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YAFFE AWARD LECTURE

A Historical Perspective on Pharmacological Studies of Gentamicin for Therapy of Neonatal Meningitis

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Much of my early career in Pediatric Infectious Disease was devoted to defining the optimal management of neonatal meningitis caused by Gram negative enteric bacteria. To accomplish this required an understanding of clinical pharmacology of antibiotics in newborn infants, an area rarely explored in the period before the late 1960s when I started my investigative career. Indeed, in 1968 Dr. Harry Shirkey, a pharmacist-turned-pediatrician, lamented the fact that infants and children were often denied use of many drugs because of the lack of data on their pharmacokinetics, safety and efficacy.[1] He termed these patients "therapeutic orphans" and encouraged the development of active programs of clinical pharmacology and drug testing in pediatric patients, especially in infants and young children. It was in this setting that I launched my investigations of the clinical pharmacology of antibiotics in newborn and young infants, particularly as they pertained to treatment of bacterial meningitis. Now, 38 years later, I am thrilled to receive the Sumner J. Yaffe Lifetime Achievement Award in Pediatric Pharmacology and Therapeutics from the Pediatric Pharmacy Advocacy Group in recognition of these and other studies that helped to clarify the safe and effective use of antibiotics in infants and children. I am honored to receive this award named for Dr. Yaffe, who was as instrumental as anyone in promoting the critical importance of clinical pediatric pharmacology. His many years of dedicated work in this arena culminated in 1992 with the creation of the Pediatric Pharmacology Research Unit (PPRU), a network of pediatric research centers in the United States, sponsored by the National Institutes of Child Health and Human Development. Dr. Yaffe has diligently worked to reduce the number of drugs that have been inadequately evaluated in infants and young children, but there remains more work to do. I am extremely proud to have my name associated with his on this award.

ABBREVIATIONS CSF, cerebral spinal fluid; IL-1β, interleukin-1 beta; LPS, lipopolysaccharide; MIC, minimal inhibitory concentration; PPRU, Pediatric Pharmacology Research Unit

During my pediatric residency at Cornell University Medical College-New York Hospital I reviewed the etiology of neonatal sepsis and meningitis at the hospital from 1954 through 1968.[2] Two changes occurred during that period. First, the number of cases in the period from 1954 through 1958 (25 cases) increased 84% in the period from 1959 through 1964 (46 cases). Secondly, the number of infants with Gram negative infection increased dramatically from 1 infant in the former to 34 cases in the latter period. The case fatality rate was 30% for newborn infants with disease caused by

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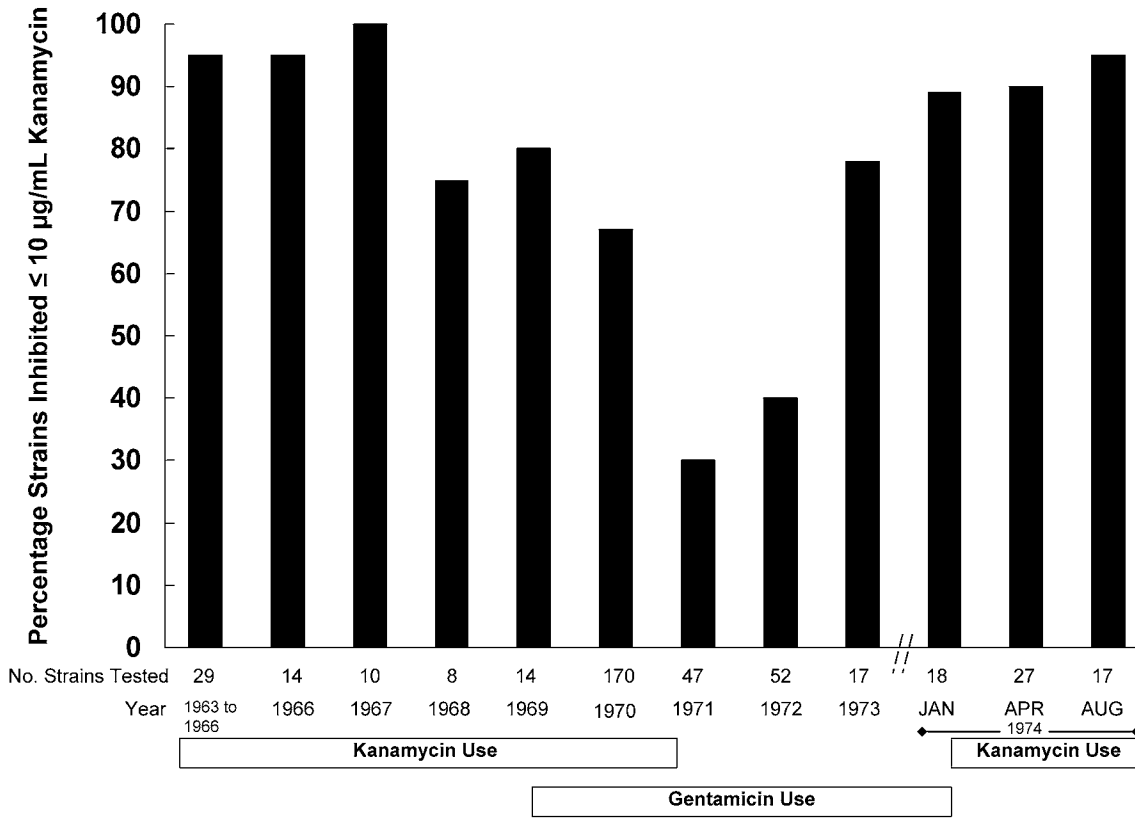

