Generic Vancomycin Products Fail In Vivo despite Being Pharmaceutical Equivalents of the Innovator

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Generic versions of intravenous antibiotics are not required to demonstrate therapeutic equivalence with the innovator because therapeutic equivalence is assumed from pharmaceutical equivalence. To test such assumptions, we studied three generic versions of vancomycin in simultaneous experiments with the innovator and determined the concentration and potency of the active pharmaceutical ingredient by microbiological assay, single-dose pharmacokinetics in infected mice, antibacterial effect by broth microdilution and time-kill curves (TKC), and pharmacodynamics against two wild-type strains of Staphylococcus aureus by using the neutropenic mouse thigh infection model. The main outcome measure was the comparison of magnitudes and patterns of in vivo efficacy between generic products and the innovator. Except for one product exhibiting slightly greater concentration, vancomycin generics were undistinguishable from the innovator based on concentration and potency, protein binding, in vitro antibacterial effect determined by minimal inhibitory or bactericidal concentrations and TKC, and serum pharmacokinetics. Despite such similarities, all generic products failed in vivo to kill S. aureus, while the innovator displayed the expected bactericidal efficacy: maximum antibacterial effect (E<sub>max</sub>) (95% confidence interval [CI]) was 2.04 (1.89 to 2.19), 2.59 (2.21 to 2.98), and 3.48 (2.92 to 4.04) versus 5.65 (5.52 to 5.78) log<sub>10</sub> CFU/g for three generics and the innovator product, respectively (P < 0.0001, any comparison). Nonlinear regression analysis suggests that generic versions of vancomycin contain inhibitory and stimulatory principles within their formulations that cause agonistic-antagonistic actions responsible for in vivo failure. In conclusion, pharmaceutical equivalence does not imply therapeutic equivalence for vancomycin.

The World Health Organization (WHO) and all drug regulatory agencies (DRA) support commercialization of generic medicines because they control costs and are irreplaceable therapeutic options in countries lacking the innovator product (10, 41). WHO defines two products as therapeutically equivalent if they are pharmaceutically equivalent and, after administration in the same molar dose, their effects with respect to both efficacy and safety are essentially the same, as determined from appropriate bioequivalence, pharmacodynamic, clinical, or in vitro studies” (41). Parenteral formulations, however, are not required to demonstrate therapeutic equivalence because it “may be considered self-evident” (41).

Such assumptions have never been challenged, but there are reasons to do so for parenteral antimicrobials. First, many antibacterials are secreted in nature by microorganisms, and industrial production of the active pharmaceutical ingredient (API) involves complex processes for biosynthesis, purification, and manufacture, hard to replicate even for the designer (22). Second, two molecules may look similar without being identical, displaying different biological effects (2). Third, makers of generic drugs do not necessarily know the nature, composition, and pharmacological interactions of excipients employed by the innovator to avoid polymorphs of the API (33). Fourth, while most medicines interact with the host only, antimicrobials also confront the invader organism, a dynamic triangle with numerous possibilities of biologic variation (3, 11, 17). Thus, mixing the exactitude of chemistry with the variability of biology could generate unpredictable effects in seriously sick patients, but differences between the generic and the innovator might pass unnoticed among the complexity of infectious diseases in which death is one of the expected outcomes.

Vancomycin (VAN) is a fermentation product of Amycolatopsis orientalis, an actinomycete discovered in 1955 in a dirt sample sent from Borneo to scientists at Eli Lilly (24, 27). Infusion reactions were common initially, but technology led the innovator to a safer product (8). Differences in composition are well known (36) and even advertised (Baxter promotional material; Baxter, Bogota, Colombia), but DRA worldwide support commercialization of vancomycin generics based on scant in vitro data claiming unaltered efficacy (9). After 50 years of unparalleled performance of vancomycin against Gram-positive pathogens, in vitro susceptibility has certainly decreased, and nowadays more than 20 clinical studies blame vancomycin for ineffectiveness and claim success for new, very expensive replacements (15). Without exception, all these studies fail to mention the manufacturer of the vancomycin products involved, despite the fact that most hospitals around

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the globe prefer generics. The present study was designed to fill the gap in evidence regarding in vivo efficacy of vancomycin generics compared with the innovator, spanned from November 2002 to November 2009, and allowed experimentation before and after Eli Lilly sold its brand name for the drug along with the secrets of manufacture (32). The null hypothesis was the assumption made by WHO and DRA, i.e., that pharmaceutical equivalence of vancomycin generics predicts their therapeutic equivalence with the innovator. Our data reject such a hypothesis.

