Bioavailability comparison study of two benzathine benzylpenicillin 1,200,000 IU intramuscular formulations in healthy male participants under fast state

Estudo comparativo da biodisponibilidade de duas formulações intramusculares de Benzilpenicilina Benzatina 1.200.000 UI em participantes saudáveis do sexo masculino em estado de jejum

Vinicius Marcondes Rezende[1], Paulo Galvinas[2], Carlos Sverdloff[1], Camila Leles Guimarães[2], Anne Silveira[3], Camila Aihara[4], Márcia Aparecida Antônio[3], Renata Di Sessa[4]

ABSTRACT

Introduction: Benzathine benzylpenicillin G is a drug present in the list of essential medicines of the World Health Organization and largely used in Brazil, where this antibiotic is used for treating pneumonias, pharyngitis, syphilis, and other infections caused by Gram-positive bacteria, being one of the most prescribed antibiotics of the public healthcare system. **Objective:** The objective of this study was to evaluate the relative bioavailability of two formulations of benzathine benzylpenicillin (Benzetacil®) 1,200,000 IU, both manufactured by Eurofarma Laboratórios S/A, by comparison of plasma levels of both drugs administered intramuscularly, evaluating the pharmacokinetic parameters: C_{max} and AUC_{0-t}, being t=672 h. **Methods:** A randomized, parallel, open-label study with one treatment and one period in 168 healthy male volunteers. Subjects received the test or reference formulations by intramuscular injection. A total of 20 blood samples were collected after administration for plasmatic quantification of the drug by LC-MS/MS along 672 h. **Results:** Both formulations were considered well tolerated, and no serious adverse event was reported during the trial. C_{max} and AUC_{0-t} were compared: the rate between test and reference formulations for C_{max} was 97.75% with confidence interval (CI) (86.34–110.67%) and power 90.55%. The rate between test and reference formulations for AUC_{0-t} was 91.15% CI (85.29–97.42%) and power of 99.99%. The rate between test and reference formulations for AUC_{0-inf} was 87.98% with CI (81.29–95.23%) and power of 99.85%. **Conclusion:** Reference and test formulations were shown to be statistically bioequivalents according to their rate and extension of absorption, based on ANVISA criteria.
Keywords: Benzathine benzylpenicillin. Penicillin G. Bioavailability. Biological availability. Pharmacokinetics.

RESUMO

Introdução: A Benzilpenicilina G Benzatina (BPGB) é um fármaco presente na lista de medicamentos essenciais da Organização Mundial da Saúde e largamente utilizado no Brasil, onde este antibiótico é utilizado para o tratamento de pneumonias, faringites, sífilis e outras infecções causadas por bactérias Gram positivas, sendo um dos mais prescritos no sistema público de saúde. **Objetivo:** Avaliar a biodisponibilidade relativa de duas formulações de Benzilpenicilina Benzatina (Benzetacil®) 1.200.000 UI, ambas fabricadas pela Eurofarma Laboratórios S/A, por meio da comparação dos níveis plasmáticos de ambos os fármacos, administrados por via intramuscular, avaliando os parâmetros farmacocinéticos C_{max} e AUC_{0-t}, sendo t=672 horas. **Métodos:** Estudo randomizado, paralelo, aberto, com um tratamento e um período em 168 voluntários sadios do sexo masculino. Os indivíduos receberam as formulações teste ou referência por injeção intramuscular. Vinte amostras de sangue foram coletadas após a administração para a quantificação plasmática do fármaco por LC-MS/MS ao longo de 672 horas. **Resultados:** Ambas as formulações foram consideradas bem toleradas e nenhum evento adverso grave foi relatado durante o ensaio. A C_{max} e a AUC_{0-t} foram comparadas: a taxa entre as formulações de teste e de referência para a C_{max} foi de 97,75% com intervalo de confiança — IC (86,34–110,67%) e poder de 90,55%. A taxa entre as formulações de teste e de referência para a AUC_{0-t} foi de 91,15% com IC (85,29–97,42%) e poder de 99,99%. A taxa entre as formulações de teste e de referência para a AUC_{0-inf} foi de 87,98%, com IC (81,29–95,23%) e poder de 99,85%. **Conclusão:** As formulações de referência e teste mostraram-se estatisticamente bioequivalentes, de acordo com sua velocidade e extensão de absorção, segundo os critérios da Agência Nacional de Vigilância Sanitária (ANVISA).
Palavras-chave: Benzatina benzilpenicilina. Penicilina G. Biomelhoradores. Disponibilidade biológica. Farmacocinética.

