Effect of DPPD (Diphenyl-para-Phenylenediamine) on Stroke and Cerebral Edema in Gerbils

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SUMMARY  Diphenyl-para-phenylenediamine (DPPD) is an antioxidant that has been shown to decrease liver damage due to the peroxidative process of carbon tetrachloride in rats and to ameliorate cold-induced cerebral edema in cats. Because lipid peroxidation disrupts the integrity of the plasma membrane, a process believed to occur in cerebral infarction, which is a major cause of cerebral edema, DPPD was tested for its protective effect against cerebral infarction. When given intraperitoneally in gerbils with unilateral ligation of the common carotid artery, DPPD had no effect on resultant incidence, morbidity, or mortality of cerebral infarction. Despite these findings, the authors believe, on the basis of what is known about free radical pathology, that DPPD and other antioxidants deserve further laboratory trials as possible drugs in the treatment of brain trauma and cerebral edema.

SIGNIFICANT brain trauma, regardless of cause (i.e., whether physical, infectious, or ischemic), is almost uniformly complicated by edema. Whether this edema develops directly through altered blood vessel walls, or indirectly through damaged astrocytic membranes, or by both mechanisms is not known. What is known is that it can have devastating consequences, including uncal herniation through the tentorial notch, tonsillar herniation through the foramen magnum, secondary herniations into the ventricles, multifocal neural dysfunctions, and decreased blood flow. These consequences have led to considerable laboratory and clinical research aimed at discovering therapeutic measures for reducing cerebral edema. These measures include administering osmotic agents, barbiturates, corticosteroids, and antioxidants. Osmotic agents appear to be effective early in the development of brain edema. It has been suggested that they: 1) reduce intracranial pressure (ICP) by dehydrating normal, non-edematous brain tissue; 2) are ineffective in areas of membrane dysfunction; and 3) may or may not have an effect on clinical improvement.1-7

Barbiturates appear to be beneficial by decreasing the incidence of cerebral infarction.8-10 Thus the injury is less severe and edema less pronounced. The mechanisms of this protection are not understood, but Smith et al.6 have provided evidence that barbiturate anesthesia, per se, is not protective. Corticosteroids are widely used in the clinical treatment of cerebral edema secondary to neoplasms and, to a lesser extent, to cerebrovascular disorders.1-7 The mechanism(s) by which they function has not yet been determined, but it is commonly believed that they "stabilize membranes." Whether they truly reduce cerebral edema and improve the clinical course is controversial.1-7, 12-13

The fourth suggested mode of therapy — antioxidant administration — has been used only experimentally. The rationale is that lipid peroxidation plays a significant role in the disruption of neural cell membrane integrity and function, with the resultant cerebral tissue damage leading to edema. It has been shown in rats that antioxidants reduce liver damage due to the peroxidative process of carbon tetrachloride poisoning14, 15 and in cats that they ameliorate cold-induced cerebral edema.7

This study was undertaken to see if an antioxidant could reduce cerebral edema from cerebral infarction. The animal model used for cerebral infarction was the Mongolian gerbil (Meriones unguiculatus) with unilateral common carotid artery ligation. The gerbil has an incomplete circle of Willis, and unilateral common carotid artery ligation produces ipsilateral cerebral infarction in about 40-60% of animals.4, 16-18 Cerebral edema develops in the infarcted hemisphere.

Methods

General Procedure

One hundred thirty-nine healthy, adult, male Mongolian gerbils, weighing 50 to 70 gm, were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg). Through a ventral midcervical incision, the left common carotid artery was isolated, doubly ligated, and transected in 100 gerbils; isolated but left intact in 19 gerbils. Twenty gerbils served as nonoperated controls. All incisions were closed with skin clips.

The dose of 1) antioxidant — N,N'-diphenyl-para-phenylenediamine (DPPD) (Eastman p5689, C6H5NHC1H4NC6HS; M.W. 260.34) suspended in 0.5 ml of corn oil (250 mg/kg) — or 2) placebo — corn oil (0.5 ml) — was injected intraperitoneally 24 hours before operation, immediately after operation, and 24 hours after operation. For nonoperated animals the drug or placebo was given in 3 doses at similar intervals.

GROUP I: Fifty-one gerbils with left common carotid artery ligation received DPPD.

GROUP II: Forty-nine gerbils with left common carotid artery ligation received placebo.

GROUP III: Ten gerbils with sham operations received DPPD.

