Multifaceted Therapy After Global Brain Ischemia in Monkeys

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SUMMARY The pathophysiology of postischemic encephalopathy is complex, and includes tissue acidosis, edema, hypoperfusion, membrane dysfunction, impaired energy production, and possibly hypermetabolism. We tested the hypothesis that this multifactorial clinical problem must be approached with multifaceted therapy, with specific treatment aimed at each of the above postischemic changes. Eighteen minutes of complete global brain ischemia was produced with a higher pressure neck cuff in pigtailed monkeys. Control treatment postischemia (n = 9): 1) Normotension (MAP ≥ 80 mmHg) restored within 2 min postischemia, 2) controlled ventilation for 24 hours with PaCO₂ = 25 mmHg, 3) normothermia, and 4) phenytoin seizure prophylaxis from 20 hours postischemia. Experimental treatment (n = 10): Control treatment plus the following modifications: 1) Hemodilution to hematocrit 25% at 1-4 min postischemia, 2) brief hypertension (MAP 130 mmHg for 5 min) after accomplished hemodilution, 3) hyperthermia for 6 hours, 4) pentobarbital 30 mg/kg i.v., 5) dexamethasone 4 mg/kg i.v. Outcome was evaluated at 96 hours postischemia by overall performance categories (OPC) (OPC I = normal, OPC V = brain death), neurologic deficit (ND) scores (100% ND = brain death, 0% ND = normal), and histologic damage scores of the brains. Results: Brain death developed in 1/9 control and 0/10 treated animals. The number of awake monkeys (OPC I and II) at 96 hours postischemia was significantly higher in the treated group (7/10) than in the control group (2/9) (p = 0.05). The median ND scores for the two groups were 16 and 35% respectively (p > 0.05). The results strongly suggest that postischemic treatment may be beneficial and that a multifaceted therapeutic approach is worth pursuing.

IN SPITE OF INTENSIVE RESEARCH, no specific therapy has so far been identified and generally accepted for use after complete global brain ischemia (GBI). Treatment with large doses of barbiturates was initially considered beneficial when given before1 as well as after2 the ischemic episode, but these encouraging results have not been reproduced in later studies,3,5 and the value of barbiturate treatment after GBI remains questionable. Research in cerebellar resuscitation has been encouraged by an improved understanding of pathophysiologic events in the brain during and after ischemia.5,7 Changes occurring after ischemia, when normal perfusion pressure is restored, are believed to cause further damage,8 and therapeutic intervention in the early postischemic period might be of value in preventing at least part of the ensuing brain damage. Identified changes in the brain after ischemia include: 1) Reflow problems, multifocal and generalized hypoperfusion,9-14 2) brain tissue acidosis,15 3) brain edema,11,16,17 4) membrane failure,5,7,18-21 5) impaired restitution of energy production,12,19-21 6) hypermetabolism with a mismatch between oxygen supply and cerebral metabolic demands,13,19,22,23 Furthermore, there may be postischemic seizures24 that may cause additional brain damage.25 With such a complex, multifactorial problem, we chose to test the hypothesis that treatment after GBI also will have to be multifaceted in order to be effective. The aim was to treat each and all of the above postischemic (PI) changes to determine if this would improve final neurologic outcome. We studied a combination of the following modes of treatment PI: 1) Hemodilution, 2) hypertension, brief and moderate, 3) hyperthermia, 4) pentobarbital anesthesia, and 5) dexamethasone. Although none of these treatment modalities alone have been shown to improve neurologic outcome after GBI, there is experimental evidence that each of these treatments has a beneficial effect on one
The soft, cuffed, endotracheal tube was changed to a stiff, non-compressible uncuffed tube. Halothane was discontinued for exactly 5 min prior to induction of ischemia, while paralysis and controlled ventilation (IPPV) with 66% N₂/O/33% O₂ were continued. Five minutes is obviously too short time for complete halothane washout, but a certain depth of anesthesia is needed in this type of experiment, and we believe that the animals were in comparable levels of anesthesia at the time of neck cuff inflation.

