The effect of steroids on gentamicin delivery to brain after blood-brain barrier disruption

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Osmotic modification of the blood-brain barrier (BBB) provides an experimental model of vasogenic edema, is totally reversible, and does not cause any structural damage. In the present communication, the effect of corticosteroids on drug delivery to normal rat brain was evaluated in this model. Intraperitoneal dexamethasone was administered at doses ranging from 12 to 48 mg/sq m for 3 days; gentamicin delivery to the brain was then evaluated after either intravenous or intracarotid administration in both control and BBB-modified animals. Only animals receiving the highest dose of dexamethasone and in which the gentamicin was given intravenously demonstrated a statistically significant decrease in drug delivery. The effect of dexamethasone over a wide range of dosages, therefore, exhibited only modest effects on drug delivery to normal brain after osmotic BBB disruption.

KEY WORDS • blood-brain barrier • steroid • drug delivery • gentamicin • cerebral edema • dexamethasone • rat

Although corticosteroids remain a useful tool in the management of cerebral edema, their effects on normal endothelial vascular permeability or, more specifically, on drug delivery, are less clear. Mixed results have been reported from investigations into the ability of steroids to inhibit the penetration of drugs and contrast materials to normal and lesioned brain in a variety of experimental animal models that produce vasogenic cerebral edema. Vasogenic cerebral edema, by definition, involves an increase in the water content of brain that is induced by damage or breakdown of the blood-brain barrier (BBB) due to injury or simply by the absence of a BBB. While corticosteroids are minimally effective in modifying brain edema associated with trauma, vasogenic edema due to brain tumor and abscess is extremely responsive to them. Osmotic BBB modification provides a model of vasogenic cerebral edema in which the edema (approximately a 1.5% increase in brain water) is reversible and without morphological sequelae.

This communication reports the effect of the corticosteroid dexamethasone on gentamicin delivery to normal brain in conjunction with BBB modification. Also of interest was the evaluation of a dose-response relationship between the use of this corticosteroid and gentamicin delivery to normal brain after osmotic BBB modification.

Materials and Methods

Modification of the Blood-Brain Barrier

Adult female Sprague-Dawley rats, each weighing approximately 250 gm, were anesthetized with sodium pentobarbital (50 mg/kg). A catheter was placed in the right external carotid artery for retrograde infusion. Evans blue dye was administered intravenously in a 2% (w/v) solution (2 ml/kg) 5 minutes prior to BBB modification to evaluate the integrity of the BBB. Mannitol (25%, w/v) was used for modification of the BBB and normal saline was given for control studies. The procedure was performed as reported previously.

Steroid Treatment and Antibiotic Delivery to Normal Brain

The rats were treated with intraperitoneal dexamethasone (4 mg/ml) at 12, 24, or 48 mg/sq m/day (1.6, 3.2, or 6.4 mg/kg/day) for 3 days prior to study; normal saline was given to the control group. Gentamicin (5 mg/kg) was infused as an intracarotid or intravenous bolus (over a period of 30 seconds) 5 minutes after mannitol or saline administration. One hour after gentamicin infusion, a serum sample was collected and the rat was perfused with 0.9% NaCl to clear the vasculature of drug. The brain was removed and dissected into gray matter, white matter, and basal ganglia for drug quantitation.
TABLE 1
Gentamicin tissue concentrations 1 hour after intravenous versus intracarotid drug delivery in saline-infused steroid-treated rats*

<table>
<thead>
<tr>
<th>Steroid Dose (mg/sq m)</th>
<th>Contralateral</th>
<th>Ipsilateral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GM</td>
<td>WM</td>
</tr>
<tr>
<td>intravenous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>24</td>
<td>0.01</td>
<td>ND</td>
</tr>
<tr>
<td>48</td>
<td>0.12</td>
<td>0.10</td>
</tr>
<tr>
<td>intracarotid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>24</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>48</td>
<td>0.10</td>
<td>0.05</td>
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* Results are expressed as ug/gm brain. Gentamicin (5 mg/kg) was infused as an intravenous or intracarotid bolus (over 30 seconds) 5 minutes after saline infusion. One hour after gentamicin administration, the rat was perfused with 0.9% NaCl to clear the vasculature of drug and the brain was dissected into gray matter (GM), white matter (WM), and basal ganglia (BG). Rats were treated with intraperitoneal dexamethasone at 12, 24, or 48 mg/sq m (one rat for each group) for 3 days prior to study. ND = beyond analytical limits.

