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Research Article

Docetaxel Pharmacokinetic Investigation In Sprague-Dawley Rats Using In-Vivo Tests After Oral And Transdermal Lipo-Nanosphere Administration

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Abstract
Background: The FDA-approved medication docetaxel (DCX), which shown promise in treating a number of cancer types.
Objective:DCX's significant gastrointestinal toxicity and high hydrophobicity, which result in low bioavailability with first-pass metabolism and P-glycoprotein efflux pumps, are the main causes of its restricted solubility, short duration of action, and instability.To increase DCX's stability and facilitate its efficient delivery to tumour tissues, a transdermal film containing docetaxel was created and placed into Lipo-Nanospheres.
Methods: 72 Sprague-Dawley male rats weighing 200±0.25g were used in this investigation. They were divided into two groups of 12 animals each. Rats received a dose of 16 mg of pure DCX suspension orally through gavage, and their skin was then coated with DCX Lipo-Nanosphere transdermal film. Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) is used to measure DCX in rat plasma.
Results: For oral routes, the maximal effect (Cmax, Tmax, and AUC0-72) was Cmax=9361±11.14, Tmax=12 hours, and 34327.433±117 ng/h/ml; for transdermal routes, it was Cmax=11263±7.4, Tmax=6 hours, and 146336.87±243 ng/h/ml. The findings indicated that there was a significant difference ($p<0.05$) in the amount of time and concentration required to get the maximal effect (Cmax and Tmax) between transdermal Lipo-Nanosphere film and DCX-oral solution. After a single dose given for 72 hours, the transdermal method's relative bioavailability was more than four times greater than that of the oral route.
Conclusions: F10 transdermal film offers a 4.26-fold higher bioavailability than DCX oral suspension made of pure powder.

Keywords: In vivo, Docetaxel, Lipo-Nanospheres, Transdermal.

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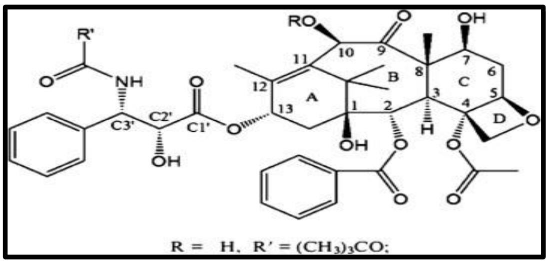
Introduction

Lipo-Nanosphere (LPNS)A is a drug encapsulation system composed of soluble solid microparticles with a diameter ranging from 0.01 to 100 micrometers. An embedding layer of phospholipid molecules stabilizes their solid hydrophobic lipid core. The bioactive substance is dissolved or dispersed by the solid lipid matrix of the internal core. Lipospheres were first introduced in the early 1990s and have since been used in many pharmaceutical applications utilizing various delivery systems. Subsequently, a self-assembling pro-nano liposphere encapsulation method provides several advantages over conventional delivery systems Figure 2 makes mention of its shape. These include enhanced physical stability, low ingredient prices, ease of production and scaling up, and high dispersibility in a water-based solution. Almost 40% of newly discovered chemical entities are lipophilic and have low water solubility, as Lipinski et al. (2001) stated 40% [(1)]. As a pharmaceutical chemical researcher, synthesizing such common water-soluble compounds for therapeutically available treatments is an important challenge. The anhydrous docetaxel (DCX) is a semi-synthetic counterpart of Paclitaxel, an anti-neoplastic medication from the second generation of the taxoid family. With the chemical formula $C_{43}H_{53}NO_{14}$, which translates to a molecular weight of 807.89 Da, In Figure., its chemical structure is discussed. DCX is generated as a semi-synthetically from;10-deacetylbaccarin-III, an inactive precursor molecule derived from the needles of a rare Pacific yew tree, Taxus baccata. The compound's log-P and PKa values of 4.1 and 10.97 indicate low membrane permeability (1 X 10^{-6} cm/second) and poor aqueous solubility (0.025 µg/ml). DTX is classified as a dissolution/permeability limited medicine under Class IV of the Biopharmaceutical categorization system (BCS) because of its poor permeability and solubility., with a melting point of (167-169 ºC) [(2,3)]



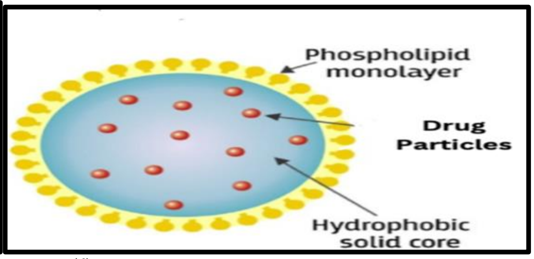


Figure 1. The chemical makeup of anhydrous docetaxel. [(4)] Figure 2. Structure of Nanoliposphere [(5)].

