MedCrave
Step into the World of Research
Journal of Microbiology & Experimentation

Research Article
Open Access
CrossMark

Sterilization with generic vancomycins of *Rattus norvegicus* Sprague Dawley hearts from experimental endocarditis by *E. faecalis* ATCC 29212

Abstract

The use of generic antibiotics creates suspicion regarding the innovator. In many countries, especially those in the developing world, these drugs are abundant, due to the cost of the innovator acquisition. However, there are many therapeutic failures that doctors report with its use. The objective of this work was to evaluate the efficacy of generic vancomycin, to sterilize the hearts of *Rattus norvegicus* Sprague Dawley from experimental endocarditis caused with the vancomycin sensitive strain *E. faecalis* ATCC 29212. Adult male specimens of *Rattus norvegicus* Sprague-Dawley were used; they were inoculated with the *E. faecalis* ATCC 29212 strain (1.5 x 10^8 CFU / ml) intravenously, to produce an experimental endocarditis. The control rat was sacrificed 48 hours after bacterial inoculation. The remaining five rats, also inoculated with *E. faecalis* ATCC 29212, received the treatment for five days intramuscularly, one rat for each vancomycin under study. Generic vancomycins were from Behrens, Celovan, Fada Pharma, Vancomax, and Vancocyn. To verify the sterility of the hearts with the generic vancomycin, they were placed in BHI broth, at 35°C. Following incubation, *E. faecalis* ATCC 29212 colony growth was verified and CFUs were counted per gram of cardiac tissue. The control rat had a bacterial growth of 1.8 x 10^{12} CFU/g of cardiac tissue. The rats treated with Behrens, Vancocyn and Celovan sterilized the hearts, while those treated with Vancomax and Fada Pharma showed bacterial growth (2.8 x 10^{12} CFU/g and 4.2 x 10^{10} CFU/g, respectively). In conclusion, the pharmaceutical equivalence of a generic vancomycin, with respect to the innovator, does not guarantee therapeutic equivalence. More studies need to be done on generic antibiotics to ensure their human clinical use.

Keywords: vancomycin, endocarditis, infection, generic, *Enterococcus faecalis*, failure, equivalence, microbiological potency

Volume 10 Issue 4 - 2022

Abadía-Patiño L,[1] Hidalgo B,[2] Rojas LM[3]
[1]Bacterial resistance laboratory, Biomedicine department, IIBCAUDO, Universidad de Oriente, Venezuela
[2]Bioanalysis department, Universidad de Oriente, Venezuela
[3]Histopathology laboratory, Biomedicine department, IIBCAUDO, Universidad de Oriente, Venezuela

Correspondence: Abadía-Patiño L, Bacterial resistance laboratory, Biomedicine department, IIBCAUDO, Universidad de Oriente, Av. Universidad, Cerro del Medio, Cumaná, Estado Sucre, Venezuela, Tel: +584148040684, Email: biociencia2013@gmail,com

Received: August 22, 2022 | **Published:** September 19, 2022

Introduction

Animal experimentation models are the fundamental pieces in medical sciences; since they are used to investigate and understand the causes, diagnoses and treatment of diseases that affect human beings and develop effective therapies for their eradication. The evaluation of animal models is a basic requirement as a step prior to clinical trials; because the information provided is essential for the efficacy and safety in the case of new drugs (basically the properties related to the pharmacokinetics and pharmacodynamics of the molecule under study) or about the loss of efficacy.[1,2] In the field of infectious diseases, these studies are relevant due to the intrinsic dynamism of bacteria that constantly evolve, generating strains resistant to the action of antibiotics and therefore to therapeutic failure.[3]

A comparative study of the *in vivo* efficacy of three generic vancomycin products in simultaneous experiments with the innovator showed that no product had differences in terms of the *in vitro* assay, lethality curve, or pharmacokinetics in infected mice. Despite these similarities, the generic products failed in the *in vivo* study, while the innovator presented therapeutic efficacy, concluding that pharmaceutical equivalence does not imply therapeutic equivalence.[4] Some authors suggest that the failure of generic antibiotics is due to the Eagle effect observed *in vivo* studies with *S. aureus* strains.[5] This study was designed to evaluate the eradication of experimental *Enterococcus faecalis* endocarditis in *Rattus norvegycus* Sprague-Dawley with generic vancomycins.

