Effect of Uridine 5'-Diphosphate on Cryogenic Brain Edema in Rabbits

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This study was undertaken to examine the effect of uridine 5'-diphosphate, administered intravenously or intraperitoneally, on cold injury–induced brain edema in rabbits. Bolus injection or continuous intravenous infusion of uridine 5'-diphosphate 26 hours after a lesion was established had adverse effects, such as increased intracranial pressure and lowered systolic arterial blood pressure and cerebral perfusion pressure for approximately 10–29 minutes, but these parameters did not change appreciably from 29 minutes to 3 hours after administration. Intraperitoneally administered uridine 5'-diphosphate did not affect these parameters appreciably during 3 hours. Thus, the intravenous administration of uridine 5'-diphosphate is harmful under neurosurgical conditions. In contrast, 10 mg/kg/day i.p. uridine 5'-diphosphate pretreatment and posttreatment, beginning 24 hours before and continuing until 24 hours after the insult, significantly reduced neurologic abnormalities, Evans blue extravasation, water content in the injured gray matter, and intracranial pressure without affecting water content in the white matter. Intravenous dexamethasone pretreatment and posttreatment in this setting significantly reduced only neurologic abnormalities. However, there were no significant differences between intraperitoneal uridine 5'-diphosphate and intravenous dexamethasone effects on cold-injured brain. (Stroke 1989;20:1694-1699)

Katunuma, Kido, and colleagues1-5 showed that derivatives of uridine nucleotide sugar may amplify the many biologic functions of glucocorticoids, such as liver enzyme induction, cytotoxicity for lymphoblast cells in vivo and in vitro, anti-inflammatory mechanisms, and protection against the development of carrageenan edema in rabbit leg. We found that uridine 5'-diphosphate (UDP) may also be protective against the development of carrageenan edema in rabbit leg (S. Yoshida, unpublished data). These two observations suggest that exogenous UDP might also amplify the actions of endogenous glucocorticoids against brain edema. Therefore, this study was designed to examine the effects of 3 hours of UDP treatment on a well-established, 26-hour-old cold lesion to determine the appropriate route and method of UDP administration and then to compare the effects of intraperitoneal UDP and intravenous dexamethasone treat-

Materials and Methods

In the delayed treatment experiment, 50 New Zealand White rabbits weighing 2.5–3.3 kg were anesthetized with 25 mg/kg i.v. sodium thiopental (Abbott Laboratories, North Chicago, Illinois) and positioned in a head frame. These rabbits were not pretreated with UDP (Sigma Chemical Co., St. Louis, Missouri). A 12.5-mm-diameter circular trephine hole was made over the left parietooccipital cortex using a standardized technique6 in all 50 rabbits. The cold injury was induced in 38 rabbits when a round stainless steel probe (area 64 mm²), previously equilibrated in a liquid nitrogen bath, was applied to the intact dura for 150 seconds. Penicillin G (Bristol Laboratories, Syracuse, New York) (50,000 unit/kg i.m.) was given prophylactically. The rabbits were then returned to their cage facilities and given food and water ad libitum.

Brief anesthesia was induced with halothane (Halocarbon Laboratories, Inc., Hackensack, New Jersey) approximately 24 hours after surgery or lesion formation. The rabbit was mechanically ventilated with a small animal respirator (Harvard Apparatus Co., Inc., Millis, Massachusetts) with a mixture of
oxygen (50%, Parsons Medical, San Diego, California), nitrous oxide (50%, Parsons Medical), and halothane (0.5%). Inspired carbon dioxide (Parsons Medical) was added to maintain Paco₂ at 38–42 torr, and an infrared heater or alcohol sponging maintained the body core temperature at 37 ± 1°C. Catheters were inserted into the femoral artery and superior vena cava for continuous monitoring of systolic arterial blood pressure and central venous pressure via an appropriate strain gauge transducer (Hewlett-Packard, Waltham, Massachusetts). A spinal needle (20 gauge) was stereotactically inserted into the uninjured (right) lateral ventricle for continuous recording of intracranial pressure (ICP). The ICP zero reference point was the interaural line. Four electroencephalographic electrodes were placed on the frontal and occipital regions of both hemispheres. All pressures, heart rate, and electroencephalogram were continuously recorded on a multichannel polygraph (Hewlett-Packard). After stabilization, approximately 26 hours after surgery or lesion formation, four different UDP regimens were started. Group A rabbits received cold injury and a bolus injection of 10 mg/kg i.v. UDP during 1 minute (n=14). Group B rabbits received cold injury and a continuous infusion of 10 mg/kg i.v. UDP for 50 minutes (n=12). Group C rabbits received cold injury and 10 mg/kg i.p. UDP (n=12). Group D rabbits received surgery but no cold injury and a bolus injection of 10 mg/kg i.v. UDP during 1 minute (n=12). The groups were further subdivided. Half of the rabbits in each group had physiological variables monitored after UDP administration, and the other half were used for the determination of either brain specific gravity or Evans blue staining. The results of ICP, systolic arterial blood pressure, and central venous pressure are expressed as mean±SD and the values before and after UDP injection were compared using the paired t test.

