Therapeutic effects of local delivery of dexamethasone on experimental brain tumors and peritumoral brain edema

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To determine if dexamethasone administered by osmotic pump directly to brain tumors would control peritumoral edema and at the same time suppress tumor growth and prolong survival, the authors studied experimental brain tumors produced in 102 rabbits by implanting VX2 carcinoma cells. Of these, 58 animals were separated into three treatment groups: Group 1 included 15 untreated rabbits; Group 2 included 18 rabbits treated with systemic dexamethasone (4 mg/kg/day); and Group 3 included 25 rabbits treated with local dexamethasone (0.24 mg/day) delivered by osmotic pump. Systemic or local dexamethasone was administered from Day 3 or Day 7 after tumor implantation, and animals were sacrificed on Day 13. A survival study was performed with 44 rabbits separated into the same treatment groups, beginning drug delivery on Day 7. Brain water content in the white matter of sacrificed animals was measured by the specific gravity method. The length and width of the brain tumors in all animals were measured and the tumor volume estimated. Findings showed that systemic and local dexamethasone administered from Day 3 or Day 7 was associated with a significant (5% level) inhibition of tumor volume as well as a mean reduction of brain edema in most tested sites. Systemic and local dexamethasone therapy also resulted in a significant (5% level) increase in survival time relative to the untreated group. These short-term results suggest that locally delivered dexamethasone may constitute a clinically important therapeutic modality.

Key Words: brain neoplasm • edema • dexamethasone • osmotic pump • drug delivery • carcinoma • rabbit

Peritumoral brain edema remains a substantial cause of mortality and morbidity in patients with brain tumors, however, the pathogenesis of this condition is still obscure. Several biochemical dysfunctions have been implicated in the genesis of peritumoral brain edema in addition to the structural changes associated with neoplasms. For the past 30 years, systemic administration of steroids has proved to be one of the few therapies effective in treating this problem; however, the use of systemic steroids is limited by many systemic side effects. In this study, we investigated the short-term therapeutic effect of the local delivery of dexamethasone to brain tumors in an attempt to determine if this mode of delivery would achieve the beneficial effects of systemic dexamethasone while potentially reducing the harmful side effects.

We used a rabbit brain tumor model established by Carson, et al. This model forms a single intracerebral tumor mass by the 7th day after surgical implantation, with rapid growth of the tumor between the 9th and 13th day. The short-term therapeutic effect on peritumoral brain edema and brain tumor growth from systemic and local dexamethasone administration was evaluated both before and after formation of the intracerebral tumor mass in the present study.

Materials and Methods

This study was performed in compliance with the Animal Care Committee of the National Institutes of Health "Guide for the Care and Use of Laboratory Animals," and was approved by The Johns Hopkins Medical Institutions Animal Services.

Tumor Implantation

Adult New Zealand White rabbits, each weighing 3.5 to 4.5 kg, were used for the study. They were anesthetized with ketamine hydrochloride (25 mg/kg) and acepromazine maleate (2.5 mg/kg). Anesthesia was maintained with a 0.5-mL bolus of thiopental sodium administered intravenously via a marginal ear vein. The heads were shaved and cleansed. Brain tumors were produced by the injection of a 25-μL suspension of 3 ×
Local dexamethasone in peritumoral brain edema

10^7 viable VX2 carcinoma cells into the right frontoparietal lobe of the rabbit brain through a 1-mm cranial burr hole.

Implantation of Osmotic Pump

The dorsal surface of the head and neck was shaved and cleansed. A 2-cm parasagittal incision was made, the periosteum was reflected and a burr hole was made with a No. 23 burr 4 mm lateral and 3 mm posterior to the bregma. A Teflon catheter was inserted to a depth of 7 mm and glued in place. An osmotic pump was then implanted subcutaneously and connected to the catheter with polyethylene 60 tubing. The incision was closed with surgical staples, and the rabbit was given antibiotic protection.

