Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v62i4.21029
Artículos originales

Comparison of *in vitro*, *in vivo*, and in silico bioavailability results of different prednisone tablet formulations to assess the feasibility of possible biowaiver

Comparación de los resultados de biodisponibilidad *in vitro*, *in vivo* e in silico de diferentes formulaciones de comprimidos de prednisona para evaluar la viabilidad de una posible bioexención

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Recibido: 19.04.2021
Aceptado: 05.09.2021
Publicado: 20.09.2021

Financiación

Sin financiación.

Conflicto de intereses

The authors declare no conflict of interest.

Resumen

Introducción: Los productos orales sólidos de liberación inmediata que contienen fármacos muy solubles y permeables son candidatos para el proceso de bioexención. Este trabajo tiene como objetivo comparar datos *in vitro*, *in silico* e *in vivo* para establecer si las formulaciones de comprimidos orales de prednisona publicadas anteriormente son candidatas a la bioexención.

Método: Para lograr este objetivo se realizaron estudios de permeación en células Caco-2. Se aplicó un estudio de bioequivalencia previo entre la formulación de prueba y el medicamento de referencia en una evaluación *in silico* utilizando Gastroplus® para evaluar la bioequivalencia de otras dos formulaciones propuestas anteriormente.

Resultados: El coeficiente de permeabilidad aparente para prednisona presentó un valor de 3,69 x 10^{-5} cm/s en 180 minutos. El estudio de bioequivalencia muestra que el producto probado y de referencia era equivalente. Las simulaciones *in silico* predijeron con éxito la farmacocinética de las formulaciones probadas y las otras dos, ya que fueron validadas con el estudio *in vivo*. Ambos exhiben los mismos perfiles de concentración plasmática frente a tiempo**.**

Conclusiones: A través de los resultados *in silico*, es posible inferir que las otras dos formulaciones ensayadas pueden ser bioequivalentes respecto al producto de referencia. Este resultado puede ser útil en la solicitud de bioexenciones. Para reducir los costos y el uso de seres humanos en los estudios de bioequivalencia, este enfoque podría ser una forma esencial de trabajar en la industria farmacéutica.

Palabras clave: prednisona; simulación *in silico*; biodisponibilidad; bioexención

Abstract

Introduction: The immediate-release solid oral products containing very soluble and permeable drugs are candidates for the biowaiver process. This work aims to compare *in vitro*, *in silico*, and *in vivo* data to establish if previously published prednisone oral tablet formulations are biowaiver candidates.

Method: To achieve this goal, permeation studies were conducted on Caco-2 cells. A previous bioequivalence study between the test and the reference drug product was applied on an *in silico* evaluation using Gastroplus® to assess the bioequivalence of two other previously proposed formulations.

Results: The apparent permeability coefficient for prednisone presented a value of 3.69 x 10^{-5} cm/s in 180 minutes. The bioequivalence study shows that the tested and reference product was equivalent. The *in silico* simulations successfully predicted the pharmacokinetics of the tested and the other two formulations since they were validated with the *in vivo* study. Both exhibit the same plasma concentration *vs.* time profiles.

Conclusions: Through the *in silico* results, it is possible to infer that the other two formulations tested may be bioequivalent concerning the reference product. This result may be helpful in biowaiver requesting. Toward to reduce costs and the use of human beings in bioequivalence studies, this approach could be an essential way to work in the pharmaceutical industry.

Keywords: prednisone; *in silico* simulation; bioavailability; biowaiver

Highlights

In silico pharmacokinetic studies could accelerate the registration process for generic drugs applying for biowaiver.

As a biowaiver candidate, the relevance of *in silico* studies for BCS class I drugs is even more critical, given the regulatory scenario.

It may induce regulatory agencies to take a more in-depth view of the dissolution aspects during stability studies and how *in silico* previsions may help develop new projects.