INTRODUCTION

Beta-lactams review

Beta-lactam antibiotics are a diverse group of medications that share a common beta-lactam ring in their chemical structure. Despite this similarity, they exhibit variations in their pharmacokinetic characteristics. Follow a valuable brief comparison:

Absorption: Beta-lactams can be administered via various routes, including oral, intravenous, and intramuscular (IM). Their absorption rates vary depending on factors such as formulation and co-administration with food. For instance, penicillins are generally well absorbed orally, while some cephalosporins may require parenteral administration due to poor oral bioavailability.

Distribution: Beta-lactams have excellent tissue penetration, with distribution into many body tissues and fluids. However, the extent of distribution varies among different beta-lactams. Factors such as protein binding and lipid solubility influence their distribution characteristics.

Metabolism: Most beta-lactam antibiotics undergo minimal metabolism in the body. They are primarily eliminated unchanged via renal excretion. However, some exceptions exist, such as the metabolism of certain cephalosporins by hepatic enzymes.

[1]ATCGen Clinical Trials – Campinas (SP), Brazil. E-mail: vinicius@atcgen.com.br; carsver@atcgen.com.br
[2]Magabi Pesquisas Clínicas e Farmacêuticas – Itapevi (SP) Brazil. E-mail: paulo.galvinas@magabi.com.br; camila.guimaraes@eurofarma.com.br
[3]Universidade São Francisco, Centro de Pesquisa, Unidade Integrada de Farmacologia e Gastroenterologia – Bragança Paulista (SP), Brazil. E-mail: anne.silveira@unifag.com.br; marcia.antonio@unifag.com.br
[4]Eurofarma Laboratórios S/A – Itapevi (SP), Brazil. E-mail: camila.aihara@eurofarma.com; renata.sessa@eurofarma.com

Half-life: The half-life of beta-lactam antibiotics varies widely, ranging from a few minutes to several hours. Short-acting penicillins like penicillin G have relatively short half-lives, requiring frequent dosing. Newer cephalosporins and extended-spectrum penicillins may have longer half-lives, allowing for less frequent dosing.

Renal clearance: Beta-lactams are predominantly eliminated by the kidneys through glomerular filtration and tubular secretion. It is clear that renal function significantly impacts their clearance rates. This is why dosage adjustments are necessary in patients with renal impairment to prevent drug accumulation and potential toxicity.

Drug interactions: While beta-lactams are generally considered to have few drug interactions, it is important to be aware that they can interact with other medications that affect renal function or compete for renal excretion.

Benzathine benzylpenicillin

Benzathine benzylpenicillin, also known as benzathine penicillin G (BPG), is an essential medicine and the first choice for treating several diseases, according to the WHO (World Health Organization) due to its efficacy, safety, and cost-benefit rate[1,2]. In Brazil, penicillin G is also listed in RENAME (Brazilian National Relation of Essential Medicines)[3], and it is a first-line treatment for streptococcal infections, syphilis, and rheumatic fever prophylaxis.

Benzylpenicillin belongs to a broad class of antibiotics called beta-lactams; this type of cyclic structure in the molecule is the major mechanism of action:

1. inhibition of transpeptidase enzymes,
2. disruption of cell wall synthesis, and
3. selective toxicity toward bacterial cells while sparing mammalian cells[4].

The administration of benzylpenicillin to the mother can effectively prevent vertical transmission. Acquired syphilis continues to affect a significant number of individuals in Brazil, with a prevalence of 99.2 per 100,000 inhabitants in 2022. If only pregnant women and newborns are considered, in 2022, syphilis was detected in 32.4 pregnant women per 1,000 live births and in 10.3 newborns per 1,000 live births[5] and can be effectively treated with BPG[1,2]. It is crucial to note that BPG is an effective treatment for syphilis, offering a high level of safety and significantly reducing the risk of transmission to the fetus. This is of particular importance in preventing negative pregnancy outcomes associated with congenital syphilis, including stillbirth, premature birth, low birth weight, and developmental abnormalities[6-8].

BPG is a crystalline powder formed by the reaction of two molecules of penicillin G with a molecule of dibenzyl ethylene diamine. Its low water solubility[9,10] is closely related to its slow absorption after IM administration, and once in the bloodstream, it is hydrolyzed to form benzylpenicillin G, resulting in a long therapeutic blood concentration and consequent greater protection against infections caused by Gram-positive bacteria[10,11]. BPG administered intra-muscularly has a half-life of approximately 336 h, and it is excreted by the kidneys[12].

Although BPG can be considered a safe drug, there is a relatively high rate of allergic reactions and anaphylactic shock after its use[7,11,13]. However, there is no consensus if the cause of the reactions is penicillin or other components of the formulation.