Complete head ischemia was produced with a combination of trimetaphan induced hypotension (MAP = 50–80 mmHg) and neck cuff inflation to a pressure of 1500 mmHg, while the non-compressible tracheal tube allowed normal ventilation to continue. F₂O₂ was 1.0 during ischemia. After about 15 min of GBI, an infusion of norepinephrine was started (0.08 mg/ml, 1–3 ml/h) to raise mean arterial pressure (MAP) towards normal just before cuff release after exactly 18 min of ischemia. Following neck cuff release, the blood pressure always dropped, but we aimed at having normotension restored (MAP ≥ 80 mmHg) within 2 min PI. Thereafter, MAP was kept at the desired level for each treatment group. This required norepinephrine infusion early PI, and sometimes trimetaphan infusion later to avoid unwanted hypertension (if MAP > 125 mmHg, trimetaphan 5 mg/ml, 0.2–2 ml/h).

Completeness of ischemia with this model has been confirmed in previous investigations with radioactive tracers, with cerebral angiography, with almost immediate EEG isoelectricity, and with the absence of intracranial hypertension. In each experiment in this study, completeness of ischemia was documented by observing EEG isoelectricity within 15 sec of cuff inflation, persisting pallor of the face and oral mucosa, and absence of retinal circulation judged by ophthalmoscopy. These observations strongly suggest that ischemia is complete, but they are not absolute proof of this.

Treatment Groups

Twenty animals underwent the described preparations and 18 min of GBI. They were randomly assigned to two different groups:

Control Group (n = 9)

1) Normotension (MAP ≥ 80 mmHg) was restored within 2 min PI, 2) paralysis/IPPV for 24 h PI with PaCO₂ at about 25 mmHg; F₂O₂ was 1.0 for the first 2 h PI, thereafter 0.5 from 2–24 h PI, with 50% N₂/O/50% O₂, 3) normothermia, 37.5 ± 1°C, 4) seizure prophylaxis with phenytoin beginning at 20 hours PI; 10 mg/kg i.v. initially, followed by 2 mg/kg i.v. every 8 h until 72 h PI. At 24 h PI, N₂O was discontinued and the residual effect of pancuronium reversed with atropin 0.15 mg and neostigmine 0.3 mg i.v. Extubation was performed when protective reflexes were adequate and blood gases normal.

Experimental Treatment Group (n = 11)

Control treatment as above, plus the following modifications:
MULTIFACETED THERAPY AFTER BRAIN ISCHEMIA/Gisvold et al

(1) Hemodilution with Lactated Ringer and 5% albumin. Hemodilution was started at 1 min PI with rapid injection of Lactated Ringer, 5 ml/kg. This was followed by an exchange of equal amounts of blood for 5% albumin, 2–3 × 10 ml/kg (depending on preischemic hematocrit). The aim was to lower hematocrit to 25% within 4 min PI. Lactated Ringer and albumin was infused through the aortic arch catheter in order to imitate an intracarotid flush. The shed blood was centrifuged and the red blood cells stored for later re-infusion to raise hematocrit to 30% at about 2 h PI if needed.

(2) Hypertension. Immediately after finished hemodilution at 4 min PI, MAP was raised to about 130 mmHg with norepinephrine infusion. MAP was kept at this level for 5 min, and thereafter reduced to normal levels at about 11–15 min PI. Normotension (MAP = 80–120 mmHg) was thereafter maintained.

(3) Hypothermia. Surface cooling with ice water bath was started at 10 min PI. The aim was to lower the oesophageal temperature to 30°C, to keep it below 32°C for at least 2 h, and then let the temperature drift upwards without active heating. During hypothermia, PaCO2 values were kept at about 25 mmHg measured in vitro at 37°C, not corrected for temperature.

(4) Pentobarbital. 30 mg/kg slowly i.v.; the first half was given over 20 min, the remaining over the next 110 min.

(5) Dexamethasone. 2 mg/kg was given i.v. between 4 and 10 min PI when hemodilution was completed. A second dose was given at 90 min PI.

