Bioavailability study of Posaconazole in rats after oral Poloxamer P188 Nano-micelles and oral Posaconazole pure drug

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ABSTRACT

Posaconazole (POCZ) is a triazole antifungal with poor solubility and low bioavailability, so this work is aimed to compare between bioavailability parameters of pure POCZ and the prepared lyophilized POCZ nano-micelles. In this study, twelve Wistar rats were used with a weight of 200 ± 20g and divided into twice groups (each group with six animals). The dose of 10mg/kg of pure POCZ and POCZ nano-micelles was administered to rats by oral gavage after reconstitution with water. The determination of POCZ in the plasma of rats is done by using (HPLC) after the construction of a spiked calibration curve with plasma and internal standard itraconazole. The bioavailability parameters for pure POCZ and POCZ nano-micelles were determined. The results show that C_{max}, T_{max}, AUC_{0-72}, and $AUC_{0-\infty}$ were 230 ± 4ng/ml, 8 ± 0.5hr, 8622 ± 127 ng.h/ml, 10050 ± 110 ng.h/ml and 2107 ± 7ng, 2 ± 0.2hr, 27261.2 ± 233ng.h/ml, 30364 ± 205 respectively for pure POCZ suspension and POCZ nano-micelles. The obtained results from this work are that the time T_{max} required for maximum concentration C_{max} was statistically different from pure POCZ and POCZ nano-micelles ($p<0.05$). The POCZ nano-micelles show relative bioavailability 3.02 times than pure Posaconazole. In conclusion, the prepared POCZ nano-micelles enhance the oral absorption and bioavailability of the drug.

Keywords: Posaconazole, Nano-micelles, Bioavailability, Pharmacokinetics

Introduction

Posaconazole (POCZ) is a triazole antifungal medication that is used to treat and prevent various fungal infections. It is known for its broad spectrum of activity against a wide range of fungal pathogens and its effectiveness against drug-resistant strains. One of the challenges in using POCZ is its poor solubility, which can limit its bioavailability and effectiveness. Several studies have been conducted to improve the solubility and bioavailability of POCZ. One study found that the addition of hydroxypropyl-beta-cyclodextrin (HPβCD) improved the solubility and

bioavailability of POCZ in rats [1]. Another study explored the use of nanostructured lipid carriers (NLCs) to improve the solubility and bioavailability of POCZ [2]. The low solubility of drugs is a major challenge in drug development and delivery, as it can limit their bioavailability and therapeutic efficacy. One approach to overcoming this challenge is the use of nano-micelles, which are nanoscale structures composed of amphiphilic molecules that can solubilize hydrophobic drugs. As drug delivery systems, nano-micelles have several benefits, including increased solubility, and improved bioavailability. One study demonstrated the effectiveness of nano-micelles in improving the solubility and bioavailability of the poorly soluble drug, paclitaxel [3]. The study found that paclitaxel-loaded nano-micelles had significantly higher solubility and improved pharmacokinetic properties compared to the free drug. Another study showed that nano-micelles could effectively solubilize the anticancer drug, curcumin, and improve its therapeutic efficacy [4]. Overall, nano-micelles offer a promising approach for improving the solubility and bioavailability of low-solubility drugs. POCZ is formulated as Nano-micelles by thin film

hydration method by using poloxamer p188 with Tween 80 with a ratio of 1:5:2 (POCZ: Poloxamer p188: Tween 80). physiologically based pharmacokinetic (PBPK) software was used for calculating the pharmacokinetic parameters of the drug [5]. The public clinical data were used to verify the model program [6]. This study aims to compare the bioavailability parameters of POCZ after oral administration of POCZ Nano-micelles versus oral administration of POCZ suspension. The research was conducted using male Wistar rats.

Materials and Methods

Posaconazole, poloxamer p188, (HangzhouHyper Chem Ltd, China), methanol, and Tween 80 (pure chemistry, Germany). Acetonitrile HPLC grade (Alpha chemika, India).

Preparation of posaconazole nano-micelles

POCZ nano-micelles were made by the thin-film hydration technique. In 30 mL methanol, all ingredients in a ratio of 1:5:2 (POCZ: Poloxamer p188: tween80) were dissolved. Next, the solvents were evaporated in a rotary evaporator at 150rpm, 50 °C for 30 minutes, at which point a thin film formed. Afterward, the deionized water (10ml) was used to hydrate the film, and the micelles solution was sonicated for 5 minutes before being magnetically stirred at 500 rpm for 20 minutes [7]. The Nano-micelles prepared were optimized and lyophilized. **Table 1** show the composition of POCZ nano-micelles.