Gentamicin Assay

The radioimmunoassay for gentamicin was performed according to the manufacturer's instructions.* Samples were homogenized in the provided buffer, and an internal standard was prepared by adding gentamicin (15 ug/gm) to normal brain; the aliquots were frozen at -60°C until used. Results were analyzed after logit transformation, and a computer-assisted standard curve was prepared by plotting the logit transformation values against the known standards on a log-log scale. The interassay coefficient of variation was 5.6%. The range of the assay is such that tissue levels can be detected at 0.05 ug/gm.

Statistical Analysis

Mean values ± standard error of the mean were calculated in all instances to summarize the data unless otherwise indicated. The Student t-test for two means was calculated for appropriate groups of data. The one-way analysis of variance for two group means was used to compare multiple groups in which the overall difference was found to be statistically significant.

Results

Rats that had been pretreated with dexamethasone at 12, 24, or 48 mg/sq m/day for 3 days and who then received intracarotid saline and subsequent intravenous or intracarotid gentamicin (5 mg/kg) had no major differences (Table 1) in either hemisphere, irrespective of route of administration or steroid treatment.

* Gentamicin supplied by Diagnostic Product Inc., Los Angeles, California.
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FIG. 2. Gentamicin concentrations (µg/gm) in various brain tissues of rats pretreated with dexamethasone and receiving intracarotid gentamicin after blood-brain barrier modification. The animals were pretreated with dexamethasone at 0, 12, 24, or 48 mg/sq m/day for 3 days prior to study. Gentamicin was administered as an intracarotid bolus 5 minutes after intracarotid infusion of mannitol (0.12 ml/sec/30 sec). The animals were sacrificed 1 hour later and the brain was dissected for study into gray matter (open bar), white matter (diagonally hatched bar), and basal ganglion (horizontally hatched bar).

There was no significant difference in mean serum drug levels in the intravenous and intracarotid drug delivery groups at any steroid level, with mean concentrations for these groups being 12.0 ± 3.1 and 20.5 ± 8.6 µg/ml, respectively.

Discussion

A common class of drugs in the treatment of lesions of the central nervous system that cause vasogenic cerebral edema is corticosteroids. Brain abscess is one such lesion upon which steroids, aside from their ability to decrease edema, have shown multiple effects in a variety of animal studies. Steroids can inhibit capsule formation, increase viability of bacteria, inhibit the inflammatory response, and ultimately decrease host survival. In a previous report from this laboratory, it was shown that steroids had little if any effect on gentamicin delivery even at a very high steroid dose (96 mg/sq m). Analogously, it has been shown in the present brain-tumor model that steroids only minimally decrease delivery of a chemotherapeutic agent (methotrexate) to tumor except after osmotic BBB modification. High-dose dexamethasone in the same study only modestly reduced methotrexate delivery to brain adjacent to tumor and brain distant to tumor (or normal brain) with or without osmotic BBB modification.

The results presented in this report correlate well with the above studies. They show only a modest decrease in delivery of gentamicin to normal brain after osmotic BBB modification despite high doses of dexamethasone. Any reduction in gentamicin concentration due to efflux of drug from brain at this time point is unlikely, since gentamicin, like methotrexate (efflux half time (t1/2) 4.8 hours), is ionized and metabolism is not a major factor. The current studies are unique in that they try to evaluate a dose-response relationship in a model of vasogenic edema. With intravenous gentamicin administration the decrease in drug delivery was more prominent and was statistically significant at a dexamethasone dose of 48 mg/sq m. On the other hand, after intracarotid gentamicin administration the effects of dexamethasone on drug delivery were less obvious, even at this high steroid dose. The reason for this is unclear.

In conclusion, the effect of dexamethasone over a wide range of dosages has only a modest effect on drug delivery to normal brain after osmotic BBB opening, irrespective of the route of administration. Yamada and coworkers have recently reviewed the effects of steroids on the BBB. As they point out, steroids can have a dramatic effect on brain edema due to tumor and abscess but have only a modest effect on edema from head injury or cerebral ischemia. As indicated in that review, the mechanism by which steroids modify capillary permeability in the production of brain edema and, thereby, the development of vasogenic cerebral edema remains unknown. They hypothesize that the steroid receptor in the glia and capillary endothelium may have an important role in the modification of capillary permeability. The current report provides a model of reversible vasogenic edema in which drug delivery is only modestly decreased after high-dose steroid administration. Klatzo has suggested that available data would be consistent with the concept that dexamethasone increases the resolution of cerebral edema rather than its rate of development. Indeed, the data in the current communication and in our previous studies of brain tumor and brain abscess would be consistent with that suggestion.

References


Manuscript received November 16, 1988. Accepted in final form July 17, 1989.

These studies were supported by the Veterans Administration, National Institutes of Health Grant CA31770, and the Preuss Foundation.

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