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Transdermal delivery of atorvastatin calcium from novel nanovesicular systems using polyethylene glycol fatty acid esters: Ameliorated effect without liver toxicity in poloxamer 407-induced hyperlipidemic rats

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ABSTRACT

Context: Atorvastatin calcium (ATV), a cholesterol-lowering agent, suffers from poor systemic availability (14%) after oral administration in addition to other side effects on the gastrointestinal tract, liver and muscle.
Objective: The goal of the present investigation was to improve ATV bioavailability and overcome complications attendant with peroral administration by developing a new nanovesicular system encapsulating ATV for its delivery *via* the transdermal route.
Methods: The vesicular systems were prepared by incorporating different polyethylene glycol fatty acid esters such as Labrasol, Cremophor EL, Gelucire 44/14 and Tween 80 as edge activators (EAs) in the lipid bilayer. The effect of the phosphatidylcholine (PC):EA molar ratio on the physicochemical properties of the vesicles was investigated. The pharmacokinetic studies of the optimized formulation were evaluated in rats. The optimized formulation was tested in poloxamer 407-induced hyperlipidemic rats. The plasma lipid profile, activity of liver enzymes, and oxidative stress parameters were measured using commercially available kits.
Results: The results revealed high ATV entrapment efficiency (EE%) ranging from 55.62 to 83.91%. The formulations that contained Labrasol showed the highest EE%. The mean diameter of the vesicles was in the range of 186–583 nm. T8 containing Gelucire 44/14 as an EA in the molar ratio of 15:1 (PC:EA) gave the smallest size and exhibited the best permeation parameters across the skin. The pharmacokinetic studies revealed that about three times statistically significant ($p < 0.05$) improvement in bioavailability, after transdermal administration of nanotransfersomal ATV gel compared to oral ATV suspension. The transdermal vesicular system exhibited a significant decrease in plasma total cholesterol, triglycerides and LDL cholesterol comparable to oral ATV. Additionally, it lowered the malondialdehyde levels in plasma and abolished the increase in liver enzyme activity.
Conclusion: The results obtained suggest that the proposed transdermal vesicular system can serve as a promising alternative means for delivery of ATV.

PubChem CID

Atorvastatin calcium	60822
Soybean lethithin	5287971
Cremophor EL	68516
Tween 80	5281955
Sodium carboxymethyl cellulose	23706213
Sodium dihydrogen phosphate	23672064
Glacial acetic acid	176
Acetonitrile	6342
2,4-Dinitrophenylhydrazine	5361189
Propylene glycol	1030
N-(1-Naphthyl)ethylenediamine	15107
Vanadium(III) chloride	62647
Sulfanilamide	5333
5,5′-Dithiobis(2-nitrobenzoic acid)	6254
Triton X-100	5590
Perchloric acid	24247

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1. Introduction

Atorvastatin calcium (ATV), a selective inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, blocks the rate limiting step in cholesterol biosynthesis in which HMG-CoA is converted to mevalonate [1]. It is used for treatment of patients with hyperlipidemia in an effort to decrease plasma levels of total cholesterol and, especially low-density lipoprotein cholesterol (LDL-C) by increasing the number of LDL receptors on hepatocytes, with subsequent acceleration in the uptake and degradation of LDL-C [2]. After oral administration, the absolute bioavailability of ATV is about 14% [3]. The low systemic availability is attributed to presystemic clearance in gastrointestinal mucosa and extensive hepatic first-pass metabolism [4]. Additionally, liver-related adverse effects of ATV mainly manifest as elevated serum transaminase activity and appear to be related to many factors such as the pharmacokinetic and physicochemical properties of ATV in addition to selective transporter-mediated liver and intestinal uptake associated with the oral route. Moreover, there are other oral-related side effects, such as muscular adverse effects, which are usually mild and reversible. However, these may precede rhabdomyolysis. Gastrointestinal (flatulence and diarrhea) complaints are also reported [5]. The aforementioned facts warrant the search for a new route of administration, like the transdermal route, to alleviate such problems experienced with oral dosing.

Transdermal delivery systems provide various distinct advantages: i) they avoid factors that influence the gastrointestinal absorption of drugs, such as enzymatic activity, pH, and drug– food interactions; ii) bypass first-pass metabolism and iii) permit rapid cessation of drug effects when necessary [6,7]. However, the barrier property of the stratum corneum (SC) is considered the greatest obstacle for transdermal delivery. Many approaches have been adopted to breach the skin barrier, such as the use of lipid vesicles to modify the SC [8].

