Inhalation delivery of proteins from ethanol suspensions

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To circumvent inherent problems associated with pulmonary administration of aqueous-solution and dry-powder protein drugs, inhalation delivery of proteins from their suspensions in absolute ethanol was explored both *in vitro* and *in vivo*. Protein suspensions in ethanol of up to 9% (wt/vol) were readily aerosolized with a commercial compressor nebulizer. Experiments with enzymic proteins revealed that nebulization caused no detectable loss of catalytic activity; furthermore, enzyme suspensions in anhydrous ethanol retained their full catalytic activity for at least 3 weeks at room temperature. With the use of Zn^{2+}-insulin, conditions were elaborated that produced submicron protein particles in ethanol suspensions. The latter (insulin/EtOH) afforded respirable-size aerosol particles after nebulization. A 40-min exposure of laboratory rats to 10 mg/ml insulin/EtOH aerosols resulted in a 2-fold drop in the blood glucose level and a marked rise in the serum insulin level. The bioavailability based on estimated deposited lung dose of insulin delivered by inhalation of ethanol suspension aerosols was 33% (relative to an equivalent s.c. injection), i.e., comparable to those observed in rats after inhalation administration of dry powder and aqueous solutions of insulin. Inhalation of ethanol in a relevant amount/time frame resulted in no detectable acute toxic effects on rat lungs or airways, as reflected by the absence of statistically significant inflammatory or allergic responses, damage to the alveolar/capillary barrier, and lysed and/or damaged cells.

Several dozen protein therapeutics have been approved for medical use or are in advanced clinical trials (1). Because of their large size and susceptibility to proteolytic degradation in the stomach, proteins usually cannot be administered orally, which is preferable, and instead are delivered by injection (2–4). This option is far from ideal, because protein drugs are rapidly cleared from circulation, injections must be frequent, and their inconvenience and pain beget poor patient compliance (5).

In principle, inhalation delivery of proteins offers an attractive, noninvasive alternative to injections and other modes of administration; lungs have a large surface area, are quite tolerant of foreign substances, are much more permeable than gastrointestinal and nasal mucosa or skin, and contain protease inhibitors preventing proteolytic breakdown (6–8). Consequently, much recent activity has focused on the pulmonary delivery of protein therapeutics (7–9), culminating in a clinical application, local delivery of Genentech's recombinant human DNase (Pulmozyme) for the treatment of cystic fibrosis (10).

There are two principal means for the deep-lung inhalation delivery of protein drugs: dry powders and aqueous solutions (11). The former afford stable formulations, low susceptibility to microbial growth, and high mass per puff; however, protein powders, at least as conventionally made (11), are liable to clump formation and poor reproducibility, pose manufacturing and packaging challenges, and require complex inhalers. [These problems are being addressed with large porous particle powders (9), as well as by a variety of manufacturing methods (12–14).] Aqueous solutions of proteins, albeit free of those drawbacks, suffer from their own, e.g., low drug loading, difficulties in creating stable formulations, and sensitivity to microbial attack. No satisfactory solution yet exists for delivering liquid protein formulations to the lungs. Therefore, alternative modalities of protein administration via inhalation are needed and actively sought (11).

In this study, we have proposed and demonstrated the initial feasibility of such an alternative, which involves the nebulization of solid proteins suspended in a common nonaqueous solvent, namely ethanol. Although proteins (e.g., enzymes) have been conventionally used in their natural aqueous media, recent research has revealed that the enzymatic activity and structure can be preserved even in suspensions in a variety of organic solvents (15). Such nonaqueous suspensions of (lyophilized or crystalline) proteins are easy to prepare, can be concentrated, and preclude microbial contamination; in addition, as illustrated by widespread successful injection therapy with aqueous suspensions of insulin (16), suspensions can be readily handled, precisely dosed, and routinely self-administered by patients. Here we demonstrate that protein suspensions in ethanol can be aerosolized with a standard compressor nebulizer with no detectable damage to biological activity. Moreover, *in vivo* experiments with laboratory rats have shown that inhaling such aerosols containing insulin suspensions lowers the blood glucose level, raises the serum insulin level, and results in a satisfactory bioavailability of the drug; inhalation of ethanol aerosols causes no detectable acute toxic effects.

Materials and Methods

Materials. Hen egg white lysozyme (47,000 units/mg solid), horseradish peroxidase (240 purpurogallin units/mg solid), and bovine pancreatic Zn^{2+}-insulin (27 units/mg) were purchased from Sigma. Absolute ethanol (200 proof) was from Pharmco Products (Brookfield, CT).

