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Enhancement of nasal absorption of large molecular weight compounds by combination of mucolytic agent and nonionic surfactant

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Abstract

For improving the nasal absorption of poorly absorbable hydrophilic compounds, the suitability of a combination of a mucolytic agent, N-acetyl-L-cysteine (NAC), and a nonionic surfactant, polyoxyethylene (C25) lauryl ether (laureth-25), was examined. Rat studies with fluorescent isothiocyanate-labeled dextran (molecular weight ca. 4.4 kDa, FD-4) as a model hydrophilic compound revealed dramatic enhancement of nasal absorption when NAC and laureth-25 were simultaneously applied. The nasal bioavailability of FD-4 in saline solution was 8.2±0.6% but increased to 40.0±5.5% when 5% NAC and 5% laureth-25 were added. This synergistic enhancement could result from the mucolytic activity of NAC in reducing mucous viscosity by which the accessibilities of FD-4 and laureth-25 to the epithelial membrane were increased. Further rat studies proved that this formulation increased nasal absorption of salmon calcitonin. Absolute bioavailability from saline solution containing 5% NAC and 1% laureth-25 was 26.8±2.2%, 3.5 times that of the commercial calcitonin nasal spray Miacalcin (7.7±2.1%). The potential of the new formulation to cause tissue damage in terms of hemolytic activity and liberation of phospholipid from the nasal membranes was nil or slight. The combination of NAC and laureth-25 appears suitable for use in development of nasal products for poorly absorbable drugs, especially peptide and protein drugs.

Keywords: Nasal absorption; Absorption enhancement; Mucolytic agent; Nonionic surfactant; Calcitonin

1. Introduction

Intranasal drug delivery has attracted a lot of interest as an alternative route for making drugs that are normally restricted to intravenous administration, such as peptide and protein drugs, systemically available [1,2]. This is because of the unique anatomical structure and physiological functions of the nasal mucosa. The large mucosal surface, highly vascularized and porous endothelial membrane, and avoidance of first-pass metabolism contribute to the attainment of adequate bioavailability for medication and quick onset of action, comparable to that of injections [3]. Moreover, the ready accessibility of intranasal administration makes it possible for patients on long-term therapy to self-medicate [4]. With these advantages, the nasal administration of drugs for systemic medication has been widely investigated in recent years, and many projects are now under clinical development.

On the other hand, there are some disadvantages with intranasal drug delivery, including the low permeability of the mucosa to large molecules, enzymatic degradation, the very small absorption capability of the nasal cavity, limiting application to drugs that can be supplied in small doses, and the rapid mucociliary clearance, which shortens the period of time available for efficient absorption and causes low bioavailability [3–5]. In attempting to improve the nasal absorption of poorly absorbable drugs, especially peptides and proteins, the use of various absorption enhancers has been tried. Nonionic surfactants [6–8], bile salts [9], chelators [10], fatty acids [11], fusidic acid derivatives [12,13], and phospholipids [14] are considered to be effective absorption enhancers. Their mechanisms of action are thought to be based on enhancement of the membrane fluidity, inhibition of proteolytic enzymes at the absorption site, or transient loosening of the tight junctions between certain epithelial tissues [15,16]. Most absorption enhancers, however, are considered to alter membrane integrity and often permanently damage the membrane [17]. Nevertheless, the potential therapeutic benefits are

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enormous, so there is still considerable interest in absorption enhancers that may be effective without causing topical or systemic toxicity following nasal administration.

One possible strategy for enhancing nasal absorption while minimizing damage to the mucosal membrane is the use of a mucolytic agent together with a permeation enhancer. N-acetyl-L-cysteine (NAC) is a potent mucolytic agent that is used clinically in bronchopulmonary diseases to reduce both the viscosity and tenacity of mucus and to facilitate its removal [18]. The long history of clinical use of this compound suggests that it is likely to have low toxicity and no local irritation. Also, NAC has been reported to have a mild absorption-enhancing effect at a high concentration (20%) [19], which may result from the increased accessibility of the drug to the epithelial membrane. When a surfactant is administered with a mucolytic agent, the mucolytic agent can be expected to reduce the viscosity of mucus, enabling the co-administered surfactant to move efficiently onto the epithelial membrane and increase membrane fluidity and the permeability of nasal mucosa. Because the accessibilities of both the target drug and the enhancer molecules to the epithelial membrane increase at the same time, the enhancing effect can be amplified. Therefore, use of an appropriate combination of mucolytic agent and penetration enhancer could result in a large synergistic enhancement of nasal absorption.

