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# Therapeutic Equivalence Requires Pharmaceutical, Pharmacokinetic, and Pharmacodynamic Identities: True Bioequivalence of a Generic Product of Intravenous Metronidazole

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**Animal models of infection have been used to demonstrate the therapeutic failure of "bioequivalent" generic products, but their applicability for this purpose requires the accurate identification of those products that are truly bioequivalent. Here, we present data comparing one intravenous generic product of metronidazole with the innovator product in a neutropenic mouse thigh anaerobic infection model. Simultaneous experiments allowed comparisons (generic versus innovator) of potency and the concentration of the active pharmaceutical ingredient (API), analytical chemistry (liquid chromatography/mass spectrometry [LC/MS]), *in vitro* susceptibility testing, single-dose serum pharmacokinetics (PK) in infected mice, and *in vivo* pharmacodynamics (PD) against *Bacteroides fragilis* ATCC 25825 in synergy with *Escherichia coli* SIG-1 in the neutropenic mouse thigh anaerobic infection model. The Hill dose-response model followed by curve-fitting analysis was used to calculate and compare primary and secondary PD parameters. The generic and the innovator products were identical in terms of the concentration and potency of the API, chromatographic and spectrographic profiles, MIC and minimal bactericidal concentrations (MBC) (2.0 mg/liter), and mouse PK. We found no differences between products in bacteriostatic doses (BD) (15 to 22 mg/kg of body weight per day) or the doses needed to kill 1 log (1LKD) (21 to 29 mg/kg per day) or 2 logs (2LKD) (28 to 54 mg/kg per day) of *B. fragilis* under dosing schedules of every 12 h (q12h), q8h, or q6h. The area under the concentration-time curve over 24 h in the steady state divided by the MIC (AUC/MIC ratio) was the best PD index to predict the antibacterial efficacy of metronidazole (adjusted coefficient of determination [AdjR$^2$] = 84.6%), and its magnitude to reach bacteriostasis *in vivo* (56.6 ± 5.17 h) or to kill the first (90.8 ± 9.78 h) and second (155.5 ± 22.2 h) logs was the same for both products. Animal models of infection allow a thorough demonstration of the therapeutic equivalence of generic antimicrobials.**

Recent data obtained with animal models for generic products of vancomycin (18), oxacillin (14), and gentamicin (21) suggest that therapeutic equivalence is not predicted by surrogate endpoints like pharmaceutical equivalence, pharmacokinetic equivalence (i.e., bioequivalence), or *in vitro* susceptibility testing identities. In addition, therapeutic failures were thoroughly demonstrated for generic vancomycin in a case report (15) and for generic cefuroxime in a large clinical trial (12). The mechanisms behind the therapeutic nonequivalence of "bioequivalent" generic products have been established for imipenem-cilastatin and meropenem (1, 2), and the fact that all these products are licensed for human use indicates that traditional tests required by drug regulatory agencies (DRA) for the approval of generic medicines could not have detected this kind of problem.

The evidence gathered so far tell us that the term "bioequivalence" is ill defined by pharmacokinetics (PK) alone and points toward animal models as the best tools to study therapeutic equivalence. However, it is necessary to determine the reproducibility of the results obtained from animal models for this purpose. If bioequivalence is to be redefined as indistinguishable PK and pharmacodynamics (PD) of two products that are also pharmaceutically equivalent, the magnitude of the PK and PD parameters generated by the animal model for a bioequivalent generic product must be statistically indistinguishable from those of the innovator product.

Metronidazole is a semisynthetic 5-nitroimidazole compound that was obtained in the late 1950s at Rhone Poulanc Research Laboratories in France. It is the first-choice therapy against pathogens as diverse as Gram-negative anaerobes, *Clostridium difficile*, *Gardnerella vaginalis*, *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Giardia lamblia*, among others (5, 8). A previous study investigating the quality of generic drugs in developing countries found that 100% of oral metronidazole products were devoid of an active pharmaceutical ingredient (API) altogether (17). Although that was a case of a counterfeit drug and should not be confused with our research problem, the cases of vancomycin and other antimicrobials justify research to determine if bioequivalent generic products of metronidazole would also fail therapeutic equivalence tests. We compared the only generic product of parenteral metronidazole available in Colombia with the innovator product, investigating the concentration and potency of the API, analytical chemistry, bioequivalence (mouse pharmacokinetics), *in vitro* activity (susceptibility testing), and *in vivo* bactericidal efficacy using a neutropenic mouse thigh anaerobic infection model after its standardization and optimization for this particular purpose. We aimed to determine if the PD index (and its magnitude) defining the *in vivo* efficacy of both products was the same. The results confirmed the identities of the generic product and the innovator product for all comparisons, demonstrating that this

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approach has excellent power and specificity to discriminate between products with and those without therapeutic equivalence.

## MATERIALS AND METHODS

**Antibacterial agents.** Metronidazole products (Metronidazol [manufactured by Corpaul in Medellin, Colombia] and Flagyl [manufactured by Baxter for Aventis Pharma in Sao Paulo, Brazil]) were bought from local drugstores and prepared for experimental use as indicated by each maker. The reference standard for analytical chemistry (metronidazole trihydrate powder) was acquired from Sigma; the solvents (ammonium acetate, acetonitrile, and formic acid) and deionized water were of high-performance liquid chromatography (HPLC) grade. Since innovator and generic products are presented as ready-to-use liquid solutions (5 mg/ml), no manipulation was required beyond dilution to obtain the complete range of doses and concentrations used during the different experiments.

