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Original Article

Pharmacokinetic parameters of ondansetron in rats after oral solution and transdermal invasomes gel: A comparison study

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ABSTRACT

This study aimed to attain a comparison in pharmacokinetic parameters of ondansetron after transdermal (as invasomes gel) and oral (as solution) routes. Ondansetron is a 5-hydroxytryptamine receptor antagonist used for chemotherapy and radiotherapy-induced nausea and emesis. The study was performed using Wistar albino rats weighing 200 ±20 g divided into two groups (6 of each). A dose of 0.28 mg of ondansetron, equivalent to 140 µl, was administered as an oral solution for the first group. Ondansetron-loaded invasomes gel was prepared, optimized, and applied on rat skin at a dose of 0.1652 mg of ondansetron, equivalent to 41 mg weight of invasomes gel for the second group. In addition, ondansetron was determined in rat plasma using HPLC apparatus and applying a modified HPLC method after validation. A comparison of primary pharmacokinetic parameters for both routes was performed. Results showed that C_{max}, T_{max}, $AUC_{0-24,}$ and $AUC_{0-\infty}$ were 58±3.4 ng/ml, 2±0.2 h., 246.25±47.6 ng.h./ml, and 259.4±57.7, respectively, for oral solution and 36±2.9 ng/ml, 5±0.5 h., 390.5±5.2 ng.h./ml and 442.8±66.1 ng.h./ml., respectively for transdermal invasomes gel. Results showed that the time and concentration needed to achieve the maximum effect (C_{max} and T_{max}) were significantly different between oral and transdermal routes ($p<0.05$). The relative bioavailability for the transdermal route was 2.9 times that of the oral route after a single dose administered for 24 h. In conclusion, the prepared invasomes gel enhanced the bioavailability of ondansetron, and transdermal delivery could be considered a vital delivery system for ondansetron.

Keywords: Invasomes gel, Ondansetron, Pharmacokinetics, Transdermal, Animal study, Bioavailability

Introduction

Compared to oral drug delivery, transdermal drug delivery has many potential benefits, such as avoiding first-pass metabolism, providing predictable and prolonged drug action with minimal side effects, enhancing physiological and pharmacological responses, decreasing the frequency of drug administration, and preventing fluctuations in plasma drug levels [1]. Lipid-based nanovesicles, made of phospholipid (as a backbone), offer an effective route for transdermal delivery due to their biocompatibility with skin lipids, allowing for the drug to be delivered via the stratum corneum (SC) with high drug permeability and therapeutic activity [2, 3]. Invasomes are vesicular systems composed of phospholipids, ethanol, and a single terpene or a combination of terpenes. When added to SC, terpenes have advantages in disturbing the SC's tight bilayers and lipid packing and facilitating drug absorption via the intercellular lipids [4]. As a relatively new method for estimating drug pharmacokinetics after delivery using modeling software, physiologically based pharmacokinetic (PBPK) modeling has garnered considerable interest in recent years [5]. The physicochemical properties of the drug can be used in PBPK modeling to predict plasma concentration-time curves. The model must first be validated against the existing public clinical pharmacokinetic data [6]. Ondansetron (ONDS) blocks the action of the serotonin receptor (5-HT3) subtype 3 in a very selective manner [7]. Ondansetron has shown remarkable efficacy in treating acute emesis without inducing unwanted side effects such as extrapyramidal responses, as observed in

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metoclopramide and prochlorperazine [8, 9]. Ondansetron is also used in the early onset of alcoholism [10]. Multiple dosage forms, including pills, solutions, injections, and intranasal sprays, are all accessible for the treatment of ONDS. Patients with dysphagia, odynophagia, geriatric chemotherapy, and young children may have difficulty swallowing the oral pill. Because of this problem, patients may not take their medications as prescribed. Some patients find the discomfort of an ONDS injection. Direct intravenous administration of a pharmacological dose results in quick onset; therefore, an overdose may develop rapidly and require immediate treatment. To give an injection to a patient experiencing nausea and vomiting while convulsing or otherwise uncooperative is problematic at best [11]. This study aimed to compare the pharmacokinetic parameters of ondansetron after transdermal (as invasomes gel) and oral (as solution) routes. The study was performed by using Wistar male rats.