Figure 1. Percentage of gram negative organisms resistant to kanamycin isolated from neonates at Parkland Memorial Hospital Nursery, Dallas, TX from 1963 to mid-1974.[4]

Gram positive organisms compared with 75% in those with disease caused by Gram negative pathogens. After locating in Dallas in 1968, I observed that neonates with meningitis cause by Gram positive organisms, principally group B streptococci, usually had sterile cerebral spinal fluid (CSF) cultures within 24 hours after starting appropriate antimicrobial therapy.[3] By contrast, CSF cultures from neonates with Gram negative coliform meningitis remained positive for an average of 3–4 days after start of therapy, and the longer the spinal fluid cultures remained positive, the poorer was the outcome. These seminal observations plus the emergence of resistant coliform organisms, mainly *Escherichia coli*, to kanamycin, the agent used routinely in the nurseries of Parkland Memorial Hospital in Dallas in1968 to1970 (Figure 1),[4] prompted us to assess the clinical pharmacology of gentamicin in newborn infants and to explore the most appropriate way to administer this aminoglycoside to newborn infants with meningitis caused by Gram negative enteric bacilli.

Because gentamicin had never been given to neonates, it was first mandatory for us to determine the pharmacokinetics and safety of the agent in infants of various birth weights and postnatal ages.[5,6] Additionally, it was necessary to develop an assay that accommodated minute quantities of plasma because the infants would require repeated heel or finger sticks for blood. The data from this investigation indicated that the peak plasma concentrations were from 1.7 to 3 µg/mL after a 1.5 mg/kg dose and varied inversely with the volume of distribution of gentamicin as well as with the birth weight and postnatal age (Figure 2). The plasma half-life values were as long as 13.9 hours in the 800–900 gram infants in the first week of life to 3.2 hours in > 3000 gram infants older than 7 days.[5,6] In addition, the excretion of gentamicin in urine was shown to be directly correlated with the creatinine clearance, which in turn correlated to the postnatal age.[7] We determined that an appropriate dosage of gentamicin in neonates was 2.5 mg/kg/dose administered every 12 hours in the first week of life and every

