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New absorption promoter for the buccal delivery: Preparation and characterization of lysalbinic acid

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Abstract

Drug delivery across the buccal mucosa is convenient and safe transport method. The efficiency of the buccal system of peptide delivery is, however, not yet satisfactory. To improve the buccal transport new absorption promoters should be developed to be sufficiently active and at the same time causing no side effects like irritation or unpleasant taste. We have found that lysalbinic acid, a product of the alkaline hydrolysis of egg albumin and a mild detergent, meets those requirements. The preparation and some physicochemical properties of lysalbinic acid are described. Hamster cheek pouch was used as a model for the penetration process studies lysalbinic acid was shown to increase significantly an oral mucosa permeability for α-interferon and insulin. So this substance of the natural origin can be applied as an absorption enhancer for the buccal delivery of peptide drugs.

Keywords: Lysalbinic acid; Absorption promoters; Buccal delivery; Buccal mucosa; Detergent

1. Introduction

There are various methods for the medicines administration. The commonly used injection method leads to the rapid increase of drug concentration in blood that causes toxic or autoimmune responses. Another way, oral delivery, has been used only for non-protein drugs. The main problem of oral route is that only compounds stable in the gastrointestinal tract can be used. Over the time, numerous attempts have been made to explore alternative routes for systemic drug administration. Development of non-injection way for proteins introduction is currently the most attractive approach. Rectal, nasal, transdermal, pulmonary and other non-injection ways for medicines introduction are popular methods now. However, all these approaches have some essential limitation, such as low bioavailability, immunoreactivity or fluctuation of drug concentration in the blood.

The buccal method is one of the most attractive ways to deliver drugs into the organism (Kamimori et al., 2002; Bousquet et al., 1992; Rygnestad, 2002; Moller et al., 2000; Sagar and Smyth, 1999; Nielsen and Rassing, 2002). It can be employed for protein drugs introduction as well (Meritet et al., 1999; Hubbard et al., 1989; Sakane et al., 1995; Squier et al., 1978; Venugopalan et al., 2001). The delivery of peptide drugs across the buccal mucosa is more convenient and safe approach than most other delivery methods. It was shown that the buccal administration of proteins like insulin, interferons, interleukins has some advantages and reduces many related side effects. For example, buccal way provides constant, predictable level of drug concentration in blood. Venugopalan et al. have shown that buccally administered insulin provided a significant hypoglycemic response without any detectable fluctuation in blood glucose profile and risk of hypoglycemia (Venugopalan et al., 2001). However, the efficiency of the buccal delivery is not currently able to compete with injection methods.

From this point of view, the role of absorption promoter for protein buccal transport is crucial. Many substances can function as absorption promoter, the most popular being detergents such as bile acids salts, sodium lauryl sulfate, etc. But many detergents have some side effects. Often they cause irritation of buccal mucosa. An additional problem is a taste of buccal composition. The most efficient absorption promoters—bile acids salts—have a strong bitter taste, so regular use of compositions containing bile acids is hardly acceptable. That is why new absorption

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promoters should be found. A good candidate for the effective absorption enhancer seems to be lysalbinic acid.

Lysalbinic acid was first described by German chemist Paal in 1902 (Paal, 1902a). It is a product of alkaline hydrolysis of albumin (egg albumin (Inoue, 1937), serum albumin (Schulz, 1941) or casein (Tyalbji, 1949)). As a protein substance, which does not contain strong cationic and anionic functional groups, lysalbinic acid can be considered a non-ionic detergent. Originally lysalbinic acid was applied as the stabilizer of metal sols for gold, silver and mercury (Tyalbji, 1949; Paal, 1902b,c,d,e; Wolvekamp, 1921, 1922; Makino, 1989). Later it was used as soft detergent in some washing liquids. Some elite cosmetic compositions contain analogues of lysalbinic acid (Bennert, 1921; Jabalee, 1976; Rousso and Wallace, 2000; End et al., 1997; Varco, 1991; Marsh et al., 1980).

Surfactant properties have caused our interest to lysalbinic acid as possible absorption enhancer for the buccal transfer of proteins. The protein nature of a preparation allowed to assume that its irritating action would be minimal, and the composition taste to be neutral. The aim of the present study was to evaluate chemical and transport enhancer properties of the lysalbinic acid.

