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Nanoemulsion strategy for olmesartan medoxomil improves oral absorption and extended antihypertensive activity in hypertensive rats

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ABSTRACT

Olmesartan medoxomil (OM) is hydrolyzed to its active metabolite olmesartan by the action of aryl esterase to exert its antihypertensive actions by selectively blocking angiotensin II–AT_1 receptor. Poor aqueous solubility and uncontrolled enzymatic conversion of OM to its poorly permeable olmesartan limits its oral bioavailability. The aim of the current study was to formulate a novel nanoemulsion of OM to improve its pharmacokinetics and therapeutic efficacy. The oil-in-water (o/w) nanoemulsion of OM was developed using lipoid purified soybean oil 700, sefsol 218 and solutol HS 15. We have characterized the nanoemulsions by considering their thermodynamic stability, morphology, droplet size, zeta potential and viscosity and *in vitro* drug release characteristics in fasting state simulated gastric fluid (pH 1.2) and intestinal fluid (pH 6.5). The thermodynamically stable nanoemulsions comprises of spherical nanometer sized droplets (<50 nm) with low polydispersity index showed enhanced permeability through the Caco-2 cell monolayer. The concentration of active olmesartan in rat plasma following oral absorption study was determined by our validated LC–MS/MS method. The result of the pharmacokinetic study showed 2.8-fold increased in area under the curve (AUC_{0-27}) of olmesartan upon oral administration of OM nanoemulsion and sustained release profile. Subsequent, *in vivo* studies with nanoemulsion demonstrated better and prolonged control of experimentally induced hypertension with 3-fold reduction in conventional dose. By analysing the findings of the present investigations based on stability study, Caco-2 permeability, pharmacokinetic profile and pharmacodynamic evaluation indicated that the nanoemulsion of OM (OMF6) could significantly enhance the oral bioavailability of relatively insoluble OM contributing to improved clinical application.

1. Introduction

Literature survey reveals that a considerable number (≈40%) of new chemical entities (NCEs) generated during drug developmental research are lipophilic in nature [1,2]. Accordingly, problems like poor oral bioavailability, erratic absorption, intra- and inter-subject pharmacokinetics variations, lack of dose proportionality are obviously associated with these NCEs [3]. Therefore, newer techniques like amorphous solid form, nanoparticles, solid dispersions, melt extrusion, salt formation [4], incorporation of lipophilic component in inert lipid vehicle [5,6], formation of microemulsion or nanoemulsions, self-emulsifying formulations, liposomes, solid lipid nanoparticles or lipid nanocarriers [7] are becoming increasingly popular to overcome these problems of lipophilic properties of compounds. Among these techniques, nanoemulsion (NE) bears much hope being isotropic, transparent (or translucent) heterogeneous mixture composed of oil droplets dispersed in aqueous media stabilized by an interfacial film of surfactant molecules [8]. NEs are advantageous to conventional microemulsions due to their enhanced thermodynamic stability of the nano sized droplets [9] and these nano sized droplets resulted in an enormous increase in interfacial areas, rapidly absorbed by internalization of the droplets into the enterocyte and thereafter transported into the systemic circulation *via* portal vein and lymphatic pathway [8]. Furthermore, long term stability, ease of preparation, solubilization and improved oral bioavailability of lipophilic drugs in NE, makes it a potential drug delivery tool.

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OM is one such newly introduced antihypertensive drug where lipophilicity is yet to be solved by improvisation of formulation techniques. OM is a prodrug which is hydrolyzed by intestinal and plasma esterases, to form its active metabolite, olmesartan following oral administration [10–14]. It is a potent, well tolerated and extensively used selective AT_1–subtype angiotensin II receptor blocker and it also reduces aldosterone secretion by angiotensin II thereby exerting its antihypertensive effect [15–17]. Recently concomitant use of OM with other antihypertensive agents is also evaluated for clinical efficacy and safety in the management of essential and diabetic hypertension [18,19]. However, very poor solubility in water and uncontrolled enzymatic conversion of OM to its poorly permeable parent molecule olmesartan in the GI fluid further limits its oral bioavailability only up to 26% [12,20]. Literature survey reveals that very few scientific works has been done to overcome this serious problem. Among these Lee et al. reported about an improved oral bioavailability up to 1.7-folds which is claimed to be achieved by self-microemulsifying drug delivery system [21]. In another report, Singh et al. developed a surface absorbed NE to solve this crisis, although specific reports on bioavailability of olmesartan are not being mentioned [10]. Furthermore, among other side effects, the incidences of diarrhea have been a serious problem with olmesartan [22]. Even FDA approves label changes to include intestinal problems (sprue-like enteropathy) linked to OM. This problem may grossly be attributed to local cell mediated immune response where physical contact of olmesartan with intestinal villi might be a trigger [23]. Therefore, avoidance of physical contact of the drug by incorporating in oil core of NE, can be expected to render protection.

