Duration of Ischemia Influences the Development and Resolution of Ischemic Brain Edema


SUMMARY. The influence of the duration of ischemia on the development and resolution of post-ischemic brain edema (SG method) was studied in anesthetized rats. Edema developed during ischemia and the amount of edema was related to the duration of ischemia (r = 0.843, p < 0.001). With recirculation to three hours, the major determinant of the amount of edema was still the duration of the preceding ischemia (p < 0.001). Resolution of brain edema only occurred following fifteen minutes ischemia. Post-ischemic blood-brain barrier breakdown ([^14]C-AIB, EB albumin) was greatest following longer ischemia. Where present, the BBB leakage was simultaneously to large and small molecules.

BRAIN SWELLING due to water accumulation is an important complication of cerebral ischemia and will frequently lead to trans-tentorial herniation and death.1–3 The amount of water accumulating during ischemia depends on the level of flow during ischemia4–5 and its duration,6,7 and on whether the ischemia is complete or incomplete.8 Restoration of blood flow to ischemic brain may improve outcome and operations to revascularize the ischemic brain have been devised. Unfortunately, reperfusion may lead to an increase in brain water, occurring either immediately9,10 or later, when it is associated with breakdown of the blood-brain barrier and leakage of protein-rich fluid into the brain interstitial space.5

We have used the model of cerebral ischemia described earlier11 to measure cerebral blood flow (CBF), brain edema, and blood-brain barrier (BBB) permeability quantitatively and on a regional basis in the same animal. This study examined the effect of increasing duration of ischemia on the development and resolution of brain edema and blood-brain barrier permeability in recirculation after temporary, severe cerebral ischemia.
Methods

Details of the preparation of animal model and measurement of cerebral blood flow may be found in the accompanying paper. In brief, we used a four-vessel occlusion model of cerebral ischemia in the paralysed, ventilated rat. Anesthesia was 70% nitrous oxide, 30% oxygen, 0.5% halothane. Systemic arterial blood pressure and body temperature were measured continuously. PaCO₂, pH and PaO₂ were measured intermittently.

Brain Edema

Brain water was measured by the specific gravity (SG) method using linearly inhomogeneous bromobenzene/kerosene columns. The columns were calibrated with potassium sulphate solutions of known specific gravity and columns were only used where the coefficient of linearity was greater than 0.9950.

Animals were killed by decapitation and the brain was rapidly removed and immersed in kerosene to prevent drying. Using the microscope, six samples, approximately 1 mm³ were dissected from each brain region, avoiding the immediate site of electrode implantation. The samples were placed immediately into the columns and the position of each sample measured under kerosene, and after removing six small samples at 3 minutes; regional SG was taken as the mean of six samples.

Blood-Brain Barrier Permeability

The blood-brain barrier was assessed qualitatively using a macromolecular tracer, and quantitatively using a low molecular weight tracer. 0.2 ml of 2% buffered Evans' Blue solution was injected intravenously 20 minutes prior to sacrifice. This dye binds to albumin (M.Wt = 70,000) which is normally excluded from the brain parenchyma by the blood-brain barrier. Its presence in the brain was scored simply as either present (EB positive) or absent (EB negative).

We also utilised a quantitative, radioisotopic technique to evaluate blood-brain barrier permeability to a small molecule. Animals received an intravenous injection of 10 uCi of alpha-aminoisobutyric acid (40-60 mCi/mM Amer- sham). Ten arterial blood samples were collected over a ten minute interval and the ¹⁴C-AIB concentration measured on 0.02 ml of plasma by liquid scintillation counting. Ten minutes after the administration of the tracer, the animals were sacrificed, the brain placed under kerosene, and after removing six small samples for measurement of brain water content (vide supra), the same brain regions were used to measure brain isotope concentration. The brain samples (50-70 mg) were placed in pre-weighed vials which were immediately re-weighed. One ml of Soluene 350 was added to each vial and vials were incubated at 55°C over-night. 10 ml of Omnifluor were added to the solubilized tis- sue plus a few drops of glacial acetic acid to neutralize the solution. Samples were left for 24 hours at room temperature before ³/²-counting was performed in a Beckman LS 3150 T spectrometer. Counts were corrected for quench and background by an external standard method.