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TABLE 1  Treatment Schedule

<table>
<thead>
<tr>
<th>Treatment Schedule</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Non treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(i.p. injection of 250 mg/kg DPPD suspended in corn oil)</td>
<td>(i.p. injection of 0.5 ml corn oil)</td>
<td>No treatment</td>
</tr>
</tbody>
</table>

Unilateral common carotid artery ligation
- Group I (51 gerbils) (49 gerbils)
- Group II (10 gerbils)
- Group III (5 gerbils) (5 gerbils)
- Group IV (9 gerbils)
- Group V (5 gerbils)
- Group VI (5 gerbils)
- Group VII (10 gerbils)

GROUP IV: Nine gerbils with sham operations received placebo.
GROUP V: Five nonoperated gerbils received DPPD.
GROUP VI: Five nonoperated gerbils received placebo.
GROUP VII: Ten gerbils subjected to neither operation nor treatment served as nonmanipulated controls (table 1).

Morbidity and Mortality Evaluation

Each animal was examined every 8 hours for 48 hours postoperation. Morbidity and mortality were evaluated and scored by an investigator who was unaware of the treatment given each animal or the group to which each animal was assigned. The double-blind method consisted of marking each cage numerically, covering the marking and then marking each station alphabetically. A computer randomly selected a station for each cage at each check. Using the computer readout, the observer arranged the cages for each check. A different sheet was marked with the station designated and was used every 8 hours for each observation period on each gerbil. All of the sheets were later compiled with each station and observation time matched for each gerbil and transferred to one master sheet for each animal.

Morbidity evaluation was based on the following: a decrease in alertness and movement, ptosis, cocked head, circling behavior, hindlimb splaying and rotation, seizures, piloerection, and tremor. A stroke index was devised that assigns a numerical weight to each of these characteristics determined for each gerbil (fig. 1). The stroke index score was then used to compare the morbidity of each group with that of every other group.

Measurement of Cerebral Edema

At the end of the observation period, all animals were killed by ether inhalation. Then, in a humid environment, a wide craniotomy was done, the cerebral peduncles were transected, and the brain removed immediately. The cerebral hemispheres were separated by sharp dissection and each was placed in a tared, aluminum weighing pan. These were weighed on a Mettler balance and heated in a laboratory oven at 100° C for 24 hours (a temperature and duration that had previously been found to be adequate for dehydration to a constant weight). After being cooled in a CaCl₂ dessicator for 30 minutes, each hemisphere was reweighed. From the wet and dry weights, the percentage water content of each hemisphere was calculated by the following formulae:

\[
\text{Percentage Water Content} = \frac{\text{Wet Weight} - \text{Dry Weight}}{\text{Wet Weight}} \times 100
\]

\[
\text{Percentage Dry Weight} = \frac{\text{Dry Weight}}{\text{Wet Weight}} \times 100
\]

"Swelling Percentage" \(= \frac{P - P'}{P} \times 100\)

Where: \(P\) = percentage dry weight of right hemisphere

\(P'\) = percentage dry weight of left hemisphere

The water content of the left cerebral hemisphere (ipsilateral to the ligated carotid artery) was compared to that of the right cerebral hemisphere by an adaptation of the method of Elliot and Jasper.8, 10, 20 This method is based on the assumption that if the water content of the left hemisphere increases after carotid ligation, its

**MORBIDITY AND MORTALITY**

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>% Water RH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Dry Wt., BH</td>
</tr>
<tr>
<td>Treatment</td>
<td>% Water LH</td>
</tr>
<tr>
<td>Date of Surgery</td>
<td>% Dry Wt., LH</td>
</tr>
<tr>
<td>Swelling  %</td>
<td></td>
</tr>
</tbody>
</table>

**STROKE INDEX**

<table>
<thead>
<tr>
<th>Stroke index</th>
<th>0</th>
<th>16</th>
<th>24</th>
<th>32</th>
<th>40</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair roughed up or tremor</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Inability to move</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Head cocked</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Eye fixed open</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Posis</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>A splayed out hind limb</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Seizures</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Seizures</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Rolling seizure</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Extreme weakness (comatose)</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

**Stroke Index Score**

X: 3

**Comments**

**Figure 1. Stroke Index Form.**
TABLE 2  Effect of DPPD on Stroke Index and Mortality

<table>
<thead>
<tr>
<th></th>
<th>Group I DPPD (% of animals)</th>
<th>Group II Placebo (% of animals)</th>
<th>Significance (p = 0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total strokes**</td>
<td>33.2</td>
<td>37.4</td>
<td>Not significant</td>
</tr>
<tr>
<td>Overall mortality</td>
<td>27.5</td>
<td>29.2</td>
<td>Not significant</td>
</tr>
<tr>
<td>Mortality (animals with stroke only)</td>
<td>82.8</td>
<td>78.1</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

*Mann-Whitney Test.
**Stroke Index Score of 5 as a minimum.

percentage dry weight (P') will be less than that preoperatively, and less than that of the right hemisphere (P). This assumption has been borne out in experimental studies. By the use of this method, each gerbil served as its own control.

