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International Journal of Pharmaceutics 153 (1997) 115–121

international journal of pharmaceutics

The effect of tazobactam on the pharmacokinetics and the antibacterial activity of piperacillin in dogs

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Received 20 December 1996; received in revised form 7 April 1997; accepted 10 April 1997

Abstract

Tazobactam (Tazosyn®) is a novel β-lactamase inhibitor belonging to a class of penicillanic acid sulfones. Tazobactam was developed to be used in combination with piperacillin against β-lactamase producing microorganisms. The current study was conducted to determine the effect of tazobactam on the pharmacokinetics and the antibacterial activity of piperacillin in dogs. Three groups of animals consisting of three beagle dogs per group were used for the study. The animals were administered a single I.V. dose of tazobactam (40 mg/kg), piperacillin (320 mg/kg) or the combination in a ratio of 1:8 (tazobactam: piperacillin). Blood samples were drawn at different time intervals. The serum bactericidal titers (SBT) were determined against a plasmid-mediated β-lactamase producing strain of *Eschericia coli* (LSU-80-8). Serum concentrations of both compounds were determined by high performance liquid chromatography.

The mean serum bactericidal titers of the combination was 1:16 against this piperacillin resistant strain of *E. coli*, 2 h after dosing as compared to less than 1:2 when piperacillin was given alone at the same dose. This indicates that serum concentrations greater than 187 μg/ml of piperacillin (SBT $<$ 1:2) were required to kill 99.9% of the piperacillin resistant *E. coli* inoculum (10^6 CFU/ml) when piperacillin was given alone. However, when piperacillin was administered in combination with tazobactam, concentrations as low as 7 μg/ml of piperacillin and 2 μg/ml of tazobactam were sufficient to exhibit the same bactericidal activity. These results indicate an in-vivo synergistic effect of tazobactam on the piperacillin activity at this dosing ratio which lasted for approximately 5 h after dosing. The coadministration of tazobactam did not appear to affect the pharmacokinetic parameters of piperacillin. However, the elimination of tazobactam was significantly inhibited when coadministered with piperacillin, resulting in a reduction of the clearance (3.5 vs. 7.5 ml/min per kg) and prolongation of the half-life (43 vs. 31 min). © 1997 Elsevier Science B.V.

Keywords: Piperacillin; Tazobactam; Pharmacokinetics; Serum bactericidal titer; Minimum inhibitory concentrations; β-lactamase inhibitors

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PII S0378-5173(97)00103-8

1. Introduction

Tazobactam, a new penicillanic acid sulfone derivative which acts as an irreversible inhibitor of many bacterial β-lactamases, was developed for coadministration with piperacillin. Tazobactam has been shown to be active against chromosome-encoded penicillinases and broad spectrum β-lactamases, plasmid mediated β-lactamases and chromosome-encoded cephalosporinases produced by *Enterobacteriaceae* (Gutman et al., 1986; Jacobs et al., 1986). Piperacillin is a broad-spectrum penicillin (Verbist, 1978) that is susceptible to hydrolysis by different β-lactamases. A number of β-lactamase inhibitors have been combined with other broad-spectrum penicillins, such as clavulanic acid with amoxicillin (Hunter et al., 1980) and sulbactam with ampicillin (English et al., 1978), which are protective of the active β-lactam. When piperacillin is combined with tazobactam, a significant synergy against β-lactamase-producing strains, such as *Staphylococcus aureus*, *Haemophilus influenza*, *Bacteroides* spp. and many of *Enterobacteriaceae*, was demonstrated (Eliopoulos et al., 1989; Kuck et al., 1989; Fass and Prior, 1989).

In-vitro studies have shown that less than 4 μg/ml of tazobactam is required to decrease the MICs of piperacillin from the resistant to the susceptible category for 90% of the *Escherichia coli*, *Bacteroides fragilis*, and *Proteus* strains tested; less than 1 μg/ml is sufficient for *Staphylococcus aureus*, *Haemophilus influenzae* and *Branhamella catarrhalis* (Eliopoulos et al., 1989). Tazobactam was proved to be highly effective in vivo in reducing the piperacillin doses required to protect mice from infections induced with β-lactamase producing bacteria (Kuck et al., 1989). This combination in ratios of 4:1 and 8:1 (piperacillin to tazobactam) was effective in reducing the MICs of piperacillin from the resistant (>128 μg/ml) to the susceptible (16 μg/ml) or moderately susceptible (32 to 64 μg/ml) range for *Escherichia coli*, *Klebsiella pneumonia*, *Proteus* spp., *Providencia* spp., *Morganella* spp., *Salmonella* spp. and *Shigella* spp. (Kuck et al., 1989).