Materials and Methods

Ondansetron, limonene, egg and soybean lecithin (Hangzhou Hyper Chemicals Limited, Zhejiang, China), absolute ethanol and carbopol 934 (Alpha chemika, India). Methylparaben (Fluka Chemical AG, Switzerland).Ondasnsetron solution (Zofran® ampoule) (2 mg/ml). Sodium hydroxide, dichloromethane potassium dihydrogen phosphate, Acetonitrile HPLC grade (Alpha chemika, India). Orthophosphoric acid (ISOLAB, Germany).

Preparation of ondansetron invasomes gel

Ondansetron invasomal dispersion was prepared, optimized, and lyophilized. Then after that, the lyophilized powder was incorporated physically with gelling base (Carbopol 934) to form 0.4% w/w to form ondansetron invasomes gel shown in **Table 1**.

Table 1. Composition of ondansetron invasomes gel (0.4% w/w)

Ingredients (mg)	Amount
Ondansetron	4 mg
Egg: Soybean lecithin	16 mg
Limonene	32 µl
Absolute ethanol	80 µl
Methylparaben	2 mg
Carbopol Q.S to	1 g

Dose calculation

The dose of ONDS-loaded invasomes gel for rats was estimated based on the body weight of the rats according to the surface area ratio. When a typical rat weighs 200 g, and the human weight is 70 kg, the surface area ratio is 56 [12]. An oral dose of ondansetron in emetogenic chemotherapy is 16 mg. Therefore, 16 mg was taken as the standard human dose, and the oral dose for a rat was calculated by using the following equation:

$$\text{Oral dose for rat X weight of rat used in the study (kg)} = \frac{\text{Human dose}}{\text{Surface area ratio}}$$

$$\text{Oral dose for rat} = \frac{16\ mg}{0.2\ kg\ X\ 56} \quad (1)$$

$$\text{Oral dose for rat} = \frac{16\ mg}{11.2\ kg} = 1.4\ \text{mg/kg}$$

The oral dose for a rat was 0.28 mg ONDS as calculated by equation 1 for a rat weighing 200 g, equivalent to 140 µl solution obtained from the ONDS solution (2 mg/ml). The transdermal dose was calculated based on the oral bioavailability of ONDS, which equals 59%. According to equation 2, the transdermal dose was 0.1652 mg of ONDS, equivalent to 41 mg weight of invasomes gel (0.4% w/w) [13].

$$\text{Transdermal dose} = \text{Oral dose X Oral bioavailability} \quad (2)$$

$$\text{Transdermal dose} = 1.4\text{mg/kg X } 59\% = 0.826\ \text{mg/kg}$$

Study design

Pharmacokinetic parameters measurements were carried out using male Wistar rats (n=12) weighing 200±20 g each, and the procedures comply with the guidelines written by the National Committee for Research Ethics in Science and Technology (NENT, Norway) [14]. Rats were divided equally into two groups. The first group received the ONDS orally by using an oral feeding tube. While ONDS invasomes gel was transdermally applied to the second group, as shown in **Figure 1**. The first group took an oral solution and fasted overnight before the experiment to avoid any food effects. At the time of administration, the two groups were anesthetized by diethyl ether, and the oral solution was administered to the first group. ONDS invasomes gel was applied on a constant shaved area of 1.767 cm^2, equivalent to the skin area used in the ex-vivo permeation study to the second group. The applied gel was occluded with adhesive plaster to avoid any loss of the gel during the time of the measurements. Time was recorded for each sampling, and a single dose for both groups was applied for the study to compare the relative bioavailability between oral and transdermal doses. Blood samples (1 ml) were collected from the heart by a puncture at predetermined time intervals from 0.5-9 h and one sample after 24 h. Blood samples were collected in EDTA-treated tubes and were separated immediately after being taken from rats. Blood samples were separated by centrifugation (Hettich Zentrifugen EBA 20, Germany) at 4000 rpm for 10 min to obtain plasma samples. Plasma samples were obtained from the supernatant, placed in Eppendorfs, and stored in the freezer for later analysis. Plasma samples were analyzed by RP-HPLC using a modified and validated method [15].

