A new model of systemic drug rescue based on permeability characteristics of the blood-brain barrier in intracerebral abscess-bearing rats

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The authors report the results of their investigation of a systemic drug rescue method using antibody to a therapeutic drug based on the differential permeability of the blood-brain barrier to low- and high-molecular-weight compounds. Rats bearing intracerebral abscesses initially received systemic [125I]-gentamicin (M, 462) followed 60 minutes later by specific gentamicin antiserum (immune group) or normal rabbit serum (nonimmune group). Animals receiving antigentamicin immunoglobulin G (IgG, M, 150,000) demonstrated 90% binding of serum gentamicin. Immunoprecipitation of serum samples with protein A-Sepharose demonstrated that the increased binding of gentamicin in immune animals was due to antigen binding with antigentamicin IgG. By contrast, the percent of gentamicin bound to antibody within the brain did not differ between immune and nonimmune groups, implying that high-molecular-weight IgG was excluded from the brain and brain lesion. Significant differences in gentamicin deliveries were noted in the abscess, brain around the abscess, brain distant from the abscess, and normal brain areas. In contrast, analysis of comparable brain areas demonstrated no significant differences in gentamicin delivery between immune and nonimmune animals, indicating that the systemic presence of gentamicin antibody did not alter central nervous system delivery of unbound drug. These findings suggest the possibility of a drug rescue method using antidrug IgG, based on the differential permeability of the blood-brain barrier to drug versus antibody. While the present work was developed in a brain abscess model, the drug rescue method may also be applicable in the management of intracerebral tumors.

Key Words • blood-brain barrier • abscess • Escherichia coli • drug rescue • gentamicin

The ability of intravenously administered drug-specific antibodies to ameliorate systemic toxicities associated with high drug dosages has been demonstrated with the cardiac glycoside digoxin. Previous studies have also suggested that the ability of compounds to penetrate intracerebral lesions may be partially dependent on molecular weight, charge, and lipid solubility. Taken together, these findings suggest that a drug rescue method could be developed and used in the management of patients with intracerebral lesions such as tumors, stroke, and infections. Theoretically, after parenteral drug administration, subsequent delayed antidrug immunoglobulin G (IgG), administered after peak drug delivery is achieved, could bind potentially toxic systemic drugs. Systemic binding, however, would not significantly affect therapeutic drug efficacy in a central nervous system lesion due to the significant exclusion of the considerably higher molecular weight of IgG (M, 150,000) from the brain lesion (Fig. 1). This system may permit increased therapeutic dosages that have not been possible because of drug-related systemic toxicity.

In the present experiments, an Escherichia coli brain abscess model was used to investigate a drug rescue method based on the differential permeability of the lesions to compounds of markedly different molecular weights. Sixty minutes after initial drug administration, we tested the hypothesis that a large percentage of systemic low-molecular-weight therapeutic drug, gentamicin, could be bound by subsequent antidrug IgG administration while the drug that had entered the intracerebral lesion would remain free due to the exclusion of the high-molecular-weight antibody. We also examined the delivery characteristics of gentamicin to the abscess both in the presence and absence of systemic drug antibody. The abscess model was used as a first
step in investigating a drug rescue method that may have application to the management of intracranial brain tumors.

Methods and Materials

*Escherichia coli* (specimen #6702) isolated from a human blood culture in the bacteriology laboratory of the Providence Medical Center, Portland, Oregon, was used in all experiments. Further development of the abscess model reported by Winn, et al.,39 has been characterized by this laboratory (JM Nazzaro, et al., unpublished data).23 Bacteria preserved in aliquots of 10% (vol/vol) glycerol in normal saline maintained at −70°C, were inoculated into trypticase soy broth and grown statically for 18 hours. The culture was centrifuged at 50,000 G for 20 minutes, the supernatant was discarded, and the bacterial pellet was washed and centrifuged again as described. The bacterial pellet was resuspended in sterile normal saline to approximately 1.8 × 10⁷ colony-forming units (CFU)/µl.