**Infecting organisms.** *Escherichia coli* clinical isolate SIG-1 was selected as a copathogen to induce a synergistic infection with *Bacteroides fragilis* ATCC 25285, the organism against which metronidazole products were tested *in vitro* and *in vivo*.

***In vitro* susceptibility testing.** Two duplicate broth microdilution assays of both products against *B. fragilis* served to compare their MIC and minimal bactericidal concentrations (MBC). The quality control organism was the same *B. fragilis* strain, as suggested by Clinical and Laboratory Standards Institute (CLSI) guidelines (4); results were compared by a Wilcoxon-Mann-Whitney test.

**Microbiological assays.** The pharmaceutical equivalence of generic metronidazole to the innovator product was determined by comparing standard curves of each product with a previously validated application of the microbiological assay (13, 20). Thioglycolate medium (Oxoid, Basingstoke, Hampshire, United Kingdom) served as the diffusion agar for the tested microorganism, *B. fragilis* ATCC 25285, plated onto a custom-designed glass plate large enough to accommodate all samples simultaneously (3, 11). Four concentrations of metronidazole were studied (32, 64, 128, and 256 mg/liter), in an attempt to span the levels attainable in the mouse after injecting the doses used for PK and PD experiments (see below). Plates were incubated for 48 h at 37°C under an anaerobic atmosphere (AnaeroGen; Oxoid), and the same researcher measured zone sizes for all assays by using an electronic caliper (20).

Standard curves were obtained after a linear regression of log-transformed concentrations ($\log_{10}$ mg/liter) plotted against their respective inhibition zones in millimeters (mean diameter of five inhibition zones per concentration). The linear regression parameter slope and intercept were then compared by curve-fitting analysis (CFA) (Prism 5; GraphPad Software, Inc., San Diego, CA). Pharmaceutical equivalence was met when the generic product displayed a regression line parallel to and overlaid with that of the innovator ($P > 0.05$ by CFA), i.e., no significant difference in terms of the nature of the API (slope) or its concentration (intercept); significant differences in one or both of these parameters implied a lack of pharmaceutical equivalence (20).

**Liquid chromatography/mass spectrometry (LC/MS).** Analytical chemistry data were obtained with an Agilent 1200 liquid chromatograph coupled to a mass spectrometer electrospray ionization VL system. At the stationary phase, a Thermo Scientific 150-mm- by 4.6-mm-internal-diameter (i.d.) Hypersil Gold 5-$\mu$m-particle-size analytical column was used (each product had its own column). The mobile phase consisted of 10 mM ammonium acetate plus acetonitrile with 0.1% formic acid at a 91:9 (volume) dilution. All preparations for reference material and pharmaceutical formulations were freshly prepared in deionized water at the moment of analysis at a metronidazole concentration of 250 $\mu$g/ml. The mobile phase was kept running in the equipment for 15 min prior to sampling; the sample volume was 20 $\mu$l.

**Single-dose serum pharmacokinetics in infected mice.** Two hours after infection, animals received a single subcutaneous injection (0.2 ml) containing the dose of metronidazole to be tested: 8, 32, or 128 mg/kg of body weight. Data were obtained from groups of 3 female mice bled to death at each of the following data points: 15, 30, 60, 120, 180, 240, 300, 360, 420, 480, and 540 min after dosing (both products tested independently). The numbers of data points (and, therefore, the numbers of groups per dose) were 6 for doses of 8 and 32 mg/kg (15 to 240 min) and 10 for a dose of 128 mg/kg (15 to 540 min, except for 180 min). Serum was obtained by blood centrifugation at 10,000 × *g* during 5 min and plated (duplicates) immediately as described above. The means of these inhibition zones were interpolated by linear regression against the standard curve of the respective product (quintuplets of each concentration ranging in doubles from 8 to 128 mg/liter), to determine serum concentrations and compute by noncompartmental analysis (NCA) the PK parameters maximal concentration ($C_{max}$), half-life of elimination ($T_{1/2}$), and area under the concentration-time curve (AUC) for each metronidazole product (WinNonlin 5.2; Pharsight Corp., Mountain View, CA). The $AUC_{all}$ (AUC from $t = 0$ to the last sampling time) of the generic product, expressed as a percentage of the innovator product, implied bioequivalence if it ranged between 80 and 125%, as accepted by DRA everywhere.

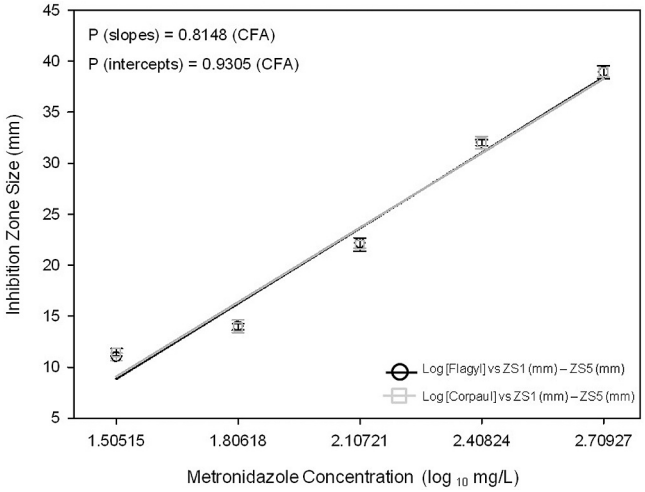


**FIG 1** Results of microbiological assays designed to obtain the standard curves of generic and innovator products of metronidazole. Each data point represents the mean of data from 3 assays with 5 concentrations per assay, plated in quintuplets. Symbols (means) of each product are not visible due to overlapping data; similarly, standard error bars are contained within symbols.