Nebulization. Nonaqueous suspensions (10 or 90 mg/ml) of lysozyme were prepared in absolute ethanol. Nebulization was performed with a reusable PARI LC Jet+ nebulizer (PARI Respiratory Equipment, Monterey, CA) in conjunction with a PARI PRONEB compressor. A starting volume of 9 ml (the maximum capacity) was charged in the reservoir of a nebulizer and nebulized for up to 10 min. Aerosol particles were collected by the impaction method (17) in a test tube immersed in an ice bath. The volume, enzymatic activity, and the protein content of the enzyme suspensions, both nebulized and remaining in the reservoir, were analyzed at 5, 7.5, and 10 min from the beginning of the operation. Lysozyme was assayed on the basis of its ability to lyse cell walls of dried *Micrococcus lysodeikticus* cells (18). The Lowry assay (19) was used to measure the protein content.

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Storage Stability. Room temperature stability of two unrelated enzymes, lysozyme and peroxidase, suspended in both absolute and anhydrous ethanol was examined for up to 3 weeks. Both enzymes were lyophilized from their 5 mg/ml aqueous solutions (pH 6.0, deionized water, in the case of lysozyme; pH 7.0, 10 mM aqueous phosphate buffer, in the case of peroxidase). The lyophilized enzyme powders were suspended at 10 mg/ml in anhydrous ethanol containing molecular sieves, unless stated otherwise, to keep the suspensions dry. Each suspension was stored in a vial sealed with aluminum foil, a cap, Teflon tape, and parafilm. Periodically, the suspensions were agitated to homogenize them, and 50-μl aliquots were withdrawn, diluted several 1,000-fold with an aqueous phosphate buffer, and assayed spectrophotometrically for the desired enzymatic activity. Lysozyme was assayed as outlined above, and peroxidase was assayed with the standard literature procedure based on the oxidation of guaiacol with H_2O_2 (20).

Insulin Suspensions in Ethanol. A 5 mg/ml suspension of bovine pancreatic Zn^{2+}-insulin in a 5 mM aqueous phosphate buffer (pH 7.4) was prepared, and then the pH was lowered with concentrated H_3PO_4 to 3.3, at which point it became a solution. Then a drop of concentrated NaOH was added with vigorous agitation to bring the pH back to 7.4, thus quickly bypassing the insulin's isoelectric point of 5.3 (21). The resultant solution was lyophilized, and the insulin powder was suspended in ethanol (10 mg/ml), followed by a 5- to 10-min sonication.

Aerosol Characterization. A 10 mg/ml ethanol suspension of insulin prepared as outlined above was used to generate aerosols (subsequently used in the *in vivo* experiments) by means of a compressed air stream with the pressure of 26 psig. The aerosol particles were directed to a chamber with three exposure ports to which animal enclosure units were later connected in the *in vivo* experiments. The aerosol particles were collected on preweighed Teflon filters with a collection flow rate of 2 l/min. [A control sample (air stream only, no aerosols) was collected to establish that no particulates were present in the air.] The filters were weighed with a Cahn 31 electrobalance in a temperature- and humidity-controlled room. The filter weight, sampling time, and sampling flow rate were used to calculate the aerosol particle concentration in the chamber [which equals (end filter weight − initial filter weight)/(sampling time) × (sampling flow rate)]. The aerosol particle density was estimated to be 1.05 g/cm^3. The aerosol particle size distribution (mass median aerodynamic diameter and geometric standard deviation) was determined with an Aerosizer DSP particle size analysis system (TSI, St. Paul).

