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Research Article

Pharmacokinetic Study of Oral Disulfiram Suspension and Topical Transdermal Nano-Invasomes Gel in Wistar Rats

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Abstract

Background: Disulfiram (DSF), an FDA-approved pharmaceutical for the management of alcoholism, has demonstrated its efficacy against several kinds of cancer. DSF has limited solubility, a fast metabolism, a short duration of action, and instability in physiological environments, mostly caused by rapid degradation in the acidic gastric environment. ***Objective***: A transdermal gel containing disulfiram, which was loaded into invasomes, was developed to improve the stability of DSF and enable its effective distribution to tumor tissues. ***Methods***: This study included 72 Wistar rats weighing 200±35 g, which were separated into two groups, each of which included 12 animals. Rats were orally provided a dose of 5 mg of pure DSF suspension via oral gavage, and DSF nano-invasomal transdermal gel was then applied to their skin. DSF is determined in rats' plasma by reverse-phase high-performance liquid chromatography (RP-HPLC). ***Results***: The results showed that the maximum effect (C_{max}, T_{max}, and AUC_{0-72}) were (C_{max}=57.3±0.2, T_{max}=3.6±0.01 and 562±3. 3ng.h/ml) for oral and (C_{max}=138±0.4, T_{max}=5.5±0.01 and 2819±6.6 ng. h/ml) for transdermal routes, respectively. Results showed that the time and concentration needed to achieve the maximum effect (C_{max} and T_{max}) were significantly different between DSF-oral suspension and transdermal invasomal gel ($p<0.05$). The relative bioavailability for the transdermal route was five times that of the oral route after a single dose administered for 72 hours. ***Conclusions***: The nano-invasomal transdermal gel filled with DSF demonstrated a more convenient way of administering DSF compared to the oral route.

Keywords: Disulfiram, Invasomes, Transdermal, Pharmacokinetic, Wistar rats.

دراسة الحركية الدوائية لمعلق ديسلفيرام الفموي وجل نانو إنفاسومات موضعي عبر الجلد في جرذان ويستار

الخلاصة

الخلفية: ديسلفيرام (DSF)، وهو دواء معتمد من قبل إدارة الغذاء والدواء لعلاج الإدمان على الكحول، فعاليته ضد عدة أنواع من السرطان. يعاني DSF من قلة الذوبان، وسرعة التمثيل الغذائي، وقصر مدة العمل، وعدم الاستقرار في البيئات الفسيولوجية، ناتجة بشكل رئيسي عن التحلل السريع في البيئة المعديّة الحمضية. **الهدف:** تم تطوير جل تحت الجلد يحتوي على ديسلفيرام، والذي تم تحميله في إنفاسومات، لتحسين استقرار DSF وتمكين توزيعه بشكل فعّال إلى أنسجة الأورام. **الطرق:** شملت هذه الدراسة اثنان وسبعون جرذا من سلالة وستر يزن كل منها ±35 جرامًا، تم تقسيمها إلى مجموعتين، كل منها يضم اثني عشر حيوانًا. تم تزويدها بجرعة 5 ملغ من تعليق DSF النقي عن طريق الفم، ثم تم وضع جل DSF نانو-إنفاسومال تحت الجلد. يتم تحديد DSF في بلازما الجرذان. **النتائج:** أظهرت النتائج أن التأثير الأقصى (T_{max}، C_{max}) و (AUC_{0-72}) كان (57.3±0.2 =C_{max}، 3.6±0.01 =T_{max} و 562±3. 3 ng.h/ml) لطريقة الفم و (138±0.4 =C_{max}، 5.5 ± 0.01 =T_{max} و 2819 ± 6.6 ng. h/ml) للطرق التحت الجلدية، على التوالي. أظهرت النتائج أن الوقت والتركيز اللازمان لتحقيق التأثير الأقصى (T_{max} وC_{max}) كانا مختلفين بشكل ملحوظ بين معلق DSF-فموي وجل DSF نانو-إنفاسوم الجلدي ($p<0.05$). كانت الامتصاصية الحيوية النسبية للطريقة التحت الجلد خمس مرات تلك للطريقة الفموية بعد جرعة واحدة معطاة لمدة 72 ساعة. **الاستنتاجات:** أظهر جل DSF نانو-إنفاسومال تحت الجلد طريقة أكثر راحة لإعطاء DSF مقارنة بالطريقة الفموية.