**Standardization of the neutropenic mouse thigh anaerobic infection model.** The neutropenic mouse thigh anaerobic infection model differs from its better-known aerobic counterpart in the use of a combination of synergistic pathogens (*E. coli* plus *B. fragilis*) to facilitate a successful, lethal infection by *B. fragilis*. To find the optimal *E. coli* inoculum for *B. fragilis* to attain maximal growth 26 h later (the duration of the model), we tested six inocula of *E. coli*, ranging from 3 to 8 $\log_{10}$ CFU/ml mixed at a 1:1 (volume) dilution with a constant amount of *B. fragilis* (8 $\log_{10}$ CFU/ml). The combination associated with the best growth of *B. fragilis* and greatest lethality near the end of the model was chosen for all experiments with metronidazole. Data were registered as $\log_{10}$ CFU/g, which in this model is the same as $\log_{10}$ CFU/thigh.

***In vivo* pharmacodynamics (dose-effect experiments).** Six-week-old specific-pathogen-free female mice of the Udea:ICR(CD-1) strain weighing 23 to 27 g were housed according to internationally accepted recommendations and allowed to eat and drink *ad libitum*. The experimental protocol was reviewed and approved by the University of Antioquia Animal Care and Experimentation Ethics Committee. Animals were rendered neutropenic by two intraperitoneal injections of cyclophosphamide (Endoxan; BMS, New York, NY) 4 days (150 mg/kg) and 1 day (100 mg/kg) before infection; all animals had zero neutrophils from the infection point to day 4 afterwards (19). To prepare the inoculum, *B. fragilis* ATCC 25285 cells were grown in thioglycolate broth (37°C in 5% $CO_2$ in