The most effective DCX-LPNS was combined with the PVA matrix to generate a film that is a simple, more affordable, and easier transdermal administration form; this ideal combination was assessed both Ex vivo and In vivo. When compared to a typical patch, simple bioadhesive films have a monolayer structure that simplifies preparation by combining the adhesive, reservoir, and baking into one layer. This drug-polymer Polyvinyl alcohol (PVA)matrix film dispersion has been modified for transdermal delivery. [(6)] The goal of this study; is that DCX as an antineoplastic medicine, is effective in treating a range of complex tumors, including ovarian and breast cancers. It was only created for intravenous infusion on the market at a dose of 100 mg/m^2 every three weeks (Taxotere®) due to its extreme gastrointestinal toxicity and high hydrophobicity, which causes low bioavailability with first-pass metabolism and P-glycoprotein efflux pumps. DCX can be better dissolved and stabilized by encasing it inside a lipid nanocarrier; these particles can decrease P-gp-mediated efflux and increase cellular absorption by using biodegradable lipids. To guarantee safety and efficacy, combining these strategies with the transdermal route will yield further benefits like prevention of first-pass metabolism, Prolonged Drug Release, Lower Infection Risk, usability, less direct adverse reactions, and enhanced quality of life for patients.

Materials and Methods

Materials

Docetaxel procured from Hangzhou Hyper Chemicals Ltd., China, polyvinyl alcohol, PEG 600 and paclitaxel provided by Hangzhou Hyper Chemicals Ltd., China,. Ethanol is 100% HPLC grade from CHEM-LAB company, Belgium.

Methods

Making the LPNS transdermal film prepared

After developing and optimizing a Lipo-Nanosphere dispersion of docetaxel (DCX). Then, DCX lipid nanocarriers was used to load onto biodegradable PVA film .Solvent casting was used to make PVA films filled with DCX lipid nanocarriers; that will be added to the transdermal film matrix, which is made up of polyvinyl alcohol(PVA10% w/v) that has been dissolved in hot DIW by employing a hot plate magnetic stirrer (JOANLAB, China) running at 1000 revolutions per minute; for three hours at 60 degrees Celsius..[(7,8)] PEG600 (polyethylene glycol 600) with lower molecular weights was more effective when the formula is plasticized, and these films also had superior optical qualities. [(9)] Ideal concentration for use as a plasticizer at 30% (w/w) [(10)]; however, to ensure uniformity and the formation of a clear solution, it must be added drop by drop using disposable syringes at a speed of 1000 RPM for 10 minutes 60° C. [(11)] to guarantee appropriate component mixing and interaction, making it more appropriate for transdermal applications· ; after cooling the solution to ambient temperature, any trapped air bubbles were removed by placing it on the sonicator(JOANLAB, China) for 10 minutes. Once the PVA polymer matrix had cooled to room temperature; the DCX-LPNS were equally distributed throughout the PVA solution for 45 minutes in a)50:50 w/w ratio(at 1000 RPM. [(12)] The prepared polymeric based on DCX-LPNS; was filled inside a clean glass petri dish with a diameter of(4cm). Turning the funnel over onto the Petri plate; regulated the evaporation rate. They were then dried for 48 hours at room temperature; when they had completely dried, covered in aluminum foil, and kept at room temperature in a desiccator until more assessment research could be conducted. [13] Table 1. lists every component of the transdermal film.

Table 1. Matrix structures for films loaded with LPNS; 50:50 (%w/w).

