Tobramycin Inactivation by Carbenicillin, Ticarcillin, and Piperacillin

HIDETOSHI KONISHI, MITSUYOSHI GOTO,* YOSHINORI NAKAMOTO, IKUYOSHI YAMAMOTO, AND HAJIME YAMASHINA

Department of Hospital Pharmacy, Tenri Hospital, Tenri City, Nara 632, Japan

Received 30 November 1982/Accepted 18 February 1983

The in vitro and in vivo inactivation of tobramycin by carbenicillin, ticarcillin, or piperacillin was investigated by the enzyme immunoassay method in clinically employed dosages. After the addition of an 80-mg dose of tobramycin to 4- to 5-g doses of a penicillin in 100 ml of 0.9% saline or distilled water, the degradation profile of tobramycin appeared to follow a biexponential pattern of decay. Remarkable losses (30 to 40%) of tobramycin combined with carbenicillin or ticarcillin were observed within 1 h, as compared with the later decline. The combination of tobramycin with piperacillin was least inactivating. When the admixture of tobramycin with carbenicillin or piperacillin used in the in vitro study was infused to six volunteers over 1 h, the observed maximum concentrations of tobramycin were on the average 66 and 74% for carbenicillin and piperacillin, respectively, of that observed after tobramycin alone was given. In contrast, the value obtained for tobramycin in combination with piperacillin was close to 90% of the control value. The elimination half-lives of tobramycin combined with the penicillins were slightly shorter than those of tobramycin alone, indicating that the interaction occurs even in patients with normal renal function.

Because of synergistic activity (1, 12, 23), tobramycin is frequently used in combination with penicillins such as carbenicillin, ticarcillin, or piperacillin to treat severe infections caused by gram-negative bacilli, particularly Pseudomonas aeruginosa. These combinations have been used even though there is an interaction between aminoglycosides and penicillins in vitro which results in a significant loss of aminoglycoside antibacterial activity (2–5, 8, 13, 15, 17, 18, 20). It is generally accepted that this inactivation is clinically significant in patients with poor renal function in whom excretion of the drug is delayed (2, 4, 5, 10, 20) or when both drugs are mixed in the same intravenous infusion bottle (8, 10, 18).

Pieper et al. recently showed that this inactivation might be due in part to in vitro degradation before and during microbiological assay (19). In most previous studies, assays of aminoglycosides were carried out some time after blood collection. The in vitro first-order inactivation rate constants of tobramycin reported by radioimmuno assay are fairly small at room temperature, ranging from approximately 0.005 to 0.03 h⁻¹ (3, 8, 15). However, these studies were carried out at relatively low concentrations. No data have been compiled on the degradation pattern of an infusion mixture, such as 4 to 5 g of a penicillin and 80 mg of tobramycin in 100 ml of solution, used in the treatment of infections. In view of the degradation in the infusion mixture on the basis of reported inactivation rate constants (3, 8, 15), there seems to be no objection to giving the two drugs through the same bottle if administered immediately after mixing.

Using the instantly measurable enzyme multiplied immunoassay technique, we have set out to (i) describe the in vitro inactivation kinetics of tobramycin by the penicillins in the infusion bottle employed clinically, (ii) estimate the effect of the in vitro degradation on serum level when given concurrently through the same bottle, and (iii) elucidate the disposition kinetics of tobramycin coupled with the penicillins in humans with normal renal function. Results are recorded in this report.

MATERIALS AND METHODS

In vitro study. Carbenicillin (lot 10562; Taito Pfizer, Tokyo, Japan), and ticarcillin disodium (lot MF4490; Fujisawa Pharmaceutical, Osaka, Japan) at doses of 5 g each were dissolved in 100 ml of distilled water, and piperacillin sodium (lot KF733A; Toyama Kagaku, Tokyo, Japan) at a dose of 4 g was dissolved in 100 ml of 0.9% saline. After mixing with tobramycin sulfate (lot TB1050; Shionogi, Osaka, Japan) at a dose of 80 mg, the mixtures were incubated at room temperature. A portion of each specimen was assayed at 0 (immedi-
ately after mixing), 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h for residual tobramycin. The stability of tobramycin at the same dose in 100 ml of distilled water and in 100 ml of 0.9% saline were simultaneously assessed.