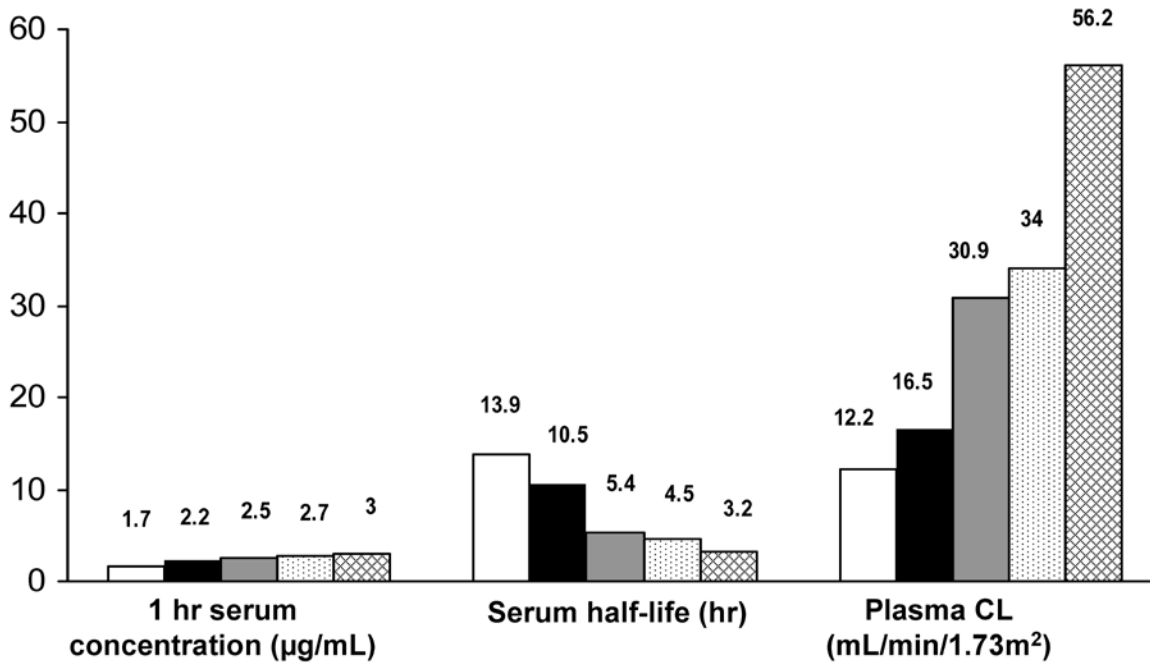

Figure 2. Pharmacokinetic values for gentamicin after intravenous administration to newborn infants of 1.5 mg/kg doses by weight and postnatal age.[5,6] □ 800-1500 g; ■ 1501-2000 g; ▒ 2001-2500 g; ▒ 2501-3500 g; ▒ infants > 1 wk.

8 hours thereafter.

In mid-1971, we started to use gentamicin routinely in newborn infants, a time when the percentage of susceptible coliform bacteria to kanamycin, especially *E. coli* strains, in the nursery was no greater than 30% (Figure 1).[4] With gentamicin being used exclusively in the nursery, the percentage of kanamycin-susceptible organisms promptly returned to > 80% by 1974. This was the first time that rotation of aminoglycoside usage in a nursery was shown to allow return of susceptible organisms to an agent when it was no longer routinely used.

In the early 1970s, routine therapy for neonatal meningitis caused by Gram negative enteric bacilli was ampicillin and gentamicin. Because the case-fatality and long-term morbidity rates exceeded 50% at that time, we sought ways to optimize gentamicin dosing. Our hypothesis was that higher concentrations of gentamicin in the CSF would result in more rapid sterilization of CSF cultures and possibly improved outcome. This tenet was based on the knowledge that aminoglycosides were more effective in killing organisms when their concentrations exceeded the pathogen's minimal inhibitory concentration (MIC) by at least 5-fold and possibly more. To accomplish this we designed a multi-centered trial that compared ampicillin and gentamicin therapy given systemically with the same regimen plus 1 mg of gentamicin given daily in the lumbar intrathecal space until CSF cultures were sterile. There were 115 infants enrolled in this first Neonatal Meningitis Cooperative Study.[8] The concentrations of gentamicin achieved in lumbar CSF at 2–4 hours after systemic plus intrathecal therapy were approximately 20-fold higher than those after systemic therapy only (Table 1). Despite higher gentamicin concentrations in the lumbar CSF, days to sterilization of CSF cultures and the case-fatality rates were similar for the two treatment groups (Table 2). Long-term outcome of the infants was also similar for the two treatment groups. We concluded from this study that higher gentamicin concentrations in the lumbar CSF space were not beneficial in neonates with coliform meningitis.

