Cerebrovascular permeability and delivery of gentamicin to normal brain and experimental brain abscess in rats

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Antibiotics vary widely in their ability to penetrate the blood-brain barrier. In studies of 70 rats, the permeability of the normal blood-brain barrier to gentamicin was shown to be poor. In experimental brain abscesses, during the cerebritic stage of development, the penetration of intravenous antibiotics was increased compared to normal brain but was very inconsistent. Antibiotic delivery to brain abscess was not significantly altered with the administration of high-dose steroids, but the macrophage and glial response was markedly decreased with high-dose steroid therapy. Reversible osmotic blood-brain barrier modification with mannitol increased the delivery of gentamicin both to brain abscess and to the surrounding brain. It also resulted in more consistent tissue drug levels. The clinical implications of these studies suggest that, because of the inconsistent delivery of gentamicin to brain abscess, the therapeutic efficacy of medical management alone may be quite variable. This mode of therapy could possibly increase the efficacy of medical management of brain abscesses, especially in patients with multiple or surgically inaccessible brain abscesses.

KEY WORDS • blood-brain barrier • experimental brain abscess • gentamicin • mannitol antibiotic delivery

DESPITE significant advances in surgical and chemotherapeutic treatment, the overall mortality rate associated with brain abscess over the last seven decades has been high, ranging from 30% to 70%.1,4,15,18,24,25,27 Surgical drainage or excision was the standard treatment of brain abscess, until it was augmented with antibiotic therapy on the introduction of antibiotics in 1940. The use of antibiotics has changed but not decreased the incidence of brain abscess, and it has decreased the mortality rate only moderately.4,18,24,25,36,40 The mortality rate appears to be more intrinsically related to the mass effect, improper identification of the offending pathogens, and failure to identify rapidly and to localize accurately the brain abscess.15,27,35

With the advent of computerized tomography (CT), the mortality rate associated with brain abscess has diminished.3,34,44 This mode of examination allows for earlier diagnosis, precise localization, identification of associated cerebral edema, and CT-guided aspiration of the brain abscess. A regimen that has met with success in selected cases with a known bacteriological diagnosis has been the use of corticosteroids and antibiotics in place of neurosurgical intervention.5,8,34,35 Many clinicians involved with the treatment of brain abscess are convinced that most brain abscesses in the cerebritic stage of development may be treated with antibiotics alone if the offending pathogen is known.5,34,35 Such a nonsurgical method of treatment is of particular importance in cases of surgically inaccessible and multiple abscesses.3,8,21,34,35 The precise criteria as to which lesions will respond to a particular type of therapy, be it treatment with antibiotics alone (with or without steroids), surgical therapy (aspiration as opposed to excision), or a combination of these, have not been established.

Antibiotics vary widely in their ability to penetrate the blood-brain barrier (BBB).22,43 However, when the normal histology of the central nervous system (CNS) is altered, such as with brain abscesses, the permeability of the BBB is increased.2,14 The mechanism and degree of this increase in permeability is uncertain, and techniques that reversibly modify the BBB may be necessary to increase the permeability of water-soluble antibiotics.

In the present study, we report experiments with rats
Experimental brain abscess

in which the PA (product of cerebrovascular permeability and capillary surface area) was determined for a water-soluble antibiotic, gentamicin, both for an intact and for an osmotically altered BBB in the normal rodent brain. Studies were then performed in the rat to determine the antibiotic delivery of gentamicin to brain abscesses during the cerebritic stage of development. High-dose steroids were also studied with respect to their effects on antibiotic delivery and the histological character of the inflammatory response. Finally, experiments were conducted to evaluate the effect of osmotic BBB modification on gentamicin delivery to a rat model of brain abscess.

