Protection Against Cerebral Damage From Intracarotid Injection of Hypaque in Animals*

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This study was designed in an attempt to investigate the adverse effects on the central nervous system of large doses of a radiopaque substance (Hypaque) injected into the carotid artery and to evaluate methods designed to prevent or counteract these effects.

Hypaque in large doses has been found to have certain toxic systemic effects1,5,21,24 and especially to have an adverse effect on the central nervous system.1,7,8,22 Studies by Bernstein et al.,3,4,2 and by Sessions et al.23 have demonstrated the efficacy of low molecular weight dextran (Rheomacrodex) (LmDx) in the reduction of these reactions. Galicich et al.10,11 and Long et al.18 have demonstrated that glucocorticoid dexamethasone (Decadron)† is effective in the reduction of cerebral edema in both clinical and experimental studies.

With this information in mind it was decided to investigate the protective action of LmDx and dexamethasone on cerebral damage produced by the intracarotid injection of Hypaque 90 per cent under experimental conditions.

Materials and Methods

Permeability of sodium fluorescein was chosen as a method of evaluation of cerebral damage because this substance normally does not pass the blood-brain barrier except in specific locations, such as the infundibulum, area postrema, choroid plexus and pineal gland.16,19

A measure of cerebral damage was devised which took into account the following factors:

- a) the extent of fluorescein stain
- b) the intensity of the fluorescein stain
- c) the presence of cerebral edema
- d) the presence of cerebral hemorrhage
- e) the survival of the animal

The extent of staining was estimated to the nearest one-fourth (quadrant) of the total area of the cerebrum and the intensity of staining was evaluated for each quadrant. The intensity of fluorescein stain was used as an index of the degree of alteration of the blood-brain barrier. Four categories of intensity of staining were used: a) no stain; b) mild (visible with ultraviolet but not ordinary light); c) moderate; and d) severe. Fig. 1 shows photographs with ultraviolet light of fluorescein stained rabbit brains following intracarotid injection of Hypaque.

Table 1 illustrates the method of scoring brain damage. This shows the score of a rabbit which survived the procedure, had no cerebral edema nor hemorrhage but showed severe staining over one cerebral hemisphere and mild staining over the other hemisphere. To chart the damage produced in this animal, each of the two quadrants of mild staining received 1 point for a total of 2 and each of the quadrants of severe staining received 3 points for a total of 6. The total cerebral damage was obtained by adding these (2 plus 6) for a total of 8. In the event there was associated cerebral edema, hemorrhage or the animal expired, an additional 1 point was added to each category thereby making a possible range of scores under this system from 0 to 16.

One hundred and thirty-nine rabbits, weighing from 2 to 3 kg., were used. Pentobarbital (30 mg./kilo) was given intravenously. The rabbits were then prepared in the following manner. Procaine (1 per cent) was injected locally over the common carotid artery. The left external jugular vein was ligated and a catheter (PE 200)

**TABLE 1**

Example of numerical assessment of brain damage
(see text)

<table>
<thead>
<tr>
<th>Intensity of fluorescein staining</th>
<th>Numerical scale</th>
<th>No. of quadrants</th>
<th>Sum of damage by quadrants</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

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† 16 alpha methyl-9 alpha fluoroprednisolone.
was inserted downward into the superior vena cava. One cc. of sodium fluorescein 20 per cent was delivered into the catheter. The left common carotid artery was then exposed, ligated proximally and a catheter (PE 300) was inserted distally. This catheter was used to inject the contrast medium. The use of a Harvard injector permitted constant injection rates. All medications given were warmed to 37°C.

Preliminary studies to determine the appropriate dose of Hypaque and the time of maximum staining of the brain were performed. These results are tabulated in Table 2 utilizing the “cerebral damage score.” The amount of damage observed is directly proportional to the dose of contrast medium used. The largest dose usually produced severe damage with a mortality rate of 40 per cent. These results agree closely with those of Bernstein and Evans who found that 50 per cent of animals died when this dose of Hypaque was given intravenously. The lowest dose produced lesions too minimal to evaluate adequately. The middle dose (2.25 cc. of 90 per cent Hypaque/kilo) appeared to be the best dosage for purposes of this experiment and it was selected as the standard test dose.

Evaluation of the effect of the interval between Hypaque injection and sacrifice revealed that the cerebral damage evident at 1 hour was the same as the cerebral damage evident at 5 minutes. At 7 hours following injection, however, the cerebral damage was markedly less and at 24 hours there was no stain demonstrable.

