad.

The right femoral artery was cannulated with polyethylene (PE-50) tubing for continuous blood pressure recording and a continuous supply of saline to prevent dehydration, as well as to obtain blood samples for serum Mg++ analysis before and 90 minutes after SAH induction. Saline or MgSO4 was administrated intravenously as a bolus injection via tail infusion.

A midline incision was made to expose the skull. Six 1.5-mm burr holes were drilled into the skull, as schematically displayed in Fig. 1, at the parietal, hindlimb, and occipital cortex areas of the ipsilateral and contralateral hemispheres. We kept the dura mater intact. After the periosteum had been removed, the burr holes were covered with an acrylic dental cement mould fixed with adhesive to anchor the electrodes within the skull. This same mould was used repeatedly in all rats. One-millimeter-diameter pellet Ag/AgCl electrodes were enclosed in polyethylene tubes measuring 10 mm long and 1 mm in diameter, which were filled with 0.9% NaCl in agar. Electrical continuity between tissue and electrodes was established by applying electrode cream. The signal from a bare Ag/AgCl electrode placed in the neck musculature served as a reference. A ground electrode was connected to the ground of an electric socket.

Induction of SAH

Subarachnoid hemorrhage was induced by advancing a sharpened No. 3.0 Prolene suture through the ligated left external CA and distally through the internal CA until the suture perforated the intracranial bifurcation of the internal CA, after which the suture was quickly redrawn. This technique was previously described by Bederson, et al., and Veelken and associates and is a modification of the endovascular suture model used for MCA occlusion. A brief decrease in blood pressure followed by a short spell of raised blood pressure gave us confidence that SAH had been induced. Four rats underwent a similar procedure, including DC potential recording; however, in these animals, the Prolene thread was kept in place for less than 2 minutes and the vessel was not perforated (sham-operated group).
Drug Treatment and Experimental Groups

Animals were randomly chosen for pretreatment with MgSO₄ or saline, which was administered 15 minutes before SAH as an intravenous bolus via tail infusion. Of the 37 surgically treated animals that were used for data analysis, 14 were pretreated with 90 mg/kg MgSO₄ (treatment group) from a 100 mg/ml solution, 19 received 0.3 ml of saline (control SAH group), and four animals underwent sham operation and received saline as well (sham-operated group).

Recording of DC Potentials

Direct current potentials were recorded before and up to 90 minutes following SAH by using a homemade six-channel electrometer–amplifier. Amplifier outputs were transferred to a personal computer and, after analog-to-digital conversion, were evaluated with the aid of a commercially available technical graphing software package.

After 90 minutes of recording, the electrodes were removed and the animal was transferred to the MR imaging magnet.

Magnetic Resonance Imaging Experiments

During the MR imaging protocol, anesthesia and maintenance of physiological parameters were achieved in the same fashion as that described earlier. The MR imaging measurements were obtained approximately 2.5 ± 0.5 hours after SAH induction by using a 4.7-tesla nuclear MR spectrometer equipped with a gradient insert of up to 220 mT/m. Each animal’s head was fixed in a stereotactic holder. A Helmholtz volume coil (85 mm diameter) was used for signal excitation, whereas an inductively coupled 20-mm-diameter surface coil was used for signal acquisition.

Multislice coronal spin-echo DW images were acquired with a single-shot, trace ADC sequence (128 × 64 data matrix, TR 2500 msec, TE 100 msec, and five b values ≤ 1781 seconds/mm). Nine contiguous 1.5-mm slices located between the cerebellar sulci and olfactory bulb were imaged. Calculation of quantitative brain maps of the ADC was performed by monoeponential fitting.

Postmortem Examination

Following the MR imaging study, the animals were killed by administration of 5% halothane and their brains were exposed and inspected for hemorrhage. The amount of blood was examined for each hemisphere separately, using scores ranging from 0 to 3; the total blood score thus could range from 0 (no SAH) to 6 (massive bilateral bleeding).

Statistical Analysis

We measured the duration of depolarizations on a personal computer by using technical graphing software. As a starting point, the beginning of the enduring depression of the DC potential baseline was taken. This time point coincided with the disappearance of the electroencephalographic signal, which was visible as a high-frequency modulation superimposed on the DC potential reading. Repolarization was assumed to be complete when the electroencephalographic signal appeared again and the DC potential had returned as a horizontal line. Due to a slow electrical drift in the measured signal, this did not necessarily occur at the same millivolt level as the pre-SAH baseline recording. The durations of depolarizations of the six electrodes were averaged to obtain the mean depolarization time per animal. The mean depolarization time was used to relate the electrophysiological results to the MR imaging data and to test the hypothesis that Mg²⁺ reduces the severity of the IDs. The amplitude of the DC potential deflection associated with ID was not taken into account, because this parameter varied strongly between experiments. The CSDs were defined as depolarizations with a spreading velocity of approximately 3 mm/minute and a duration of approximately 1 to 2 minutes.