Correlation between the pharmacokinetics of both tazobactam and piperacillin to the in-vivo activity were not performed for any of the previous studies. Therefore, it was necessary to characterize the pharmacokinetic and pharmacodynamic profiles of tazobactam and piperacillin in different animal models and to determine the drug–drug interaction between the two compounds.

In the current study, we evaluated the effect of tazobactam on the pharmacokinetics and antimicrobial activity of piperacillin in male beagle dogs following intravenous administration of this combination in a ratio of 8:1 of piperacillin to tazobactam.

2. Materials and methods

2.1. Dosing solution

Tazobactam (YTR-830) and Piperacillin were obtained from Lederle Laboratories (Pearl River, NY). Tazobactam was dissolved in sterile water for injection containing an equimolar concentration of sodium bicarbonate (28 mg $NaHCO_3$/100 mg of tazobactam). The dosing solution was prepared immediately before dosing at a concentration of 145 mg/ml. Each dog received 40 mg/kg of the drug. Piperacillin was dissolved also in sterile water for injection and sonicated for 5 min in a Branson Sonifier. The final concentration of the solution was 470 mg/ml. Each dog was administered 320 mg/kg of the drug in approximately 5 ml of the solution.

Tazobactam and piperacillin solutions were mixed to form homogenous mixture. The final concentration of piperacillin and tazobactam were 530 and 67 mg/ml, respectively. Each dog received 5–7 ml of this dosing solution in which a ratio of 8:1 of piperacillin to tazobactam was maintained. The dogs were dosed intravenously, with the different treatments, slowly over a 1 min period in the cephalic vein.

2.2. Animals

Nine male beagle dogs, weighing 8.6–11.8 kg were used for this study. Dogs were divided into three groups, with three animals in each group. Group I, II and III received tazobactam (40 mg/kg), piperacillin (320 mg/kg) and the combination, respectively. The dogs were housed individually in stainless steel metabolism cages in windowless, air

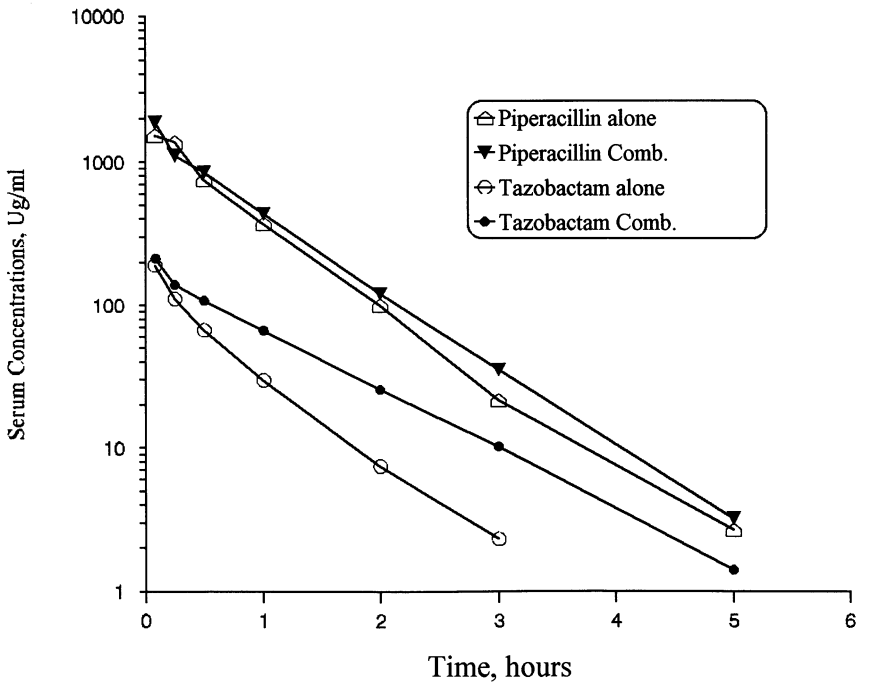

Fig. 1. Mean serum concentrations of piperacillin and tazobactam following intravenous administration of each compound alone and in combination.

conditioned facilities, that are illuminated for 12 h per day. All dogs were fasted 16 h prior to and for 6 h after dosing. Water was available ad libitum.