In Brazil, the active principle of BPG is imported, and consequently, at risk of punctual shortages. Recently, there were two threats of this shortage becoming a public health problem, in 2015 and 2017, once the number of cases of syphilis got higher. In 2014, the number of reported cases of acquired syphilis in Brazil was 50,579. In 2015, this number was 69,319, and in 2017, it was 122,172 cases (an increase of 70% compared to 2015)[5]. However, different efforts have been made by the regulatory agencies and pharmaceutical industries to supply the necessities of medicine, for example, searching for different suppliers and optimizing the distribution of the product in the Brazilian territory, among others[14].

The objective of this study was to evaluate if a new formulation of BPG 300,000 IU/mL (Benzetacil®) suspension for deep IM injection developed by Eurofarma Laboratórios S/A reached equivalent plasmatic levels as the currently commercialized medicine by the same industry (Benzetacil® 300,000 IU/mL suspension for injection).

Main uses of benzathine penicillin G

Syphilis treatment

In the realm of sexually transmitted infections, benzathine benzylpenicillin serves as the gold standard for syphilis therapy. Its sustained activity enables a single IM injection to effectively treat early-stage syphilis. In the case of late-stage or neurosyphilis, prolonged courses or higher doses may be indicated. Benzathine benzylpenicillin's efficacy in eradicating *Treponema pallidum* underscores its critical role in combating this global health concern[2].

Streptococcal infections

Benzathine benzylpenicillin remains a cornerstone in the management of streptococcal infections, including pharyngitis, tonsillitis, and cellulitis. By targeting susceptible strains of *Streptococcus*, it alleviates symptoms, prevents complications, and curtails community spread. Its use in streptococcal endocarditis prophylaxis is also noteworthy, particularly in individuals with predisposing cardiac conditions[15].

Rheumatic fever prophylaxis

Benzathine benzylpenicillin plays a pivotal role in the prevention of recurrent attacks of acute rheumatic fever (ARF) and rheumatic heart disease. Administered via IM injection at regular intervals, typically every 3–4 weeks, it effectively suppresses *Streptococcus pyogenes* infections, the causative agent of ARF. This prophylactic regimen significantly reduces the risk of developing carditis, the most serious complication of rheumatic fever[16].

Other applications

In addition to its traditional applications, benzathine benzylpenicillin has been demonstrated to be efficacious in a number of clinical

scenarios. It can be employed prophylactically in certain surgical procedures to reduce the risk of postoperative infections. Moreover, benzathine benzylpenicillin has been demonstrated to be efficacious in the management of dermatological conditions such as erysipelas and cellulitis, where its antimicrobial spectrum aligns with the causative pathogens[17].

Formulation

BPG formulation has as excipients: sodium citrate, povidone, disodium EDTA, propylparaben, methylparaben, sodium metabisulfite, and water for injections, presented as a total of 1,200,000 IU of BPG per ampoule (300,000 IU/mL, 4 mL), as described in its insert label[14]. This presentation is for deep IM injection, and due to its low solubility, it has a slow release in the bloodstream[8,9].

Generally, BPG is well tolerated when the patient does not have an allergy to penicillins and presents as the most common adverse events (>1/100 and <1/10): headache, oral moniliasis, nausea, vomiting, diarrhea, vaginal, and/or vulvar moniliasis. Uncommon reactions (>1/1,000 and < 1/100): cutaneous eruptions, rash, pruritus, urticaria, rare reactions: fluid retention edema, anaphylactic reactions, serum sickness-like reaction, laryngeal edema, and hypotension. Rare reactions (>1/10,000 and <1/1,000): Stevens-Johnson syndrome, toxic epidermal necrolysis, erythema multiforme, mental confusion, convulsions, venous thrombosis, thrombophlebitis, drug hepatitis, pseudomembranous colitis, acute interstitial nephritis, crystalluria, acute renal failure, hypokalemia, hemolytic anemia, thrombocytopenia, leukopenia, agranulocytosis, eosinophilia, and coagulation disorders. Local symptoms: tumors, lesions, and pain at the injection site.

Patients in treatment with penicillins, as well as patients with multiple allergens hypersensitivities, reported serious hypersensitivity, which eventually proved fatal.

OBJECTIVE

The study aimed to determine key pharmacokinetic parameters to provide evidence of the relative bioavailability between two formulations of BPG injectable suspension. Each formulation was administered at a dose of 1,200,000 IU of BPG, with each ampoule containing 300,000 IU/mL in a total volume of 4 mL. The entire content of the ampoule was administered via IM injection. Plasma concentrations of the drug were measured in subjects for each formulation. The pharmacokinetic parameters, specifically the maximum plasma concentration (C_{max}) and the area under the plasma concentration-time curve from time 0 to 672 h (AUC0-672), were then statistically evaluated.