Table 1. Composition of Posaconazole nano-micelles

Ingredients	Amount (mg)
Posaconazole	100
Poloxamer p188	500
Tween 80	200

Bioavailability parameters after oral administration

Study design

The methods used to assess bioavailability parameters complied with the suggestions made by the National Committee for Research Ethics in Science and Technology (NENT, Norway) [8]. The bioavailability parameters were measured using male Wistar rats (n = 12), and each rat weighed between 200 ±20 grams. Rats were split up into two groups with an equal number in each (n=6). All rats were administered 10 mg/kg of POCZ pure and POCZ nano-micelles orally., after being fasted for the previous night and only being given access to water thereafter. Depending on the amount of POCZ in the formulation, the lyophilized formulation and pure POCZ powder were dissolved in distilled water and then orally administered by gavage tube to rats. (0.5ml) of the blood samples were withdrawn from the Retroorbital venous plexus 5, 15, 30, 1, 2,4, 6, 8, 12, 24, 48, and 72 hours after oral administration. A blood sample (0.5ml) was collected in microcentrifuge tubes containing the anticoagulant (EDTA). The collected plasma blood samples were centrifuged at 4500 rpm for 15 minutes in a refrigerated environment before being stored at -25°C until being analyzed by HPLC. The experiment was done in agreement with the ethical code of the World Medical Association (Declaration of Helsinki).

Detection of POCZ in plasma sample by HPLC

The method of HPLC was suggested for the quantification of POCZ in the plasma of rats. Detection of POCZ by HPLC first includes several steps which include precipitation of protein by methanol, column separation, and ultraviolet absorbance at 262 nm. The POCZ plasma concentration quantification is calculated using spiked calibration curves. Drug-free plasma was combined with the proper amounts of the standard POCZ solution in acetonitrile to create the calibration and quality control samples. The calibration curves ranged between 3 and 20 µg/mL ($r2 = 1$). In brief, a 100 µL aliquot consists of 50 µL of internal standard (IS) solution (Itraconazoleb5 µg/mL) and 50 µL of POCZ solution. After 30 seconds of vertexing, 500 µL of methanol is added for protein precipitation and vortexed for 2 minutes. The solution was centrifuged at 7000rpm for 15 minutes (Ohaus cooling centrifuge). The filtration of supernatant by a 0.22 µm filter membrane syringe and 20µL of the filtered mixture was injected into the HPLC system. The mobile phase flow rate was 1 mL/min and consisted of 0.09Mammonium phosphate monobasic acetonitrile-triethylamine (55: 45 v/v)[9]. In the case of detection of an unknown concentration of POCZ in rat plasma which is collected during the procedure, the extraction of plasma samples is done by using the same method, and the plasma sample is spiked with 100 µl of mobile phase containing 5µg/ml of IS. The unknown concertation of POCZ was calculated using the equation obtained from the spiked calibration curve. After measurements of POCZ concentration in plasma with time, the analysis by using the non-compartmental technique to calculate pharmacokinetic parameters using PK-SOLVER. The drug's maximum plasma concentration (Cmax) and time to Cmax (Tmax) were determined. AUC0-72 is the area under the plasma concentration-time curve from 0 to 72 hours [10, 11].

Statistical analysis

All obtained values of pharmacokinetics parameters represent a mean result of the study (±SD; n = 3). When a $p<0.05$ statistically significant difference was considered. The bioavailability parameters, Cmax, Tmax, AUC0-72, and $AUC_{0-\infty}$ were analyzed statistically using a student t-test [12].

Results and Discussion

Relative peak of POCZ to an itraconazole calibration curve of plasma samples

The calibration curve construction was made by using the developed method for the spiked plasma and a solution with a known amount of POCZ in it as shown in **Figure 1**. **Figure 2** shows the HPLC graph of blank plasma and was not affected by any components of the plasma [9]. The method of HPLC analysis was sensitive, and specific enough to detect POCZ in the mobile phase and in rats plasma samples that had been spiked. The HPLC spiked plasma's graph showed that the POCZ, which had a retention time (Rt) of 3 minutes, was completely different from the itraconazole, which had a signal at 7.11 minutes so there is no interpretation between POCZ and internal standard as shown in **Figure 3**.
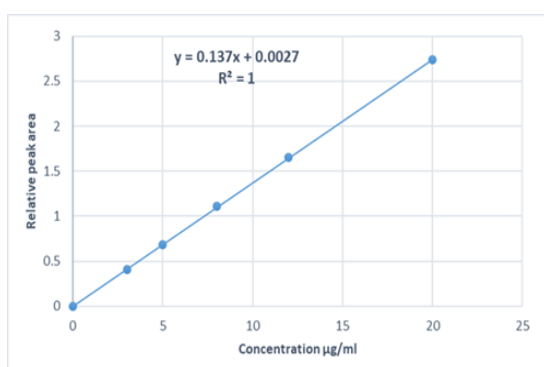
**Figure 1.** Calibration curve of spiked rat plasma samples.
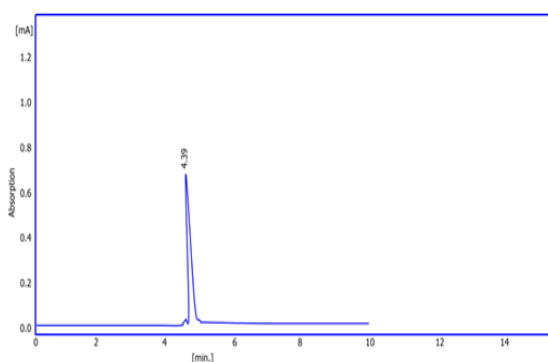
Figure 2. HPLC graph of blank plasma.
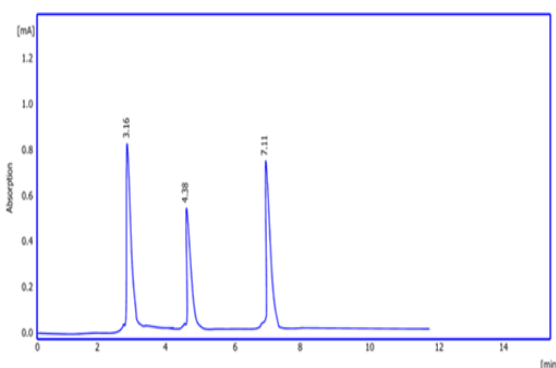
Figure 3. HPLC graph of plasma specimen with POCZ and internal standard (IS) itraconazole.

