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Therapeutic Equivalence (TE) of 5 Generic Products (GP) of Cefotaxime (TAX) Compared with the Original Compound (OC) in the Neutropenic Mouse Thigh Infection Model (NMTIM)

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ABSTRACT

Background: Contrary to accepted dogma, recent animal model data with GP demonstrated that pharmaceutical equivalence (PE) does not predict TE for penicillin G, ampicillin, oxacillin, amikacin, and lincomycin. Here, we report PE and TE of 5 GP of TAX legally marketed in Colombia.

Methods: PE was determined by microbiologic assays using Antibiotic Medium 1 as seeding agar and *S. aureus* ATCC 6538p as testing organism, comparing their standard curves against the OC by curve fitting analysis (CFA). MIC / MBC by broth microdilution against *E. coli* SIG-1 and *P. aeruginosa* ATCC 27853 were compared by Kruskal-Wallis test. For the NMTIM, we used 6 week-old, 25±2 g MPF female mice of the strain Udea:ICR(CD-1) infected with *E. coli* SIG-1. Primary pharmacodynamic parameters (PDP) E_{max}, ED_{50}, and Hill's slope were calculated by least squares nonlinear regression (NLR) applied to the sigmoid dose-response model and used to compute secondary PDP bacteriostatic dose (BD) and the doses needed to kill 1 (1LKD) and 2 logs of bacteria (2LKD). TE was determined comparing each PDP as well as whole NLR curves.

Results: 3 GP available for testing failed PE; 2 had greater concentration than the OC (up to 146-149%; P≤0.0375) and 1 had different potency. Despite of this, MIC / MBC of all 5 GP were not different compared with the OC (P=0.1437). Mice had $10^{7.37-8}$ CFU/g before treatment during 24h with TAX 0.586-150 mg/kg/day divided q1h. At the end of therapy, untreated controls had $10^{9.23-9.43}$ CFU/g (24h growth=1.8-1.9 log_{10} CFU/g). Even though all 3 tested GP failed PE, in vivo efficacy of 5 GP was no different compared with the OC: Emax = 4.25-5.09 vs 4.65 log_{10} CFU/g; BD = 0.9-1.9 vs 1.67 mg/kg; 1LKD=2.1-4.8 vs 4.0 mg/kg (P>0.1018).

Conclusion: In vivo efficacy of TAX GP was no different from the OC. However, GP were not " equivalent" because they had significantly greater concentration of active principle or different potency respect to the OC.

INTRODUCTION

In the past decades, concern about reducing health care cost has led to attempts to increase the dispensing of generic drug products. In fact, state anti-substitution laws were modified around the world to permit or even mandate generic substitution. Today, demonstration of pharmaceutical equivalence -and bioequivalence for pharmaceutical forms other than intravenous– is enough to presume therapeutic equivalence [1]. We argue that, in the case of infectious diseases, such assumption could be wrong, because it overlooks the complex pharmacological relations between host, drug and microorganism. The presence of the third factor, the bug, places infectious diseases in a different therapeutic dimension, because it involves the attack to a living agent inside the patient.

In the last version of ICAAC, we presented data using the animal model to demonstrate that pharmaceutical equivalence does not predict therapeutic equivalence for penicillin G, ampicillin, oxacillin, amikacin, and lincomycin. Here, we challenge again this dogma by comparing the bactericidal efficacy of generic products with the innovator cefotaxime, using the neutropenic murine thigh infection model (NMTIM).

