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Modulating intestinal uptake of Atenolol using niosomes as drug permeation enhancers

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Abstract

It is well established through the last decade that niosomes have potential applications as drug carriers either to improve drug permeation across membranes or targeting to specific tissues. Having a considerable ability to improve the permeability of drugs through lipoid membranes, niosomes have been utilized as carriers to enhance atenolol absorption from the gastrointestinal tract. Two methods have been adopted to prepare niosomes, the proniosome-derived method (A) and the conventional film hydration method (B). The products from the two methods were compared in terms of their morphology, vesicle size, drug encapsulation efficiency, in vitro drug release and enhancement effect on drug permeation across the intestinal membrane using an everted sac technique. Proniosome-derived niosomes were smoother and exhibited a smaller (5 μm) vesicle size compared to those prepared by conventional methods (12 μm). High encapsulation efficiencies of 98.6% and 93.4% were achieved by methods A and B, respectively. In vitro drug release has been significantly retarded from both types of niosomes. Comparing to pure drug, which dissolved completely in 15 min, only 8.9% and 9.9% of the entrapped drug was released in the same time period. The drug release kinetics showed non-Fickian (anomalous) behavior. Permeation through an everted

intestinal sac showed a significant enhancement effect (more than 4 fold) for both types of niosomes compared to untrapped drug; however, the difference between the two types of niosomes was not significant.

Key Words

Atenolol, niosomes, everted sac, intestinal absorption, permeation enhancer

Introduction

In recent years, pharmaceutical modification by inclusion complexation has been extensively developed to improve drug absorption and bioavailability. Non-ionic surfactant vesicles known as niosomes are microscopic lamellar structures formed on admixture of a non-ionic surfactant, cholesterol and lecithin with subsequent hydration in aqueous media. Niosomes are biodegradable, biocompatible, non-toxic and capable of encapsulating large quantities of material in relatively small volumes of vesicles [1-3].

It has been shown in many publications that niosomes significantly improve transdermal delivery of drugs [4-6]. However, little research related to the effect of niosomes on drug permeation through the intestinal membrane could be identified, although promising results have been reported [7-9].

Atenolol is a β-adrenergic receptor blocking agent used for treatment of hypertension, either alone or with other antihypertensives such as thiazide diuretics. It is reported that the bioavailability of 50 mg atenolol tablets is only about 63 % [10]. Among the ways to improve the bioavailability and minimize the side effects of atenolol is its formulation for transdermal delivery [11].

The aim of this present work was to investigate the suitability of niosomal preparation as a drug carrier for the antihypertensive drug, atenolol, which could be used as an aid to enhance the intestinal absorption of the drug using the everted sac technique.

The everted sac technique has been a simple and useful in-vitro method to study drug absorption [12]. The system would provide information on drug

absorption through testing the drug content in the intestine and that transported through the intestinal tissue. It has been used to study the uptake of lipid vesicles [13], proteins and macromolecules with oral drug delivery potential.

Experimental

Chemicals

Span 60 was supplied from Koch-Light Laboratories Ltd. (Colebrook Bucks, England). Acetonitrile and methanol (both HPLC grade), potassium dihydrogen phosphate, and disodium hydrogen phosphate were purchased from BDH Laboratory Supplies (BDH Chemicals Ltd., Poole, UK). Lecithin was obtained from Merck Company (Darmstadt, Germany). Cholesterol and ethanol were supplied from Riedel Dehaën (Darmstadt, Germany). Atenolol was generously donated from Al-Hikma Pharmaceutical Industry (Amman, Jordan). Metoprolol was purchased from USP Pharmacopoeia (New York, NY, USA). Dodecyl sulfate sodium salt (SDS) was purchased from Sigma Chemicals Company (St. Louis, MO, USA). All other chemicals were of analytical grade.