A blood-to-brain transfer constant, Ki, was derived:

\[
Ki = \frac{Ci}{\int_0^T Cp \cdot dt}
\]

where \(Ci\) is the isotope concentration in the brain at the end of the experiment and a correction is applied for the amount of tracer present in the vascular compartment at the end of the experiment. The denominator is a calculated integral of the tracer activity in the plasma (Cp) during the experiment.

Experimental Protocol

The same 48 rats described in the accompanying paper were used. In addition, 12 rats were sacrificed to measure pre-ischemic brain SG and BBB permeability under the experimental conditions. The three experimental groups were as before: Group A — 15 minutes ischemia; Group B — 30 minutes ischemia; Group C — 60 minutes ischemia.

Animals were sacrificed at specific times in recirculation: 0 minutes (i.e. end-ischemia, no recirculation); 30 minutes recirculation; 60 minutes recirculation; 180 minutes recirculation.

Again three cortical regions, frontal, parietal and occipital were assessed (‘site’). The study design was therefore a four x three x three matrix. Each cell contained data from four animals (each with six cortical samples).

Statistical Analysis

The specific gravity data were compared by analysis of variance with repeated measures using the BMDP programme package.

Eight variables were used (Group, Time plus six regions for each animal). The SG data were trans- formed to give mean regional data.

The design dependants were therefore three x three (Groups A, B, C x frontal, parietal, occipital) with four reperfusion times (0, 30, 60, 180 minutes). The differences between the dependant means were compared to an error term. The degrees of freedom were calculated for individual cases.

Specific intergroup comparisons were made using a Students T-test.

The quantitative transfer constants for ¹⁴C-AIB were assessed by comparing the data for each experimental group with a comparable pre-ischemic value, using the Mann-Whitney, U-test.

Results

General Physiological Parameters and Cerebral Blood Flow

These have been detailed in tables 2 and 3 of the accompanying paper.
Table 1  Brain Specific Gravity in Recirculation after Ischemia

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group A</td>
<td>1.0470 ± .0005</td>
<td>1.0455 ± .0008</td>
<td>1.0462 ± .0003</td>
</tr>
<tr>
<td>group B</td>
<td>1.0463 ± .0008</td>
<td>1.0453 ± .0005</td>
<td>1.0459 ± .0004</td>
</tr>
<tr>
<td>group C</td>
<td>1.0422 ± .0009</td>
<td>1.0446 ± .0015</td>
<td>1.0449 ± .0010</td>
</tr>
<tr>
<td>Parietal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group A</td>
<td>1.0469 ± .0006</td>
<td>1.0462 ± .0009</td>
<td>1.0468 ± .0008</td>
</tr>
<tr>
<td>group B</td>
<td>1.0465 ± .0008</td>
<td>1.0457 ± .0010</td>
<td>1.0460 ± .0005</td>
</tr>
<tr>
<td>group C</td>
<td>1.0448 ± .0005</td>
<td>1.0449 ± .0011</td>
<td>1.0450 ± .0010</td>
</tr>
<tr>
<td>Occipital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group A</td>
<td>1.0469 ± .0007</td>
<td>1.0461 ± .0007</td>
<td>1.0464 ± .0008</td>
</tr>
<tr>
<td>group B</td>
<td>1.0457 ± .0007</td>
<td>1.0454 ± .0008</td>
<td>1.0454 ± .0017</td>
</tr>
<tr>
<td>group C</td>
<td>1.0448 ± .0009</td>
<td>1.0450 ± .0005</td>
<td>1.0440 ± .0017</td>
</tr>
<tr>
<td>Mean Cortical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group A</td>
<td>1.0469 ± .0006</td>
<td>1.0459 ± .0008</td>
<td>1.0465 ± .0006</td>
</tr>
<tr>
<td>group B</td>
<td>1.0462 ± .0008</td>
<td>1.0455 ± .0007</td>
<td>1.0458 ± .0006</td>
</tr>
<tr>
<td>group C</td>
<td>1.0446 ± .0008</td>
<td>1.0448 ± .0012</td>
<td>1.0446 ± .0016</td>
</tr>
</tbody>
</table>