MATERIALS AND METHODS

Bacteria, media, and antibiotics. Experiments were performed with *Escherichia coli* SIG-1, a clinical strain with intermediate resistance to ampicillin (MIC=16 mg/L) and ampicillin/sulbactam (MIC=8 mg/L). It was cultured in Mueller-Hinton broth (MHB) and agar (MHA) for susceptibility tests, and in Trypticase Soy broth (TSB) and agar (TSA) for in vivo studies (Becton Dickinson, Sparks, MD). *Pseudomonas aeruginosa* ATCC 27853 was used as quality control organism for all susceptibility tests, as recommended by Clinical Laboratory Standards Institute (CLSI). *Staphylococcus aureus* ATCC 6538p was the testing organism for microbiological assays performed with Difco™ Antibiotic Medium No. 1. The antibiotics used in all studies included 5 generic products (Table 1) and the original compound (Claforan®, Aventis Pharmaceutical, Mexico). Antibiotics were bought from local drugstores as needed and prepared at required concentrations following the manufacturer instructions for clinical use. To facilitate data illustration, we used code numbers to replace the manufacturers names.

In vitro susceptibility tests. We determined the MIC and MBC of all products commercially available by broth microdilution, following CLSI criteria (1-3 assays, each by duplicate) [2]. For this study, the only comparator employed in statistical analysis was the original compound. Significance of the difference between geometric means of all products as a group and the comparator was calculated with the Kruskal-Wallis test (KW) followed by Dunn's test to identify the generic products responsible for such difference (Prism 4®, GraphPad Software Inc., San Diego, CA).

Microbiologic assays. The potency and concentration of 3 generic products and the original compound (TAX-innovator) were determined with a standard microbiologic assay using the test strain and media described above, following guidelines of US Pharmacopoeia [3]. Two generic products (TAX-25, TAX-26) were not available at the time of the assays. Drug concentrations (log_{10} mg/L) were plotted against the diameter (mm) of their inhibition zones to produce 10-datapoints standard curves for each product. To prevent inter-assay variation, a special apparatus (giant glass plate) was designed that allowed simultaneous runs of all assays needed for TAX by duplicate. Selected concentrations were 4-256 mg/L; the limit of detection was 4 mg/L. The same researcher measured zone-sizes for all assays using an electronic caliper (Mitutoyo Corp., Kawasaki, Japan). Linear regressions of the results produced standard curves which intercepts (concentration) and slopes (potency) were compared by Curve Fitting Analysis (CFA, Prism 4®, GraphPad Software Inc., San Diego, CA).

MATERIALS AND METHODS (CONT...)

Determination of therapeutic efficacy with the NMTIM. Six-week-old, murine-pathogen-free female Udea:ICR[CD-1] mice, weighting 23-27 g, were rendered neutropenic (0 neutrophils/µL) by intraperitoneal injections of cyclophosphamide 4 days (150 mg/kg) and 1 day (100 mg/kg) before infection. Two hours before starting treatment, ether-anesthetized mice were inoculated with 0.1 mL of a log-phase culture of *E. coli* SIG-1 into each thigh. Cefotaxime treatment ranged from minimal to maximum effect and at least 5 total doses ranging from 0.586 to 150 mg/kg/24h were studied in groups of 10 mice per product. Each 24h total dose was given to subgroups of 2 mice and administered q1h by 0.2 ml SQ injections, trying to optimize the PK/PD index responsible for maximum bactericidal efficacy in mice with normal renal function. Two inoculated but untreated control mice were sacrificed right after inoculation (-2h), at the onset (0h), and at the end of therapy (24h). Treated mice were sacrificed at the end of therapy (24h) and their thighs dissected under aseptic technique, homogenized independently, serially diluted, plated by duplicate on appropriate solid media, and incubated at 37°C for 18 hours under air atmosphere. The limit of detection was 100 CFU/thigh (1 thigh = 1 g); data was registered as log_{10} CFU/g. To determine net antibacterial effect, the number of CFU remaining in the thighs after 24 h of treatment was subtracted from the number of CFU that grew in the thighs of control mice during the same period (24h control CFU/g minus 0h control CFU/g). This way, net bacterial death or growth produces negative or positive values, respectively. A sigmoid dose-response model (E_{max} model) was used to characterize the dose-response relationship.

