Effect of Osmotic Blood-Brain Barrier Disruption on Gentamicin Penetration into the Cerebrospinal Fluid and Brains of Normal Rabbits

LARRY J. STRAUSBAUGH* AND GARY S. BRINKER

Department of Medicine, Division of Infectious Diseases, University of Missouri School of Medicine, Columbia, Missouri 65212

Rapid infusion of hyperosmolar solutions into the internal carotid artery transiently disrupts the blood-brain barrier, permitting entry of substances that are ordinarily excluded from the nervous system. This study compared gentamicin concentrations in the cerebrospinal fluid (CSF) and brain tissue of rabbits receiving intracarotid infusions of 2 molal mannitol with those in three groups of control animals. After catheter placement and intravenous gentamicin administration (20 mg/kg), rabbits received either 2 molal mannitol in the internal carotid artery, 2 molal mannitol intravenously, 0.9% saline in the internal carotid artery, or 0.9% saline intravenously. Mannitol and saline were administered in 8-ml bolus injections over 40 s. After 2 h, serum, CSF, and brain specimens were obtained for antibiotic assay. Gentamicin concentrations in serum were comparable in all groups (mean concentrations ranged from 14.6 to 17.9 μg/ml at 60 min and from 5.7 to 7.4 μg/ml at 135 min), but gentamicin concentrations in CSF and brains were significantly higher in animals in the group receiving mannitol in the internal carotid artery. In this group the mean gentamicin concentration in CSF, 5.3 μg/ml, was twofold greater than those in the other three groups (range, 1.7 to 2.6 μg/ml). Similarly, the mean gentamicin concentration in the brains of animals in the group receiving mannitol in the internal carotid artery, 2.3 μg/g was significantly higher than those in the other groups (mean of measurable values, 1.4 μg/g, in all three control groups). Osmotic disruption of the blood-brain barrier enhanced the penetration of gentamicin into CSF and brain tissue.

Rapoport has described a novel technique that transiently disrupts the integrity of the mammalian blood-brain barrier. The technique involves infusions of hyperosmolar solutions into the internal carotid artery (14). Solutions of urea, mannitol, arabinose, and similar substances have been utilized to open the blood-brain barrier to Evan blue dye and other compounds that are normally excluded from cerebrospinal fluid (CSF) and brain tissue. The passage of hyperosmolar solutions through the carotid circuit appears to leech out intracellular water from cerebrovascular endothelial cells, causing them to shrink. This shrinkage widens the tight junctions between cells, which in turn disrupts their barrier function. The effect of one intracarotid injection lasts several hours, after which time the integrity of the barrier is completely restored. Clinical evaluation of the animals involved in these experiments and histological examination of their brains have, for the most part, failed to detect any harmful or lasting effects from the intracarotid infusions.

The potential applications of this technique in the therapy of central nervous system infections are of considerable interest. If osmotic opening of the blood-brain barrier facilitates passage of antimicrobial agents into CSF and brain tissue, then it may prove to be a useful adjunctive measure in the treatment of patients infected with organisms that are susceptible only to agents with poor penetration properties. Accordingly, the purpose of this study was to examine the effect of osmotically opening the blood-brain barrier on gentamicin penetration into CSF and brain tissue in normal rabbits. Three control groups were utilized to assess the relative contributions of the intracarotid injection and the hyperosmolar solution of mannitol.

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MATERIALS AND METHODS

Antibiotics. Gentamicin was supplied by Schering-Plough Corp., Kennilworth, N.J. All experimental animals received a single dose of 20 mg/kg of body

weight administered rapidly (in less than 5 sec) as a single bolus through the femoral venous catheter.

Rabbit model. At 1 or 2 days before each experiment, 2-kg New Zealand white rabbits were fitted with helmets made of dental acrylic (4). This helmet allowed fixation of the head of the animal in a modified stereotaxic frame for cisternal puncture. On the day of each experiment, rabbits received a 50-mg dose of sodium pentobarbital administered intravenously via a marginal ear vein. Polyethylene catheters were then inserted into the femoral artery and vein. A solution of 0.9% sodium chloride was continuously administered through the femoral venous catheter, which was used for anesthesia maintenance, gentamicin administration, and, in some cases, mannitol administration. The femoral arterial catheter was periodically flushed with heparinized saline to maintain patency; blood specimens for assays to measure antibiotic concentration in serum were collected from this catheter.

Rabbits were further prepared with the placement of catheters into the right common carotid artery. Atropine sulfate (0.1 mg) was administered intramuscularly before carotid artery manipulation to counteract the effects of vagal stimulation. After the right common carotid artery was isolated, a ligature was placed distal to the origin of the internal carotid artery to occlude the external carotid artery. Thus, the catheter inserted into the common carotid artery below this ligature provided direct access to the right internal carotid artery. This was confirmed by demonstration of retinal blanching when the carotid catheter was flushed with 0.5 ml of 0.9% saline.