Sham Experiment (n = 1)

One animal underwent the same preparations and treatment as described for the control group, but was not subjected to brain ischemia. 24 hours of paralysis/IPPV with 50% N2O was followed by weaning, observation and outcome evaluation as in the other groups. The main purpose of this sham experiment was to see if the brain was judged to be normal on the blinded histologic examination which was performed at the end of the experiment.

General Intensive Care

All animals received optimal life support for 96 h PI, including control of blood pressure and body temperature at normal levels after the initial treatment; PaCO2 was kept at about 25 mmHg in both groups during IPPV; PaO2 was kept above 100 mmHg during IPPV and above 80 mmHg thereafter by adjusting FIO2 or using PEEP if necessary. The i.v. infusion was increased if necessary to compensate for urine loss. Furosemide 1 mg i.v. was given if mean urine flow was below 1 ml·kg⁻¹·h⁻¹ in any 4 h period, aiming for a zero fluid balance. If seizures occurred PI in spite of phenytoin prophylaxis, small doses of diazepam were given i.v. (increments of 0.5 mg), but never after 60 h PI. Tetracycline was given prophylactically, 50 mg i.m. every 12 h starting at 12 h PI. Monitoring lines were usually removed at 48–72 h PI, depending on the extent of recovery, to allow the animals to move more freely and enable better observation of behaviour. Careful oral feeding was started as soon as possible after 48 h PI.

Eliminations

Animals that did not follow protocol were eliminated from final analysis according to predetermined criteria. These included incomplete ischemia, hypotension, hyperthermia, hypoxemia, and hypercapnia.5, 27 Ischemia was judged to be incomplete if EEG was not isoelectric within 20 sec of neck cuff inflation, if there were signs of facial/oral congestion during ischemia, or if circulation could be seen on ophthalmoscopy. Animals dying a primary brain death during the observation period in spite of life support according to protocol were included in final analysis.

Outcome evaluation was done in 3 different ways.5, 26, 27

1) Overall performance categories (OPC). At 96 h PI, all animals were clinically evaluated and assigned to one of five OPC’s. OPC I = normal, OPC II = awake, but with some definite neurologic damage, OPC III = stuporous, OPC IV = vegetating, OPC V = brain death. (See Table 3, Results.)

2) Neurologic deficit (ND) scoring. ND was determined at 6, 12, 24, 48, 72, and 96 h PI. This is a detailed clinical neurologic examination where the result is quantified and expressed in percent (100% ND = brain death, 0% ND = normal). The ND scoring was independently performed by two of the investigators, who both knew which treatment had been given.

3) Histopathologic examination. After final clinical evaluation, the animals were killed by perfusion-fixation. The brains were removed and examined by two neuropathologists who did not know the treatment. The results were quantified and expressed as histopathologic damage (HD) scores.5, 25, 26, 27 Eleven brains were examined, five from each of the two groups plus the brain from the sham experiment. These brains were not selected at random, but were deliberately chosen from animals with different final ND scores. The result of the histopathologic examination in terms of HD scored was then ranked from best to worst to see if this correlated with the clinical ND ranking. Since we did not have the capacity to examine all brains histologically, this method cannot be used alone for comparing the outcome between the groups. However, if the two ways of ranking the outcome correlate well, the histologic results will be a confirmation of our clinical outcome evaluations.

Statistical Analysis

Laboratory results are given as mean values ± standard deviations, and the data from the two groups are compared by Student’s t-test for independent samples. The final ND scores from the two groups are compared with a Wilcoxon rank test for non-parametric data. The results of the overall performance categorization in the two groups were compared by a Fisher exact test. The same test was used to compare the number of awake animals postischemia in the seizure vs. the nonseizure
group. The ranking of the final ND scores was correlated with the ranking of the HD scores using Kendall’s correlation coefficient. The EEG return times were correlated with final ND scores using Pearson’s correlation coefficient.