For statistical analysis and characterization of in vivo antimicrobial efficacy of each product, we applied the sigmoid dose-response model with the Hill equation, and calculated PD parameters (PDP) by least squares nonlinear regression (SigmaPlot 8.0®, SPSS Inc., Chicago, IL). Primary PDP included maximum effect (E_{max}), the dose needed to reach 50% of the Emax (ED_{50}), and slope (N). From these, we computed three secondary parameters portraying more biological sense, specifically, the doses (mg/kg/24h) required in vivo to reach a net bacteriostatic effect (BD) and to kill the first (1LKD) and second (2LKD) logs of bacteria per gram of tissue in 24h. To compare antibacterial efficacy of each generic against the original compound, we employed CFA of their nonlinear regressions under the null hypothesis that the data for both compounds came from the same population, i.e., no difference in effect between generic and innovator (Prism 4®, GraphPad Software Inc., San Diego, CA). In case of violation of normality and parametric assumptions, the non-parametric tests KW followed by Permutation One-Way ANOVA with General Scores (POWAGS) were applied to raw data. If significant differences were found between all generics and the innovator compound, the WMW test was applied to each dataset (generic versus original) to identify the products responsible for such difference (StatXact-5®, Cytel Software Corp., Cambridge, MA). Exact P values were calculated in all cases, and statistical significance accepted when two-sided P<0.05.

RESULTS

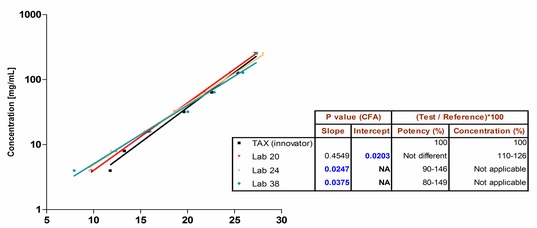
Table 1 lists the products, lot number and country of manufacture. The generic products of cefotaxime for human use were manufactured in Colombia (4) and Portugal (1).

Table 1. Characteristics of the Cefotaxime Products

Product code	Manufacturer	Country of Manufacture	Batch
TAX (innovator)	AVENTIS P	Mexico	DAX0010, FCX3001
TAX-20	LA SANTE	Colombia	0002, O202
TAX-24	BIOCHEMIE (NOVARTIS)	Portugal	200055.3, 203058.3
TAX-25	OPHALAC	Colombia	IMA199-02
TAX-26	PENTACOOP	Colombia	01764
TAX-38	VITALIS (CARLON)	Colombia	C100134, C100283

Figure 1 shows the standard curves of 3 generic products and the comparator. Coefficients of determination (r^2) for these standard curves ranged between 0.9826 and 0.9967. All generics tested were statistically different compared with the original compound (80-149, P < 0.0375). One generic product, TAX-20, had significantly different intercept with similar slope, and the other two generic products, TAX-24 and 38, showed significantly different slopes suggesting modifications in the pharmaceutically active ingredient (PAI) that induce alteration in the biological behavior (potency).

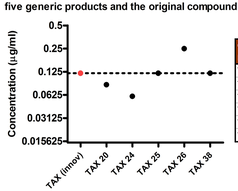
Figure 1. Standard curves of three generic products of cefotaxime compared with the original compound. Microbiological assay with Difco™ Antibiotic Medium 1 as diffusion agar and *S. aureus* ATCC 6538p as test organism. Limit of detection: 4 mg/L



	P value (CFA)		(Test / Reference)*100	
	Slope	Intercept	Potency (%)	Concentration (%)
TAX (innovator)			100	100
Lab 20	0.4549	0.0203	Not different	110-126
Lab 24	0.0247	NA	90-146	Not applicable
Lab 38	0.0375	NA	80-149	Not applicable

The significant differences in quality of the PAI found with the microbiological assay were not detected when efficacy was studied in vitro in terms of MIC, MBC or MBC/MIC ratios of the generic products and the original compound (Figure 2).

Figure 2. Geometric mean values for Minimal Inhibitory (MIC) and Bactericidal (MBC) Concentrations of five generic products and the original compound aganist *E. coli* SIG-1.