2.3. Sample collection

Blood samples were obtained from the jugular vein and collected in red top vacutainers at 0.0, 0.08, 0.25, 0.5, 1, 2, 3, 5 and 8 h after dosing. Immediately following sampling, blood samples were centrifuged at 4°C and the serum was harvested and divided into two separate tubes for both bactericidal titers determination and HPLC analysis.

2.4. Sample analysis

2.4.1. Serum bactericidal titers

The serum bactericidal titer for the dog serum samples collected at 1, 2, 3, and 5 h after dosing was determined using the standard NCCL (National Committee for Clinical Laboratory Standards) guidelines. A strain of *Escherichia coli* (LSU-80-8); that produces characterized plasmid-mediated β-lactamase enzyme (Kuck et al., 1989) and is piperacillin-resistant; was used as the indicator organism. Titration of serum was done in a microtiter system by using a 1:1 mixture of Mueller–Hinton broth and normal human serum as the diluent (Reller and Stratton, 1977). The inoculum concentration was 10^6 CFU/ml and the sampling for bactericidal determination was conducted by subculturing 10 μl of each well onto drug-free agar. The criterion for the bactericidal titer was a 99.9% killing of the original inoculum (Pearson et al., 1980). Results were expressed as the mean bactericidal titer at each given time after dosing.

2.4.2. HPLC assay of piperacillin and tozobactam

The remaining serum samples were kept frozen at -70°C until analyzed for unchanged drug. The concentrations of each compound in serum were determined using a gradient elusion, reversed-phase, high-performance liquid chromatography (HPLC) method with ultraviolet detection (Ocampo et al., 1989). Briefly, serum samples were mixed with potassium benzyl peni-

Table 1
Mean(± S.D.) pharmacokinetic parameters following I.V administration of single dose of piperacillin (320 mg/kg), tazobactam (40 mg/kg) or the combination of both antibiotics in beagle dogs

Parameters	$t_{1/2}$ (min)	$AUC_{0-\infty}$ (μg h/ml)	CL (ml/min per kg)	V_d (l/kg)	MRT (h)
Piperacillin					
Alone	34.5 ± 0.4	1041 ± 110	5.2 ± 0.6	0.26 ± 0.02	0.78 ± 0.02
Combination	37.0 ± 5.9	1216 ± 189	4.1 ± 0.5	0.23 ± 0.04	0.86 ± 0.06
Tazobactam					
Alone	31.0 ± 4.5*	91 ± 21*	7.5 ± 1.5*	0.33 ± 0.04	0.71 ± 0.12
Combination	43.0 ± 7.6	184 ± 27	3.5 ± 0.3	0.24 ± 0.06	1.1 ± 0.08

* Statistically significant ($P<0.05$).

cillin (as internal standard) dissolved in 0.05 M sodium phosphate buffer (pH 6.0). Acetonitrile was added for protein precipitation and the samples were vortexed for 30 s followed by centrifugation at 2500 rpm for 10 min. The supernatants were decanted and 2 ml of methylene chloride were added, and the tubes were vortexed, centrifuged and the upper aqueous layer was transferred to the auto sampler vial inserts.

2.5. Data analysis

2.5.1. Pharmacokinetic analysis

The pharmacokinetic parameters (using the HPLC data) for the unchanged tazobactam and piperacillin were calculated by model independent methods. The elimination rate, β was determined from the log-linear regression of the terminal portion of the serum concentrations versus time curve. The area under the serum concentration time curve (AUC_{0-t}) was calculated by the linear trapezoidal method for the pre-peak data and the logarithmic trapezoidal method for the post-peak data. Extrapolation to infinity was made using the equation:

$$AUC_{(t-\infty)} = C_{t/\beta}$$

where C_t is the last data point.

The area under the first moment curve from time zero to infinity (AUMC), mean residence time (MRT), steady state volume of distribution (V_{dss}) and systemic clearance (CL) were calculated using the following equations:

$$MRT = AUMC_{(0-\infty)}/AUC_{(0-\infty)}$$

$$AUMC_{(0-\infty)} = AUMC_{(0-t)} + t \cdot C_{t/\beta} + C_{t/\beta^2}$$

$$V_{dss} = dose \cdot MRT/AUC_{(0-\infty)}$$

and

$$CL = dose/AUC_{(0-\infty)}$$

An unpaired Student's t-test was used to determine the significant difference between the pharmacokinetic parameters of tazobactam and piperacillin when given alone and in combination.