Introduction

As in the USA and Europe, the generic drug registration in Brazil requires bioequivalence (BE) studies. Pharmaceutical equivalents drug products are considered bioequivalent and, therefore, interchangeable when the bioavailability is not statistically different between the products after administration at the same dose and under similar experimental conditions in a BE study[1].

Prednisone is a glucocorticoid extensively used in clinical practice for inflammatory diseases. It is considered borderline class I in the Biopharmaceutical Classification System (BCS), which is highly soluble and permeable. The drug has a plasma protein-binding rate of approximately 90%, bioavailability around 80%, and volume of distribution ranges from 0.4 to 1.0 $L.kg^{-1}$. It has a biological half-life time of about 3.6 hours, and maximum serum concentration (C_{max}) is reached between one and three hours after oral administration. The permeability rate of prednisone in artificial phospholipid membrane is 0.3×10^{-6} cm/s[2].

In Brazil, prednisone is marketed as immediate-release oral tablets at concentrations of 20 mg and 50 mg. The reference medicine is named Meticorten®, produced by Merck Sharp & Dome®[3]. Numerous generic formulations of prednisone registered in the Brazilian market prove the BE with the reference product. These tests require resources and time in the formulation development process. Thus, one of the main discussions among the academic, industrial, and regulatory arena today revolves around biowaivers.

A biowaiver account that relative bioavailability or BE tests are not required for drug registration by the regulatory agency when a suitable *in vitro* assay could replace it[4]. The need for the pharmaceutical industry to obtain a safe tool that allows an *in vitro-in vivo* correlation (IVIVC) for a possible biowaiver is increasingly higher. The concept and application of this correlation have been focused attention on universities, pharmaceutical industries, and regulatory sectors.

The correlation of the *in silico* simulation with the *in vitro* and *in vivo* studies can predict the absorption, distribution, metabolism, and elimination of several drugs[5]. Biowaiver procedure could involve an evaluation through computational simulations that delineate a natural or laboratory process. For *in silico* simulation, some software such as SIMCYP™, PK-sim™, CLOEPK™, and GastroPlus® are currently used. However, to obtain a correlation, this software needs some active pharmaceutical ingredient (API) input data such as permeability rate, pKa, logP, logD, and solubility[6].

Gastroplus® was used in a previous study to predict oral absorption and BE of two formulations of glyburide[7]. Also, the *in vitro* results combined with *in silico* simulation to justify a biowaiver for etoricoxib[8]. Even though it is handy in drug bioavailability prediction in the development phase, Brazilian legislation currently does not consider the *in silico* results to grant exemption or replace bioequivalence studies. On the other hand, the biowaiver could be required based on the Biopharmaceutical Classification System (BCS)[4,9].

In a previous paper, we presented a pre-formulation study by which a prednisone raw material supplier was chosen. Tablet formulations were prepared, and a stability study was conducted to select which one should be scaled up. Moreover, we monitored dissolution and dissolution profiles during stability study, a not common approach in pharmaceutical literature. Considering the reduction of dissolution during stability studies, it could eventually impact the *in vivo* performance of the product over time[10].

This work compared *in vitro*, *in silico*, and *in vivo* data to establish if previously published prednisone oral tablet formulation is an effective candidate for the biowaiver process. Simultaneously, this paper brings a wholly new perspective of biowaiver attempt helping to understand this process better.

Methods

Permeation studies on Caco-2 cells

Cytotoxicity studies

Caco-2 cells (passage 57) were seeded in a 96-well plate, 32,000 cells/well, in a total volume of 200 microliters/well, using Dulbecco's Modified Eagle Medium (DMEM, pH 7.40) supplemented with 10% (v/v) fetal bovine serum, 1% benzylpenicillin (160 IU/ml) streptomycin sulfate (100 µg/ml) and 1 % of non-essential amino acids was used as the culture medium. Cell cultures were maintained at 37 °C, 95% air atmosphere, 5% CO_2, and 95% relative humidity[11,12] as a culture medium. Subsequently, cell substrates were taken to the incubator at 37 °C and 5% CO_2 for 24 hours for cell growth[13]. After 24 hours, the culture medium was removed from wells, and cells were placed in contact with a 31.5 µg/mL solution of prednisone in Hank's Balanced Salt Solution (HBSS) + 5% ethanol (pH 6.8). HBSS and HBSS + 5% ethanol solutions, both at pH 6.8, were used as control groups.

