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COMMUNICATIONS

EFFECTS OF FORMULATION VARIABLES ON THE PULMONARY DELIVERY OF INSULIN

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ABSTRACT

The effects of some formulation variables on the pulmonary absorption of insulin in presence of a bile salt, sodium glycocholate (NaGC), have been described in this report. Relationship between intratracheally administered insulin dose and pharmacodynamic response has been established. The data obtained from this study revealed that an increase in the viscosity of an aqueous formulation administered to pulmonary sacs can facilitate insulin absorption probably due to reduced mucocilliary clearance. A hypertonic formulation produced similar overall hypoglycemic effect to the isotonic solution. On the other hand, hypotonicity elicited significantly improved hypoglycemic response, probably due to membrane damage. The cumulative hypoglycemic effect of intratracheally administered insulin has been linearly correlated with the logarithmic doses.

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INTRODUCTION

In a previous investigation reported from this laboratory (1), insulin at a dose level of 1U/kg with different concentrations of NaGC has been delivered to the pulmonary sacs of the rat . A significant hypoglycemic effect has been achieved and the absolute bioavalability after intratracheal instillation of 1U/kg insulin with 20 mM NaGC reached approximately 78%, indicating that the lungs may be a suitable alternative site for noninvasive protein drug delivery. The possible mechanisms of bile salt-mediated pulmonary insulin absorption have been suggested to be the dissociation of insulin oligomers, dilation of tight junction, direct membrane bilayer effect, etc.

The rate and extent of insulin absorption from the pulmonary route could be influenced by formulation physicochemical properties, such as pH, molecular size, surface activity, and solubility. Some of these very basic factors i.e., viscosity and osmolarity have not yet been addressed with respect to pulmonary protein absorption. It is, therefore, the purpose of this study to characterize the viscosity effect relative to insulin pulmonary absorption and the osmolarity effect relative to toxicity. In addition, the insulin pharmacodynamic dose-response relationship has been also established following intratracheal administration.

MATERIALS AND METHODS

Materials

Porcine zinc insulin (26.3 IU/mg) was kindly donated by Eli Lilly and Company (Indianapolis, IN). Sterile saline solution (Abbott Laboratories, North Chicago, IL) was used to dissolve insulin and to replace the blood volume taken during sampling. A sodium heparin injection (1000 U.S.P. U/ml, Lyphomed®, Resemont, IL) was utilized after proper dilution. Sodium glycocholate (NaGC) was purchased from Sigma Chemical Co. (St. Louis, MO). Hydroxypropyl methylcellulose 4000 cps (HPMC) was obtained from the Dow Chemical Company (Midland, Michigan). Sodium Chloride was purchased from J. T. Baker Inc. (Phillipburg, NJ). Deionized double-distilled water was used throughout the study.

Methods

Preparation of Insulin Solution

Crystalline zinc insulin powder was dissolved with 2-3 drops of 0.1 N HCl solution to which sterile saline solution was added. The solution pH was subsequently adjusted to the physiological value of 7.4 by the addition of 0.1 N NaOH. In order to study the viscosity effect, different concentrations of HPMC saline solutions were used instead of saline alone. In case of osmolarity study, NaCl was used to generate different osmolarity values.

Pulmonary Administration of Insulin and Measurement of Blood Glucose

Male Sprague-Dawley rats, weighing 170-230 g, were fasted 18-24 hours prior to an experiment. Ninety mg/kg ketamine hydrochloride and 10 mg/kg xylazine were administered to maintain anesthesia of the animals. The body temperature was kept close to 37^0C by laying the animals on a platform above a water bath and a light bulb was also placed above the platform.

After the animal was secured on the board, jugular vein and tracheal cannulations were performed. The detailed procedures have been described in our previous report (1). For the administration of the drug into the lungs, approximately 0.1 ml of a solution was instilled into the lungs through a plastic tubing (PE-20). Blood samples were withdrawn from the jugular vein at predetermined time intervals. Blood glucose levels were determined by the Chemstrip bG® and AccuChek IIm® Blood Glucose Monitor (Boehringer Mannheim Corporation, Indianapolis, IN).