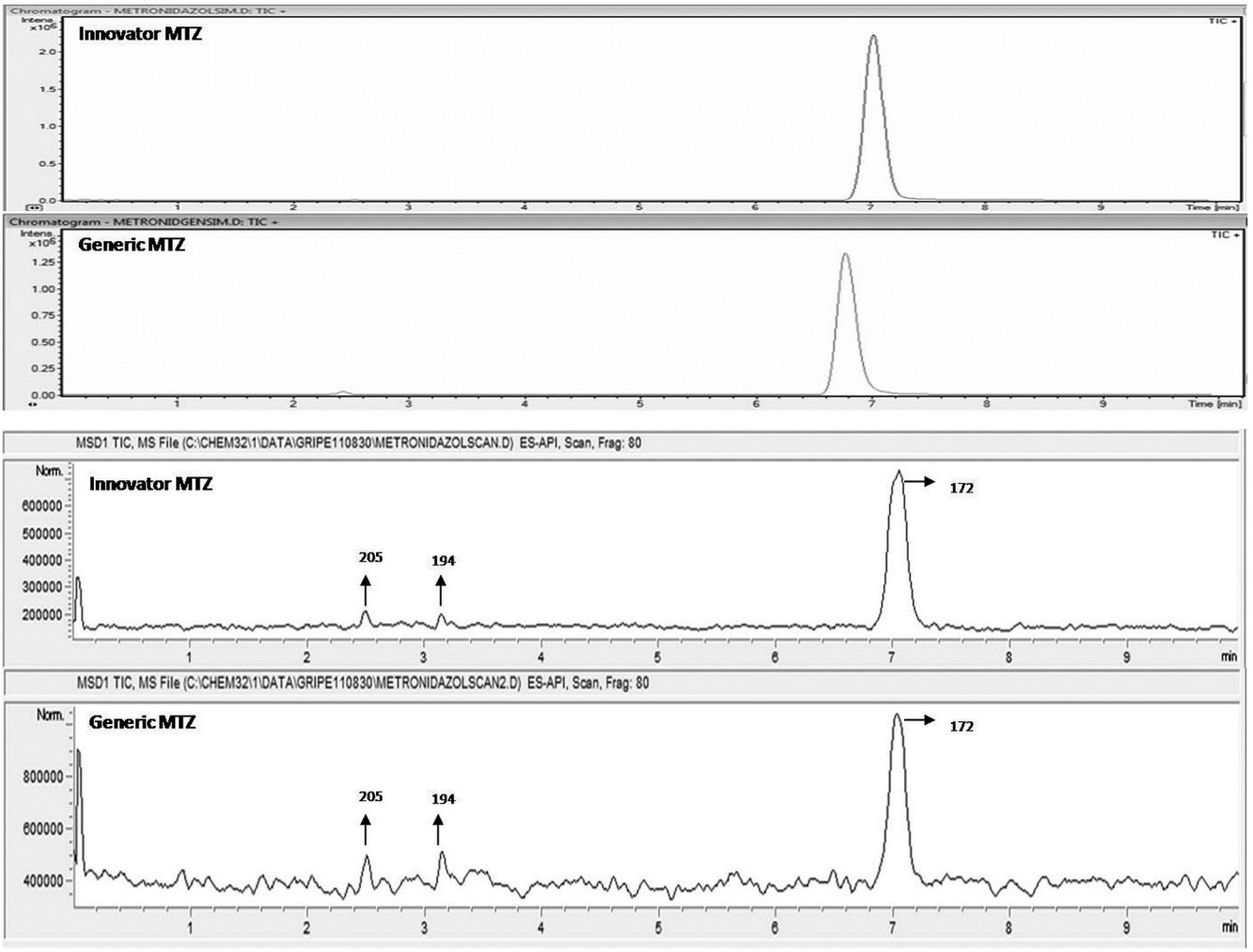


**FIG 2** LC/MS graphic data for a generic product of metronidazole (MTZ) compared with its innovator at 250 μg/ml. No differences were detected in the chromatograms (top) or spectrograms (bottom) of the reference product (not shown), the innovator, or the generic product.

an anaerobic atmosphere), and *E. coli* SIG-1 cells grown in BBL Trypticase soy broth (Becton Dickinson & Co., Sparks, MD). For colony counting, the following culture conditions allowed the visualization of each strain in solid medium without interference by the other strain: (i) blood agar plus 16 mg/liter gentamicin under an anaerobic atmosphere for *B. fragilis* and (ii) Trypticase soy agar under an aerobic atmosphere for *E. coli*. Broth cultures were adjusted to $10^8$ and $10^6$ log-phase CFU/ml for *B. fragilis* and *E. coli*, respectively, and then mixed at a 1:1 (volume) dilution. Infection was induced by the intramuscular injection of 100 μl of this mix into each thigh. Groups of 10 mice per product received 6 different total doses ranging from no effect to a maximum effect, allocating subgroups of 2 animals to each of the doses of 10, 20, 40, 80, 160, and 320 mg/kg per day. Treatment started at 2 h postinfection, lasted 24 h, and was administered by subcutaneous injections of 200 μl containing the corresponding dose for each schedule, which included all 6 of the above-mentioned daily doses distributed every 3, 6, 8, 12, or 24 h. Untreated controls were sacri-

**TABLE 1** Results for single-dose serum PK in neutropenic mice infected with *B. fragilis* plus *E. coli*[a]

| Metronidazole dose (mg/kg) | Formulation | $T_{1/2}$ (range) (min) | $C_{max}$ (range) (mg/liter) | $AUC_{all}$ (range) (h · mg/liter) |
|---|---|---|---|---|
| 8 | Generic | 124 (117–131) | 51.3 (50.5–52.6) | 45.6 (45.2–46.4) |
| | Innovator | 136 (110–149) | 44.7 (43.6–46.1) | 39.9 (39.3–40.6) |
| 32 | Generic | 179 (178–181) | 137.7 (136.7–138.8) | 331.8 (330.7–333.5) |
| | Innovator | 192 (181–204) | 125.3 (124.3–126.2) | 338.2 (336.8–340.7) |
| 128 | Generic | 352 (326–369) | 171.2 (168.3–174.3) | 851.7 (849.5–854.2) |
| | Innovator | 397 (362–461) | 156.4 (150.4–161.2) | 814.5 (812.2–818.5) |

[a] There was no difference between generic and innovator antimicrobials (the AUC of the generic product was within 80 to 125% the AUC of the innovator at all doses).

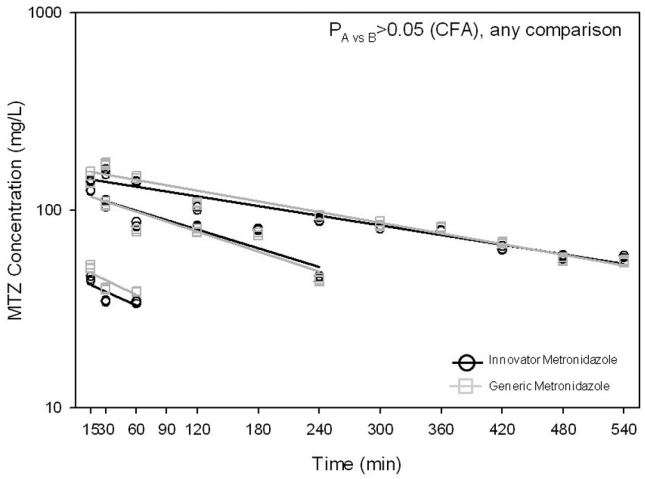


**FIG 3** Single-dose pharmacokinetics of a generic product and the innovator product of metronidazole at three different subcutaneous doses in infected mice (8, 32, and 128 mg/kg). All three doses generated linear PK indistinguishable by curve-fitting analysis ($P > 0.05$ for any assessment), demonstrating the bioavailability of generic metronidazole after subcutaneous injection. PK parameters are described in Table 1.

ficed in groups of 2 mice right after inoculation (hour −2) to confirm the inoculum size and at the time of starting (hour 0) and ending (hour 24) therapy. Treated animals were sacrificed at hour 24, and their thighs were dissected aseptically, homogenized, serially diluted, plated by duplicate, and incubated at 37°C for 48 h. After colonies were counted for each thigh, data were registered as $\log_{10}$ CFU/g in an Excel database; the limit of detection was 2.0 $\log_{10}$ CFU/g. To determine the 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. All experiments included the original compound and the generic product simultaneously.