Materials and Methods

Modification of the Blood-Brain Barrier

Adult female Sprague-Dawley rats, each weighing 250 to 300 gm, were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg). Catheters were placed in the femoral vein and artery and the right external carotid artery for retrograde infusion. Evans blue dye was administered intravenously in a 2% solution (2 ml/kg) 5 minutes prior to barrier modification to evaluate the integrity of the BBB. Mannitol (25%) was used for modification of the BBB, and normal saline for control studies. The procedure was performed as reported previously. The cerebrovascular permeability of gentamicin was examined under three different experimental conditions using the technique of Ohno, et al. In Group 1, three animals received an intravenous bolus of gentamicin (5 mg/kg) 5 minutes after the intravenous infusion of Evans blue dye (2%, 2 ml/kg). The control animals were not perfused with either saline or mannitol. Subsequently, 0.2 ml of femoral arterial blood was collected at 1-minute timed intervals for 10 minutes, after which the animals were sacrificed by barbiturate overdose. The brains were removed and dissected into gray matter, white matter, and basal ganglion. The brain homogenates were then assayed for determination of gentamicin concentration.

In Group 2, gentamicin (5 mg/kg) was administered to three animals as an intravenous bolus 10 minutes after intravenous administration of Evans blue dye, and 5 minutes after intracarotid infusion of either mannitol or saline. Femoral arterial blood samples were obtained, and the animals sacrificed. The brains were dissected as described above.

In Group 3, six rats received gentamicin as an intracarotid bolus (5 mg/kg), again 10 minutes after intravenous Evans blue dye infusion and 5 minutes after intracarotid infusion of either mannitol or saline. Samples were collected as described above. At sacrifice, all animals were evaluated for Evans blue staining in the cerebral hemispheres and quantitated as described previously. Only those animals that displayed Grade 2+ or 3+ staining (over 80%) after BBB modification with mannitol were used for further study. Binding of gentamicin to blood proteins was determined to be 10% by passing iodine-125-labeled gentamicin incubated with rat serum over a G-25 Sephadex column.

Calculations

The determination of cerebrovascular permeability in BBB-modified and control brains to gentamicin were evaluated by the method described by Ohno, et al. The cerebrovascular permeability of gentamicin is described as,

\[ \frac{dC_{\text{brain}}}{dt} = 0.9 \times \left( PA \right) \times \text{Cart}, \]  

where \( PA \) (sec \(^{-1}\)) = cerebrovascular permeability \( P \) (cm \( \cdot \) sec \(^{-1}\)) \times capillary surface area \( A \) (cm\(^2\) \cdot gm\(^{-1}\) brain, or cm\(^{-1}\)), \( \text{Cart} \) (\( \mu \)g/ml) = femoral artery plasma concentration, \( C_{\text{brain}} \) (\( \mu \)g/gm) = brain parenchymal concentration, 0.9 = fraction of unbound gentamicin in plasma, and \( t = \) time. Equation 1 can be integrated to time of death, \( T \), to give PA in terms of the plasma concentration integral:

\[ PA = \frac{C_{\text{brain}} (T)}{0.9 \times \int_0^T \text{Cart} \, dt}. \]  

Brain Abscess Production

Preparation of Microorganisms. The bacterium used was a strain of *Escherichia coli* that was isolated from a blood culture in the bacteriology laboratory of the Dallas Veterans Administration Medical Center. The method of preparation of the bacterial inoculum was adapted from the technique described by Enzmann, et al., and Winn, et al.

On the day of animal inoculation, 3 ml of a lightly inoculated trypticase soy broth culture grown for 18 hours statically at 35°C was centrifuged at 3000 rpm for 15 minutes. The broth was discarded and the bacterial pellet was resuspended in 2 ml of sterile normal saline. The suspension was centrifuged again at 3000 rpm, the supernatant discarded, and the pellet resuspended with 2 ml of normal saline. From this suspension, a 1:40 dilution was prepared using normal saline as the diluent. This suspension contained approximately \( 10^4 \) colony-forming units (CFU/ml), measured with standard microbiological techniques. Each animal was inoculated with 1 ml of this suspension containing \( 10^5 \) CFU. The minimal inhibitory concentration (MIC) and minimal bacteriocidal concentration (MBC) of gentamicin for this strain of *Escherichia coli* was determined by standard broth dilution methods.

