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Brain delivery of baclofen as a hydrophilic drug by nanolipid carriers: Characteristics and pharmacokinetics evaluation

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Glyceryl trioleate (PubChem CID: 5497163)
Tween 80 (PubChem CID: 86289060)
Trehalose dehydrate (PubChem CID: 181987)
Soy lecithin (PubChem CID: 94190)

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ABSTRACT

Formulation of baclofen in lipid-based nanoparticles may be helpful to overcome its difficulties in order to reach the site of action in the CNS. This study reports production, characterization and pharmacokinetics evaluation of baclofen loaded lipid nano-carriers. Lipid nano-carriers containing baclofen were prepared by using double emulsion solvent evaporation method and physicochemical characteristics of prepared nanoparticles were evaluated. The selected formulation was administered on rat model to be compared with an aqueous drug solution. Plasma and brain concentrations of drug and pharmacokinetics parameters were compared. Nanostructured lipid carriers (NLC) formulation showed better physicochemical properties than solid lipid nanoparticles (SLNs). Also, the formulation of baclofen by NLC increased the half-life of drug in plasma and brain up to 10 and 1.5 times respectively compared to the solution formulation. Results revealed a more significant and prolonged effect of baclofen-NLC in comparison with baclofen solution in both plasma and brain mediums that emphasize and prove theories on the formulation of hydrophilic drug in NLCs can be a solution for target drug delivery to the brain.

1. Introduction

Severe spasticity is a disabling disorder, usually originated by prior CNS malfunctions or injuries to the spine [1]. Both invasive and non-invasive medical approaches are being used to deal with this problem [2]. Several peripheral or central relaxant agents are suggested as the non-invasive pharmacotherapy alternatives [3]. One of these medicines is baclofen, a selective $GABA_b$ receptor agonist, which promotes different pharmacological effects such as central muscle relaxation [4–6]. Baclofen is being used widely in the treatment of multiple sclerosis and severe spasticity [7,8]. However, the effect of oral baclofen is limited due to its polar nature, which creates insufficient concentrations in the brain and cerebrospinal fluid (CSF). Therefore, the reference route for treatment of severe spasticity is an intrathecal injection of baclofen by using a surgically implanted pump [9]. But there are some drawbacks in the use of this method, such as the surgery itself, high expenses, catheter dysfunctions, risk of infections and several other disturbing side effects [10]. These complications in the treatment and lack of proper efficacy of drug emerges studies for revised methods and new drug delivery systems. In this manner, incorporating drug into the lipid based nanoparticles may be reasonable to overcome aforementioned defects [11].

Solid Lipid Nanoparticles (SLNs) are colloidal systems based on solid lipids, including high-melting point glycerides or waxes [12,13]. However, SLNs may have some limitations in drug loading capacity or drug loss during storage due to the lipid crystallization into the stable β-modification [14]. Nanostructured lipid carriers (NLCs), the next generation of SLNs, are produced by the

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incorporation of liquid lipids into the solid lipid structures to overcome possible limitations related to the SLNs [15].

In a previously reported research, baclofen loaded SLNs were prepared and optimized by this group using double emulsion solvent evaporation method [16]. In the present study, mentioned formulation and modified form of NLCs were prepared and *in vivo* performance of carriers was evaluated in rat model.

2. Materials and methods

2.1. Materials

Baclofen was provided by Chemidaru (Tehran, Iran). Glyceryl monostearate (GMS), glyceryl distearate (GDS) and glyceryl trioleate (GTO) were kindly donated by Gattefossé (France). Tween 80 was purchased from Sigma-Aldrich (Germany). Trehalose dehydrate, soy lecithin (SL) and all solvents (HPLC graded) were purchased from Merck (Germany).

2.2. Preparation of baclofen-loaded nanoparticles

In the previously published research, formulation of Baclofen-loaded SLNs were optimized [16]. SLNs were prepared by a double emulsion-solvent evaporation technique (DESE). Briefly, lipid(s) mixed with SL was dissolved in 5 mL dichloromethane according to Table 1 (oil phase). Inner aqueous phase (W_1) was prepared by dissolving 15 mg of the drug in 0.5 mL of 0.1 M hydrochloric acid solution. The first aqueous phase (W_1) was emulsified in the oil phase by using a high-shear homogenizer (IKA, Germany) at 20,000 rpm for 1 min. The W_1/O emulsion was added into 25 mL of an aqueous solution of Tween 80 under high-shear homogenization (20,000 rpm) using a syringe pump (Kd Scientific, USA) at rate of 3 mL/min. Ultimately, the organic solvent in the emulsion was evaporated by a rotary evaporator (Büchi, Switzerland) to produce lipid nanoparticles.