Peritumoral Brain Edema Study

Fifty-eight tumor-bearing rabbits were separated into three groups: Group 1 included 15 untreated rabbits; Group 2 included 18 rabbits treated with systemic dexamethasone (4 mg/kg/day); and Group 3 included 25 rabbits treated with local dexamethasone (0.24 mg/day) delivered by osmotic pump. Systemic or local dexamethasone administration was begun on Day 3 or Day 7 after tumor implantation, with sacrifice on Day 13. The brains were removed and the placement of the implanted catheter in the tumor mass was visually confirmed. Brain water content was measured by a specific-gravity method. Brain samples, approximately 1 cu mm, were excised and placed onto a kerosene/monobromobenzene column. The position of a brain sample was read 5 minutes after placement of the sample onto the column. Columns were calibrated with NaCl solutions of known specific gravity. The eight sampling areas are illustrated in Fig. 1.

Survival Study

Another group of 44 tumor-bearing rabbits were separated into three groups: Group 1 included 16 untreated rabbits; Group 2 included 14 rabbits treated with systemic dexamethasone (4 mg/kg/day); and Group 3 included 14 rabbits treated with local dexamethasone (0.24 mg/day) delivered by osmotic pump. Treatment was started from the 7th day after tumor implantation. Length of survival was measured with the day of tumor implantation considered as Day 0. The brains were removed to ascertain whether brain tumors had been successfully produced, and estimated tumor volumes were calculated.

Tumor Volume Study

We estimated tumor volumes in all 102 rabbits. Tumor volume was determined by measuring tumor dimensions along the long axis of the tumor and across the tumor at the widest point perpendicular to the long axis, and substituting the values into the following equation: estimated tumor volume (cu mm) = (length (mm) × width (mm)) ÷ 2.

Statistical Analysis

Hypothesis tests were performed as a measure of the therapeutic impact of the various treatment strategies, based upon the following considerations. We hypothesized that if dexamethasone treatment is therapeutic, animals receiving treatment should have reduced mean brain edema or higher specific gravity (SG), as reported in Table 1, than control animals. Conversely, if the treatment has no effect, mean brain edema is expected to be similar in the treated and untreated animals. In no case did we hypothesize the treatment to increase mean brain edema in the animals. Moreover, dexamethasone therapy may have enough of a measurable effect that treated animals' mean brain edema is found to be significantly different from control animals' mean brain edema at a given significance level (5%, for instance). Therefore, we used one-sided hypothesis tests of the following generic form: null hypothesis, SGc = SG_r; and alternative hypothesis, SG_c < SG_r, where subscripts C and T refer to control and treated animals, respectively.

Rejection of the null implies acceptance of the alternative, and rejection of the null is deemed to be convincing evidence for the short-term therapeutic impact of dexamethasone. We note in general that, although for a given significance level it may not be possible to reject the null, if mean SG in the treated animals is greater than mean SG in the control group by any amount whatsoever, it is possible to find some significance level at which the null will be rejected against a one-sided alternative. Therefore, rather than report only test sites at which the null was rejected at an arbitrarily chosen (5%) significance level for each site where mean edema was reduced in the treated animals, we also report the p value, the significance level at which the null would be marginally rejected against a one-sided alternative. Nonparametric tests using approximate (asymptotic) confidence intervals were used. Critical values were obtained from the standard normal distribution.
TABLE 1
Specific gravity values of eight sampling areas in tumor-bearing rabbits, comparing untreated with local and systemic dexamethasone-treated groups