Female Sprague-Dawley rats, each weighing 250 to 275 gm, were anesthetized with intraperitoneal ketamine (11.6 mg/kg) and xylazine (1.16 mg/kg). They were then stereotactically25 inoculated with 3 µl (5.4 × 10⁷ CFU) of the bacteria/saline preparation into the right caudate putamen, using a 10-µl Hamilton syringe with a No. 30 needle mounted to a micromanipulator.* Control animals were injected with 3 µl of sterile saline. Inoculations were performed using a Burleigh Inchworm apparatus† with a total infusion time of 90 minutes. Following surgery, animals had access to food and water *ad libitum*; all experiments were conducted 6 days after surgery. We are reporting elsewhere the histopathological and quantitative culture evidence of consistent abscess production using this method (JM Nazzaro, et al., unpublished data).

**Gentamicin Antiserum Preparation**

Rabbit antiserum to gentamicin‡ was further purified by sequential affinity chromatography on blue Sepharose CL-6B and protein A-Sepharose.§ This procedure gave a four- to fivefold increase in antibody titer over crude antiserum. Nondenaturing gel electrophoresis in 7% acrylamide demonstrated that the concentrated antiserum did not contain any high-molecular-weight IgG aggregates.

**Determination of Differential Permeability**

Following induction of anesthesia with pentobarbital (50 mg/kg intraperitoneally), the right external carotid artery was cannulated and the femoral vein exposed in 37 animals. These animals received an intravenous injection of 0.5 ml normal saline containing 20 × 10⁶ cpm [³²P]-gentamicin(2 (1005 μCi/µg, < 1% free [³²P]), I fluorescein (10% vol/vol, 0.5 ml/kg, M, 376), and Evans blue dye (2% vol/vol, 2 ml/kg, M, 64,560 Evans blue:albumin complex). Sixty minutes after injection of the radiolabeled gentamicin, a 0.5-ml blood sample was taken; an intravenous injection of 75 µl (0.75 mg) gentamicin antiserum (immune group) in phosphate-buffered saline (PBS) or 75 µl (3 mg) normal rabbit serum in PBS (nonimmune group) was then given. Fifteen minutes after antiserum injection in 12 rats from the immune group ("15-minute immune group") and six rats in the nonimmune group ("15-minute nonimmune group"), or 60 minutes after injection in five rats from the immune group ("60-minute immune group"), a second 0.5-ml blood sample was taken and the animals were subsequently perfused intravenously with normal saline at 37°C in order to clear the vascular bed of [³²P]-gentamicin. Perfusion was terminated upon cessation of cardiac activity and a final 0.5 ml of perfusate was withdrawn. Five animals forming the sham-operated group were inoculated with 3 µl sterile saline into the right caudate putamen, received gentamicin antiserum 60 minutes after intravenous administration of radiolabeled gentamicin, and were perfused 15 minutes following antiserum administration.

Serum and tissue samples were immediately frozen at −70°C and analyzed within 48 hours. The samples were partially thawed, then abscess, brain around the abscess (BAA), brain distant from the abscess (BDA), and contralateral normal brain, kidney, and liver were sectioned. From a 5-mm thick coronal section of whole

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* Micromanipulator manufactured by David Kopf Instruments, Tajunga, California.
† Burleigh inchworm apparatus manufactured by Burleigh Instruments, Fishers, New York.
‡ Gentamicin antiserum supplied by Chemicon, El Segundo, California.
§ Protein A-Sepharose supplied by Sigma Chemical, St. Louis, Missouri.
[³²P]-gentamicin supplied by New England Nuclear, Boston, Massachusetts.
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brain a block of tissue was obtained measuring approximately 1.5 mm in coronal diameter and centered on the target area (abscessed brain) or the contralateral normal brain. Surrounding ganglionic brain in the inoculated hemisphere representing the BAA and BDA was sectioned from the brain 2 mm peripheral to the original coronal section. In the sham-operated group, brains were also sectioned as described and specimens were classified according to the above terminology (abscess in this group represented the central inoculation area).

