Effects of Hypothermia on Evoked Potentials, Magnetic Resonance Imaging, and Blood Flow in Focal Ischemia in Rabbits

Eng H. Lo, PhD, and Gary K. Steinberg, MD, PhD

Background and Purpose: Mild hypothermia has been shown to ameliorate neuronal damage due to cerebral ischemia. In our study, the influence of mild-to-moderate hypothermia was examined in a rabbit model of focal cerebral ischemia.

Methods: After 4 hours of permanent ischemia induced by occlusion of the anterior and middle cerebral and internal carotid arteries, somatosensory evoked potentials and regional cerebral blood flow were measured. Ex vivo magnetic resonance imaging scans were also obtained to determine the degree of ischemic brain injury. Three temperature (temporalis muscle) groups were studied: 37°C, 33°C, and 30°C (n=5 per group). An additional two animals were used to confirm that temporalis muscle temperatures were well correlated with brain temperature. Rectal temperatures were kept constant (37.5°C) in all groups.

Results: After 4 hours of focal ischemia, evoked potentials in the normothermic animals remained depressed (2.2±2.1% [mean±SEM] preocclusion values). Recovery of potentials was significantly enhanced in both hypothermic groups (p<0.05): 18.2±6.5% (33°C) and 43.6±12.2% (30°C). Quantitative magnetic resonance measurements showed that T1 and T2 relaxation times were increased in the core ischemic regions within the cortex (20.4±4.0% and 25.3±5.9%, respectively). These elevations in T1 and T2 were reduced by hypothermia. However, blood flow was not improved by lowered temperature; in fact, flow in the 30°C group was significantly decreased compared with the other groups (p<0.01). There was no statistically significant correlation between specific cerebral blood flow values and T1 or T2 elevations.

Conclusions: These results demonstrate that hypothermia can improve evoked potentials and magnetic resonance parameters in permanent focal ischemia. However, moderate hypothermia (30°C) appears to also significantly decrease blood flow in the ischemic brain.

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KEY WORDS • cerebral blood flow • cerebral ischemia • hypothermia • magnetic resonance imaging • rabbits

It has been known for some time that temperature can profoundly influence the ability of nervous tissue to withstand injury.1-5 For example, complete cessation of cerebral blood flow may be withstood for up to an hour by lowering brain temperature to 6°C.6 The majority of studies that demonstrate the efficacy of hypothermia have been conducted with global ischemia models.1,2,4,5 The evidence for hypothermic protection in focal ischemia is not as clear; some studies demonstrate protection,2 whereas others show that hypothermia may not protect or, in fact, may be detrimental.10

Although there has been a revived interest in the therapeutic use of hypothermia, the underlying mechanisms that mediate the beneficial effects of lowered temperature are not well defined, especially in focal ischemia. In this study, we used a rabbit model of focal cerebral ischemia. Previous studies have shown that this model results in a large and reproducible region of decreased flow and abolition of somatosensory evoked potentials (SEPs).11,12 The aims of the present study are threefold: 1) to examine the effects of hypothermia on functional recovery using somatosensory evoked potentials (SEPs) as a quantitative measure of electrophysiologic injury and recovery, 2) to measure the effects of mild and moderate hypothermia on regional cerebral blood flow (rCBF) patterns in and around the ischemic core, and finally 3) to further investigate the protective mechanisms of hypothermia by correlating ex vivo measurements of magnetic resonance imaging (MRI) parameters of cerebral injury with rCBF values in focal ischemia.

Materials and Methods

We used a rabbit model of focal cerebral ischemia that has been described previously.11,12 Briefly, male New Zealand White rabbits (2.8-3.2 kg) were anesthetized with halothane (3% induction and 0.5-1.0% maintenance) and artificially ventilated with a mixture of 0.5 l/min oxygen and 4.5 l/min air. Systemic parameters including mean arterial pressure, end-tidal CO2, and...
arterial blood gases were continuously monitored. Base
deficits were corrected as necessary with intravenous
sodium bicarbonate. A transorbital approach was used
to selectively occlude the left anterior, middle cerebral,
and internal carotid arteries with microaneurysm clips.
A permanent occlusion for 4 hours was used for this
study.