In vivo study. Six adult male volunteers participated in this study after informed written consent had been obtained. Their ages ranged from 28 to 59 years (mean ± standard deviation, 40.3 ± 13.4 years), and their body weights ranged from 45 to 65 kg (57.0 ± 7.1 kg). None had taken any drug during the month before the investigational period. Results of prestudy physical examination and pre- and post-laboratory findings were normal. In view of concern over the nephrotoxicity of aminoglycosides, it is noteworthy that their serum creatinine values ranged from 0.9 to 1.3 mg/100 ml (1.1 ± 0.1 mg/100 ml) during the study period.

Each of the volunteers received an 80-mg dose of tobramycin (1.42 ± 0.18 mg per kg of body weight) dissolved in 100 ml of 0.9% saline intravenously over 1 h with an automatic infusion pump (type AIP-2H; Atom Apparatus Co., Tokyo, Japan). Each also received the mixture of tobramycin and penicillins, prepared as described above, with 3-week intervals separating the respective doses. Tobramycin was added to the penicillins just before infusion. Treatments were randomized and delivered in a crossover fashion. Subjects fasted overnight before each study; food was also withheld for 4 h after dosing.

Blood samples (5 ml each) were drawn from an arm vein at 0 (immediately after intravenous infusion ceased), 0.1, 0.25, 0.5, 1, 2, 3, 4, 5, and 7 h after each administration. Samples were always withdrawn from the arm contralateral to that used for infusion. Serum was separated as soon as clotting had occurred.

The postinfusion serum concentration data (Ct) for tobramycin were fitted to the following biexponential equation (6, 7): 

\[ C_t = R \cdot e^{-\alpha t} + S \cdot e^{-\beta t}, \]

where \( R \) and \( S \) are coefficients (micrograms per milliliter), \( \alpha \) and \( \beta \) (per hour) denote the hybrid constant, and \( t \) denotes the postinfection time. Based on the serum level when tobramycin alone was infused, the steady-state volume of distribution was directly calculated by the method of Wagner (22), with the above equation modified by application of the Loo and Riegelman correction (11).

Since we have previously demonstrated that the kinetic parameters for aminoglycosides are fairly reproducible within the same individuals (7), the tobramycin inactivation rate constant (\( K_i \)) by the penicillins in serum was estimated by the following equation: 

\[ K_{i,CB} \ (or \ K_{i,TI} \ or \ K_{i,T1}) = \frac{\beta - \beta_{CB} \ (or \ \beta_{TI} \ or \ \beta_{T1})}{\beta_{CB} \ (or \ \beta_{TI} \ or \ \beta_{T1})}, \]

where \( K_{i,CB} \), \( K_{i,TI} \), and \( K_{i,T1} \) are the values of \( K_i \) and \( \beta_{CB} \), \( \beta_{TI} \), and \( \beta_{T1} \) are the values of \( \beta \) when combined with carbenicillin, ticarcillin, and piperacillin, respectively.

The best values of the various pharmacokinetic parameters were calculated by the least-squares method in conjunction with a Toshiba model TOSBAC 40 computer (Tokyo Shibaura Electric Co., Ltd., Tokyo, Japan).

**Tobramycin assay.** Tobramycin concentrations were determined by the enzyme multiplied immunoassay technique (Syva Corp.) in triplicate. For measurement of concentrations below 1 \( \mu g/ml \), only single dilutions were performed (9). Known tobramycin control standards were measured periodically throughout the assay procedure to confirm the accuracy of the assay.

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**RESULTS**

Figure 1 shows the time course of tobramycin levels for mixtures containing 40 to 50 mg of penicillin per ml. The residual concentration of tobramycin in combination with carbenicillin, ticarcillin, and piperacillin was 63.2, 71.6, and 89.2%, respectively, of the initial concentration at 1 h, 32.4, 50.0, and 83.8%, respectively, at 8 h, and 20.5, 38.3, and 78.2%, respectively, at 24 h. Although the inactivation rate by piperacillin is in accordance with those obtained in other combinations at low concentrations (3, 8, 15), marked losses of tobramycin levels were observed with the mixture of carbenicillin and ticarcillin. The rapidity of degradation within 1 to 2 h seems surprising in comparison with the later decay curves, including that with piperacillin. In particular, loss of activity exceeding 50% occurred as early as 2 h with carbenicillin.