Surgical Procedure. A total of 35 adult female Sprague-Dawley rats, each weighing 250 to 300 gm, were injected intracerebrally according to the method described by Winn, et al. The rats were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg)

\* G-25 Sephadex column manufactured by Pharmacia Fine Chemicals, 800 Centennial Avenue, Piscataway, New Jersey.
and placed in a stereotaxic frame,† and the top of the head was shaved with electric shears. A 2-cm midline incision was made to expose the frontal bone where a 2-mm burr hole was made just posterior to the coronal suture and 4 mm lateral to the sagittal suture line. A 10-μl syringe with a No. 30 needle was fitted to a micromanipulator.‡ The needle was lowered just enough to pierce the dura and then slowly lowered (over 10 minutes) to a depth of 2.5 mm into the white matter of the frontal lobe. After positioning, 1 μl of normal saline containing a known amount of bacteria was inoculated over 30 minutes using a Burleigh Inchworm injection device.§ The needle was withdrawn slowly over 10 minutes, and the wound was irrigated with normal saline. The burr-hole was covered with bone wax and the skin was closed in a single layer. In four control animals, 1 μl of sterile normal saline was inoculated using the above procedure.

Antibiotic Delivery to Brain Abscess. Experiments examining the delivery of gentamicin to the brain abscess were carried out under four different conditions. Six days after bacterial inoculation, all animals were anesthetized, and Evans blue dye was administered as described earlier. Animals were sacrificed 1 hour after drug administration, serum was collected, and the brains were dissected into four different regions: the abscess (2 to 3 mm in diameter), brain around the abscess (2 to 3 mm of brain around abscess), brain distant from the abscess (occipital pole of cerebrum), and the contralateral hemisphere (occipital pole of cerebrum).

In this section of the study, 10 Group 1 animals were given intravenous gentamicin (5 mg/kg, over 30 seconds). Ten Group 2 rats received dexamethasone (96 mg/sq m/day in two divided doses) for 3 days, starting 3 days after bacterial inoculation, and then were given intravenous gentamicin. Four Group 3 (sham-operated) animals were inoculated with sterile saline and received intravenous gentamicin on the day of study. In Group 4, 6 days after bacterial inoculation, 10 animals underwent BBB modification with mannitol and were then given intracarotid gentamicin (5 mg/kg, over 30 seconds) 5 minutes after mannitol infusion. In five control animals, 0.9% NaCl instead of mannitol was infused and intracarotid gentamicin was then administered.

Gentamicin Assay. The radioimmunoassay (for gentamicin) was performed according to the manufacturers’ instructions.¶ Rat brains were homogenized (1:10 weight:volume) in Tris buffer (0.05 M) with a Polytron PT-10 homogenizer* for 30 seconds. An internal standard was prepared by adding gentamicin (15 μg/gm) to normal brain and liver tissue homogenates, and the aliquots of standard were frozen at -60° until used. As further controls, spiked tissue homogenates of cerebritic and normal brain were assayed to evaluate differences in assay characteristics. The gentamicin recovered was quantitated and was equal with both tissues.

Results were analyzed after logit transformation as follows:

\[
\ln \left( \frac{B/B_0}{1 - B/B_0} \right)
\]

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 5.8%. The range of the assay is such that tissue levels can be detected at 0.05 μg/gm.

Statistical Analysis. Mean values ± the standard error of the mean (SEM) 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 utilized to compare multiple groups, followed by multiple Winer comparisons, in which the overall difference was found to be statistically significant.45

Macroscopic and Microscopic Study

All brains underwent macroscopic evaluation and were observed for swelling, midline shift, discoloration, and areas of Evans blue staining. A coronal section was made through the inoculation site in the appropriate

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† Stereotaxic frame manufactured by David Kopf Instruments, 7324 Elmo Street, Tujunga, California.
‡ Micromanipulator manufactured by David Kopf Instruments, 7324 Elmo Street, Tujunga, California.
¶ Radioimmunoassay kit manufactured by Diagnostic Products Corp., 5700 West 96th Street, Los Angeles, California.
* Polytron PT-10 homogenizer manufactured by Brinkman Instruments Co., Cantiague Road, Westbury, New York.
Experimental brain abscess