Preparation of niosomes

Method A

Proniosomes were prepared using a modified literature method [6]. Using a wide mouth glass tube, 100 mg of atenolol with surfactant, lecithin and cholesterol was mixed with 2.5 ml absolute ethanol. The open-end of the glass tube was covered with a lid and the tube was warmed in a water bath at 65 ± 3 °C for 5 min. Then 1.6 ml of phosphate buffer (pH 7.4) was added and the mixture was further warmed in the water bath for about 2 min so that a clear solution was obtained. The mixture was allowed to cool to room temperature until the dispersion was converted to proniosomal gel. The proniosomal gel was then hydrated with 10 ml phosphate buffer (pH 7.4), and the formed niosomes were separated by centrifugation at 25000 rpm at 20 °C for 30 min. The resulting niosomes were dried in an oven at 40 °C.

Method B

Niosomes were prepared by a rapid hydration method [14] as follows: to 20 ml of atenolol solution (10 mg/ml) in a chloroform/methanol mixture (1:1), 900 mg Span 60, 900 mg lecithin and 100 mg cholesterol were added and vortex mixed. The mixture was transferred to a 500 ml round bottom flask and attached to a rotary evaporator (Buchi Rotavapor RE 120). The organic solvents were removed under reduced pressure at 60 °C to form a thin dry film on the wall of the flask. The film was then hydrated with 20 ml of phosphate buffer (pH 7.4) at 60 °C at a rotation speed of 150 rpm for 1 hour. The dispersion was sonicated for 3 min. The dispersion of the formed niosomes was then centrifuged at 25000 rpm at 20 °C for 30 min, the supernatant decanted, and the separated niosomes were dried in an oven at 40 °C.

Analysis of Atenolol

Concentrations of atenolol were assayed using a modified literature method [15], in which reversed phase high performance liquid chromatographic (HPLC) and fluorescence detection (λ_{EX} = 258 nm, λ_{EM} = 300 nm) were employed. The HPLC system consisted of a Waters Model 515 HPLC pump, a Waters autosampler Model 717 plus and a Waters scanning fluorescence detector Model 474 (Waters Inc., Bedford, MA, USA) governed by a microcomputer running Millennium® version 32 software.

Briefly, the mobile phase consisted of acetonitrile/methanol/0.01 M phosphate buffer with a pH adjusted to 6.0 with NaOH, containing 0.01% dodecyl sulfate sodium salt delivered at a flow rate of 1.2 ml/min at ambient temperature through a Lichrosorb analytical column, 250 x 4.6 mm ID, 10 µm RP18 (Merck), protected by a guard column (Security-guard; Phenomenex, CA, USA). To 500 µl of each sample, 100 µl of metoprolol of 5 µg/ml in methanol/water (1:1) and 200 µl of NaOH (0.25 M) were added. After a brief vortex mixing, 3.5 ml of the extraction solvent (hexane/ n-butanol, 1:1) was added. The tubes were then centrifuged (14000 rpm at 25 °C for 10 minutes) and the organic layer was transferred to another set of

clean tubes to back extracted with 250 µl of 0.1 M hydrochloric acid (vortex mixing for 20 seconds). The tubes were then centrifuged (14000 rpm at 25 °C for 10 minutes), the organic layer was discarded, and the aqueous phase was transferred to a clean tube to be evaporated to dryness at 40 °C, under a N_2 stream. The residue was dissolved with 100 µl of the mobile phase, transferred to the injection vials, and a 20 µl aliquot was injected into the chromatographic system.

Atenolol encapsulation efficiency

To 0.2 g of the dried niosomes, weighed in a glass tube, 10 ml phosphate buffer (pH 7.4) was added. The aqueous suspension was sonicated in a sonicator bath (Transonic T460/H, Elma, Germany) for 5 min. The atenolol-containing niosomes were separated from untrapped drug by centrifugation at 25000 rpm at 20 °C for 30 min. The supernatant was recovered and assayed by HPLC method for atenolol content. The percentage of drug encapsulation (EP (%)) was calculated by the following equation:

$$EP\ (\%) = [(C_t - C_r) / C_t] \times 100\%$$

Where C_t is the concentration of total atenolol and C_r is the concentration of free atenolol.