Control Groups. Hypaque-M 90 per cent was given to 37 rabbits by the carotid catheter at the rate of 0.33 cc./min. Seven animals received 1.50 cc./kilo, 25 animals received 2.25 cc./kilo and 5 animals received 3.00 cc./kilo of Hypaque-M 90 per cent. In order to determine any possible effect of intracarotid injection of LmDx or of saline, 8 animals received 5 cc./kilo of LmDx 15 per cent and 8 animals received 5 cc./kilo of normal saline into the carotid artery at the rate of 0.33 cc./min.

To evaluate the relationship of cerebral damage with time, 2 of the animals were sacrificed at intervals of 5 minutes, 1 hour, 7 hours and 24 hours after injection of the test dose of Hypaque.

### Table 2

Cerebral damage from intracarotid injection of 90 per cent Hypaque

<table>
<thead>
<tr>
<th>Hypaque cc./kilo</th>
<th>No. of Animals</th>
<th>Assessed Mean Cerebral Damage</th>
<th>Mortality per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.50</td>
<td>7</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>2.25</td>
<td>25</td>
<td>6.5</td>
<td>24</td>
</tr>
<tr>
<td>3.00</td>
<td>5</td>
<td>9.0</td>
<td>40</td>
</tr>
</tbody>
</table>

**Experimental Groups.** Intravenous premedication with LmDx was evaluated at 2 dose levels. Twenty rabbits received 10 cc./kilo of LmDx 15 per cent and 6 received 20 cc./kilo of LmDx 15 per cent. Eleven rabbits received 10 cc./kilo of normal saline and 6 received 20 cc./kilo of normal saline. The infusion rate was 0.5 cc./min. A period of 10 minutes to permit equilibration was maintained prior to injection of the Hypaque.

In 8 animals, immediately preceding the Hypaque injection, LmDx 15 per cent, 5 cc./kilo was injected by way of the carotid artery at a rate of 0.33 cc./sec.

Intravenous premedication with dexamethasone was given to 17 animals as a “loading dose” of 0.2 mg. t.i.d. for two days and 0.4 mg. 1 hour prior to Hypaque injection. Another group of 7 animals received a single intravenous injection of 0.4 mg. of dexamethasone, one hour preceding the injection. To determine the effect of utilizing both premedication techniques, 11 animals received the loading dose of dexamethasone and LmDx 15 per cent (10 cc./kilo) intravenously.

**Observations and Data Recording.** The animals’ immediate responses (mouth movements, screaming, convulsions, etc.) were recorded as mild, moderate or severe. Pupillary response was measured and recorded and the conjunctival capillary bed was observed through a dissecting microscope. The animals were observed for 45–60 minutes. If still alive, sodium thiameyal (1 cc.)
was given intravenously and the animals sacrificed by a rapid thoracotomy and transection of the great vessels. A craniectomy was performed and the presence of cerebral edema was evaluated by observing whether or not the brain tended to herniate through a dural incision 1 cm. in diameter. The mesencephalon was then transected and the entire forebrain was removed intact. The brain was observed with ordinary and ultraviolet light. The intensity and extent of fluorescein staining of the cerebral hemispheres were recorded. Unusual features such as subarachnoid hemorrhage were also noted. The brains were quick-frozen and later re-evaluated without knowledge of the treatment received. Continual alternation of experimental and control animals permitted more objective evaluation and had a neutralizing effect on minor improvements in technique.

Results

The pupillary response was uniformly that of constriction when Hypaque was injected. The constriction was usually more pronounced in those animals subsequently proven to have cerebral damage. The immediate response of the rabbit was difficult to evaluate; it seemed to vary more with the level of anesthesia than with cerebral damage. Severe sludging of the blood and localized vasospasm was seen in the conjunctival vessels upon injection of Hypaque into the common carotid. Premedication with LmDx markedly improved conjunctival microcirculation as determined by observation with a dissecting microscope.

Table 3 demonstrates that the cerebral damage for those receiving Hypaque alone was over twice the score of those animals premedicated with LmDx. These results are statistically significant at the 1 per cent level. The fact that animals pretreated with equal amounts of saline had a higher cerebral damage score than the Hypaque group eliminates the possibility that this may be an effect of hemodilution. The mortality rate of the 3 groups was strikingly different, the LmDx group showing the lowest rate.

To determine the relationship between the amount of LmDx given and the protection afforded, one group of animals received twice the original dose of LmDx. Amounts of 20 cc./kilo provided less protection than 10 cc./kilo. The average cerebral damage may be misleading in this instance because the brains were either perfectly normal or showed severe staining with edema. Animals having severe staining and cerebral edema died from pulmonary edema precipitated by the injection of the large dose of Hypaque. This also occurred in animals that received similar doses of saline. It is believed the increased fluid load could not be tolerated when given over a short period of time.