Samples were stayed connected with Caco-2 cells for 3 hours and then aspirated. Thus, the cells were treated with 2.5 mg/mL 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide solution (MTT solution in phosphate buffer solution - PBS, pH 7.4), adding 100 µL of HBSS (pH 6.8) and 25 µL of MTT solution per well. The plate was incubated for an additional 3 hours at 37 °C and 5% CO_2. MTT solution was aspirated, and the cells were washed with PBS (pH 7.4) two times. Then 100 µL/well of dimethylsulfoxide (DMSO) were added for formazan crystals solubilization, generating a violet coloration according to the degree of cellular viability. Absorbance readings from the wells were performed on Microplate Absorbance Reader (iMARKTM, USA), at 570 nm, using 690 nm as referencing, after vigorous shaking for 60 seconds[14,15].

Caco-2 cells monolayer permeability studies

Samples were subjected to permeability tests through Caco-2 cell monolayers during passage 57. Cells were seeded onto polycarbonate filters (0.33 cm^2, 6.5 mm internal diameter, and 0.4 µm pores) in 24 well plates at a density of 2.5 x 10^5 cells/cm^2. The cells' filters were maintained with cell culture medium changes for 21 days after seeding and were used for transepithelial electrical resistance and transport assays[14-16]. The media used for API dilution was HBSS + 5% ethanol (pH = 6.8). This solution was used in the apical compartment (donor). HBSS (pH 7.4) mimics the microenvironment under the intestinal epithelium in the basolateral compartment (receptor). Prednisone solution at 31.5 µg/mL was prepared in HBSS + 5% ethanol (pH 6.8). Two hundred microliters of this solution were added into the apical compartment of the cell monolayer. At fixed times (30, 60, 120, and 180 minutes), each filter and its apical compartment were repositioned on a new well containing 600 µL of the fresh basolateral medium. All receptor solutions were collected and analysed directly (without dilution) by a UV spectrophotometer (λ = 239 nm). The apparent permeability coefficient (P_{app}) was calculated using Eq. (1).

$$Papp = \frac{dQ}{dt/(A * 60 * C_0)} \qquad (1)$$

Where dQ/dt is the permeability rate (amount of prednisone permeated per minute), A is the diffusion area of the cell monolayer, C_0 is the initial concentration of prednisone in the apical compartment[14-16]. During the experiment, the integrity of the monolayer was evaluated by Transepithelial Electric Resistance (TEER) at fixed intervals (along with collection times) using the Millicell-ERS system (Millipore Corp., USA)[14,15]. Monolayer integrity was considered when voltages above 350 Ω.cm^2 were observed[11].

Bioequivalence study

The bioequivalence study between the 20 mg prednisone tablets produced by our group (biobatch P0020020910) and the reference drug product (Mantercorp® Pharmaceutical) was under the responsibility of the Nucleus of Bioequivalence and Clinical Trials (NuBEC) of the Federal University of São Paulo.

An open, randomized, cross-over study was conducted with two treatments, two sequences, and two periods, in which volunteers received, in each period, the test or the reference formulation. An amount of 26 healthy volunteers, men and women aged between 18 and 50 years, and body mass index ≥ 19 and ≤ 28.5 $kg.m^{-2}$ receive one tablet containing 20 mg prednisone fasted orally, uniquely with 200 mL of non-gas mineral water. The confinement period was 24 hours. Blood samples for determination of plasma concentration of prednisolone were collected before administration and up to 24 hours later at pre-established intervals. Plasma concentrations of prednisolone were determined by HPLC-MS[17].