We analyzed parametric ADC images by using an image-analysis software package. The area of the acute ischemic lesion was calculated from the ADC maps by thresholding, using mean ADC values of sham-operated animals (0.76 ± 0.08 × 10⁻³ mm²/second). An ADC value was considered pathological if it was two SDs below the mean ADC level of brain-tissue H₂O in sham-operated animals. We determined total lesion volume as well as lesion volume in the ipsilateral (left) and contralateral (right) hemispheres.

We used the independent-samples t-test to compare means for the control SAH group and the Mg²⁺-treated group. Data are presented as means ± SDs. Linear regression analysis was used to correlate lesion volumes with electrophysiological data. Data are presented as correlation coefficients with significance levels; probability values lower than 0.05 were considered significant.

Sources of Supplies and Equipment

Bison Kit Powerglue, purchased from Bison International (Goes, The Netherlands) was used to anchor the electrodes. The Ag/AgCl electrodes were manufactured by Harvard Apparatus, Inc. (South Natick, MA). Redux electrode cream was obtained from Hewlett-Packard Co. (Palo Alto, CA). The technical graphing software used to evaluate DC potential recordings was KaleidaGraph version 3.0.9, which was developed by Synergy Software (Reading, PA). The nuclear MR spectrometer and the ImageBrowser image-analysis software package were acquired from Varian (Palo Alto, CA).

Results

General Results

Fifty-two rats underwent surgery. In three animals we failed to achieve SAH. One animal in the control SAH group and the Mg²⁺-treated group. Data are presented as means ± SDs. Linear regression analysis was used to correlate lesion volumes with electrophysiological data. Data analysis was confined to the 37 animals in which we completed electrophysiological and MR imaging measurements, among which there were four sham-operated animals.

Serum Mg²⁺ Levels

The mean pretreatment serum Mg²⁺ level in all animals was 0.91 mmol/L. There was no difference in the pretreat-
Magnesium reduces ischemic depolarization and SAH volume

Fig. 3. Apparent diffusion coefficient maps from DW MR images obtained in three different animals demonstrating ADC reductions in the cortex of both hemispheres (upper), in the ipsilateral cortex (center; most common), and in cortical plus hippocampal areas (lower).

ment serum level of Mg$^{++}$ between the two groups. Ninety minutes after SAH induction, the Mg$^{++}$ level in the control SAH group was 0.88 mmol/L and the level in the treatment group was 1.29 mmol/L, which was 47% higher (p = 0.001).

**Direct Current Potentials**

Within 10 to 20 seconds after SAH induction, all electrodes simultaneously demonstrated a small decline in the DC potential, which recovered in 20 to 30 seconds in all cases. In all animals this was followed by depolarization within 1 minute after SAH induction, as indicated by the massive decline in the DC potential.

Except for one animal, in which the electrodes in the contralateral parietal and occipital regions did not show a depolarization, all electrodes were involved in depolarization in all animals. A typical example of a DC potential recording is shown in Fig. 2. In 19 (58%) of the 33 animals with SAH the decline in the DC potential was first seen at the ipsilateral parietal electrode, but in 12 animals (36%) it was observed at several electrodes simultaneously. In 17 animals (52%) depolarization started ipsilaterally, in nine animals (27%) contralaterally, and in seven animals (21%) in both hemispheres at the same time. The mean time until all electrodes were depolarized was 110 seconds, ranging from 0 to 666 seconds, and was similar for the control SAH group (111 seconds) and the Mg$^{++}$-treated group (120 seconds).

In 26 (79%) of 33 animals repolarization of the DC potential occurred at all electrodes during the 90-minute observation period. We found a CSD-like depolarization, spreading 3 mm/minute from one electrode to the others in only two animals, both of which belonged to the control SAH group. In both instances, however, this occurred after repolarization of the ID.