**Statistical analysis.** Data from dose-effect experiments modeled by nonlinear regression (NLR) and fitted to the Hill equation produced curves and pharmacodynamic parameters (PDP) for both products. The sigmoid dose-response model is described by the Hill equation, $E = [(-E_{max}) \times D^N]/(ED_{50}{}^N + D^N)$, where $E$ is the net antibacterial effect ($\log_{10}$ CFU/g) after 24 h of treatment, $D$ is the metronidazole dose (mg/kg per day), $E_{max}$ is the maximum effect ($\log_{10}$ CFU/g), $ED_{50}$ is the effective dose reaching 50% of the $E_{max}$ (mg/kg per day), and $N$ is Hill's slope, describing the sensitivity of the dose-effect relationship. The last three PDP are the primary PDP, calculated by least-squares nonlinear regression. NLR curves and the PDP $E_{max}$, the bacteriostatic dose (BD), and the doses required to kill the first log of bacteria (1LKD) and the second log of bacteria (2LKD) were compared by CFA with SigmaPlot 9.01 (Systat Software, Point Richmond, CA) and Prism 4.03 (GraphPad, San Diego, CA). The PD index best defining the efficacy of each metronidazole product was that with the highest adjusted coefficient of determination ($AdjR^2$). If, as expected, the same PD index defined efficacy for both products, the magnitudes of each product's index required for bacteriostatic (BD) and bactericidal (1LKD and 2LKD) efficacy were compared by CFA (9, 10).

## RESULTS

***In vitro* activity.** The MIC against *B. fragilis* ATCC 25285 equaled the MBC for the generic and innovator products (2.0 mg/liter), with identical results in two different assays, leading to an MBC/MIC ratio of 1 for both products. Quality control results remained within CLSI-accepted ranges.

**Microbiological assay.** Figure 1 shows the standard curves generated by the linear regression of generic and innovator products as well as CFA results (data from 3 experiments); both products were indistinguishable in their intercepts ($P = 0.8148$) and slopes ($P = 0.9305$). Based on these data, one curve better describes the underlying population than two individual curves, demonstrating that the API is present in both products with the same concentration and potency.

**Analytical chemistry (LC/MS).** The chromatograms (single ion monitoring [SIM] mode) of the reference, innovator, and generic forms did not show differences in retention times, the peaks of the analyte, or other peaks (Fig. 2, top). The SCAN mode (range, $m/z$ 50 to 300) detected the same main masses (172, 194, and 205 atomic mass units) in both products (Fig. 2, bottom).

**Single-dose serum PK in infected mice.** Both products displayed identical linear pharmacokinetics (PK) in the serum of infected mice after the subcutaneous administration of 8, 32, and 128 mg/kg as single doses, encompassing those used in dose-effect experiments (Table 1 and Fig. 3). The $AUC_{all}$ values for the generic product relative to the innovator product at each dose level were 114%, 98%, and 105%, respectively, all within the range of 80 to 125% accepted by DRA. These data demonstrate the bioequivalence of this generic product of metronidazole.

**Standardization of the animal model.** Results for the standardization of the animal model are summarized in Table 2. We found that an *E. coli* inoculum of $10^6$ CFU/ml mixed with $10^8$ CFU/ml of *B. fragilis* led to a better growth of the latter in the thighs than with all other inocula, and mortality occurred at the optimal time (around the end of the model): animals started to die 22 h after infection with this inoculum, and 100% were dead by 26 h.

**TABLE 2** Standardization of the neutropenic mouse thigh anaerobic infection model (*B. fragilis* plus *E. coli*)

| *E. coli* inoculum ($\log_{10}$ CFU/ml) | Mouse | Time to mouse death (h after infection) | *B. fragilis* growth 26 h after infection ($\log_{10}$ CFU/g) | |
|---|---|---|---|---|
| | | | Each mouse | Mean ± SEM |
| 3 | 1 | 44 | 9.43 | 9.38 ± 0.37 |
| | 2 | 44 | 9.73 | |
| | 3 | 44 | 9.49 | |
| | 4 | 44 | 8.87 | |
| 4 | 5 | 56 | 9.42 | 9.54 ± 0.21 |
| | 6 | 56 | 9.40 | |
| | 7 | 68 | 9.49 | |
| | 8 | 68 | 9.85 | |
| 5 | 9 | 23 | 8.37 | 8.51 ± 0.20 |
| | 10 | 23 | 8.38 | |
| | 11 | 26 | 8.81 | |
| | 12 | 26 | 8.49 | |
| 6 | 13 | 23 | 9.32 | 8.99 ± 0.23 |
| | 14 | 23 | 8.78 | |
| | 15 | 23 | 8.95 | |
| | 16 | 23 | 8.90 | |
| 7 | 17 | 18 | 7.13 | 8.42 ± 1.34 |
| | 18 | 18 | 7.39 | |
| | 19 | 21 | 9.70 | |
| | 20 | 21 | 9.44 | |