TABLE 2

Gentamicin tissue concentrations for intravenous versus intracarotid bolus administration in control and barrier-modified rats*

<table>
<thead>
<tr>
<th>Mode of Gentamicin Administration</th>
<th>No. of Rats</th>
<th>Left Hemisphere</th>
<th>Right Hemisphere</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GM</td>
<td>WM</td>
<td>BG</td>
</tr>
<tr>
<td>intracarotid saline</td>
<td>3</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>intravenous bolus</td>
<td></td>
<td>0.03 0.03</td>
<td>0.01 0.01</td>
<td>0.05 0.05</td>
</tr>
<tr>
<td>intracarotid bolus</td>
<td></td>
<td>0 0</td>
<td>0.01 0.01</td>
<td>0 0</td>
</tr>
<tr>
<td>intracarotid mannitol</td>
<td></td>
<td>0 0</td>
<td>0.01 0.01</td>
<td>0 0</td>
</tr>
</tbody>
</table>

* Gentamicin (5 mg/kg) was administered as an intravenous or right intracarotid bolus (over 30 seconds) 5 minutes after infusion of either isotonic saline or mannitol 25% (0.12 ml/sec for 30 seconds) into the right internal carotid artery. The animals were sacrificed after 10 minutes and the brains divided into left hemisphere (unperfused) and right hemisphere (perfused), and then sectioned for drug quantitation (gray matter (GM), white matter (WM), and basal ganglion (BG)). Mean ± standard error of the mean values are expressed as µg/gm tissue or µg/ml of serum. Tissue values are corrected for intravascular drug activity.
† Statistically significant increase (p < 0.05) from intracarotid compared to intravenous administration.

Results

Cerebrovascular Permeability of Gentamicin in Normal Rats

In saline-perfused rats as well as in uninfused rats (three rats each), the cerebrovascular PA was near 0 for both hemispheres. This indicated that gentamicin does not permeate a normal barrier.

In three barrier-modified animals, the mean PA for the perfused hemisphere was 15.3 ± 4.2 × 10⁻³ sec⁻¹ as compared to 0.02 ± 0.02 × 10⁻³ sec⁻¹ in the contralateral hemisphere (Table 1). When PA's from the mannitol-perfused hemisphere were compared to the saline-perfused hemisphere, there was an elevation in brain permeability from near 0 to 15.

Tissue Levels of Gentamicin in Normal Rat Brain

Table 2 presents gentamicin tissue concentrations in rats injected with intravenous or intracarotid gentamicin (5 mg/kg) 5 minutes after intracarotid infusion of mannitol or saline (0.12 ml/sec, for 30 seconds). All animals were sacrificed 10 minutes after drug administration.

In three animals given intravenous gentamicin, the mean concentration in the mannitol-perfused hemisphere was 1.35 ± 0.36 µg/gm, while that in three animals given intracarotid drug was 3.11 ± 0.95 µg/gm. In both groups the unperfused contralateral hemispheres showed no appreciable differences in mean concentrations. The ratio of gentamicin concentrations at 10 minutes in perfused hemispheres from intracarotid versus intravenous routes of administration indicated that delivery of drug to brain was augmented two- to fivefold by intracarotid gentamicin administration. As described by Ohno, et al., a cerebral blood volume of 2% was taken into account and brain concentrations were corrected for intravascular drug activity.

Gentamicin Brain Levels in Rats with Experimental Brain Abscess

Tables 3 and 4 represent the mean gentamicin concentrations in tissue and serum determined 1 hour after gentamicin (5 mg/kg) was infused as an intravenous bolus in rats 6 days post-inoculation with 1 µl of bacteria (10⁵ CFU) or sterile saline. In the four saline-injected (noninfected) animals (Table 3), the mean concentration in the mannitol-perfused hemisphere was 1.35 ± 0.36 µg/gm, while that in three animals given intracarotid drug was 3.11 ± 0.95 µg/gm. In both groups the unperfused contralateral hemispheres showed no appreciable differences in mean concentrations. The ratio of gentamicin concentrations at 10 minutes in perfused hemispheres from intracarotid versus intravenous routes of administration indicated that delivery of drug to brain was augmented two- to fivefold by intracarotid gentamicin administration. As described by Ohno, et al., a cerebral blood volume of 2% was taken into account and brain concentrations were corrected for intravascular drug activity.
Effect of BBB Modification on Antibiotic Delivery