Tissue specimens were homogenized* in 0.5 ml normal saline or PBS (1:10 wt:vol) for 15 seconds. The percent of delivered [\(^{251}\)I]-gentamicin was calculated according to the following equation:

\[
\text{cpm/gm tissue} \div \text{administered dose } [\^{251}\text{I}]-\text{gentamicin} \times 100.
\]

Bound and free [\(^{251}\)I]-gentamicin were separated by the addition of isopropl alcohol to each tissue homogenate (75% final concentration). Samples were then vortexed and centrifuged at 1200 G for 15 minutes, then the resultant supernatant and pellet fractions were counted for radioactivity in a gamma counter.†

Additional experiments were conducted in a separate group of abscess-bearing animals (four immune and three nonimmune) to more specifically define the total bound gentamicin in serum and brain. Tissue homogenates were centrifuged at 12,000 G for 15 minutes and both supernatant and particulate fractions were counted for radioactivity. Subsequently, both fractions were incubated overnight at 4°C with protein A-Sepharose to quantitatively and specifically bind gentamicin/antigentamicin IgG complexes. The protein A was pelleted at 1200 G for 15 minutes and washed three times with PBS. After counting for radioactivity, non-specifically bound [\(^{251}\)I]-gentamicin remaining in the supernatant was precipitated with isopropyl alcohol as described above. Pre-antibody serum samples were analyzed in two ways to evaluate nonspecific precipitation of [\(^{251}\)I]-gentamicin.

First, to account for precipitation of [\(^{251}\)I]-gentamicin potentially bound to low-molecular-weight peptides,28 bound [\(^{251}\)I]-gentamicin was precipitated with 90% isopropl alcohol for 15 minutes and the pellet and supernatant fractions were counted for radioactivity. Second, serum gentamicin which potentially could have bound to endogenous rat IgG was quantitated by incubating 100-µl serum samples with 5 µg biotinylated anti-rat IgG2 overnight at 4°C and then equilibrated with 50 µl avidin-agarose affinity gel.31 Samples were centrifuged at 1200 G for 15 minutes, and the pellets were washed three times with PBS and counted for radioactivity. Gentamicin/antigentamicin IgG complexes were then quantitated with protein A-Sepharose as described for brain.

Control experiments to determine the extent of free [\(^{251}\)I] or [\(^{252}\)I]-gentamicin binding to unconjugated Sepharose 4B or protein A-Sepharose were performed by adding 100 µl of Sepharose 4B or protein A-Sepharose to 0.5 ml PBS containing 0.14 µCi [\(^{251}\)I]- or 0.15 µCi [\(^{252}\)I]-gentamicin. Tubes were incubated for 1 hour, centrifuged, and counted as above. Possible free [\(^{251}\)I] binding to gentamicin antisera was evaluated by incubating 20 µg of antisera in 0.5 ml PBS with 0.14 µCi [\(^{251}\)I]- for 1 hour, then adding 100 µl protein A-Sepharose (rotated at 4°C overnight). The samples were then centrifuged and counted.

**Statistical Analysis**

Values were calculated as cpm/gm tissue or cpm/ml of serum. Bound values represent [\(^{251}\)I]-gentamicin bound to brain, biotinylated anti-rat IgG, protein A-Sepharose, or isopropanol-precipitated fractions. These were expressed as a percentage of total counts present corrected for tissue weight or serum volume. Mean values ± standard error of the mean were calculated to summarize the data unless otherwise indicated. Statistical analysis was performed using the repeated measures analysis by the Neuman-Keuls' multiple comparison when the overall difference was statistically significant.

**Results**

**Fluorescein and Evans Blue Staining**

To test the permeability of brain abscess to low- and high-molecular-weight compounds, animals were inoculated with fluorescein and Evans blue dye. In bacteria-inoculated animals, the center of the lesion characteristically demonstrated a light green stain, indicating the presence of both low-molecular-weight fluorescein and some high-molecular-weight Evans blue albumin. Areas corresponding to the BAA generally showed strong yellow and only trace green staining upon examination with ultraviolet light, while more peripheral areas were characterized by less intense yellow staining. No discoloration was found in normal brain.

