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Investigation of betahistine dihydrochloride biocompatibility and nasal permeability *in vitro*

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ABSTRACT

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Introduction

Betahistine dihydrochloride (BET) is a histamine-like agent used for the symptomatic treatment of vestibular disorders associated with Meniere's syndrome. Generally, the drug is prescribed in the form of tablets (8, 16 and 24 mg) or a solution (8 mg/dose) for oral administration three to four doses daily. BET is known for its short plasma half-life (3–4 h) which necessitates frequent administration and may lead to noncompliance, especially in elderly patients (Lacour et al., 2007). Moreover, BET acts like histamine agonist on H_2 receptors localized on secretory cells of the gastric mucosa (Della Pepa et al., 2006). Therefore, oral administration by patients suffering gastritis, ulcer, etc. should be avoided. On the other hand, typical of Meniere's syndrome symptoms such as persistent nausea and vomiting, or neurovegetative disorders such as swallowing difficulty and also patient incapability to move are other limitations for the oral administration of BET. These facts could serve as a strong argument for studying alternative routes for BET application in the treatment of Meniere's syndrome.

Nasal delivery with its undeniable advantages and various areas of application is a reasonable alternative as it provides fast systemic drug absorption and rapid onset (Bitter et al., 2011; Jadhav et al., 2007). To reduce the duration and frequency of attacks related to the disease and to achieve favorable prognosis regarding hearing, the rapid onset is of considerable importance. Not exclude the possibility of absorption of BET in the cerebrospinal fluid and achieving rapid onset through the mechanism of direct "nose-to-brain" delivery via the olfactory neurons (Bommer, 2007).

An adhesive system for transdermal administration of BET was developed (Heda et al., 2010) as an approach to overcome the problems related to oral administration and the potential for detailed studies on its transdermal absorption was uncovered. Transdermal delivery of BET encapsulated in microemulsion formulations was studied as a promising and reliable alternative (Hathout and Nasr, 2013). Literature does not reveal any data about investigations on other alternative routes of administration of BET, including intranasal. It was thus advantageous to evaluate biocompatibility and nasal permeability of BET.

In vitro cell culture models have gained significant importance in biocompatibility and drug transport studies since they allow a rapid analysis of a large number of substances at a reasonable price and also suggest a possibility for the simultaneous study of different variables avoiding the controversial usage of animal models (Dimova et al., 2005; Lin et al., 2007, 2005). RPMI 2650 cell line is the only commercially available nasal human cell line. It was derived from pleural effusion of 52-year old male with nasal septum carcinoma (Moorhead, 1965). RPMI 2650 epithelial model has exhibited features such as high stability throughout culturing, production of mucoid material on the cell surface and ability to form a tight barrier layer with a transepithelial electric resistance (TEER) close to physiological conditions (Kreft et al., 2015). RPMI 2650 has been widely explored for its suitability as a permeation study model (Harikarnpakdee et al., 2006; Kreft et al., 2015; Kurti et al., 2013; Reichl and Becker, 2012; Wengst and Reichl, 2010).

The ability of the living cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide dye (MTT) to formazan product after treatment with xenobiotics is a routine technique

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applied for the assessment of substances' toxicity (Mosmann, 1983). This test is widely used in many studies to demonstrate the toxic effect of drugs on different cell lines. No data was found in the scientific literature on studies of BET epithelial toxicity using *in vitro* nasal models.

The aim of our research was to study *in vitro* biocompatibility and permeability of BET incorporated in bioadhesive chitosan microspheres as a potential drug delivery system for nasal administration.

Materials and methods

Betahistine dihydrochloride, chitosan (from shrimp shells, low viscosity, degree of deacetylation >75%), sorbitan monooleate 80 (Span 80), petroleum ether, fetal bovine serum (FBS), trypsin, EDTA, dimethyl sulfoxide (DMSO) and penicillin were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). RPMI 2650 cell line and Eagle's Minimum Essential Medium (EMEM) were purchased from ATCC (USA). MTT and streptomycin were obtained from AppliChem (Germany). All other reagents and solvents were provided by Alfa Aesar (Germany).

Preparation of BET loaded microspheres

Microsphere formulations were prepared by single emulsion/solvent evaporation method. Four models labeled M1–M4 were developed by varying drug/polymer concentration and ratio. The formulated microspheres were analyzed for size, shape, drug loading, entrapment efficiency, drug/polymer compatibility and mucoadhesion. The obtained results have been extensively described in our previous research paper (data not present). The evaluated formulations M1–M4 varied in size, drug loading and entrapment efficiency as given in Table 1.