Results are mean ± SD, n = 4 each cell. Pre-ischemic SG = 1.0484 ± .0005.

Specific Gravity (table 1, fig. 2)
The mean cortical SG in non-ischemic rats was 1.0484 ± 0.0005 (± SD, n = 12), there was no regional difference in SG and electrode implantation did not alter cortical SG.

During ischemia, there was a fall in SG in all cortical regions and in all three experimental groups. The amount of water accumulating during ischemia was significantly correlated to the duration of ischemia, r = 0.843, p < 0.0001 (fig. 1). The specific gravity data obtained in recirculation are shown in table 1. ANOVA demonstrated a highly significant Group effect (df = 2, F = 49.20, p < 0.001) which was independent of both site (site/group interaction; F = 0.35, p = 0.846) and reperfusion time (group/time interaction, F = 1.32, p = 0.274).

There was a site effect (df = 2, F = 5.24, p = 0.0076) and this was independent of reperfusion time (site/time interaction, F = 1.85, p = 0.012). There was significant resolution of brain edema only occurred following 15 minutes ischemia. Significant resolution of brain edema only occurred following 15 minutes ischemia. Group A. • = 30 minutes ischemia. Group B. • = 60 minutes ischemia. Group C. * = significant group effect, p < 0.001. ⋆ = significant resolution of brain edema, p < 0.05.
was no site/time interaction (df = 12, F = 1.55, p = 0.127).

There was no overall effect of recirculation (df = 3, F = 2.32, p = 0.092). However, within group analysis of the SG data demonstrated that the increase in mean cortical SG, seen in Group A, between 30–180 minutes reperfusion, was statistically significant (df = 7, t = 2.99, p = 0.02).

In summary, the main determinant of the amount of brain water in ischemia and recirculation was the duration of ischemia. A significant resolution of ischemic edema was only found following fifteen minutes ischemia.

**Blood-Brain Barrier Permeability (table 2, fig. 3)**

The mean cortical Ki for 14C-AIB in the anesthetised rat was 3.20 ± 0.7 ml/g/min x 10^-3 (mean ± SG, n = 12). There was no significant regional difference and electrode implantation did not change the measured Ki.

BBB permeability was measured in the animals that were reperfused to 180 minutes (n = 12) and the three experimental groups were as described earlier (n = 4 each group). At 180 minutes recirculation after 15 minutes ischemia (Group A) there was no EB staining and no difference between the mean cortical Ki values obtained pre-ischemia and at 180 minutes recirculation (p > 0.05, Mann-Whitney). At 180 minutes recirculation after 30 minutes ischemia (Group B) seven of 24 cortical regions (29%) showed EB staining. The cortical Ki were increased in all regions but the differences between the mean values were not significant when compared to the comparable mean pre-ischemic values (p > 0.05, Mann-Whitney). At 180 minutes recirculation after 60 minutes ischemia (Group C) there were nine of 24 (37%) EB positive cortical regions. In all regions, the Ki for 14C-AIB was significantly greater than the pre-ischemic values (p < 0.01, Mann-Whitney).

Barrier opening, where present, seems to involve an increase in permeability to both small and large molecules (fig. 3).