**Results**

Of the 21 experiments, 1 animal in the treated group was excluded from final analysis due to intractable pulmonary edema and hypoxemia, starting at about 8 h PI; 1 was the sham experiment, 9 followed protocol in the control group, and 10 in the experimental treatment group. The two groups were comparable with regard to preischemic variables which might influence neurologic outcome^4^ - ^5^ (table 1): PaO₂, PaCO₂, hematocrit, esophageal temperature, and blood glucose. The blood glucose levels in the two groups immediately preischemia were 114 ± 22 mg/dl and 130 ± 28 mg/dl. The groups were also comparable with regard to body weight and sex. Age was difficult to assess, but we have reason to believe that the groups were comparable also in this respect.

**Brain Ischemia**

Hypotension prior to neck cuff inflation was achieved with trimetaphan 20–50 mg i.v. in all animals, EEG isoelectricity was observed within 14 sec of cuff inflation, shortly thereafter, facial pallor and bloodless retinal vessels on ophthalmoscopy were observable.

**Hemodilution Postischemia** (fig. 1)

In the treatment group, hemodilution was finished immediately post PI. Hematocrits were checked immediately thereafter, and the treated group had an hematocrit of 26 ± 2%. Animals with hematocrit below 28% at this point were given red blood cells at 2–3 h PI to raise hematocrit to approximately 30%. This was done in 6 of 11 animals in the treatment group.

**Hypertension Postischemia** (fig. 1)

After an initial drop of blood pressure following neck cuff release, rapid normalization was achieved in both groups. MAP of 80 mmHg was reached at 81 ± 12 sec and at 79 ± 12 sec PI in the two groups. Thereafter, the blood pressure pattern was different in the two groups. In the treated group, the blood pressure was significantly higher than in the control group at 4, 6, 8, and 10 min PI (p < 0.001). We used norepinephrine infusion to achieve these blood pressure patterns. The total dose of norepinephrine needed was different in the two groups: In the control group a total dose of 128 ± 24 µg was needed, compared to 480 ± 128 µg in the treatment group (p < 0.001).

**Hypothermia Postischemia** (fig. 1)

In the treated group, surface cooling was started at 10 min PI. Esophageal temperature dropped quickly, and we took the animals out of the bath at 14–16 min PI, when the temperature was 32.5°C. Thereafter, the temperature dropped to about 29°C at approximately 45 min PI, and then started to drift slowly upwards again. It remained below 32°C for just over 2 h in all experiments, and was about 36°C at 6 h PI. Except for moderate bradycardia no arrhythmias were seen during hypothermia.

**Pentobarbital** (fig. 1)

Pentobarbital, 30 mg/kg i.v. during hypothermia was well tolerated. There was a tendency to moderate hypotension which was controlled with norepinephrine.

**Postischemic Lab Values** (table 1)

Blood glucose values tended to rise during the PI-period in both groups, with a more pronounced rise early PI in the treated group (p < 0.05 at 2½ and 12 h PI). The two groups were otherwise comparable with regard to the most important lab values through the postischemic period.

**Postischemic Recovery**

**EEG Activity**

The first sign of cerebral recovery after ischemia was the start of EEG burst suppression (BS) activity. For the survivors in the control group (n = 8), this was observed at 64 ± 12 min PI (range 50–90 min). In the one monkey of this group which later died a brain death, BS activity started at 270 min PI. EEG activity was continuous at 129 ± 23 min PI in this group (range 65–210 min). In the treated group the corre-

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**Table 1 Laboratory Values Pre- and Post-ischemia**

<table>
<thead>
<tr>
<th>Group</th>
<th>Control values</th>
<th>Time post-ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
<td>2½ h</td>
</tr>
<tr>
<td>Blood glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>114 ± 22</td>
<td>149 ± 30</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>130 ± 28</td>
<td>193 ± 36*</td>
</tr>
<tr>
<td>PaO₂</td>
<td>163 ± 13</td>
<td>415 ± 27</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>175 ± 14</td>
<td>426 ± 37</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>29.5 ± 1.5</td>
<td>24.6 ± 2.0</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>29.1 ± 1.8</td>
<td>25.0 ± 1.4</td>
</tr>
<tr>
<td>Se-osmollality</td>
<td>297 ± 5</td>
<td>301 ± 8</td>
</tr>
<tr>
<td>(mosmol/kg)</td>
<td>294 ± 9</td>
<td>303 ± 15</td>
</tr>
</tbody>
</table>

