Inactivation of Gentamicin by Penicillins in Patients with Renal Failure

F. RICHARD ERVIN, WARD E. BULLOCK JR.,* AND CHARLES E. NUTTALL
Division of Infectious Diseases, Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky 40506

Received for publication 22 December 1975

Kinetics of gentamicin inactivation by carbenicillin and ticarcillin were studied in vitro and in 17 patients with renal failure. In vitro, the half-life of carbenicillin in human serum at 37°C is longer (19.2 ± 0.7 h) than ticarcillin (7.2 ± 0.6 h). Thus, incubation of gentamicin with equal concentrations of ticarcillin or carbenicillin results in greater inactivation of aminoglycoside activity by the latter. If concentrations of the two penicillins are held equal by repetitive addition, rates of gentamicin inactivation are the same.

The serum half-life of gentamicin in patients serving as their own controls was significantly reduced by administration of either penicillin. After carbenicillin, the half-life decreased from 46 ± 8 h to 22 ± 3 h (P < 0.02). The constant for inactivation of gentamicin (k) by carbenicillin was 0.02 h⁻¹. The results indicate that gentamicin requirements are underestimated by methods currently employed to calculate dosage for patients with renal failure who receive carbenicillin concurrently. Adjustment of gentamicin dosage in such cases by application of the k for gentamicin is suggested.

The antibiotic combination of an aminoglycoside and a penicillin or cephalosporin is frequently administered to provide broad antibacterial therapy for patients with sepsis. In particular, the combination of gentamicin and carbenicillin is employed for treatment of infections caused by Pseudomonas aeruginosa. Although clinical proof is lacking for the superiority of combined over single agent therapy in these infections, the simultaneous use of both antibiotics is favored by many clinicians (2).

Most, if not all, penicillins have the capacity to inactivate gentamicin in vitro at a rate that is dependent upon temperature, concentration, and medium composition (20, 22). The reaction is thought to occur via nucleophilic opening of the β-lactam ring, probably by the methylamino group of gentamicin with concomitant formation of a biologically inactive amide (3, 27; P. G. Daniels, personal communication). As a rule, penicillins must be present for several hours in concentrations at least fivefold greater than those of gentamicin before inactivation of the latter can be demonstrated (20).

In clinical practice, such inactivation is observed most frequently when gentamicin and a penicillin are inadvertently mixed in the same bottle of parenteral solution, which is then hung for slow intravenous (i.v.) infusion. Of the penicillins administered concurrently with gentamicin, carbenicillin is most frequently given in sufficient dosage to achieve peak serum levels of at least 100 µg/ml, thereby exceeding concentrations of the aminoglycoside by more than fivefold. Nevertheless, little or no destruction of aminoglycoside activity can be demonstrated in patients with normal renal function if the two antibiotics are given by separate routes. In patients with renal failure, however, the serum half-lives (t1/2) of gentamicin and carbenicillin are greatly prolonged (13, 28). Thus, repetitive administration of carbenicillin to patients requiring reduction of gentamicin dosage because of renal failure may lead to clinically significant inactivation at body temperature. To explore this question more fully, we have utilized an in vitro model to study the kinetics of gentamicin inactivation by carbenicillin and ticarcillin, a new penicillin more active against P. aeruginosa (26). In addition, we have studied the decay of serum gentamicin activity with and without concomitant administration of carbenicillin and ticarcillin in 17 patients with severe renal failure.

MATERIALS AND METHODS

Serum antibiotic assay. Gentamicin reference powder (kindly supplied by Schering Corp., Kenilworth, N.J.) was desiccated, weighed, and solubilized in 0.2 M phosphate buffer, pH 8.0, in a concen-
tration of 1,000 μg/ml. Aliquots of this stock solution were kept frozen at −20 °C until use. They were then filtered (0.22-μm pore size) and diluted in pooled human serum to make up working standards in concentrations of 10, 5, 2.5, 1.25, and 0.62 μg/ml. Carbencillin and ticarcillin reference powder (kindly supplied by Beecham Pharmaceuticals, Bristol, Tenn.) was weighed and solubilized in 0.2 M phosphate buffer (pH 7.0) in a concentration of 10,000 μg/ml. Aliquots of this stock solution were frozen at −70 °C until use. After filtration (0.22 μm), the appropriate penicillin was diluted in pooled human serum to provide working standards in final concentrations of 200, 100, 50, 25, and 12.5 μg/ml.