<table>
<thead>
<tr>
<th>Study Group</th>
<th>No. of Rabbits</th>
<th>Specific Gravity Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area 1</td>
<td>Area 2</td>
</tr>
<tr>
<td>untreated</td>
<td>15</td>
<td>1.0469±0.0017</td>
</tr>
<tr>
<td>local</td>
<td>15</td>
<td>1.0467±0.0010</td>
</tr>
<tr>
<td>(Day 3)</td>
<td>p value</td>
<td>none</td>
</tr>
<tr>
<td>systemic</td>
<td>11</td>
<td>1.0465±0.0009</td>
</tr>
<tr>
<td>(Day 3)</td>
<td>p value</td>
<td>none</td>
</tr>
<tr>
<td>local</td>
<td>10</td>
<td>1.0466±0.0017</td>
</tr>
<tr>
<td>(Day 7)</td>
<td>p value</td>
<td>none</td>
</tr>
<tr>
<td>systemic</td>
<td>7</td>
<td>1.0458±0.0007</td>
</tr>
<tr>
<td>(Day 7)</td>
<td>p value</td>
<td>none</td>
</tr>
</tbody>
</table>

* Values are expressed as the mean ± standard error of the mean. The eight sampling areas are shown in Fig. 1.

Results

Peritumoral Brain Edema Study

The findings of the brain edema study are presented in Table 1. The mean amount of brain edema was reduced or unchanged relative to the control rabbits in seven of the eight brain sites tested in rabbits receiving local drug therapy from Day 3, with both gray-matter sites in the ipsilateral and one gray-matter site in the contralateral hemisphere exhibiting a mean reduction in edema. The mean amount of brain edema was lower or unchanged in five of eight sites tested in rabbits receiving systemic drug therapy from Day 3, with only one gray-matter site in the contralateral hemisphere exhibiting a mean reduction in edema. The mean amount of brain edema was less in four of eight sites tested in rabbits receiving local drug therapy from Day 7, with only white-matter sites exhibiting a mean reduction. Similarly, the mean brain edema was reduced in four of eight sites tested in rabbits receiving systemic drug therapy from Day 7, with only white-matter sites exhibiting a mean reduction. White-matter sites showed a mean reduction in edema across all treatment groups. Gray-matter sites showed a reduction in edema only in the groups with treatment beginning on Day 3, and the group with local drug delivery showed a mean reduction of gray-matter edema in more sites than the systemic treatment group. In the Day 3 treatment groups, mean white-matter edema in all sites was found to be significantly different from mean edema in the corresponding sites in the control rabbits at the 5% significance level. In the group with local drug delivery beginning on Day 7, mean white-matter edema in one site in the contralateral hemisphere was found to be significantly different from mean edema in the corresponding site in the control rabbits at the 5% significance level. In the Day 7 systemic drug delivery group, mean white-matter edema in three sites (two in the contralateral and one in the ipsilateral hemisphere) was found to be significantly different from mean edema in the corresponding sites in the control rabbits at the 5% significance level. In no group was gray-matter edema found to be significantly different from the corresponding sites in the control rabbits at the 5% significance level.

Tumor Volume

Tumor volume data are illustrated in Fig. 2. In all treatment groups, dexamethasone therapy resulted in a lower mean tumor volume than in the control rabbits. In the Day 3 treatment groups, rabbits receiving local drug therapy exhibited lower mean tumor volumes than either the control rabbits or the rabbits receiving systemic drug therapy. Conversely, in the Day 7 treatment groups, rabbits receiving systemic drug therapy exhibited lower mean tumor volume than either the control rabbits or the rabbits receiving local drug therapy. In all treatment groups, mean tumor volumes were found to be significantly different from the mean tumor volume of the control rabbits at the 5% significance level.

Survival Study

Both treatment groups in the survival study (those receiving systemic or local dexamethasone treatment beginning on Day 7) exhibited an increase in mean survival time relative to the untreated group (Table 2). Moreover, the difference in survival time was found to be statistically significant at the 5% significance level.

Discussion

Study Rationale

Peritumoral brain edema is responsible for most secondary effects of brain tumors. The development of peritumoral brain edema complicates surgery, radiotherapy, and chemotherapy. For these reasons, a large amount of research energy has been directed toward the elucidation of the pathogenic and therapeutic aspects of this problem and has resulted in a number of
Local dexamethasone in peritumoral brain edema

Fig. 2. Graph showing tumor volume in tumor-bearing rabbits. C = untreated group; S = systemic dexamethasone-treated group; T = local dexamethasone-treated group. 3S and 3T = treatments given beginning 3 days after tumor implantation; 7S and 7T = treatment given beginning 7 days after tumor implantation. Values are mean ± standard error of the mean. Asterisks: p < 0.05, compared with control values.