Vesicle physical characteristics

The shape, surface characteristics and size of the niosomes were observed by scanning electron microscopy. A 0.2 g sample of the dried niosomes was placed in a glass tube and then diluted with 10 ml phosphate buffer (pH 7.4). The niosomes were mounted on an aluminium stub using doubled-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (BOC Edwards, Wilmington, MA, USA) and examined using a scanning electron microscope (JEOL, JSM-5510, Tokyo), equipped with a digital camera, at 20 kV accelerating voltage.

In vitro release study

A 0.2 g sample of dried niosomes prepared by method A or B was spread on a circular glass disk (5.04 cm^2 diameter), then covered by cellophane dialyzing membrane (with molecular weight cut-off 8000, Spectrum Medical Inc., Los Angeles, USA) which was securely mounted on the disk by a rubber band. The disk was placed on the bottom of a glass tube fitting the disk diameter. Fifty ml of phosphate buffer (pH 7.4) was poured on the membrane surface. The whole assembly was immersed in a water bath maintained at 37 ^{o} C. The buffer solution was continuously circulated over the membrane surface in a closed circuit at a rate of 5 ml/min using a Watson-Marlow peristaltic pump. Drug release was monitored using an automated monitoring system which consisted of an IBM computer and PU 8605/60 dissolution software, a Philips Vis/UV/NIR single beam eight cell model PU 8620 spectrophotometer and an Epson Fx 850 printer. For each preparation, drug release was studied in triplicate, with absorbance at 275 nm recorded automatically up to 6 hours, and the percentage of drug released was calculated.

In vitro transport of atenolol across rat everted gut sac

Everted intestinal sacs were prepared from male Sprague-Dawley rats (250-275 g) obtained from the Animal Care Center, King Saud University. They were maintained on commercial feed obtained locally. After overnight fasting, rats were anesthetized under ether anesthesia and the intestine (jejunum) was isolated, rinsed with ice-cold Tyrode solution (sodium chloride 800 mg, potassium chloride 20 mg, magnesium chloride 10 mg, sodium acid phosphate 5 mg, anhydrous calcium chloride 20 mg, sodium bicarbonate 100 mg, anhydrous dextrose 100mg, water to 100 ml) [10] and everted. This segment was tied at one end with a silk suture, filled with oxygenated (95% O_2 and 5% CO_2) Tyrode solution and then tied at the other end. The resultant large sac was divided into smaller sacs (3 cm each) by tying at intervals. Each sac contained about 0.5 ml oxygenated Tyrode solution. The rats were finally sacrificed by overexposure to ether.

The sacs were placed in oxygenated Tyrode solution until the incubation started. Three ml of each of the three samples (each equivalent to 1 mg of atenolol per ml of Tyrode solution) control (atenolol solution), niosomes A suspension (prepared by method A) and niosomes B suspension(prepared by method B) were placed in small and narrow test tubes to ensure full exposure of the whole sac to the drug. The studies were completed in triplicates and kept at 37 °C using a shaking water bath. After 5 min, one sac was placed in each tube to start incubation. The whole solution inside the sacs was taken at 15, 30, 60, 90, and 120 min. At the end of each time point, at which time the corresponding everted sacs were removed from the tube, rinsed with Tyrode solution three times, blotted dry and weighed. Then, the contents of the sacs were collected and the sacs were weighed again to adjust for the content volume. The drug concentration in the sac content was determined by the HPLC method.

Statistical Analysis

Data were expressed as the mean of three experiments ± the standard deviation (SD) and were analyzed using the unpaired Student's *t*-test on a microcomputer statistical package (Statgraphics Plus, Manugistics, Inc., Rockville, MD, USA). The differences were considered significant at $p \leq 0.05$.

Results and Discussion

Results from scanning electron microscopy of niosomes prepared from proniosomes (A) and by conventional rotary evaporator method (B) are presented in figure 1, which shows a slight difference in the appearance of the surfaces. Niosomes prepared using method A appear to be smoother compared to those prepared by method B.