Pharmacokinetics/Bioavailability. Male Sprague–Dawley rats weighing between 250 and 300 g (Taconic Farms) were handled in accordance with the National Institutes of Health guidelines for the care and storage of laboratory animals. Rats were placed in Plexiglas restraining tubes that served as head-only exposure flow plethysmographs (22, 23). The tubes were fitted with silicone rubber gaskets designed to fit snugly around the animal's neck and seal the head from the rest of the body. Once the animal was in the tube, a large piston was moved into place behind the animal. The piston served to prevent the animal from moving and to seal the body chamber from the outside air. Air displaced at the body surface as the animal breathed passed across a pneumotachograph (8 mm in diameter fitted with a screen filter) attached to a differential pressure transducer (model 163PC01D75; Omega Engineering, Stamford, CT). The resulting flow signal was analyzed by a BUXCO computer program, which computed minute ventilation, tidal volume, breathing frequency, and inspiratory and expiratory times on a breath-by-breath basis and reported the average of each of these values every minute. The cranial end of the tube was inserted through a port in the exposure chamber into which aerosols were introduced. The rats were first exposed to filtered air for 20–30 min. The initial 15 min of this period was used to adapt the animals to the plethysmographs. Baseline values were the average ones obtained in the last 5–15 min of this 20- to 30-min period. Then either the animals continued to be exposed to the filtered air or the air in the exposure chamber was switched to the aerosols. Exposure to the aerosols then proceeded for an additional 40 min. For every rat, averages of each ventilatory parameter were computed minute by minute. Based on the minute volumes, the insulin aerosol concentration in the chamber, the exposure time, and the estimated fraction (10%) deposited in the lungs after aerosol head exposure (24), the insulin dose for each animal was calculated to be $\approx$100 μg. After the exposure, the rats were anesthetized by inhalation of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Halocarbon Products, Hackensack, NJ). Blood samples (500 μl) were periodically withdrawn from the tail artery for 5 h. Those from the control animals, which were exposed to room air, were also taken over the same period. A commercial glucose meter (ref. 25; Glucometer Elite, Bayer, Elkhart, IN) was used for the analysis of glucose in the whole blood samples. An RIA (ref. 26; Linco Research Immunoassay, St. Charles, MO) was used for the analysis of insulin in the serum.

To ascertain the bioavailability of insulin delivered by our inhalation method, an s.c. injection of the same suspension of insulin in ethanol was carried out as a control. Ten microliters of a 10 mg/ml ethanol suspension of insulin was diluted with 340 μl of PBS (VWR Scientific), and this solution was injected into the scruff of the rat's neck. The rats were anesthetized, and blood samples were periodically withdrawn from the tail artery for up to 5 h and analyzed for glucose and insulin as outlined above.

Ethanol Toxicity Studies. Twenty rats were randomly divided into four equal groups (control, as well as 2, 6, and 24 h). The rats were exposed for 10 min to aerosols obtained from ethanol as in the aforementioned *in vivo* experiments. Thereafter, the rats were euthanized with an overdose of sodium pentobarbital (65 mg, i.p.) (Veterinary Laboratories, Winnipeg, MB, Canada), and bronchoalveolar lavage was performed on each rat through a tracheal incision with four 5-ml washes with PBS. The fluids recovered were combined and centrifuged at 350 × *g* for 10 min at 4°C, cell pellets were resuspended in 1 ml of PBS, and total cell numbers were counted with a hemacytometer after dilution with a trypan blue solution. The cell type was determined with the use of modified Wright—Giemsa-stained cytocentrifuge preparations; 200 cells were counted per sample. The supernatant was clarified at 14,300 × *g* for 30 min at 4°C and used to analyze the lactate dehydrogenase (27) and β-glucuronidase (28) activities, as well as the total protein content (29).

Statistical Analysis. Student's *t* test for multiple comparisons was used. The criterion for statistical significance was set at $P < 0.05$.

Results and Discussion

Human consumption of alcoholic beverages, with few ill effects if in moderation, has taken place for centuries. Therefore, we selected ethanol [the daily worldwide consumption of which in such beverages today exceeds 2 ml per person (30)] as a low-toxicity solvent (31) in which to suspend proteins for inhalation delivery. It is worth noting that ethanol has been used as an excipient in some marketed nebulized (e.g., Tornalate) and metered dose inhaler (e.g., Azmacort, Decadron Respihaler, and Bronkometer) formulations (8), none of which involve protein drugs, however.

The solubility of proteins in ethanol, as in nearly all other organic solvents (32), is very low and even under optimal conditions is usually far below 1 mg/ml (33). In this work, a