METHODS

This study was designed to obtain relevant pharmacokinetic parameters for statistical comparison to provide evidence of relative bioavailability between two formulations of BPG injectable suspension, both in a dose of 1,200,000 IU of BPG (each ampoule had 300,000 IU/mL, ant total volume of 4 mL); all the ampoule content was used in the intra-muscular application. The evaluation was made by obtaining the plasma concentration of the drug in subjects for each formulation and statistically evaluating the pharmacokinetic parameters C_{max} and AUC_{0-t}, with t=672 h.

Study design and drug administration

This was an open, randomized, parallel study with a single treatment in one period, with planned 168 healthy male subjects.

The pharmacokinetic parameters (C_{max} and AUC_{0-t}) were obtained directly from the plasmatic concentration of the active principle of the medications, based on the application of a non-compartmental model, adequate for the evaluation of concentrations after a 1,200,000 IU IM single administration.

Considering the half-life of the drug administered via intra-muscular injection is 336 h[18], a truncated 672-h study was performed (AUC_{0-672})[19].

Study population and inclusion/exclusion criteria

Bioequivalence studies commonly recruit healthy adult subjects to ensure a homogeneous population with similar characteristics, such as age and body mass index (BMI). This approach minimizes interference from differences in body composition, metabolism, or previous diseases on the pharmacokinetic profiles, particularly in parallel designs. By doing so, we can confidently draw conclusions about the bioequivalence of the tested products.

Participants selection

A total of 168 male subjects were selected for this trial. All participants were 18–50 years old and had to have a BMI between 18.5 and 29.9 kg/m^2. Subjects who participated in any other clinical trial in the last 6 months prior to the initiation of the study were excluded, as well as the ones with a history of alcohol or drug abuse and a history of allergy to benzathine benzylpenicillin, which was evaluated by the clinical board during the medical interview. Additionally, a backup hospital was available during all the trial confinement to treat eventual emergencies. Other exclusion criteria were the use of any medication within 14 days before the study, a positive result for hepatitis B, C, or HIV, or any clinically significant alteration of laboratory exams. In addition, volunteers with medical conditions that could interfere with their participation or who were hospitalized in the last 8 weeks were not selected.

Ethical considerations

All participants signed an Informed Consent Form, which was approved by the Sao Francisco University Ethics Committee under the registry number CAAE 43145215.9.0000.5514, along with the clinical protocol JPJ14JPJ14/15 version 1.0, elaborated by the Clinical Center and approved by the Principal Investigator and Sponsor. Further, all procedures of the study were conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines and local ethics regulations[20,21]. For this study, submission to CONEP (Brazilian National Committee of Ethics in Research) is not applicable.

Trial conduction

The formulations were administered via IM in a single dose, followed by blood sampling until 672 h after administration. Trial subjects remained under 9 h fasting before and 4 h for solid meals after administration, being permitted water 2 h after, and they remained confined for approximately 36 h.

Trial subjects received a single IM 1,200,000 IU injection of either test or reference formulations, according to a randomization list, which was generated by the online application at http://www.randomization.com/, on March 19, 2015, with formulation balanced.

Sample collection

Blood samples of 8.5 mL were collected through a catheter in a superficial forearm vein. A total of 20 samples were collected in a tube containing lithium heparin as an anticoagulant. Sampling times were 0:00; 1:00; 2:00; 4:00; 8:00; 12:00; 14:00; 16:00; 18:00; 20:00; 24:00; 36:00; 48:00; 72:00; 120:00; 168:00; 216:00; 336:00; 504:00; and 672:00 h after administration. The selection of collection times was based on the pharmacokinetic properties of the drug, including the time required to reach C_{max}. To ensure accurate characterization, more collections were conducted around the expected time, with additional collections conducted until the last time point (672 h) for evaluation, which allowed for the description of the elimination phase of the drug.

Blood samples were centrifuged at 3,000 rpm for 10 min, and the plasma obtained from this process was separated into two aliquots in cryogenic tubes and stored at -70°C.

Bioanalytical analysis

The analysis of the plasmatic concentrations of BPG was conducted in an HPLC system (Agilent 1200 Series RRLC System, Germany) coupled to a mass spectrometer (Sciex, API5500 Qtrap, Canada). The internal standard used was ampicillin.

For the extraction of the samples, 50 µL of plasma was placed in 2-mL Eppendorf tubes, and 50 µL of internal standard was added (1 µg/mL of ampicillin), along with 400 µL of acetonitrile. The tubes were vortex-mixed for 30 s and centrifuged at 20,800 RCF for 5 min. The supernatant was transferred to a test tube with 2 mL of water and vortex-mixed for 30 s, and finally, 600 µL was transferred to 96 deep-well plates to load the autosampler.