Results

Morbidity and Mortality

As shown in table 2, there was no significant difference in the total number of clinically evident strokes or mortality between the DPPD-treated and placebo-treated ligated animals (Groups I and II, respectively). No strokes developed in the other animals (Groups III through VII), and none of those animals died during the study period.

A further amplification of the morbidity of Groups I and II is shown in figure 2. Note that not only is there no significant difference in the overall morbidity, but there is also no significant difference at any of the 8-hour check points.

Cerebral Edema

Within and between the control Groups III through VII, there was no significant difference between the water content of the 2 hemispheres. The data from all 5 groups were combined, therefore, and considered normal (± 1 sd): % water content of the right hemisphere = 79.26 ± .401; % dry weight of the right hemisphere = 20.76 ± .406; % water content of the left hemisphere = 79.42 ± .516; % dry weight of the left hemisphere = 20.58 ± .524; swelling percentage = 0.0%.

For Groups I and II, the % water content of the left hemisphere, the % dry weight of the left hemisphere, and the swelling percentage were significantly different (p = 0.05) from those factors for the 5 control groups, but all 3 factors were not significantly different when Groups I and II were compared. Animals with stroke in both Groups I and II developed cerebral edema on the left side, as evidenced by the calculated swelling percentages, and the animals that developed cerebral edema had a high stroke index score.

Discussion

Theories of free radical pathology have been discussed by many authors,1, 2, 3, 6, 7, 13, 21-28 This discussion applies those theories to the development of cerebral damage secondary to ischemia.

Cerebral ischemia causes cellular damage in the area of distribution of the occluded artery, distal to the occlusion. It may be that this damage leads to a loss of capillary endothelial integrity with a consequent extravasation of red blood cells and fluid into the ischemic area. As the neurons, glial cells, and extravasated red blood cells break down, iron and copper complexes (heme and cytochromes) would be released into the tissue spaces to act as catalysts to initiate free radical reactions with the remaining O2 in the hydrophobic midzone area of the membrane (fig. 3). The presence of the free radicals would deplete cholesterol, and there would be massive cellular destruction that might not have occurred under different conditions. The purpose of this study was to alter those conditions by adding an antioxidant.

Demopoulos et al.3 have shown that barbiturates can diminish free radical damage to simulated CNS membranes. They suggest that control of free radicals may be the mechanism whereby barbiturates protect

![Cerebral ischemia](http://stroke.ahajournals.org/)

![Possible role of free radical pathology in acute cerebral ischemia](http://stroke.ahajournals.org/)
the brain in experimental cerebral ischemia. Furthermore, of the barbiturates, the shorter acting ones have the greatest protective capacity and are also the most lipophilic.27

Brain edema secondary to vascular disorders has shown a variable response to corticosteroids. It is known that plasma cortisol falls to subnormal levels 24 to 48 hours after ligation of one common carotid artery in gerbils.28 This is also the period of maximal cerebral edema.11, 15, 29 It is possible that both endogenous and exogenous corticosteroids function in much the same way as cholesterol, that is, they "stabilize" the cell membranes. Thus, there may be a common antioxidant characteristic inherent in agents used to treat cerebral edema.

No antioxidant function has been attributed to osmotic agents themselves. However, it might be that use of these agents with adjunctive antioxidant would be followed by less pronounced membrane destruction than occurs when these agents are used alone. In the presence of intact membranes, these agents could be more effective in the damaged hemisphere.

Although it is not clear exactly how antioxidants function, they do sequester free radicals, decompose peroxides, and chelate metals. For this study of the effect of a known antioxidant on cerebral edema, we chose DPPD because: 1) it is lipophilic and therefore can penetrate into the hydrophobic midzone area where lipid peroxidation occurs;15 2) it is absorbed intraperitoneally;14, 15 and 3) it has been shown to ameliorate cold-induced cerebral edema in cats.7 When given intraperitoneally in gerbils it appeared to have no effect on morbidity, mortality, and cerebral edema following unilateral common carotid artery ligation. Perhaps the use of another dose schedule, another route of administration, or another animal model might have confirmed the therapeutic effect of DPPD already shown in open cerebral lesions as well as in fatty degeneration of the liver of rats.14, 16

On the basis of the probable mechanism of antioxidant protection just discussed, we believe that further study of the effect of DPPD on cerebral lesions is justified. Furthermore, studies on the metabolism of DPPD are indicated so that an effective therapeutic blood level can be established. With so few studies having been done to date, we do not believe that DPPD should be rejected as a possible therapy for brain trauma and the resultant cerebral edema.

References

15. Di Luzzio NR, Costales F: Inhibition of the ethanol and carbon tetrachloride induced fatty liver by antioxidants. Exp Mol Pathol 4: 141, 1965
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