Material and methods

Effectiveness of vancomycins for the eradication of experimental endocarditis in an animal model Based on the Code of Ethics of the National Fund for Science, Technology and Innovation (FONACIT) and on the methodology of Lafaurie,[6] modified in the IIBCAUDO Bacterial Resistance Laboratory, specimens of *Rattus norvegicus* Sprague-Dawley, males, were used. adults about 0.3 kg; they were anesthetized by administering 0.02 ml/kg of the solution of the active compound Xylazine (Hydrochloride) (2-(2,6-xylidino)-5,6-dihydro-4H-1,3 thiazine hydrochloride) via intramuscularly, to inoculate them with the strain *E. faecalis* ATCC 29212 (1.5x10^8 CFU/ml) intravenously. To do this, each of the rats was held horizontally and with a 1 ml insulin syringe, 120 µl of the inoculum was placed through the lateral tail vein to produce an experimental endocarditis. The control rat was sacrificed 48 hours after bacterial inoculation.

The remaining five rats, also inoculated with *E. faecalis* ATCC 29212, were treated intramuscularly for five days, one rat for each vancomycin under study at a dose of 15 mg/kg body weight every 12 hours.

The rats were sacrificed with an intramuscular injection of Succinylcholine 50 mg/ml, eight hours after the last dose of treatment. Using aseptic techniques, the chest was opened with a dissection team; Then, with sterile tweezers, the heart of each of the rats was extracted and placed in sterile Petri dishes to be weighed on the analytical balance. Next, each of the hearts was dissected separately with the help of a sterile scalpel and placed in 6 ml of BHI broth; they were incubated in a bain-marie at 35°C. Dilutions were made depending on the turbidity of the tube containing each of the hearts. 100 µl of the last three dilutions and the undiluted tube were seeded on BHI agar plates with the help of a previously sterile glass rod and incubated for 24 hours at 35°C. After that time, the ability of each of the vancomycins to sterilize the heart was verified by counting

colonies on plates. The dosage regimen was chosen on the basis of plasma concentrations similar to those obtained in humans, and the standard dose of vancomycin corresponds closely to the standard human dosage of 1 gram every 12 hours. The experiments were performed in triplicate.[6] Finally, the colony forming units (CFU) was performed on each plate, an average was applied to them, and the CFU per gram of heart tissue was calculated.[7] The formula used was the following:

$$\frac{CFU}{g} = \frac{\frac{Colony\ numbers}{Seeded\ \ volume(ml)} x \frac{1}{Dilution} x \left\lfloor Tube volume(ml) + \text{Weight(g)} \right\rfloor}{Weight(g)}$$

Statistic analysis

The results of this research are presented in a table, in addition, a non-parametric statistical analysis (Cochran's Q test) was carried out to verify the existence or not of significant differences with a significance level of 1%.[8]

Results

In this work, inocula of 1.5 x 10^8 CFU/ml of the *E. faecalis* strain ATCC 29212 (MIC vancomycin 4 µg/ml) were used to induce bacterial endocarditis in rats and it was observed that three of five non-original vancomycins had bacterial eradication, at eight hours' post-treatment Table 1.

Table 1 Effect of generic vancomycins for eradication of experimental endocarditis in *Rattus norvegicus* sprague-dawley specimens, origin, and batch number of vancomycins

Vancomycins	CFU/g	Source	Batch number
Behrens	–	Mexico	53005
Vancocyn	–	India	V061420
Vancomax	2.8 x 10^{12}	Argentina	K9303
Celovan	–	India	7600236
Fada Pharma	4.2 x 10^{10}	Argentina	27109

In the results of the study of therapeutic efficacy of experimental endocarditis induced in rats with the strain *E. faecalis* ATCC 29212, it was possible to show that the control rat, which was sacrificed 48 hours after being inoculated with 1.5 x 10^8 CFU/ml with the strain mentioned above, there was bacterial growth of 1.8 x 10^{12} CFU/g of heart tissue.