Magnesium treatment led to a reduction in depolarization time for all electrodes. The mean depolarization time for both hemispheres was 31 minutes (range 1–89 minutes) in the treatment group and 41 minutes (range 5–89 minutes) in the control SAH group (difference 10 minutes [25%], p = 0.31). In the ipsilateral hemisphere, the mean depolarization time was 35 minutes (range 1–90 minutes) in the Mg$^{++}$-treated group compared with 45 minutes (range 7–90) minutes in the SAH control group (difference 10 minutes, p = 0.4). In the contralateral hemisphere, the mean depolarization time was 27 minutes (range 1–89 minutes) compared with 37 minutes (range 3–89 minutes) in the SAH control group (difference 10 minutes, p = 0.2).

**Lesion Volumes on DW MR Images**

Following the DC potential recordings, DW MR imaging was performed 2.5 ± 0.5 hours after SAH induction to measure the volume of tissue at risk for irreversible injury. Typical examples of ADC data are displayed in Fig. 3. The water ADC maps often showed hypointensities at multiple variable locations, but predominantly in the ipsilateral cortex. All animals subjected to SAH were found to harbor lesions in both hemispheres. In 10 animals (30%) lesion volume was even larger on the contralateral side than on the ipsilateral side. In circumscribed lesions the mean ADC value (0.54 × 10⁻³ mm²/second) was comparable with that of reported values in infarction areas after MCA occlusion (0.49 × 10⁻³ mm²/second). As expected, sham-operated animals did not display any ADC abnormalities.

The mean (+ SD) total lesion volume in the saline-treated control animals was 0.32 ± 0.42 ml and the mean volume in Mg$^{++}$-treated animals was 0.11 ± 0.06 ml, a reduction of 66% (p = 0.045, 95% confidence interval 0.005–0.4) (Fig. 4). In the ipsilateral hemisphere the reduction was 67% (from 0.18 ± 0.22 ml in the SAH control group to 0.06 ± 0.04 ml in the treatment group; p = 0.037, 95% confidence interval 0.008–0.2), and in the contralateral hemisphere the reduction was 64% (0.14–0.05 ml, p = 0.068).

**Correlation Between Depolarization Time and Lesion Volume on DW MR Images**

The Pearson correlation coefficient between depolarization time and lesion volume was 0.39 (p = 0.025) (Fig. 5). When one considers only the ipsilateral side, the correlation coefficient increased to 0.46 (p = 0.007).
Postmortem Findings

There was no evidence of SAH in any sham-operated animal. In all other animals, however, extensive SAH was identified, with blood distributed around the circle of Willis and a thin layer overlying the cortex and around the brainstem (Fig. 6). The amount of subarachnoid blood was equal in the two groups in which SAH was induced.

Discussion

Our data demonstrate that prolonged depolarizations occur immediately after SAH and that the duration of these depolarizations is related to the extent of ischemic lesions observed on MR images. Moreover, we found that pretreatment with Mg reduces the extent of the ischemic lesions.

Direct current potential deflections can be divided into two types: CSDs, which are inherently transient in nature and have a very characteristic migration pattern, and IDs, which, depending on regional perfusion status, may be transient or permanent. The spreading velocity, duration, and pattern of depolarizations in our study are typical of IDs. Cortical spreading depressions were rarely detected in our study and, therefore, seem to be of minor importance for the development of ischemic lesions during the acute phase following SAH. Two other studies in which DC potential recording was used after acute SAH detected depolarization and transient depression in electrocortical activity, but the method used to imitate an SAH was direct application of blood or artificial cerebrospinal fluid containing the hemo- lysis products K and hemoglobin on the cerebral cortex. Using the same endovascular filament method to induce SAH as we did, Beaulieu and associates detected CSD in an indirect manner, namely the occurrence of transient ADC reductions in the ultraacute phase following SAH. Nevertheless, ADC changes can occur before membrane depolarization and thus it is not certain that these transient reductions are actually CSDs.

The absence of CSDs after SAH may be explained by the extent of hypoxia. The ischemic lesions that occur after SAH are the result of a global hypoxia, in contrast with the regional area of hypoperfusion with penumbra seen in models of MCA occlusion, from which these CSDs are considered to originate. The global ischemia and absence of a zone of marginally perfused tissue probably preclude the occurrence of CSDs in the acute phase following SAH. The IDs that we observed demonstrate that the intensity of the ischemia is sufficient to induce CSDs. The appearance of depolarizations in both hemispheres indicates that the impact of SAH from a ruptured artery affects the whole brain and not only the region of the ruptured artery. This is also supported by our finding that the lesion volume on the contralateral side was almost identical to that on the side of the ruptured artery.