In order to separate drug-loaded nanoparticles, the ultimate mixture was centrifuged at 22,000 rpm (Sigma, Germany) for 40 min at 10 °C and then washed two times with double deionized water. Then, the retrieved nanoparticles were re-suspended in 2 mL of 10% (w/v) trehalose and after freezing at −20 °C were lyophilized (Christ, Germany) at −40 °C for 48 h.

2.3. Characterization of nanoparticles

2.3.1. Determination of particle size, polydispersity index and zeta potential

The particle size, polydispersity index (PDI) and zeta potential were measured by photon correlation spectroscopy using a Zetasizer NanoZS (Malvern Instruments, UK) at 25 °C. All of the samples were diluted with double distilled water to make a suitable obscuration before analysis.

2.3.2. Entrapment efficiency

A defined amount of nanoparticles was added to 10 mL of dichloromethane in order to dissolve lipid base. The same volume of HCl solution (pH 1.2) was added into the previous solution and agitated for 5 min to extract the drug.

The amount of drug in the aqueous phase was assayed using a UV spectrophotometer (Spekol, Germany) at 219 nm. The entrapment efficiency was calculated according to the following equation:

$$\text{Entrapment efficiency}\,(\%) = \frac{\text{mass of initial drug} - \text{mass of free drug}}{\text{mass of initial drug}} \times 100$$

2.3.3. In vitro release study

In vitro drug release from lyophilized formulations was performed in phosphate buffer (pH 6.8). A known amount of formulation was dispersed in deionized water (5 mL) and poured into a dialysis tube (cut off 12 KD, Spectrum Laboratories, USA) and sealed at both ends. Then the dialysis tube was dipped into the dissolution medium. At predetermined time points, samples were taken and the same volume was replaced with fresh dissolution medium. The cumulative release of baclofen was assayed using the spectrophotometry method as described previously.

The release data from the best formulation was fitted to different kinetic models such as zero order, first order and korsmeyer-Peppas, to elucidate the mechanism of drug release from the formulations.

2.3.4. Thermal analysis

Thermal behavior of baclofen, GMS, GDS, GTO, their physical mixture and NLCs were analyzed using differential scanning calorimeter (Mettler Toledo, Switzerland). Approximately, 6 mg of samples were placed in aluminum pans and heated at the scanning rate of 10 °C/min from 25 to 280 °C. Indium and zinc were used as the standard reference materials to calibrate the temperature and energy scale of the DSC instrument.

2.3.5. Scanning electron microscopy (SEM)

Lyophilized NLCs were spread on an aluminum stub and sputter-coated for 90s with gold–palladium (Bal-Tec, Germany). The morphology of nanoparticles was examined by a scanning electron microscope (Philips, Netherlands) at an acceleration voltage of 17 kV.

2.4. In vivo and drug extraction protocol

A Formulation with the lowest size and minimum size change during lyophilization process, acceptable entrapment efficiency and sustained release profile was selected as a suitable formulation for *in vivo* studies. For this experiment, male Sprague–Dawley rats (weighing 260–270 g) were maintained in a clean room under 12 h light-dark cycle, controlled environmental temperature between 20 and 23 °C, a relative humidity of 50% and free access to standard laboratory chow and water. The study was approved by the "institutional review board of the pharmaceutical research center in Tehran University of Medical Sciences". The animals were randomly divided into two groups: sterilized lipid nanoparticles incorporated with baclofen (NLB) and baclofen aqueous solutions as control group (n = 12 in each group).

The optimum dose which was well-tolerated by the animals and produced a linear kinetic model, was determined by applying increasing doses on animals. The animals were received 7.5 mg/kg doses of formulation in NLB group and baclofen aqueous solution in the control group, intraperitoneally. In both groups, animals were sacrificed by CO_2 in predetermined times: 1, 2 and 4 h after

Table 1
Different formulation of lipid based particles.

Run No	Lipid (mg)			Lecithin (mg)	Tween 80 (%)
	GMS	GDS	GTO		
F_1	130	–	–	130	0.5
F_2	65	65	–	130	0.5
F_3	65	–	65	130	0.5