The Protective Effect of Combined Administration of Anti-Oxidants and Perfluorochemicals on Cerebral Ischemia

JIRO SUZUKI, M.D., SHUNICHI FUJIMOTO, M.D., KAZUO MIZOI, M.D., AND MASATOSHI OBA, M.D.

SUMMARY We previously published results of investigations which indicated that the combination of mannitol, which acts as a free radical scavenger, and perfluorochemicals (PFC), which have a strong oxygen-carrying capacity, can be therapeutic in cases of brain infarction. The present experiment tested the hypothesis that the effectiveness of such treatment could be increased by an optimal combination of such scavengers and other chemicals.

Fifty-two dogs were used, employing the “canine model of a completely ischemic brain regulated with the perfusion method.” A total of six drugs with free radical scavenger capacities were tested: mannitol, vitamin E, vitamin C, Nizofenone* (Y-9197), dexamethasone (DEXA) and sulcotidil† (MY-103). These drugs were administered intravenously 15 minutes prior to the production of ischemia, when cerebral blood flow was reduced to one-tenth its normal volume. After one hour, recirculation was allowed and the recovery of electrical activity of the brain observed for three hours. Judged by the degree of recovery of brain electrical activity, five drugs were considered to have protective effect against brain ischemia: mannitol, vitamin E, MY-103, DEXA and Y-9197.

Among these five drugs, mannitol, vitamin E and DEXA are known to be safe and easily used clinically. The combined administration of these three drugs, together with PFC, was also investigated. It was found that the speed and degree of recovery of brain electrical activity were greater when these drugs were given together than when one was administered alone. Particularly with administration of PFC, it was found that electrical activity was not greatly attenuated, even during the period of brain ischemia, and the recovery of electrical activity was rapid following recirculation. In these experiments, the combined administration of mannitol, vitamin E, DEXA and PFC appeared to be more effective in the treatment of the ischemic brain than mannitol and PFC alone.

HAVING BECOME AWARE of the possible protective effects of 20% mannitol on the ischemic brain, we have reported clinical studies relating to this1-3 and have investigated the effects of mannitol on ischemia over the past decade, using various canine models.4-6 We have also demonstrated that the administration of perfluorochemicals (PFC), originally developed as an artificial blood substitute, and of mannitol, further increases the anti-ischemic capabilities.7-9 These effects have also been demonstrated by us in clinical cases when these drugs are administered in the acute phase of cerebral infarction.10

In recent years, a new hypothesis concerning the involvement of the lipid peroxidation caused by active oxygens which occurs at CNS cytomembranes in the process of cerebral damage, has attracted considerable attention.11,12 We surmised that the protective effects of mannitol on the ischemic brain may in part be due to the capacity of mannitol to act as a scavenger of active oxygens.

In the present study, we have made a comparative investigation of the protective effects of six agents, primarily mannitol; vitamin E; vitamin C; nizofenone (Y-9179) an imidazole derivative;13 dexamethasone (DEXA); and sulcotidil (MY-103).14 From those agents which were found effective in protecting the ischemic brain, we have administered three: mannitol, vitamin E and DEXA; we also gave PFC, together with three drugs, to other experimental animals. Here we report the results of these investigations and briefly discuss the possible mechanisms involved.

Materials and Methods

A. The Experimental Model (fig. 1)

Fifty-two adult mongrel dogs, weighing approximately 10 kg each, were used. For production of brain ischemia, we used the “canine model of a completely ischemic brain regulated with the perfusion method,” details of which have been reported previously.6 Briefly, under the intravenous administration of thiopental sodium (25 mg/kg), an intratracheal tube was inserted, the animal was immobilized by I.V. administration of pancuronium bromide (0.2 mg/kg), and regulated respiration was instituted. Blood gases and blood pressure were maintained within normal limits. A small bone window in the left parietal region was then opened and two stainless steel electrodes of 1 cm diameter each were placed on the dura mater. Using this bipolar recording technique, the electrical activity of the brain was recorded and a power spectral analysis was undertaken, using a signal processor (San’Ei 7T07). Then the intracranial procedure was begun by performing a right temporal craniotomy. The bilateral ethmoidal
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FIGURE 1. Occluded points of the main arteries of the canine brain and blood supply of the cerebral cortex (→).

arteries, bilateral ophthalmic arteries, right posterior cerebral artery, right superior cerebellar artery and basilar artery were occluded by cauterization, and the right middle cerebral artery (MCA) was cannulated. Finally, the bilateral internal carotid arteries were occluded using aneurysm clips, and autologous blood was infused to the MCA from the femoral artery using a Harvard peristaltic infusion pump (Model 1310). In this manner, the blood volume flowing to the left cerebral hemisphere could be regulated at will (fig. 2).