C = control group (n = 9); T = treatment group (n = 10). All values are mean ± SD. Control samples were drawn immediately pre-ischemia with FiO₂ 0.33. At 30 min PI, FiO₂ was 1.0. At 2½ h PI, FiO₂ was 0.5. Thereafter the animals breathed room air with O₂ on a face mask. *p < 0.05.
TREATMENT POST-ISCHEMIA

**Figure 1.** This figure shows how the experimental treatment was given. The upper graph shows that hemodilution was performed between 1 and 4 min PI in the treatment group (○—○). Hematocrit was lowered to 25%, while it remained normal in the control group (■—■). Red blood cells were reinfused after two hours PI, if necessary, to raise hematocrit towards 30%. The middle graph shows the blood pressure pattern PI. After an initial drop, MAP is normalized before 2 min PI in both groups. The treatment group had a significantly higher MAP from 4-10 min PI (P < 0.001), followed by normalization. The lower graph shows the hypothermia which started at 10 min PI. The temperature was below 32°C for just over two hours, and was close to normal at 6 hours PI. Dexamethasone was given at 5 and 90 min PI, and pentobarbital was given during the hypothermic period.

Table 2: EEG Return Pattern Post-ischemia in the Control Group: Relation to Clinical Outcome

<table>
<thead>
<tr>
<th>Study</th>
<th>Start of EEG start burst suppression (min PI)</th>
<th>Start continuous EEG activity (min PI)</th>
<th>EEG recovery speed (min PI)</th>
<th>Final NDS 96 h PI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF 3</td>
<td>50</td>
<td>65</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>MF 11</td>
<td>60</td>
<td>85</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>MF 7</td>
<td>55</td>
<td>115</td>
<td>60</td>
<td>29</td>
</tr>
<tr>
<td>MF 14</td>
<td>60</td>
<td>140</td>
<td>80</td>
<td>33</td>
</tr>
<tr>
<td>MF 6</td>
<td>65</td>
<td>145</td>
<td>80</td>
<td>35</td>
</tr>
<tr>
<td>MF 2</td>
<td>90</td>
<td>130</td>
<td>40</td>
<td>36</td>
</tr>
<tr>
<td>MF 20</td>
<td>70</td>
<td>140</td>
<td>70</td>
<td>36</td>
</tr>
<tr>
<td>MF 15</td>
<td>60</td>
<td>210</td>
<td>150</td>
<td>43</td>
</tr>
<tr>
<td>MF 10*</td>
<td>270</td>
<td>720</td>
<td>450</td>
<td>100</td>
</tr>
</tbody>
</table>

Correlation: 
- r = 0.48, p > 0.10
- r = 0.90, p < 0.005
- r = 0.80, p < 0.01

EEG recovery speed is the time from start of burst suppression until EEG activity is continuous.

*The data from study MF 10 are not included in the correlation analysis, the high values from this one animal would influence the correlations unduly.

Sponding times were obviously much later, since these animals were hypothermic and received pentobarbital. Start of BS was at 6 ± 1 h PI, and EEG activity was continuous at 8.5 ± 2 h PI. When looking at the control group separately (9 animals with no hypothermia or CNS-depressant drugs early PI), there was a strong correlation between the time pattern of EEG return early PI, and the clinical outcome (ND score) at 96 h PI (table 2). Correlation coefficients were calculated for ND scores vs. the time in minutes PI when EEG BS started (r = 0.48, p > 0.10), for ND scores vs. min PI when EEG activity was continuous (r = 0.90, p < 0.005), and for ND scores vs. the time from start of EEG BS activity until the EEG activity was continuous (speed of EEG recovery) (r = 0.80, p < 0.01).

Weaning from Controlled Ventilation was uneventful at 24–26 h PI. Spontaneous breathing was adequate in all animals, and extubation was performed when feasible according to usual clinical criteria.