In silico model absorption for prednisone immediate-release tablet

The *in silico* absorption model was developed using the software GastroPlus® (Version 9.5, Simulation Plus Inc., USA). The model was built with the integration between the Advanced Compartmental Absorption and Transit (ACAT) and pharmacokinetic (PK) model (1, 2, and 3-compartment model). The ACAT model describes the gastrointestinal tract like compartments considering drug (unreleased drug, dissolved drug, precipitated drug) and physiology (luminal volume, pH, transit time) characteristics. Once integrated into the PK information, like distribution volume, clearance, and half-life time, it is possible to observe the simulated drug kinetic absorption and exposition in the gastrointestinal tract.

The software has three main input modules: compound, physiology, and pharmacokinetics. The physicochemical (solubility, permeability, pKa) and drug dosage form (tablet, dose, particle size) properties were inputted at the compound module. These data were taken from the literature or experimentally determined (**Table 1**). The effective gastrointestinal permeability (P_{eff}) of prednisone (biobatch P0020020910) was predicted based on the conversion of the apparent permeability (P_{app}) with Caco-2 cells using a built-in correlation of the GastroPlus®. The solubility data was determined in pH 1.2, 5.5, and 6.8 aqueous media[8,18,19].

Table 1. Input parameters used for the prednisone IR tablet absorption model.

Parameters	Value	Reference
Molar mass (g/mol)	358.43	(ANVISA, 2019)
Log P	1.46	(Paixão, Gouveia, & Morais, 2012; Vogt *et al*., 2007)
Dose (mg)	20	BE
Dosage form	Tablet IR	E
Dose volume (mL)	200	BE
Solubility (mg/mL)		
pH 1.2	4.48	(Toehwé *et al*., 2017)
pH 5.5	4.53	
pH 6.8	4.55	
P_{app} in Caco-2 (cm/s x 10^{-5})	3.69	E
Mean precipitation time (s)	900 s	DV
Drug particle radius (g/ml)	1.2	DV

Parameters	Value	Reference
Particle radius (µm)	25	DV
Physiology	Fasted	E
AFS (Model)	SA/V 6.1	DV
Body mass (kg)	66.4	BE
Blood/Plasma ratio	1.0	E
Distribution volume (L/kg)	0.69	E
Clearance (L/h)	11,51	E
$t_{1/2}$ (h)	2.86	E
Simulation Time (h)	24	E

P_{app} = apparent permeability coefficient; BE = bioequivalence study; E = experimental; DV = GastroPlus® default value

In the physiology module, the Opt logD Model SA/V 6.1 estimates the GI tract permeability. The absorption gradient coefficient C1-C4 was used to calculate the absorption scale factors (ASF) and scale the effective permeability to account for absorption rate variations that differ from one compartment to another[18,20].

In the pharmacokinetic module, the used data were obtained from the bioequivalence study of the biobatch P0020020910, as described above (item 2.3)[10,17]. The *in vivo* plasma concentration versus time data was loaded into the PKPlus™ module (integrated module of GastroPlus®) and evaluated by the software according to non-compartmental, 1-, 2-, and 3-compartment PK models. The best model parameters were then imported into the pharmacokinetic module to enable the *in silico* plasma concentration *versus* time data[8,18,21]. To develop the prednisone absorption model, the "IR tablet mode" was selected in the compound module that refers to immediate-release tablets[20,21]. Through Eq. (2) it was calculated the percent prediction error (%PE) to compare observed and simulated PK parameters[19].

$$\%PE = 100 * \left[\frac{(observed - predicted)}{observed}\right] \quad (2)$$

Results

Permeation studies on Caco-2 cells

Cytotoxicity and Caco-2 cells monolayer permeability studies

The cytotoxicity assessment was performed to determine whether concentrations of 31.5 µg/mL of API and HBSS pH 6.8 + 5% ethanol medium used in the Caco-2 cell permeability test would harm the cells. The cell viability was almost 100% for all evaluated media (data not shown), so no cytotoxic effect was observed among the tested samples.