The rabbits in the delayed treatment groups used for specific gravity determination were killed with an overdose of sodium thiopental and exsanguinated after 3 hours of UDP therapy 29 hours after surgery or lesion formation. Cortical gray and subcortical white matter samples were obtained via a reproducible method from the brain surrounding the site of the cold lesion. Homologous samples were then taken from the contralateral hemisphere. Specific gravity of each sample was obtained by microgravimetric techniques. The results are expressed as mean±SD and were analyzed using the Bonferroni method.

Each rabbit in the delayed treatment groups scheduled for Evans blue staining received 0.34 ml/kg i.v. 3% Evans blue (Sigma) immediately after surgery or lesion formation. All rabbits were killed with an overdose of sodium thiopental and were perfused with 5% formalin via the carotid arteries after 3 hours of UDP therapy. The fixed brains were coronally sectioned at 2.8-mm intervals. A clear Mylar grid (1.4 x 1.4 mm) was placed directly over each slice, and the stained area was determined. The sum of the stained areas from six slices was converted to square centimeters. The results are expressed as mean±SD and were analyzed using the Bonferroni method.

In a subsequent experiment (pretreatment plus posttreatment), standard cold lesions were formed in 161 New Zealand White rabbits using the same method as in the delayed treatment experiment. The rabbits were divided into eight groups. Those in the control normal group received surgery, but no cold injury and no therapy (n=18). Those in the untreated injured group received cold injury and 2 ml i.p. saline (n=37). Those in the UDP-treated injured groups received cold injury and 100 (n=12), 10 (n=26), or 1 (n=13) mg/kg/day i.p. UDP dissolved in 2 ml saline. Those in the UDP-treated normal group received surgery and 10 mg/kg/day i.p. UDP dissolved in 2 ml saline, but no cold injury (n=18). Those in the dexamethasone treated groups received cold injury and 1 (n=17) or 0.1 (n=20) mg/kg/day i.v. dexamethasone sodium phosphate (Carter-Glogau Laboratories, Inc., Glendale, Arizona). In all treatment groups (including the untreated injured group) three doses were administered, one 24 hours before the lesion formation, one at the moment of lesion formation, and the third 24 hours after lesion formation.

Twenty-four hours after surgery or lesion formation, approximately one third of the rabbits in six of the pretreatment plus posttreatment groups (control normal, untreated injured, 10 mg/kg/day UDP-treated injured, 10 mg/kg/day i.v. dexamethasone, and 0.1 mg/kg/day dexamethasone) were prepared for the monitoring of ICP, systolic arterial blood pressure, central venous pressure, and electroencephalogram as described for the delayed treatment groups. The physiological variables were recorded continuously from 26 to 27 hours after surgery or lesion formation. The results are expressed as mean±SD and were analyzed using the Bonferroni method.

Specific gravity was measured in one fourth to one half of the rabbits in all eight and the area of Evans blue staining was measured in approximately one third of the rabbits in four (untreated injured, 10 mg/kg/day UDP-treated injured, 1 mg/kg/day dexamethasone, and 0.1 mg/kg/day dexamethasone) pretreatment plus posttreatment groups 26 hours after surgery or lesion formation. The methods and analysis of results are as described for the delayed treatment groups.