The methodology for the current study was validated for the following parameters: selectivity, calibration curve, precision and accuracy of the lower limit of quantification, precision and accuracy of the quality control samples, and control of dilution, matrix effect, and residual effect. Stability tests for post-processing, long and short duration, freeze and thaw, analyte in solution, and internal standard in solution were performed. The limit of quantification was obtained as the lowest level able to be determined with acceptable precision and accuracy (equal or inferior to 20% in CV), and it was 1.00 ng/mL of BPG in plasma.

The concentrations of the analyte in the samples were determined from calibration curves, defined by a linear regression model, whose points were obtained from blank human plasma, contaminated with known concentrations of the analyte (BPG) and of the internal standard (ampicillin).

The chromatography was performed in a Phenomenex column, Onyx, Monolithic C18, 5 µm (50×4.6 mm) using a gradient of acetonitrile and 23 mM of formic acid solution in a flow of 1.0 mL/min, with a total run time of 5 min.

The mass spectrometer operated in a negative electrospray ionization mode. The resulting ions of the BPG molecules were (m/z): precursor ion: 333.1, product ion: 191.9, and of ampicillin: precursor ion: 348.1, product ion: 207.1; all of them were monitored in multiple reaction monitoring mode.

The temperature of the source was 600°C, with an ion spray voltage of -4,500 V and medium collision gas. Collision energy was -20 V for BPG and -17 V for ampicillin.

Standard reference materials used were BPG (lot K0F132) and ampicillin (lot K1M4933), both of them from US Pharmacopeia.

Calculation of sample concentration by software

Concentrations were calculated by Analyst software version 1.5.2. The function applied to the samples of the calibration curve was calculated by a system of weighted linear regression, using the rate of the area of the analyte and the area of the internal standard (response) from the respective chromatograms. This function was previously validated, according to the current legislation. The samples of blank, zero, or rejected by criteria of acceptance/rejection were not used for the construction of the calibration curve. The responses from every sample were then interpolated by the software in the calibration curve to inform the concentration of the compound of interest.

Statistical analysis

To conduct relative bioavailability analyses between the two medications, the following variables were considered primary: C_{max} and AUC_{0-t}. The bioequivalence of the components was assessed by means of analysis of variance and calculating standard 90% CIs for the ratio test/reference using log-transformed data for C_{max} and AUC_{0-t}. The components were considered bioequivalent if the confidence interval for the ratio of the means fell within the interval of 0.80–1.25, or equivalently, if the 90% confidence interval for the difference in the means on the natural log-transformed data fell within the interval of 80–125% (equivalent to a ratio of 0.80–1.25).

The statistical analysis of the data was done with Phoenix WinNonlin™ version 6.3, Microsoft® Excel® version 97, and Microsoft® Word® version 97. All calculations of pharmacokinetic parameters were done with plasma concentration obtained from analytical determinations for each collected sample.

RESULTS

Tolerability and safety analysis

A total of 167 subjects ended the study; one participant was withdrawn due to an adverse event (AE) (sore throat during confinement). Clinical exams post-study did not show modifications in the general health or well-being of the participants that could be attributed to the study products. Both formulations were well tolerated in the administered dose, and no serious adverse events were reported.

In general, it can be cited that most of the AEs were alterations in laboratory exams, such as small alterations in hemogram, leukogram, and biochemistry, including liver enzymes or total blood protein. Considering the time of study (56 days), these AEs may not even be related to the use of the product, as they did not represent any physiological interference caused by the product. In addition, we can cite that, during the confinement, only seven headaches and one sore throat were observed. All other events were observed after hospital discharge. **Table 1** displays the most frequent AEs reported from a total of 80 AEs. The causality and the quantity of AEs that were observed during the hospitalization or after discharge are presented in **Table 2**.

Table 1. Adverse events observed during the study (including the dropped-off participant).

Adverse events (summarized)	Quantity of observed AE
Headache	13
Leukogram alterations	13
Urine alterations	10
Erythrogram alterations	10
Lipid profile alterations	10
Liver enzymes alterations	8
Glycemic profile alterations	4
Blood protein profile alterations	3
Sore throat	2
Abdominal pain	1
Low back pain	1
Malaise	1
Tonsillitis	1
Upper respiratory tract infection	1
Pain in the local of application	1
Conjunctivitis	1
Total	80

AE: adverse event.

Table 2. Quantity of adverse events observed during the hospitalization and after discharge and quantity by causality.