MATERIALS AND METHODS

Antibacterial agents. Antibiotics were bought from local drugstores and prepared following label instructions for clinical use (Table 1). Before 2004, four vancomycin (VAN) products were commercialized in Colombia: the innovator (Vancocin CP; Eli Lilly, Indianapolis, IN) (here called VAN-Lilly) and three generics manufactured by Abbott Laboratories (Chicago, IL), American Pharmaceutical Partners (APP; Los Angeles, CA), and Proclin Ltda. (Laboratorios Northia, Argentina) (here called VAN-Abbott US, VAN-APP, and VAN-Proclin, respectively). By November 2004, Eli Lilly terminated vancomycin production and sold its brand name to several manufacturers worldwide (32), a deal that generated these changes in the Colombian market: Baxter (Deerfield, IL) started commercialization of Vancocin CP (20) (here called VAN-Baxter), the vancomycin from Abbott in Chicago gave way to a product manufactured in France (here called VAN-Abbott France), VAN-APP was discontinued (APP, press release, 2003, and P. J. Vollmerhaus, ViroPharma, press release, 2004), and VAN-Proclin remained unchanged. By 2008, Abbott introduced additional changes, restarting manufacture in Chicago and commercialization under the brand name Hospira (here called VAN-Hospira). We kept enough provision of frozen at 10°C.

TABLE 1. Characteristics of vancomycin products

<table>
<thead>
<tr>
<th>Vancomycin product</th>
<th>Form</th>
<th>Label</th>
<th>Batch/lot no.</th>
<th>Manufacturer</th>
<th>Importer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lilly (innovator)</td>
<td>0.5 g powder for i.v. injection</td>
<td>Vancocin CP</td>
<td>A050370, A048213, A014744</td>
<td>Eli Lilly &amp; Compañía de Mexico SA de CV</td>
<td>Eli Lilly Interamericana Inc., Bogotá, Colombia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vancocina CP</td>
<td>5MJ42M, 5MT38P, 5MT66 M</td>
<td>Eli Lilly &amp; Company, Indianapolis, IN</td>
<td>Eli Lilly Interamericana Inc., Bogotá, Colombia</td>
</tr>
<tr>
<td>Abbott</td>
<td>0.5 g powder for i.v. injection</td>
<td>Sterile vancomycin hydrochloride, USP</td>
<td>18879Z7, 95826Z27</td>
<td>Abbott Laboratories, North Chicago, IL</td>
<td>Abbott Laboratories de Columbia SA, Bogotá, Colombia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vancomicina IV</td>
<td>19236TB21, 22S26TB21, 83855Z7</td>
<td>Abbott France, France</td>
<td>Abbott Laboratories de Columbia SA, Bogotá, Colombia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vancomicina IV</td>
<td>85739Z7, 03703Z7, 09993Z7</td>
<td>Abbott Laboratories, North Chicago, IL</td>
<td>Abbott Laboratories de Chile Ltda., Santiago, Chile</td>
</tr>
<tr>
<td>APP</td>
<td>0.5 g and 1 g powder for i.v. injection</td>
<td>Vancomycin hydrochloride, USP</td>
<td>121384, 120331, 120740</td>
<td>American Pharmaceutical Partners Inc., Los Angeles, CA</td>
<td>Comedica Ltda., Bogotá, Colombia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vancomicina 500 mg</td>
<td>6679, 8872, 8690, 8441, 11471, 10049</td>
<td>Laboratorios Northia, S.A.C.I.F.I.A., Argentina</td>
<td>Proclin Pharma SA, Bogotá, Colombia</td>
</tr>
</tbody>
</table>

**Materials**

**Antibiotics**

- Antibiotics were bought from local drugstores and prepared following label instructions for clinical use (Table 1).
- Before 2004, four vancomycin (VAN) products were commercialized in Colombia: the innovator (Vancocin CP; Eli Lilly, Indianapolis, IN) (here called VAN-Lilly) and three generics manufactured by Abbott Laboratories (Chicago, IL), American Pharmaceutical Partners (APP; Los Angeles, CA), and Proclin Ltda. (Laboratorios Northia, Argentina) (here called VAN-Abbott US, VAN-APP, and VAN-Proclin, respectively).
- By November 2004, Eli Lilly terminated vancomycin production and sold its brand name to several manufacturers worldwide (32), a deal that generated these changes in the Colombian market: Baxter (Deerfield, IL) started commercialization of Vancocin CP (20) (here called VAN-Baxter), the vancomycin from Abbott in Chicago gave way to a product manufactured in France (here called VAN-Abbott France), VAN-APP was discontinued (APP, press release, 2003, and P. J. Vollmerhaus, ViroPharma, press release, 2004), and VAN-Proclin remained unchanged. By 2008, Abbott introduced additional changes, restarting manufacture in Chicago and commercialization under the brand name Hospira (here called VAN-Hospira). We kept enough provision of VAN-Lilly and generics (VAN-Abbott US, VAN-APP, and VAN-Proclin).