B. The Experimental Procedures

Immediately following production of the model, arterial blood was infused at a speed of 20 ml/min and the normal electrical activity was maintained for one hour. In the middle of this period, the infusion pump was temporarily stopped for 30 seconds in order to confirm that normal cerebral circulation could be achieved using only the pump. Electrical activity became completely flattened within 30 seconds after interruption of perfusion. Subsequent resumption of perfusion immediately produced complete recovery of electrical activity (fig. 3). Following this procedure, the drugs were administered intravenously about 15 minutes prior to the onset of ischemia. Next, blood flow was reduced to 2 ml/min for one hour. Blood flow was then made to return to 20 ml/min and the changes in electrical activity were observed for three hours thereafter.

C. Drugs and Doses

Eight groups of animals were treated using the following doses of each drug:

I. Untreated control group (7 dogs)
II. 10 mg/kg vitamin E (5 dogs)
III. 30 mg/kg vitamin E (5 dogs)
IV. 2 g/kg mannitol (5 dogs)
V. 3 mg/kg MY-103 (5 dogs)
VI. 1 mg/kg DEXA (5 dogs)
VII. 3 mg/kg Y-9179 (5 dogs)
VIII. 1000 mg/kg vitamin C (5 dogs)

Mannitol was administered by intravenous drip infusion (over approximately 10 minutes). All other drugs were administered intravenously. For the vitamin E group, the emulsion of dl-alpha-tocopheryl nicotinate was used.

Tests were also done on two additional groups of dogs in which various combinations of drugs were given:

IX. 1 mg/kg DEXA, 10 mg/kg vitamin E and 2 g/kg mannitol (5 dogs)
X. Following administration of the above 3 drugs, 20 ml/kg of PFC by intravenous drip (5 dogs)

For the PFC, 20% Fluosol-DA (Green Cross Corporation, Osaka, Japan) was used. Due to the inhalation of 100% O₂, the arterial O₂ pressure (P aO₂) rose to 400–500 mmHg. In all dogs of this group, a test dose of 3–5 ml of PFC was given intravenously prior to surgery.

FIGURE 2. Experimental system of the model.

FIGURE 3. EEG record following temporary interruption and resumption of perfusion.
The species-specific transient fall in blood pressure and the subsequent spontaneous recovery of normal pressure occurred in all the dogs. In those dogs which showed severe decreases, an adequate dose of hypertensive agent (Ethylephrine Hydrochloride, Effortil®) was given intravenously.

D. Electroencephalography (EEG) Recording

In the analysis of EEG, the following three points were studied:

1) Survival time, i.e., the time required from the start of brain ischemia until complete attenuation of electrical activity.
2) Degree of recovery, i.e., the degree of recovery of electrical activity following three hours of recirculation, based upon a five-stage classification: Grade 0, complete attenuation; Grade 1, less than 30 μV; Grade 2, 30–50 μV; Grade 3, 50–100 μV; and Grade 4, more than 100 μV (fig. 4).

Statistical analyses were performed using Student’s t test and chi square test.

Results

A. The Control Group

In the untreated control group (I), the EEG survival time was 7.4 ± 4.3 min. In five of the seven dogs, recovery of electrical activity was not seen subsequent to recirculation. In the two remaining dogs, electrical activity reappeared very slightly after two and 30 minutes. But three hours after the start of recirculation, six dogs were Grade 0 and one dog was Grade 1.

B. The Drug Group

In the vitamin E group given 10 mg/kg (II), the survival time in the EEG was 9.4 ± 6.1 min, which was not significantly longer than the time seen in the control group. The recovery time was 32.5 ± 17.9 minutes and, after three hours of recirculation, one dog was grade 4, one was Grade 2, two were Grade 1 and one was Grade 0.