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INTRODUCTION

Transdermal drug delivery has a number of advantages over oral drug delivery. These include the ability to release drugs over a long period of time at a consistent rate, the ease of stopping drug administration by removing the device, the ability for self-application, and most importantly, the ability for the drug to bypass hepatic first-pass metabolism and gastrointestinal incompatibility. Transdermal drug delivery tries to control how molecules move through the different layers of skin. This has a lot of potential for treating long-term illnesses because it makes sure that the effects last while keeping the drug concentration under control [1]. Invasomes are liposomal vesicular systems that are very flexible and malleable. They consist of a combination of safe, natural components such as phospholipids (PC), a

terpene or a mix of terpenes, and ethanol. In comparison to liposomes, invasomes have enhanced skin permeability capabilities. The combination of terpenes, ethanol, and phospholipids (PC) has a synergistic effect that enhances the flexibility of vesicles. This leads to increased penetration through the stratum corneum, as both ethanol and terpenes act as enhancers of permeation. Therefore, invasomes have a far higher rate of penetrating the skin compared to liposomes and ethosomes. Many people consider this method beneficial for improving transdermal delivery [2,3]. Disulfiram (DSF), a widely recognized medication for treating alcoholism, has demonstrated significant effectiveness in treating aggressive forms of colon, breast, lung, prostate, ovarian, cervical, and brain cancers. People have safely used this drug for over 70 years. Moreover, this medication selectively and efficiently eradicates drug-resistant cancer stem cells and counteracts chemoresistance. [4]. Plasma concentration-time curves can be predicted using the drug's physicochemical properties in physiologically based pharmacokinetic models. Validating the model with publicly available clinical pharmacokinetic data is a must before implementation [5]. This study aims to compare the bioavailability characteristics of disulfiram when applied using a transdermal DSF nano-invasomal hyaluronic acid gel and when taken orally as a DSF suspension.

METHODS

Materials

Disulfiram was obtained from Hyperchem for Chemicals, China. Absolute ethanol, HPLC-grade methanol, KH_2PO_4, and NaOH were purchased from Chem-Lab, Belgium. Carvacrol was purchased from Bide Pharmaceutical Ltd. (China). Soybean phosphatidylcholine SPC90 (SPC), which has a purity of >90% (China), was used. Hyaluronic acid (HA): Hyperchem for Chemicals, China. Diphenhydramine (DPH) (internal standard, IS) was supplied by the AL-Kindi CO. Pharmaceutical Industry in Baghdad, Iraq.

Preparation of disulfiram nano-invasomes gel

As shown in Table 1, a nano-invasomal dispersion of disulfiram was produced and optimized.

Table 1: Composition of disulfiram invasomes gel

Ingredient	Amount (%)
Disulfiram	0.5
SPC	2
Carvacrol	1
Ethanol	40
Hyaluronic acid	3

Subsequently, hyaluronic acid 3% (a gelling agent) was immersed in the invasomal dispersion. The components specified in Table 1 of the disulfiram-loaded HA gel formulation were combined and agitated using a magnetic stirrer at a speed of 300 rpm for a whole night at room temperature until a partially solid gel with a consistent distribution of the DSF invasomal dispersion was achieved [6,7].

In vivo pharmacokinetic study

This study employed 72 female Wistar rats, aged three months, with an average weight of 200±35 g. The rats were provided unrestricted access to both food and water.

In vivo pharmacokinetic study

This study employed 72 female Wistar rats, aged three months, with an average weight of 200±35 g. The rats were provided unrestricted access to both food and water. The *in vivo* experiments performed on the rats were authorized by the Research Ethics Committee for Experimental Investigations, College of Pharmacy, Baghdad University, Iraq, under the protocol number RECAUBCP482023M.

Study design

The pharmacokinetic parameters were assessed in a group of twelve female Wistar rats, following the guidelines set by the National Committee for Research Ethics in Science and Technology (NENT, Norway) [8]. The rats were evenly distributed into two groups. The initial group received an oral dose of 5 mg (0.5 ml) of pure disulfiram suspended in acacia 2%, which was administered using an oral gavage. The second group received transdermal administration of the DSF nano-invasomal gel. The DSF invasome gel was applied topically to the hairless area of the rat's skin. The DSF invasome gel was applied topically to the hairless area of the rat's skin. To prevent any loss of gel during the time of measurements, the applied nano-invasomal gel was coated with sticky plaster. The duration of each sampling period was quantified, and a singular dosage was delivered to both groups in order to evaluate the comparative bioavailability of oral and transdermal dosages. Two ml of blood samples were obtained from the heart via puncture at certain time intervals spanning from 0 to 72 hours. Rat blood samples were obtained using EDTA-treated tubes and immediately separated. Plasma samples were acquired using the centrifugal process (Hettich, Germany), where blood samples were spun at a speed of 4500 revolutions per minute for a duration of 5 minutes. Plasma samples were obtained from the clear portion, transferred into Eppendorf tubes, and stored in the freezer for further analysis [9,10]. A modified HPLC analysis method was used to determine DSF in plasma samples. This method was validated in terms of linearity, specificity, precision, accuracy, lower limit of detection, lower limit of quantification, and stability. The HPLC system by SYKAM (Germany) has a UV detector, a Phenomenex Luna C18 4.6 × 150 mm column with a 5 µm particle size (Phenomenex, Torrance, CA, USA), and a micro-volume double plunger pump (10 µl/stroke). 80% HPLC-grade methanol and 20% HPLC-grade water constitute the