Cerebral arteries have been shown to respond to SAH with a biphasic constriction pattern. An acute constriction begins within minutes after the bleeding, whereas delayed vasospasm develops 48 hours later. Although the significance of delayed vasospasm for ischemic brain damage after SAH is recognized, the contribution of acute vasconstriction is less clear. The CBF decreases rapidly and ischemic injury occurs after SAH in both experimental and clinical studies. Bederson and colleagues described a pattern in which CBF reduced to 16.5% of baseline within 5 minutes and remained reduced to 44% 60 minutes after experimental SAH. This reduction in CBF is initially due to raised ICP, but later is accompanied and followed by acute vasoconstriction of large cerebral arteries. The duration of the ID we found is in line with the aforementioned pattern of CBF changes, which recovered to values above the ischemic threshold within 60 minutes.

Magnesium treatment led to a reduction in lesion volume, although its effect on the duration of the depolarizations was not statistically significant. This might be caused by the fact that measurements of DC potentials were restricted to 90 minutes after SAH induction. At this time
Magnesium reduces ischemic depolarization and SAH volume

point, persistent depolarization was found on some electrodes in six animals in the control group and in only one in the Mg\(^{++}\)-treated group. This difference in the distribution of the two groups can partly be obviated by the use of a non-parametric (Mann–Whitney) test. When this test was used, Mg\(^{++}\) therapy led to a reduction in the duration of depolarization time that was nearly significant (\(p = 0.053\)).

Depolarization time, as detected by DC potential measurements, was found to correlate with lesion volumes on DW MR images. Similar observations have been made in rat models of ischemic stroke, indicating that IDs may play a role in the pathophysiology of SAH.\(^10\)

Our data support the concept that acute ischemia-induced reductions in the ADC primarily reflect cellular swelling (that is, cytotoxic edema), because the depolarization-induced ion shifts are accompanied by intracellular H\(_2\)O accumulation.\(^{15,21,28,41}\)

Magnesium is readily available and inexpensive, and has a well-established clinical profile in obstetric and cardiovascular practice. It is a promising agent for suppressing cell membrane depolarization during ischemia, and it passes the blood–brain barrier.\(^13\) Neuroprotective mechanisms of Mg\(^{++}\) include inhibition of the release of excitatory amino acids and blockade of the NMDA–glutamate receptor.\(^{33}\) The excitatory amino acid glutamate is released in excess during brain ischemia and is a reliable predictor of outcome in experimental SAH.\(^3\) The NMDA receptors are activated by glutamate and other excitatory amino acids, after the voltage-dependent removal of channel-blocking Mg\(^{++}\). The influx of Ca\(^{++}\) ions via the NMDA receptor is an important mechanism in the pathogenesis of ischemic cerebral injury.\(^7\) Magnesium delays anoxic depolarization, in contrast to the potent NMDA-receptor antagonist MK-801.\(^{38}\) This implies that Mg\(^{++}\) does not exert its anoxic depolarization–postponing effect through NMDA receptor blocking alone. Magnesium is also a noncompetitive antagonist of voltage-dependent Ca\(^{++}\) channels, displays cerebrovascular dilatory activity,\(^{31}\) can reverse delayed cerebral vasospasm after experimental SAH in rats,\(^2\) and is an important cofactor of cellular adenosine triphosphatases, including the Na\(^+/K^+\)-adenosine triphosphatase.

Our results on the neuroprotective value of Mg\(^{++}\) are in agreement with those of others who reported reduction in infarction volume in rats that were pretreated with 90 mmol/L MgSO\(_4\) before being subjected to 1.5 hours of MCA occlusion.\(^{29}\) In patients with SAH, not only initial ischemia but also secondary ischemia plays an important role. The pathogenesis of secondary ischemia remains to be elucidated. In our model, which closely resembles aneurysm rupture in humans, the mortality rate from the initial bleeding was 20%, which is lower than expected. Because of the relatively low case fatality rate, this model probably can also be used for long-term survival experiments after SAH to study delayed ischemia associated with SAH. The aim of the present experiments was to investigate whether this model is suitable to test the effect of promising neuroprotective agents by means of both electrophysiological and MR imaging data. The possibility of detecting an effect is maximum when this medication is given before hemorrhage occurs; however, this design is not in accordance with the clinical situation. The results of this study show that our method of inducing SAH is valid. To approach the clinical situation, additional studies are needed.

These should include extended observation periods after the SAH and administration of Mg\(^{++}\) after induction of SAH.

**Acknowledgment**

Gerard van Vliet is gratefully acknowledged for his expert technical assistance.

**References**


Address reprint requests to: W. M. van den Bergh, M.D., Department of Neurosurgery, Room G03.124, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands. email: w.m.vandenbergh@neo.azu.nl.