**Discussion**

This study examined the changes in brain edema and BBB permeability consequent upon recirculation after temporary cerebral ischemia. The model has been characterised in the accompanying paper.4 The micro-gravimetric technique is a sensitive method of measuring brain edema in a quantitative manner. We feel that it accurately reflects water content during ischemia and early recirculation.4 The BBB has classically been assessed with macromolecules, such as Evans’ Blue, albumin,101-albumin (RISA) or horse-radish peroxidase, yielding a qualitative estimate of permeability. Recently, the use of small, radioisotopically labelled molecules has permitted a quantitative measure of barrier permeability.16 The 14C-AIB methodology assumes a unidirectional flux of tracer from blood to brain and this is true in the normal brain where the cells concentrate and ‘trap’ AIB intracellularly.17 In order to ensure that the tracer flux is unidirectional after ischemia, when cellular uptake may be reduced, a suitable experimental time constant must be selected and a ten minute experiment has been considered appropriate.14 In our experiments, the tracer concentration in blood at sacrifice was still 2–3 times the concentration in the brain, which will ensure an essentially unidirectional flux, even if there is no cellular uptake. Any small reflux would lead, at worst, to a small underestimate of Ki in the pathological groups.

Ischemic brain edema develops when CBF is reduced below a threshold of about 20 ml/100 g/min and the amount of edema increases as CBF is further reduced.4,3 The four-vessel occlusion model used in this study produced severe, incomplete hemispheric ischemia,14 and this would represent a near maximal stimulus to ischemic edema formation. We found that brain edema developed during ischemia and that the amount of edema formed increased progressively with increasing duration of ischemia (fig. 1). The stimulus to this water movement is the change in brain osmolarity that occurs during ischemia.20 Failure of oxidative metabolism leads to the generation of small molecules such as H^+, pyruvate and lactate by anaerobic metabolism and

**TABLE 2 Influence of Duration of Ischemia on Ki14C-AIB**

<table>
<thead>
<tr>
<th>Pre-ischemic</th>
<th>180 Minutes Reperfusion</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal</td>
<td>3.40 ± 0.70</td>
<td>4.00 ± 1.32</td>
<td>4.31 ± 1.61</td>
<td>5.51 ± 1.20*</td>
</tr>
<tr>
<td>Parietal</td>
<td>2.81 ± 0.42</td>
<td>2.78 ± 0.30</td>
<td>3.21 ± 0.70</td>
<td>4.21 ± 0.91*</td>
</tr>
<tr>
<td>Occipital</td>
<td>3.39 ± 0.68</td>
<td>3.62 ± 0.88</td>
<td>4.18 ± 1.49</td>
<td>5.88 ± 1.39*</td>
</tr>
<tr>
<td>Mean cortical</td>
<td>3.20 ± 0.70</td>
<td>3.46 ± 1.30</td>
<td>3.90 ± 2.51</td>
<td>5.20 ± 2.43*</td>
</tr>
</tbody>
</table>

Results are mean ± SD ml/g/min x 10^-3.

n = 4 each cell.

*p < 0.01

fp < 0.001 compared to pre-ischemic values (Mann-Whitney).

**FIGURE 3. Post-ischemic blood-brain barrier permeability.** At 180 minutes recirculation after temporary cerebral ischemia, the degree of BBB leakage was related to the duration of ischemia. Where there was an increase in BBB permeability, this leak was simultaneously to both large and small molecules. A = 15 minutes ischemia, B = 30 minutes ischemia, C = 60 minutes ischemia, • EB -ve regions, □ EB +ve regions. Results are mean cortical Ki ml/g/min x 10^-3 with standard error bars.
brain osmolarity rises. The distribution of this edema will, initially, be predominantly intracellular (cytotoxic), in response to the intracellular accumulation of Na+, Ca2+ and Ca2++, resulting from failure of energy dependant ion pumps. The development of brain edema during ischemia also requires a 'functioning' vascular compartment. This is why, in complete cerebral ischemia, there is, paradoxically, less edema formed than in incomplete ischemia. The absence of residual flow in complete ischemia, limits water movements into the brain and there is an increase in brain osmolarity rather than the formation of brain edema. Our work would confirm that where there is any residual flow, however small, brain edema will develop during ischemia.