Transfersomes are ultraflexible lipid bilayer vesicles used as alternative vehicles for transdermal and topical drug delivery, which are able to invade the intact skin. On the contrary, conventional liposomes usually stay restricted to the upper layer of the SC and accumulate in the skin appendages as a result of lack of flexibility and large vesicle size, which leads to reduced penetration to deeper tissues [9]. Each transfersome is composed of one or more inner aqueous cores that are surrounded by a lipid bilayer with specially-tailored characteristics arising from the incorporation of edge activators (EAs) into the vesicular membrane [10]. EAs are single chain components with a high radius of curvature which are able to increase the deformability of the bilayer by influencing the interfacial tension of the vesicles. Upon addition of EA, deformed vesicles with oval or irregular structures have been demonstrated *via* transmission electron microscopy [11]. Ultradeformable liposomes are essentially different from conventional ones in that the greater hydrophilicity of the former permits their elastic membrane to swell more compared to the conventional lipid bilayer. The higher membrane elasticity and hydrophilicity prevent fusion and aggregation of ultradeformable vesicles under osmotic stress that represents an obstacle with conventional liposomes. EAs usually incorporated in ultradeformable liposome preparation include Tween 20, Tween 60, Tween 80, Span 60, Span 65, Span 80, sodium cholate, sodium deoxycholate and dipotassium glycyrrhizinate [12]. In the present study, polyethylene glycol (PEG) fatty acid esters such as Labrasol, Cremophor EL, Gelucire 44/14, and Tween 80 were used as EAs. They are reported to be bioenhancers as they ameliorate the bioavailability of absorbed compounds by aiding paracellular and transcellular absorption [13].

Elastic vesicles squeeze themselves through intercellular regions of the SC under the effect of the transepidermal water activity gradient upon application on the skin surface. The hydrophilicty of the phospholipid layer allows the vesicles to exist in a maximal swollen state on the skin surface and then migration to the deeper skin strata following the local hydration gradient to avoid a dry skin layer (xerophobia) [14]. Also, transfersomes exhibit a series of stress-dependant modifications of the local carrier structure to decrease the hindrance to their motion through the confining channel that permits noninvasive and reproducible transport of drugs [12]. Moreover, transfersomes provide effective protection of the drug from undesirable rapid clearance into cutaneous blood vessels and are able to retain the drug for enough time on, in, and below the skin barrier. Furthermore, they can cross the SC irrespective of drug concentration [15]. Transfersomes have been largely used as carriers for different compounds, some examples of which are valsartan [16], insulin [17], diclofenac sodium [18], corticosteroids [19,20], celecoxib [21] and anticancer drugs [22,23].

Based on the above considerations, we hypothesized that the proposed transdermal ATV-loaded vesicular system may be effective in restoring disturbances associated with hyperlipidemia without hepatic, muscular or gastrointestinal side effects.

In this work, new phospholipid based nanotransfersomes encapsulating ATV for its transdermal delivery were developed after incorporating different PEG fatty acid esters as EAs. *In vivo* studies in rats were performed comparing the pharmacokinetic parameters of ATV after oral and transdermal administration. The pharmacodynamic effects were testable in a well-established rodent model of hyperlipidemia. Thus, such drug delivery system opens the door for potential transdermal delivery of a variety of drugs that are normally given by oral administration but for which non-enteral administration would be more appropriate due to concerns with either organ toxicity, poor and erratic absorption resulting from a high first-pass effect, or intestinal membrane diffusion/permeation.

2. Materials and methods

2.1. Materials

Atorvastatin calcium was received as a gift sample from Eipico (Cairo, Egypt), Labrasol (PEG-8 glyceryl caprylate/caprate), Gelucire 44/14 (Lauroyl polyoxyl-32 glycerides) were kindly supplied by Gattefossé (St-Priest, France). Soybean lecithin (L-α-phosphatidylcholine), Cremophor EL (PEG-35-castor oil), Tween 80 (Polyoxyethylene sorbitan monooleate), propylene glycol, carbopol 971P (polyacrylic acid), poloxamer 407, sodium carboxymethyl cellulose (Na CMC), acetonitrile (HPLC grade), glacial acetic acid (HPLC grade), 2,4-dinitrophenylhydrazine, sulfanilamide and *N*-(1-naphthyl)ethylenediamine dihydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dialysis bags with a molecular weight cut off of 12,000 Da were purchased from SERVA Electrophoresis GmbH (Heidelberg, Germany). 5,5′-Dithiobis(2-nitrobenzoic acid) and vanadium(III) chloride were purchased from Acros Organics (Geel, Belgium). Triton X-100 and perchloric acid were purchased from Loba Chemie (Mumbai, India). All other ingredients used were of analytical grade.

2.2. Preparation of ATV-loaded transfersomal vesicles

Different ATV transfersomal vesicles were prepared according to the thin film hydration technique [24]. One hundred milligrams of PC and specific weights of EA (Labrasol, Cremophor El, Gelucire 44/14 and Tween 80) in different molar ratios were added to a constant weight of drug (10 mg) and dissolved in chloroform: methanol, 2:1 v/v in a round bottom flask. The organic solvents were removed under vacuum in a rotary evaporator (Stuart rotary evaporator, RE300, Wolf Laboratories, North Yorkshire, UK with Stuart vacuum pump, RE3022C, Wolf Laboratories, North Yorkshire, UK) at 40°C for 20 min to form a thin film on the wall of the flask and kept in a desiccator under vacuum for 2 h to ensure total removal of trace solvents. Ten milliliters of phosphate buffer saline (PBS) pH 7.4 were then added and complete hydration of the dry film was performed by rotation at 60 rpm for 1 h at room temperature. Sonication of the resulted vesicles was carried out