2.5.2. The bactericidal activity curve

Results were expressed as the mean serum bactericidal titer (titers are defined as the dilution of serum that killed more than 99.9% of the inoculum of 10^6 CFU/ml of the test organism) for each drug regimen at any given time after dosing. Synergy was defined as at least four-fold dilution difference between the titers obtained with piperacillin alone and the titers obtained with piperacillin–tazobactam combination at each sampling time.

The reciprocal of the bactericidal titers for each compound and the combination were plotted against time for each individual dog. The area under the bactericidal activity (AUBC) curves were calculated by the trapezoidal rule as an attempt to use the AUBC as a measure of in-vivo synergism.

Unpaired Student's t-test was used to determine if the difference between AUBC of the sum of each compound was statistically significant from the AUBC of the combination.

Table 2
Serum bactericidal titers and area under the serum bactericidal activity curves for piperacillin, tazobactam and the combination against *E. Coli* LSU-80-8

Compound	Animal no.	Sampling time (h)				AUBC
		1	2	3	5	
Piperacillin	574 856	<1:2	<1:2	<1:2	<1:2	9
	576 051	<1:2	<1:2	<1:2	<1:2	9
	585 181	<1:2	<1:2	<1:2	<1:2	9
Mean ± S.D.		<1:2	<1:2	<1:2	<1:2	9 ± 0.0
Tazobactam	536 831	<1:2	<1:2	<1:2	<1:2	9
	501 557	<1:2	<1:2	<1:2	<1:2	9
	749 460	<1:2	<1:2	<1:2	<1:2	9
Mean ± S.D.		<1:2	<1:2	<1:2	<1:2	9 ± 0.0
Piperacillin/tazobactam	633 402	1:16	1:16	1:4	<1:2	40
	582 000	1:64	1:16	1:8	<1:2	94
	584 177	1:32	1:16	1:4	<1:2	50
Mean ± S.D.		1:37	1:16	1:5	<1:2	61.3 ± 28.7

Titer is defined as: the dilution of serum that killed more than 99.9% of the inoculum of 100 CFU/ml of test organism. AUBC, area under the bactericidal titer reciprocal-time curve calculated by the trapezoidal rule.

3. Results

The mean serum concentrations of piperacillin and tazobactam given alone and in combination are depicted in Fig. 1. As shown in the Fig., the elimination of piperacillin was not affected by coadministration of tazobactam. However, piperacillin significantly decreased the elimination rate of tazobactam when given in combination as presented in Table 1. The mean half-life ($t_{1/2}$) of tazobactam was significantly longer ($P < 0.05$) when given in combination than when administered alone (43 ± 7.6 vs. 31 ± 4.5 min). Coadministration with piperacillin also reduced the clearance of tazobactam significantly ($P < 0.05$) from 7.5 ± 1.5 to 3.5 ± 0.3 ml/min per kg. The mean residence time of tazobactam was significantly longer ($P < 0.05$) when coadministered with piperacillin (0.71 ± 0.1 vs. 1.1 ± 0.08 h). This inhibitory effect of piperacillin on the elimination of tazobactam was documented before in human (Wise et al., 1991). The serum bactericidal titers and the area under the serum bactericidal activity curves (AUBC) for each compound are summarized in Table 2 and represented graphically in Fig. 2. The mean AUBCs for piperacillin and tazobactam administered alone were 9.0 ± 0.0. However, the mean AUBCs for the combination was 61.3 ± 28.7, which was significantly greater than the sum of the AUBC for each of the compounds given alone. The use of the AUBC for measurement of the pharmacodynamics of the antimicrobials allows for a meaningful correlation of the in-vitro activity of the drugs with the in-vivo pharmacokinetic disposition. The serum bactericidal titer of the combination was 1:16 against the piperacillin-resistant strain of *E. coli* 2 h after drug administration as compared to less than 1:2 for piperacillin when given alone at the same dose level as shown in Fig. 2. Therefore, the coadministration with tazobactam resulted in a significant increase in the serum bactericidal titer (eight-fold) of piperacillin against that strain of *E. coli*.