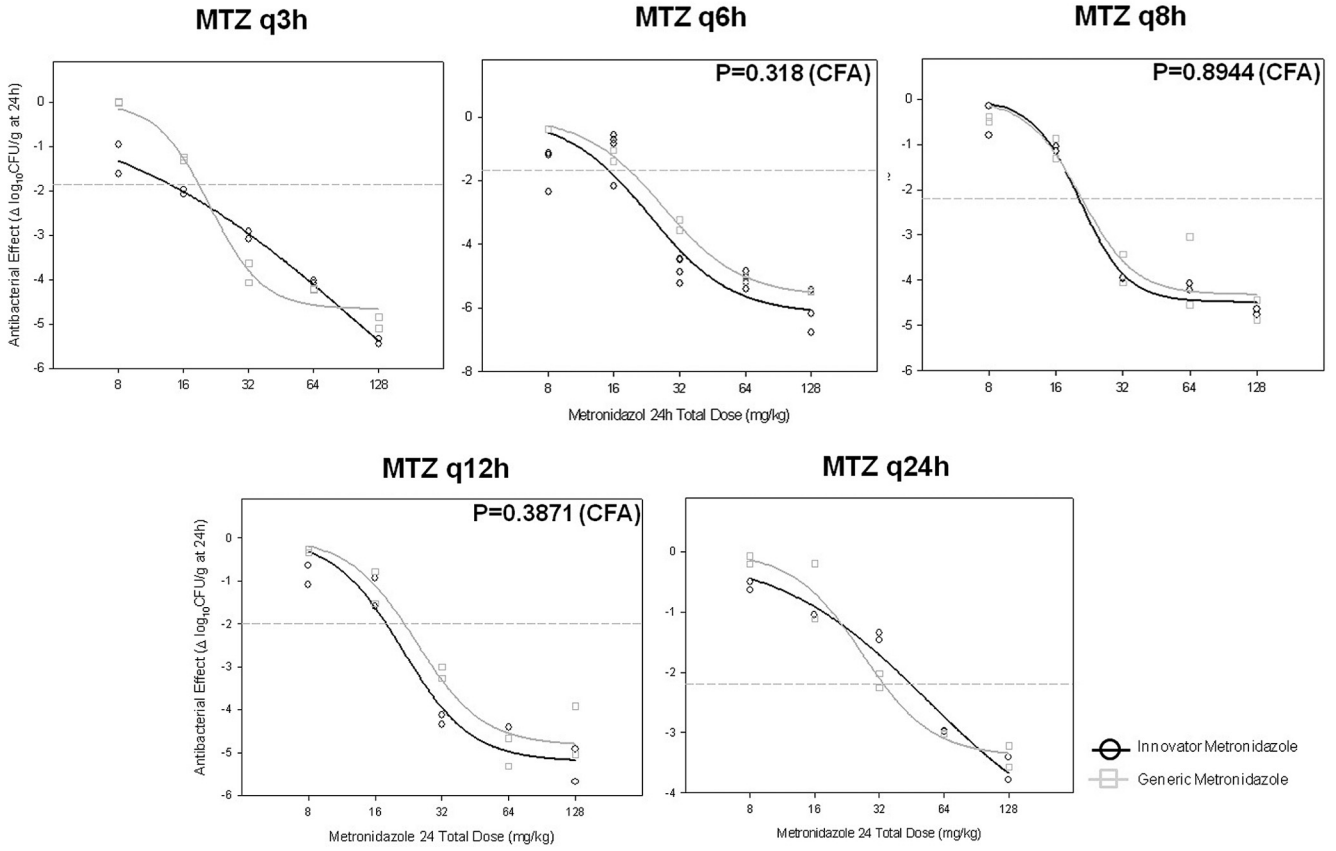


**FIG 4** Dose-effect relationships of a generic product and the innovator product of metronidazole against *B. fragilis* in a neutropenic mouse thigh anaerobic infection model testing the same dose range (from ineffective to maximally effective) divided into 5 different dosing intervals. Curve-fitting analysis was not applied to the q3h and q24h schedules because they resulted in pharmacodynamic profiles that did not fit the Hill model.

**Therapeutic equivalence.** Mice had $10^{6.61}$ to $10^{7.26}$ *B. fragilis* CFU/thigh when treatment began; by the end of therapy, untreated controls had $10^{8.48}$ to $10^{9.05}$ CFU/g (24 h of growth = 1.68 to 2.19 $\log_{10}$ CFU/g). Figure 4 illustrates the NLR and the respective CFA for every dosing schedule of generic and innovator products of metronidazole, and Table 3 details the PDP for treatments divided every 6 h (q6h), q8h, and q12h. The dosing of metronidazole q24h and q3h resulted in PD profiles that did not fit the Hill model, probably due to excessively long and short dose intervals, respectively, for the mouse in this uniformly lethal model of infection, which was a reason for not including such data in PD index-versus-effect comparisons. The other three dosing schedules (q6h, q8, and q12h) fit perfectly to the Hill model, and no difference was found in the efficacies ($E_{max}$), relative potencies ($ED_{50}$), affinities for the molecular target ($N$), or absolute potencies (BD, 1LKD, and 2LKD) of these products, confirming the therapeutic equivalence of generic metronidazole to the innovator product ($P > 0.05$ for all comparisons). These data demonstrate that the pharmacodynamic behavior of equivalent generic products is identical to that of the innovator product, with indistinguishable dose-effect NLR curves.