As for the five remaining rats after 48 hours of exposure to the aforementioned bacteria, five days of treatment with generic vancomycins (15 mg/kg every 12 hours) were applied; when they were sacrificed after five days of treatment, it was determined that Behrens, Vancocyn and Celovan managed to eliminate the experimental endocarditis by sterilizing the heart, since there was no evidence of the presence of bacteria, while with Vancomax and Fada Pharma bacterial growth was determined with an average of 2.8 x 10^{12} CFU/g and 4.2 x 10^{10} CFU/g, respectively; presenting only Fada Pharma lower bacterial counts than those found in the control Table 1.

Bacterial counts higher than the initial inoculum (1.5 x 10^8 CFU/ml) that was used to induce experimental endocarditis were observed both for the control and for the two vancomycins (Vancomax and Fada Pharma) that failed to eradicate said infection.

Despite not having worked with neutropenic rats, the success of treatment with generic vancomycins (Behrens, Vancocyn, and Celovan) obtained in this work is not due to the immune response of rats, since there were cases in which vancomycins did not work (Vancomax and Fada Pharma). Neither of the two vancomycins from Argentina (Vancomax, and Fada Pharma) were effective *in vivo*. Those that were synthesized in India (Vancocyn and Celovan) and Mexico (Behrens) were effective *in vivo* Table 1.

This allows health centers to be suggested to refrain from purchasing vancomycins from Argentina, since their pharmacological equivalence will not guarantee therapeutic equivalence, bringing with it therapeutic failure in patients treated with these vancomycins.

The statistical analysis allowed to support the validity of the results obtained in this study, since when applying Cochran's Q Test it was determined that there were significant differences (Q0.01=72.5), with a confidence level of 1% between the five vancomycins that were used to treat experimental endocarditis, between those that failed with those that did not.

Discussion

The increase in drug costs has become a transcendent aspect in the health policy of many countries, including Venezuela. The presence of generic drugs in the pharmaceutical market has been favored in order to replace innovative drugs that have a high cost.[7] One of the factors that has slowed down the progress of generic drugs is the lack of confidence in these drugs. What has been said so far reinforces the need to adjust the definitions of generic drugs (PAHO, 2006).

In this way, it is recommended to improve not only acceptance, but also the strict regulation of quality and effectiveness requirements when promoting the replacement of the prescribed drug by a lower-priced alternative to the general public.[9]

In other studies, the inoculum of the *S. aureus* GRP-0057 strain was lower (10^4 CFU/ml) with a vancomycin MIC of 0.5 mg/l and none of the generic vancomycins marketed in Colombia worked *in vivo*, despite having equivalence pharmaceutical.[10] In this study, the strain of *Enterococcus faecalis* that is sensitive to vancomycin and with a higher inoculum was used. The reference MIC for vancomycin for this strain varies between 1 to 4 mg/L. MICs vancomycin with these generic vancomycins it was 0.5 mg/L for Behrens and Celovan, while with the other vancomycins it was 1 mg/L.[11]

These variations could be due to the fact that study used different parameters, which would explain part of the discrepancies in the results.[6] used New Zealand white rabbits (2 to 2.5 kg) as animal model in their study, their inoculation route was through an ear vein through a polyethylene catheter, however, in this study used the lateral tail vein in *Rattus norvegicus* Sprague-Dawley white rats (0.3 kg). The treatment was not applied intravenously in this study, as its administration in humans is established, due to its difficult handling; this difficulty could be avoided by anesthetizing the rats but increases study costs.