Analytical method

A modified HPLC analysis method was used to determine ONDS in plasma samples. The method was validated in terms of (linearity, specificity, precision, accuracy, lower limit of detection, lower limit of quantification, and stability) [16]. To perform the studies, an HPLC nucleosil column C-18 (10 x 50 mm I.D., particle size 2 µm; Sykam, Germany) was used with a 20 µL autosampler volume. The 0.05 M potassium dihydrogen phosphate buffer and acetonitrile in a 70:30 ratio made up the mobile phase. Orthophosphoric acid was used to alter the pH to 3.0. At all times, the mobile phase was filtered through a 0.22 µm microfilter and degassed with a sonicator before being pumped in isocratic mode at a flow rate of 1 ml/min while the eluent was monitored at 216 nm. Using several dilutions of stock solutions by mobile phase, working standard solutions of ONDS were created with a concentration range of 5–100 ng/ml to construct a calibration curve [17].

All of the measurements were done in triplicate. The internal standard (IS) metoclopramide was used to construct the calibration curve at a constant concentration (10 ng/ml). The relative peak area of ondansetron to the internal standard was determined. A calibration curve was constructed by plotting the relative peak area of the spiked plasma sample versus the concentration of ONDS. From this calibration curve, unknown ONDS in plasma samples could be measured. The unknown plasma concentration of ondansetron was calculated using the regression equation based on these relative peaks. The detailed steps to obtain the calibration curve involved taking the blank plasma sample, 300 µl in volume, spiked with 100 µl of mobile phase containing a known concentration of ONDS for 30 sec. The samples were vortexed - mixed before being used in the liquid-liquid extraction process. The plasma sample was then treated with 0.2 ml of sodium hydroxide and 6 ml of dichloromethane for protein precipitation and then centrifuged for 10 min at 3000 rpm and vortexed for 15 min. Dichloromethane extract was evaporated to dryness at 40°C under a stream of nitrogen. The residue was reconstituted in 100 µl of mobile phase containing 10 ng/ml IS, and a volume of 20 µl was injected into the HPLC for analysis [18]. In the case of plasma samples containing an unknown concentration of ONDS taken from rats during the experiment, the plasma samples were extracted by the same extraction process, and the dried sample spiked with 100 µl of mobile phase containing 10 ng/ml IS and from the relative peak area of the HPLC measurements, the unknown concertation of ONDS could be calculated. Non-compartmental analysis was applied to compute primary pharmacokinetic parameters using PK-SOLVER after measuring ONDS plasma concentration with time for the two groups. The first-order terminal elimination rate constant (k) was calculated using linear regression to calculate Auc_{last} ($Auc_{last} = C_{last}/k$). The maximum plasma concentration of the drug (C_{max}) and time for C_{max} (T_{max}) was determined. The area under the plasma concentration-time curve from time 0 to 24 h (AUC_{0-24}) and from 0 to infinity was also calculated ($AUC_{0-\infty}$) [19].

a)

b)

Figure 1. a) administration of an oral solution, b) Application of transdermal invasomes gel

Statistical analysis

Results were expressed as mean values (±SD; n = 3). A statistically significant difference was considered when $p<0.05$. The pharmacokinetic parameters, C_{max}, T_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ were analyzed statistically using a student t-test [20]. The following equation was used to assess ondansetron's relative bioavailability (F) following transdermal versus oral dosing [11]:

$$\%F=\frac{\text{AUC Transdermal x oral dose}}{\text{AUC oral x Transdermal dose}} \times 100 \quad (1)$$

Results and Discussion

Calibration curve of spiked plasma samples

The calibration curve was obtained by applying the suggested procedure for the spiked plasma with a standard solution of known concentration of ONDS. As shown in **Figure 1**, the results of HPLC analysis showed no endogenous components were interfering with the chromatogram of blank plasma. The method was precise, specific, and sensitive for determining ONDS in the mobile phase standard solutions and spiked plasma samples. The chromatogram of spiked plasma showed complete

separation of ONDS, which showed retention time (R_t) at 4.3 min from the internal standards (IS) metoclopramide which showed a signal at 7.6 min as shown in **Figures 2 and 3**.