**Delivery of [\(^{251}\)I]-Gentamicin**

In abscess-bearing animals, percent delivery of the administered dose of [\(^{251}\)I]-gentamicin differed significantly according to the area of brain sampled (Fig. 2). Animals sacrificed 15 minutes after gentamicin antisera administration demonstrated a significantly greater (p < 0.01) drug delivery to abscessed brain as compared with the BAA, BDA, or normal brain, suggesting increased permeability in central lesion areas. A significant difference was also found (p < 0.01) in drug delivery to the BAA as compared with deliveries to the BDA and normal brain. Similarly, the 15-minute nonimmune animals demonstrated significantly greater de-
treated with a single-step alcohol precipitation; gentamicin antiserum was administered 60 minutes after abscess-bearing rats. Delivery was determined in brain samples described in the text. Gentamicin antiserum was significantly greater than deliveries to other brain areas: BAA 0.048 ± 0.012, p < 0.01; BDA 0.025 ± 0.006, p < 0.01; and normal brain 0.014 ± 0.005, p < 0.01. Drug delivery to the sampled brain areas in the 60-minute immune group did not significantly differ from deliveries to comparable lesion areas found in the 15-minute immune and nonimmune groups. Furthermore, in two abscess-bearing animals that received saline instead of drug-specific antiserum or normal rabbit serum, a similar percent of gentamicin delivery was found in the abscessed area.

By contrast with the deliveries found in abscess-bearing animals, increased drug delivery to the inoculation site was not found in sham-operated control animals: abscessed brain 0.025 ± 0.007; BAA 0.022 ± 0.007; BDA 0.016 ± 0.005; and normal brain 0.010 ± 0.005. Drug delivery to abscessed areas as well as to the BAA in both immune and nonimmune animals was significantly greater (p < 0.01) than deliveries to these corresponding areas in sham-operated animals. Delivery to the BDA and normal brain in abscess-bearing animals did not significantly differ from delivery to these respective brain areas in the sham-operated group.

Taken together, these findings suggest that drug delivery differs depending on the lesion area sampled and that the systemic presence of drug antibody does not significantly influence intralesion drug delivery.

There was no significant difference between the 15-minute immune, 60-minute immune, nonimmune, or sham-operated immune groups in the delivery of gentamicin to kidney or liver when respective organs were examined. In all groups, delivery of drug was significantly higher in the kidney than in the liver (p < 0.01).

**Binding of [125I]-Gentamicin in Brain**

There were no significant differences between the 15-minute immune and nonimmune groups in the percent of free versus bound gentamicin when homogenates from respective brain sections were compared (Fig. 3). The [125I]-gentamicin binding in serum and brain samples was calculated as:

\[ \text{cpm}_{\text{total}} - \text{cpm}_{\text{supernate}} \times 100 \]

corrected for weight or volume, and

\[ \text{cpm}_{\text{bound}} = \text{cpm}_{\text{total}} - \text{cpm}_{\text{supernate}} \]

By contrast to our drug delivery findings, the percent of gentamicin in the bound fraction did not differ significantly when different brain areas within a single experimental group were compared. In addition, the 60-minute immune animals as well as the sham-operated animals demonstrated similar percentages of gentamicin in the bound fraction (data not shown) that did not differ significantly from the fraction of bound [125I]-gentamicin found in the 15-minute immune and nonimmune groups.

The levels of bound gentamicin in abscess-bearing animals injected with saline rather than gentamicin antiserum or normal rabbit serum was not significantly different from those found in any other experimental group. These findings suggest that gentamicin antibody was significantly excluded from the lesion and may be

![Fig. 2. Bar graph showing percent delivery of [125I]-gentamicin to brain in immune and nonimmune intracerebral abscess-bearing rats. Delivery was determined in brain samples treated with a single-step alcohol precipitation and calculated for abscessed brain (AB), brain around abscess (BAA), brain distant from abscess (BDA), and normal brain (NB) as described in the text. Gentamicin antiserum (75 μL to the immune group) or normal rabbit serum (75 μL to the nonimmune group) was administered 60 minutes after [125I]-gentamicin. Data represent animals sacrificed 15 minutes after gentamicin antiserum or normal rabbit serum administration. Significantly greater deliveries were found in AB (p < 0.01) compared with BAA, BDA, or NB, as well as to BAA (p < 0.01) compared with BDA or NB. There were no significant differences in delivery between the immune and nonimmune groups when comparable brain areas were analyzed.](image2)

![Fig. 3. Bar graph showing percent bound [125I]-gentamicin in serum and brain of immune and nonimmune abscess-bearing animals. Bound values are defined by alcohol precipitation (see text). Serum was collected immediately prior to and 15 minutes after administration of gentamicin antiserum or normal rabbit serum as in Fig. 1. A statistically significant increase (p < 0.0001) in the percent of gentamicin bound in serum was observed in animals receiving gentamicin antiserum (* = postadministration of antiserum). See text for further analysis of results.](image3)
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contrasted with our drug delivery data that indicated delivery of the low-molecular-weight gentamicin to the lesion.