In the vitamin E group given 30 mg/kg (III), the survival time was 12.0 ± 6.8 minutes and the recovery time was 3.6 ± 1.7 minutes, which was significantly faster than the recovery time in the vitamin E (10 mg/kg) group (p < 0.05, Student’s t-test). The degree of recovery after three hours was as follows: three dogs were grade 4 and two dogs were Grade 3.

In the mannitol group (IV), the survival time was 10.0 ± 5.7 minutes, which was longer than that of the control group, but not statistically significant. Recovery time was 29.0 ± 20.1 minutes, and the Grades after three hours of recirculation were: Grade 4 in two dogs, grade 3 in one dog and Grade 2 in two dogs.

In the MY-103 group (V), survival time was 16.4 ± 11.5 minutes. This figure was not significantly different from the control group, but this survival time was the longest seen among the six different drug groups. The recovery time was 24.0 ± 22.2 minutes. After three hours of recirculation, one dog was Grade 3, two dogs were Grade 2 and one was Grade 1.

In the DEXA group (VI), the survival time was 4.8 ± 1.9 minutes, and the recovery time was 58.0 ± 34.9 minutes. After recirculation, one dog was Grade 3, two were Grade 2 and two were Grade 1.

In the Y-9179 group (VII), the survival time was 13.0 ± 8.9 minutes, the recovery time was 23.3 ± 12.5 minutes and the Grades after three hours of recirculation were: one Grade 1 animal, one Grade 2 animal, one Grade 3 animal and two Grade 0 animals.

In the vitamin C group (VIII), the survival time was 10.0 ± 5.6 minutes and the recovery time was 32.6 ± 33.8 minutes. After recirculation, one dog was Grade 1, three were Grade 1 and one was Grade 0.

In the group of dogs given mannitol, vitamin E and DEXA (IX), the survival time was 12.0 ± 9.4 minutes. Recovery time was 8.0 ± 2.8 minutes and all five dogs were judged as Grade 4 after three hours of recirculation.

In the group given mannitol, vitamin E, DEXA and PFC (X), four of the five animals showed some attenuation of voltage and slowing of frequency in the EEG, but complete attenuation was not seen during ischemia. In only one animal was there complete attenuation after 20 minutes of ischemia. After recirculation, the recovery time was rapid in all five dogs. All five were judged as Grade 4, continuously showing normal electrical activity after three hours of recirculation, throughout the recirculation period. As shown in fig-

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**Figure 4.** Grading of functional recovery following recirculation in the experimental dogs. Grade 0: EEG is completely flat. Grade 1: EEG potentials are less than 30 μV. Grade 2: EEG potentials are between 30 and 50 μV. Grade 3: EEG potentials are between 50 and 100 μV. Grade 4: EEG potentials are above 100 μV.
ure 5, there was a clear distinction between the power spectra of groups IX and X.

The results in the six single-drug groups and the two groups given a combination of drugs are summarized in tables 1 and 2 and figure 6. When compared to the untreated control, there were statistically significant beneficial effects (p < 0.05, chi square test) in the Vitamin E (30 mg/Kg) group, the mannitol group and the two combination therapy groups.

Discussion

In treatment during the acute period of brain ischemia, the improvement of brain metabolism and the suppression of brain edema have thus far been the primary concerns. For over a decade, we have undertaken experimental and clinical studies of the protective effects which mannitol can have on the ischemic brain. In recent years, we have investigated the protective effects which mannitol can have on the ischemic change, the development of ischemic brain edema, and hemorrhagic infarction. Furthermore, we have found in clinical cases that this combined treatment can indeed be effective.

The hypothesis has been advanced that tissue damage in cerebral ischemia occurs due to the lipid peroxidation of cell membranes caused by the release of free radicals, but subsequent work has demonstrated the involvement of a free radical reaction after recirculation rather than during ischemia itself. Many problems remain, however, in the experimental methods for proving the presence of the free radical reaction.

The actual mechanism involved in mannitol's protective effect has remained uncertain, but it has become known that this drug has biochemical properties that allow it to act as a scavenger of hydroxyl radicals, which are a form of highly reactive oxygen. We have consequently studied the mechanism of mannitol in brain ischemia from this perspective.