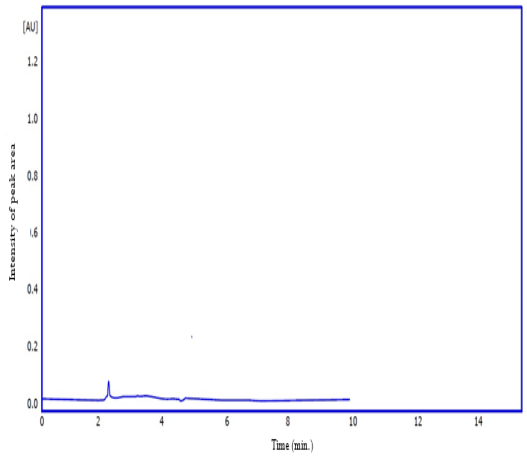

Figure 2. Chromatograms of blank plasma

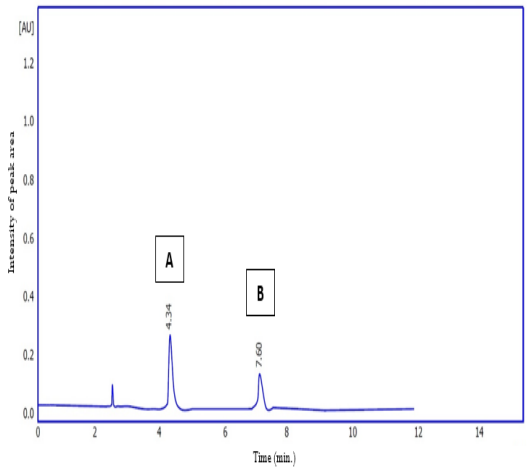

Figure 3. Chromatogram of test sample showing ondansetron (A), and internal standard (metoclopramide) (B)

The constructed calibration curve shown in **Figure 4** illustrates a linear relationship between ondansetron concentration and the relative peak area of ONDS to metoclopramide, with a high correlation coefficient (R^2= 0.997) for eight concentration points in the range of 5,10,20,30,40,50,80 and 100 ng/ml. HPLC method was validated to measure the ondansetron concentration in rat plasma. All validation parameters were within acceptable criteria [16]. When HPLC-validated parameters were used, ondansetron was successfully determined. Retention lasted for 4.3 minutes. Eight concentration was used to assess the method's linearity and determination the lower limit of quantification, which was 5 ng/ml [21].

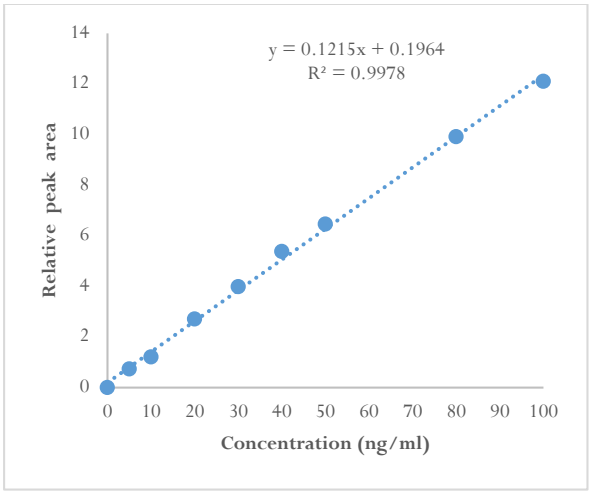

Figure 4. Calibration curve of spiked plasma samples with mobile phase containing ONDS and constant concentration of the internal standard (IS) (10 ng/ml)