After 21 days of culture, the mean transepithelial resistance (TEER) of the Caco-2 cell monolayer was 711.2 ± 37.05 Ω.cm^2. **Figure 1** presents the permeation data of prednisone and the mean TEER (Ω) as a function of time. At 180 minutes, prednisone permeation reached 65.84% of the initial mass (6.3 µg) deposited in the apical compartment.

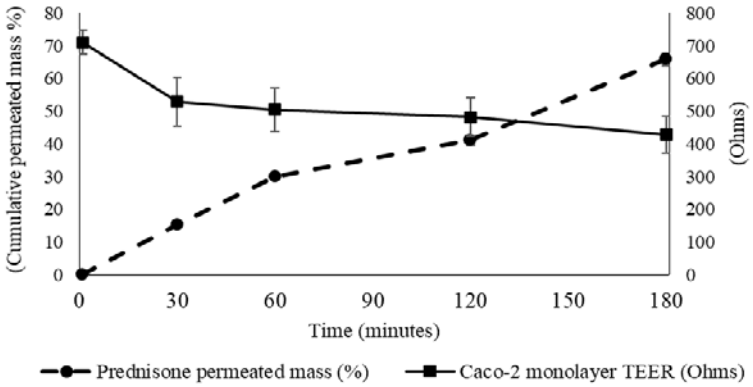

Figure 1. Cumulative permeated mass (%) of prednisone across Caco-2 monolayer and TEER values (Ohms) after 180 minutes.

The apparent permeability coefficient (P_{app}) for prednisone was calculated from the mean of 5 determinations of the permeability test and presented a value of 3.69 x 10^{-5} cm/s in the accumulated time of 180 minutes. It should be observed that this value of P_{app} was used in the simulation of bioavailability by GastroPlus®.

Bioequivalence studies

The mean plasma concentration *vs.* time curve of prednisolone (active prednisone metabolite) in volunteers using the biobatch P0020020910 and reference drug product (R023) is shown in **Figure 2**. The pharmacokinetic parameters analyzed in the study are described in **Table 2**.

Table 2. Pharmacokinetic parameters obtained from the mean plasma concentration *versus* prednisolone time curve.

Parameters	P0020020910				R023			
	Average	SD	Mini	Maxi	Average	SD	Mini	Maxi
AUC_{0-t} (ng.h/mL)	563.22	120.70	365.38	972.94	598.02	186.89	365.33	1208.02
AUC_{0-inf} (ng.h/mL)	598.50	127.27	380.56	985.52	628.25	187.50	379.12	1222.93
C_{max} (median/range) (h)	126.08	27.36	78.36	197.49	131.65	31.52	101.14	247.72
T_{max} (median/range) (h)	1.52	0.86	0.75	4.00	1.56	0.78	0.50	3.00
K_{el} (L/h)	0.25	0.05	0.15	0.33	0.26	0.04	0.14	0.37
$t_{½}$ (median/amp) (h)	2.82	0.56	2.07	4.49	2.77	0.59	1.86	4.92

SD = standard deviation; Mini = minimum; Maxi = maximum

According to the estimated geometric means ratio between C_{max} and the AUC_{0-t} for batches P0020020910, and R023 the results obtained for these parameters were 104.24% (99.12%, 109.62%) and 100.60% (96.59%, 104.78%), respectively, considering a 90% confidence interval. These results compile with the range established by the Guide for tests of relative bioavailability/bioequivalence of medicines[22]. Therefore, the formulations under study are bioequivalent[17].