Further characterization of bound [\(^{125}\)I]-gentamicin in brain is shown in Table 1. Centrifugation of the brain homogenates, prior to the addition of a precipitation agent, showed that approximately 25% of the gentamicin was bound to tissue in both immune and nonimmune animals. Treatment of the resultant supernatants with protein A-Sepharose demonstrated no significant difference in immune and nonimmune animals, indicating no specific binding of nonbrain-bound gentamicin by antieoprotein IgG. Analysis of brain samples of comparably treated animals revealed no significant differences between total bound gentamicin (\(\text{brain}_{\text{bound}} + \text{protein A}_{\text{bound}} + \text{alcohol}_{\text{precipitate}}\)) and the percent of drug in the bound fraction defined by antibody precipitation alone. Control experiments, in which free \(^{125}\)I was incubated with normal brain homogenates, demonstrated only 2% binding of \(^{125}\)I.

The percent of drug bound in kidney and liver did not differ significantly between the 15- and 60-minute immune groups. However, percent binding in each of these groups differed significantly from that found in nonimmune kidney (\(p < 0.01\)) and liver (\(p < 0.01\)) specimens when respective organs were compared, suggesting considerable antibody-drug complex precipitation.

**Binding of \(^{125}\)I-Gentamicin in Serum**

Sixty minutes after \(^{125}\)I-gentamicin administration but prior to the administration of gentamicin antiserum or normal rabbit serum, alcohol precipitation of serum samples demonstrated that 38.50% ± 1.45% of the gentamicin present in serum was bound by plasma constituents (Fig. 3). Sera analyzed from animals receiving normal rabbit serum demonstrated that 40.33% ± 1.36% of the circulating gentamicin was in the bound state, indicating no significant binding of gentamicin by normal rabbit serum. By contrast, following gentamicin antiserum administration, 15-minute immune animals demonstrated 93.42% ± 0.50% \(^{125}\)I-gentamicin binding, indicating significant drug binding by gentamicin antibody (Fig. 3). Similarly, the 60-minute immune group demonstrated 95.60% ± 0.60% binding of gentamicin in serum. In each immune group, the percent of gentamicin bound in serum following antiserum administration was significantly greater than baseline pre-antiserum values and also significantly differed from posttreatment values of nonimmune animals.

Of note, immune animals typically showed an increase in \(^{125}\)I-gentamicin counts in both the bound and free serum fractions following gentamicin antiserum administration which was dependent on antibody concentration (data not shown). While the nature of this elevation is not clear, these findings suggest that, when excess gentamicin antibody was administered, the increase in total serum radioactivity reflected extraction of \(^{125}\)I-gentamicin from peripheral pools and/or delayed urinary clearance. Despite the differences in serum \(^{125}\)I-gentamicin, we found no difference between immune and nonimmune animals.

In order to further characterize the total bound \(^{125}\)I-gentamicin in serum, samples were incubated sequentially with biotinylated anti-rat IgG/avidin-agarose, protein A-Sepharose, and isopropyl alcohol. Separate serum samples from this group of animals were also analyzed according to the single-step alcohol precipitate method. Anti-rat IgG, used to specifically bind potential endogenous rat IgG-\(^{125}\)I-gentamicin complexes, failed to demonstrate significant binding (<1%), suggesting absence of \(^{125}\)I-gentamicin to endogenous rat IgG (Table 2). When anti-rat IgG serum supernatants were incubated with protein A-Sepharose, animals receiving normal rabbit serum failed to demonstrate significant binding (<5%) of the \(^{125}\)I-gentamicin. However, in animals receiving gentamicin antiserum, 61.9% ± 5.0% of the \(^{125}\)I-gentamicin was bound by protein A-Sepharose, indicating that this fraction of the genta-