mobile phase. The injection volume was 20 µL, and the flow rate was 1 mL/min. UV detection was carried out at a wavelength of 275 nm [11]. reversed-phase high-performance liquid chromatography (RP-HPLC) was used to evaluate the plasma samples. The maximum plasma concentration (C_{max}) of the DSF was measured in this study, as was the amount of time it took for the medication to reach its C_{max} and T_{max}. By calculating the integral of the plasma concentration-time curve from time 0 to 72 hours and from time 0 to infinity, respectively, The AUC_{0-72} and $AUC_{0-\infty}$ were calculated [12,13]. The PK-Solver® software was utilized to determine the pharmacokinetic parameters [14].

Statistical analysis

The results were presented as mean values with their standard deviation ±SD (n=3). A difference was considered statistically significant if the P-value was below 0.05. The pharmacokinetic parameters, including C_{max}, T_{max}, and AUC_{0-72}, undergo a statistical analysis using a student's t-test [15,16].

RESULTS

As shown in Figure 1, we constructed the calibration curve using the proposed procedure for the spiked plasma and a solution containing a known quantity of DSF with a high correlation factor (r^2=0.9999) for concentrations ranging from 5 to 100 ppb.

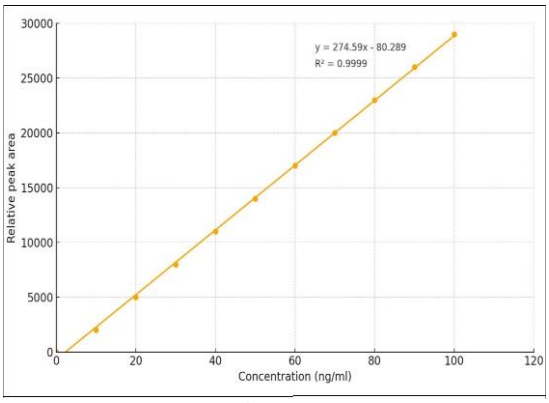

Figure 1: Calibration curve of spiked plasma samples with mobile phase containing disulfiram and 10 ppb of diphenhydramine.

The HPLC analysis demonstrated that no endogenous components interfered with the blank plasma chromatogram. Figure 2 illustrates the RP-HPLC graph of blank plasma, demonstrating that it remained unaffected by any plasma components. The spiked plasma chromatogram demonstrated a clear distinction between DSF and internal standards, with DSF having a retention time (Rt) of 5.75 minutes. The DPH compound exhibited a signal at 7.88 minutes, indicating that there is no interpretation between DSF and DPH (the internal standard), as depicted in Figure 3. The following method was precise, specific, and sensitive for determining DSF in the mobile phase standard solutions and spiked plasma samples. Each validation parameter was within the permitted limits.

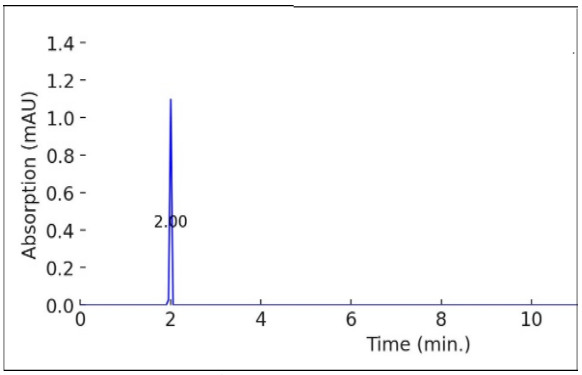

Figure 2: HPLC graph of blank plasma.

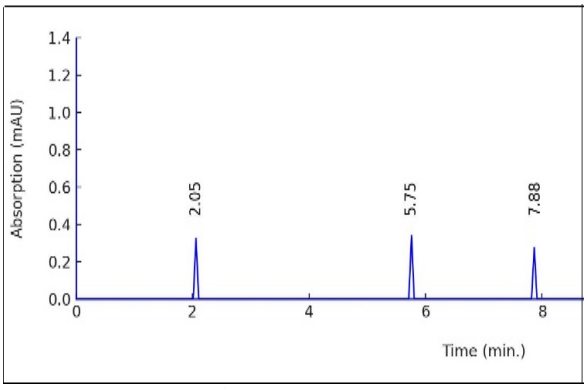

Figure 3: HPLC graph of plasma specimen with DSF and internal standard DPH.