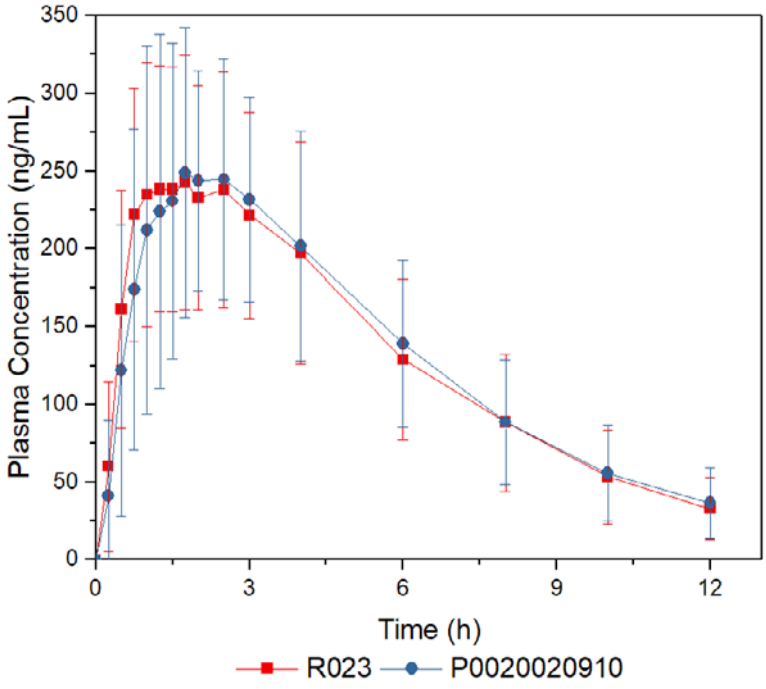

Figure 2. Mean plasma concentration of prednisolone versus time in volunteers following a single dose administration of prednisone 20 mg tablets of biobatches P0020020910 and R023.

In silico absorption model for prednisone immediate-release tablet

The absorption model was developed for prednisone (biobatch P0020020910) immediate-release tablet. The fraction of the amount of drug absorbed was 96.4%. **Table 3** presents the prednisone simulated compartmental absorption along to the GI tract. According to the ACAT model, this simulation confirmed the high absorption in the proximal region (~93,7%).

The simulated and experimental PK parameters after oral administration of 20 mg prednisone immediate-release tablets (biobatch P0020020910) are presented in **Table 4**. The predicted outputs and bioavailability *in vivo* were almost similar. The predicted errors were lower than 10% for all parameters, except for $t_{1/2}$ in the reference product.

GastroPlus® was used to simulate plasma concentration-time curves for all the products (R023, P0020020910, MEST01, and MEST 02). The resulting profiles were then compared to the reference product (R023) curve plotted with *in vivo* experimental values to evaluate the proposed formulations' *in vivo* performance (**Figure 3**). According to the predicted plasma concentration-time curves, the biobatch P0020020910 show an *in vivo* performance equivalent to the reference product, as proven by the BE studies (**Figure 2**). Furthermore, the MEST01 and MEST02 formulations would show equally *in vivo* performance compared to the reference product.

Table 3. Simulation of the percentage absorbed of prednisone (biobatch P0020020910) along with the compartments of the gastrointestinal tract.

Compartments	Prednisone (%)
Stomach	0
Duodenum	45.8
Jejunum 1	37.7
Jejunum 2	10.2
Ileum 1	3.1
Ileum 2	1.0
Ileum 3	0.4
Caecum	1.3
Ascending colon	0.4
Total	96.4