Serum levels of gentamicin were measured by the method of Alcid and Seligman (1) utilizing Staphylococcus epidermidis (ATCC 27626), an assay organism resistant to 500 μg of carbencillin or ticarcillin per ml. Wells containing the five gentamicin standards and the unknown serum samples were arranged circumferentially and equidistant from the center of the assay plate. By placing pairs of standards or unknowns in opposite wells, the error introduced by variations in agar depth and convexity of plastic petri dishes was minimized. In similar manner, serum levels of carbencillin and ticarcillin were assayed as total antibiotic activity by an agar overlay-well technique using P. aeruginosa (ATCC 10490) as the assay organism. Unknown sera found to contain greater than 200 μg/ml were diluted in serum and reassayed. Each standard or unknown was assayed in quadruplicate, and data from the assay of standards were used to calculate a reference curve by means of the least squares method of linear regression (25). The concentration of each unknown was then derived from the reference curve. The error of the assay for both gentamicin and the penicillins, expressed as the coefficient of variation, was ±0.13.

Study patients. Seventeen patients with end-stage renal disease volunteered to participate in the study by giving informed consent to an investigational protocol approved by the University of Kentucky Human Investigation and Studies Committee. The creatinine clearances (Ccr) of 14 patients ranged from 0.9 to 8.0 ml/min; two patients were surgically anephric and one was functionally anephric. All required dialysis to prevent uremia. Sera separated from serial bleedings of 1.0 ml were immediately frozen and stored at −70 °C until assay. Tubes of serum standards containing 10 μg of gentamicin per ml and 100 μg of carbencillin or ticarcillin per ml were also frozen, stored at −70 °C for 6 months, and then assayed; there was no loss in activity of either the gentamicin or the penicillins.

Calculation of antibiotic t1/2. The t1/2 of gentamicin and of each penicillin in serum was computed by the formula: \[ t_{1/2} = \frac{\ln 2}{K} \] where \( K \) is the slope of the linear regression line, determined by the method of least squares for serum concentration, \( \\log(100) \), plotted against time (12). Clinically, i.v. administration of gentamicin is followed by a rapid phase of distribution, the t1/2 of which is approximately 28 min (5). Subsequent elimination obeys the rule of first-order kinetics (5) and, therefore, the serum t1/2 of gentamicin in vivo was calculated from the monoexponential portion of the decay curve (12). The significance of differences between groups was determined by the Student t test (25).

RESULTS

In vitro studies. Preliminary experiments were performed to assess the stability of carbencillin and ticarcillin in human serum at body temperature. In three studies, 10 ml of sterile serum containing 500 μg of either antibiotic per ml was incubated at a pH of 7.3 to 7.4 for 70 h. Aliquots were removed at frequent intervals and assayed immediately for residual penicillin activity. The decay in activity of both ticarcillin and carbencillin proceeded in a monoexponential fashion throughout the 70-h period. However, the mean t1/2 of ticarcillin was 7.2 ± 0.6 (standard error of the mean) h, whereas the t1/2 of carbencillin was 19.2 ± 0.7 h. Thus, ticarcillin appears to be the less stable in human serum.

An experiment was next performed to measure the inactivation of gentamicin in serum at 37 °C by adding equal amounts of ticarcillin or carbencillin. At zero time, the initial concentration of gentamicin in test serum was 10 μg/ml, alone or in combination with 500 μg of ticarcillin or carbencillin per ml. The decay curves of each penicillin in serum containing 10 μg of gentamicin per ml are illustrated in Fig. 1A, where it can be seen that the loss of ticarcillin over 70 h of incubation is again more rapid. The corresponding decay curves of gentamicin activity when present in serum alone or in combination with either penicillin are shown in Fig. 1B. Clearly, gentamicin was inactivated more extensively by carbencillin since only 35% of the initial gentamicin activity remained at 70 h, whereas 91% of the initial activity remained in a control tube containing gentamicin alone. Gentamicin inactivation by ticarcillin was less marked with 53% recovery of the initial concentration. Undoubtedly, the latter observation is explained by the more rapid decay of ticarcillin in serum. With either penicillin, however, the rate of gentamicin inactivation slowed considerably as the respective concentrations fell below levels of 50 to 100 μg/ml.