**Antimicrobial agents**

- Antibiotics were bought from local drugstores and prepared following label instructions for clinical use (Table 1).
- Before 2004, four vancomycin (VAN) products were commercialized in Colombia: the innovator (Vancocin CP; Eli Lilly, Indianapolis, IN) (here called VAN-Lilly) and three generics manufactured by Abbott Laboratories (Chicago, IL), American Pharmaceutical Partners (APP; Los Angeles, CA), and Proclin Ltda. (Laboratorios Northia, Argentina) (here called VAN-Abbott US, VAN-APP, and VAN-Proclin, respectively).
**In vivo pharmacodynamics (PD): the animal model.** We used the neutropenic mouse thigh infection model to determine PD of each product. Mice were bred and housed in a murine pathogen-free barrier facility (Micro-Isolator system: Lab Products, Seaford, DE), with free access to sterile water plus vitamin K (Sigma-Aldrich, St. Louis, MO) and sterile mouse chow (Zeigler Bros., Gard- ners, PA). The University of Antioquia Animal Experimental Ethics Committee approved each experimental procedure involving mice. For the model, 6-week-old Udea:ICR(CD-1) females weighing 23 to 27 g were rendered neutropenic by 2 intraperitoneal injections of cyclophosphamide (Cytoxan; BMS, New York, NY), 4 days (150 mg/kg) and 1 day (100 mg/kg) before infection; ≤10 neutrophils/μl were confirmed from infection point to day 4 afterwards (43). Infection was induced by intramuscular inoculation of 100 μl log-phase bacteria in each thigh; treatment started 2 h later and lasted 24 h. At least 5–24 h total doses (24hTD), spanning from no effect to maximum effect, were studied per product. Each 24hTD was given to groups of 2 mice (10 mice to test five 24hTD) and administered by 200-μl subcutaneous injections given every 1 h (q1h). Although the area under the concentration-time curve over 24 h in the steady state divided by the MIC (AUC/MIC ratio) is the pharmacodynamic index that predicts its efficacy, vancomycin is actually a time-dependent antibiotic with prolonged persistent effects. For instance, to maintain maximum kill rates, serum levels should constantly exceed the MIC (12). Since its half-life in the mouse (~30 min) is approximately 12 times shorter than that in humans (~360 min), the q1h dosing schedule was selected to constantly exceed the MIC, as happens with q12h dosing in adult humans (12). Untreated controls were sacrificed in groups of 2 mice right after inoculation (hour –2, to confirm inoculum size) and at the time of starting (hour 0) and ending therapy (hour 24). Treated animals were also sacrificed at hour 24, and their thighs were rendered neutropenic, serially diluted, plated in duplicate, and incubated at 37°C for 24 h. Detection of antibiotic carryover was part of the protocol in every experiment, but it was found only with doses of ≥2,400 mg/kg per day (used once, thighs were washed three times to eliminate vancomycin from tissues; no trace of vancomycin was detected after this procedure, and no signs of carryover effect were seen on plates). After colonies were counted for each thigh, data were stored in an Excel database (Microsoft, Seattle, WA). Each data point in the figures represents the mean of both thighs from one mouse, unless indicated otherwise. The limit of detection was 100 CFU per thigh, and each thigh in this model weighs 1 g; therefore, any thigh with zero colonies was entered in the database as 100 CFU/g.

**Statistical analysis.** All experiments included the innovator (gold standard) and at least one generic product; tests to assess the magnitude and significance of the differences between groups varied according to the parameters involved. Comparisons of concentration and potency of the API as well as protein binding of each product required curve fitting analysis (CFA) with Prism. Primary population PK parameters volume of distribution and clearance were computed under a two-compartment model (Kinetica) and used to calculate secondary PK parameters of each product to determine its PD profile without the influence of the others, and fixing the null hypothesis is correct (generic vs. innovator). Accepting a 5% chance for a type I error under CFA (a specialized ANOVA), the expression 10 log[ED50 (3)] in equation 4 corresponds to the logarithmic form in which the dose is introduced in all dose-response relationships: [4] is the independent variable, represented here by the 24hTD. Since Basal is zero (CFU/guntreated = 0 without treatment), Range equals EDin our Gaussian model:

$$E = E_{max} \times e^{-\left[0.5(e^{2/\text{Slope}} - 1)\right]}$$  

If any generic and the innovator fit different PD models, their effects are not “essentially the same”; therefore, there is not therapeutic equivalence. To establish which model appropriately described the dose-effect relationship of each product, the individual probability of the Hill and Gaussian model being correct was computed by Akaike’s information criterion (AICc) with Prism. Besides, we ran all products simultaneously under the Hill model (multiple NLR [M-NLR]), fixing the E_{max} to the innovator’s value, a strategy that permits calculation of hypothetical ED_{max} and N values for generic products assuming that the null hypothesis is correct (generic = innovator). The experimental design (inclusion of the innovator product in every experiment to guarantee simultaneous comparisons with all generics) allows this approach, giving significant PD for all products without violating NLR assumptions, an absolute requirement for valid comparison of PD by CFA (23). Simple NLR permits independent analysis of each product to determine its PD profile without the influence of the others, and M-NLR allows comparison of several generics against the innovator, assuming that all have identical proportions of the same chemical entities (the null hypothesis). Accepting a 5% chance for a type I error under CFA (a specialized ANOVA), the treatment of 10 animals per product to compare 3 generics with the innovator (one experiment with 40 treated and 6 untreated mice) confers 96.6% power to reject the null hypothesis if the magnitude of the difference in antibacterial efficacy between generics and innovator is ≥1 log_{10} CFU/g. Such difference represents in this model a net bactericidal effect of ≥0.1 million bacterial cells, a threshold value several orders of magnitude greater than what would be considered important in clinical medicine.