Both mortality and neurologic scores for all rabbits in each pretreatment plus posttreatment group (except six rabbits each in the high- and low-dose UDP-treated injured groups) were calculated and compared 26 hours after surgery or lesion formation. We calculated neurologic scores according to the following scale: motor system (3, normal; 2, hemiparesis; 1, hemiplegia or tetraparesis; 0, tetraplegia), head tilting (1, negative; 0, positive), level of consciousness (3, normal; 2, lethargic; 1,
TABLE 1. Mortality and Neurologic Score in Eight Groups of Rabbits Treated With UDP Before and After Cold Injury

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg)</th>
<th>n</th>
<th>Mortality (%)</th>
<th>Neurologic score (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control normal</td>
<td>—</td>
<td>18</td>
<td>0</td>
<td>10.0±0*</td>
</tr>
<tr>
<td>Untreated injured</td>
<td>0</td>
<td>37</td>
<td>21.6</td>
<td>5.6±2.7</td>
</tr>
<tr>
<td>UDP treated injured</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>8.5±0.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>26</td>
<td>7.7</td>
<td>7.4±2.6†</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>8.4±0.5†</td>
</tr>
<tr>
<td>UDP treated normal</td>
<td>10</td>
<td>18</td>
<td>0</td>
<td>10.0±0*</td>
</tr>
<tr>
<td>Dexamethasone injured</td>
<td>1</td>
<td>17</td>
<td>7.6</td>
<td>6.9±2.5†</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>20</td>
<td>10.0</td>
<td>7.7±2.6*</td>
</tr>
</tbody>
</table>

UDP, uridine 5'-diphosphate.
*p<0.005, 0.05, respectively, different from untreated injured by Kruskal-Wallis rank test.

Results

In seven rabbits of Group A there was a significant increase in ICP (maximum 20.4±3.7 torr, p<0.001 vs. pretreatment values) for 16.5±5 minutes and a significant decrease in systolic arterial blood pressure (minimum 80±9 torr, p<0.01) for 17±7 minutes almost immediately after the injection, but these parameters did not change appreciably from 17 minutes to 3 hours after treatment (Figure 1). After UDP administration there was a transient increase in central venous pressure. On the electroencephalograms, there was a reduction of high-voltage slow waves and a marked increase in fast wave activity for approximately 5 minutes almost immediately after the injection in five of the seven rabbits. The intravenous bolus injection of UDP increased the heart rate immediately in all but one rabbit for 11–27 minutes. In six rabbits of group B there was a slight increase in ICP for 29±1 minutes and a significant decrease (p<0.01) in systolic arterial blood pressure for 10±4 minutes (Figure 2). Central venous pressure and electroencephalogram did not change appreciably. In six rabbits of Group C there were no gross changes in these parameters. Among the remaining rabbits of Groups A, B, and C, neither specific gravity nor area of Evans blue staining differed significantly.

In six rabbits of Group D there was a significant increase in ICP (maximum 11.7±4.7 torr, p<0.01) for 23±10 minutes and a significant decrease in systolic arterial blood pressure (minimum 77±10 torr, p<0.001) for 11±2 minutes almost immediately after the injection. Central venous pressure and heart rate changed as in Group A. There was a reduction in electroencephalographic voltage almost immediately after the intravenous injection in one of the six rabbits for approximately 5 minutes.

In the pretreatment plus posttreatment experiment there were no significant differences in mortality among the groups (Table 1). However, there was a trend toward lower mortality in the four UDP-treated groups, as well as in the 0.1 mg/kg/day dexamethasone group. Neurologic scores in both 10 mg/kg/day UDP, in the 1 mg/kg/day UDP, and in both dexamethasone groups were significantly higher than that in the untreated injured group (Table 1). There were no significant differences in neurologic scores between the UDP and dexamethasone groups, nor between the sham-operated groups (control normal and UDP-treated normal).
In the pretreatment plus posttreatment experiment, specific gravity in the injured gray matter decreased significantly (water content increased) in the untreated injured group compared with control normals (Table 2). In contrast, specific gravity was near normal and significantly increased in both 10 mg/kg/day UDP groups compared with the untreated injured group. However, there were no significant differences between the UDP and dexamethasone groups, nor between the sham-operated groups. In the injured white matter, specific gravity decreased significantly in the untreated injured group compared with control normals. There were no significant differences among the injured groups (untreated, UDP-treated, and dexamethasone-treated) or between the sham-operated groups. However, there was a slight increase in specific gravity in the left injured hemisphere and it decreased to 4.3±0.8 cm² (n=10) 26–27 hours after injury were stable in all six groups in which physiologic variables were monitored. ICP in the 10 mg/kg/day UDP injured group was significantly lower than that in the untreated injured group (p<0.05, Table 3), and ICP in the untreated injured group was significantly higher than that in the control normal group (p<0.005). There were no differences in ICP between the UDP and dexamethasone groups, nor between the sham-operated groups. There were no differences in systolic arterial blood pressure or central venous pressure among the six groups. There were no differences in systolic arterial blood pressure or central venous pressure among the six groups. There were no differences in electroencephalograpic findings among the injured groups or between the sham-operated groups.