Gentamicin level at the site of inoculation was 0.23 ± 0.03 µg/gm and was not significantly different from that in the other brain samples. In 10 bacteria-injected nonsteroid-treated animals, there was a marked increase in gentamicin uptake at the site of the abscess (Table 4), that being 1.41 ± 0.29 µg/gm. Serum and liver gentamicin levels were not significantly different between the two groups.

In comparing 10 steroid- versus 10 saline-treated animals with abscesses, there were no appreciable differences at the site of the abscess (Table 4). There was, however, a significant increase (p < 0.05) in gentamicin levels in brain distant from abscess in steroid-treated animals as compared to nonsteroid-treated rats. There were no significant differences in the contralateral hemisphere, liver, or serum.

There was marked variability of gentamicin delivery to abscesses of infected animals, irrespective of steroid administration (Fig. 1). Gentamicin concentrations ranged from 0.61 to 3.65 µg/gm in the nonsteroid-treated animals and from 0.46 to 3.91 µg/gm in the steroid-treated animals. The MIC and MBC of gentamicin for the Escherichia coli bacteria tested were both 0.5 µg/ml.

Effect of BBB Modification on Antibiotic Delivery to Brain Abscess

Gentamicin concentrations were measured in four different brain regions: the abscess, the brain around the abscess, the normal-appearing brain far from the abscess and the contralateral (left) hemisphere after osmotic BBB modification. The abscess-bearing rats tolerated the procedure well with a mortality rate of less than 5%. Figure 2 compares the gentamicin concentrations for the four different brain regions of interest. After osmotic barrier modification, both intravenous and intracarotid gentamicin administration groups showed drug levels in all three regions of the ipsilateral abscess-bearing hemisphere that were significantly higher when compared to the nonperfused and saline-infused control animals. As shown in Fig. 2, there was approximately a two-fold increase in gentamicin brain concentrations (C) seen between the mannitol-infused intravenous gentamicin group (Group III) and the mannitol-infused intracarotid gentamicin group (Group IV). Contralateral hemisphere levels (D) were not markedly different under any of the four treatment regimens described.

Effects of Mannitol on Serum Gentamicin Concentration

The serum gentamicin level after 1 hour was increased in both the groups infused with mannitol as compared to the saline-infused animals (Table 5). This increase in serum gentamicin correlated with the diuretic effect of intra-arterial mannitol as reported before. To provide a control group for this diuretic effect, osmotic barrier modification was performed in the nonabscess-bearing (left) hemisphere, thereby reproducing the diuretic effect of the mannitol on the serum gentamicin level without altering the BBB of the abscess. The concentration of gentamicin in the abscess of animals in which the BBB was opened in the abscess-bearing (left) hemisphere was markedly higher than in the animals in which the barrier was opened on the contralateral (left) side (Table 5), despite similar serum levels of gentamicin in both groups. Blood-brain barrier modification therefore results in an increased delivery of gentamicin to the abscess, brain around the abscess, and brain far from the abscess, and this increase is

<table>
<thead>
<tr>
<th>Source Tested</th>
<th>Saline-Treated Group</th>
<th>Steroid-Treated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>abscess</td>
<td>1.41 ± 0.29</td>
<td>1.70 ± 0.42</td>
</tr>
<tr>
<td>brain around abscess</td>
<td>0.60 ± 0.15</td>
<td>0.75 ± 0.22</td>
</tr>
<tr>
<td>brain distant to abscess</td>
<td>0.32 ± 0.06</td>
<td>0.66 ± 0.11†</td>
</tr>
<tr>
<td>contralateral hemisphere</td>
<td>0.29 ± 0.07</td>
<td>0.50 ± 0.32</td>
</tr>
<tr>
<td>liver</td>
<td>1.30 ± 0.19</td>
<td>1.65 ± 0.42</td>
</tr>
<tr>
<td>serum</td>
<td>3.30 ± 0.38</td>
<td>3.51 ± 0.71</td>
</tr>
<tr>
<td>no. of rats</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