**TABLE 1**

Characterization of brain \(^{125}\)I-gentamicin binding in a rat model

<table>
<thead>
<tr>
<th>Mode of Treatment†</th>
<th>Abscessed Brain</th>
<th>BAA</th>
<th>BDA</th>
<th>Normal Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonimmune</td>
<td>Immune</td>
<td>Nonimmune</td>
<td>Immune</td>
</tr>
<tr>
<td>centrifugation</td>
<td>24.19 ± 0.54</td>
<td>22.15 ± 4.06</td>
<td>25.52 ± 1.99</td>
<td>22.05 ± 2.44</td>
</tr>
<tr>
<td>protein A-Sepharose</td>
<td>2.31 ± 0.61</td>
<td>5.29 ± 1.67</td>
<td>2.47 ± 0.69</td>
<td>5.08 ± 1.61</td>
</tr>
<tr>
<td>isopropanol precip-</td>
<td>4.65 ± 1.38</td>
<td>5.95 ± 2.01</td>
<td>7.59 ± 1.23</td>
<td>3.87 ± 0.81</td>
</tr>
<tr>
<td>ation</td>
<td>31.15 ± 2.53</td>
<td>33.93 ± 7.74</td>
<td>35.58 ± 3.91</td>
<td>31.00 ± 4.86</td>
</tr>
</tbody>
</table>

* BAA = brain around the abscess; BDA = brain distant from the abscess; ND = not determined.
† Samples were sequentially centrifuged at 12,000 G, incubated with protein A-Sepharose, and precipitated with isopropanol as described in the text. Nonimmune animals received 75 \(\mu\)l rabbit serum and immune animals received 75 \(\mu\)l gentamicin antiserum. Mean ± standard error of the mean values are expressed as percent bound \((\text{cpm}_{\text{bound}}/\text{cpm}_{\text{total}}) \times 100\), where \(\text{cpm}_{\text{bound}} = \text{cpm}_{\text{total}} - \text{cpm}_{\text{unbound}}\). There were no significant differences between immune and nonimmune animals in percent bound gentamicin when comparable brain sections were analyzed.
‡ Total bound gentamicin values, calculated as \(\text{brain}_{\text{bound}} + \text{protein A}_{\text{Sepharose}} + \text{isopropanol precipitation}_{\text{bound}}\), were similar to those obtained by a single-step alcohol precipitation.

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micin present in serum was specifically bound by antidrug IgG. The addition of alcohol to the protein A supernatant precipitated the remaining nonspecifically bound gentamicin.

In a subset of immune animals, the single-step isopropanol precipitation method demonstrated that 87.06% ± 5.32% of the serum gentamicin was bound and the sum total percentage of gentamicin precipitable in these experiments (biotinylated anti-rat IgG bound + protein A bound + alcohol precipitable, 80.22% ± 8.89%) for each respective group did not significantly differ from the total percentage of gentamicin bound, defined by alcohol precipitation alone. These data indicate that the increased level of bound gentamicin in immune animal serum may be attributable to drug binding by the administered drug-specific antibody.

Control experiments, in which [125I]-gentamicin was incubated with unconjugated Sepharose 4B or protein A-Sepharose in the absence of gentamicin antiserum, demonstrated less than 2% binding, indicating that [125I]-gentamicin was not interacting nonspecifically with the agarose matrix to a significant extent.

Discussion

Differential Blood-Brain Barrier Permeability

Our studies have used the brain abscess model as a method to investigate the possibility of a drug rescue system based on the proposed differential permeability of the lesions produced.19 Our data support the hypothesis that molecular weight may be an important variable contributing to the delivery of systemically administered drug to intracerebral abscesses.5,24 The fluorescein/Evans blue stain data, although only qualitative, suggest such differential delivery, while our gentamicin delivery and binding data present quantitative support for this concept. Precipitation of antibody-bound gentamicin demonstrated approximately 90% binding in serum following gentamicin antibody administration, in contrast to no significant increase of antibody-bound gentamicin levels when brain lesion samples were assayed. Characterization with protein A-Sepharose demonstrated that a highly significant proportion of the total serum binding reflected serum [125I]-gentami-