From the HPLC data, the bactericidal titers of 1:16 (at 2 h after dosing) corresponds to a concentration range of 5–21 μg/ml piperacillin and 1–3 μg/ml of tazobactam when given in combination. However, a mean serum concentration of greater than 187 μg/ml of piperacillin alone is needed for the same bactericidal effect. The concentrations of different drugs associated with the titers is esti-

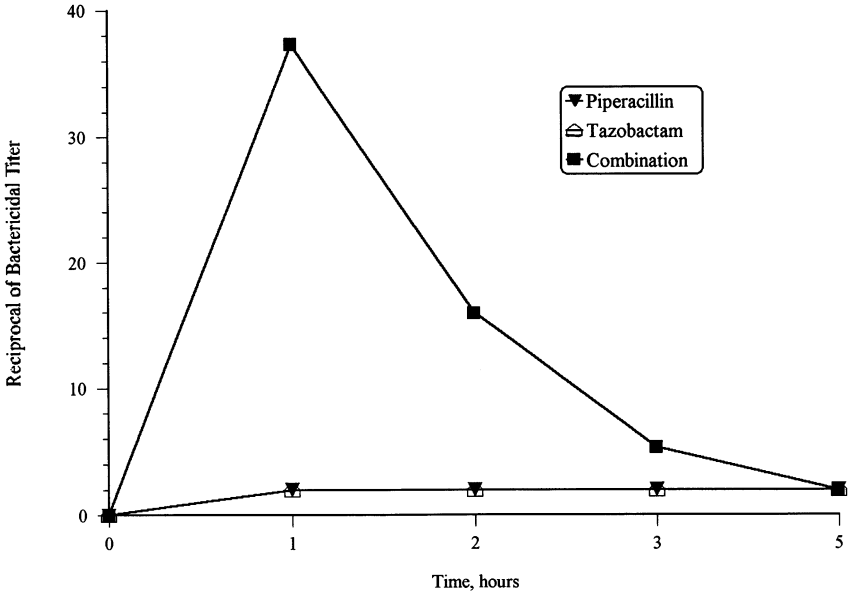

Fig. 2. Area under the bactericidal titer reciprocal-time curve for piperacillin, tazobactam and the combination against piperacillin resistant *E. Coli* following intravenous administration of each compound alone and in combination.

mated by dividing the concentrations obtained from the HPLC assay for each sample by the titer. These results agree with the in-vitro tests (Kuck et al., 1989) using the same strain which have shown that a concentration range of 4–16 μg/ml of piperacillin and 0.5–2 mg/ml of tazobactam is required to kill 99.9% of the inoculum with this strain when given in combination, however a concentration of greater than 256 mg/ml of piperacillin alone is required for the bactericidal effect.

4. Discussion

The purpose of the present investigation was to evaluate the pharmacokinetic profile and the efficacy of the parenterally administered piperacillin used alone and in combination with the new β-lactamase inhibitor tazobactam in the dog. The efficacy was measured by determination of the serum bactericidal titer (SBT) of each antibiotic alone and in combination for each serum sample taken following the intravenous infusion in the dog. The area under the bactericidal titer was used as a measure of the in-vivo synergy between the two compounds over the whole duration of activity. In addition, synergy was also defined by a greater than four-fold dilution difference between the titers of piperacillin alone and the combination at each sampling time.

The major advantage of this method in prediction of synergism over the in-vitro testing are that it accounts for the individual pharmacokinetic parameters of each animal as well as other parameters such as protein binding (Van der Auwera and Klastersky, 1990a), formation of active metabolises and the influence of serum at clinically relevant concentrations (Van der Auwera and Klastersky, 1990b).

The pharmacokinetic parameters determined in the dog in the current study were similar to human pharmacokinetic parameters (Ganes et al., 1991) which makes the dog an excellent model for tazobactam pharmacokinetics and pharmacodynamics. Since the primary route of elimination of tazobactam is via the kidney and its clearance in dogs exceeds the glomerular filtration rate ($\sim$3 ml/min per kg), these results suggest the possibility that tazobactam is excreted in part by tubular

secretion. Piperacillin has been proven to be eliminated by tubular secretion (Batra et al., 1979). Therefore, the inhibitory effect of piperacillin on tazobactam (53% reduction in systemic clearance and 39% increase in elimination half life) could be due to competitive inhibition of the active transport processes involved in tubular secretion. This inhibitory effect has been seen in human studies (Sorgel and Kinzing, 1993).

In conclusion, the use of serum bactericidal titers to evaluate the efficacy of piperacillin against β-lactamase producing bacteria proved to be very advantageous over in vitro testing. This ex-vivo method account for the individual pharmacokinetic parameters at the clinically relevant serum concentrations. In addition, the calculation of the AUBC can be used to quantitate the overall activity throughout the duration of activity especially in case of synergy.

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