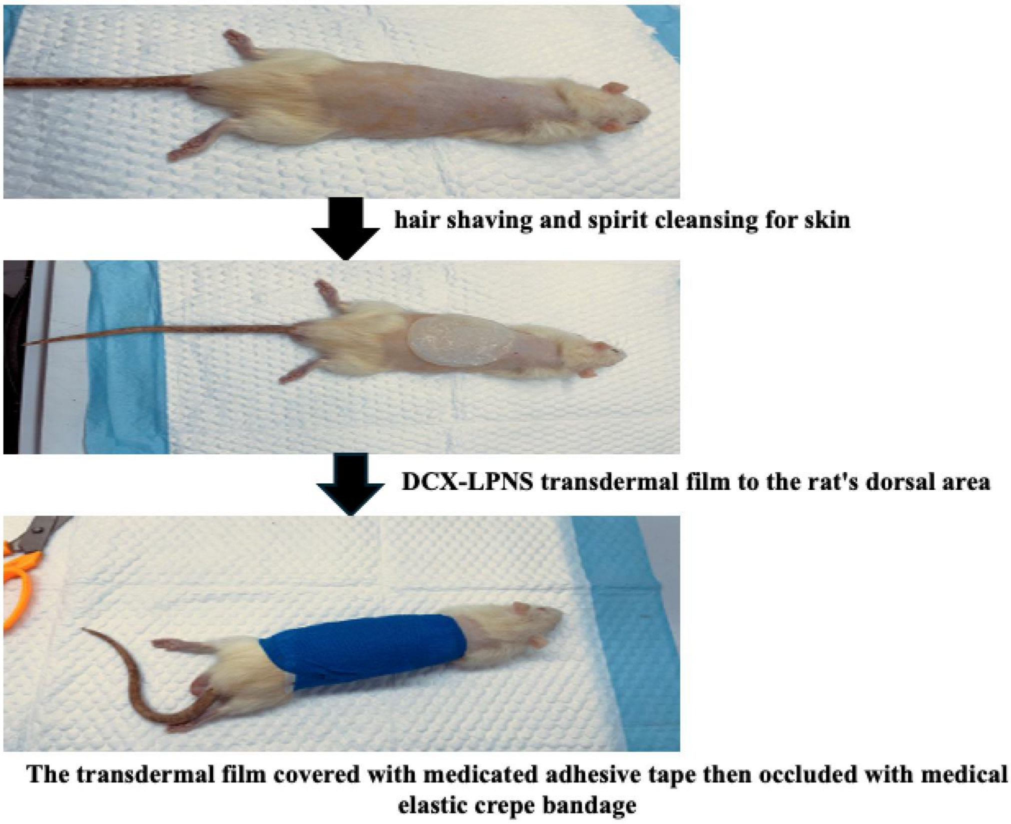
Ingredient	Amount (%)
DCX-LPNS	18 ml
PVS	10%
PEG600	30%
DIW	q.s.*

***q.s. stands for an adequate amount of DIW deionised water to create 100 ml**

Research of in vivo biodistribution

To begin with, male Sprague-Dawley rats; aged 10-12 weeks can provide blank plasma, which can be used as a negative control in high-performance liquid chromatography (HPLC) studies. After that, male rats weighing 200±0.25g ;72 rats each were divided into two groups, with (n=12) rats in each; Group I and Group II comprise in vivo evaluation of the optimized DCX Lipo-Nanospher loaded in transdermal films in contrast to DCX oral suspension; these groups were randomly selected from among the rats; the rats were kept in a room with consistent humidity, temperature (25 ± 1° C) and light; the rats were not fed any food the night before the trial was conducted; they were simply allowed to drink water. Every animal was treated humanely and followed the guidelines for the use and care of laboratory animals. The US National Institutes of Health released the handbook (No 85–23, revised 1996); all efforts were made to reduce the level of suffering experienced by the animals used in the experimental technique. By applying a DCX-LPNS transdermal film to the rat's dorsal area after hair shaving and spirit cleansing. With specific surface area of the transdermal film was 12.5 cm^2 (diameter 5cm); was applied on the shaved skin back of the rats and covered with medicated adhesive tape then occluded with medical elastic crepe bandage (85%)cotton size 15cm; and each treated animal was housed in a separate cage ; as seen in Figure 3. The animals in groups I and II were gently sedated with diethyl ether inhalation, and at intervals of (5 minutes., 15 minutes,30 minutes, 1, 2, 4,,6, 8, 12, 24, 48, and 72 hours);[14] with using a disposable syringe, 5 ml of blood was extracted from a heart puncture; this method allows for the extraction of sizable amounts of blood and sufficient plasma from a single rat. [15]To prevent clotting, the blood sample was placed in a microcentrifuge tube containing EDTA. Following sample collection, the plasma was separated using a micropipette and centrifuged at 4300 rpm for 10 minutes. The samples were then stored at -20 °C for further analysis. [16]The Research Ethics Committee for Experimental Investigations, College of Pharmacy, Baghdad University, Iraq, approved the in vivo tests conducted on the rats, with protocol number REACUBCP16-4-2024A.

Figure 3. transdermal film investigation in vivo employing rats



Examination of the amount of docetaxel in plasma

The HPLC system S600-Sykam GmbH (Germany) equipped with a hyper-silicon-BDS C18 column (250 X 4.6 mm,3.5 µm) for separation was used to perform the analysis. One of the conditions for chromatography is the mobile phase acetonitrile: Water (60:40 v/v) with 0.1% v/v orthophosphoric acid flow rate: 1.0 mL/minute; injection volume: 25 µL; column temperature: room temperature (25 ± 2 C); DCX wavelength detected: 232 nm. [17]