<table>
<thead>
<tr>
<th>Method of Serum Analysis</th>
<th>Nonimmune</th>
<th>Immune</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>biotinylated anti-rat IgG/avidin agarose</td>
<td>1.03 ± 0.24</td>
<td>0.87 ± 0.28</td>
</tr>
<tr>
<td>protein A-Sepharose</td>
<td>4.64 ± 1.32</td>
<td>2.28 ± 0.17</td>
</tr>
<tr>
<td>isopropanol precipitation</td>
<td>36.04 ± 3.25</td>
<td>41.24 ± 4.16</td>
</tr>
<tr>
<td>total percent bound gentamicin</td>
<td>41.11 ± 4.81</td>
<td>44.39 ± 4.61</td>
</tr>
</tbody>
</table>

* Serum samples were incubated sequentially in biotinylated anti-rat immunoglobulin G (IgG)/avidin agarose, protein A-Sepharose, and isopropanol alcohol as described in Methods. Pre = before and Post = after administration of normal rabbit serum and gentamicin antiserum to nonimmune or immune animals. Means ± standard error of the mean values were expressed as described in Table 1. See text for analysis of results.

Drug Delivery

In agreement with previous studies using the abscess model,16 we found that gentamicin delivery varies according to the area of the lesion sampled. Our measurement of significantly greater drug delivery to the center of the abscess suggests increased permeability in this area of the lesion. Although permeability characteristics of lesions produced in various abscess and tumor experimental paradigms often vary,15 laboratory as well as clinical evidence suggests that drug delivery may often markedly differ at the proliferating border of intracerebral lesions in contrast with central areas.1,14,15,16,32,33,37 Indeed, one of the most crucial research questions challenging the neuro-oncologist pertains to the increased delivery of antimitotic drugs to such peripheral areas, which, as the current data imply, can be accomplished.

Effect of Gentamicin Antibody

It is also important to note that there was no significant difference between immune and nonimmune animals in delivery of drug to the brain lesion. These data suggest that antidrug IgG may be used to bind a large percentage of circulating drug without significantly affecting intralesion drug delivery. If host antibody production directed against antidrug IgG is to be avoided, an anti-antibody may be needed to accelerate clearance and, thereby, avoid a host immune response.29
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As noted, our binding data show almost complete binding of serum gentamicin by the drug antibody. The high specificity of protein A, coupled with our control data, permits differentiation between specific and nonspecific binding. However, the precise nature of the remaining nonspecifically (nonantidrug IgG) bound \([^{125}\text{I}]\)-gentamicin is not clear at this time. While studies using assay methods similar to those employed in this report approximate our results concerning the binding of gentamicin by endogenous serum constituents,\(^{20}\) other studies using gel filtration\(^{16}\) and equilibrium dialysis\(^4\) have reported nonspecific gentamicin binding in serum of only 10% and less than 20%, respectively.

**Clinical Applicability**

Although multiple factors may contribute to intracerebral drug delivery,\(^{7,9,23}\) our findings suggest the importance of molecular weight and lead support to the theory that a drug rescue method may be developed based on differential permeability. The present experiments were not designed to test the ability of gentamicin antibody to ameliorate systemic toxicities associated with that drug nor was the question of drug efficacy in brain explored. While our ultimate goal is to use the proposed drug rescue method in the treatment of intracerebral tumors, particularly if this method permits higher than usual systemic drug dosages, the abscess model was used in the present work specifically because it may afford a simple and inexpensive model to drug delivery before proceeding to tumor models.

**Direction of Future Studies**

Intracerebral drug efficacy in the presence of systemic antidrug IgG needs to be examined in abscess-bearing rats by quantitative bacterial cultures. In addition, to demonstrate antibody rescue of specific drug-induced systemic toxicities,\(^{6,7,27,30,31}\) experiments are also needed to test the ability of gentamicin antisera to ameliorate drug-induced nephrotoxicity\(^{12,13}\) via the binding of circulating drug by systemically administered gentamicin antibody. Experiments with larger drug dosages than those reported here may be needed to address the possibility of an antibody-induced serum sickness. Finally, since differential permeability to fluorescein and Evans blue-albumin has been reported previously in nude rats bearing intracerebral human lung tumor xenografts, experiments have been initiated to test this therapeutic means to increase drug delivery in a tumor model.\(^{20}\)

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