**RESULTS**

**Microbiological assays.** The concentration and potency of VAN-APP and VAN-Proclin were indistinguishable from those of the innovator. VAN-Abbott US displayed equivalent potency (parallel slopes, P = 0.9434) but contained a greater concentration of API (124.7%, different intercepts, P = 0.0085). Vancomycin protein binding in mouse serum ranged from 22.7 to 27.2% (64 mg/liter) and from 24.2 to 36.4% (16 mg/liter) for all products, without difference between generics (VAN-Hospira and VAN-Proclin) and the innovator available.
at the time of this assay (VAN-Baxter) or between the concentrations tested (mean protein binding, 28.4%).

**Single-dose serum PK in infected mice.** Table 2 contains primary and secondary population PK parameters for VAN-Lilly, VAN-Abbott US, VAN-APP, and VAN-Proclin after one subcutaneous injection of 50 mg/kg. Prediction curves for population PK parameters were highly correlated with observed data for all products ($r^2 = 0.979$ for VAN-Lilly and $>0.999$ for generics). As expected from its pharmaceutical nonequivalence, VAN-Abbott US exceeded significantly serum AUC (123%), while pharmaceutically equivalent generics VAN-APP (99%) and VAN-Proclin (103%) remained indistinguishable from VAN-Lilly.

**In vitro susceptibility testing.** Vancomycin products did not differ in MIC, MBC, or MBC/MIC ratio against S. aureus GRP-0057 or ATCC 29213. Geometric means of MIC and MBC against the first strain ranged from 1.19 to 1.41 and from 1.00 to 1.19 log10 CFU/g, respectively (Table 3).

**Time-kill curves (TKC).** Before addition of vancomycin, MHB cultures had (S. aureus GRP-0057) 105.56 to 106.19 CFU/ml; untreated controls grew up to 108.68 to 109.13 CFU/ml by 24 to 48 h (growth, 2.49 to 3.57 log10 CFU/ml). Bacteriostatic and bactericidal concentrations acted as expected, but no product or concentration achieved culture sterilization. In all polymicrobial MHB cultures had ($V_0$ = 105.56 to 106.19 CFU/ml) 108.68 to 109.13 CFU/ml by 24 to 48 h (growth, 2.49 to 3.57 log10 CFU/ml). Bacteriostatic and bactericidal concentrations acted as expected, but no product or concentration achieved culture sterilization. $I_E$ comparisons showed no differences between generics and the innovator ($P \geq 0.22$ for all vancomycin concentrations).

**In vivo pharmacodynamics.** We obtained identical results from three independent experiments in the neutropenic mouse thigh infection model designed to compare the dose-effect curves of VAN-Abbott US, VAN-APP, and VAN-Proclin against those of VAN-Lilly. Untreated control mice from these three experiments had on average ($\pm$ standard error of the mean [SEM]) 104.30 $\pm$ 0.16 and 107.82 $\pm$ 0.11 CFU per thigh at hours 0 and 24, respectively (24-h growth range, 3.39 to 3.65; weighted growth mean, 3.47 $\pm$ 0.08 log10 CFU/g). As expected, there was no difference among the three dose-effect curves of the innovator ($P = 0.2594$), allowing the combination of the data in a single NLR. Equation 1 (Hill model) described VAN-Lilly's dose-effect relationship with an excellent fit, producing multicollinearity-free, very significant PDP and a sound NLR fulfilling normality, constant variance, and independence assumptions (Table 4). $E_{max}$ was 5.65 $\pm$ 0.07 log10 CFU/g, and $ED_{50}$ was 62.7 $\pm$ 1.61 mg/kg per day. The steep N ($5.6 \pm 0.70$) suggests that vancomycin-receptor interaction is an all-or-none phenomenon, exquisitely dose dependent. ILKD (79.6 $\pm 1.54$) was only 16.7% greater than BD (68.2 $\pm 1.26$ mg/kg/day), as expected from highly bactericidal antibiotics (low MBC/MIC ratios).