The ultimate goal of our study program is to establish a new formulation strategy for improving the nasal absorption of poorly absorbable hydrophilic compounds by combining a mucolytic agent and a nonionic surfactant. In our present study, we evaluated the synergistic enhancement of nasal absorption in rats by using NAC as a mucolytic agent and polyoxyethylene (C25) lauryl ether (laureth-25) as a nonionic surfactant. Furthermore, we used biochemical assessments to evaluate the capacity of this formulation system to cause tissue damage. To eliminate the influence of enzymatic degradation in the nasal cavity, we initially used fluorescent isothiocyanate-labeled dextran (molecular weight ca. 4.4 kDa, FD-4) as the model hydrophilic compound. We then examined the feasibility of the proposed formulation system by using salmon calcitonin as an example of a peptide drug and compared it with the commercial calcitonin nasal spray Miacalcin.

2. Materials and methods

2.1. Materials

NAC, FD-4, and glycocholate sodium were purchased from Sigma-Aldrich (Tokyo, Japan). Laureth-25 was a gift of Nikko Chemicals (Tokyo, Japan). Salmon calcitonin was obtained from Bachem Bioscience (Philadelphia, PA, USA). The salmon calcitonin nasal spray Miacalcin was purchased from Novartis Pharmaceuticals (East Hanover, NJ, USA). Salmon calcitonin enzyme immunoassay kits were obtained from Peninsula Laboratories (Belmont, CA, USA). Phospholipid assay kits were purchased from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of reagent grade and were obtained commercially.

2.2. Preparation of nasal formulation

To investigate the synergistic effect of a combination of mucolytic agent and nonionic surfactant on the nasal absorption of FD-4 or salmon calcitonin, four different liquid formulations were given intranasally to rats: formulation-N, containing NAC as a mucolytic agent; formulation-L, containing laureth-25 as a nonionic surfactant, formulation-LN, containing both NAC and laureth-25, and formulation-C, containing no enhancer. Nasal formulations were prepared by dissolving 25 mg of FD-4 or 1.0 mg of salmon calcitonin in 50 μl of saline with or without NAC and/or laureth-25. To investigate the synergistic enhancement of FD-4 absorption in more detail and to obtain the optimum blending ratio of mucolytic agent and penetration enhancer, nasal administration studies were performed with varying doses of NAC or laureth-25 (0%, 1%, 2%, or 5%).

2.3. Animal experiments

The rat in vivo experiment was performed according to Hirai's method [20], with small modifications. Male Wistar rats (Nippon SLC, Hamamatsu, Japan) weighing 200 to 250 g were fasted for 20 h before the experiment and anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg). The rats were then tracheotomized and the outlets of the nasal cavity were sealed by another blind-ended cannula placed in the esophagus to prevent drainage of the drug into the esophagus or trachea and thus avoid overestimation of drug absorption. Five microliters of nasal formulation (corresponding to 2.5 mg FD-40 or 100 μg salmon calcitonin) was administered into the nasal cavity with a micropipette. For comparative study, 10 μl of Miacalcin (corresponding to 10 μg salmon calcitonin) was administered. Another group of male Wistar rats were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg). A dose of 50 mg/kg FD-4 or 100 μg of salmon calcitonin was given into the jugular vein in order to estimate absolute bioavailabilities. Blood samples (100 μl) were taken from the jugular vein with heparinized syringes at predetermined time intervals and centrifuged at 12,000 rpm for 3 min to obtain plasma. Plasma samples were stored frozen at −20 °C until assayed.

2.4. Detection of FD-4 and salmon calcitonin in rat serum

Plasma samples (20 μl) were diluted with 680 μl of 0.1 N sodium hydrogen carbonate. FD-4 concentrations in the plasma were determined with a fluorometer (excitation wavelength 495 nm; emission wavelength 515 nm). Plasma concentrations of salmon calcitonin were assayed with salmon calcitonin enzyme immunoassay kits.

2.5. Data analysis

The areas under the individual plasma concentration–time curves for 120 min (AUC_{0-120}) for both FD-4 and salmon calcitonin were calculated using the linear trapezoidal rule.