Data Analysis

The percent blood glucose remaining was plotted as a function of time. Then the areas above the % blood glucose remaining vs. time curve (AAC) were calculated by the linear trapezoidal method (2).

Measurement of Viscosity

Hydroxypropyl methylcellulose was dissolved in saline solution. Zinc insulin powder was dissolved by adding 2-3 drops of 0.1 N HCl, then a HPMC solution was subsequently added, and the pH was adjusted to 7.4 with 0.1 N NaOH solution. The final volume was adjusted with HPMC solution. The viscosities of 0.35%, 0.6%, and 0.8% HPMC solutions was determined by Brookfield Digital Viscometer (Brookfield Engineering Labs, Inc. Stoughton,

MA) at room temperature (23.5^{0}C). All percentages have been expressed on a weight/volume basis. The spindle speed was set at 30.

Measurement of Osmolarity

Osmolarities of different solutions were measured by an OSMETTE S Automatic Osmometer (Precision Systems, INC., Natick, MA).

RESULTS AND DISCUSSION

Figure 1 schematically illustrates the pharmacodynamic response following intratracheal instillation of insulin at different dose levels in the presence of 10 mM NaGC. The selection of 10 mM NaGC for further studies was based on our previous report (1) that this trihydroxy bile salt optimally improves pulmonary insulin uptake at this concentration. At the insulin dose range of 0.2-2.0 U/kg, significant hypoglycemic effect was observed (Fig. 1) with maximum glucose depression occurring at 120-180 minutes post insulin administration. The degree of the pharmacodynamic response, however, tends to be dose-dependent, such that the maximum glucose depression can be correlated directly with insulin dose. The cumulative pharmacodynamic effect, expressed as the area above the % blood glucose remaining versus time (AAC) from 0 to 240 minutes, has been calculated and again plotted against insulin dose as shown in Fig. 2. Fig. 2 depicts the semilogarithmic plot describing the pharmacodynamic-dose relationship following intratracheal administration of insulin in the presence of 10 mM NaGC. A linear log dose-cumulative area above the curve has been obtained within the studied dose range. For most drugs the pharmacological effect (E) is found to be proportional to the logarithm of the dose. The log-dose response curve is usually linear within a dose range of 20-80% of the maximum pharmacological effect (3). Mathematically, in this case the relationship can be expressed by the equation E = 100521 logDose + 14075. Doses higher than 2U/kg could not be administered due to the death resulting from hypoglycemia.

Previous reports have indicated that inhalation of nonisotonic saline induces a significant reduction in forced expiratory volume in one second (FEV_1) in asthmatic patients, while no response is observed in normal subjects (4). Mann et al. have also reported that hypotonicity is the main mechanism for bronchoconstriction induced by nebulised ipratropium bromide (5). Thereby they suggested that the osmolarity of a solution could be an important determinant of

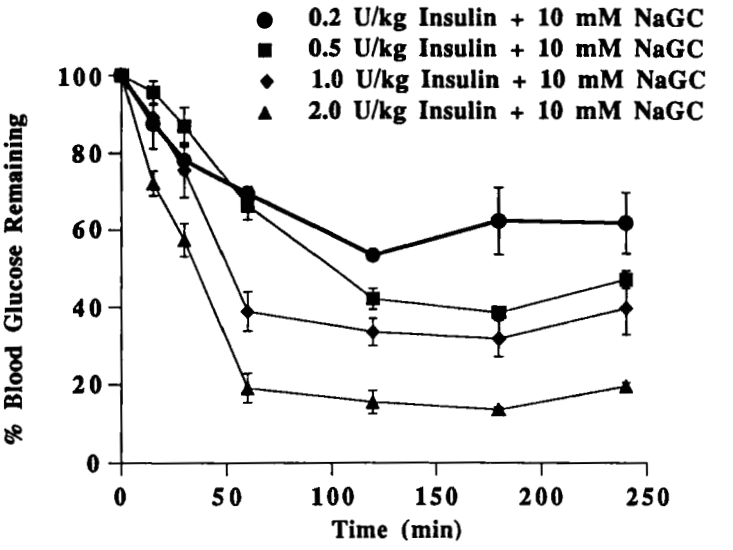

FIGURE 1
Pharmacodynamic Response Following Pulmonary Delivery of Insulin at Different Doses in the Presence of 10 mM NaGC