Posaconazole nano-micelles and posaconazole suspension pharmacokinetics

After reconstitution with water, the relative bioavailability of POCZ oral nano-micelles was calculated in comparison to the oral suspension of pure POCZ. Following administration of the oral POCZ nano-micelles and POCZ suspension, the drug plasma concentration with time profiles is shown in **Figure 4**. Using PK-Solver, the pharmacokinetic parameters were calculated. **Table 2** displays the parameters for both oral POCZ nano-micelles and POCZ suspension, where all the obtained results show measurements in triplicate, and the level of significance for each value was evaluated [13]. The t-test statistical analysis shows that the concentration and time needed for achieving maximum effect (Cmax and Tmax) for oral POCZ nano-micelles were (Cmax=2107 ± 7, Tmax=2 ± 0.2) and (Cmax=230 ± 4, Tmax=8 ± 0.5) for POCZ pure suspension, respectively. The obtained results show a ($p<0.05$) which is considered a statistically significant difference between these values. Due to the high solubility of the drug in nano-micelles, POCZ nano-micelles have a high Cmax and a low Tmax. The AUC0-∞ for the POCZ pure suspension was 10050 ± 110ng.h/ml significantly ($p<0.05$) lower than that of the POCZ nano-micelles, which was 30364 ± 205 ng. h/ml. The relative bioavailability of POCZ nano-micelles was 3.02 times more bioavailable than pure POCZ oral suspension. The results demonstrated a statistically significant effect ($p<0.05$), indicating that the POCZ nano-micelles were significantly more bioavailable than the oral suspension dose; this was due to the fact that POCZ nano-micelles enhance the solubility of POCZ, resulting in rapid dissolution [9]. The results revealed a statistically significant difference between these values ($p<0.05$). Due to the high solubility of the drug in nano-micelles, POCZ nano-micelles have a high Cmax and a low Tmax. The AUC0-72 for the oral POCZ pure suspension was 8622 ± 127 ng. h/ml which is significantly ($p<0.05$) less than the POCZ nano-micelles, which was 27261.2 ± 233 ng. h/ml.
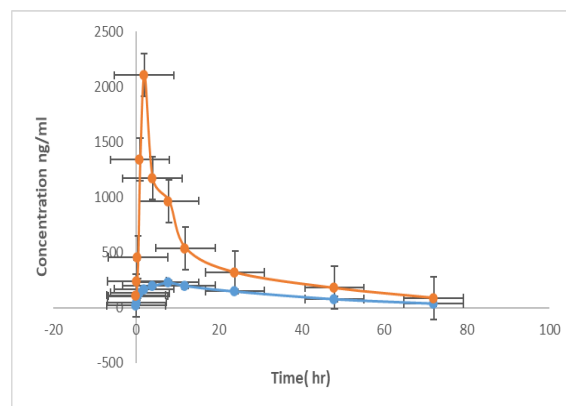
Figure 4. Mean Plasma Concentration of Posaconazole Nano-micelles and Pure Posaconazole after the Same Dose (2mg).

Table 2. Bioavailability parameters of POCZ nanomicelles versus POCZ pure suspension

Parameter	Pure POCZ suspension	POCZ nano-micelles
Cmax	230±4ng/ml	2107±5ng/ml
Tmax	8hr	2hr
T1/2	24.75±0.4 hr	23.8±0.6hr
AUC 0-72	8622±127ng.h/ml	27261.2±233ng/ml
AUC 0-∞	10050±110ng.h/ml	30364±205ng.h/ml

Conclusion

In conclusion according to bioavailability parameters obtained from the study, Posaconazole nano-micelles have 3..02 folds in relative bioavailability in comparison with pure Posaconazole suspension, so POCZ nano-micelles considered a promising approach in the preparation of Posaconazole as oral drug delivery system with excellent bioavailability in comparison with POCZ pure suspension.

Ethics statement: The committee protocol in the College of Pharmacy/University of Baghdad approved this study, which complied with the ethics as reported in the guidelines written by the National Committee for Research Ethics in Science and Technology (NENT), Norway.

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