It should be noted that the pharmacokinetic profile of antimicrobials in experimental animals often differs markedly from that in humans, because antibiotics are eliminated faster in animals.[12,13] Some authors have established guidelines in experimental infections in relation to dosage and efficacy in order to simulate pharmacokinetic parameters in humans. Generally, the dose must be related to the serum concentration and the antimicrobial half-life dosing intervals, since the half-life of the drug in animals is shorter, because its metabolism is higher, these factors can affect the pharmacodynamics.[14,15]

Physicochemical characterization was performed on these vancomycins,[11] all vancomycins presented 9 to 10 functional groups

of the 23 contained in the original molecule and those found are responsible for the intermolecular interactions of vancomycin with the peptidoglycan precursor (D-Ala-D-Ala), to form the five bridges that confer stability to the bacteria-antibiotic domain. The number of molecules necessary to bind to D-Ala-D-Ala was estimated and all of them present the molecules necessary to achieve therapeutic success, Celovan being the one with the most molecules.

There are associations of bacteria and antibiotics that work very well *in vitro*, however, *in vivo*, this is not the case. A case published in the international literature, with a cephalosporin, which is highly effective *in vitro*, but does not work in *S. aureus* endocarditis.[16] It is also known that vancomycin does not always result in the eradication of the microorganism, even when its sensitivity profile is demonstrated in the laboratory.[17]

The *in vitro* effectiveness study of these generic vancomycins against certified strains of *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* Mu50 and *Enterococcus faecalis* ATCC 29212, *E. faecalis* V583, was carried out to determine their behavior and the five generic vancomycins studied presented microbiological potency.[8] These generic vancomycins work very well on these certified strains *in vitro*, even if we saw a clinical failure *in vivo* with Fada Pharma and Vancomax.

It has been described that the failure of antibiotic treatments with generics, due to bioavailability in critical patients, is a risk associated with the under dosing of antibiotics that induce intra treatment bacterial resistance,[19] regardless of the *in vitro* results of the bacteriology laboratories that compare the generics with the innovator.

When comparing various vancomycins, which were similar with respect to MICs, and CMBs (concentration minimum bactericidals), intra-treatment developed resistant mutants, etc., they found pharmaceutical equivalence.[20] However, pharmaceutical equivalence (*in vitro*) does not imply therapeutic equivalence (*in vivo*), as was shown in a study conducted with several generic vancomycins in Colombia, when treating neutropenic mice infected with *S. aureus* strains.[21] Not only were the vancomycin generics ineffective in combating the infection in mice, but they also allowed the recovery of resistant subpopulations of *S. aureus* while under antibiotic therapy.[22]

Generic antibiotics may contain contaminating particles that damage the microcirculation of critically ill patients, causing multiple organ dysfunction syndrome. Microcirculatory dysfunction, caused by ischemia, brings with it the deterioration of the patient and is not precisely due to failure of the action of the antibiotic on the microorganism.[23-26]

A study carried out with different generics of cefotaxime demonstrated the presence of foreign particles retained on a filter membrane. These generic contaminating particles are heterogeneous populations that can range from elongated to small, dense or spherical fibers. Intravenous injection of these particles compromises blood microcirculation, demonstrated by histological analysis of capillary lumens with embedded particles.[27]

The possible presence of contaminating particles in generic antibiotics is a risk to the health of patients, since it can cause mechanical blockage of the microcirculation. The formation of salts from drugs generates instability and impurities.[28] Among the impurities described in vancomycin vials are: pentanoic acid, (2RS)-2-ethylpentanoic acid, (2RS)-2-(1-methylethyl)pentanoic acid, 2,2-dipropylpentanoic acid, pentanamide (valeramide), 2-propylpentanamide, 2,2-dipropylpentanamide, pentanenitrile (valeronitrile), 2-propylpentanenitrile, 2,2-dipropylpentanenitrile, N-dimethyl vancomycin B, desamido vancomycin B, agluco vancomycin B, desvancosaminil vancomycin B, product of degradation or fermentation processes of vancomycin molecules; this is due to environmental contamination by microorganisms capable of producing these processes in medicines, which is why the detection of endotoxins present in each vial is recommended.[29] There are studies that say that the amount of impurities present in vancomycin should not exceed 4%.[30]

Crystalline degradation products occur by deamination of the amino acid asparagine from the vancomycin structure.[31] This transformation is accelerated in the presence of alkaline pH and high temperatures, resulting in changes in the solubility of the molecule and, therefore, its inter-relationship with the precursors of the peptidoglycan of bacteria. The hypothesis is that the impurities act as antagonists, affecting the potency and efficacy of the antibiotic.[32-34]

Conclusion

The pharmaceutical equivalence of a generic vancomycin, with respect to the innovator, does not guarantee therapeutic equivalence. More studies need to be done on generic antibiotics to ensure their human clinical use in countries where it is expensive to acquire innovative drugs.