Immediately after catheter placement, gentamicin was administered intravenously. Then, animals were randomly assigned to one of the following experimental groups: (i) the intravenous saline group received an 8-ml infusion of 0.9% saline through the femoral venous catheter over 40 s; (ii) the intracarotid saline group received an 8-ml infusion of 0.9% saline through the common carotid catheter over 40 s; (iii) the intravenous mannitol group received an 8-ml infusion of a 2 molal mannitol solution through the femoral venous catheter over 40 s; and (iv) the intracarotid mannitol group received an 8-ml infusion of a 2 molal mannitol solution through the common carotid catheter over 40 s. Mannitol and saline infusions were administered with a syringe infusion pump (model 355; Sage Instruments Inc., Cambridge, Mass.) 15 min after gentamicin administration. There were 10 to 15 rabbits in each group.

Serum samples were obtained 60 and 135 min after gentamicin administration. After the second serum samples were collected, rabbits were placed in a modified stereotaxic frame for cisternal punctures. A spinal needle mounted on the geared electrode introducer of the frame was guided without trauma through the posterior atlantooccipital membrane into the cisterna magna. Through this needle 0.5- to 1.0-ml samples of CSF were collected. Rabbits were then sacrificed with an overdose of sodium pentobarbital, and their entire brains were removed. After removal, brains were rinsed in 0.9% saline and frozen at −70°C, as were serum and CSF specimens. All specimens were assayed for gentamicin content within 7 days; storage at −70°C did not affect assay results within this time period.

Antibiotic assays. Concentrations of gentamicin in serum, CSF, and brain tissue were determined by an agar well diffusion assay with *Bacillus subtilis* as the test organism (2). Streptomycin assay agar with yeast extract was used throughout the study. Brain specimens were processed by the method described by Beam and Allen (1). The entire brain was removed and homogenized in Mueller-Hinton broth at a ratio of 1:2 (wt/vol). No attempt was made to remove CSF from the ventricles before homogenization. Gentamicin assays were performed on supernatants of the brain homogenate because supernatant fluid contained virtually all of the free drug activity. All samples and standards were tested in quadruplicate. Serum, CSF, and supernatant from brain homogenates obtained from normal rabbits produced no zones of inhibition in this assay system. Serum specimens with gentamicin concentrations of >20 µg/ml were diluted in pooled normal rabbit sera and reassayed. Other serum specimens and CSF and brain supernatant specimens were tested without dilution. Serum standards for the assay were also diluted in pooled normal rabbit sera. Standards for CSF and brain tissue assays were prepared in 0.9% sodium chloride. Previous experiments indicated that standards prepared in normal rabbit CSF and in supernatant from brain homogenates had zone sizes that closely approximated (within ±5%) those of standards prepared in 0.9% saline. Brain concentrations were corrected for blood contamination by the method described by Lowry and Hastings (9). The assay detected gentamicin concentrations of ≥0.50 µg/ml in CSF and ≥0.80 µg/g in brain tissue.

Analysis of data. Differences observed among the four groups in gentamicin concentrations in serum, CSF, and brain tissue were analyzed for statistical significance by the Kruskal-Wallis one-way analysis of variance (6). Differences in these values for any of the individual groups were analyzed for statistical significance with Dunn's technique for multiple comparisons by rank sums (5). The experimental error rate (alpha) in these latter comparisons was 0.05.

RESULTS

Gentamicin concentrations in serum after the 20-mg/kg intravenous injection were comparable in the four groups of rabbits at both sampling times. The mean ± standard deviation of the drug concentration in serum at 60 min ranged from 14.6 ± 3.3 µg/ml in the intravenous mannitol group to 17.9 ± 5.6 µg/ml in the intracarotid mannitol group (Table 1). These groups differences were not statistically significant ($H = 3.7$; $P = 0.30$). At 135 min, the mean ± standard deviation of the drug concentration in serum ranged from 5.7 ± 1.5 µg/ml in the intracarotid saline group to 7.4 ± 1.9 µg/ml in the intracarotid mannitol group. These group differences were not statistically significant ($H = 5.4$ [Kruskal-Wallis one-way analysis of variance]; $0.20 > P > 0.10$).

In contrast, mannitol infusions in the internal carotid artery had a profound effect on gentamicin concentrations in CSF and brain tissue. Gentamicin concentrations in CSF were highest in animals in the intracarotid mannitol group.

TABLE 1. Serum, CSF, and brain concentrations of gentamicin in the four treatment groups

Expt group (no. of rabbits)	Mean ± SD gentamicin concn in:			
	Serum (µg/ml) at:		CSF (µg/ml)	Brain tissue (µg/g)
	60 min	135 min		
Intracarotid mannitol (15)	17.9 ± 5.6	7.4 ± 1.9	5.3 ± 2.9[a]	2.3 ± 0.4
Intracarotid saline (10)	16.1 ± 4.7[a]	5.7 ± 1.5	2.6 ± 1.6	1.4 ± 0.7[b]
Intravenous mannitol (10)	14.6 ± 3.3	6.2 ± 1.5	2.4 ± 1.7[a]	1.4 ± 0.5[b]
Intravenous saline (10)	14.7 ± 5.6	7.0 ± 2.9	1.7 ± 1.0[c]	1.4 ± 0.7[d]

[a] Specimens were obtained from only nine animals.
[b] Undetectable gentamicin concentrations in brain tissue of three animals.
[c] Undetectable gentamicin concentrations in CSF of two animals.
[d] Undetectable gentamicin concentrations in the brain tissue of seven animals.