Seizures were observed clinically and on EEG in 3 of 9 monkeys in the control group, and in 4 of 10 monkeys in the treatment group in spite of phenytoin...
TABLE 3  Overall Performance Categories (OPC) at 96 h PI

<table>
<thead>
<tr>
<th>OPC</th>
<th>Corresponding clinical picture</th>
<th>Control (n)</th>
<th>Treatment (n)</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fully awake, moderate motor deficits. Can sit and feed themselves.</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>II</td>
<td>Fully awake, with severe motor deficits. Can not sit or feed themselves. May eat and drink when fed.</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>III</td>
<td>Not awake. May show slight awareness off and on. Mostly quiet, but can move all limbs.</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>IV</td>
<td>No awareness. Often abnormal body position, e.g., overall extension. Usually adequate spontaneous respiration. May open eyes, yawn and chew. Abnormal reaction to pain.</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>V</td>
<td>Totally unresponsive. Brain dead at 96 h PI or dying a brain death during the observation period.</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*p = 0.05.

The seizures were usually of a slow, clonic and generalized type. They always started between 24 and 40 h PI, and responded well to small doses of diazepam (total dose 1–2 mg) in all except 2 animals in the treated group. These two were the only ones with seizures after 50 h PI. They received a total dose of 5 and 6 mg of diazepam respectively up to 60 h PI. Of the animals with seizures PI, only 1 of 7 had a normal level of consciousness at 96 h PI; of the animals without seizures, 8 of 12 were awake at 96 h PI. This difference is statistically significant (P = 0.04, Fischer exact test).

Polyuria was observed after ischemia in 2 of 9 in the control group and 3 of 10 in the treated group. The urine output was far in excess of fluid input, and careful attention was required to avoid clinically significant hypovolemia. We were able to counteract this phenomenon with aggressive fluid replacement, and the problem subsided after 40–50 h PI. Urine electrolytes in these cases were in the following range: Na⁺ 55–85 mmol/l, K⁺ 5–15 mmol/l. Two animals in the control group and one in the treatment group had seizures and polyuria and ended up with severe neurologic deficits, whereas polyuria alone did not consistently forecast a bad neurologic outcome.

Clinical Outcome (table 3, fig. 2)

The overall performance categorization (OPC) showed a significantly better outcome for the treated group than the control group, when we compared the number of awake animals at 96 hours postischemia. In the treated group 7 of 10 reached outcome category I or II being fully awake, whereas only 2 of 9 were awake at 96 h PI in the control group (p = 0.05). By awake we mean animals that were looking around, following events in the room and showing emotions in response to various stimuli. They were always able to chew and swallow food. In the control group 1 of 9 developed brain death (OPC V), none in the treated group. The median ND score at 96 hours PI was 16% in the treated group vs. 35% in the control group (fig. 2). This difference is not statistically significant with a Wilcoxon rank test, p > 0.05.

Histologic Outcome (table 4)

The blinded histologic examination supported the results of the clinical evaluation. The brain from the sham experiment appeared normal with an histologic deficit (HD) score of 0 points. All other brains examined showed some degree of histologic damage, predominantly in the cerebellum, hippocampus, thalamus and midbrain, while the neocortex was affected to a lesser degree. When the brains were ranked from best to worst according to HD scores, this ranking correlated well with the clinical ranking based on ND scores (r
have made a change in the system that will increase the sensitivity of the scale in the clinically critical range of 20-35%. This is done by putting more relative nu-
tance, with a median ND score of 16% in the treated control group. The last point is worth emphasizing. There is no doubt that the quality of the postischemic treatment makes a difference in the sense that bad care may worsen the outcome;5,27 the subject of the present discussion is whether one can improve upon good intensive care. Although our results were not totally unequivocal, we do feel that they support this hypothesis.