Permeation study

Cell cultivation

In vitro cell culture model was applied using RPMI 2650 cell line derived from human nasal septum carcinoma. Cells were seeded onto matrices at density 2×10^6 cells/mL and cultivated in Eagle's minimal essential medium enriched with 10% FBS, 100 IU/mL penicillin and 100 mg/mL streptomycin at 37 °C, 5% CO_2 and high humid conditions. At 80–90% confluence the cells were trypsinized using trypsin – EDTA solution, suspended in EMEM to obtain density 2×10^6 cells/mL and used for the prospective studies.

RPMI 2650 cell culture model

100 μL of the initial RPMI 2650 cell suspension at a density of 2×10^6 cells/mL were cultivated onto Thincert® filter inserts (polyethylene terephthalate membrane, 0.4 μm pore size, 10.34 mm diameter, 33.6 mm^2 surface area, Greiner bio-one, Germany) for 9 days to attain confluence. Cultivation medium was replaced in apical and basolateral chambers of the inserts every two days.

TEER measurement

Measurement of transepithelial electric resistance (TEER) is a routine technique in various *in vitro* and *in vivo* studies. As an indicator for tight junctions' permeability for sodium ions in culture conditions, it is widely applied for rapid and reliable assessment of monolayer integrity (Benson et al., 2013; Lin et al., 2007).

The barrier role of cell monolayer was established by daily measuring of TEER with specially designed equipment. The appliance was completed with a couple of thin (0.8 mm) Ag/AgCl electrodes. It used alternating current in order to overcome the adverse polarizing effects of the constant electric field. In sterile conditions the inserts were allowed to reach room temperature, TEER values were measured and after filtering and analog-to-digital conversion TEER changes were recorded in a two-dimensional array: resistance (Ω) – time (s). The obtained values were expressed relative to the surface area of cell monolayers (Ω cm^2). Drug transport was evaluated after stable, unchanging TEER values were obtained.

Drug transport study

The test was performed after formation of a confluent monolayer confirmed by TEER measurement. Cell cultures were washed with tempered medium prior to each experiment. All transport studies were carried out in HBSS (Hank's balanced saline solution). A certain amount of microspheres, corresponding to 750 μg BET, was added to the apical chamber of the inserts containing 350 μL HBSS. At predetermined time intervals baso-lateral compartment content (1200 μL) was withdrawn and was replaced with fresh transport medium. BET concentration was analyzed spectrophotometrically using an Ultrospec 3300 pro UV/Visible Spectrophotometer (Biochrom Ltd., Cambridge, UK) at a wavelength of 261 nm with calibration correlation coefficient 0.9999. Also, permeability study was performed for BET not incorporated in polymer particulate delivery system as a standard. For the purpose 350 μL BET solution at concentration 850 μg/mL was added to the apical compartment of the inserts. Similarly, drug transport was studied across cell-free filter inserts.

Tight junction integrity study

To evaluate polymer influence on the tight junction integrity 0, 15, 30, 60, 120, 180 and 240 min after treatment in the permeability test changes in TEER were monitored. As a negative control, untreated filter insert with confluent monolayer was used. The study was performed in HBSS at room temperature.

Permeation coefficient calculation

The apparent permeation coefficients of BET through the cell monolayer (P_{app}) were calculated according to the equation:

$$Papp = \frac{dQ}{dt} \frac{1}{CoA}$$

where *dQ/dt* is the cumulative drug content in the basolateral compartment after a certain time interval (μg/s), A is the surface area (cm^2), C_0 is the initial donor concentration of the substance (μg/mL).

Table 1
Composition and physicochemical properties of BET loaded microspheres (*n = 6).

Model	Drug concentration (%)	Polymer concentration (%)	Drug/polymer ratio	Drug loading (% ± SD)*	Entrapment efficiency (% ± SD)*	Mean particle size (μm± SD)*
M1	1	1	1:1	44.78 ± 0.78	69.37 ± 0.91	3.82 ± 0.14
M2	1	1.5	1:1.5	41.55 ± 1.34	93.02 ± 0.98	4.49 ± 0.24
M3	1	2	1:2	33.97 ± 0.89	93.85 ± 2.51	4.52 ± 0.33
M4	2	2	1:1	58.78 ± 1.76	98.27 ± 0.73	7.69 ± 0.33

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