Generics’ PD were completely different from those of the innovator. $E_{max}$ was statistically different from zero under equation 1 for all three generics, but their magnitudes were much lower, killing ~445,000 fewer microorganisms per gram of tissue than did VAN-Lilly ($P < 0.0001$). VAN-Abbott US could not reach bacteriostasis ($E_{max} = 2.04 \pm 0.07 \log_{10} CFU/g$), allowing bacterial growth even at maximal dosing (1,200 mg/kg per day; AUC/MIC ratio, 1,068 h) and therefore preventing computation of $ED_{50}$ and N. VAN-APP had significant PDP but marginal antibacterial efficacy ($E_{max} = 2.59 \pm 0.18 \log_{10} CFU/g$), violating the constant variance assumption under the Hill model. VAN-Proclin performed best among generics ($E_{max} = 3.48 \pm 0.27 \log_{10} CFU/g$), but the data did not fit the Hill model either, violating the constant variance assumption and giving nonsignificant values for $ED_{50}$ and N (Table 4).

Analysis of dose-effect relationships explains generics’ unfitfulness to the Hill model (Fig. 1). VAN-Abbott US was completely ineffective; it is shown in comparison with the innovator in a separate graph because it was not pharmacologically equiv-
TABLE 4. In vivo efficacies of three generics and the innovator product of vancomycin

<table>
<thead>
<tr>
<th>Model PDP (unit) or statistical test</th>
<th>Vancomycin product PDP magnitude ± SE</th>
<th>P value by CFA (IP vs GP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hill’s $E_{\text{max}}$ (log$_{10}$ CFU/g)</td>
<td>Lilly 5.65 ± 0.07$^b$</td>
<td>&gt;0.9999 Hill, &gt;0.9999 Gaussian, &gt;0.9999 Gaussian, &gt;0.9999 Gaussian, &gt;0.9999 Gaussian, &gt;0.9999 NA</td>
</tr>
<tr>
<td>Gaussian $E_{\text{max}}$ (log$_{10}$ CFU/g)</td>
<td>APP 2.04 ± 0.07$^b$</td>
<td>&gt;0.9999 NA</td>
</tr>
<tr>
<td>Hill’s ED$_{50}$ (mg/kg/day)</td>
<td>Proclin 2.59 ± 0.18$^b$</td>
<td>&gt;0.9999 NA</td>
</tr>
<tr>
<td>Gaussian logED$_{50}$</td>
<td>APP 3.28 ± 0.13$^b$</td>
<td>&gt;0.9999 NA</td>
</tr>
<tr>
<td>Hill’s slope ($N$)</td>
<td>Proclin 5.07 ± 0.39$^b$</td>
<td>&gt;0.9999 NA</td>
</tr>
<tr>
<td>Gaussian slope</td>
<td>Proclin 5.60 ± 0.70$^b$</td>
<td>&gt;0.9999 NA</td>
</tr>
<tr>
<td>Hill’s BD (mg/kg/day)</td>
<td>Proclin 62.7 ± 1.61$^b$</td>
<td>&gt;0.9999 NA</td>
</tr>
<tr>
<td>Hill’s LD$_{50}$ (mg/kg/day)</td>
<td>Proclin 1.90 ± 0.04$^b$</td>
<td>&gt;0.9999 NA</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>Proclin 1.50 ± 0.04$^b$</td>
<td>&gt;0.9999 NA</td>
</tr>
<tr>
<td>$\Delta_{\text{ED}}$</td>
<td>Proclin 1.09 ± 0.04$^b$</td>
<td>&gt;0.9999 NA</td>
</tr>
</tbody>
</table>

$^a$ Simple nonlinear regression analysis of each product based on the pharmacodynamic equation (Hill or Gaussian) best fitting its dose-effect relationship (all equations passed normality and constant variance tests). Abbreviations: GP, generic product; IP, innovator product; NA, nonapplicable; NC, not computable; NS, nonsignificant PDP (the PDP value was not significantly different from zero). Data in bold refer exclusively to the model best fitting each vancomycin product.

$^b$ P < 0.0001 (other PDP had P values between 0.0001 and 0.050).

alent to VAN-Lilly; thus, the two products have different AUC/MIC ratios despite their identical dosing regimens (Fig. 1A). VAN-APP and VAN-Proclin achieved bacteriostasis or killed 1 log at 75 to 150 mg/kg (fAUC/MIC, 66.8 to 133.5 h), but greater doses caused paradoxical bacterial growth in a U-shaped, Gaussian pattern (Eagle effect) (Fig. 1B). AICc model comparison confirmed that while the dose-effect relationships of VAN-Lilly and VAN-Abbott US fitted equation 1 (the Hill model) with a probability of correctness of >0.9999, those of VAN-APP and VAN-Proclin fitted equation 6 (the Gaussian model) with probabilities of correctness of >0.9999 and 0.9793, respectively (Table 4). Comparison of all generic products with the innovator under Hill’s model (M-NLR) demonstrated that 2.1 (VAN-Proclin), 4.3 (VAN-APP), and infinite (VAN-Abbott US) dose increments would be required to reach the innovator’s efficacy (Table 5).