To compensate for the more rapid decay of ticarcillin in serum, a second in vitro model was used whereby each penicillin was added repetitively to separate tubes of serum to maintain the concentration at levels ranging from 50 to 100% of the concentration at zero time. The initial concentration of both penicillins was 250 μg/ml. The concentration of gentamicin at zero
time was 10 μg/ml, thereby approximating the serum level achieved in vivo after administration of a loading dose to patients with renal failure. At the expiration of each penicillin \( t_{1/2} \) an aliquot of serum was assayed for residual penicillin and gentamicin activity. Twenty-five microliters of ticarcillin or carbenicillin in concentrated solution was immediately added to the remaining serum, an aliquot of which was also assayed for "peak" levels of penicillin. This procedure was repeated throughout a test period of 70 h. As a control, phosphate buffer was added repetitively to the tube of serum containing gentamicin alone. The results of this experiment showed that, when serum levels of ticarcillin or carbenicillin were maintained between 125 and 250 μg/ml for 70 h at 37 C, 61% of the gentamicin was inactivated by carbenicillin and 66% by ticarcillin. Conversely, the loss of gentamicin activity in control tubes was less than 10% of the initial concentration. Thus, the rates of gentamicin inactivation by ticarcillin and carbenicillin are equal when serum concentrations of the two antibiotics are maintained at equal levels.

The results of an experiment in which "high" and "low" levels of ticarcillin were maintained by repetitive addition to serum containing gentamicin are shown in Fig. 2. The high levels ranged from 115 to 285 μg/ml (Fig. 2A) and, thus, were equivalent to serum concentrations that may be expected in patients with renal failure who are treated with 6 g of carbenicillin or ticarcillin per day (8, 13). The low levels ranged from 52 to 147 μg/ml (Fig. 2B). This range is achieved in the serum of patients with end-stage renal failure who receive 2 to 3 g of ticarcillin per day (11). The rate of gentamicin inactivation is proportional to the concentration of ticarcillin (Fig. 2C). At low levels,
ticarcillin inactivated 42% of the initial gentamicin concentration within 70 h. At high levels, it inactivated 69% of the gentamicin as compared with a 10% loss in control tubes.

**Patient studies.** To one group of eight patients in renal failure gentamicin was administered i.v. over 20 min in a loading dose of 1.0 mg/kg of body weight (minimum 60 mg) by an infusion pump at the termination of a hemodialysis. The first blood sample was drawn 10 min after completion of the infusion (0.5 h). Additional samples were obtained at 1, 4, and 8 h. Subsequently, samples were drawn at 8-h intervals until the next dialysis (approximately 40 h). Upon completion of dialysis, 1 mg of gentamicin per kg was again infused. Immediately thereafter, ticarcillin was given i.v. over 10 min in a dosage of 40 mg/kg. Additional ticarcillin doses of 13 mg were given i.v. at 8 and 16 h after the initial infusion. One patient also received a dose at 24 h and two received additional doses at 24 and 32 h.

The decay curves of serum gentamicin concentration are presented in Fig. 3. The mean peak level of gentamicin measured after infusion was 6.6 ± 0.8 μg/ml at 0.5 h, and the highest individual concentration measured was 10.0 μg/ml (not shown). In the control study, the serum level at 1 h was 4.3 ± 0.4 μg/ml (Fig. 3A). After readministration of gentamicin, the levels at 1 h were somewhat higher (5.2 ± 0.5 μg/ml) since there was a small amount of residual activity (1.2 ± 0.5 μg/ml) in serum after the interim dialysis. By the 24th h after infusion of gentamicin alone or in combination with ticarcillin, the mean levels had fallen 2.5 ± 0.3 μg/ml. At 40 h, the gentamicin level was 1.7 ± 0.3 μg/ml in the control study and 1.5 ± 0.3 μg/ml in the presence of ticarcillin. The mean serum concentration of ticarcillin at 0.5 h after infusion was 169 ± 17 μg/ml. At 24 h it was 95 ± 18 μg/ml, and at 40 h it was 56 ± 18 μg/ml.