We studied a total of 17 rabbits. Three temperature
groups were used: 37°C, 33°C, and 30°C (n=5 per
group). Temporalis muscle temperature was measured
using a 16-gauge copper-constantan thermocouple
probe (Omega). Local temperatures were maintained at
the desired levels with heat lamps and a cooling fan.
The distance of the heat lamp from the animal’s head
was changed to maintain the proper temperatures for
the 37°C and 33°C groups. For the 30°C rabbits, the
lamp was turned off, and a cooling fan was used to lower
the temperature. In addition, two other animals were
used to correlate temporalis muscle temperature with
brain temperature. A hole was drilled 1 cm lateral to
bregma, and a 33-gauge brain thermocouple probe
(Omega) was lowered 4 mm into the brain to measure
cortical brain temperature. Temperatures were then
varied between 27°C and 39°C over the course of 4
hours, and brain and temporalis muscle measurements
were monitored. Rectal core temperatures were mea-
sured with a Yellowsprings Instrument probe and kept
constant at 37°C with a warming pad placed under the
animal.

The SEPs were measured with bone screws inserted 5
mm lateral to bregma. Electric stimuli (10 mA, 0.25
msec, 2.1 Hz) were delivered using needle electrodes
placed over left and right median nerves. One hundred
responses were averaged for each measurement, and
SEPs were measured before and subsequently every
half hour after occlusion. SEP amplitudes were mea-
sured as the difference between the first positive peak
and the first major negative trough11 and analyzed as
percentages of preocclusion values.

The rCBF was measured after 4 hours of permanent
focal ischemia using 15-μm radioactive microspheres. A
bolus injection of the microspheres into the left atrial
appendage was followed by a reference blood withdrawal
rate of 3.6 ml/min from the femoral artery. Rabbits were
then killed, brains were removed, and MRI scans were
performed. After scanning, brains were dissected into
separate regions for radioactive counting in a well-type
gamma counter. Cortical areas were dissected as de-
scribed previously12 to correspond with MRI scanning
levels. The rCBF values were calculated using standard
techniques.13 Frequency distribution analysis was used to
analyze cortical blood flow values.12 Briefly, each brain
was dissected into 10 cortical regions per hemisphere.
The cumulative distributions of rCBF were plotted for
each temperature group. Comparisons of flow between
the three groups were performed by comparing the averages
of these frequency distributions.

We performed MRI scanning with a proton coil in a
1.5-T General Electric Sigma Imaging System. Scans
were obtained using a 256×256 data matrix and 5-mm
slice thickness with no interslice spacing in the coronal
plane. MRI scan parameters were varied accordingly
for T1-weighted (repetition time [TR] 600 msec, echo time
[TE] 25 msec) and T2-weighted (TR 2,500 msec, TE
25×4 msec) scans, respectively. T1 and T2 relaxation
times were measured using standard methods that have
been described elsewhere.14 Regions-of-interest (ROI) 1×1
mm2 were placed over cortical areas corresponding to
tissue samples dissected for microsphere counting. The method of cortical dissection has been
described previously.11 In this way, correlations between
relaxation parameters and rCBF could be performed.
Calibration vials were not used for relaxation time
calculations; some variance between scans would be
expected. To normalize for possible interscan vari-
ations, analysis of MRI relaxation times were performed
using percentage increases in T1 and T2 compared with
a corresponding ROI placed in the contralateral
hemisphere.