* Gentamicin (5 mg/kg) was infused as an intravenous bolus in rats inoculated with 1 µl of bacteria (10⁶ colony-forming units) at 6 days post-inoculation. Mean gentamicin values ± standard error of the mean were measured 1 hour after administration and are expressed in µg/gm of tissue or µg/ml of serum. Tissue values were not corrected for intravascular drug. Dexamethasone (96 mg/sq m/day, in two divided doses) was administered intraperitoneally 2 to 3 days prior to the day of study. Non-steroid treated rats were given intraperitoneal saline.

† Statistically significant increase (p < 0.05) as compared to the corresponding non-steroid group.
Experimental brain abscess

![Graph](image)

Fig. 2. Gentamicin concentrations (µg/gm) in brain tissues of 25 abscess-bearing rats, in the abscess (A), in brain around the abscess (B), in brain far from the abscess (C), and in the cerebral hemisphere contralateral to the abscess (D). Four different treatment regimens were used: I, intravenous gentamicin in 10 rats; II, intracarotid saline then intracarotid gentamicin in five rats; III, intracarotid mannitol then intravenous gentamicin in five rats; IV, intracarotid mannitol then intracarotid gentamicin in five rats. Gentamicin (5 mg/kg) was infused as a 30-second bolus either through the right femoral vein or into the right internal carotid artery catheter, beginning 5 minutes after intracarotid infusion of saline (Group II) or mannitol (Groups III and IV). Animals were sacrificed 1 hour after the infusion of gentamicin. There were statistically significant (p < 0.05) increases in Groups III and IV when compared to control treatment Groups I and II. Also, Group IV levels were significantly increased over Group III levels except in the cerebral hemisphere contralateral to the abscess. Values in the ipsilateral and contralateral hemispheres were not corrected for intravascular drug.

### Table 5

<table>
<thead>
<tr>
<th>Source Tested</th>
<th>Saline, Ipsilateral</th>
<th>Mannitol, Ipsilateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>abscess</td>
<td>1.59 ± 0.45</td>
<td>1.52 ± 0.40</td>
</tr>
<tr>
<td>brain around</td>
<td>0.95 ± 0.18</td>
<td>1.19 ± 0.25</td>
</tr>
<tr>
<td>brain distant</td>
<td>0.69 ± 0.19</td>
<td>0.90 ± 0.26</td>
</tr>
<tr>
<td>non-abscess</td>
<td>0.23 ± 0.06</td>
<td>0.56 ± 0.90</td>
</tr>
<tr>
<td>bearing (left)</td>
<td></td>
<td>0.98 ± 0.19†</td>
</tr>
<tr>
<td>serum</td>
<td>6.35 ± 1.56</td>
<td>17.4 ± 1.70†</td>
</tr>
</tbody>
</table>

* Gentamicin (5 mg/kg) was infused into the internal carotid artery as a 30-second bolus, beginning 5 minutes after saline or mannitol, and the animals were sacrificed 1 hour later. Mean gentamicin values ± standard error of the mean are expressed in µg/gm of tissue or µg/ml of serum. Tissue values were not corrected for intravascular drug. Ipsilateral = abscess-bearing (right) hemisphere; contralateral = non-abscess-bearing (left) hemisphere.

† A statistically significant increase (p < 0.05) over the control (saline-treated) group.

**Morphological Effects of Steroids on Cerebral Abscesses**

Seven rats injected with bacteria were examined morphologically. Rats that were pretreated with dexamethasone showed large foci of necrosis which extended from the injection site into the cerebral hemisphere. Figure 3 upper shows that the ventricular system was filled with cells that were identified at higher magnification as polymorphonuclear cells. The abscess as demonstrated in Fig. 3 lower consisted of a central region of acute necrotic debris and degenerated polymorphonuclear cells. The neuropil adjacent to the abscess was vacuolated, and polymorphonuclear cells were scattered throughout the edematous neuropil.