The Ccr of each patient and the serum t½ of gentamicin before and after infusion of ticarcil-
lin are presented in Table 1, group A. The mean $t_{1/2}$ of gentamicin in the control study was 33 ± 6 h; however, after infusion of ticarcillin, the $t_{1/2}$ was shortened significantly to 23 ± 3 h ($P < 0.05$).

Higher doses of gentamicin and carbenicillin were given to a second group of nine patients according to the protocol outlined above. Each patient received gentamicin in a dosage of 1.5 mg/kg of body weight. Upon termination of the control period by interim dialysis, the gentamicin dosage was repeated with the addition of carbenicillin, 75 mg/kg. Supplemental doses of carbenicillin (25 mg/kg) were given at 8 and 16 h; two patients received a third dose at 24 h. In this group, the mean peak serum level of gentamicin measured at 0.5 h was 10.0 ± 0.7 µg/ml. The highest individual level was 14.2 µg/ml and in only two patients did it exceed 12 µg/ml. In the control study, the serum level 1 h after gentamicin administration was 6.3 ± 0.6 µg/ml and it was 8.0 ± 0.7 µg/ml after readministration (Fig. 3B). At 24 h, the level was 4.1 ± 0.4 µg/ml in the control study and, in the presence of carbenicillin, it was 3.6 ± 0.3 µg/ml. By the 40th h, the levels had declined to 3.1 ± 0.3 µg/ml and 2.1 ± 0.2 µg/ml, respectively. The mean serum level of carbenicillin 0.5 h after infusion was 308 ± 41 µg/ml. At 24 h it was 160 ± 20 µg/ml, and at 40 h it was 107 ± 15 µg/ml.

The $C_{cr}$ of each patient and the $t_{1/2}$ of gentamicin in serum before and after infusion of carbenicillin are presented in Table 1, group B. In this study, the $t_{1/2}$ of gentamicin in serum was sharply reduced from 46 ± 8 h to 22 ± 3 h ($P < 0.02$) after infusion of carbenicillin.

Inactivation constant ($k_r$) for gentamicin. Clinical application of the above findings is facilitated by calculation of the $k_r$ for gentamicin. Although very small amounts of gentamicin may be sequestered (17) or metabolized (28), its elimination essentially follows the rule of first-order kinetics (5), where the serum concentration after zero time is proportional to $e^{-k_t}$ (12). Assuming a single compartment model, the distribution volume of gentamicin was calculated as $V_D = dose/serum concentration$ at zero time as extrapolated from the terminal portion of the slope of the decay curve for each patient (5, 12). The $V_D$ was 26.0 ± 2.0% of body weight, which is close to previously reported values (28). Total body elimination ($K$) may be considered as the sum of all individual routes of elimination. Therefore, $K = k_{nr} + k_r + k_i$, where $k_{nr}$ is nonrenal elimination, $k_r$ is renal elimination, and $k_i$ is elimination through inactivation by a penicillin. The $k_{nr}$ and $k_r$ were
assumed to be constant because each patient served as his own control. Since the \( k_i \) in control studies was zero, it follows therefore that \( \Delta K = k_i \). In this study, the \( k_i \) for gentamicin in patients receiving carbenicillin (75 mg/kg) was 0.016 ± 0.003 h\(^{-1}\). In addition, we calculated the \( k_i \) from data supplied by other authors who have studied gentamicin inactivation by carbenicillin (8; L. Riff, personal communication). The \( k_i \) calculated from this data base was 0.029 ± 0.004 h\(^{-1}\). Thus, the average \( k_i \) for gentamicin = 0.02 h\(^{-1}\) in patients treated with the regimen recommended for use in renal failure. The \( k_i \) for gentamicin in patients with renal failure to whom we gave ticarcillin in lower dosage (40 mg/kg) was 0.01 h\(^{-1}\).