The mean value for this group, 5.3 µg/ml, was twice that observed in the three control groups; these group differences were highly significant ($H = 12.4$; $0.01 > P > 0.005$). Individual comparisons of one group with another established a statistically significant difference between gentamicin concentrations in CSF in the intracarotid mannitol group and the intravenous saline group, but the other individual comparisons did not reveal statistically significant differences. There was some overlap in gentamicin concentrations in CSF among animals in the four groups. Gentamicin concentrations in CSF ranged from 1.2 to 10 µg/ml in the intracarotid mannitol group, from 0.7 to 5.2 µg/ml in the intravenous mannitol group, from 0.6 to 4.6 µg/ml in the intracarotid saline group, and from <0.5 to 3.1 µg/ml in the intravenous saline group.

Gentamicin concentrations in brain tissue were also highest in animals in the intracarotid mannitol group. The mean value for this group, 2.3 µg/g, was almost twice that for detectable concentrations in the other groups. These group differences were highly significant ($H = 23.9$; $P < 0.0005$). Individual comparisons of one group with another also established statistically significant differences between gentamicin concentrations in brain tissue of animals in the intracarotid mannitol group and each of the three control groups. As with gentamicin concentrations in CSF, there was some overlap in concentrations in brain tissue among animals in the four groups.

DISCUSSION

In this study, three control groups were used to differentiate the relative contributions of the hyperosmolar solution and the intracarotid infusion. From the results, it seems clear that intracarotid infusion of the hyperosmolar solution is essential for disruption of the blood-brain barrier. Neither intracarotid infusion of normal saline nor intravenous administration of mannitol were sufficient to increase gentamicin concentrations in CSF and brain tissue.

The enhanced penetration of gentamicin into brain tissue observed in rabbits subjected to mannitol infusions in the internal carotid artery parallels observations made by Rapoport and associates. They have previously demonstrated in rabbits that 2 molal solutions of urea transiently open the blood-brain barrier on the perfused side to Evan blue dye (14). This dye, which is tightly bound to albumin, will not stain cerebral tissue when injected intravenously under other circumstances. Our choice of a 2 molal concentration was based on their observation. Mannitol was selected instead of urea because of its common use in clinical medicine, but not before dye studies in our laboratory confirmed its potential for barrier disruption.

Neuwelt and his colleagues have used internal carotid infusions of 25% (1.37 molal) mannitol to increase methotrexate concentrations in the brains of dogs (11, 12). These investigators also noted a 10-fold increase in mean methotrexate levels in the CSF of dogs receiving intracarotid mannitol infusions in the internal carotid artery but noted some overlap with control values in specimens obtained 1 h after barrier disruption. In the present study, there was some overlap in gentamicin concentrations in CSF and brain tissue in the four treatment groups. This observation suggests that barrier function may vary somewhat from one animal to another and that the effects of barrier disruption are not uniform.

Although it may be premature to speculate about the clinical application of this technique, several comments are in order. First, there is a need for methods that would enhance the penetration of antimicrobial agents like gentamicin into CSF and brain tissue. Recent discussions of therapy for gram-negative bacillary meningitis have repeatedly emphasized the problems associated with aminoglycoside antibiotics in terms of both penetration and toxicity (3, 7, 13, 16). Second, the demonstration by Neuwelt and associates that osmotic barrier disruption can be safely undertaken in humans provides motivation for pursuing this line of approach. They

carried out osmotic blood-brain barrier disruption 33 times in eight patients (10). Last, the evaluation of the potential of this technique will need to address the toxic potential of high antibiotic concentrations in CSF and the brain. The neurotoxic potential of penicillin has been appreciated for some time (8). The neurotoxicity of aminoglycoside antibiotics was first noted in early experiences with intrathecal streptomycin administration (18). Several fatalities were reported in patients who received large intrathecal doses. In time, it became apparent that only dosages of <100 mg could be safely administered intrathecally to adult patients. More recently, neurotoxicity has been ascribed to gentamicin. A case report and experimental study have described the development of brain lesions after prolonged exposure to high gentamicin concentrations in CSF (17). The ototoxicity of gentamicin makes evaluation of vestibular and auditory function of prime importance too. Does the osmotic disruption technique increase perilymph or endolymph aminoglycoside concentrations? Will it hasten hair cell loss in the organ of Corti? Future evaluations of osmotic disruption will need to focus on these potential toxicities as well as on those toxicities inherent in the technique itself.

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