All data shown so far were obtained before November 2004; results from experiments carried out after 1 December 2004 are shown below (this is relevant to understanding why some products did and others did not change their PD profile during the execution of this study). Despite the fact that serum PK results from experiments carried out after 1 December 2004 demonstrated comparable absorptions from subcutaneous space for all products, we ruled out any impact of the injection site or the inoculum size on the results, adapting the thigh model for intravenous (i.v.) treatment and increasing the number of microorganisms per thigh at hour 0. After infection with...
S. aureus GRP-0057, groups of 15 mice received 2 h later VAN-Abbott US or VAN-Lilly q8h i.v., in 5 doses ranging from 75 to 1,200 mg/kg per day (3 mice per dose), starting when mice had 6.74 ± 0.12 log10 CFU per thigh (24-h growth, 1.52 ± 0.21 log10 CFU/g). Confronting this higher inoculum by the i.v. route, both products became less potent (VAN-Lilly, 3.5-fold; VAN-Abbott, 3.8-fold) and showed the Eagle effect, but it was more conspicuous in VAN-Abbott US (Fig. 2), which displayed minimal efficacy compared to the innovator ($E_{\text{max}} = 3.82 \pm 0.40$ versus 5.35 ± 0.15, respectively; $P < 0.0001$). This lot of VAN-Abbott US was manufactured and imported directly from Chicago by the maker (labeled in English), while all lots employed in previous experiments came from Chile (labeled in Spanish but also manufactured in Chicago). We also determined if this new lot of VAN-Abbott US was equivalent to VAN-Lilly by other routes (intraperitoneal and subcutaneous) or under different dosing regimens (q1h, q3h, q6h, and q12h) at 1,200 mg/kg per day: it had some efficacy independently of these variables but always less than that of the innovator. M-NLR analysis confirmed the significant inferiority of VAN-Abbott US ($P < 0.001$) related to the product itself ($P = 0.003$), not to the route ($P > 0.05$) or schedule ($P > 0.05$) of administration (not shown).

We found during in vivo TKC experiments with S. aureus GRP-0057 that VAN-Abbott France displayed essentially the same efficacy as did VAN-Lilly (not shown), in contrast to data from VAN-Abbott US. This was confirmed by repeating the thigh model with S. aureus ATCC 29213, a strain more susceptible in vivo to vancomycin than is S. aureus GRP-0057. VAN-APP was not tested because it became unavailable in Colombia in 2005; VAN-Baxter had bought the manufacturing secrets from Eli Lilly and introduced the same brand name as the innovator (Vancocin CP). Four groups of 10 animals received subcutaneous treatment q1h (18.75 to 300 mg/kg per day), starting when mice had 4.13 log10 CFU per thigh (24-h growth, 4.58 log10 CFU/g). There was no difference ($P = 0.7681$) in efficacy between VAN-Abbott France, VAN-Baxter, and VAN-Lilly, all three fitting Hill’s model. VAN-Proclin, however, differed significantly from VAN-Lilly, displaying again the paradoxical U-shaped pattern, with 99.7% probability of better fit to the Gaussian model than to the Hill model by AICc (Fig. 3). To determine if extreme bacterial inocula could impact in vivo results, we repeated the thigh model with a very high inoculum of S. aureus GRP-0057 and tested all vancomycin products available in Colombia during 2008. VAN-Baxter (substituting for the innovator due to discontinuation of VAN-Lilly) was compared with VAN-Proclin and VAN-Hospira (new generic made by Abbott in Lake Forest, IL, commercialized under the brand name Hospira). Low inoculum (4.07 log10 CFU/g per thigh when treatment started) led to minimal bacterial growth (4.17 log10 CFU/g, $P < 0.0001$) compared to VAN-Baxter, and therapeutic success of VAN-Hospira and VAN-Baxter ($E_{\text{max}} = 5.43 \pm 0.10$ and 5.60 ± 0.15 log10 CFU/g, respectively, $P = 0.3497$) (Fig. 4A). Very high inoculum (8.34 log10 CFU per thigh when treatment started) led to minimal bacterial growth (1.12 log10 CFU/g in 24 h) and decreased efficacy of both products, but VAN-Proclin still differed significantly from VAN-Baxter ($P = 0.0021$), particularly in terms of the exact potency of each vancomycin (BD = 274.2 ± 17.3 and

