Serum standards for the bioassay of aminoglycosides in cerebrospinal fluid

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SUMMARY Four diluents were compared as reference standards for the assay of gentamicin in cerebrospinal fluid (CSF): human CSF, human serum, distilled water, and 150 mmol NaCl/4.5 mmol CaCl₂. Standards prepared in pooled human serum were the best alternative to CSF for the assay of gentamicin and were also useful for the assay of tobramycin, netilmicin, amikacin, and sisomicin. The pH (6.0-9.8) of CSF did not alter the results of the assay.

The accurate determination of microbiologically active gentamicin in cerebrospinal fluid (CSF) is often critical in the management of patients with Gram-negative bacterial meningitis. Several clinical studies have reported CSF drug concentrations; however, standards for the assay were not specified (Kaiser and McGee, 1971; McCracken, 1972; McCracken and Mize, 1976). Although CSF would seem to be the best diluent for standards, it is often not available. A recent report described an alternative solution for CSF standards (Deacon, 1976a). We compared this artificial diluent (150 mmol NaCl/4.5 mmol CaCl₂) with distilled water, human CSF, and pooled human serum. Since cerebrospinal fluid pH may vary markedly, the effect of pH was also examined. Unlike Deacon's report, our results indicate that CSF or serum is a better diluent for gentamicin standards than the artificial solution or water. Serum was found to be a reliable alternative to CSF for four newer aminoglycosides as well.

Material and methods

Microbiologically active gentamicin was measured by the Bennett agar diffusion assay method using a serum-resistant gentamicin-sensitive *Klebsiella pneumoniae* as the test organism (Bennett *et al.*, 1966). Bacto antibiotic medium 11 (Difco) was substituted

for nutrient agar as the former produced clearer zones of inhibition. A 30-mm square agar plate was flooded with a 1/100 dilution of an overnight growth of the test organism in nutrient broth. The excess inoculum was removed by pipette and the plate was dried by incubation for approximately 45 minutes at 37°C with the lid ajar. Wells (2 mm diameter) were cut in the seeded plate and numbered so that samples were distributed randomly on the plate. Gentamicin standards were prepared in concentrations of 20, 10, 5, 2.5, and 1.25 µg/ml in the various test fluids. Four replications of each sample were performed for each of three tests, and zones of inhibition (mm) were recorded and averaged after 18-20 hours' incubation. Standard curves were plotted on semilog paper. The same method was employed when four other aminoglycosides were assayed to compare CSF and serum as diluents.

CSF was obtained from hospital patients without leptomeningitis or ventriculitis. CSF 1 was from a hydrocephalic patient; CSF 2, 3, and 4 were CSF pools obtained from diagnostic lumbar puncture in febrile infants without meningitis. Human serum was collected from adult volunteers. Glucose, protein, and cell count of the CSF were determined by standard methods. CSF 1, 2, and 3 and different pools of human serum were used for each of three experiments involving gentamicin (Table 1). The NaCl/CaCl₂ solution was prepared as recommended by Deacon (1976a).

The effect of pH was examined with CSF 4. Using 2% HCl and 4% NaOH the samples were adjusted to pH 6, 7, 8.2, 9, and 9.8. Gentamicin was added to each sample to yield a final concentration of 5 µg/ml.

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Table 1 Analyses of four cerebrospinal fluids

	CSF 1	CSF 2	CSF 3	CSF 4
Glucose (mg/100 ml)	39	64	64	47
(mmol/l)	2.2	3.6	3.6	2.6
Protein (g/l)	0.72	0.14	0.09	0.38
Cell count/mm ³	53 polys 21 lymphs	0 polys 1 lymph	0 polys 1 lymph	1 poly 0 lymph

Results

The average of the results of the standard curves of three experiments with gentamicin diluted in Deacon's solution, distilled water, and pooled human serum is compared to that obtained with pooled human CSF (Figure). The mean difference in zones of inhibition (CSF-alternative diluent) for gentamicin diluted in distilled water when compared to CSF was 1.65 mm (range 1.0 to +2.25 mm), for Deacon's solution 1 mm (range 0.75 to +1.75 mm), and for serum 0.05 mm (range 0 to +0.25 mm). The most accurate approximation of the CSF curve was obtained with standards prepared in pooled human serum (Figure). The zones of inhibition were in exact agreement for one run (CSF 1) and insignificantly different (mean differences CSF-serum +0.35, -0.35 mm) in two other runs using different CSF and serum pools. Pooled human CSF standards of 5 µg/ml gentamicin with a pH of 6, 7, 8.2, 9, and 9.8 gave identical zone sizes. Newer aminoglycosides were assayed to compare standards prepared in CSF

and serum. The results of a single experiment with four replications are listed in Table 2 and demonstrate negligible differences in zone diameters between the standards.

Table 2 Difference in zones of inhibition between standards prepared in CSF and serum

Antibiotic	CSF-serum (mm)	
	Mean difference	Range
Tobramycin	0.25	0 to +1.0
Netilmicin	0.24	0 to +0.6
Amikacin	0.21	-0.3 to +0.5
Sisomicin	0.22	-0.2 to +0.8

Discussion

The activity of gentamicin in serum is subject to various physiological conditions, that is, effect of pH, ionic strength, protein, serum, etc. (Rubenis *et al.*, 1963; Deacon, 1976b; Shanson and Daniels, 1975). We found no report comparing gentamicin activity in pooled human serum and CSF. It would be technically convenient to be able to assay both serum and CSF gentamicin concentrations using one set of reference standards. The results of our studies indicate that this is valid for gentamicin as well as for four newer aminoglycosides and that the use of distilled water or electrolyte solutions may lead to considerable inaccuracies. The reasons for the lack of agreement of our results with those of Deacon (1976a) are unclear. Technical differences such as the use of a single assay plate for all samples in our study versus the use of several separate plates by Deacon (1976a) may be responsible. Shanson and Daniels (1975) reported that ionic content affected serum standards when the pH was altered from 7.5 to 6.7. On testing this phenomenon by comparing serum and CSF standards at pH 6.4, 8, and 9.3, no effect was demonstrable. This difference in results is probably due to the extreme sensitivity of Shanson and Daniels' assay organism and the use of 7-mm rather than 2-mm wells. The advantages in sensitivity offered by their method may be outweighed by the inaccuracies incurred through the use of separate plates for each sample. In conclusion, using the assay method described in this study, drug standards prepared in pooled human serum are reliable for the microbiological assay of CSF gentamicin concentrations as well as those of tobramycin, sisomicin, amikacin, and netilmicin.

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Figure Comparison of average zones of inhibition of various gentamicin concentrations prepared in four diluents

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