Effect of increasing the plasma oxygen diffusivity on experimental cryogenic edema

JAMES V. GAINER, JR., M.D., AND G. ROBERT NUGENT, M.D.

Division of Neurosurgery, West Virginia University Medical Center, Morgantown, West Virginia

Mongrel cats with experimental cryogenic brain lesions were treated with the carotenoid compound crocetin. It has been shown that crocetin increases the diffusion speed of oxygen through plasma, and should provide a net increase in available oxygen to the capillary endothelial cell. The treated group of animals showed a significant reduction in edema as compared to a comparable control group. It is suggested that oxygen availability is an important factor in vasogenic edema.

KEY WORDS • cerebral edema • oxygen diffusivity • crocetin • carotenoid compound • cryogenic edema

We have previously demonstrated in our laboratory (unpublished data) that one of the properties of dexamethasone in the concentration range of 0.5 to 4.0 µg/ml is to increase the net amount of oxygen dissolved in plasma (Fig. 1). Since this property should provide an increase in the total available oxygen at the level of the capillary cell, we have postulated that it may play some part in the cell's mechanism of action in vasogenic edema. This concentration range is approximately the same as would be obtained in an adult patient receiving an initial dose of 10 mg to start and 4 mg every 6 hours. To further test the hypothesis that an increase in available oxygen to the capillary cell would decrease vasogenic edema, we have studied the effect of increasing the diffusion speed of oxygen through the plasma in animals with cryogenically-induced cerebral edema. In this way, a net increase in oxygen availability to the capillary endothelial cells was produced by a mechanism different from that of dexamethasone. Gainer and Chisholm have determined that crocetin will produce an increase in the diffusion speed (diffusivity) of oxygen through plasma of approximately 80%. Crocetin is a water-soluble carotenoid; when dissolved in plasma it will cause an increase in oxygen diffusivity. Other carotenoids, even those with a close chemical structure, have not been found to have this effect. Using the cryogenic lesion proposed by Klatzo, et al., we treated a group of animals with this compound to determine if it would cause a reduction of edema similar to that of dexamethasone.

Materials and Methods

Fifty-seven mongrel cats of both sexes were anesthetized with 44 mg/kg of ketamine and allowed to breathe spontaneously. Two ml of 2½% solution of Evans blue were injected intravenously. The cats' heads were then placed in a stereotaxic frame and the operative site shaved and prepped as a sterile field. A bone button 15 mm in diameter was removed over
the midportion of the right middle suprasylvian gyrus. The center of the craniotomy was located 8 mm to the right of the midsagittal line and 15 mm anterior to the interauricular line. The dura was opened in a cruciate fashion. A Cooper cryosurgical unit* with a modified probe that had a flat, circular tip 10 mm in diameter was used to give a cold lesion on the exposed cortex. The tip temperature was maintained at -50 °C and was applied for 20 seconds. The tip was disengaged with a flush of normal saline. The dura was laid back over the lesion but was not closed. The bone button was replaced and the temporal muscle and fascia closed over it. The skin edges were approximated with silk suture, and a spray-on dressing applied. The animals were returned to their cages and maintained in a routine manner, with no special considerations. The animals were divided into groups as follows.

Pretreated Crocetin Group
Crocetin was given to 10 cats in a dose of 0.2 mg intramuscularly twice daily for 24 hours prior to the lesion and twice daily until sacrifice. Animals in this group were sacrificed at 24 and 48 hours after the lesion was made.

Pretreated Dexamethasone Group
In eight cats, dexamethasone was started in a dose calculated to give a blood level of 2 to 4 µg/ml, assuming total absorption of the drug, 24 hours prior to making the lesion. The initial dose was four times the maintenance dose. The drug was administered intramuscularly every 6 hours until sacrifice at 24 or 48 hours after the lesion was made.

Posttreated Crocetin Group
An initial dose of 0.2 mg of crocetin was given to eight cats intravenously 30 minutes after the lesion was produced. Thereafter 0.2 mg was given intramuscularly twice daily until sacrifice 24 or 48 hours later.

Posttreated Dexamethasone Group
The initial dose was given to eight cats intravenously 30 minutes after lesion production, and subsequent doses were given intramuscularly every 6 hours until sacrifice. The dose for each cat was calculated to give a blood level of 2 to 4 µg/ml, assuming total absorption of the drug. This is similar to the regimen used clinically for humans. The initial intravenous dose was a loading dose four times the size of the maintenance dose. The initial doses ranged from 0.8 mg to 2.4 mg and the maintenance doses from 0.2 mg to 0.6 mg.

Posttreated Combined Dexamethasone and Crocetin Group
The same dosage regimen for the separate crocetin and dexamethasone groups was used in combination in this group of eight cats.

Control Group
These 15 animals received no treatment; 11 were sacrificed at 24 hours and four at 48 hours after the lesion.