Table 3 also shows the results of LmDx

### Table 3

<table>
<thead>
<tr>
<th>Premedication</th>
<th>Type</th>
<th>Dose cc. kilo</th>
<th>No. of Animals</th>
<th>Assessed Mean Cerebral Damage</th>
<th>Mortality Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>25</td>
<td>6.5</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>LmDx 15 Per Cent</td>
<td>10</td>
<td>20</td>
<td>3.0*</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Normal saline</td>
<td>10</td>
<td>11</td>
<td>8.4</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>LmDx 15 Per Cent</td>
<td>20</td>
<td>6</td>
<td>4.7</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Normal saline</td>
<td>20</td>
<td>6</td>
<td>7.4</td>
<td>33</td>
</tr>
<tr>
<td>Intracarotid</td>
<td>LmDx 15 Per Cent</td>
<td>5</td>
<td>8</td>
<td>2.9</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>(No Hypaque)</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Normal saline</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* P < .01
given intra-arterially. The protection afforded was not greater than when given intravenously. When the dextran was given intra-arterially, the animals manifested discomfort. However, the animals did not react to intracarotid saline. The brains of those animals which received only intra-arterial LmDx or saline were not stained nor were they edematous.

**Dexamethasone.** Table 4 summarizes the results in those animals pretreated with intravenous dexamethasone. The mean cerebral damage of those given a loading dose was approximately one-half that of the Hypaque group and this is statistically significant at the 1 per cent level. In addition the mortality rate is much less than in the control group. A single dose of dexamethasone, however, seemed to have no protective effect as the mean cerebral damage for this group was the same as that for the control group.

**LmDx and Dexamethasone.** Pretreatment with both LmDx and dexamethasone was superior to treatment with either separately (significant at 1 to 2 per cent level). Pretreatment with both drugs was vastly different from the control group and significant at the .001 per cent level. Table 5 graphically summarizes the pertinent results. It especially shows the striking differences between control and experimental groups.

**Discussion**

These experiments demonstrate that large doses of contrast medium given into the carotid artery result in increased permeability to fluorescein, accompanied by brain swelling and even death. This is consistent with previous studies of Broman and Olsson,\(^3\) Bassett et al.,\(^1\) and McIntosh et al.\(^19\) Broman\(^6\) demonstrated that cerebrovascular permeability was not altered by prolonged anoxia, moderate change in osmotic pressure, moderate pH change, increased venous pressure or non-lethal doses of intravenous air. He postulated that the barrier was a function of the permeability of the vascular endothelium.

Read\(^21\) has shown experimentally with various contrast media that the toxic effects are directly proportional to the osmotic activity of the contrast medium. Read also demonstrated red blood cell aggregation and “sludging” of the blood following intravenous administration of hypertonic solutions including contrast media. Sessions et al.\(^20\) believes that sludging *per se* is not the basis of toxicity of contrast media since this predicates that tissue anoxia is the basic mechanism of injury. They state that the onset of the toxic reaction in the central nervous system is too rapid to be secondary to anoxia and suggest that the observations are more indicative of a chemical irritant or cytotoxic effect than of anoxia. Experimental animals in this study responded actively within seconds to intracarotid contrast medium and frequently remained hyperirritable as demonstrated by convulsions for some time after injection. The hypothesis that this is a cytotoxic rather than an anoxic effect is further supported by the fact that

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**TABLE 4**

*Cerebral damage from intracarotid Hypaque after dexamethasone and after a combined premedication of both LmDx and dexamethasone*

<table>
<thead>
<tr>
<th>Type</th>
<th>Dose</th>
<th>Duration</th>
<th>Sample Size (No. of rabbits)</th>
<th>Assessed Mean Cerebral Damage</th>
<th>Mortality Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>—</td>
<td>25</td>
<td>6.5</td>
<td>24</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Loading dose</td>
<td>48 hrs.</td>
<td>17</td>
<td>3.9*</td>
<td>6</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Stat dose</td>
<td>1 hr.</td>
<td>7</td>
<td>6.6</td>
<td>0</td>
</tr>
<tr>
<td>Dexamethasone and LmDx</td>
<td>Loading dose</td>
<td>48 hrs.</td>
<td>11</td>
<td>0.8†</td>
<td>0</td>
</tr>
</tbody>
</table>

* P<.01 † P<.00001
TABLE 5
Cerebral damage from intracarotid injection of Hypaque 90% with and without premedication.