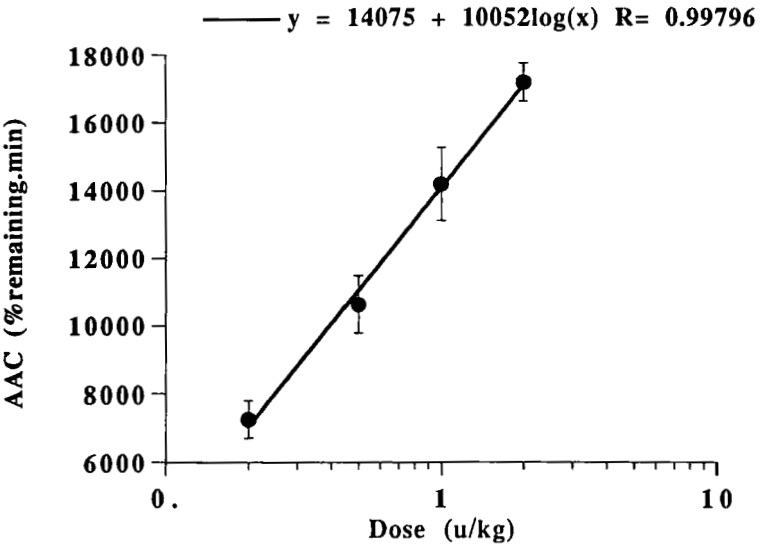

FIGURE 2
Dose Response Effect in Pulmonary Delivery of Insulin

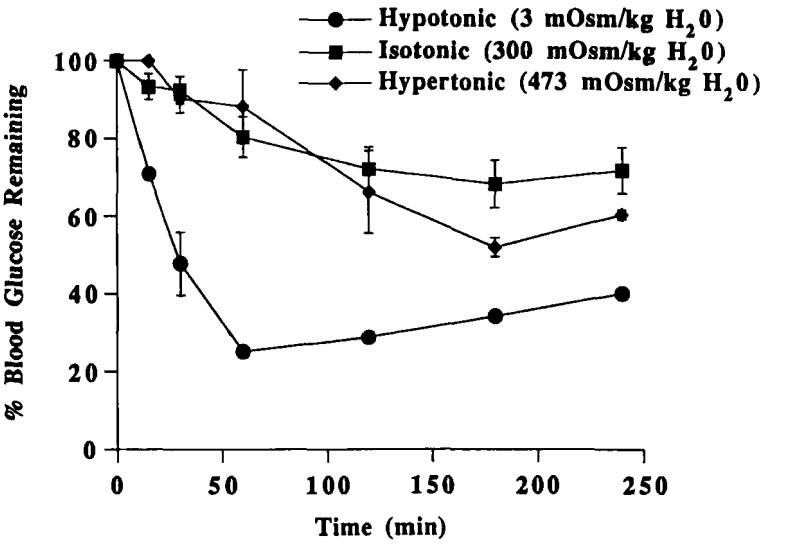

FIGURE 3
Hypoglycemic Effect Following Pulmonary Delivery of Insulin at Different Osmolarities

the airway drug response. In our experiment, the osmolarity effect on pulmonary delivery of insulin was studied at a dose level of 1 U/kg, and the results are presented in Figure 3. Hypotonic insulin solution produced a significant hypoglycemic effect. Such a high magnitude of response could probably be attributed to the high membrane damaging effect by low osmotic pressure solution on the pulmonary epithelial membrane. On the other hand, hypertonic insulin solution showed a slightly reduced hypoglycemic effect compared to that of the control, although not statistically different from one another ($P > 0.05$). These results are also consistent with the observations from the nasal delivery of nonisotonic aqueous solutions (6). Figure 4 depicts the cumulative hypoglycemic effect in terms of blood glucose response (AAC) following intratracheal administration of the three formulations, once again suggesting that isotonic or slightly hypertonic solutions are compatible with lung tissue.

