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A Clinical Trial of Tobramycin with Pharmacological and Microbiological Studies

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Tobramycin, a new aminoglycoside antibiotic, was shown to be effective in vitro against Pseudomonas, Klebsiella, Staphylococcus aureus, Enterobacter, Proteus mirabilis, indole-positive Proteus, and Escherichia coli. It was shown to produce serum and urine concentrations similar to gentamicin when administered intramuscularly. Preliminary animal studies showed this agent to have nephrotoxic and ototoxic effects similar to gentamicin. In our studies, tobramycin was well tolerated and produced only two transient elevations in serum creatinine and no abnormalities in audiograms and vestibular function tests. It was effective in 10 of 12 susceptible infections, including 2 of 4 with Pseudomonas endocarditis. Tobramycin compared favorably with gentamicin and may be superior in treatment of susceptible Pseudomonas infections.

TOBRAMYCIN is a new stable, water soluble, aminoglycoside antibiotic derived from the Streptomyces tenebrarius. Growing in fermentation medium, this organism produces eight chemically related compounds known collectively as nebramycin. Tobramycin is factor six of this group, and has the greatest activity and spectrum in vitro.

Preliminary studies showed that tobramycin was similar to gentamicin in spectrum and adverse effects. In vitro tobramycin was consistently more active against Pseudomonas strains than gentamicin, and strains resistant to gentamicin were also resistant to tobramycin.

The present investigation of tobramycin was undertaken to determine the following: (1) in vitro activity against gram positive and gram negative organisms from our institution and comparison of selected Pseudomonas strains with gentamicin, (2) serum and urine concentrations in healthy volunteers after single and multiple intramuscular doses, and (3) therapeutic efficacy and toxicity in patients with infections due to susceptible microorganisms.
Materials and Methods

Test Antibiotic: Tobramycin (Eli Lilly and Company) was supplied in 2 ml vials (50 mg/ml), and administered intramuscularly.

For in vitro studies, tobramycin was supplied as standard laboratory solution and in 10 μg discs for disc diffusion tests by Dr. R. S. Griffith of the Lilly Research Laboratories.

Standard laboratory solution and 10 μg discs were also used for gentamicin.

Biological Activity: The minimal inhibitory concentration (MIC) of tobramycin for 24 strains of Pseudomonas, 24 strains of Klebsiella, 21 strains of Staphylococcus aureus, 22 strains of Streptococcus pneumoniae, 24 strains of Enterobacter, 24 strains of Proteus mirabilis, 27 strains of indole-positive Proteus, and 23 strains of Escherichia coli, as well as the clinical isolates from patients treated with tobramycin, was determined by the replica plate method of Steers, Foltz, and Graves on Mueller-Hinton agar containing twofold increments of tobramycin (.195, .39, .78, 1.56, 3.125, 6.25, 12.5, 25, and 50 μg/ml). A 10<sup>-5</sup> dilution of an overnight (18 to 24 hour) culture in Trypticase soy broth was used as the inoculum.

Disc diffusion susceptibility was performed by the standardized disc technique on all test organisms and clinical isolates using Mueller-Hinton agar.

Seventeen strains of Pseudomonas were compared with gentamicin using the same methods.

Volunteer Studies: Ten healthy normal subjects, who gave written voluntary consent, were given 100 mg of tobramycin intramuscularly every 12 hours for seven days. Audiograms, vestibular function tests, and serum creatinine were performed prior to, during, and one week after administration. Additional audiograms and vestibular function tests were performed three months later.

Serum specimens were obtained at 0, ½, 1, 2, 4, 6, and 8 hours after the initial dose. Two-and eight-hour post injection serum specimens were also taken randomly through the seven-day period. The serum was frozen at -20°C until tested. Tobramycin was assayed by the modified cup-plate method with Bacillus subtilis (ATCC 6633) as the test organism. Undiluted (100%) human pooled serum was used as the diluent for the assay of drug in serum.

Urine was collected in three fractions (0-2, 2-4, and 4-6 hour) after the initial intramuscular dose and antibiotic concentration determined in the manner described for serum except .1 molar phosphate buffer, pH 8, was used as the diluent.

Sera and urine from patients treated with tobramycin were similarly tested to determine their respective concentrations. The serum antibacterial titers against the causative organism were also measured.

Clinical Study: The therapeutic efficacy and toxicity of tobramycin was evaluated in adult patients (ages 24-72) with infections susceptible to this agent. Pregnant females were excluded from this study. Individuals with bacteremia, endocarditis, and septic arthritis received an intramuscular dose of 100 mg every eight hours while those with urinary tract infections were given 100 mg every twelve hours.
A Clinical Test of Tobramycin with Studies

Figure 1

Antibacterial Activity: The in vitro studies of tobramycin against the test organisms (Figure 1) showed that the most sensitive organism was Staphylococcus aureus. The mean MIC was .25 μg/ml and the range was .097 to .78 μg/ml. The Streptococcus pneumoniae were resistant. Most gram negative organisms tested were sensitive to 5 μg/ml or less tobramycin, ie, 96% of Pseudomonas, 92% of Enterobacter, 91% of indole-positive Proteus, 100% of Escherichia coli, Proteus mirabilis, and Klebsiella tested.

