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Pharmacological and histological examination of atorvastatin-PVP K30 solid dispersions

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ABSTRACT

The objective of the present study was to investigate the pharmacological efficiency of orally administered atorvastatin calcium (ATV) solid dispersions (SDs). SDs of ATV were prepared using polyvinylpyrrolidone K30 (PVP) through the solvent evaporation method. Physicochemical characteristics of the prepared formulations were assessed benefitting scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and powder X-ray diffractometry (PXRD). The drug dissolution rates (under sink and non-sink conditions) as well as solubility studies were also examined. Serum lipid levels, liver index and histological analysis of the liver tissue in hyperlipidemic rats were considered to evaluate the pharmacological efficiency of prepared SDs. According to our findings, the drug crystallinity was reduced and the drug dissolution characteristics were improved in the prepared SDs. In vivo studies revealed that oral administration of ATV (3 mg/kg/day) in the SD form (ASDT) for 14 days along with high fat diet (HFD) to the hypercholesterolemic rats led to a significant decline ($P < 0.05$) in serum level of total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C). Moreover, ASDT exhibited more beneficial effects on the liver steatosis compared to ATV physical mixture (APMT) and hyperlipidemic control (HC) groups. In the present study, it was concluded that the SDs of ATV with improved physicochemical characteristics provided an increased therapeutic potential for management of hyperlipidemia compared to the corresponding physical mixtures (PMs).

1. Introduction

Atorvastatin calcium (ATV), a lipid-lowering agent, acts by the reversible inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [1]. ATV is administered for treatment of hypercholesterolemia and other dyslipidemic disorders alone or in conjunction with other anti-hyperlipidemic drugs. It is also helpful in primary prevention of cardiovascular disease and reducing the risk of myocardial infarction [2,3]. ATV belongs to class II of biopharmaceutical classification system (BCS) [4] and tends to exhibit solubility or dissolution rate limited absorption. The marketed products of ATV suffer from poor oral bioavailability (~12%) [5]. Poor aqueous solubility, pre-systemic clearance in the gastrointestinal mucosa and/or hepatic first-pass metabolism are responsible for the low oral bioavailability of ATV [6]. Achieving appropriate therapeutic effects is possible by using higher doses of drug which results in dose related undesirable adverse effects. Moreover, development of ATV formulation due to its poor aqueous solubility and insufficient bioavailability after oral administration is challenging [5]. Therefore, developing effective approaches to minimize the associated problems could be highly advantageous. Several techniques have been applied to enhance the solubility and dissolution rate of ATV, including nanosuspension formulation [7], preparation of the self-emulsifying drug delivery systems (SEDDS) [8,9], complexation with hydroxypropyl-β-cyclodextrin [6], preparation of the solid dispersions (SDs) [10–12], applying supercritical antisolvent (SAS) process [13,14] and spray drying technique [15]. SDs are used as one of the most practical and effective strategies in order to increase the dissolution behavior of poorly water-soluble drugs. The advantages of SDs include particle size reduction (possibly to molecular level), increase in wettability and porosity, decrease of drug crystallinity and sometimes conversion into amorphous state [16]. Accordingly, the drug substance could disperse as separate molecules, amorphous particles or crystalline particles, whereas, the carrier might present in the crystalline or amorphous state. Improvement in drug solubility and dissolution rate through SD technique has been shown by numerous studies [16]. Oral bioavailability of some poorly water-soluble drugs [17]

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such as ibuprofen [18], carbamazepine [19], naproxen [20] and ritonavir [21] has also been enhanced using SD technique. According to the conducted researches, polyvinylpyrrolidone (PVP)-based SDs of ATV exhibited noticeable increase in ATV absorption following oral administration [10]. Therefore, in this study, SDs of ATV were prepared using PVP K30 as an amorphous carrier, via the solvent evaporation method. Regarding literature review, evaluation of the therapeutic efficiency of ATV SDs (especially in liver tissue) via in vivo study has not been considered by other researchers. Although, the majority of researches are focused on effects of hypercholesterolemia on atherosclerosis and heart disease; however, toxic effects of cholesterol on the liver are also of particular importance [22]. Thus, in this work, after preparation and physicochemical characterization of ATV SDs, in order to investigate the therapeutic efficiency of prepared formulations in the diet induced hyperlipidemic rats, serum lipid levels, liver index and histopathological examination of the rat livers were evaluated.

2. Materials and methods

2.1. Materials

ATV was purchased from Abidi pharmaceutical company (Tehran, Iran). PVP K30 and sodium hydroxide were supplied from Merck (Germany). Methanol was of high-performance liquid chromatography (HPLC) grade (Caledon Labs, Ontario, Canada). All other chemicals were of analytical grade.