The mean serum levels of tobramycin after a 1-h intravenous infusion of the 80-mg dose with or without penicillin are shown in Fig. 2. The kinetic data of tobramycin alone, calculated by a two-compartment model methodology, showed that tobramycin is eliminated the biexponentially from serum, with a \( \beta \) of 0.331 ± 0.052 h\(^{-1}\) and a steady-state volume of distribution of 0.190 ± 0.032 liters/kg, values which are consistent with those of previous work (16). Although the elimination profile of tobramycin alone appeared to be almost identical to that of tobramycin combined with piperacillin, the tobramycin serum levels with carbenicillin or ticarcillin were fairly lower than those in the control study over 8 h. Postinfusion maximum concentrations (\( C_{max} \)) are shown in Table 1. The \( C_{max} \) values obtained with the penicillins were lower than those obtained without the penicillins. Combining tobramycin with carbenicillin or ticarcillin resulted in a marked decrease of \( C_{max} \) for tobramycin, whereas piperacillin had less influence on this value. The faster the in vitro inactivation rate, the lower the \( C_{max} \) value. This in vivo observation corresponds to the in vitro degradation profile of tobramycin by the penicillins, indicating that the in vitro degradation directly affects the in vivo serum level and that the physical mixing of these drugs, especially in combination with carbenicillin or ticarcillin, should be avoided.

Elimination rate constants and inactivation rate constants for tobramycin are also shown in Table 1. The values of \( \beta_{CB} \), \( \beta_{TI} \), and \( \beta_{P} \) are slightly lower than those of \( \beta \), indicating that the inactivation could occur in patients with normal renal function as well as in patients with renal functional insufficiency. The \( K_{i,CB} \), \( K_{i,TI} \), and \( K_{i,P} \) values ranged from 0.012 to 0.122 (mean, 0.047), 0.018 to 0.095 (mean, 0.042), and 0.006 to 0.036 (mean, 0.021) h\(^{-1}\), respectively. Thus, the
TOBRAMYCIN INACTIVATION BY PENICILLINS

FIG. 1. Tobramycin (800 μg/ml) inactivation by carbenicillin (50 mg/ml), ticarcillin (50 mg/ml), and piperacillin (40 mg/ml) at room temperature. ○, Tobramycin alone in 0.9% saline; ●, tobramycin alone in distilled water; △, tobramycin with carbenicillin in distilled water; □, tobramycin with ticarcillin in distilled water, ×, tobramycin with piperacillin in 0.9% saline.

K_1^{CB} values were close to the constant for gentamicin inactivation by carbenicillin reported in patients with impaired renal function (2, 4).

DISCUSSION

It has not been reported that aminoglycosides are inactivated biexponentially by penicillins. In recent work by Edwards and Schentag concerning such interaction (3), however, the decay profile for tobramycin appeared to follow a biexponential decline rather than a monoeXponential decline. Unfortunately, these authors do not discuss the degradation reaction. This inactivation is regarded as the consequence of the formation of a biologically inactive amide resulting from the nucleophilic opening of the β-

FIG. 2. Tobramycin serum level (mean ± standard deviation) after intravenous infusion of tobramycin alone (●), tobramycin with a 5-g dose of carbenicillin (○), tobramycin with a 5-g dose of ticarcillin (△), and tobramycin with a 4-g dose of piperacillin (□). The same 80-mg dose of tobramycin was infused over a 1-h period.
lactam ring (8, 20), implying that the intact penicillin molecule is responsible for the interaction and that the inactivation could be prevented if β-lactamase were immediately added. Contrary to this postulated mechanism, Kradjan et al. have reported that the gentamicin activity in plasma samples to which β-lactamase is added immediately does not significantly differ from those to which β-lactamase had not been added (10). Similar data had also been shown previously by McLaughlin et al. (13). There are, moreover, no reports stating that cephalosporins having a β-lactam ring in the molecule have the capacity to inactivate aminoglycosides in vitro. These studies indicate the existence of other inactivation process(es), except for the proposed mechanism. Our own data, exhibiting multieponential decay, might result from such an unknown mechanism.

The difference in electron density among the carbonyl groups in the β-lactam ring of these penicillins may not be significant because none of the side chains of the penicillins appear to influence the distant carbonyl group from the standpoint of the molecular model. Piperacillin has a large side chain, such as the 4-ethyl-2,3-dioxo-1-piperazinocarboxamido group, in comparison with the other penicillins, and the activity of piperacillin itself was maintained at more than 80% for at least 24 h even at 35°C (21). The relative stability of tobramycin in combination with piperacillin can be attributed to steric hindrance. In addition, the different solvent may also contribute to the relative stability of tobramycin in combination with piperacillin, because the loss of tobramycin in distilled water is most remarkable (20).