**DISCUSSION**

The present study demonstrates that therapeutically dosages of carbenicillin or ticarcillin significantly reduce the serum \( t_{1/2} \) of gentamicin when given concurrently to patients with renal failure. Although preliminary studies in vitro had indicated that carbenicillin was the more potent inactivator of gentamicin, this proved to be an artifact arising from its greater stability in serum at 37 C. We are not aware of any published literature pertaining to the stability of either penicillin in serum at 37 C. However, such information is available for the older penicillins. As shown by Eagle (10), the in vitro loss of penicillin G in human serum is 9%/h at 37 C, equal to a \( t_{1/2} \) of 9.5 h. The reasons for the shorter \( t_{1/2} \) of penicillin G and ticarcillin in serum as compared with the \( t_{1/2} \) of carbenicillin are unknown. However, it is possible that penicillin G and ticarcillin are more susceptible to the effects of trace metal ions in serum which may hydrolyze the \( \beta \)-lactam ring to penicilloic acid, either directly or indirectly through penicilloic acid formation (15). Indeed, the relative instability of ticarcillin in serum may partially account for its shorter \( t_{1/2} \) in patients with renal failure (13, 20) as well as increased excretion of the penicilloic acid metabolite in the urine of normal subjects (6).

In an in vitro model designed to mimic the conditions of repetitive antibiotic dosing to patients with renal failure, both ticarcillin and carbenicillin were maintained at equal concentrations in serum containing gentamicin. The rates of gentamicin degradation under these conditions were equal. Thus, given in equivalent dosage to patients with renal failure, ticarcillin and carbenicillin can be expected to shorten the \( t_{1/2} \) of gentamicin by approximately the same order of magnitude. Evidence supporting this hypothesis has been published recently by Davies et al. (8), who observed reductions in the \( t_{1/2} \) of gentamicin averaging 56 and 66%, respectively, in patients with renal failure who were receiving 6 g of either ticarcillin or carbenicillin per day by i.v. administration. Likewise, Riff and Jackson (22) noted a reduction of approximately 60% in the \( t_{1/2} \) of gentamicin among four patients to whom carbenicillin and gentamicin were administered concomitantly in a dosage ratio of 80:1.

In our studies, maintenance of serum ticarcillin levels within a range considered to be "therapeutic" (56 to 169 \( \mu \)g/ml) resulted in considerably less inactivation of gentamicin in vitro and in vivo than observed with a therapeutic range of carbenicillin (107 to 308 \( \mu \)g/ml). Although the reduction of gentamicin \( t_{1/2} \) by ticarcillin in patients with renal failure was statistically significant, the actual mean reduction was only 26%. Furthermore, there was essentially no change in the \( t_{1/2} \) of gentamicin among three of eight patients given ticarcillin. Since it is likely that ticarcillin will be administered in dosages that are 30 to 50% lower than the recommended dosages of carbenicillin (8), the degree of gentamicin inactivation by this antibiotic in clinical use will probably be less than that experienced with carbenicillin.

Unfortunately, the documentation of gentamicin inactivation in vivo by carbenicillin and ticarcillin further complicates the already complex pharmacology of aminoglycoside therapy for patients in renal failure. Gentamicin serum concentrations are quite unpredictable after intramuscular administration (14) and, even after i.v. administration, wide scatter in the serum antibiotic concentration has been reported (18). Likewise, we have noted that serum levels of gentamicin 1 h after infusion of 1 mg/kg ranged from 2.4 to 6.0 \( \mu \)g/ml; after infusion of 1.5 mg/kg, levels ranged from 4.1 to 9.1 \( \mu \)g/ml. In addition, the effects of hemodialysis (5), binding of gentamicin to erythrocytes (23), and variations in residual renal function (5) all serve to challenge the clinician who elects to treat serious infections with gentamicin.