**EEG Return**

The pattern and timing of EEG return PI seems to be of value in the early prediction of outcome (table 2). The earlier the EEG returned to a continuous activity PI, the better the clinical outcome. And vice versa: If EEG activity was not continuous by approximately 2 hours PI, a good recovery never occurred (in this study). This is in agreement with previous observations,27 and suggests, in the absence of life support complications, that the long term clinical outcome is largely determined within a few hours postischemia. Possibly, the EEG return pattern alone, may be useful as a method of outcome evaluation in short term screening studies, when testing non-CNS-depressant therapies after GBI.
Seizures

Seizures PI strongly indicated a bad clinical outcome: Six of seven animals with seizures PI never regained consciousness, whereas 8 of 12 animals without seizures PI were awake at 96 hours PI ($P = 0.04$). This is in agreement with previous observations, and suggests primarily that seizures PI reflect severe brain damage, which is also suggested by the histologic examination in the present study. But this is not always the case, and since seizures in themselves may cause additional damage, one may want to include early seizure prophylaxis as a routine measure postischemia. Indeed, Todd et al found reduced mortality in thiopental treated cats PI, probably due to suppression of seizure activity early PI. 4

Potential for Therapeutic Response — Relation to the Severity of the Insult

In the present study we used 18 min of ischemia, compared to 16 min in previous studies. This was done to cause more severe brain damage in control animals, in the hope that any beneficial therapeutic effect would be more sharply defined. However, this point is debatable. It may well be that only brains with lesser injuries have the potential for responding to therapy. One must assume that some neurons are irreversibly damaged during the ischemic episode, while some still have the potential for survival when circulation and oxygen supply is restored. The latter population of neurons is the main target for our therapy, and this population may be greater in ischemic insults of only moderate severity. The relationship between these two populations of neurons at the end of the ischemic episode, may express the “potential for responding to therapy.” To some extent, this potential must be time-dependent. Thus, if the insult is too prolonged, one may lose the possibility of the beneficial response to therapy all together.

Choice of Therapeutic Components

None of our five therapeutic components have been properly tested and shown to improve neurologic outcome postischemia. So far, this has not been reliably done with any “single” therapy. However, there is a lot of evidence that each of these therapeutic components have a beneficial effect on one or more of the pathophysiologic changes we set out to treat. The rationale for the choice of therapy is discussed below.

Hemodilution

Impaired reperfusion is potentially a serious problem after periods of more than 5 min of cerebral circulatory arrest, in spite of normal blood pressure. After an initial (10–20 min) increase in total cerebral blood flow (CBF), there is a pronounced decrease to below 50% of pres ischemic control values. It remains unclear whether this is caused primarily by sludging, clotting and pericapillary edema, or whether it is largely a vasospastic disorder. Hemodilution leads to increased CBF with and without preceding ischemia, and promotes a more even recirculation PI. Reports on clinical and experimental focal ischemia, suggest that postischemic hemodilution may be beneficial. However, oxygen transport to the brain may suffer when the hematocrit drops below 30%. We therefore decided to lower the hematocrit to 25% immediately PI, and when the temperature rose above 32°C, to raise the hematocrit to 30% by reinfusing red blood cells, thereby hoping to achieve a significant rheologic benefit early PI. Lactated Ringer was given as an initial flush (5 ml/kg) through the catheter in the ascending aorta, to get a very low hematocrit in the blood perfusing the brain early PI (in pilot studies this caused a brief hematocrit reduction to 10% in the blood going to the brain). Also, a slight blood volume expansion might offer a hemodynamic and rheologic benefit. This method of hemodiluting was used in a previous study in dogs; combined with heparinization and hypertension PI, this gave promising results, while i.v. hemodilution alone in a separate study did not seem beneficial. In the latter study, however, hemodilution was done later and more slowly PI than in the present study, possibly an important difference.

Hypertension

Hypotension PI has clearly been shown to be harmful, and should be avoided. Hypertension, on the other hand, may offer a certain benefit. Safar et al combined hypertension with hemodilution and heparinization after cardiac arrest in dogs, with promising results. Nemoto et al found that some brain areas needed higher than normal blood pressures to be adequately reoxygenated after ischemia, and Fischer et al found moderate hypertension PI to improve the evenness of recirculation. However, hypertension after an insult to the brain is a two-edged sword; CBF autoregulation is probably lost, and severe repetitive hypertension PI has been shown to be deleterious. Consequently, we decided on a brief period of moderate hypertension PI in this study. The timing was such that the 5 min of hypertension coincided with maximal hemodilution, in order to get an optimal rheologic effect and possibly “open up” the microcirculation before hypothermia was induced at 10 min PI (fig. 1).