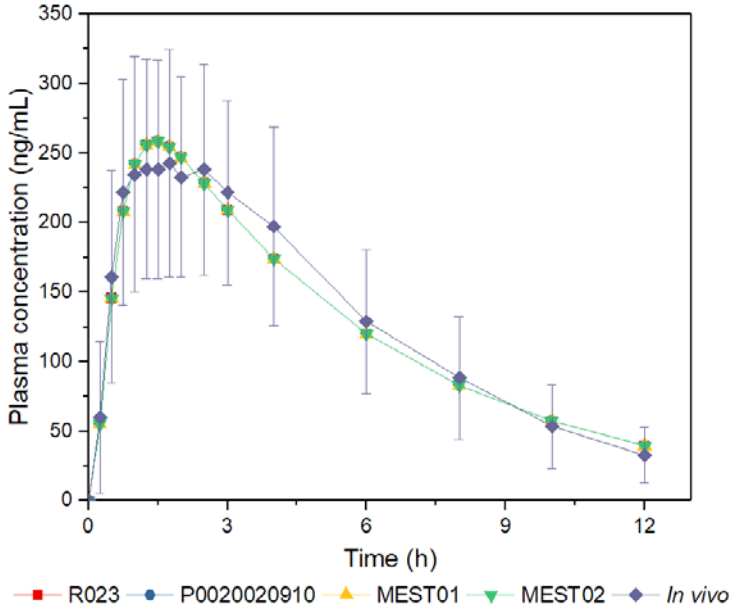

Figure 3. Plasma concentration *vs*. time curve of the reference product (R023) based on *in vivo* experimental values and of products P0020020910, MEST01, and MEST 02 created using simulated values given by GastroPlus® based on *in vitro* dissolution data[32]. The error bars represent the standard deviation for the reference plot.

Discussion

Permeation studies on Caco-2 cells

MTT test demonstrated that both drugs and media did not cause cell damage since its viability at the end of the experiment was around 100%. This result follows Yamashita and co-workers, which conclud-

ed that ethanol up to 5% (v/v) does not cause significant permeability and transepithelial resistance (TEER). The results also show that HBSS (pH 6.8) and HBSS + 5% ethanol (pH 6.8) media are not altering cell viability, as expected[23].

TEER values were compatible with those reported in the literature for mature monolayers[12,15]. In **Figure 1**, it is possible to observe that the most significant permeate fraction occurred in the interval between 120 and 180 minutes and that TERR remained above 350 Ω, confirming that monolayer cells remained intact throughout the experiment[11].

As stated by the literature, drugs that show absorption between 70% and 100% should have P_{app} greater than 10.0 x 10^{-6} cm/s[24]. A study that evaluated the correlation between biopharmaceutics classification system (BCS) and P_{app} value (cm/s) argued that drugs with Papp greater than 9.75 x 10^{-6} cm/s show a high, and less than 5.00 x 10^{-6} cm/s, a low permeability. As previously mentioned, prednisone belongs to BCS class I. The P_{app} obtained in this study for this drug (3.69 x 10^{-5} cm/s) corroborates with BCS classification[25].

According to Vogt and collaborators[2], there are very limited studies reporting the *in vitro* permeability of prednisone since it is a prodrug. However, the FDA Guidance[1] states that the prodrug permeability should be measured when the conversion occurs after GI membrane permeation. So, the results about Caco-2 cells prednisone permeability could be beneficial in a biowaiver decision.

In silico absorption model for prednisone immediate-release tablet

According to the drug solubility of previous studies and permeability analyzed in this work, prednisone is highly soluble and absorbed in the intestinal tract. These characteristics added to an immediate-release dosage form shown a dissolution profile of ≥85% in 15 minutes[10], suggesting, like other class I drugs, that the prednisone can be highly absorbed in the proximal region of the intestine[21,26].

The evaluation of the *in vivo* plasma concentration *vs.* time curve by GastroPlus® present that prednisone kinetics follow a one-compartmental model. It means that the drug promptly occupies the central compartment, and the plasma concentration decrease depends only on the elimination rate constant, which follows a first-order process[27,28].

The predicted outputs and BA *in vivo* (**Table 4**) suggest that the *in silico* absorption model based on the **Table 1** data successfully simulate the *in vivo* bioavailability of prednisone IR tablets. The lower %PE values prove that the model could mimic the *in vivo* PK parameters. Duque and co-workers successfully used the ACAT model in BA simulated of fluconazole, a BCS class I drug, showing that the prediction model was also valuable for generic and reference drugs[21]. Also, this absorption model was widely applied in BCS class II drugs[18,20].