**TABLE 3** Results of dose-effect experiments comparing generic and innovator products of metronidazole using the neutropenic mouse thigh anaerobic infection model[a]

| Dose | Formulation | Mean ± SEM for PDP[b] | | | |
|---|---|---|---|---|---|
| | | $E_{max}$ ($\log_{10}$ CFU/g) | BD (mg/kg day) | 1LKD (mg/kg day) | 2LKD (mg/kg day) |
| MTZ q6h | Generic | 5.64 ± 0.13 | 18.9 ± 0.63 | 25.8 ± 0.68 | 38.8 ± 1.1 |
| | Innovator | 6.19 ± 0.50 | 15.2 ± 2.1 | 20.8 ± 2.1 | 27.5 ± 3.1 |
| MTZ q8h | Generic | 4.33 ± 0.33 | 20.9 ± 2.3 | 27.6 ± 4.2 | NC |
| | Innovator | 4.49 ± 0.18 | 20.3 ± 1.2 | 25.6 ± 2.0 | 39.2 ± 5.7 |
| MTZ q12h | Generic | 4.84 ± 0.34 | 21.7 ± 2.1 | 29.1 ± 3.0 | 42.3 ± 6.3 |
| | Innovator | 5.21 ± 0.37 | 18.0 ± 1.9 | 23.8 ± 2.5 | 32.7 ± 2.3 |

[a] Results are measured based on the bactericidal effect on *B. fragilis*. The other pathogen (*E. coli*) was used only to increase the virulence of the anaerobe.
[b] The *P* value was >0.05 for any comparison between generic and innovator products (CFA). PDP, pharmacodynamic parameter; MTZ, metronidazole; NC, not computable.

**PD index.** The AUC over 24 h in the steady state divided by the MIC (AUC/MIC ratio) was superior ($AdjR^2 = 0.846$) to the $C_{max}$/MIC ratio ($AdjR^2 = 0.808$) and $T$>MIC ($AdjR^2 = 0.796$) for predictions of the antibacterial efficacies of both metronidazole products (Fig. 5). The fitting of the AUC/MIC-versus-effect curve by NLR with the Hill model demonstrated that the generic and innovator products actually behaved as a single population, thus allowing the computation of the magnitudes of the AUC/MIC ratio needed for a bacteriostatic or bactericidal effect, which were statistically indistinguishable for both products (Table 4).

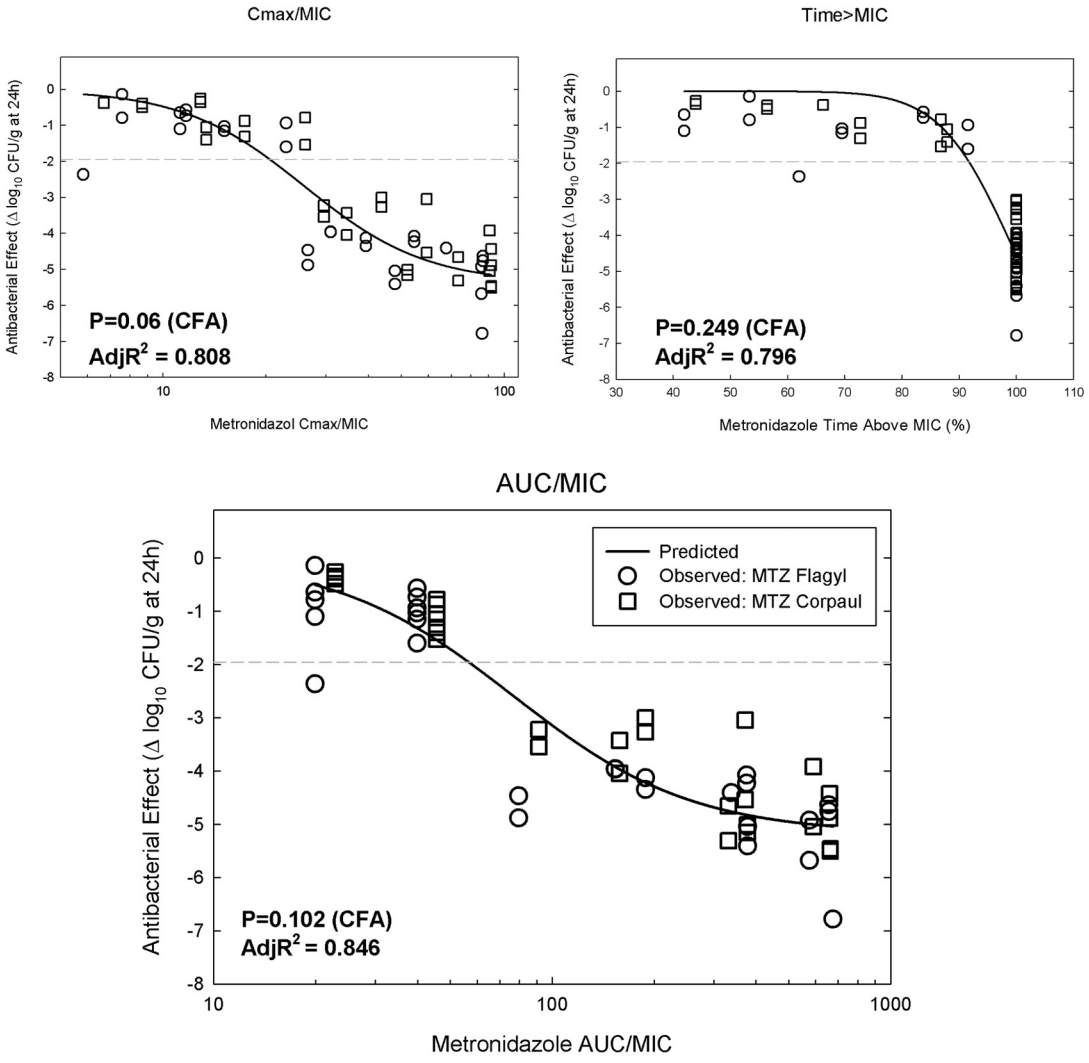


**FIG 5** Influence of pharmacodynamic indices on the antimicrobial effect of metronidazole on *B. fragilis* in a neutropenic mouse thigh anaerobic infection model. Only one curve is depicted because the data belong to a single population despite the fact that they were obtained after treatments of different groups of animals with a generic product or the innovator. The AUC/MIC ratio drives the antibacterial efficacy of metronidazole.