Hypothermia

Hypothermia has a well documented protective effect against brain anoxia, while reports are more inconclusive regarding the value of hypothermia started after brain ischemia. Conn et al have been using therapeutic hypothermia in children after near-drowning with seemingly good results; unfortunately, they lack proper control groups. However, deleterious effects of prolonged hypothermia have also been reported in cats, monkeys, and dogs. We settled for a temperature of 30–32°C, which has been done frequently in the past with no cardiovascular problems. The question of duration of hypothermia was more difficult, mostly because it is unclear how long the postischemic hypoperfusion and hypermetabolism last. This combination results in a mismatch between oxygen supply and demand which may worsen the
brain tissue acidosis and cause further neuronal damage. This problem may be beneficially influenced by hypothermia and barbiturates; in fact, the combination may have a more than additive depressant effect on cerebral metabolism and oxygen demand. We decided on short term hypothermia, assuming that this problem is more pronounced during the early hours PI. Indeed, a study in patients seems to show that after 2 hours PI, there is no mismatch between oxygen supply and demand in the brain. The postischemic hypermetabolism is actually questioned by several investigators. The increased blood viscosity during hypothermia should be counterbalanced by the hematodilution. Besides, hypothermia was not induced until the rheologic flush was over at 10 min PI.

Barbiturates

Pentobarbital and pancuronium were used for anesthesia during hypothermia to prevent shivering and hypermetabolism and to promote poikilothermia. Also, barbiturates reduce cerebral oxygen demand during the period of PI hypoperfusion, and edema and elevated intracranial pressure if present, may be counteracted. However, in a previous study, prolonged thiopental anesthesia PI was not alone found to be beneficial.

Corticosteroids

There were two reasons for including a steroid: 1) ATP synthesis may recover faster PI with dexamethasone treatment. The impairment of ATP synthesis PI depends on the duration of ischemia and may occur inhomogenously in the brain. After moderately severe insults, ATP levels may return to near normal within minutes, but recovery of ATP production does not necessarily forecast functional recovery. On the other hand, without ATP recovery PI, normal function cannot be restored. Interestingly, energy production may lag behind also in areas of the brain that are adequately reperfused, thereby suggesting that the PI hypoperfusion is secondary to energy failure and not vice versa. 2) Steroids may stabilize membranes and have a beneficial influence on the PI cerebral edema. However, the degree of edema and its clinical significance after brain ischemia is unclear.

Hyperventilation

Moderate hyperventilation with PaCO₂ of 25 mmHg was included as part of the protocol for both groups PI. Animals hyperventilate spontaneously when permitted to do so PI, presumably in an attempt to counteract the low brain pH due to lactic acidosis. Thus, it seems natural to hyperventilate at least to the same degree when ventilation is controlled. We adjusted ventilation according to the PaCO₂ values measured in vitro at 37°C, uncorrected for temperature, which now seems to be accepted and possibly also more correct.

Seizure Prophylaxis

Phenytoin was also part of the control treatment, since seizures frequently are seen after ischemia, and may cause further damage. In previous experiments with monkeys, we did not see seizures before the animals were weaned from controlled ventilation and N₂O was discontinued. The seizures seemed related to increased input of stimuli during weaning and waking up. In the present study, therefore, we started seizure prophylaxis at 20 h PI, before the weaning process at 24 h PI. Earlier phenytoin therapy might in itself influence neurologic function beneficially.

Concluding Remarks

The present study was largely an attempt to attack the postischemic hypoperfusion with a combined rheologic and metabolism-depressant approach, assuming that the postischemic hypoperfusion is a limiting factor in recovery. The study supports the hypothesis that treatment started postischemia may influence the outcome favourably. However, considering the complexity of this clinical problem, it seems unlikely that one single therapy will be the treatment for postischemic encephalopathy. More likely, future therapy will be composed of an increasing number of therapeutic components. Following this line of thought, studies should be done with certain prostaglandins as well as Ca⁺⁺ channel blockers. These substances might offer a rheologic benefit, and might in addition protect against hypoxic damage at the cellular level.

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