Acknowledgments

The researchers would like to thank Dr. Lily Figueroa, pharmacist in charge of pharmacy at the Antonio Patricio de Alcalá University Hospital, for supplying the generic vancomycins to carry out this study.

Conflicts of interest

The authors of the work declare that they have no conflict of interest with the pharmaceutical laboratories that produce generic vancomycins.

References

1. Von-Hoosier S. The age of biology: opportunities and challenges for Laboratory Animal Medicine. *Scand J Lab Anim Sci*. 1999;26(4):176–184.
2. Zuñiaga J, Tur M, Milocco S, et al. *Ciencia y tecnología en protección y experimentación animal*. Editorial McGraw-Hill Interamericana. México, 2001;682.
3. Obrink K, Rehbinder C. Animal definition: a necessity for the validity of animal experiments? *Lab Anim*. 2000;34:121–130.
4. Vesga O, Agudelo M, Salazar B, et al. Generic vancomycin products fail *in vivo* despite being parmaceutical equivalenst of the innovator. *Antimicrob Agents Chemother*. 2010;54(8):3271–3279.
5. Agudelo M, Franco S, Cardeno J, et al. Therapeutic failure of a bioequivalent generic product of imipenem against Pseudomanas aeruginosa in a murine meningo encephalitis model. 50th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). Boston, USA, 2010;(abstract A1–1385).
6. Lafaurie M, Périchon B, Lefort A, et al. Consequence of VanE-type resistance on efficacy of glycopeptides *in vitro* and in experimental endocarditis due to Enterococcus faecalis. *Antimicrob Agents Chemother*. 2001;45(10):2826–2830.
7. Siegel S, Castellan, *J. Estadística no paramétrica aplicada a las ciencias de la conducta*. 2da ed. Editorial Trillas. Distrito Federal-México,1995.
8. Laosa O, Guerra P, López J, et al. Estudios de bioequivalencia: la necesidad de establecer la fiabilidad de los medicamentos genéricos. *Rev. Peru. Med. Exp. Salud Pública*. 2009;26(4):553–562.

9. Vacca C, James F, Fitzgerald J, et al. Definición de medicamento genérico ¿un fin o un medio? análisis de la regulación en 14 países de la Región de las Américas. *Rev Pan Salud Pub*. 2006;20(5):314–323.

10. C, Agudelo M, Cataño J, et al. Potential therapeutic failure of generic vancomycin in a liver transplant patient with MRSA peritonitis and bacteremia. *J Infect*. 2009;59(4):277–280.

11. Abadía-Patiño L, Hidalgo B, Mosqueda S, et al. Caracterización fisicoquímica de vancomicinas adquiridas en Cumaná, estado Sucre, Venezuela. Saber. 2020;32:54–62.

12. Mizen L, Woodnutt G. A critique of animal pharmacokinetics. *J Antimicrob Chemother*. 1988;21:273–278.

13. Zak O, Reilly OT. Animal models in the evaluation of antimicrobial agents. *Antimicrob Agents Chemother*. 1991;35:1527–1531.

14. Vogelman B, Gudmundsson S, Legget, J, et al. Correlation of antimicrobial pharmacokinetics parameters with therapeutic efficacy in an animal model. *J Infect Dis*. 1988;158(4):831–847.

15. Leggett J, Ebert S, Fantin B. et al. Comparative dose-effect relations at several dosing intervals for beta-lactam, aminoglycoside and quinolone antibiotics against Gram-negative bacilli in murine thigh infection and neumonitis models. *Scand J Infec Dis Suppl*. 1991;7:179–184.