<table>
<thead>
<tr>
<th>PDP (unit)</th>
<th>Lilly</th>
<th>Abbott</th>
<th>APP</th>
<th>Proclin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{max}}$ (log10 CFU/g)</td>
<td>$-5.65 \pm 0.25$ (&lt;0.0001)</td>
<td>$-5.65 \pm 0.25$ (&lt;0.0001)</td>
<td>$-5.65 \pm 0.25$ (&lt;0.0001)</td>
<td>$-5.65 \pm 0.25$ (&lt;0.0001)</td>
</tr>
<tr>
<td>ED50 (mg/kg/day)</td>
<td>62.7 ± 6.21 $^b$</td>
<td>1.877 ± 1.684 $^d$</td>
<td>270.7 ± 112.6</td>
<td>129.5 ± 33.6</td>
</tr>
<tr>
<td>N (Hill slope)</td>
<td>0.55 ± 2.67 $^c$</td>
<td>0.41 ± 0.14 $^c$</td>
<td>0.74 ± 0.25 $^b$</td>
<td>0.77 ± 0.16 $^b$</td>
</tr>
<tr>
<td>BD (mg/kg/day)</td>
<td>68.2 ± 1.26 $^b$</td>
<td>No bacteriostatic effect</td>
<td>No bacteriostatic effect</td>
<td>No bacteriostatic effect</td>
</tr>
<tr>
<td>1LKD (mg/kg/day)</td>
<td>78.6 ± 1.57 $^b$</td>
<td>No bactericidal effect</td>
<td>No bactericidal effect</td>
<td>No bactericidal effect</td>
</tr>
</tbody>
</table>

$^a$ Abbreviations: GP, generic product; IP, innovator product; NA, nonapplicable.

$^b$ $P < 0.0001$.

$^c$ $P$ between 0.0001 and 0.050.

$^d$ $P = 0.268$.

![FIG. 2. In vivo efficacy against S. aureus GRP-0057 (year 2004) at a high inoculum (6.74 log10 CFU per thigh when intravenous treatment q8h started). VAN-Abbott US was compared with the innovator (VAN-Lilly) after intravenous administration (75 to 1,200 mg/kg per day) but with 2.5-log increases in the inoculum size. The greater bacterial load required four times more vancomycin to reach maximum effect (600 mg/kg; AUC/MIC: 53.41 h) and caused the Eagle effect in both products, but the efficacy of VAN-Abbott US was significantly inferior to that of VAN-Lilly ($E_{\text{max}} = 3.82 \pm 0.33$ versus 5.35 ± 0.13, respectively; $P < 0.0001$). Note that despite the use of identical dosing regimens, the AUC/MIC ratio of VAN-Abbott US is 124% of that of VAN-Lilly due to pharmaceutical nonequivalence.](image-url)
151.2 ± 23.1 mg/kg, respectively, P = 0.0006) (Fig. 4B). Of note, these lots of VAN-Proclin did not show an Eagle effect.

**DISCUSSION**

These data indicate that, before 2005, all generic versions of vancomycin commercialized in Colombia were ineffective in vivo, i.e., they lacked therapeutic equivalence with respect to the innovator. The findings were consistently reproduced under diverse conditions in neutropenic mice infected in the thighs with two wild-type clinical strains of *S. aureus* and occurred independently of the manufacturer’s reputation. Unexpectedly, two products (VAN-APP and VAN-Proclin) were indistinguishable from the innovator in terms of concentration and potency of the API, protein binding, MIC, MBC, MBC/MIC ratios, standard TKC, and PK profiles, and the only product that differed (VAN-Abbott) had 125% of the API concentration and 123% of the AUC of VAN-Lilly, but none of it made these generics effective. One uncomfortable aspect uncovered by this study is that all these tests have been used for decades to guarantee therapeutic equivalence of generic drugs, except *in vivo* pharmacodynamics. On the positive side, we also found that some generic products evolved and reached therapeutic equivalence after 2005, and one maker was able to produce effective vancomycin (VAN-Baxter) right from the beginning after buying manufacturing secrets from the innovator.

Two potential limitations deserve consideration. Determination of pharmaceutical equivalence was based on microbiological assays, a nonchemical technique unsuitable for finding fermentation impurities or degradation products that probably explain therapeutic failure of generic vancomycin (see below). However, the microbiological assay was better suited to the exploratory nature of this study because it gives accurate estimates of potency (besides concentration), a specific requirement from DRA for pharmaceutical equivalence (21). Another limitation was the use of the maximum dose (50 mg/kg) as the only level at which PK parameters were obtained (we lost to technical errors the data from the other two dose levels, 12.5 and 3.125 mg/kg). One dose level is enough to establish bioequivalence (31), but additional dose levels would have pro-

![FIG. 3. In vivo efficacy against *S. aureus* ATCC 29213 (year 2005) at a low inoculum (4.13 log_{10} CFU per thigh when subcutaneous treatment q1h started), after some makers of generics acquired manufacturing secrets from Eli Lilly. Vancomycin generic products were compared with the innovator (VAN-Lilly) in dose-effect experiments (18.75 to 300 mg/kg per day) using the neutropenic mouse thigh infection model (each data point represents the mean CFU/g of both thighs from a single mouse). VAN-Abbott France, VAN-Baxter, and VAN-Lilly fitted to the Hill model and were indistinguishable (P = 0.7681). VAN-Proclin, on the other hand, displayed again the Eagle effect, fitting the Gaussian instead of the Hill model, as happened before 2005.](image-url)