Because ventriculitis is an integral part of neonatal meningitis we speculated that the large concentrations of gentamicin observed in the lumbar CSF did not distribute throughout the CSF space and especially to the ventricles, the sites of initial infection as a result of seeding of the choroid plexus following bacteremia. The Second Neonatal Meningitis Cooperative

Table 1. Concentrations of gentamicin in lumbar csf after systemic (2.5 mg/kg) administration or systemic and intrathecal (1 mg) administration of gentamicin. Taken from the Neonatal Meningitis Cooperative Study[8]

Serum concentration (μg/mL)	Systemic Therapy	Systemic Therapy plus 1 mg IT	
		2–4 hr*	18–24 hr*
Mean	1.6	30	1.6
Range	0.3–3.7	18–40	0.3–3.4

IT, intrathecal
** time after IT dose*

Table 2. Clinical outcome in neonates with gram negative bacillary meningitis enrolled in the neonatal meningitis cooperative study comparing systemic gentamicin and ampicillin therapy with or without 1 mg gentamicin given intrathecally[8]

	Systemic Therapy	Systemic Therapy plus 1 mg IT
Number of patients	65	50
Days Positive CSF Culture	3.3 ± 0.6	3.9 ± 1.2
Deaths	22 (34%)	15 (30%)
Sequelae	15 (35%)	10 (29%)

IT, intrathecal

Table 3. Clinical outcome in neonates with gram negative bacillary meningitis enrolled in the second neonatal meningitis cooperative study comparing systemic gentamicin and ampicillin therapy with or without 2.5 mg gentamicin inoculated intraventricularly[9]

	Systemic Therapy	Systemic Therapy plus 2.5 mg IV
Number of patients	28	24
Days Positive CSF Culture	4.2 ± 1.2	3.0 ± 0.9
Deaths	3 (12.5%)*	12 (42.9%)
Normal, mild, moderate on follow-up	16 (57%)	11 (46%)

IV, intraventricular
** P = .016*

Study was designed to assess the effectiveness of intraventricular gentamicin therapy.[9] Systemic ampicillin and gentamicin therapy was given to one group of infants and the same regimen plus a 2.5 mg dose of gentamicin given daily into the ventricles was randomly assigned to the other group. The concentrations of gentamicin in the ventricles were 10 to 130 μg/mL (mean, 48 μg/mL) at 2–4 hours and 1 to 24 μg/mL (mean, 8.1 μg/mL) at 16–24 hours after the intraventricular gentamicin dose. Thus, the peak concentrations in the ventricles exceeded the MIC values for the Gram negative enteric bacilli by at least 30- to 50-fold. However, after only 52 patients were enrolled, the case-fatality rate in those infants receiving the intraventricular regimen was significantly higher than in those given systemic therapy only (43% vs 12.5%, P = .016) (Table 3). The trial was halted and at that time we were unable to explain why those given intraventricular therapy did poorly.

During the decade of the 1980s we directed our research efforts to understanding the molecular pathophysiology of meningitis using animal models. We showed that inoculating the lipooligosaccharide (endotoxin) of *Haemophilus influenzae* directly in the cisterna magnum of rabbits resulted in a brisk but transient CSF inflammatory response.[10] Further, we showed that in untreated rabbits with experimental *H influenzae* meningitis, the lipooligosaccharide is released slowly from the organisms at 6 through 10 hours after inoculation and the concentration of *H. influenzae* in CSF increased by approximately 10 CFU/mL.[11] By contrast, when chloramphenicol was administered intravenously, the concentration of *H. influenzae* in CSF fell by more than 100 CFU/ml during that 4-hour period, and the endotoxin concentration in that compartment increased more than 4-fold (Figure 3). Inflammation in

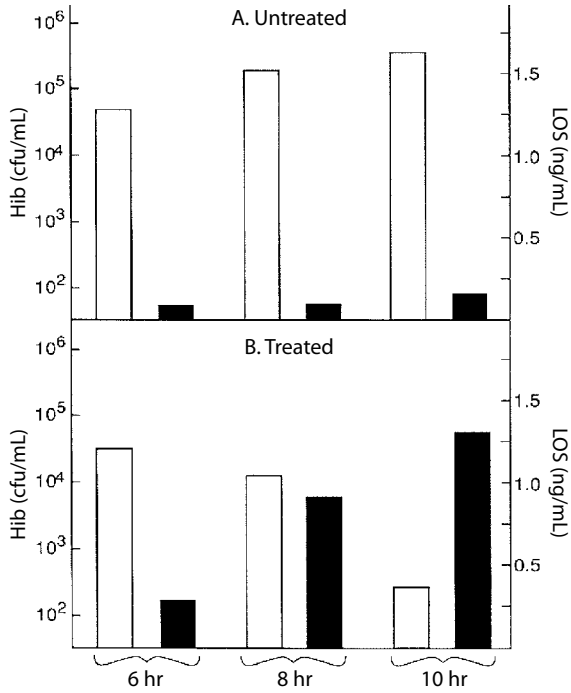

Figure 3. Mean concentrations of *Haemophilus influenzae* type b (in CFU/ml) and lipooligosaccharide (LOS) in CSF of untreated and cloramphenicol-treated rabbits at 6 to 10 hours after intracisternal inoculation.[12] □ Hib (cfu/mL); ■ LOS (ng/mL)