2. Materials and methods

2.1. Preparation of lysalbinic acid

Lysalbinic acid was prepared by modification of the original method (Paal, 1902a). One hundred grams of egg albumin powder (Biolar, Latvia) was added slowly with stirring and water bath heating to the solution of 15 g NaOH in 500 ml of water. The resulting viscous solution was heated at boiling water bath for 1 h, cooled to the ambient temperature and filtered through paper filter. Diluted sulfuric acid (40–50% aqueous solution) was added slowly to the filtrate until pH reached 5. The precipitate was filtered off through paper filter and washed by water (2×20 ml). The filtrate was dialysed (Spectra/Por 6 membrane, Cole-Parmer) against water for 3 days. To the dialysate containing lysalbinic acid sulfate, aqueous solution of barium hydroxide was added carefully by small portions until the filtered probe contained neither SO_4^{2-} nor Ba^{2+} ions. $BaSO_4$ was filtered off, and the foamy solution of free lysalbinic acid was lyophilised at $-20\,^{\circ}C$ at Jouan Heto DW8-85 freeze drier. The obtained powder was washed with ethanol (2×10 ml) and dried in vacuum. Typical yield of lysalbinic acid is 20–25 g per 100 g of albumin.

2.2. Determination of molecular weight of lysalbinic acid

It was carried out by gel filtration on Sephadex G-75 (Reanal, Hungary) (2.5 cm × 53 cm, $V_o = 70$ ml). Column was calibrated using following molecular weight markers: glucose (180 Da), actinomycin (1200 Da), myoglobin (17.8 kDa), bovine serum albumin (68 kDa). Electrophoresis in 16% polyacrylamide gel in the presence of molecular markers (Fermentas, Lithuania) was, also, used for molecular weight determination.

2.3. Surfactant characteristics of lysalbinic acid

They were obtained by standard physicochemical methods (Dominguez et al., 1997). Concentration dependence of the surface tension (σ) and electric conductivity (x) of lysalbinic acid solutions were determined at ambient temperature. Surface tension and conductivity data were plotted versus the concentration of the surfactant, and critical micelle concentration (CMC) of the substance was found from the plots (Fig. 2). Reported data represent the results of three measurements. The standard deviation in the experiments was below 5%.

2.4. The effect of lysalbinic acid on protein buccal penetration

It was investigated on hamster cheek pouch model. Cheek pouches were removed from ether–anaesthetised hamsters and washed with a Ringer solution (0.154 M NaCl, 0.154 M KCl, 0.11 M $CaCl_2$, 0.154 M KH_2PO_4, 0.154 M $MgSO_4$, 0.154 M $NaHCO_3$) (Dawson et al., 1986). The cheek pouch was turned inside out, filled with 1 ml of Ringer solution and dipped into 10 ml of Ringer solution containing a substance to be studied (peroxidase, α-interferon or insulin). Incubation was carried out at 37 °C with constant slow stirring. The amount of protein permeating from the mucosal side of the cheek pouch to serosal side was measured over time. Probes were taken from a cheek pouch in certain time intervals (1–15 min) and analysed by colorimetric, electrophoretic or fluorescence methods.

2.5. Probe analysis by colorimetric method

The concentration of peroxidase (Sigma) was determined using the reaction with 3,3′,5,5′-tetramethylbenzidine (TMB, Amersham) following the manufacturer's protocol. Starting peroxidase concentration in the Ringer solution was 4.5×10^{-5} mM, that of lysalbinic acid was in the range 1–10%. Peroxidase solution without lysalbinic acid was used as a control. A 50 μl probe was mixed with an equal volume of TMB solution and in 1 min reaction was quenched by 50 μl of 0.05 M sulfuric acid. Concentration was determined at Multiscan MCC/340P instrument at 450 nm.

2.6. Probe analysis by gel electrophoresis

Quantitative analysis of α-interferon was performed by disc electrophoresis in 16% polyacrylamide gel according to the published protocol (Westermeier, 1997). Initial interferon concentration in Ringer solution was 3.1×10^{-4} mM, lysalbinic acid content was 0.1–10%. Interferon solution without lysalbinic acid was used as a control. A 50 μl probes were taken for the analysis. Gels were stained with Coomassie R250 and scanned with HP Deskjet Professional scanner. Protein amounts were determined using TotalLab 2.01 software.