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Research article

INVIVO PHARMACOKINETIC INVESTIGATION OF ITRACONAZOLE MICROEMULSION IN ALBINO WISTAR RATS

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ABSTRACT

The aim of this research is to create microemulsion formulations to increase the bioavailability of Itraconazole that are poor in bioavailability. Itraconazole microemulsion (10 formulations) is prepared and optimised by phase titration technique and screened Itraconazole microemulsion is selected for in vivo pharmacokinetic performance assessment in albino wistar rats from this optimization study results. Invivo pharmacokinetic tests showed positive findings in the formulation of IM9. This research shows an improvement in Cmax in Itraconazole microemulsion, which shows greater bioavailability than the branded Itraconazole capsule (Sporanox ® Capsule). This research shows an increase in AUC0- ∞; Tmax; Microemulsion has been concluded to be a promising method for improving the bioavailability of drugs such as Itraconazole.

Key Words:- Bioavailability, *In vivo* pharmacokinetic, Microemulsion, Itraconazole.

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INTRODUCTION

Itraconazole is used to treat a range of fungal infections. It belongs to a family of antifungal azoles. It functions by preventing fungi from developing. Itraconazole has the same mechanism of action as other azoles antifungals: it prevents the fungal-mediated synthesis of ergosterol by inhibition of lanosterol 14 alpha-demethylase. Itraconazole is a type II (low solubility / high permeability) compound with a weakly basic Biopharmaceutical Classification Scheme (BCS). Its bioavailability is about 55 percent, with further urinary excretion (35 percent) and faeces (54 percent) with around 99.8 percent high protein binding. All the above pharmacokinetic parameter leads to select the Itraconazole for microemulsion to enhance the bioavailability of Itraconazole [11]. The key hypothesis of this research is to increase the bioavailability of Itraconazole by microemulsion formulation in albinowistar rats.

METHODOLOGY

In-vivo pharmacokinetic studies of Itraconazole microemulsion

Using PK solver software, the pharmacokinetic (PK) output of Microemulsion after oral administration was studied. Healthy male Wistar adult albino rats weighing 180-250 gm were used. The single dose analysis was split into 2 classes, each comprising 6 numbers as follows.

Animals were fasted 24 hours before medication formulations were delivered but had free access to liquids. As seen in Table No:1, the drug dosage form was delivered orally with the aid of an oral feeding needle. Blood samples of approximately 1 ml volume were obtained at intervals of 0.5, 1, 2, 3, 4, 5, 6h, following oral administration by retro-orbital puncture. Samples

were obtained from retro orbital puncture into heparinized glass tubes containing anticoagulant ammonium oxalate (0.5 percent solution) with the aid of capillary tubes. The plasma was automatically separated at 10000 RPM with the aid of micro centrifugation and deposited at -10°C before the HPLC analysis was conducted. The study was submitted to and approved by IAEC ethics committee (SVCOP/IAEC/2019-23) [2-14].

Sample preparation from Itraconazole plasma drug concentration

At 5000 RPM for 10min, 1ml of collected animal blood was centrifuged and 0.5ml of transparent supernatant plasma was collected into an eppendorf tube. 0.5ml of 20µl methanol was applied to this batch. The substance was removed from plasma by vortexing at 4°C for 35min. In order to measure Itraconazole, the 20 µl supernatant solution or aliquot was siphoned out and 5µl injected into HPLC.

Quantification of Itraconazole in Plasma

Quantification of Itraconazole in Plasma was carried out by using HPLC (Schimadzu, Japan)

Column: Reversed phase C18 column (250 mm X 4.6 mm i.d., Particle size - 5 µm)

Mobile Phase: 0.5% KH_2PO_4 (pH 6.0)-acetonitrile (30:70, v/v) and degassed in an ultrasonic bath

Flow rate : 0.5 ml/min

Injection Volume: 5 µl

Detection of wavelength maxima of itraconazole was performed by using following criteria with a run time of 10 – 20 min and sample was detected at 260 nm. Calibration curve (Concentration in µg/ml on X axis Vs. Peak area in % on Y axis) was performed for 8 solutions of Itraconazole in phosphate buffer solution pH 7.4 with the concentration ranging from 0.04-0.32 µg/ml at 260 nm and the regression value was found to be r^2=0.998. The Unknown concentration was determined by picking the unknown sample peak area and interpolation it to X-axis to get the concentration of sample.

Pharmacokinetic data analysis

The time vs. plasma drug concentration data that derived from HPLC method are plotted in PK solver software. From the individual plasma concentration time profile peak plasma concentration (Cmax), time of its occurrence to attain peak plasma concentration (tmax), AUC_{0-t} and $AUC_{0-\infty}$ were read directly. The other PK parameters, e.g. biological half-life (t1/2), MRT are also calculated by using PK solver software. The differences in various PK parameters were evaluated statistically by ANOVA.