Statistical analysis of rCBF and MRI data was per-
formed using completely randomized analysis of vari-
ance (ANOVA) followed by post hoc Student's t tests
and Tukey’s honestly significant difference tests. SEP
data were analyzed using the nonparametric Kruskal-
Wallis test followed by post hoc Mann-Whitney tests.
Linear regressions were performed to examine correla-
tions between parameters of interest.

Results

In the two unoccluded animals, brain temperature was
found to be well correlated with temporalis muscle tem-
perature (r=0.849, p<0.01). The following equation de-
scribed the relation between the two temperature para-
eters: (brain temperature)=8.178 ± 0.814 x (temporalis
muscle temperature). In the ischemic animals, there were no
differences in systemic parameters between the three
temperature groups (Table 1).

SEPs were abolished within 5 minutes of arterial occlu-
sion in all temperature groups. Recovery of SEP amplit-
itudes was demonstrated to be significantly influenced by
temperature (Table 2). After 4 hours of permanent focal
ischemia, SEP was 2.2±2.1% (mean±SEM) of preocclu-
sion values in the normothermic (37°C) animals. Kruskal-
Wallis tests demonstrated that there were significant dif-
fferences in SEP recovery between the three temperature
groups (p<0.05). Mann-Whitney tests showed that SEP
recovery was significantly improved (p<0.05) to
18.2±6.5% (33°C) and 43.6±12.2% (30°C). There was,
however, no statistically significant difference between the
two hypothermic groups.

Consistent with previous findings in this model, is-
chemia-induced MRI alterations were primarily found
in the anterior and ventral cortical regions in the
 distributions of the left internal carotid, middle cere-
TABLE 2. Evoked Potentials and Quantitative MRI Parameters by Temperature Group

<table>
<thead>
<tr>
<th>Temperature group</th>
<th>Evoked potentials (percent preocclusion values)</th>
<th>MRI scan T1-weighted (percent increase)</th>
<th>MRI scan T2-weighted (percent increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>2.2±2.1</td>
<td>20.4±4.0</td>
<td>25.3±5.9</td>
</tr>
<tr>
<td>33°C</td>
<td>18.2±6.5</td>
<td>9.8±2.9</td>
<td>10.1±1.2</td>
</tr>
<tr>
<td>30°C</td>
<td>43.6±12.2</td>
<td>10.1±1.2</td>
<td>16.0±2.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=5 rabbits in each temperature group.

bral, and anterior cerebral arteries. Quantitative MRI analysis demonstrated that both T1 and T2 relaxation times were increased by 4 hours of permanent focal ischemia compared with values in the contralateral hemisphere (Table 2). Percentage increases in T1 and T2 were well correlated (r=0.708, p<0.01), and showed a significant dependence on temperature. ANOVA between the various groups revealed p=0.032 (T1) and p=0.028 (T2). Post hoc t tests showed that T1 relaxation times were improved by hypothermia (p<0.05); in the ischemic cortex, T1 was increased by 20.4±4.0% (mean±SEM) in the normothermic animals versus 9.8±2.9% (33°C) and 10.1±1.8% (30°C) in the hypothermic groups. T2 was increased by 25.3±5.9% in the normothermic ischemic cortex versus 10.0±1.2% (33°C) and 16.0±2.3% (30°C) in the hypothermic animals. The variation in T2 measurements was greater than those for T1 measurements so that, although ANOVA demonstrated that there were significant differences between the three temperature groups, post hoc t tests showed significance (p<0.05) only between 37°C and 33°C groups. The difference between 37°C and 30°C rabbits approached significance (p=0.06). The more conservative Tukey's honestly significant difference test revealed similar conclusions, although the actual probability values were less impressive: T1, p<0.05 (37°C versus 33°C) and p=0.06 (37°C versus 30°C); T2, p<0.05 (37°C versus 33°C) and p=0.09 (37°C versus 30°C). There were no differences in other brain regions examined (striatum, thalamus, and hippocampus).