16. Chambers H, Mills J, Drake T. Failure of a once-daily regimen of cefonicid for treatment of endocarditis due to Staphylococcus aureus. *Rev Infect Dis*. 1984;6(4):870–874.

17. Castellano I, González P, Castillo M, et al. Vancomycin dosing in hemodialysis patients. *Nefrología.* 2008;28(6):607–612.

18. Abadía-Patiño L, Hidalgo B, Mosqueda S. Efectividad *in vitro* de vancomicinas genéricas expendidas en Venezuela, en cepas certificadas de los géneros Enterococcus y Staphylococcus. *Rev. Soc. Venezol. Microbiol*. 2019;39:15–20.

19. Pea F, Viale P. Bench-to-bedside review: appropriate antibiotic therapy in severe sepsis and septic shock-does the dose matter? *Crit Care*. 2009;13(3):214.

20. Diaz J, Silva, E, Arias M, et al. Comparative *in vitro* study of the antimicrobial activities of different commercial antibiotic products of vancomycin. *BMC Clin Pharmacol*. 2011;11:9.

21. Vesga O, Agudelo M, Salazar B, et al. Generic vancomycin products fail *in vivo* despite being parmaceutical equivalenst of the innovator. *Antimicrob Agents Chemother*. 2010;54(8):3271–3279.

22. Rodríguez C, Agudelo M, Zuluaga A, et al. Generic vancomycin enriches resistant subpopulation of Staphylococcus aureus after exposure in neutropenic mouse thigh infection model. *Antimicrob Agents Chemother*. 2012;56(1):243–247.

23. Kirkpatrick C, Bittinger F, Klein C, et al. The role of the microcirculation in multiple organ dysfunction syndromes (MODS): a review and perspective. *Virchows Arch*. 1996;427:461–476.

24. Lehr H, Bittinger F, Kirkpatrick C. Microcirculatory dysfunction in sepsis: a pathogenetic basis for therapy? *J Pathol*. 2000;190:373–386.

25. Peters K, Unger R, Brunner J, et al. The molecular basis of endothelial dysfunction in sepsis. *Cardiovasc Res*. 2003;60(1):49–57.

26. Ghanaati S, Webber M, Unger R, et al. Dynamic *in vivo* biocompatibility of angiogenic peptide amphiphile nanofibers. *Biomaterials*. 2009;30:6202–6212.

27. Lehr H, Brunner J, Rangoonwala R. et al. Particulate matter contamination of intravenous antibiotics aggravates loss of functional capillary density in postischemic striated muscle. *Amer J Res Cri Care Med*. 2002;165:514–520.

28. Meredith P. Potential concerns about generic substitution: bioequivalence versus therapeutic equivalence of different amlodipine salt forms. *Curr Med Res Opin*. 2009;25:2179–2189.

29. European pharmacopoeia 5.0. Vancomycin hidrochloride. 2005;01:1058.

30. Harris C, Kopecka H, Harris T. Vancomycin: structure and transformation to CDP-I. *J Amer Chem Soc*. 1983;105:6915–6922.

31. Duquesne S, Destoumieux-Garzón D, Peduzzi J, et al. Microcins, gene-encoded antibacterial peptides from enterobacteria. *Nat Prod Rep.* 2007;24(4):708–734.

32. Selsted M, Ouellette A. Mammalian defensins in the antimicrobial immune response. *Nat Immunol*. 2005;6(6):551–557.

33. De Smet K, Contreras R. Human antimicrobial peptides: defensins, cathelicidins and histatins. *Biotechnol Lett*. 2005;27:1337–1347.

34. Fischbach M, Walsh C. Assembly-line enzymology for polyketide and nonribosomal Peptide antibiotics: logic, machinery, and mechanisms. *Chem Rev*. 2006;106(8):3468–3496.

Citation: Abadía-Patiño L, Hidalgo B, Rojas LM. Sterilization with generic vancomycins of *Rattus norvegicus* Sprague Dawley hearts from experimental endocarditis by *E. faecalis* ATCC 29212. *J Microbiol Exp*. 2022;10(4):142–145. DOI: 10.15406/jmen.2022.10.00365