RESULTS AND DISCUSSION

***In-vivo* pharmacokinetic studies of Itraconazole microemulsion**

A calibration curve was designed using various concentrations of Itraconazole to calculate the unknown plasma drug concentration. By plotting the peak area and nominal concentration of Itraconazole, the linearity of the calibration curve was calculated. Eight different doses of Itraconazole have been analysed for linearity tests (0, 0.04, 0.08, 0.12, 0.16, 0.2, 0.24, 0.28, 0.32µg / ml). The peak area response was found to be linear over the concentration range studied. The HPLC calibration curve has been successfully used to determine the pharmacokinetic data from the unknown plasma drug concentration followed by single dose administration of CMC with Itraconazole ® Sporanox capsule and CMC with lyophilized Itraconazole microemulsion particle (IM9). From the peak area of the injected sample the unknown concentration was determined. The mean plasma concentration of Itraconazole as a function of time has been plotted as shown in Figure 1 and the comparative studies on *In-vivo* plasma drug concentration profile between CMC with Itraconazole ® Sporanox capsule; CMC with lyophilized Itraconazole microemulsion particle (IM9) was tabulated in Table 2. It was observed that CMC with lyophilized Itraconazole microemulsion particle (IM9) enhance the release as well as the pharmacokinetic parameters when compared to the Sporanox capsule. There was a significant difference in 'p' value as < 0.05 between the pharmacokinetic parameters of CMC with Itraconazole ® Sporanox capsule and CMC with lyophilized Itraconazole microemulsion particle (IM9) with T_{max} of 2 hrs and 2.2 hrs; and the maximum peak plasma concentration (C_{max}) of 0.261 µg/ml and 0.205 µg/ml respectively. Area Under Curve ($AUC_{0-\alpha}$) was found to be 7.268µg/ml/h, and 24.834 µg/ml/h respectively. From the *in-vivo* pharmacokinetic data it was concluded that increase in $AUC_{0-\infty}$, T_{max} with decrease in C_{max} in Itraconazole Microemulsion when compared to marketed Sporanox Capsule. On calculating the relative bioavailability by keeping marketed formulation as standard, it has been confirmed that the Itraconazole loaded Microemulsion showed the enhancement of bioavailability of about 3.41 points.

Table 1. Grouping of albino wistar rats for pharmacokinetic studies of Itraconazole microemulsion

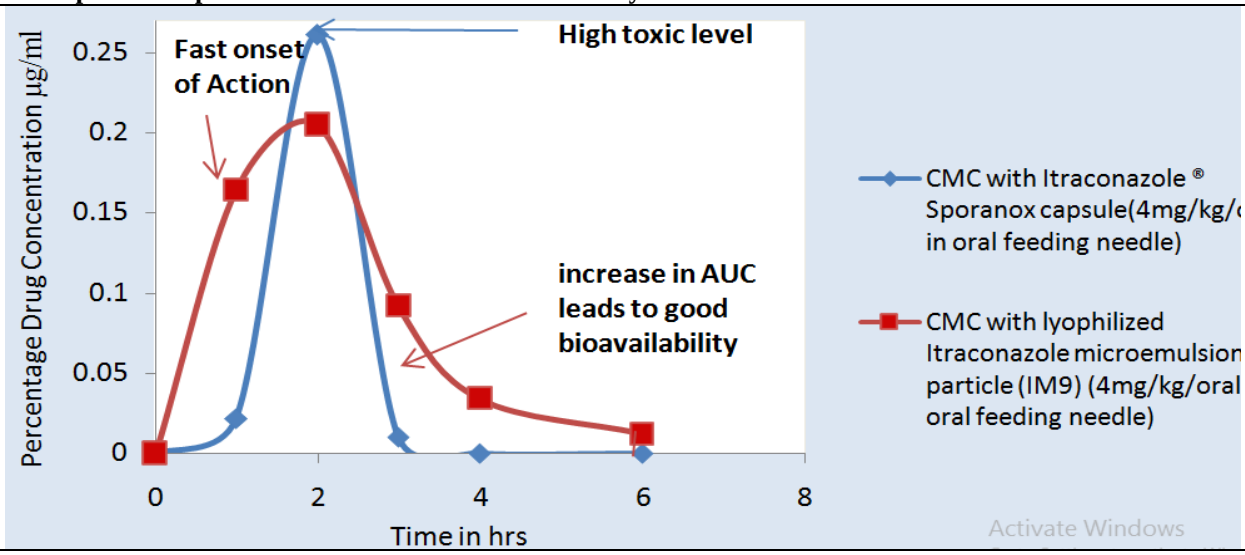
Group code	Treatment
A	CMC with Itraconazole ® Sporanox capsule (4mg/kg/oral in oral feeding needle)
B	CMC with lyophilized Itraconazole microemulsion particle (IM9) (4mg/kg/oral in oral feeding needle)

Table 2. Comparative in-vivo pharmacokinetic studies data between Itraconazole formulations treatment groups

Parameter	CMC with Itraconazole ® Sporanox Capsule (4mg/kg/oral in oral feeding needle)	CMC with lyophilized Itraconazole microemulsion particle (IM9) (4mg/kg/oral in oral feeding needle)
Tmax (h)	2	2.2
Cmax (µg/ml)	0.261	0.205
AUC 0-α (µg/ml/h)	7.268	24.834
F rel= $\frac{(AUC)_{drug}.(Dose)_{std}}{(AUC)_{std}.(Dose)_{drug}}$		Bioavailability enhanced by 3.41point

Note: Increase in $AUC_{0-\infty}$; Tmax; Decrease in Cmax in Itraconazole microemulsion shows better bioavailability than marketed Itraconazole capsule

Fig: 1 Graph of Comparative *in-vivo* Pharmacokinetic study data for Itraconazole formulations



CONCLUSION

This invivo pharmacokinetic analysis indicates a rise in $AUC_{0-\alpha}$ Tmax; a decline in Cmax in Itraconazole microemulsion compared to the branded Itraconazole capsule (Sporanox ® Capsule), which demonstrates a boost in bioavailability of approximately 3.41 points. Microemulsion has been concluded to be a promising method for improving bioavailability of drugs such as Itraconazole.

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