The continued formation of brain edema with longer ischemia argues for a continuing osmolar change as ischemia continues. Ionic redistribution in the cortex is likely to have been rapid, as the ischemic flows recorded were well below accepted thresholds for membrane pump failure. In incomplete ischemia, there is a continued supply of substrate and a continued generation of idioigenic osmoles. Our data suggests that this anaerobic cellular metabolism continues with up to 60 minutes of severe, incomplete ischemia. Recirculation after cerebral ischemia may lead either to an increase or to resolution of brain edema. This study demonstrated a tendency for brain edema to increase in the first 30 minutes after recirculation. The effect was not significant and should, perhaps, be ignored, however, it is of interest that the change in brain edema was related to the level of post-ischemic hyperemia. A number of authors have suggested that post-ischemic hyperemia will increase brain edema by increasing capillary hydrostatic pressure. This is an important consideration and limitation of post-ischemic hyperemia may be necessary to limit early post-ischemic brain edema. A controlled study of the relationship of post-ischemic blood flow and brain edema would seem to be required.

By three hours recirculation, resolution of brain edema had only occurred following 15 minutes ischemia. It seems, therefore, that the duration of ischemia is a determinant of whether ischemic edema will resolve. In the gerbil model, Iannotti and Hoff described a CBF threshold (10 ml/100 g/min, 60 minutes ischemia) below which ischemic edema did not resolve. Presumably these two determinants, ischemic flow and ischemic duration, act in a similar manner. That is, the potential for resolution of edema, depend upon the severity of the ischemic insult. A similar link between ischemic flow and duration has been described for neurological and neuropathologic outcome following ischemia in primates. Resolution of brain edema implies a return of cell volume homeostasis. Recirculation leads to a burst of aerobic metabolism and a rapid return of cellular energy, provided that there has been no irreversible cellular damage. This study suggested that severe cerebral ischemia is compatible with recovery of cell homeostasis, provided the duration of ischemia is short.

We also demonstrated that opening of the BBB was dependant upon the duration of ischemia. An increase in BBB permeability is well recognised after ischemia and has led to ischemic edema being classified separately. In permanent ischemia, the BBB remains intact, at least for 6-9 hours. Recirculation after ischemia accelerates BBB damage. We found that the barrier was damaged to a greater extent following a longer ischemic insult. This confirms the data of Nishimoto et al and extends it by quantitating the degree of barrier permeability. Recent workers have suggested that there may be an early, post-ischemic increase in barrier permeability to small molecules only, in the absence of a protein leak. Measuring barrier permeability at 180 minutes recirculation, we found, by contrast, that BBB breakdown, where present, was to both protein and low molecular weight substances together (fig. 3). This is the classic vasogenic phase of post-ischemic edema.

BBB opening should accelerate post-ischemic edema. We were unable to correlate regional specific gravity with Ki. This probably reflects the measurement of barrier permeability at a single point in time when the barrier change is a process in evolution. However, it remains possible that the failure of edema to resolve following prolonged ischemia is due to a secondary water increase related to BBB breakdown. Brain edema and associated BBB breakdown are serious consequences of cerebral ischemia. With severe, incomplete ischemia both the degree of brain edema and the extent of BBB breakdown are proportional to the duration of the ischemic insult. From our studies we would suggest that there is no 'safe' period of ischemia, but rather ischemic damage progressive with the duration of ischemia.

Acknowledgments
The authors are grateful for the technical help of Miss Kathryn Allen and Miss Caroline Williams.

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INFLUENCES OF DURATION OF ISCHEMIA ON ISCHEMIC BRAIN EDEMA/Todd et al


Duration of ischemia influences the development and resolution of ischemic brain edema.
N V Todd, P Picozzi, A Crockard and R W Russell

Stroke. 1986;17:466-471
doi: 10.1161/01.STR.17.3.466

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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