There were no differences in striatal, thalamic, hippocampal, and cerebellar blood flow (left and right hemisphere) between the various temperature groups. A frequency distribution analysis was conducted by plotting the probability distribution functions for all cortical regions in each temperature group. Blood flow values in the cortex ranged from approximately 9 to 90 ml/100 g/min in the occluded hemispheres (Figure 1), demonstrating the large range and gradients of flow present in focal ischemia. Moderate hypothermia significantly affected cortical blood flow in the ischemic hemisphere (Figure 1). In general, flow was lower across all rCBF values in the ischemic cortex of the 30°C group compared with both the 37°C and the 33°C groups (p<0.01). To compare between groups, the mean flow value for each distribution was calculated. Mean rCBF (±SEM) was decreased in the 30°C group (33.3±1.9 ml/100 g/min) compared with the other rabbits (44.2±2.9 ml/100 g/min [37°C] and 43.8±3.0 ml/100 g/min [33°C]). A similar but less consistent reduction in rCBF was observed in the contralateral hemisphere: 64.5±2.0 ml/100 g/min (30°C), 71.0±3.0 ml/100 g/min (37°C), and 79.0±2.0 ml/100 g/min (33°C). Statistical significance was achieved only between the 30°C rabbits and the 33°C rabbits (p<0.01); rCBF differences compared with the 37°C group did not reach statistical significance (p=0.12). There was no correlation between rCBF values in the various cortical regions and the loss/recovery of SEP. There was also no significant correlation between individual rCBF values and MRI relaxation times in the ischemic cortex. However, there was a trend that showed that T1 was inversely related to rCBF values (r=0.55, p<0.05).

Discussion

Previous studies conducted in our laboratory with this model of focal ischemia in the rabbit have shown that reproducible histological lesions are obtained.11,16

FIGURE 1. Graph of cumulative probability distribution of regional cerebral blood flow (rCBF) in the ischemic cortex demonstrating that flow is lower in rabbits in the 30°C group (dashed line) compared with the other two temperature groups, 37°C (solid line) and 33°C (dotted line).
Regions of ischemic neuronal damage observed in the striatum and neocortical regions were characterized by moderate-to-severe neuronal shrinkage, increased nuclear basophilia, nuclear pyknosis, and neuropil palor. In the present study, the effects of hypothermia on SEPs, MRI, and rCBF were examined in this model of focal cerebral ischemia. It was found that hypothermia appeared to promote the recovery of SEPs after 4 hours of permanent ischemia. The recovery of evoked potential amplitudes may reflect the return of electrophysiological function in the affected neurons. However, other studies argue that there may be poor correlation between electrophysiological activity and clinical recovery. Other studies have shown that lowered temperature slowed conduction times in synaptic and axonal pathways, which may also have an impact on clinical status. Recovery of SEPs may in fact be related to the degree of subcortical injury to afferent white matter tracts; it was not possible to measure white matter rCBF given the spatial limitations of the microsphere technique. Despite these possible variations in the interpretation of improved evoked potentials, the data suggest that the return of electrophysiological function may be enhanced by lowered temperature, which may be beneficial regardless of the exact site of action.

Thus far, hypothermic protection against ischemic injury has been primarily measured using histological correlates. Although microscopic evidence of morphological damage is still the most accurate method of quantifying brain injury, other indexes of damage may be more relevant clinically. In our study, we have used quantitative MRI scanning to measure ischemic injury. Hypothermia reduced the ischemia-induced elevations in both T1 and T2 relaxation times. Although the exact relation between MRI relaxation times and cellular injury is not clearly defined, other investigators have shown that alterations in MRI parameters most likely correlate with increases in total water content as well as cellular water compartmentation. Previous studies have demonstrated that N-methyl-D-aspartate antagonists also prevented MRI alterations after focal ischemia that corresponded to protection against histological correlates of edema and neuronal injury. The advantage of MRI is that it provides a clinically applicable index for assessing various strategies of treatment for focal ischemia. The drawback in our study was that MRI scans were performed ex vivo. However, studies have shown close correlation between ex vivo and in vivo measurements of relaxation times.