Physiological effects of vitamin E are known from the work of Lucy to include a stabilizing effect on cytomembranes. As far as this anti-oxidant action is concerned, in addition to the scavenging effects of the alkyl radicals produced during the autoxidation of lipids, studies have found that this drug has biochemical properties known that this drug has biochemical properties.
TABLE 1 (Continued)

<table>
<thead>
<tr>
<th>Case</th>
<th>Treatment</th>
<th>Survival time</th>
<th>Recovery time</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.</td>
<td>Y-9179 3 mg/kg</td>
<td>10 min</td>
<td>—</td>
</tr>
<tr>
<td>34.</td>
<td>6 kg F</td>
<td>3 min</td>
<td>40 min</td>
</tr>
<tr>
<td>35.</td>
<td>10 kg M</td>
<td>7 min</td>
<td>10</td>
</tr>
<tr>
<td>36.</td>
<td>6 kg F</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>37.</td>
<td>10 kg M</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>VIII. Vit. C 1 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38.</td>
<td>6 kg M</td>
<td>3 min</td>
<td>40 min</td>
</tr>
<tr>
<td>39.</td>
<td>13 kg M</td>
<td>7 min</td>
<td>4 min</td>
</tr>
<tr>
<td>40.</td>
<td>13 kg M</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>41.</td>
<td>12 kg M</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>42.</td>
<td>11 kg M</td>
<td>7</td>
<td>60</td>
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<tr>
<td>IX. DEXA + Vit. E + Mannitol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43.</td>
<td>8 kg M</td>
<td>7 min</td>
<td>4 min</td>
</tr>
<tr>
<td>44.</td>
<td>8 kg M</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>45.</td>
<td>8 kg M</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>46.</td>
<td>13 kg M</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>47.</td>
<td>13 kg F</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>X. DEXA + Vit. E + Mannitol + PFC</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>48.</td>
<td>7 kg F</td>
<td>60 min</td>
<td>0 min</td>
</tr>
<tr>
<td>49.</td>
<td>11 kg M</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>50.</td>
<td>6 kg F</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>51.</td>
<td>16 kg M</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>52.</td>
<td>12 kg M</td>
<td>60</td>
<td>0</td>
</tr>
</tbody>
</table>

Significant differences were calculated with Student's \( t \)-test. 
* \( p < 0.005 \): comparison with untreated control (chi square test).
† \( p < 0.05 \): comparison with Vit. E 10 mg/kg treated dogs.

TABLE 2 Summary of Grading in Each Group

<table>
<thead>
<tr>
<th>Agents</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Non treatment</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
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<tr>
<td>II. Vit. E 10 mg/kg</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
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<tr>
<td>III. Vit. E 30 mg/kg*</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
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<tr>
<td>IV. Mannitol*</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
</tr>
<tr>
<td>V. MY-103</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
</tr>
<tr>
<td>VI. DEXA</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
</tr>
<tr>
<td>VII. Y-9179</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
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<tr>
<td>VIII. Vit. C</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
</tr>
<tr>
<td>IX. II. + IV. + VI*</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
</tr>
<tr>
<td>X. IX. + PFC*</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
<td>○○○○</td>
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</tr>
</tbody>
</table>

* experimental animal.
† \( p < 0.05 \): comparison with untreated control (chi square test).

Vitamin C is known to act as a scavenger of superoxide anion (O\(_2^-\)), but in vitro experiments show its capacity to quench O\(_2^-\) is considerably weaker than that of superoxide dismutase.34 Furthermore, although it is found that vitamin C, when present in high con-

ids,29 it has recently been emphasized that there is a quenching effect of singlet oxygen.31 It is thought that, due to these actions, the peroxidation reaction is suppressed. As to the stabilizing effect on cytomembranes, it is thought that, due to the physical binding of vitamin E to membrane phospholipods,30 or to the stabilization of nonheme proteins,32 various abnormal reactions, such as the peroxidation reaction, are prevented.

In the present experiments, the concentration of vitamin E in the blood was about three times higher in the dogs administered 30 mg/kg than in those given 10 mg/kg for the entire three-hour period following recirculation.33 The recovery time was significantly shorter in the former group and the degree of recovery was also better. We consequently believe that intravenous administration of a large dose of vitamin E is effective due to its transport from the blood to its site of action, the cellular membrane.

FIGURE 6. EEG activity at three hours after recirculation in all experimental dogs.
centrations, can suppress free radical reaction, when present in low concentrations, it has been reported to act to the contrary, as an initiator of free radical formation. At the concentration used in the present experiments, vitamin C did not have a protective effect on the ischemic brain.