As mentioned, the biowaiver is requested for BCS class I drugs when using immediate-release tablets. In this context, a predictive *in vitro* dissolution could replace the bioequivalence study[29]. Once the *in silico* absorption model was developed for prednisone IR tablets, it becomes possible that the bioavailability simulation helps during the optimization of the formulation process. By evaluating the *in vivo* and *in silico* data, it is possible to infer that an *in vivo* study is probably unnecessary for these, suggesting the bioequivalence between the MEST 01, MEST 02, R023, and biobatch P002002910.

Table 4. Pharmacokinetic parameters obtained in the *in vivo* study and simulated by GastroPlus® for biobatch P0020020910 and reference product R023.

Parameters	Simulated	R023	%PE	P0020020910	%PE
C_{max} (ng/mL)	318.9	298	-7.01%	313	-1.88%
T_{max} (h)	1.36	1.17	16.2%	1.50	9.33%
ASC_{0-t} (ng.h/mL)	1730.5	1685.7	2.65%	1718.5	-0.69%
ASC_{0-inf} (ng.h/mL)	1737.1	1769.6	1.83%	1811.6	4.11%

%PE = percent prediction error

The Brazilian regulatory agency recommendations for the waiver of BE studies according to the BCS state that IR solid oral products must have rapid drug dissolution (> 85% in 30 min) in 0.1 HCl, pH 4.5, and pH 6.8[4]. Previously, the dissolution tests were conducted in purified water, as recommended by the Brazilian compendia[10]. All the products that meet the requirements of the dissolution rate still showed an *in silico* performance equivalent to the reference product. The very rapid dissolution (85% in up to 15 minutes) of the tested prednisone tablets makes the gastric emptying rate the limiting factor for *in vivo* drug absorption, and no more the dissolution rate[30]. In some cases, the *in vitro* dissolution method was not discriminative for the *in vivo* process. Al-Tabakha and co-workers evaluated different products containing amoxicillin and potassium clavulanate that was considered bioequivalent but presenting differently *in vitro* dissolution profiles[31].

On the other hand, to declare that the tested formulations are bioequivalent to the reference product, more predictive dissolution studies will be necessary to discriminate the formulation's performance. The use of purified water as dissolution media, although recommended for quality control tests[10], may not be adequate for evaluating pharmaceutical equivalence.

Although the results present by this study provide a biowaiver favorable data based on the *in silico* pharmacokinetic study and the BCS, prednisone is at the borderline of the current criteria of BCS class I. The biowaiver of prednisone must be carefully evaluated considering its indication, therapeutic index, pharmacokinetic performance, and the possibility of drug-excipients interaction[2]. Due to many marked generic products of prednisone and other class I drugs, the use of different formulation excipients could modify their dissolution and oral absorption. Therefore, *in vivo* bioequivalence studies are still needed.

Conclusion

The present study evaluates the *in silico* bioavailability simulations comparing these data with *in vivo* ones in order to establish if previous published prednisone oral tablet formulation is a valid candidate for the biowaiver process. The *in silico* bioavailability performed for prednisone in the GastroPlus® software was successful since the predicted PK parameters were similar to *in vivo* data. The simulated plasma concentration *vs*. time curve based on the previous dissolution data allows us to infer that the proposed formulations MEST01 and MEST02 could be bioequivalent with the reference product. In order to confirm this hypothesis, a predictive dissolution study should be applied.

This work turns available the bioavailability profile obtained in human volunteers and compares reference and tests drug products, which is not common in the offered publications. More than that, it presents a viable *in silico* model to simplify post-approval changes in the regulatory scenario. Prednisone is classified as a BCS class I or borderline class I drug and a promising candidate for biowaiver. However, a careful evaluation of the excipients used is necessary to claim it. Toward to reduce costs and the use of human beings in bioequivalence studies, this approach could be an essential way to work in the pharmaceutical industry.

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