Particle size analysis of niosome preparations shows that the conventionally prepared niosomes (by method B) are larger and slightly more heterogeneous than those derived from proniosomes (by method A). The average size of niosomes form

proniosomes (method A) is approximately 5 µm, while that of the conventional niosomes (method B) is about 12 µm.

On determining the encapsulation efficiency, niosomes from method A entrapped 98.6 ± 0.33 % while niosomes from method B entrapped 93.4 ± 0.52 % of the drug.

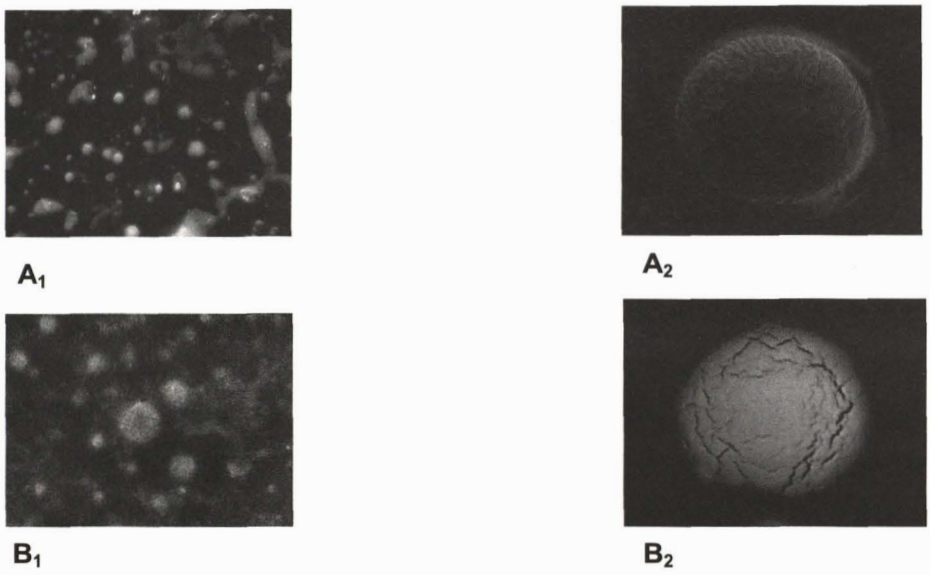

Fig. 1. Scanning Electron Microscopy (SEM) images of different niosomal preparations: A_1 and A_2: niosomes prepared by method **(A)**; experimental conditions: magnifications = X 200 and 2000, respectively, Acc.V 20K, signal SEI, WD 16 mm, ___ 10.00µm. B_1 and B_2: niosomes prepared by method rotary evaporator method **(B)**; experimental conditions: magnifications = X 200 and 800, respectively, Acc.V 20K, signal SEI, WD 16 mm, ___ 2.11µm.

Figure 2 shows the in vitro release profile of atenolol from each type of niosomes compared to the dissolution rate of the pure drug. It is obvious that a considerable retardation of drug release has been exhibited by each type of niosomes. The pure drug dissolved completely within 15 min, while only 8.9% and 9.9% of encapsulated drug was released from niosomes, prepared by method A and B, respectively, at the same time. Parameters associated with the release kinetics from the niosomes are computed and are shown in table 1.