A regression line was drawn using the method of least squares. Correlation of in vitro tobramycin susceptibility by the standardized disc technique and agar dilution method (Figure 2) along with the results of our clinical trials with tobramycin indicates that the zone diameter of 15 mm or more using a 10 μg tobramycin disc would differentiate susceptible from resistant organisms.

The mean MIC of seventeen strains of Pseudomonas was 2.97 μg/ml (range .024 to 6.25 μg/ml) with gentamicin as contrasted to .51 μg/ml (range 0.24 to .78 μg/ml) with tobramycin. (Table I) At least four times as much gentamicin as tobramycin was required for inhibition of 82% of the strains tested while 12% needed twice the amount. In one strain, the MIC was equivalent for both gentamicin and tobramycin.
Serum Concentration and Urinary Excretion - Volunteer Studies:

Peak serum concentration of tobramycin after the first of a series of 100 mg intramuscular doses in different normal subjects ranged from 2.96 to 6.60 μg/ml with a mean peak level of 4.87 μg/ml. Serum levels of tobramycin at eight hours ranged from .13 to .64 μg/ml, and the mean concentration was .44 μg/ml in the ten subjects studied. (Table II) The serum levels on the last day of administration, or day seven, showed a mean two-hour level of 3.68 μg/ml with range 2.50 to 4.70 μg/ml in nine volunteers tested. This data indicated no evidence of tobramycin serum accumulation on this dosage regimen. One volunteer, age 54, (Subject no. 8, Table II) was dropped from the study group on day six when he developed tinnitus. On that day, his two-hour serum level was 6.40 μg/ml and eight-hour 1.70 μg/ml — distinctly elevated over the remainder of the study group. Also, his serum creatinine rose to 1.6 mg/100 ml on day six of administration and was normal (1.3 mg/100 ml) on recheck 24 hours later. Normal results were obtained in this man and the rest of the study group for audiograms and vestibular function tests prior to, during, and one week and
A Clinical Test of Tobramycin with Studies

### TABLE I
COMPARISON OF THE SUSCEPTIBILITY OF 17 STRAINS OF PSEUDOMONAS TO GENTAMICIN AND TOBRAMYCIN

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gentamicin</th>
<th>Tobramycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disc Diffusion Method</td>
<td>Agar Diffusion Dilution MIC</td>
</tr>
<tr>
<td>CT-248</td>
<td>20 mm 1.56 μg/ml</td>
<td>28 mm .39 μg/ml</td>
</tr>
<tr>
<td>CT-229</td>
<td>22 1.39</td>
<td>29 .195</td>
</tr>
<tr>
<td>CT-232</td>
<td>17 3.125</td>
<td>23 .78</td>
</tr>
<tr>
<td>CT-238</td>
<td>18 3.125</td>
<td>26 .39</td>
</tr>
<tr>
<td>CT-243</td>
<td>20 3.125</td>
<td>28 .39</td>
</tr>
<tr>
<td>CT-246</td>
<td>19 3.125</td>
<td>28 .39</td>
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<td>15 3.125</td>
<td>24 .78</td>
</tr>
<tr>
<td>CT-249</td>
<td>30 .024</td>
<td>30 .024</td>
</tr>
<tr>
<td>CT-239</td>
<td>20 3.125</td>
<td>29 .195</td>
</tr>
</tbody>
</table>

three months after administration. No significant changes in serum creatinine were found in the other subjects.

The mean six-hour urinary excretion of the initial 100 mg intramuscular dose of tobramycin was 43% with a range of 22% to 54%.

**Clinical Results:** Tobramycin was administered to a total of thirteen patients. The dosage of tobramycin was 100 mg intramuscularly every twelve hours in patients with urinary tract infections and 100 mg every eight hours in other infections. One patient with *Pseudomonas* endocarditis and septic arthritis of the knee received 100 mg every six hours. In a few instances other antibiotics were administered prior to tobramycin, but were discontinued when clinical and bacteriological data indicated that an aminoglycoside antibiotic was more appropriate.

### TABLE II
TOBRAMYCIN SERUM LEVELS μG/ML (VOLUNTEERS)

| Day | Hour | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | Ave. |
|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| 1   | 0    | 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00 |
| 1/2 | 1/2  | 3.60| 3.10| 3.20| 4.00| 5.92| 5.92| 5.92| 5.92| 5.92| 5.92| 5.92| 5.92 |
| 1   | 1    | 4.80| 4.20| 3.48| 3.40| 3.80| 3.80| 3.80| 3.80| 3.80| 3.80| 3.80| 3.80 |
| 2   | 2    | 3.84| 3.36| 3.10| 4.50| 2.50| 2.50| 2.50| 2.50| 2.50| 2.50| 2.50| 2.50 |
| 4   | 4    | 1.24| 2.00| 1.53| 2.24| 1.05| 1.05| 1.05| 1.05| 1.05| 1.05| 1.05| 1.05 |
| 6   | 6    | .78 | .61 | .84 | 1.00| .50 | .50 | .50 | .50 | .50 | .50 | .50 | .50 |
| 8   | 8    | .58 | .27 | .39 | .64 | .13 | .26 | .60 | .50 | .48 | .54 | .44 |

A = 28 hr. level
B = 32 hr. level
There were eight patients with infections due to *Pseudomonas aeruginosa* and five due to other gram negative organisms. Five of the eight patients with *Pseudomonas* were cured, including two of four treated for endocarditis, two who had septic arthritis, and one with urinary tract infection and bacteremia.