In contrast, rats that were not pretreated with dexamethasone (Fig. 4 upper) showed abscesses of similar size, but few developed ventriculitis. Microscopically, the acutely necrotic centers were filled with polymorphonuclear cells, but the regions adjacent to the less edematous neuropil were filled with macrophages (Fig. 4 lower). Examination of routine H & E sections as well as sections stained for GFAP demonstrated that reactive glial cells were present only in the neuropil adjacent to abscesses in the nonsteroid-treated rats. Connective tissue proliferation was absent in both groups of animals.

**Discussion**

Gentamicin is a water-soluble aminoglycoside antibiotic that has been used successfully for the last decade in the treatment of Gram-negative bacillary infections. Little is known about the penetration of gentamicin into brain tissue, but it is generally thought to be poor.¹⁴ No data either clinically or experimentally have been published regarding gentamicin levels in normal brain. However, gentamicin levels obtained from aspirated brain abscesses in patients who were given a normal systemic dose (1 to 1.5 mg/kg/dose) have been reported to range between 0.5 and 1.0 µg/ml.¹⁴ Gentamicin was chosen for evaluation in our model not because it is the best agent for the treatment of brain abscesses, but because it is a very effective agent against Gram-negative pathogens, crosses the BBB poorly, and can be easily assayed. As expected, in our study gentamicin was found to penetrate the normal
brain poorly if at all, as quantitated by its PA (permeability × capillary surface area) value determined by the method of Ohno, et al.32 This low PA value corresponds to other comparative studies demonstrating the poor permeability of water-soluble substances such as methotrexate and carbon-14 (14C)-sucrose across the normal BBB for which the PA values are 3.6 × 10⁻³ sec⁻¹ and 1.1 × 10⁻⁵ sec⁻¹, respectively.32 The PA values were not determined in the rat abscess model because of the unknown variables, such as blood flow and blood volume.

Clinically more pertinent is the actual antibiotic levels of gentamicin obtained in the various areas of the brain. As expected, from the low PA value and the clinical literature, gentamicin levels in normal brain tissue are approximately 1% to 10% of the serum concentration. Since gray matter has a greater vascularity than white matter, gentamicin levels were somewhat higher in the gray matter as compared to the white matter. Tissue levels of gentamicin in the basal ganglia were lower than in the white matter.

The value of augmenting drug delivery to the normal brain using osmotic barrier modification was then evaluated. The elevation in the PA of gentamicin from near 0 to 15 following osmotic BBB modification corresponds to findings in other reported studies using identical experimental conditions but different water-soluble agents, such as 14C-sucrose and methotrexate.32 Drug delivery can be further improved using the intracarotid route of administration. In our study, intracarotid gentamicin was augmented two- to fivefold after 10 minutes in cerebral regions supplied by the perfused internal carotid artery, compared with gentamicin delivered intravenously. Contralateral regions of either the saline- or mannitol-infused animals were not appreciably augmented (Table 2). Thus, intracarotid gentamicin infusion after osmotic barrier modification can augment brain uptake by a factor of 30 to 45 as compared to uptake with intravenous gentamicin infusion in normal unaltered brain.

In the second part of our study, experiments were performed in the rat to determine the antibiotic delivery
of gentamicin to brain abscess during the cerebritic stage of development and to determine what effects high-dose corticosteroids have on altering the effect of this delivery. Gentamicin levels in the abscess (cerebritic stage) were markedly increased as compared with normal brain. Even so, despite the fact that we were able to produce what we believe was a controlled and homogeneous model, gentamicin tissue levels varied widely. The decreased gentamicin level at the abscess-brain interface as compared to the abscess center is also pertinent. This observation concurs with studies performed with experimental brain tumors in which a marked variability in drug uptake in tumor as well as a decrease in drug level at the tumor periphery has been reported. Walker and Weiss posed a “sink effect” to explain the lower concentration of drug at the tumor periphery; that is, the drug that enters the periphery of the tumor via an altered BBB, rapidly diffuses into the drug-free surrounding brain.