In comparison, delayed treatment with intravenous UDP increased ICP and decreased systolic arterial blood pressure and cerebral perfusion pressure for 10–29 minutes immediately after injection. However, delayed treatment with intraperitoneal UDP did not change these parameters during 3 hours. In contrast, pretreatment plus posttreatment with 10 mg/kg/day i.p. UDP significantly increased specific gravity in the gray matter and decreased the areas of Evans blue staining and the ICP compared with the untreated injured group. Pretreatment plus posttreatment with intravenous dexamethasone did not change these parameters. However, we could not demonstrate significant differences in all of the comparisons of UDP and dexamethasone.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg)</th>
<th>n</th>
<th>Injured (left) hemisphere</th>
<th>Uninjured (right) hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gray</td>
<td>White</td>
</tr>
<tr>
<td>Control normal</td>
<td>—</td>
<td>9</td>
<td>1.0439±0.0006*</td>
<td>1.0429±0.0007*</td>
</tr>
<tr>
<td>Untreated injured</td>
<td>0</td>
<td>9</td>
<td>1.0415±0.0013</td>
<td>1.0350±0.0028</td>
</tr>
<tr>
<td>UDP-treated injured</td>
<td>100</td>
<td>6</td>
<td>1.0425±0.0004</td>
<td>1.0340±0.0015</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9</td>
<td>1.0433±0.0007*</td>
<td>1.0367±0.0022</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>1.0423±0.0008</td>
<td>1.0349±0.0023</td>
</tr>
<tr>
<td>UDP-treated normal</td>
<td>10</td>
<td>6</td>
<td>1.0442±0.0007*</td>
<td>1.0426±0.0007*</td>
</tr>
<tr>
<td>Dexamethasone injured</td>
<td>1</td>
<td>6</td>
<td>1.0426±0.0012</td>
<td>1.0330±0.0015</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>9</td>
<td>1.0423±0.0015</td>
<td>1.0354±0.0017</td>
</tr>
</tbody>
</table>

UDP, uridine 5'-diphosphate. Data are mean±SD.

*tp<0.005, 0.05, respectively, different from untreated injured by Bonferroni method.

TABLE 2. Specific Gravity of Brain Regions in Eight Groups of Rabbits Treated With UDP Before and After Cold Injury

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg)</th>
<th>n</th>
<th>Pressure (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control normal</td>
<td>—</td>
<td>6</td>
<td>4.7±1.8*</td>
</tr>
<tr>
<td>Untreated injured</td>
<td>0</td>
<td>10</td>
<td>9.7±2.5</td>
</tr>
<tr>
<td>UDP-treated injured</td>
<td>10</td>
<td>10</td>
<td>6.8±2.1†</td>
</tr>
<tr>
<td>UDP-treated normal</td>
<td>10</td>
<td>6</td>
<td>4.5±1.5*</td>
</tr>
<tr>
<td>Dexamethasone injured</td>
<td>1</td>
<td>6</td>
<td>8.6±1.7</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>7</td>
<td>9.4±3.7</td>
</tr>
</tbody>
</table>

UDP, uridine 5'-diphosphate. Data are mean±SD.

*tp<0.005, 0.05, respectively, different from untreated injured by Bonferroni method.

TABLE 3. Intracranial Pressure in Six Groups of Rabbits Treated With UDP Before and After Cold Injury
Discussion