Beginning of AE		
During confinement, after administration	8	7 Headache and 1 sore throat
After hospital discharge	72	Others
Causality		
Unlikely	45	
Unrelated	4	
Possible	31	

The most common AE possibly related to the treatment was headache, with 16.25% of all 80 events. The most common AEs not related to the treatment were alterations of post-study laboratory exams, with a total of 72.5% of all events in this class. All AEs were resolved without sequels during the follow-up.

Statistical results

Table 3 shows the most important statistical results of the pharmacokinetics parameters C_{max}, AUC_{0-t} and $AUC_{0-inf.}$

The mean plasmatic concentration of BPG after the administration of each formulation can be viewed in **Figure 1**.

The calibration curve used to validate the method was linear, within the range of 1,000–500,000 ng/mL, with a regression coefficient ≥0.99 and a non-significant intercept.

DISCUSSION

Generalities and pharmacokinetics results

The current study reveals that the two studied formulations have very similar concentration curves. Scientific evidence indicates that the concentration of BPG after a 1,200,000 IU dose is about 20 ng/mL on the 28th day after IM administration, data that was also observed in a previous study[12]. These data are also indicative that the analytical method used for the evaluation of dosing was adequate.

Adverse events

BPG presents as the most important AE, the sensitization and hypersensitivity reactions. The importance is because of its possible complications and even the occurrence of anaphylactic cases.

It must be mentioned that this study showed no serious AEs, although there are some trials that point out BPG as a drug with a significant index of sensitization[22]. The reactions of hypersensitivity are particularly important and occur in 1/100 to 1/1,000 of the cases, according to the clinical trials and postmarketing reported events. On the contrary, there are studies in which the incidence of AEs related to hypersensitivity is not only low but, in many times, not related to

Table 3. Geometric means, confidence intervals, and p-values obtained by ANOVA.

Statistical results by pharmacokinetic parameter			
	C_{max} (ng/mL)	AUC_{0-t} (ng×h×mL^{-1})	AUC_{0-inf} (ng×h×mL^{-1})
Geometric means obtained by minimum square methods			
Reference (R)	84.38	20683.08	29009.38
Test (T)	82.48	18853.12	25522.68
Confidence intervals obtained for rate between treatments (transformed data)			
Rate (T/R)	97.75	91.15	87.98
Inferior limit	86.34	85.29	81.29
Superior limit	110.67	97.42	95.23
Power a *posteriori* (%)			
T/R	90.55	99.99	99.85

$C_{max:}$ Maximum concentration; $AUC_{0-t:}$ area under curve 0 to 672h; $AUC_{0-inf:}$ area under curve 0 to infinite; T: test formulation; R: reference formulation.

penicillin[23-25]. Kaya et al. have published a study[24] on 535 children with acute rheumatic fever who would receive BPG to prevent endocarditis. Eleven of the 535 children were suspected to have had allergic reactions, in a total of 17,641 injections. After detailed evaluation, only one child had confirmation of penicillin allergy (0.18%). Garcia et al. have published a prospective study on pregnant women with syphilis and labeled as allergic to penicillin. They were divided into two groups (low risk and high risk of reaction) — low-risk patients with negative skin testing and negative serum-specific IgE to penicillin underwent a drug provocation test. The remaining patients underwent desensitization. Allergy to penicillin was confirmed in 7.69% of pregnant women labeled as allergic[23].

In the current trial, the low incidence of AEs related to the use of the drug, mainly hypersensitivity, can be attributed to the homogeneity of the population (healthy adults with similar BMI and no diseases) and the control of their feeding, daily habits, and activities during the confinement. Additionally, the participants with allergies to penicillins or related drugs were excluded as a criterion in the screening phase.

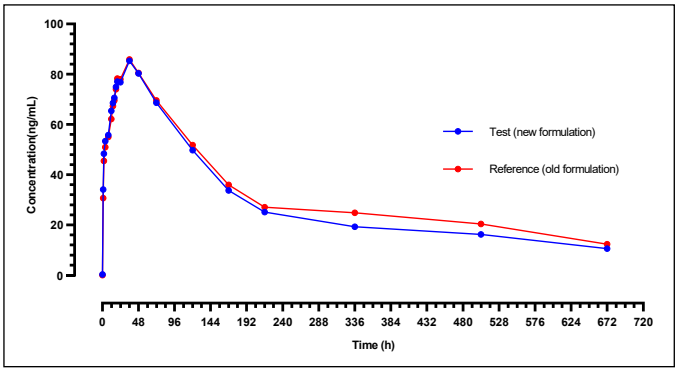

Figure 1. Mean plasmatic concentrations of benzylpenicillin benzathine versus time.

In this BA study, none of the 168 subjects presented a reaction to the studied drug, which means that the formulations are safe. The most common AE possibly related to the treatment was headache, with 16.25% of all the events. The most common AEs not related to the treatment were alterations of post-study laboratory exams, with a total of 50% of all events of this class. All AEs were resolved without sequels.