TABLE 2
Survival study of rabbits receiving treatment from Day 7 after tumor implantation*

<table>
<thead>
<tr>
<th>Study Group</th>
<th>No. of Rabbits</th>
<th>Length of Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated</td>
<td>16</td>
<td>13.25 ± 1.00</td>
</tr>
<tr>
<td>systemic dexamethasone</td>
<td>14</td>
<td>17.57 ± 0.58†</td>
</tr>
<tr>
<td>(4 mg/kg/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>local dexamethasone</td>
<td>14</td>
<td>17.92 ± 0.79†</td>
</tr>
<tr>
<td>(0.24 mg/day)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data are expressed as the mean ± standard error of the mean. † Significance of difference vs. untreated group: p < 0.05.

valuable findings. There is no question that steroids are effective in reducing peritumoral brain edema both experimentally and clinically,^{11,12,21,26,44,45} while steroids have not been shown to be effective in human cerebral trauma,^{37} cerebral infarction,^{28} or cerebral hemorrhage.^{32} Possible mechanisms that have been discussed include a reduction of vascular permeability,^{6,17,29,30,34} a cerebrospinal fluid reduction effect,^{47} a metabolic effect, and an oncolytic effect. We discuss the latter more fully below. The prolonged use of high-dose steroid therapy is associated, however, with adverse reactions such as steroid myopathy, aseptic bone necrosis, osteoporosis, peptic ulcers, poor wound healing, onset of a cushingoid state, and impairment of the immune system.^{14,24,25,31,41,46,48} Therefore, the development of alternative methods to ameliorate peritumoral brain edema appears warranted.

It is with this in mind that we investigated the delivery of steroids locally as opposed to systemically. Local steroid delivery is an established treatment modality for a number of disorders, including arthritic problems, bursitis, and inflammatory conditions of the skin. Given available technology, there is no obvious reason to exclude the brain from the list of potential sites for the local delivery of steroids. Some authors^{22} have documented the efficacy of local chemotherapy in the treatment of malignant brain tumors. Moreover, one recent report demonstrated that polymer-mediated interstitial delivery of dexamethasone in the brain of rats implanted with the 9L gliosarcoma is associated with a reduction of both mean brain edema and mean tumor volume.^{43} This is an important study, and differs from the present in several respects.

Tamargo and colleagues^{42,43} used the 9L gliosarcoma, which is appropriate for modeling human primary brain tumors, whereas we have used the VX2 carcinoma, a model more appropriate for metastatic brain tumors. Tamargo, et al., measured aggregate brain water content as a proxy for brain edema, whereas we have measured brain water content at two gray-matter sites and two white-matter sites in the hemisphere ipsilateral to the tumor, as well as the corresponding sites in the contralateral hemisphere. This latter technique suggests that dexamethasone has different effects in these anatomically distinct tissues. Tamargo, et al., used polymer-mediated delivery of dexamethasone to brain-tumor beds, whereas we have used an osmotic pump and catheter to deliver dexamethasone into the tumor mass itself. The basic similarity of results despite the disparity of techniques is convincing evidence for the biological effectiveness of locally delivered dexamethasone, at least in the short term.