## DISCUSSION

These data demonstrate that equivalence between generic and innovator antimicrobials, when present, can be thoroughly demonstrated with this experimental approach. The data also show that the magnitude of the PD index required for efficacy is the same for generic and innovator antimicrobials. The pursuit of such a level of identity between generic and innovator products might appear to be excessive, but the available data suggest that the approach

**TABLE 4** Comparison of the magnitudes of the AUC/MIC index needed for efficacy of generic and innovator products of metronidazole[a]

| PDP | Mean value for formulation ± SEM | | | *P* value for generic vs innovator (CFA) |
|---|---|---|---|---|
| | Generic | Innovator | Generic and innovator as a single population | |
| $E_{max}$ ($log_{10}$ CFU/g) | 4.95 ± 0.29 | 5.37 ± 0.41 | 5.17 ± 0.27 | 0.5324 |
| AUC/MIC ratio for reaching 50% of $E_{max}$ | 84.5 ± 12.5 | 67.1 ± 14.3 | 76.4 ± 10.6 | 0.4816 |
| *N* (Hill's slope) | 1.74 ± 0.33 | 1.66 ± 0.46 | 1.66 ± 0.29 | 0.9674 |
| AUC/MIC ratio for bacteriostasis | 66.1 ± 6.51 | 48.1 ± 6.87 | 56.6 ± 5.17 | 0.1019 |
| AUC/MIC ratio for killing the 1st log | 106.0 ± 12.5 | 75.9 ± 12.6 | 90.8 ± 9.78 | 0.1019 |
| AUC/MIC ratio for killing the 2nd log | 187.4 ± 28.9 | 124.7 ± 28.0 | 155.5 ± 22.2 | 0.1019 |

[a] Shown are primary and secondary pharmacodynamic parameters derived from the AUC/MIC-versus-effect curve of generic and innovator metronidazole formulations against *B. fragilis* in the neutropenic mouse thigh anaerobic infection model. Products were undistinguishable by all pharmacodynamic parameters (PDP).

used so far by DRA has failed. Such an approach consists of assuming that pharmaceutical equivalence predicts therapeutic equivalence, but evidence suggests that the central assumption is wrong unless the meaning of the expression "pharmaceutical equivalence" implies absolute chemical identity (12, 14, 18, 21). "Exact pharmaceutical equivalence" implies the same concentration of the API in an identical state of purity, potency, contaminants, and degradation products, all within the same excipient. Only identical products can lead to reliably similar PK (taken today as proof of bioequivalence) and, therefore, to therapeutic equivalence (true bioequivalence). As shown here, "true bioequivalence" requires the integration of PK and PD (in opposition of an exclusively PK approach), otherwise, it is risky to consider generic drugs as indistinguishable from their gold standards.

We included the only generic product (manufactured locally) available in Colombia during the time of the study, and it displayed the same concentration, potency, chromatographic retention times and peaks, spectrometry masses, pharmacokinetics, and pharmacodynamics as those of the innovator product. No significant differences were found despite 5 dosing schedules being tested, and the magnitude of the AUC/MIC ratio required for efficacy was the same for both products. To our knowledge, this is the first time that PD indices have been used to demonstrate therapeutic equivalence. When using PD indices, the researcher has to be careful with the model at hand, because in small animals like mice, it is not feasible to use extreme periods of time between doses (too short or too long) and still obtain the reproducibility obtained with moderate periods of time. Concentration-dependent antibiotics would not behave as expected with very short dosing intervals, and both time- and concentration-dependent drugs would give unreliable results with very long dosing intervals (6, 7). The concentration dependence of metronidazole forced the exclusion of data from the q3h and q24h dosing schedules in order to obtain reliable parameters.

In a large study of the generic product's quality in Nigeria by Taylor et al., 100% of 5 suspension and 72% of 36 tablet metronidazole products failed (17). While the suspensions were all fake medicines, tablets without pharmaceutical equivalence had a slightly higher concentration of the API (114%) than allowed by the British Pharmacopoeia (95 to 105%). Although Taylor et al. did not determine therapeutic equivalence, the literature has several reports of "bioequivalent" generic products of other antimicrobials failing in patients and in animal models of infection (12, 14, 15, 18, 21). The demonstration of the therapeutic equivalence of one intravenous generic product of metronidazole provides the necessary trustfulness for patients and physicians to use it.

Despite the fact that therapeutic equivalence has never been required for antimicrobials, it is a vital issue in cases where the severity of the infection calls for optimal, not borderline, antimicrobial efficacy. Similarly, the fact that suboptimal efficacy usually selects for resistant mutants implies that ignorance about the efficacy of generic antimicrobials could have been adding to the problem of resistance (16). The experimental approach presented here might be useful for obtaining insightful data on the benefits and potential problems surrounding the massive use of generic antibiotics.

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We have no conflicts of interest.

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