Pharmacokinetics comparison between the most common beta-lactams

It is worth mentioning the low inter-subject variability between both formulations for pharmacokinetic parameters, as described in **Table 3**. Previous research had already shown similar results with a lower sample size[18].

The ANOVA test did not show a significant statistical difference between both formulations for product effect and sequence effect for the parameters C_{max} and T_{max}.

Considering the drug half-life of 336 h[18], a truncated 672 h study was performed (AUC_{0-672})[19], and according to the data shown in **Table 3** and in **Table 4**, no significant differences were observed between the test and reference formulations for AUC_{0-t} and AUC_{0-inf}.

The pharmacokinetic parameters of beta-lactams vary considerably depending on the route of administration, dose, and pharmaceutical form. These characteristics, in conjunction with an understanding of the pathophysiology of the infection, the characteristics of the target tissue, and the microorganism involved, inform the selection of the optimal alternative in each case. **Table 5**[4,25] illustrates the various characteristics of the beta-lactams most commonly used in therapy.

Bioanalytical method

The LC-MS/MS method described here for the quantification of the drug presented high sensitivity, specificity, and high recovery

Table 4. Mean concentrations of each sampling time for all participants by formulation. Values of concentration in ng/mL. Amplitude values as minimum and maximum. Dispersion reported in CV%.

Time (h)	Test (new formulation) n=84					Reference (old formulation) n=83				
	Mean	SE	Min	Max	CV%	Mean	SE	Min	Max	CV%
0	0.075	0.075	0.000	6.186	911.043	0.290	0.235	0.000	19.047	741.695
1	30.666	3.137	3.175	132.887	93.197	34.115	3.159	2.634	104.161	84.880
2	45.522	3.433	8.673	158.103	68.711	48.417	3.697	7.002	131.316	69.984
4	51.010	3.372	13.283	154.066	60.224	53.419	3.845	10.622	135.055	65.963
8	55.091	3.502	10.682	189.486	57.917	55.824	4.167	10.441	221.975	68.412
12	62.202	3.552	16.957	184.220	52.032	65.435	4.625	16.456	251.224	64.786
14	67.358	3.787	20.767	191.465	51.216	68.570	4.407	19.081	237.923	58.911
16	69.607	3.913	22.551	210.202	51.212	70.636	4.510	19.186	240.124	58.514
18	74.073	4.169	24.766	215.685	51.275	74.999	4.783	21.424	267.507	58.450
20	78.265	4.217	26.326	201.408	49.084	77.104	4.876	20.593	268.612	57.958
24	78.009	4.032	26.948	172.104	47.091	76.786	4.753	17.861	242.185	56.730
36	85.844	4.234	32.603	200.067	44.938	85.281	5.082	18.161	242.769	54.290
48	80.494	3.606	26.440	198.097	40.817	80.306	4.244	17.444	200.015	48.441
72	69.552	3.046	24.931	165.173	39.899	68.626	3.353	18.337	173.757	44.776
120	51.775	1.989	19.140	127.652	34.996	49.756	2.748	10.682	231.810	50.616
168	35.941	1.267	15.242	72.164	31.921	33.706	2.557	3.851	230.472	69.536
216	27.060	0.845	10.516	60.308	28.445	25.176	0.744	2.840	42.555	27.102
336	24.863	1.739	7.809	150.711	63.728	19.318	0.693	0.000	40.963	32.878
504	20.385	0.968	3.226	50.971	43.263	16.274	0.754	0.000	38.379	42.459
672	12.354	0.686	0.000	29.844	50.552	10.585	0.617	0.000	25.056	53.421

Mean: average of individual values; SE: standard error; Min: minimum value; Max: maximum value; CV: coeficiente of variation in %.

Table 5. A comparison of pharmacokinetic parameters for the most common beta-lactam antibiotics is presented. The data are based on information from several parts of textbooks[4,25] and serve only as a direction because there are several options in the market with the same active ingredients, leading to different results from the parameters.