2.2. Preparation of the solid dispersions

SDs of ATV were prepared by PVP K30 in different drug to polymer ratios, using the common solvent evaporation method. Briefly, different weight ratios (1:1, 1:3, 1:5 w/w) of drug:polymer combinations were dissolved in minimum volume of methanol and stirred (Heidolph, MR Hei-Tec, Germany) at 600 rpm for 15 min at room temperature. After complete dissolving, the solvent was evaporated using rota-evaporator (Heidolph, Laborota 4010 digital, Germany) at room temperature, 50 rpm for 12 h. Then the residue was pulverized by mortar and pestle, sieved (mesh size 60) and then kept in a desiccator. Recrystallized ATV was also prepared through recrystallization of ATV from methanol.

2.3. Scanning electron microscopy (SEM)

The morphology of prepared samples was observed using scanning electron microscopy (SEM; Tescan, Brno Czech) operating at 20 kV. The samples were directly dispersed on a double-sided tape and coated by gold in a vacuum condition, before the examination.

2.4. Differential scanning calorimetry (DSC)

Thermal behavior of the SDs, PMs as well as pure drug and polymer was evaluated using differential scanning calorimeter (DSC60 Shimadzu, Kyoto, Japan). Accurately weighed samples (3 mg) were placed into crimp sealed aluminum pans. The measurement was performed at a heating rate of 20 °C/min from 50 to 210 °C. The aluminum oxide and indium powders were applied as reference and standard, respectively.

2.5. Powder X-ray diffraction (PXRD)

X-ray diffraction patterns of the pure drug, polymer, PMs as well as prepared SDs were obtained by the X-ray diffractometer (Siemens D5000, Munich, Germany). Samples were scanned over the range of 2θ from 2 to 40°, under Cu Kα radiation, at a voltage of 40 kV and a current of 30 mA.

2.6. Fourier transformed infrared spectroscopy (FTIR)

A Shimadzu 43000 FTIR spectrophotometer was employed for FTIR analysis. Samples were examined in the transmission mode and prepared using the KBr disk method (1 mg sample in 100 mg KBr powder). A spectral range of 400–4000 cm^{-1} and a resolution of 2 cm^{-1} were used in order to obtain the spectra.

2.7. Solubility of ATV

Solubility measurements were performed according to the Higuchi and Connors' method [23]. Briefly, an excess amount of ATV was added into the test flasks containing various concentrations of PVP-K30 in the dissolution medium (10 mL, phosphate buffer, pH 6.8). The samples were sonicated (Metler Electronics, model ME5.5, USA) for 30 min at room temperature. Afterward, the sealed test flasks were shaken at 25 °C (room temperature) for 48 h on a rotary shaker incubator (Heidolph unimax 1010, Inkubator 1000). The suspensions were centrifuged at 10,000 rpm for 5 min (Eppendorf 5810R centrifuge) and filtered through a 0.45 μm membrane filter. After suitable dilution, the samples were analyzed spectrophotometrically at a wavelength of 241 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The polymer did not interfere with the UV measurement and no significant adsorption of the drug to the filter membranes was detected. All solubility experiments were performed in triplicate and the obtained results were presented as mean ± SD.

2.8. Dissolution study under sink condition

Dissolution study was performed using USP apparatus II, paddle stirrer (Erweka dissolution tester). The pure drug, SDs and PMs, all equivalent to 20 mg ATV, were added to the 900 mL dissolution medium (phosphate buffer, pH 6.8) under stirring rate of 75 rpm at 37 ± 0.5 °C. At appropriate time intervals, 2 mL of sample was withdrawn and filtered through 0.45 μm membrane filter. The removed volume was refilled with the fresh medium at 37 °C. The filtrate was suitably diluted and analyzed spectrophotometrically at 241 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The obtained results were deducted from a standard calibration curve of ATV and the percent of the dissolved drug was plotted against the time. The reported results were the average value of at least three replicates ± SD.

2.9. Dissolution study under non-sink condition

Non-sink condition represented an insightful dissolution situation to further evaluation of the dissolution phenomena [24]. In this condition, dissolution medium is not enough for dissolving the total amount of drug [25]. The apparent dissolution profiles of SDs were investigated under non-sink condition as well. An accurately weighed amount of the SDs, each equivalent to 10 mg drug, was added to test flasks containing 10 mL phosphate buffer and shacked at 37 °C, 100 rpm (Heidolph unimax 1010, Inkubator 1000). The experiment was performed on five time points with three replicates. The samples were centrifuged at 10,000 rpm for 3 min and then filtered using 0.45 μm membrane filter. The supernatant was then further diluted and analyzed spectrophotometrically at 241 nm.

2.10. Animal study

2.10.1. Animals

Male Wistar rats were purchased from Pasteur Institute of Iran (Tehran). Following their arrival to the animal care facility center, they were housed for one week prior to examination. Afterward, rats weighing 250 ± 20 g were divided into four groups and employed in this research. Rats were kept in the Animal House of Tabriz University of Medical Sciences at a controlled ambient temperature of 25 ± 2 °C