More recent studies in which human serum is used have demonstrated that tobramycin inactivated by piperacillin showed a decline profile similar to those reported in other combinations (17, 18). Although these observations appear to conflict with ours, the inactivation rate by piperacillin observed in our in vitro study is in good agreement with those reported in other combinations (3, 8, 15). Since the rate of the interaction depends on temperature (13, 15), concentration (5, 8, 17, 18, 20), and solvent (20), it is likely that, under our experimental conditions, carbenicillin and ticarcillin inactivated tobramycin at a faster rate than that reported previously. In any case, further investigation is necessary to elucidate the detailed mechanism of the interaction between aminoglycosides and penicillins.

The view is widely held that such interaction does not take place in patients with normal renal function if the two drugs are given separately (2, 10, 17, 18, 20). Farchione, on the other hand, reported that unpredictable increases of aminoglycoside serum levels occur immediately in patients with normal renal function when penicillin is stopped (5). Similar results have also been shown by Murillo et al. (14). In passing, it should be stated that the elimination rate constant (β) consists of the renal elimination rate constant, (Kf), the nonrenal elimination rate constant (Knr), and the inactivation rate constant (Ki) when combination therapy is performed (β = Kf + Knr + Ki) (4). The constitution of this equation should not change essentially, even in patients with normal renal function, insofar as the two drugs coexist in serum. However, the values of renal elimination rate constant occupy the larger portion of β in subjects with normal renal function, the values of nonrenal elimination rate constant being very small. Therefore, it seems to be difficult to determine the Ki value in subjects with normal renal function by the analytical method reported previously. We now believe that only in renally impaired patients, in whom the renal contribution to the plasma clearance has become so small as to be negligible, can Ki values be estimated with the methodology used in previous studies.

The dosing regimen of tobramycin taken into account in the in vivo Ki is recommended for its safety and effectiveness, particularly in patients with severe renal function who are being treated with tobramycin combinations.

ACKNOWLEDGMENTS

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### TABLE 1. Observed maximum concentrations, elimination rate constants, and inactivation rate constants after infusion of tobramycin with carbenicillin, ticarcillin, or piperacillin over 1 h

<table>
<thead>
<tr>
<th>Combination</th>
<th>C_{max} (µg/ml)</th>
<th>β (h^{-1})</th>
<th>K_i (h^{-1})^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobramycin alone</td>
<td>7.7 ± 1.2</td>
<td>0.331 ± 0.052</td>
<td>0.047 ± 0.039</td>
</tr>
<tr>
<td>Tobramycin + carbenicillin</td>
<td>5.1 ± 0.7</td>
<td>0.378 ± 0.061</td>
<td>0.042 ± 0.026</td>
</tr>
<tr>
<td>Tobramycin + ticarcillin</td>
<td>5.7 ± 0.8</td>
<td>0.372 ± 0.051</td>
<td>0.021 ± 0.011</td>
</tr>
<tr>
<td>Tobramycin + piperacillin</td>
<td>6.8 ± 0.7</td>
<td>0.351 ± 0.052</td>
<td></td>
</tr>
</tbody>
</table>

^a Numbers represent means ± standard deviations. The doses used were: tobramycin, 80 mg; carbenicillin, 5 g; ticarcillin, 5 g; and piperacillin, 4 g.

^b Calculated by the equation K_{i,CB} (or K_{i,T} or K_{i,P}) = β - β_{CB} (or β_{Ta} or β_{Pa}).
LITERATURE CITED


22. Wagner, J. G. 1976. Linear pharmacokinetic equations allowing direct calculation of many needed pharmacokinetic parameters from the coefficients and exponents of polyexponential equations which have been fitted to the data. J. Pharmacokinet. Biopharm. 4:443–467.

ERRATUM

Tobramycin Inactivation by Carbenicillin, Ticarcillin, and Piperacillin

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Department of Hospital Pharmacy, Tenri Hospital, Tenri City, Nara 632, Japan

Volume 23, no. 5, p. 653, abstract, line 9: “piperacillin” should read “ticarcillin.”
Page 653, abstract, line 12: “piperacillin” should read “ticarcillin.”