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Research paper

Arachidonic acid with taurine enhances pulmonary absorption of macromolecules without any serious histopathological damages

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ABSTRACT

Therapeutic peptides and protein are being used in several indications; however, their poor permeability still remains to be solved. This study focused on the pulmonary route of macromolecules. First, the effects of arachidonic acid (AA) as an absorption enhancer on drug serum concentration, after intratracheal administration, were investigated in rats. Second, the safety of AA was assessed in rats in an acute toxicity study for 7 days. AA enhanced the exposure of both interferon-α (IFN-α) and fluorescein isothiocyanate 4000 (FD-4). In addition, the histopathological analysis indicated that AA caused alveolitis and bronchitis in rats. In combination with Taurine (Tau), these lung injuries were prevented through the histopathological analysis. The combined use of Tau with AA did not show any changes in the pharmacokinetics of FD-4. From these results, we suggest the combined use of AA with Tau as a novel formulation on the pulmonary route of macromolecule drugs. This formulation could improve the bioavailability of macromolecule drugs without any serious local damage to the lungs.

1. Introduction

Therapeutic peptides and protein are being used in several indications such as diabetes, osteoporosis, and hypercalcemia [1,2]. These drugs have large molecular weights and hydrophilic characteristics, which lead to poor membrane permeability. Therefore, intravenous administration is a primary administration route for these drugs. In order to make the administration and the treatment for outpatients easier, the development of other administration routes is needed in clinical settings.

The lung is recognized as a route for systemic delivery of therapeutic proteins and peptides, since alveolar epithelium is an adjacent area of the air-blood pathway [3,4]. The large surface area of alveolar epithelium supports the absorption of the drug effectively. However, alveolar epithelium has various absorption barriers, such as alveolar lining fluid layers, macrophages, and alveolar epithelial cells [5–7]. These absorption barriers result in low bioavailability of drugs after the pulmonary route. Therefore, the poor permeability of therapeutic peptides and protein drugs in alveolar epithelium still remains to be solved.

The pulmonary surfactant prevents airspace collapse by reducing surface contractile forces at low volumes in the air/liquid interface of alveoli [8]. It is also known to prevent small airway collapses [9–11]. Therefore, the pulmonary surfactant was expected to have potential as an absorption enhancer. The enhancer effects of the pulmonary surfactant were investigated in order to improve the low bioavailability of therapeutic peptides and proteins through the pulmonary route. The component of pulmonary surfactants is a complex mixture of lipids and proteins [12]. Specifically, lipids mainly consisted of phospholipids. Arachidonic acid (AA) is a polyunsaturated fatty acid present in the phospholipids. Since AA is the starting material of arachidonate cascade, it is a ubiquitous component in the body. Therefore, this study examined the effects of AA, as one of the main components of phospholipids in the pulmonary surfactant, on the absorption enhancement of therapeutic peptides and proteins.

Considering the clinical application, the safety of the absorption enhancer is required, in addition to the improvement of low

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bioavailability of therapeutic peptides and proteins through the pulmonary route. A potential issue is that the absorption enhancers might have local toxicity in alveolar epithelial cell layers [13–16]. On the contrary, pulmonary surfactants were reported to have several properties related to the maintenance of a normal pulmonary condition: protecting inflammation [17]; facilitating mucus clearance [18]; preventing pulmonary infections [17]; scavenging extracellularly generated oxyradicals [19]; and enhancing intracellular antioxidant enzyme content [19]. AA was expected to have low toxicity, since it was one of the main components of phospholipids in the body; however, the toxicity effects after continuous administration remain to be clarified. Therefore, in this study, the safety of AA was assessed in an acute toxicity study in rats for 7 days. In addition, Taurine (Tau) is known to show cytoprotection action in the large intestine from histopathological findings in rats [20]. Then, we examined the protection effect of Tau on the local toxicity in alveolar epithelial cell layers caused by absorption enhancers.

In the present study, interferon-α (IFN-α) and fluorescein isothiocyanate 4000 (FD-4) were chosen as model macromolecules with different molecular weights: IFN-α, 13,000–21,000; FD-4, 3000–5000. FD-4 is known to show low absorption without any biological effects. This allows us to compare results between pharmacokinetics study and acute toxicity study. On the other hand, IFN-α is one of endogenic factors. IFN-α was used as a biological medicine with large molecule available on the market in order to assess only the effect of AA on the absorption enhancement, since it can't deny the possibility that IFN- α has biological effects, including cytoprotection effect. This study examined the effects of AA on the absorption enhancement of these drugs in rats after intratracheal administration. Histopathological evaluation was conducted as the protective effect of Tau on the pulmonary toxicity of AA was observed.