The animals were again anesthetized with 44 mg/kg of ketamine. The right chest was opened, and 20 ml of 5% buffered formalin was injected immediately into the left ventricle, causing instant death. The brain was then removed intact down to the midbrain and photographed (Fig. 2). The hemispheres were divided, and four coronal sections were taken of the right hemisphere through the area of the lesion. Measurements of dye extravasation were made in two planes, using the coronal section which demonstrated the largest area of dye spread. A section of brain was then taken from the area of the lesion; the gray matter was dissected free from the white matter, and samples of each were placed on tared aluminum foil and weighed. Similar gray and white matter samples were obtained from a comparable area of the left hemisphere and weighed. The samples were placed in a drying oven for 24 hours at 75 °C and dried to a constant weight. Wet and dry weights were determined for the right- and
Increase of plasma O₂ diffusivity on cryogenic edema

TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cats</th>
<th>Mean Edema (gm/gm of Brain Tissue)</th>
<th>Standard Error</th>
<th>Percent Changes</th>
<th>Significance (p)</th>
<th>Mean Dye Extravasation (sq cm)</th>
<th>Standard Error</th>
<th>Percent Changes</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sacrificed at 24 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control crocetin (pretreated)</td>
<td>4</td>
<td>.016 ± .013</td>
<td>-80</td>
<td>.025</td>
<td>1.02</td>
<td>± 0.28</td>
<td>-37</td>
<td>.07</td>
<td></td>
</tr>
<tr>
<td>dexamethasone (pretreated)</td>
<td>4</td>
<td>.041 ± .019</td>
<td>-49</td>
<td>.15</td>
<td>.96</td>
<td>± 0.30</td>
<td>-41</td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>crocetin (posttreated)</td>
<td>4</td>
<td>.045 ± .017</td>
<td>-44</td>
<td>.15</td>
<td>1.01</td>
<td>± 0.33</td>
<td>-38</td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>dexamethasone (posttreated)</td>
<td>4</td>
<td>.052 ± .007</td>
<td>-36</td>
<td>.2</td>
<td>1.08</td>
<td>± 0.18</td>
<td>-34</td>
<td>.07</td>
<td></td>
</tr>
<tr>
<td>dexamethasone and crocetin (posttreated)</td>
<td>4</td>
<td>.045 ± .010</td>
<td>-44</td>
<td>.2</td>
<td>.79</td>
<td>± 0.20</td>
<td>-52</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>sacrificed at 48 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control crocetin (pretreated)</td>
<td>4</td>
<td>.096 ± .013</td>
<td>-86</td>
<td>.005</td>
<td>.46</td>
<td>± 0.19</td>
<td>-76</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>dexamethasone (pretreated)</td>
<td>4</td>
<td>.065 ± .013</td>
<td>-32</td>
<td>.2</td>
<td>1.03</td>
<td>± 0.09</td>
<td>-45</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>crocetin (posttreated)</td>
<td>4</td>
<td>.052 ± .016</td>
<td>-48</td>
<td>.05</td>
<td>.91</td>
<td>± 0.25</td>
<td>-52</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>dexamethasone (posttreated)</td>
<td>4</td>
<td>.076 ± .014</td>
<td>-21</td>
<td>.3</td>
<td>1.04</td>
<td>± 0.24</td>
<td>-45</td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>dexamethasone and crocetin (posttreated)</td>
<td>4</td>
<td>.045 ± .017</td>
<td>-58</td>
<td>.05</td>
<td>.62</td>
<td>± 0.28</td>
<td>-67</td>
<td>.01</td>
<td></td>
</tr>
</tbody>
</table>

left-side samples. The left-hemisphere samples were considered the normal value, and the right-side samples were expressed as a percentage increase or decrease compared to them. The values were then converted to grams of water per gram of brain tissue.

Results

No significant effect was produced in gray-matter edema with crocetin or dexamethasone. Pretreatment with crocetin resulted in a significant reduction of white-matter edema at both 24 and 48 hours (Table 1). This reduction was most marked in the 48-hour group, but was also significant at 24 hours. Pretreatment gave better results than posttreatment. Pretreatment with dexamethasone also reduced edema, but not as effectively as crocetin.

The posttreated animals also showed a significant reduction of edema, to a some-what lesser degree. Posttreatment with crocetin gave a more significant degree of edema reduction than did dexamethasone in the dosages employed. The combination of dexamethasone and crocetin was no more effective than crocetin alone in reducing edema.

In the 24-hour group, the combination of dexamethasone and crocetin in the posttreated animals was more effective in preventing dye extravasation than either drug alone. It was also more effective than pretreatment with crocetin. This combination was also slightly more effective in the posttreated group sacrificed at 48 hours in reducing dye extravasation than the drugs used singly. However, pretreatment with crocetin gave the best reduction of dye extravasation at 48 hours.

In the numbers of cats used, the reductions in edema obtained with dexamethasone were not statistically significant. However, the reductions in dye extravasation were signifi-
FIG. 2. Photograph of cat brain shows lesion in right hemisphere.

cant. The decrease in edema obtained with crocetin was significant, as was the reduction in dye extravasation.

Comment

As noted previously, it has been established that crocetin will increase the diffusion speed of oxygen through plasma. This mechanism should result in a net increase in the available oxygen at the capillary-endothelial level. We have postulated that oxygen availability at this level is a critical factor in the development of vasogenic edema, and an increase in oxygen availability to the capillary cell should decrease vasogenic edema. Our results tend to indicate that this theory is true. Treatment with crocetin was significantly effective in reducing edema in our series. It is not certain if this effect is primarily due to free radical scavenging by oxygen or if crocetin provides a means of increasing the metabolic rate of the capillary cells and hastening repair. Probably both effects occur. Further study is required to determine if crocetin is useful as a clinical agent for the treatment of cerebral edema. Experimentally, it appears to have promise.

References


Address reprint requests to: James V. Gainer, Jr., M.D., 114 McDonald Street, Kingwood, West Virginia 26537.