Nizofenone (Y-9197) is one of the imidazole derivatives and, during ischemia, is known to suppress the consumption of energy and O2. It is also said to have an anti-oxidant action. In the present experiments, however, it was not so effective in protecting the ischemic brain.

Among the steroid drugs, DEXA in particular has been known to have anti-edemic properties. It has been postulated that the actual mechanisms of DEXA action in cerebral edema may be related to its efficacy in stabilizing cytomembranes and scavenging radicals. The value of DEXA in ischemic brain edema has been re-examined by various researchers in both experimental and clinical studies, but conclusions as to its benefits are still controversial.

In the present experiment, a large dose of DEXA (1 mg/kg) was administered prior to ischemia, and moderate effects were obtained, when compared with those produced by the other five drugs.

Suloctidil (MY-103) is a new drug with various known pharmacological properties. It produces improvements in the cycle of aerobic metabolism, has anti-platelet aggregative action, and has vascular anti-spasmodic effects. Recently we have found that the protective effects of MY-103 include its role as a free radical scavenger. Details of the work on the chemiluminescence of hypoxic brain will be reported elsewhere. In our experiments, the effects of MY-103 varied widely, indicating that further study of this drug is required.

In the investigation of EEG survival time, no significant differences were found between the control group and each of the groups given one of the drugs. Over, those animals with long survival times did not consistently show favorable degrees of recovery following recirculation. In contrast, although no significant differences were found in the recovery time of the various drug groups, it was seen that the shorter the recovery time (i.e., the faster the return of electrical activity following recirculation), the better was the ultimate degree of recovery of such activity. Particularly for the animals with an EEG recovery time of less than 10 minutes, most showed recovery to Grade 3 or 4. It is of interest that the degree of recovery after recirculation correlated more closely with the recovery time than with the survival time. We surmise that the favorable effects on the degree of recovery are because irreversible histological changes during ischemia are prevented by the drugs, and the metabolic structures are thereby preserved, and can effectively and rapidly utilize the oxygen and glucose supplied due to blood recirculation.

In light of the above results concerning the administration of each of these drugs alone, the following five were considered to have protective effects against brain ischemia: mannitol, vitamin E, MY-103, DEXA and Y-9197. However, the results in the MY-103, the DEXA and the Y-9197 group were not statistically significant since the number of animals was too small. The fact that the drugs which have anti-oxidant actions show protective effects on the brain suggests that free radical reaction is involved in the neuronal cell damage at the ischemic focus. Although vitamin C also has anti-oxidant property, it was not found to have therapeutic effect. It should be noted, however, that in the present study a single dose of each drug (except vitamin E) was used. Therefore, further examination of the effects of dosage using these and other drugs will be required.

Finally, examination was also made of the combined administration of three of the drugs (mannitol, vitamin E, and DEXA, which were found effective when administered singly and which are known to be safe and easy to use clinically. It was found that their combined administration produced shorter recovery times and better final grades of recovery following recirculation, than the administration of one of the drugs alone. It is particularly noteworthy that addition of PFC to these three drugs prevented complete attenuation of EEG activity during one hour of brain ischemia in four of the five dogs tested, although slowing and moderate attenuation did occur. Also, immediately following recirculation, the electrical activity of the brain returned rapidly to nearly a pre-ischemic state. We believe these results clearly indicate that the combined administration of mannitol, vitamin E, DEXA and PFC produces results which are superior to the administration of only mannitol and PFC together, which we had previously advocated.

In the present experiments, some control studies were not performed because of the following reasons.

1) Control study for the use of oxygen inhalation. With regard to this, we had previously investigated the effect of oxygen inhalation alone using the same experimental schedule as that of the present study, and reported that no recovery of electrical activity was seen following recirculation in all of the control group subjected to oxygen inhalation.

2) Control study for the hemodilution. In the animals of group X, there was about a 20% decrease in hematocrit after intravenous administration of both 20% mannitol (10 ml/kg) and PFC (20 ml/kg). In our previous study, some of the animals were given 30 ml/kg of Ringer's solution intravenously as a control for the group given mannitol and PFC. In these animals there was also a 15–20% decrease in hematocrit, but no recovery of EEG was observed following recirculation.