The marked variability in gentamicin delivery in our rat brain abscess model also confirms a few uncontrolled clinical studies that noted the variability of antibiotic delivery to human brain abscesses after parenteral administration. In the current report, although the abscess levels were variable, they averaged around 1.5 μg/gm of tissue for a 5-mg/kg dose of gentamicin, which is approximately three times the normal single intravenous dose for humans (1 to 1.5 mg/kg). However, since the adult rat has five to six times the surface area per kilogram of weight than does an adult human, the dose used in the current report was not excessive (35 to 40 mg/sq m for both). The concentration of gentamicin at the abscess site might then be on the low end for an adequate in vitro bacteriocidal concentration, which is 0.5 to 5 μg/ml for most susceptible organisms. High brain drug levels may be necessary since, as demonstrated by Strausbaugh and Sande, in order to kill bacteria in the CNS, antibiotic levels often need to be 10 to 30 times the MBC.

One recent report showed that short-term treatment with corticosteroids in dogs with experimental brain abscess will reduce the degree of contrast enhancement shown on CT in the cerebritic stage of abscess development. Later stages of encapsulation were unaffected. It was suggested from that study that, since the degree of enhancement (increased CT number) can be a reliable indicator of BBB breakdown, the reduction in CT number seen with the steroid-treated animals might reflect lowered antibiotic penetration at the inflammatory site. In a limited study by Klinger, et al. dexamethasone therapy in a cat brain abscess model decreased delivery of a cephalosporin (Cefazedon) to brain and cerebrospinal fluid. Even so, these same investigators advocated a course of steroids based on a review of 73 brain abscess patients at their institution.

In the present report, no significant difference in gentamicin delivery to brain abscess (cerebritic stage) was observed between animals that were pretreated with corticosteroids and those that were not. However, there was a statistically significant increase in gentamicin levels in brain distant from the abscess in the steroid-treated animals. This observation might suggest that the steroid-treated animals were not able to limit the spread of infection as compared to the nonsteroid-treated animals, thus causing infectious cerebritic spread and further breakdown of the BBB. This hypothesis is consistent with our neuropathological studies which showed that steroids markedly inhibit the macrophage and glial response to infection. However, steroids are more lympholytic in rodents than in man, and this steroid dose was a high clinical dose.

Finally, the role of osmotic BBB modification in gentamicin delivery to brain abscess was evaluated. There was a marked and consistent increase in the delivery of gentamicin to the abscess and brain surrounding the abscess after BBB modification with intracarotid mannitol. Associated with this increase in drug delivery was a concomitant increase in the gentamicin serum level due to an osmotic diuresis caused by the intracarotid mannitol. However, as shown in Table 5, it is the BBB modification and not the diuretic-induced elevation of serum gentamicin concentration that is responsible for most of the increased antibiotic delivery to the abscess and surrounding brain. After osmotic BBB modification, gentamicin levels in both the abscess and the surrounding brain are not only markedly increased but also more consistent than after intravenous administration alone.

The consistency and increased drug levels reported in these studies suggest that osmotic BBB opening may have a role in the treatment of brain abscesses. However, further pharmacological studies, such as on the half-life of the active drug in abscesses and brain around abscesses, as well as survival and efficacy studies are needed to evaluate the role that osmotic BBB opening may have in the treatment of brain abscess. Increasing drug delivery does not solve the problem that aminoglycosides are most active under conditions normally not present in brain abscess, including an alkaline pH, and an elevated pO2. Nor does it solve the problem that purulent tissue can bind gentamicin and other antibiotics rather firmly. Increasing drug delivery may prove to be efficacious, particularly in multiple and surgically inaccessible brain abscesses, because osmotic BBB opening is tolerated fairly well clinically.

Addendum

Another report on the effects of steroid therapy on brain abscesses in the rat in regard to survival and bacterial growth, including detailed pathological correlations, will be published soon (Neuwelt EA, Lawrence MS, Blank NK: The effect of gentamicin and dexamethasone on the natural history of the rat E. coli brain-abscess model with histopathological correlations. Neurosurgery 15, In press, 1984).
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