Thus in the present study an attempt has been made to design, develop and characterize a novel, thermodynamically stable o/w NE of OM by spontaneous method intended for oral use. Subsequently, preclinical pharmacokinetic and pharmacodynamic evaluation of the novel formulation in rat models has also been performed keeping the procedures and standards comparable with the existing regulatory norms.

2. Materials and methods

2.1. Materials

OM was gifted from Burgeon Pharmaceuticals (Chennai, India). The various oil phases were provided by the following companies: propylene glycol mono caprylic ester (Sefsol 218) (Nikko Chemicals (Tokyo, Japan)); lipoid purified soyabean oil 700 (Lipoid GmbH, Ludwigshafen, Germany). Solutol HS 15 (surfactant) was obtained from Sigma–Aldrich.

All chemicals and reagents used were of HPLC grade and were purchased from Merck Pvt. Ltd. (Mumbai, India). HPLC-grade water (resistivity of 18.2 MΩ cm was generated from a Milli-Q gradient system of Millipore, Elix 3, Milli-Q A10 Academic, Molsheim, France).

2.2. Preparation of NE

2.2.1. Selection of the NE components

Solubility of OM in various oils, surfactants and in mixture of oils and surfactants were reported in our previous article [24]. On the basis of solubility study in various combinations of oils and surfactants, the combination comprising of lipoid purified soybean oil 700:sefsol 218:solutol HS 15::1:1:2 was selected for further formulation development study due to highest solubilization of OM (25.54 ± 1.33 mg/mL) than the other combinations at 25 ± 1.0 °C.

Table 1
Observation table for evaluation of dispersibility test.

Observations	Grade
Rapidly forming NE (within 12 min) (producing a clear or slight bluish appearance)	A
Rapidly forming, slightly less clear NE (having bluish appearance)	B
Fine milky emulsion that formed within 2 min	C
Dull, grayish white emulsion having slightly oily appearance (slow to emulsify)	D
Formulation exhibiting either poor or minimal emulsification with large oil globules present on the surface	E

2.2.2. NE preparation

During developmental stage, OM was dissolved initially in the mixture of lipoid purified soybean oil 700 and sefsol 218 (1:1). The surfactant (solutol HS 15) was added to the mixture and mixed. Water was added to the final mixture gradually with vigorous vortexing (Cyclomixer, CM 101, REMI Equipments Ltd., India) and emulsification was achieved by phase inversion technique [25]. The mixing was continued for 10 min after complete addition of the aqueous phase.

2.3. Thermodynamic stability study

In order to assess the physical stability of the selected formulations following tests were performed:

i. Centrifugation test: The formulations were centrifuged (Remi Equipments Ltd., India) at 5000 rpm for 30 min and observed for phase separation, creaming or cracking.
ii. Heating-cooling cycle: Qualified samples were stored at refrigerator temperature (4 °C) and 40 °C (six cycles each) for 48 h at each temperature.
iii. Freeze thaw cycle: Passed formulations were subjected to three freeze thaw cycles between −20 °C in deep freezer (Celfrost, Celsius Refrigeration Pvt. Ltd., India) and at room temperature (25 ± 2 °C) with storage of 48 h at each temperature.

2.4. Dispersibility test

Thermodynamically stable samples were further evaluated for the efficiency of self-emulsification capacity of the formulated NEs. This study was performed by mixing 1 mL of the formulated NEs in 500 mL of water and in 0.1 N HCl (separately) maintained at 37 ± 0.5 °C using a standard dissolution apparatus (USPXXII) with the paddle rotated at a speed of 50 rpm for gentle mixing [6,26,27]. The results of the *in vitro* performance of the formulations were evaluated visually as per Table 1 and the formulations passed in Grade-A were selected for further studies.

2.5. Characterization of NE

2.5.1. Percentage transmittance

Percentage transmittance was done for the thermodynamically stable NEs using UV–visible spectrophotometer (V-630 Spectrophotometer, Jasco Analytical Instruments, Japan). Diluted samples (100 times in de-ionized water) were analyzed at 500 nm [5].

2.5.2. Refractive index

The refractive index of the selected formulations and their corresponding placebo (NE without drug) was measured by using an Abbe refractometer (Scientific Engineering Corporation, Delhi, India) by making a film of the formulation on the slide in triplicate at 25 °C.