No Premedication

Premedication with 15 per cent LmDx, 10 cc./kilo I. V.

Premedication with dexamethasone loading dose

Premedication with both 15 per cent LmDx and dexamethasone

Mortality 24 per cent

Mortality 5 per cent

Mortality 6 per cent

Mortality 0 per cent
sodium fluorescein staining was present within minutes after injection. Broman\(^6\) has demonstrated that cerebrovascular permeability does not change even after 2 hours of anoxia.

The mechanism of the protective action of LmDx on adverse reactions to contrast media is not known, however, this mechanism may be mediated through LmDx's profound effect on the suspension stability of blood. Gelin has written extensively on the rheologic disturbances of blood.\(^2,12,13,14\) He has shown that the suspension stability of the blood, as indicated by an increased erythrocyte sedimentation rate, is altered by many conditions such as burns, crush injury with fat embolism, toxic shock, oliguria, thrombosis, acute arterial insufficiency, vascular surgery, extracorporeal circulation and large doses of contrast media. He and many other authors have demonstrated an improvement in the suspension stability of blood and improvement in the above conditions following the administration of LmDx.\(^2\)

Cyрус et al.\(^9\) concluded that the administration of LmDx prior to occlusion of the middle cerebral artery in dogs diminished significantly both the size of the infarct and residual neurologic deficit. This was confirmed in a separate study by Hardin et al.\(^15\) Bernstein et al.\(^4\) using large doses of Hypaque intravenously, showed histologic damage to the lung, liver, spleen and kidney. In this study it was shown that this damage was less when the animals were premedicated with LmDx. Sessions et al.\(^23\) injected Urokon into the aorta and produced damage to the spinal cord with resultant paraplegia and hyperreflexia. Significant protection against this damage was afforded by the use of LmDx.

The effectiveness of LmDx in protecting against cerebral damage from the intracarotid injection of Hypaque has not been previously investigated. This study demonstrates definite protection against cerebral damage when LmDx is given prior to injection of Hypaque. This protection is afforded irrespective of whether the LmDx is given intravenously or intra-arterially. The intravenous route is preferred since discomfort was apparent when it was given intra-arterially.

Prados et al.\(^20\) have demonstrated experimentally that ACTH as well as adrenal cortical extract can inhibit the cerebral edema which results from prolonged exposure of the brain to air. Ingraham et al.\(^17\) have observed clinically that the use of prophylactic corticosteroids in the removal of tumors (craniopharyngiomas) led to an unusually benign postoperative course which they attributed, in part, to lack of cerebral edema. Glucocorticosteroids have also been accredited by Russek et al.\(^22\) with a decrease in cerebral edema and with mental and neurological improvement following cerebral infarction. Galicich et al.\(^10,11\) have demonstrated a reduction in intracranial pressure and in neurological deficit in 18 of 21 patients with brain tumors who were treated with dexamethasone, a potent synthetic glucocorticoid. In 2 cases angiographic evidence of a decrease in the size of the intracranial mass was seen.

The mechanism of the action of dexamethasone is not clear. Corticosteroids have many effects on membrane permeability and water balance. Dexamethasone may act on vascular endothelial permeability, allowing it to withstand the insult of the contrast media. This investigation demonstrates that the effect of dexamethasone is not immediate and that a period of premedication is necessary to obtain a protective effect.

Premedication with a combination of LmDx and dexamethasone resulted in almost complete protection from cerebral damage and death. The additive effect of the two drugs suggests that the mechanism of protection afforded by dexamethasone is different from that provided by LmDx.

This animal study is of course intended to simulate many aspects of clinical cerebral angiography. It has been done in a controlled manner so that abnormal reactions could be produced consistently and evaluated. The dose and concentrations of contrast medium were necessarily greater than that used in clinical angiography. In spite of these dissimilarities it seems reasonable to
expect that premedication with LmDx and dexamethasone may provide protection from certain complications of clinical cerebral angiography. The use of these drugs may prove to be particularly useful in patients with compromised cerebral blood flow or in patients requiring the use of large amounts of contrast medium.

Summary

Cerebral damage and death resulting from injection of large doses of Hypaque into the carotid artery were studied experimentally in the rabbit. Premedication with low molecular weight dextran or with dexamethasone resulted in a significant reduction in cerebrovascular permeability to dyes, concomitant with less cerebral edema and a lower mortality rate. Premedication with both drugs simultaneously produced almost complete protection against cerebral damage from the intracarotid injection of Hypaque.

References