Cefotaxime Product Code	MIC±SD (mg/l)	MBC±SD (mg/l)	MIC/MBC ratio	P value (KW)
TAX-innovator	0.120 ± 0.000	0.173 ± 0.000	1.44	0.144
TAX-20	0.085 ± 0.000	0.085 ± 0.000	1.00	
TAX-24	0.060 ± 0.000	0.060 ± 0.000	1.00	
TAX-25	0.120 ± 0.000	0.173 ± 0.000	1.44	
TAX-26	0.250 ± 0.000	0.250 ± 0.000	1.00	
TAX-38	0.120 ± 0.000	0.120 ± 0.000	1.00	

Mice had $10^{7.26-7.58}$ CFU/g (mean both thighs) when treatment with cefotaxime started. At the end of therapy, untreated controls had $10^{9.23-9.43}$ CFU/g (24h growth = 1.85-1.97 log_{10} CFU/g). NLR analysis of TAX data from the animal model fit better with a simple E_{max} model than with the Hill model; the simpler model has two instead of three parameters (E_{max} and ED_{50}) because it fixes the Hill's slope to the unity (N = 1).

In vivo, CFA of the whole NLR found no difference (P = 0.3636) in efficacy (E_{max}) or potency (ED_{50}) between generics and the original product of cefotaxime (Figure 3). Independent analysis of primary (Maximum Effect or E_{max}) or secondary (Bacteriostatic Dose or BD, 1 Log-Kill Dose or 1LKD, and 2 Logs-Kill Dose or 2LKD) pharmacodynamic parameters did not show significant differences either (Table 2).

Figure 3. In vivo efficacy against *E. coli* SIG-1 of 5 generic products of cefotaxime, compared with the original compound in the netropenic mouse thighinfection model (dosing q1h by SQ injections)

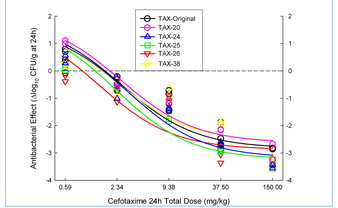


Table 2. Pharmacodynamic parameters of 5 generic products and the innovator of cefotaxime

TAX Product	Pharmacodynamic Parameter (95%CI)			
	Emax	BD	1LKD	2LKD
TAX-Original	4.65 (4.29 to 5.02)	1.67 (1.17 to 2.18)	4.00 (2.86 to 5.14)	11.79 (7.73 to 15.8)
TAX-20	4.74 (3.80 to 5.70)	0.90 (0.09 to 1.70)	2.12 (0.31 to 3.93)	5.93 (0.42 to 11.4)
TAX-24	5.09 (4.52 to 5.65)	1.25 (0.68 to 1.82)	2.80 (1.58 to 4.02)	6.76 (3.79 to 9.73)
TAX-25	4.25 (3.34 to 5.16)	1.84 (0.25 to 3.43)	4.82 (0.89 to 8.76)	21.19 (-13.6 to 56.0)
TAX-26	4.82 (4.17 to 5.47)	1.88 (0.90 to 2.86)	4.38 (2.22 to 6.53)	11.77 (5.41 to 18.1)
TAX-38	4.60 (4.11 to 5.09)	1.66 (0.95 to 2.37)	4.01 (2.39 to 5.64)	12.27 (6.14 to 18.4)
P value (CFA)	0.3636	0.5988	0.4086	0.1018

CONCLUSIONS

- In contrast to findings presented at 44th ICAAC with other antibiotics, we found that TAX generic products do not have pharmaceutical equivalence but are therapeutically equivalent. However, more experiments with greater number of mice and different microorganisms are required to detect smaller differences in efficacy, because our design has 90% power to detect differences greater than 1.0 log_{10} CFU/g, but only 50% power to detect differences of 0.5 log_{10} CFU/g between generics and the original compound.

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