3) Control study for the use of Effortil®. In some animals of group X which showed severe decreases in blood pressure following the test administration of PFC, an adequate dose of Effortil® was given. There were no animals given Effortil® in the control group, however, because blood pressure was maintained within a normal range throughout the experiment.

It is impossible to draw final conclusions from these
experiments alone, but it may be that the effectiveness of this combination therapy is due to the joint effects of:

1. Mannitol and vitamin E, which work as anti-oxidants

2. Mannitol alone, but it may be that the effectiveness of mannitol and perfluorochemicals and to apply these methods in clinical cases in the acute stage of brain ischemia.

In future research, it would be desirable to investigate the effects of dosage and method of administration of mannitol, vitamin E, DEXA and PFC, and to apply these methods in clinical cases in the acute stage of brain ischemia.

References


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Calcium Antagonist PY 108–068: Demonstration of Its Efficacy in Various Types of Experimental Brain Ischemia

N. WIERSPERGER, PH.D., P. GYGAX, PH.D., AND A. HOFMANN, M.Sc.

SUMMARY The protective properties of PY 108–068 against ischemic disturbances in the brain were investigated in vitro and in vivo. The calcium antagonistic effects were demonstrated on isolated, calcium-loaded arterial rings of feline cerebral arteries. Determination of brain tissue oxygenation with \( \text{PO}_2 \) microelectrodes showed that PY 108–068 improved oxygen supply in the cortex of cats subjected to epicerebral arterial occlusion. In a model of cerebral vasospasm induced by autologous blood superfusion, PY 108–068 reversed the spastic state of cortical microvessels. In a chronic stroke model (microsphere embolization in rats) PY 108–068 led to a significant reduction in mortality and in the severity of neurological symptoms, whereby the peroral efficacy of the drug could be demonstrated. The efficacy of PY 108–068 in a variety of ischemic insults makes this drug a promising aid in the treatment of cerebrovascular accidents.

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THE IMPORTANCE OF CALCIUM, its homeostasis and its implication in a variety of pathological processes related to vascular diseases has recently become a major area of research. Calcium is considered to be involved in a variety of biochemical processes leading to contraction of smooth muscle cells, inhibition of mitochondrial respiration and activation of phospholipases with a concomitant increase in the concentration of free fatty acids and prostaglandin synthesis.15

Due to the growing importance attributed to calcium in physiology and biochemistry, efforts have also been made to develop drugs able to interfere with the actions of calcium. Among the various drugs having been claimed to counteract the disturbances in calcium homeostasis, dihydropyridines belong to the most potent calcium antagonists known. Whereas calcium antagonists were first utilized in the treatment of cardiovascular diseases, there has been growing interest in using such drugs for the management of cerebrovascular disorders. This approach is substantiated by the fact that brain vessels seem to be more sensitive to calcium and its inhibitors than peripheral vessels.15

We describe here the beneficial effects of PY 108–068, a potent calcium antagonist of the dihydropyridine type,1 on the cortical microcirculation in various models of brain ischemia. The following experimental approaches were utilized:

a) isolated cerebral vessels in vitro
b) incomplete, focal brain ischemia
c) spastic brain ischemia
d) chronic multiembolization of one rat brain hemisphere

Methods

1. In Vitro Isolated Brain Arteries

The calcium antagonistic effect of PY 108–068 was investigated on isolated ring segments \( (n = 4–5) \) of the cat middle cerebral artery, according to a modification of the protocol by Edvinsson et al.1 The ring segments (length: 4 mm, diameter: 0.2–0.3 mm) were immersed in a depolarizing oxytocsin-saturated Krebs medium of following composition: mM \( \text{NaCl} = 65, \text{KH}_2\text{PO}_4 = 1.2, \text{NaHCO}_3 = 25, \text{glucose} = 1, \text{pH} = 7.4 \). The rings were strained with a mass of 500 mg. PY 108–068 was added to the bath at a dosage of \( 10^{-6} \text{ M} \). Then, contraction of the vessel segments was induced by addition of \( \text{CaCl}_2 \) to the medium in increasing concentrations \( (3 \times 10^{-5} \text{ to } 3 \times 10^{-3} \text{ M}) \). The isometric force which developed was registered on a Texas Instrument
The protective effect of combined administration of anti-oxidants and perfluorochemicals on cerebral ischemia.

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