Analysis of statistics

The mean values and their standard deviation (±SD; n=3) were used to present the results. If a difference's P-value was less than 0.05, it was deemed statistically significant. Cmax, Tmax, and AUC0-72 are among the pharmacokinetic parameters that are statistically analysed using a student's t-test.[18,19]

Findings and Discussion

Analysis and discussion of the quantity of DCX in plasma

Using the technique for the spiked plasma, an internal standard (paclitaxel) and DCX standard solution with a known concentration, the calibration curve was created. R, the correlation coefficient, was 0.9998. The second approach was sensitive and accurate. Blank plasma, DCX, and the internal standard (paclitaxel) all had retention periods of 3.84 and 6.05 minutes, respectively. Table 2 and Figure 4 ;the transdermal film of DCX-LPNS was found to be significantly more bioavailable than the oral suspension dose, as demonstrated by the statistically significant effect ($p<0.05$) in the results. This can be attributed to the enhanced solubility of DCX caused by DCX Lipo-Nanospheres, which leads to rapid dissolution and a low Tmax. Comparing the oral DCX pure suspension to the transdermal film, the AUC0-72 for the former was substantially ($p<0.05$) lower at 34327.433±117 ng .h/ml. the latter's value was 146336.87±243 ng .h/ml. DCX LPNS transdermal film has a 4.26-fold better bioavailability than DCX pure oral suspension, according to the results obtained

Figure 4. DCX-LPNS transdermal film and DCX oral suspension plasma concentration time curve

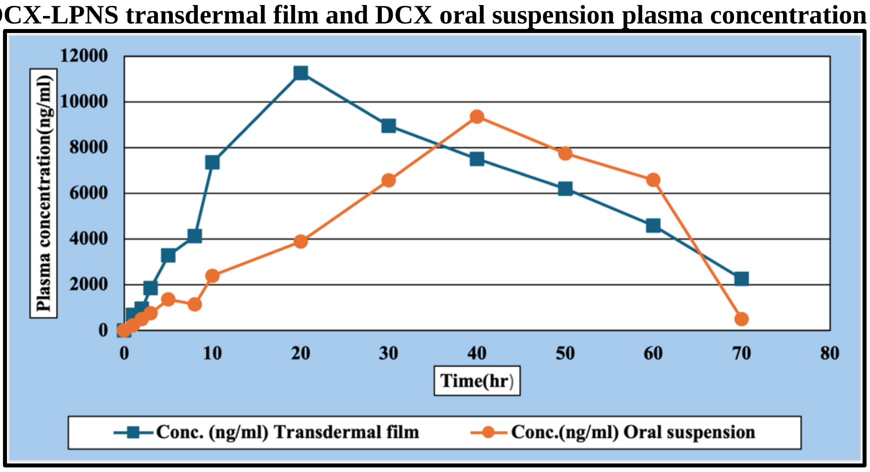


Table 2: Pharmacokinetic characteristics for F10 transdermal film and oral suspensionof DCX

Parameters	Oral suspension DCX	DCX-LPNS transdermal film
C_{max} (ng/ml)	9361±11.14	11263±7.4
T_{max}(hr)	12	6
AUC_{0-72} (ng.hr/ml)	34327.433±117	146336.87±243

Where the results expressed as (mean±SD, n=12))

Conclusion

Small lipid nanoparticles enter the lipid domains of corneocytes and hair follicles and offer a delayed release of drug, facilitating systemic absorption. Lipid nanoparticulates ensure intimate contacts and occlusion of skin and increase drug partitioning into stratum corneum.[20] The understanding that the oral bioavailability of lipophilic, poorly water-soluble drugs—specifically, class IV BCS, which has limited solubility and permeability ;may be improved when taken concurrently with a high-fat meal has sparked a growing interest in the formulation of these drugs inside lipids as a way to improve drug permeability and solubilisation .[21] Lipid suspensions, nanostructure lipid carrier ,solid lipid nanoparticle , and other methods have all been tried to improve the in vivo drug bioavailability; verify the viability of the concept to incorporate a lipophilic medication into a drug delivery system based on nanolipids.like ; DCX is more soluble in soy oil. *K. Gao et al.* created a submicron lipid emulsion of DCX, and the resulting in emulsions that are physically and chemically stable form [22]

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Conflicts of Interest

The authors stated no conflict of interest in the manuscript.

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Ethics Statements
The Research Ethics Committee for Experimental Investigations, College of Pharmacy, Baghdad University, Iraq, approved the in vivo tests conducted on the rats, with protocol number REACUBCP16-4-2024A.

Author Contribution
The author's responsibilities are described as follows: Preparation, collecting, and analyzing data: Maysam M. Abass. Designing, reviewing, and supervising the project : Shaima Nazar Abd Al Hammed. All authors reviewed the results and approved the final version of the manuscript

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