The application of PR-HPLC-validated parameters satisfactorily identified DSF, with a retention time of 5.82 minutes. We determined the comparative bioavailability of the DSF-loaded invasome gel versus the oral DSF suspension. Figure 4 displays the mean plasma drug concentration-time profiles following oral-free DSF suspension and DSF transdermal nano-invasome gel delivery.

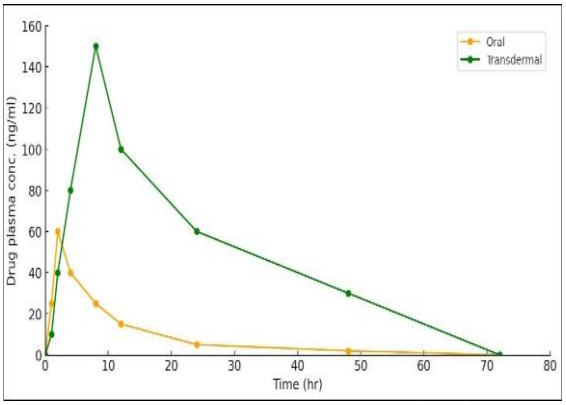

Figure 4: Mean plasma concentration-time profile of oral DSF-suspension and transdermal DSF-invasomes gel after the same (Dose =5 mg) of disulfiram.

Table 2 shows the parameters for both oral and transdermal methods. We have evaluated the significance level for each value in these triplicate measurements. A *t*-test study showed that the maximum concentration and time needed to get the best effect (C_{max} and T_{max}) were (C_{max}= 57.3±0.2, T_{max}= 3.6±0.01) for oral routes and (C_{max}= 138±0.4, T_{max}= 5.5±0.01) for transdermal routes. Results showed a significant difference between these values

(p<0.05). A *t*-test showed that the highest concentration (C_{max}) and time (T_{max}) needed to have the most impact were 57.3±0.2 ng/mL for the free DSF suspension and 3.6±0.01 hours for the DSF-invasomal gel. Both were 138±0.4 ng/mL and 5.5±0.01 hours, respectively.

Table 2: Bioavailability parameters of disulfiram transdermal invasomes gel versus oral pure suspension

Pharmacokinetic parameters	Oral disulfiram suspension	Transdermal nano-invasomal gel
C_{max} (ng/ml)	57.3±0.2	138±0.4
T_{max} (h)	3.6±0.01	5.5±0.01
AUC_{0-72} ng.h/ml	562±3.3	2819±6.6
$AUC_{0-\infty}$ ng.h/ml	561±3.3	2837±9.07

DISCUSSION

The transdermal DSF nano-invasomal gel had a much higher DSF bioavailability than the DSF-free suspension that was administered orally. Significant improvements in the blood relative bioavailability of DSF were achieved fivefold (based on the estimated $AUC_{0-\infty}$ values). Many factors contribute to this enhancement: I) the hydrophobic character of the vesicular system, which enhances its propensity to cross the skin; and II) the synergistic effects of terpene and ethanol in enhancing skin permeability. III) The small particle size (nano size), large surface area, and flexibility allow close contact and penetration through the layers of the skin. IV) This prevents the liver from metabolizing the substance during its initial journey through the body. Our study showed that the DSF contents have a signal at 5.75 min that doesn't interfere with the internal standard. This result was similar to that reported by Najlah *et al*. (2027) [11]. Moreover, we evaluated the linearity of the following method using six concentrations, with a lower limit of quantification equal to 2.0 ng/ml, which aligns with the findings of Al-Akkam *et al*. (2013) [17]. In this study, the result demonstrates a statistically significant difference between these formulations, which seems comparable to that reported by others [18]. The transdermal route offers several advantages that can improve the bioavailability of DSF. Firstly, it allows the drug to directly enter the systemic circulation, bypassing the portal circulation and the first-pass metabolism. Secondly, it ensures a steady and controlled release of the drug, resulting in consistent drug levels in the blood. This not only reduces the frequency of dosing but also minimizes associated side effects with improved patient compliance [19–21].

Conclusion

The generated DSF-loaded nano-invasomal transdermal gel has a much higher relative bioavailability compared to the oral-free DSF suspension. Therefore, the nano-invasomal transdermal gel filled with DSF demonstrated a more convenient way of administering DSF compared to the oral route. Future studies may recognize transdermal administration as a crucial method of delivering DSF to enhance its bioavailability.

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Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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