Clinical Implications of the Present Study

Different techniques elicit different results. However, Tamargo and colleagues^{42,43} have made important progress toward understanding the implications of tissue and plasma dosage in systemic versus local delivery of dexamethasone. The current study, however, suggests that brain edema is more consistently reduced in white-matter sites than in gray-matter sites with steroid therapy. It also demonstrates that, in the Day 3 treatment groups, local steroid delivery is more effective than systemic treatment in inhibiting tumor growth, as well as in reducing brain edema in the gray matter. Conversely, in the Day 7 treatment groups, systemic delivery is more effective than local treatment in inhibiting tumor growth and reducing brain edema in the white-matter sites, whereas reduction of edema was not observed in the gray matter after formation of a gross tumor mass in the present study. We believe this differential reduction in edema between gray and white matter and between Day 3 and Day 7 treatment groups has important implications for clinical practice, if the impact of dexamethasone can be demonstrated to be effective during longer courses of therapy.
In the clinical setting, brain edema is a problem usually associated with white-matter areas, so reduction of white-matter edema is paramount in this context. By this standard, both local and systemic drug delivery methods were effective in reducing brain edema in the Day 3 and the Day 7 treatment groups. Since results in the Day 3 treatment groups show that local delivery is more effective against both edema and tumor mass, local delivery would be the preferred strategy in cases where tumor cells can be presumed present prior to gross tumor formation. Moreover, there are clearly some patients with an existing tumor mass who would benefit more from a moderate reduction of edema and protection from steroid toxicity than from a more dramatic reduction of edema and possible severe steroid side effects. Generally, aggressive systemic treatment would seem to be indicated in such cases. We provisionally suggest, therefore, that the Day 3 treatment groups offer insight for the use of adjuvant steroid therapy after gross resection of a tumor mass, while correspondingly the Day 7 groups offer insight for primary therapy in situations where long-term steroid toxicity is not a concern.

Accordingly, we offer the following provisional rubric based on these results, which may help to focus future research questions. We do not, however, recommend it at this time as a basis for clinical practice.

Adjuvant steroid therapy should be local, and primary therapy should be systemic. If, however, the dominant objective of reduction of edema and avoidance of long-term steroid toxicity is important, there is a rationale for local delivery of steroids in primary therapy, although the results are not expected to be as dramatic as with the systemic modality.

In sum, our results suggest that aggressive early treatment is important for the decisive reduction of both brain edema and tumor volume with either systemic or local delivery. We conclude that local dexamethasone administration displays similar therapeutic effectiveness in the short-term to systemically administered dexamethasone. This conclusion is further reinforced in light of the finding that both systemic and local dexamethasone delivery result in a significant increase in survival relative to the untreated group. The precise reason for the increase in survival remains unclear; however, we assume it is related to the observed reduction in tumor volume.

Two Possible Effects of Dexamethasone

The lower tumor volumes found in all treatment groups may be an indirect cause of edema reduction. That dexamethasone should have this effect suggests that its biological mechanism is not limited to the well-established reduction of vasogenic edema, but that it also possesses a direct cellular action. The possible mechanisms whereby steroids may inhibit tumor growth have been considered in some previous studies.6-10,16,35 Sherbet, et al.76 confirmed the inhibitory effect of steroids on the growth of human astrocytoma cells in culture but also concluded that these results cannot be extrapolated to in vivo tumor growth. Freshney12 reported that glucocorticoids in vitro studies have a cytostatic effect on glioma cells that may be mediated via a membrane-altering cell-to-cell interaction. Tamargo and colleagues6-23 found that tumor volumes were reduced even in treatment groups in which the mean blood plasma level of dexamethasone was only 0.12 μg/ml, but which had mean brain tissue concentrations of 1.50 μg/gm. This is additional evidence for the existence of a direct cellular locus for the mechanism of dexamethasone. It remains for future studies to elucidate more clearly both the existence and the relative strengths of these two possible effects.

Further research is also desirable to investigate the brain tissue distribution and optimal therapeutic dose of locally delivered dexamethasone. It is acknowledged, moreover, that we have not positively demonstrated that local delivery of dexamethasone to brain tumors is successful in reducing the toxicity associated with systemic steroid therapy; measurement of serum cortisol in treated animals may help to elucidate this. We believe nonetheless that this study is a significant contribution, and has potential implications for clinicians involved in the treatment of brain tumor patients.

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References

Local dexamethasone in peritumoral brain edema

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