One endocarditis patient received only tobramycin for six weeks and was cured. One other endocarditis patient remained febrile and bacteremic after five days of tobramycin therapy. Carbenicillin (30 gm daily) and probenecid (2 gm daily) were instituted, and the dose of tobramycin was increased to 100 mg every six hours. These three agents were continued through the remainder of the six weeks of treatment, and this patient was also cured.

The other patients with *Pseudomonas* endocarditis failed. One of these patients was admitted with blood cultures positive for *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Treatment with tobramycin and cefazolin was given for 23 days. Because the patient experienced a rise in serum creatinine, therapy was changed to gentamicin, carbenicillin, and cephalothin. These were given for an additional 16 days. However, fever and *Pseudomonas* bacteremia recurred four weeks later. Six weeks of treatment with gentamicin and carbenicillin, plus tricuspidectomy, resulted in a cure. The other patient with endocarditis, who was not cured with antibiotics, received various treatment programs that included gentamicin and carbenicillin with probenecid, then subsequently tobramycin and carbenicillin with probenecid. He was cured when a tricuspidectomy was performed. *Pseudomonas aeruginosa* was cultured from a vegetation on the valve.

One patient with urinary tract infection and blood culture positive for *Pseudomonas aeruginosa* responded to a 14-day course of treatment.

Two patients (heroin addicts) with acute sternoclavicular arthritis responded to a six-week course of tobramycin that followed surgical drainage of the joint space.

One of the eight patients with *Pseudomonas* infections was admitted for congestive heart failure and respiratory insufficiency secondary to chronic obstructive lung disease. During his hospitalization, he developed a gram negative necrotizing pneumonia. Sputum culture grew *Pseudomonas aeruginosa*. Three days after therapy was instituted, the patient expired. Progress cultures were not obtained and we were unable to evaluate tobramycin in this patient.

There were no failures among the five patients with infections due to other gram negative organisms. Four patients with lower urinary tract infections responded promptly to the agent. Among these infections were two with *Escherichia coli*, one with *Klebsiella*, and one with a mixed culture of *Escherichia coli* and *Klebsiella*. The remaining patient had three blood cultures with *Escherichia coli* in addition to a positive urine culture for the same organism. Twenty-four hours after therapy was initiated, the patient was afebrile. Cultures remained negative.

Serum and urine concentrations and the MIC of organisms cultured from these 13 patients were similar to the in vitro and volunteer studies noted above.

Tobramycin generally was well tolerated in these patients. The injection was essentially non-painful, and there were no local reactions. One patient experienced a rise in serum creatinine to 3.0
A Clinical Test of Tobramycin with Studies

mg/100 ml. This was probably secondary to tobramycin. No abnormalities were seen in pre- and post-treatment and audiograms and vestibular function tests completed on six of fourteen patients.

Discussion

These in vitro studies demonstrated that tobramycin was an effective agent against Pseudomonas, indole-positive Proteus, Proteus mirabilis, Escherichia coli, Enterobacter, Klebsiella, and Staphylococcus aureus. The relative susceptibility of Pseudomonas to gentamicin and tobramycin paralleled each other, but the mean MIC for 17 strains tested was six times greater for gentamicin than tobramycin. Our findings are consistent with earlier published reports comparing these antibiotics.9

Our pharmacology studies with this agent indicated that a 100 mg intramuscular injection gave therapeutic serum and urine levels. Peak mean serum concentration of 4.87 μg/ml and 43% recovery in the urine after six hours are similar to those reported for equivalent doses of gentamicin 10,11, but the peak serum level in our group is somewhat higher than that reported by others for tobramycin.12

Studies in animals showed that tobramycin has potential nephrotoxic and ototoxic effects although the nephrotoxic effects may be less than with equivalent doses of gentamicin.12 In our study, one volunteer experienced tinnitus and associated rise in serum creatinine, while another patient being treated with this agent developed an elevated serum creatinine. Like other aminoglycoside antibiotics, reduction in dosage may be necessary in older patients and those with pre-existing renal disease. Further evaluation in this area should be carried out.

The failure of two patients with Pseudomonas endocarditis was not unexpected, and these data agree with those of Reyes et al where 13 of 23 cases required surgery for unremitting bacteremia.13 In their study, they also cite the need for multiple antibiotics when medical cure was achieved.

Our study showed tobramycin to be an effective agent in the treatment of susceptible microorganisms. In this respect, it appears to be equivalent to gentamicin. Since it is more potent in vitro, it may be superior in the treatment of Pseudomonas infections.

Acknowledgments

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References