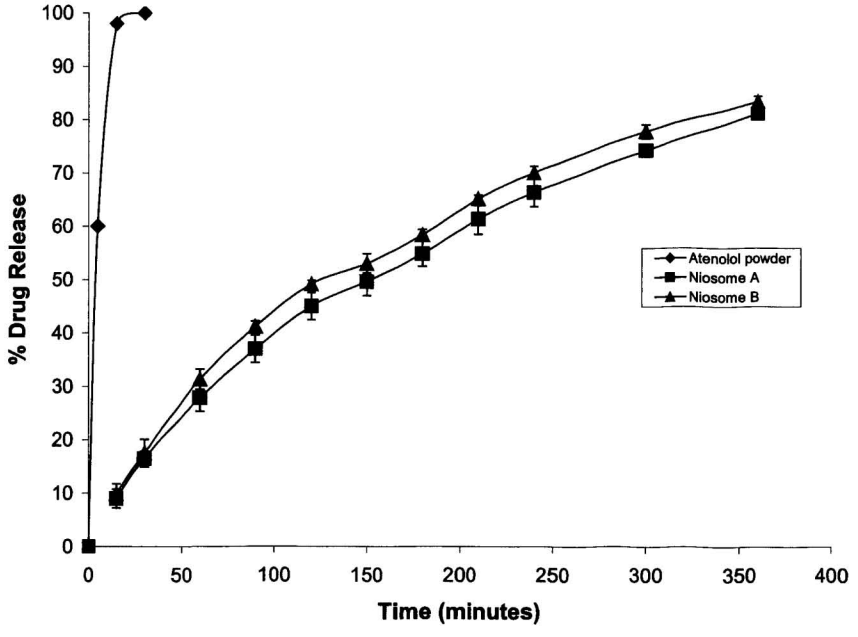

Fig. 2. In vitro release profile of atenolol from both types of niosomes compared to the dissolution rate of the pure drug.

Table 2 illustrates the amount of atenolol permeated through the intestinal wall using the everted sac technique. The results show about a 4-fold increase ($p < 0.05$) in drug permeation from both types of niosomes compared to the pure drug. On the other hand, there is no significant difference between both niosomes as permeation enhancers ($p > 0.05$).

It is obvious from the mathematical treatment of the data of in vitro release presented in table 1 that the release kinetics follow a first order model, based on the values of the correlation coefficient (r). According to Peppas equation [16]:

$$M_t / M_\infty = k \cdot t^n$$

Where M_t / M_∞ is the fractional release of the drug, t is the release time, k is a constant incorporating structural and geometric characteristics of the controlled release device and n is the release exponent, an indicative of the mechanism of drug release. When n = 0.5, Fickian diffusion is observed and the release rate is dependent on $t^{1/2}$, while 0.5 > n < 1.0 indicates anomalous (non- Fickian) transport and the release approaches zero order when n = 1.0.

From the slopes of the plots of the log fraction of drug released against log time and computing n values from the slope of the plots (0.765 for niosomes prepared by method A and 0.758 for niosomes from method B) indicates anomalous (non- Fickian) release behavior [16].

On determining the amount of atenolol permeated through the intestinal wall using the everted sac technique, it is expected that most niosomes accumulate in the mucus before they reach the intestinal wall. The main ingredients of mucus are mucoserous polysaccharides and proteins. Niosomes may be adsorbed or combined with the mucoserous polysaccharides and proteins, resulting in longer contact of the niosomes with the intestinal wall [9] which would promote the expected effect of niosomes as permeation enhancers.

Several mechanisms could explain the ability of niosomes to modulate drug transfer through the intestinal wall. These include (i) adsorption and fusion of niosomes to the intestinal wall, facilitating drug permeation, (ii) vesicles acting as penetration enhancers (due to surfactant and lecithin content) to reduce the barrier properties of the lipoid intestinal membrane, and (iii) the intercellular lipid barrier in the intestinal wall becoming far looser and more permeable following treatment with niosomes, as reported in transdermal studies [17].

The slight difference in effect on drug permeation between the two types of niosomes (non-significant $p > 0.05$) could also be attributed to the difference

in vesicle size. The smaller size of niosomes prepared by method A compared to B makes their mobility, adhesion and fusion to a mucosal membrane and the lipid barrier of intestinal tissue more effective.

In conclusion, the results showed that niosomes could be utilized as drug carrier to enhance atenolol absorption though gastrointestinal tract. Besides providing the controlled delivery of atenolol, the proposed delivery system is more stable, possesses high entrapment efficiency and significantly improves the drug intestinal uptake. The proposed niosomal preparation may be a promising candidate and could be used with a potential application. Further study will be carried out to examine the oral bioavailability of such formulation.

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