Beta-lactam antibiotic	Absorption	Distribution	Metabolism	Half-life	Renal clearance
Penicillin G	Good oral absorption; variable IM absorption	Widely distributed; poor CNS penetration	Minimal metabolism	Short (30 min to 1 h)	Predominantly renal
Amoxicillin	Good oral absorption	Widely distributed	Minimal metabolism	Short (1 h)	Predominantly renal
Ceftriaxone	Not orally bioavailable; IV/IM administration	Excellent tissue penetration including CNS	Minimal metabolism	Long (7 h)	Predominantly renal
Cefuroxime	Good oral absorption	Widely distributed	Minimal metabolism	Intermediate (1-1.5 h)	Predominantly renal
Meropenem	IV administration	Widely distributed including CNS	Minimal metabolism	Intermediate (1)	Predominantly renal

IM: intra-muscular injection; CNS: central nervous system; IV: intra-venous.

of the drug from the sample, which are essential for pharmacokinetic and bioequivalence studies. In conclusion, a specific, sensitive, simple, and widely applicable HPLC-MS/MS analytical method has been developed for the determination of benzylpenicillin in human plasma. The chosen HPLC-MS/MS technique is currently considered the gold standard for the quantification of drugs in biological fluids and has been demonstrated to be robust in quantification. Despite the high cost of this equipment, there is a great demand for specialized laboratories, which promotes constant technological updating, allowing the development and validation of increasingly challenging methods.

Bioequivalence studies

In the pharmaceutical industry, bioequivalence studies are of critical importance in ensuring that a generic drug has the same bioavailability as its brand-name counterpart. These studies assess whether the generic drug releases its active ingredient into the bloodstream at a similar rate and extent to the original drug.

Bioequivalence studies (BES) are the most commonly employed method to ensure therapeutic equivalence between generic or non-brand-name drugs and their brand-name counterparts (reference formulation).

Strengths

As strengths of bioequivalence, we can consider that BES are generally less costly and time-consuming than full clinical trials, and their conduct is governed by regulatory standards. The development of a generic formulation is facilitated once it has only the objective of producing a pharmacokinetic equivalent formulation. Finally, the generic drugs promote market competition and generally lead to a reduction in final price for customers[26,27].

Limitations

The limitations of BES include a restricted focus on pharmacokinetics (PK), the variability in study populations, the limited applicability of some drugs, and differences in regulatory landscapes across the world. The BES primarily focuses on PK parameters with a single-dose scheme and does not explore other aspects of the drug, such as efficacy and safety. The populations used in bioequivalence studies are often small and homogeneous, typically healthy volunteers, limiting the challenge of the drug among variable populations. This approach may not accurately reflect the broader patient population, potentially overlooking differences in drug metabolism and response in diverse groups. Despite the existence of guidelines, differences in regulatory requirements across different regions can complicate the study design and approval process, leading to delays and increasing the production costs for some studies[26,27].

These considerations are consistent with the experience of this group of authors, not only in the present work but also in their long-term experience. They represent the reality of the most strengths and limitations of BES.

CONCLUSION

The rate and extension of absorption were considered, as required by regulatory agencies FDA (Food and Drug Administration) and ANVISA (Brazilian National Health Surveillance Agency). The rates of means of minimum squares and confidence intervals of 90% derived from the analysis of pharmacokinetic measurements (log-transformed) of AUC_{0-t}, AUC_{0-inf}, and C_{max} for BPG are within the limit of 80–125%. Thus, it can be concluded that the analyzed formulations are bioequivalent.

Based on the results of AE reporting and severity assessments, as well as clinical examinations, electrocardiograms, and laboratory assays, both products were deemed to be well tolerated by the participants. The AE profiles observed were consistent with those reported in the literature and referenced in the product insert.

Finally, the use of generic drugs in clinical practice should be encouraged as an alternative to public health systems to reduce costs and maintain the quality of offered treatments, provided that bioequivalence trials demonstrate interchangeability between generic and reference products.

Approval by the Human Research Ethics Committee

Prior to the commencement of any study-related procedures, including subject enrollment, the protocol and the informed consent form were reviewed and approved by the ethics committee, *Comitê de Ética em Pesquisa da Universidade São Francisco, Bragança Paulista, SP*, Brazil. The committee's approval was granted under the registry number CAAE: 43145215.9.0000.5514.

Participation of each author

VMR: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. PG: Formal analysis, Methodology, Validation. CS: Writing – original draft, Writing – review & editing. CLG: Formal analysis, Methodology, Validation. AS: Investigation,

Project administration, Supervision. CA: Project administration. MAA: Investigation. RDS: Conceptualization, Supervision.

Funding

This study was sponsored by Eurofarma Laboratórios S/A. and conducted in partnership with Unifag.

Conflict of interest

The authors declare that, with the exception of Camila Kaori Aihara and Renata Gebara de Grande Di Sessa, who are associates of Eurofarma Laboratórios S/A, there are no conflicts of interest in this study or publishing work.

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Address for correspondence
CARLOS SVERDLOFF
Rua General Marcondes Salgado, 130 – sala 11
Campinas (SP), Brazil
CEP: 13015-220
E-mail: carsver@atcgen.com.br

Received on: 06.14.2023
Approved on: 04.29.2024