2. Materials and methods

2.1. Materials

OIF®, which is the clinical lyophilized formulation containing 10 million IU native human interferon-α (IFN-α), was obtained from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Fluorescein isothiocyanate 4000 (FD-4; average molecular weight from 3000 to 5000, arachidonic acid (AA) and taurine (Tau) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium laurate (C12) was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). All other reagents were of analytical grade commercial products.

2.2. Animals

The male Sprague-Dawley (SD) rats (Japan SLC, Hamamatsu, Japan), weighting 250–280 g, were maintained at 23 °C and 60% humidity. The rats were allowed free access to standard laboratory chow (Oriental Yeast Co. Ltd., Tokyo) and water prior to the experiments. The rats were randomly assigned for pharmacokinetics assays and toxicity assays. Animal experiments in this study were performed after approval by our local ethics committee at Otsuka Pharmaceutical Co., Ltd. and in accordance with "*Principles of Laboratory Animal Care (NIH publication # 85–23)*".

2.3. Pharmacokinetics study

The drugs used in this study were dissolved in distilled water to the final concentration of 25 million IU/mL for IFN-α and 100 mg/mL for FD-4. For each drug, the solution was intratracheally administered to the rats at a dose of 5 million IU/0.2 mL/kg for IFN-α and 40 mg/0.4 mL/kg for FD-4, according to the method of Ho et al. [21] under isoflurane anesthesia. The compositions of solution administrated intratracheally are exhibited in Table 1. In order to calculate bio availability (F) of drugs, pharmacokinetics study after intravenous administration was also conducted in this study. For each drug, the solution was intravenously administered to the rats at a dose of 0.5 million IU/0.2 mL/kg for IFN-α and 40 mg/0.4 mL/kg for FD-4. Blood samples after intratracheal administration were collected from the jugular vein at the following time points: 0.25, 0.5, 1, 2, 4, 6 and 8 h for IFN-α; 0.083, 0.25, 0.5, 1, 2 and 4 h for FD-4 under isoflurane anesthesia. Blood samples after intravenous administration were collected from the jugular vein at the following time points: 0.083, 0.25, 0.5, 1, 1.5 and 2 h for IFN- α; 0.033, 0.083, 0.25, 0.5, 1, 2 and 4 h for FD-4 under isoflurane anesthesia. The collected blood samples were centrifuged at 14,000 rpm for 10 min at 4 °C to obtain serum fraction (100 μL). All obtained serum samples were stored at −30 °C until analysis.

2.4. Analytical method for the measurement of drug in serum

2.4.1. IFN-α

IFN-α concentration in serum was determined using the sandwich enzyme-linked immunosorbent assay (ELISA) kit, according to the method recommended by the manufacturer (Japan Immunoresearch Laboratories. Ltd., Takasaki, Japan). Briefly, calibration samples (1.56–100 IU/mL) and 50 μL of rat serum samples, which were diluted to 10 and/or 100 times by a buffer in the assay kit, were transferred to each well in a microtiter plate, which was coated with an anti-IFN-α monoclonal antibody (mAb). Then, the each samples was mixed well in each well. After 2 h of the plate incubation, the wells were washed five times with the wash solution in the assay kit to remove unbound IFN-α. Peroxidase (POD)-linked anti-IFN-α mAb solution (100 μL) was added to each well and the plate was incubated for a further 2 h. The wells were washed five times with the wash solution to remove POD-linked anti-IFN-α mAb. 100 μL of o-phenylenediamine (OPD) containing 0.015% (v/v) hydrogen peroxide was added to each well for col-

Table 1
Compositions of solution administrated intratracheally to the rats.

Ingredient	Control	AA (1, 5, 25 mM)	C12 (25 mM)	Tau (50 mM)	AA (25 mM) + Tau (50 mM)
IFN-α (IU) or FD-4 (mg)	25 million IU/mL or 100 mg/mL	25 million IU/mL or 100 mg/mL	25 million IU/mL or 100 mg/mL	25 million IU/mL or 100 mg/mL	25 million IU/mL or 100 mg/mL
Water (mL)	1.0	1.0	1.0	1.0	1.0
AA (mg)	0	0.3, 1.5, 7.6 mg (1, 5, 25 mM)	0	0	7.6 mg (25 mM)
C12 (mg)	0	0	5.6 mg (25 mM)	0	0
Tau (mg)	0	0	0	6.3 mg (50 mM)	6.3 mg (50 mM)

AA, C12 and Tau mean arachidonic acid, sodium laurate and taurine, respectively.