Ondansetron invasomes gel Pharmacokinetics

The ondansetron-loaded invasomes gel's relative bioavailability was calculated compared to the oral solution. **Figure 5** shows the mean plasma drug concentration-time profiles following administration of the oral solution and ONDS invasomes gel. The pharmacokinetic parameters were calculated using PK-Solver. The parameters for both oral and transdermal routes are shown in **Table 2**, where these values represent triplicate measurements, and the significance level was studied for each value [22]. Statistical analysis using a t-test revealed that the time and concentration needed to achieve the maximum effect (C_{max} and T_{max}) were (C_{max}=58±3.4, T_{max}=2±0.2)for oral and (C_{max}=36±2.9, T_{max}=5±0.5) for transdermal routes, respectively. Results showed a significant difference between these values (p<0.05). Low C_{max} and high T_{max} with transdermal formulation are attributed to the skin's barrier properties. The $AUC_{0\text{-}24}$ for oral dose was 246.25 ng.h/ml was significantly lower (p<0.05) than $AUC_{0\text{-}24}$ for transdermal dose, which was 390.5±5.2 ng.h/ml. The relative bioavailability for the two formulations showed that the transdermal route had 2.9 times more available than the oral route after a single dose administered for 24 h. Results showed a statistically significant effect (p<0.05), and this would refer that the transdermal dose was highly bioavailable when compared to the oral dose, and this was due to the oral route exposed to factors that may reduce the bioavailable dose to exert therapeutic effects like first-pass metabolism [23]. The transdermal delivery for ONDS showed an alternative to oral therapy for the treatment of emesis [24].

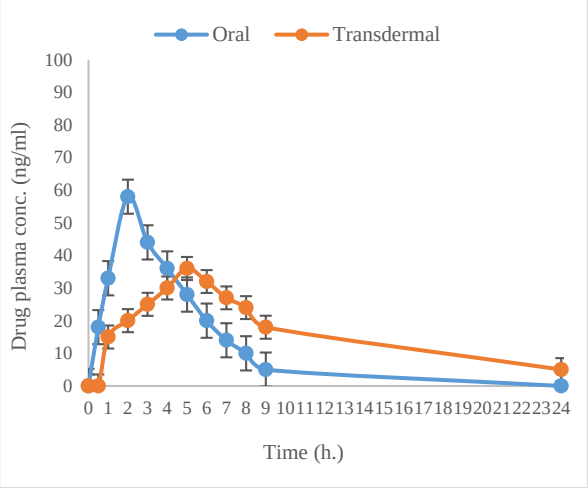

Figure 5. Mean plasma concentration-time profile of oral solution (Dose= 0.28mg) and transdermal invasomes gel (Dose =0.1652 mg) of ondansetron in male rat plasma

Table 2. Pharmacokinetic parameters of ONDS from oral solution and transdermal invasomes gel

Parameter	Unit	*Oral solution	*Transdermal ONDS invasomes gel
C_{max}	ng/ml	58±3.4	36±2.9
T_{max}	h.	2±0.2	5±0.5
AUC_{0-24}	ng.h./ml	246.25±47.6	390.5±5.2
$AUC_{0-\infty}$	ng.h./ml	259.4±57.7	442.8±66.1

*Mean± SD (n= 3)

Conclusion

In conclusion, a higher relative bioavailability of the prepared ONDS invasome gel compared to the oral solution (F_{rel}=290 %) showed a more convenient dosage form for the administration of ONDS than the oral route for the treatment of vomiting. In future studies, transdermal delivery could be considered a vital delivery system for ondansetron.

Future studies

Preparation of ONDS invasomes as a transdermal patch by using gel base as a reservoir for drug release and calculating the surface area and dose of the prepared patch depending on the required flux to be studied *in-vivo* in humans.

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Conflict of interest: None

Financial support: None

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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