Figure 5 demonstrates the viscosity effect on pulmonarily delivered insulin (0.5 U/kg) with 10 mM NaGC. An enhancement in the viscosity of the solutions lead to significantly improved hypoglycemic effect. This effect could be due to the fact that the retrograde mucus transport was slowed down by the viscous

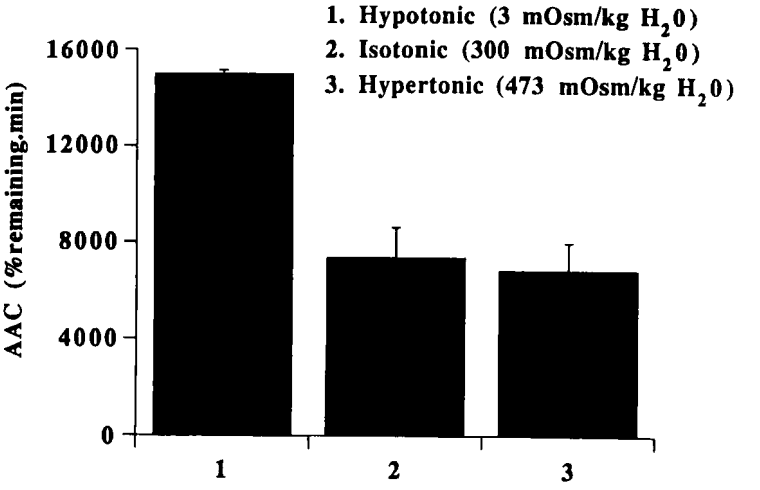

FIGURE 4
Osmolarity Effect in Pulmonary Delivery of Insulin

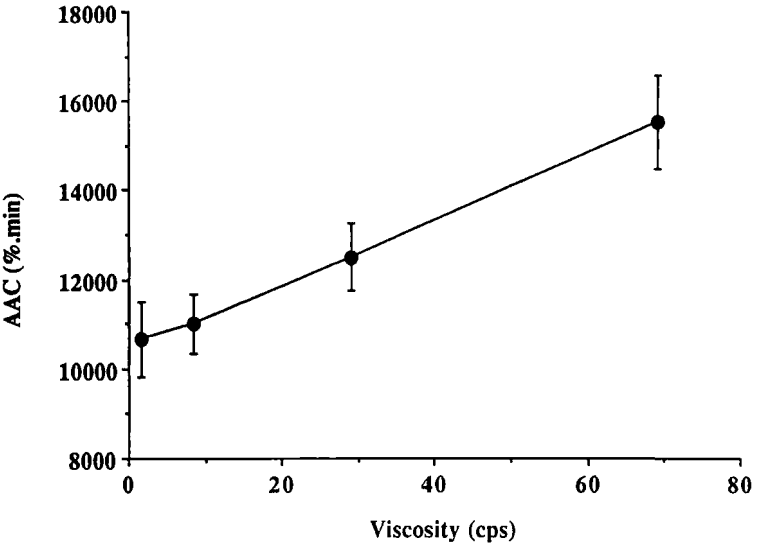

FIGURE 5
Viscosity Effect on Pulmonary Delivery of Insulin

solutions, thereby reducing mucociliary clearance of insulin. An increased viscosity of insulin solution may also depress the cilia movement, thus prolonging the residence time of the insulin solution. Therefore, a combination of two factors i. e., lower retrograde mucous movement and lower ciliary clearance may have increased the residence time of the peptide in the alveolar sac and as a result may have enhanced the drug transport into the systemic circulation.

In conclusion, the in vivo pulmonary absorption results obtained in this study revealed that hypoglycemic response of intratracheally administered insulin is dose dependent. A hypotonic solution appears to increase hypoglycemic response of insulin significantly possibly due to epithelial cell damage whereas hypertonic and isotonic solutions seem to have very little effect. Increases in viscosity of the formulation may decrease mucociliary clearance and prolong the residence time of insulin solution in the pulmonary sacs thereby facilitating insulin absorption.

ACKNOWLEDGMENTS

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