CSF increased briefly after chloramphenicol therapy in these animals. This observation prompted us to examine the possibility that intraventricular gentamicin therapy in the Second Neonatal Meningitis Cooperative Study had enhanced the killing of coliform bacilli in the ventricles, thereby increasing endotoxin concentrations and secondary inflammation.[12] Specimens from the ventricles had been frozen at -70°C and were available for measuring the markers of inflammation. As anticipated from the results of our rabbit studies mentioned above, the infants who received intraventricular gentamicin therapy had significantly larger amounts of interleukin-1 beta (IL-1β), lipopolysaccharide or endotoxin (LPS), white blood cells and protein compared with those who received systemic therapy only (Table 4). Thus, these data clearly illustrated that direct inoculation of gentamicin into the ventricles resulted in greater killing of organisms and release of endotoxin in the ventricles that fueled inflammation resulting in a higher case-fatality rate.

In light of the above observations, we speculated whether dexamethasone would be effective in reducing the secondary inflammation that results from the bactericidal effect of antibiotics on organisms in the CSF space of neonates with meningitis. In the rabbit model we demonstrated that when dexamethasone was given before ceftriaxone therapy in experimental *Haemophilus influenzae* meningitis, the secondary inflammatory response in CSF was modulated as a result of a block in transcriptional signaling of the seminal cytokines, tumor necrosis factor alpha (TNFα) and IL-1β.[13] This was also clearly illustrated in our study of infants and children with bacterial meningitis in which those patients who received dexamethasone before ceftriaxone therapy had significantly improved CSF inflammatory indices, and TNFα, IL-1β and platelet activating factor (PAF) values in CSF were significantly reduced by 75%, 90% and 85%, respectively.[14] Long-term outcome was significantly better in those given dexamethasone compared to those who received placebo.[14] Presently it is unknown whether these data are applicable to management of neonates with bacterial meningitis.

In this communication I have tried to illustrate the setting in which I entered academic life in pediatric infectious diseases. In the late 1960s there were very few clinical investigators interested in the pharmacology of drugs in infants and children; most drugs used at that time in pediatrics had not been adequately evaluated with regard to appropriate dosage and safety, and properly designed and conducted trials to determine efficacy were rare. In 1968 Dr. Harry Shirkey was correct that infants and children were "therapeutic or pharmaceutical orphans."[1] Pediatrics was fortunate that Dr. Sumner Yaffe took the lead at that time to develop clinical pharmacology in this age group, and because of his insight and perspicacity the Food and Drug Administration enacted legislation that encouraged pharmaceutical companies and pediatricians to investigate drugs to ensure their safety and effectiveness in children. This eventuated in the Pediatric Rule in 1994, the Food and Drug Administration Modernization Act in 1997, the Final Pediatric Rule in 1998 and the Better Pharmaceuticals for Children Act in 2002. Perhaps most important was the creation of the PPRU in 1992 as a result of Dr. Yaffe's leadership. I am proud to have played

Table 4. Markers of inflammation in ventricular fluid of infants with gram negative bacillary meningitis who received systemic or systemic plus intraventricular gentamicin therapy (Second Neonatal Meningitis Cooperative Study)[12]

	Systemic Therapy (n = 11)	Systemic Therapy plus 2.5 mg IV (n = 10)	P value
IL-1β (pg/mL)	87 ± 30	5025 ± 1840	.0015
LPS (ng/mL)	22 ± 21	78 ± 64	.023
WBC (per mm^3)	2194 ± 1560	5970 ± 2918	.043
Protein (mg/dL)	109 ± 32	868 ± 194	.007

IL-1β, interleukin-1 beta; IV, intraventricular; LPS, lipopolysaccharide

a small part in the evolution of clinical pharmacologic studies of antimicrobials in infants and children and honored to have my name associated with Dr. Yaffe in this award.

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