UDP is a pyrimidine nucleotide that is constantly present in the brain and other tissues. Although the precise role of exogenous UDP is not yet known, it is a vasoactive substance. This probably explains the variable effects by routes of UDP administration. However, there are probably species differences regarding UDP-induced relaxation of pial vessels. In human brain circulation, UDP induces either powerful transient dilatation or stable constriction, depending on whether the agent reaches the luminal (endothelium) or the abluminal (smooth muscle cells) side of the vessel wall. In contrast, in rabbits, dogs, and cats UDP may be a powerful, long-lasting constrictor of isolated cerebral arteries, with no detectable dilatory components. In contrast to these in vitro findings, the rapidity with which ICP initially increased after intravenous UDP injection in our in vivo study suggests that transient dilatation of cerebral vessels might account for the transient ICP increases because intravenous UDP comes into direct contact with the endothelium in a short time. This probably explains the simultaneous decrease in systolic arterial blood pressure in our study. In contrast to this finding, MacDonald et al. found that intravenous UDP (0.0202–0.1616 mg/kg/min) may increase systolic arterial blood pressure when infused into intact anesthetized rats. This difference in blood pressure responses between studies can probably be accounted for by the dosage range and species differences. Our results indicate that intravenous UDP is harmful under neurosurgical conditions. Thus, we administered UDP to the rabbits intraperitoneally during the pretreatment plus posttreatment experiment. It was understood that intraperitoneally administered UDP does not come into direct contact with the endothelium or smooth muscle cells of the vessel wall. Thus, intraperitoneal UDP was not harmful, as shown in the delayed treatment experiment. On the other hand, the absorption of steroids can be capricious after intraperitoneal injection; therefore, we administered dexamethasone to the rabbits intravenously.

There were disparities in the effect of UDP on cold-injured brain in the pretreatment plus posttreatment experiment. Especially, we failed to find any significant effect of UDP on specific gravity in the white matter in the injured groups. This may be explained by several possibilities. First, UDP may indeed have no effect on brain edema in the white matter. In general, edema fluid in vasogenic edema accumulates predominantly in the white matter (Table 2), in agreement with the observations of many investigators. However, Long speculated that brain edema in rabbits represents cortical swelling rather than swelling of the deep white matter.

Second, the thin layer of white matter (approximately 1 mm) in rabbits, dogs, and cats may have precluded our detecting a decrease in edema. It was very difficult to obtain softened white matter because there was a loss of clear demarcation between gray and white matter. Thus, the SD of the specific gravity values from white matter was much greater than that from gray matter (Table 2). Moreover, there were trends toward edema improvement in the white matter of the 10 mg/kg/day UDP injured group. Consequently, we cannot rule out the possibility of a beneficial UDP effect in the white matter.

Third, the dosage schedule or the timing of our determination of UDP effect may have been inappropriate. We do not know the optimal timing of UDP therapy. Herrmann and Neuenfeldt demonstrated that peak edema caused by cold injury occurs approximately 24 hours after the insult in rabbits. In most studies, an effect of steroids on brain water content cannot be demonstrated quantitatively until 24 hours after injury, with the greatest reduction being seen 24–48 hours after the injury. Furthermore, a striking feature observed in several cold injury studies is the definite effect of steroids given 24–48 hours before injury. Since we assume that exogenous UDP may also amplify the action of endogenous glucocorticoids against brain edema, these observations suggested our timing of UDP pretreatments and posttreatments and could explain the variable effects derived from the timing of UDP administration.

Another possible mechanism to explain its effects on cold-injured brain is the metabolic effect of UDP. Pathologic alterations in cerebral energy and carbohydrate metabolism in various forms of experimental brain edema, such as cold injury, have been observed. UDP plays an important role in brain carbohydrate metabolism as follows: UDP glucosyl + glycogen (n residues) → UDP + glycogen (n + 1 residues). Glycogen is the largest single energy reserve when glucose levels fall, and local carbohydrate reserves are important for brain functions. Thus, UDP might participate in the regulation of an altered carbohydrate metabolism. However, there is no sufficient evidence to explain these two possible mechanisms and, therefore, further studies are needed to clarify them.

The discrepancy between the effects of UDP and those of dexamethasone can be partly explained by differences in the number of subjects. Of all our evaluations, the 10 mg/kg/day UDP injured group had the most subjects (n=9) when compared with the dexamethasone groups (n=6). This bias toward a significant effect is seen when comparisons are made with the dexamethasone groups. Our data may show negative results, but they do demonstrate trends toward improvement in both dexamethasone groups. Thus, we could not reject the hypothesis of a beneficial effect of dexamethasone in this model.

In conclusion, the intravenous injection of UDP is harmful under neurosurgical conditions. In contrast, 10 mg/kg/day i.p. UDP pretreatment and posttreatment improved neurologic abnormalities and decreased the water content of injured gray
matter, Evans blue extravasation, and ICP but did not change the water content of white matter. Since there were disparities in the effectiveness of intraperitoneal UDP pretreatments and delayed treatments, our results are inconclusive and further studies are needed to clarify the effect of intraperitoneal UDP on cold-injured brain.

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References


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References


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