In our present study, a frequency distribution analysis demonstrated that moderate hypothermia to 30°C resulted in decreased cortical blood flow when compared with temperature levels of 37°C and 33°C. These subtle alterations in rCBF were not observed in other brain regions, including striatum, thalamus, hippocampus, and cerebellum. Frequency distribution analysis of rCBF is a useful method of assessing alterations in rCBF in focal ischemia in which there are sharp gradients in rCBF and intersubject variations in the anatomical distribution of flow values. These differences may prevent significant changes in rCBF from being detected with a simple region-by-region comparison between groups. The use of frequency distribution analysis is also especially useful in this study, as the sample sizes are small (n=5 per group). In such a case, this technique allows for a greater power in detecting small differences in rCBF. Of course, there is a trade-off because increased statistical power does not come free: frequency distribution analysis may be able to detect small but significant differences in flow values, but it is not able to identify the anatomical location of these improvements in flow. Because the high heterogeneity of rCBF in this focal model of cerebral ischemia results in small regions of low flow (<20 ml/100 g/min), and overall flow values are likely to be in the 30-40 ml/100 g/min range, this method of analysis has a tremendous drawback in its inability to identify the region of flow improvements. Also unclear is why there is a decrease in blood flow at 30°C but not at 33°C. If the decreased rCBF were related to temperature-mediated reductions in global metabolism, one would expect a graded response. Furthermore, the changes in the contralateral hemisphere did not show consistent statistical significance and may be due to variability in the normothermic group. It is possible that the decrease in rCBF at 30°C may be related to functional alterations similar to the nonlinear SEP response observed in hypothermia experiments. It has been well documented that evoked potential amplitudes are not decreased when temperature is lowered from 37°C to 28-30°C. However, when the temperature is lowered below this threshold, evoked potential amplitudes rapidly decrease. Busto et al. showed that hypothermia to 30°C showed a greater degree of protection than to 33°C, even though both levels of lowered temperature resulted in similar degrees of reduction in excitotoxic neurotransmitter efflux. Therefore, it is possible that additional mechanisms of cerebral protection may play important roles at different degrees of hypothermia. It is unclear how a reduction in flow might be beneficial. However, it has also been found that protective doses of MK-801 decrease rCBF in a rat model of focal ischemia. It is possible that the decreased blood flow may reflect decreased excitotoxic activity caused by the ischemic insult.

It is important to recognize that the degree of rCBF reduction, and hence the level of hypothermia, is crucial; excessive decreases in rCBF could have potentially adverse effects in focal ischemia, as capillary sludging may decrease collateral blood supply to the penumbral regions. However, in this study, there appeared to be no detrimental effects, and evoked potentials and MRI parameters were equally improved at 33°C and 30°C. On the other hand, it is possible that delayed processes of damage could emerge much later than the time scale of the present study. Finally, our data differ from that of Busto et al. that showed no change in rCBF in global ischemia during hypothermia. Focal ischemia may result in different spatial alterations in rCBF from global ischemia where blood flow is uniformly reduced. These differences may result in pathophysiologic states that respond differently to temperature changes.

In conclusion, our data suggest that mild-to-moderate hypothermia improves SEP and MRI parameters in a rabbit model of permanent focal ischemia. However, there was evidence that moderate hypothermia (30°C) induced a decrease in rCBF that was not seen in mild hypothermia (33°C). These additional effects on blood flow may significantly influence the degree of hypothermia that would be...
therapeutic in focal ischemia. Temperature effects on electrophysiological function may underlie the decreased rCBF and further contribute to neuroprotection at 30°C, but excessive reductions in rCBF may adversely affect collateral flow in the penumbral zones. The underlying mechanisms of these alterations are not well defined and warrant further investigation.

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