![FIG. 4. In vivo efficacy of vancomycin products available in Colombia during 2008 against *S. aureus* GRP-0057 at low (A) and very high (B) inocula (4.07 and 8.34 log_{10} CFU per thigh when subcutaneous treatment q1h started, respectively). After VAN-Lilly was discontinued, VAN-Baxter replaced it as the innovator product; both panels show its dose-effect relationship compared with those of the newest version of VAN-Abbott (commercialized under the brand name Hospira) and VAN-Proclin. At a low inoculum, VAN-Hospira was indistinguishable from VAN-Baxter while VAN-Proclin was again ineffective; the very high inoculum had a marked impact on vancomycin pharmacodynamics, but VAN-Proclin remained inferior despite losing its Eagle effect.](image-url)
vided a more accurate extrapolation of PK parameters to the lowest dose used in the animal model (0.78 mg/kg).

The animal model demonstrated that the innovator of vancomycin required an AUC/MIC ratio of 133.5 h for maximal efficacy. Generic products, in contrast, would fail in the clinical setting if such a target were attained, because VAN-Abbott US and VAN-APP would not even reach bacteriostatic effect, while VAN-Proclin would kill ~445,000 fewer microorganisms per gram than would the innovator. Efficacy would never be obtained with VAN-Abbott US, independently of the dose prescribed by the physician (Fig. 1). If, on the other hand, VAN-APP and VAN-Proclin are prescribed to reach the commonly recognized target for maximal efficacy of vancomycin (400 h), an even less effective response would ensue due to the Eagle effect (Fig. 1). This paradoxical PD profile, reported in 1948 for S. aureus exposed to increasing concentrations of penicillin (18), is described by the Gaussian model, which is used to fit concentration-response curves with both inhibitory and stimulatory components (6).

Vancomycin had so many fermentation impurities that it was nicknamed “Mississippi Mud” 50 years ago (30). After several attempts, Eli Lilly developed a chromatographic purification method that led to a product with at least 92% factor B and less than 4% impurities (Vancocin CP). Such impurities, known as crystalline degradation products or CDP-1 (minor and major fractions) (25), explained the greater frequency of adverse reactions reported for generics elsewhere (36) and, we propose, the Eagle effect found here. Antibacterial efficacy depends entirely on factor B (5, 29, 39), but CDP-1 binds ß-Ala-ß-Ala (36) with less affinity (<1,000×) and efficacy (7 to 14×) (5, 29, 39). Generics have less factor B (84% at most) and two to three times more CDP-1 than does the innovator (9, 36). A vancomycin agonist-antagonist pharmacodynamic pattern is also evident with the innovator, but only at the greatest dose and by the intravenous route, without impact on efficacy compared with generic products (Fig. 2). It is not surprising if we consider that a 25-g mouse will pass through its body ~1 × 10^{18} molecules of vancomycin after 100 mg/kg per day and that one S. aureus cell has 10^6 (false cell wall) and 10^3 to 10^5 (periplasmic space) ß-Ala-ß-Ala targets. If bacterial growth reaches 10^5 CFU/g, the number of false targets will be 10^{15}. That would leave only 10^3 molecules of vancomycin in the mouse to confront 10^{12} to 10^{13} vital ß-Ala-ß-Ala targets per gram of tissue (26). Under this adverse balance, every molecule of vancomycin counts, and so protein binding, renal clearance, and, of course, concentrations of factor B and its antagonist CDP-1 become critical for in vivo efficacy. It explains why vancomycin is so susceptible to inoculum size of S. aureus in vivo (Fig. 4), as was demonstrated by Craig et al. (13). Based on this interpretation of the data, we postulate that the lower efficacy of generics in vivo is due to a relative absence of free factor B molecules, wasted in binding more ß-Ala-ß-Ala to counteract the agonistic competence of CDP-1. This hypothesis would explain the PD profile of generics displaying the Eagle effect, not that of VAN-Abbott US (before November 2004) and VAN-Proclin (from 2008), devoid of in vivo efficacy altogether. For these products, one potential explanation would be faster degradation of factor B in vivo, but we have no data to substantiate such a claim.

The dogma proclaiming that pharmaceutical equivalence predicts therapeutic equivalence is not true for vancomycin. In vivo failure of generics was consistent, independently of their route of administration or the dosing schedule. Although the poor quality of generic products has always been a matter of concern worldwide (37), we describe here an entirely different situation: good quality, as currently defined (41), does not imply efficacy in vivo, and this has clinical implications (34). “Similar” standards seem as insufficient to guarantee therapeutic equivalence as do pharmaceutical equivalence and so-called “bioequivalence” (1, 16, 35). Given their medical importance and the variety of epidemiological consequences emerging from their improper use, all antimicrobials deserve the same scrutiny as that presented here for generic products of vancomycin.

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