Satisfactory responses to gentamicin therapy in *Pseudomonas* sepsis caused by susceptible organisms have been correlated with peak serum concentrations of at least 4 \( \mu \)g/ml (21), although successful treatment of pneumonia may require peak levels of 8 \( \mu \)g/ml (19). Therefore, it seems reasonable to maintain serum gentamicin levels within the range of 4 to 8 \( \mu \)g/ml for treatment of life-threatening sepsis caused by susceptible *Pseudomonas* strains in patients with renal failure (24). To do so, actual
measurement of serum gentamicin levels is highly desirable. However, in the event that facilities for aminoglycoside assay are not available, one of several methods for modifying gentamicin dosage must be utilized. Unfortunately, all of these methods fail to account for the fact that the inactivation of gentamicin by carbenicillin, $k_i = 0.02 \text{ h}^{-1}$, is nearly three times greater than the elimination by nonrenal mechanisms ($k_{nr} = 0.007 \text{ h}^{-1}$) in the anuric patient (9). Thus, serum levels of gentamicin are likely to be suboptimal in patients with renal compromise who also receive carbenicillin.

Treatment regimens for patients with renal failure in which a constant dosage of gentamicin is given at varying intervals commonly employ 1 to 2 mg/kg as the loading dose (7). This dose is repeated every third $t_{1/2}$ or, alternatively, one-half the loading dose may be given at each $t_{1/2}$. If hemodialysis is required, a loading dose of gentamicin is given followed by 1 mg/kg after each dialysis. We can suggest no precise mechanism by which these schedules may be supplemented if carbenicillin is to be given concurrently. However, Riff and Jackson (22) have observed that, in patients receiving hemodialysis, additional doses of gentamicin (0.3 to 0.5 mg/kg) given 24 to 36 h after the postdialysis dose have boosted the serum concentration to an adequate therapeutic level.

Gentamicin therapy in renal failure may also be modified by giving variable doses at constant intervals. Such methodology is embodied in a nomogram formulated by Chan et al. (4) in an attempt to avoid a severe peak and valley effect in the serum levels of gentamicin. It also tends to underestimate the gentamicin dosage required for patients with renal compromise who are treated concurrently with carbenicillin. However, this nomogram does list the elimination constant for gentamicin (designated as $K_z$) through a wide range of renal function and, therefore, it may be corrected to provide more adequate dosage of gentamicin for patients treated concurrently with carbenicillin. In theory, such a correction can be made as follows. First, the value of $K_z$ corresponding to the $C_{cr}$ is read from the nomogram. As the nomogram is difficult to read for a $C_{cr}$ of less than 2 ml/min, a $K_z$ value of 0.01 may be used in such cases since $K_z = k_{nr} = 0.007 \text{ h}^{-1}$ in patients who are anuric. If the patient is receiving carbenicillin, the $k_i$ for gentamicin (0.02) can then be added to $K_z$ to give $K'_z$. In the event of ticarcillin therapy, a $k_i$ of 0.01 would be added to $K_z$. The "corrected" amount of gentamicin per kilogram of body weight to be administered every 8 h may then be read from the nomogram by drawing a horizontal line from $K'_z$ to the dosing schedule. Clinical evaluation of this suggested correction for the nomogram of Chan et al. (4) is currently in progress within our hospital.

Although this investigation has focused upon the inactivation of gentamicin, it should be emphasized that other aminoglycosides, including tobramycin, may be inactivated by the penicillins in vitro (18; B. Lynn and A. Jones, Proc. Int. Congr. Chemother., 7th, Prague, p. 701-705, 1971). Thus, partial inactivation of any aminoglycoside may occur in vivo when it is given to patients with renal failure in conjunction with high-dose penicillin therapy. Newer penicillins with greater potency against $P. \text{ aeruginosa}$ are being synthesized and, in the future, it may well be possible to treat with a penicillin in dosages low enough to avoid aminoglycoside inactivation completely. Until that time, however, it is necessary to remain cognizant of the problem as typified by current experience with gentamicin.

ACKNOWLEDGMENTS

We wish to express our appreciation to Catherine Fine for editorial assistance and to Donald Conrod, Thomas Foster, and Louis Dittert for helpful critical comments. It is a pleasure also to acknowledge the valuable assistance of nurses in the hemodialysis unit.

This work was supported in part by a grant from the Beecham Pharmaceutical Company, Bristol, Tenn.

LITERATURE CITED

GENTAMICIN INACTIVATION BY PENICILLINS

1011


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