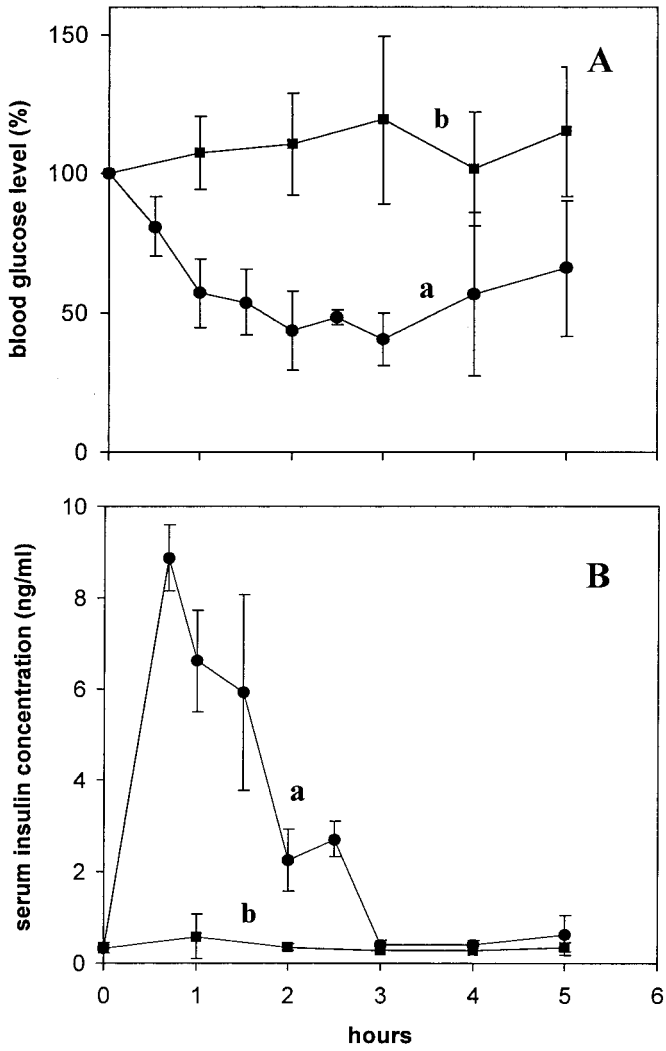

Fig. 1. Rat blood glucose concentration (*A*) and serum insulin concentration (*B*) as a function of time after the animals inhaled for 40 min the aerosols created by nebulizing a 10 mg/ml suspension of Zn^{2+}-insulin in absolute ethanol. Curve a refers to the exposed animals (2–11 rats); curve b corresponds to the control animals that breathed room air instead of insulin/EtOH aerosols (5–7 rats). The insulin dose inhaled by a rat was calculated to be ≈100 μg. For other experimental conditions, see *Materials and Methods*.

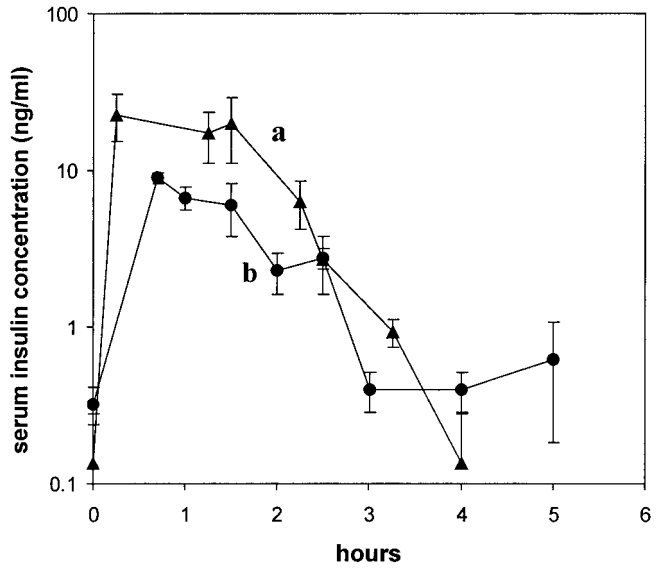

Fig. 2. The rat serum insulin concentration as a function of time after a s.c. injection of 10 μl of a 10 mg/ml suspension of Zn^{2+}-insulin in absolute ethanol diluted with 340 μl of PBS (curve a) or a 40-min inhalation administration of aerosols of the same suspension (curve b). For the s.c. injections, four or five rats were used. For other experimental conditions, see the legend to Fig. 1 and *Materials and Methods*.

protein concentration in ethanol of 10 mg/ml (1%, wt/vol), resulting in a thick suspension, was used, unless mentioned otherwise. Of the two types of standard commercial nebulizers (8), compressor (air jet) and ultrasonic, we chose the former to avoid damage to the proteins by cavitational ultrasound.

In a typical *in vitro* experiment, a protein suspension in ethanol was placed in the nebulizer container, compressed air was applied to it, and the resultant aerosols were collected by condensation in a connected test tube immersed in an ice bath. The condensate was then assayed for protein content and, in the case of enzymes, for catalytic activity.

The initial work was carried out with the well-investigated model enzymic protein hen egg-white lysozyme (34), the enzymatic activity of which was used as a sensitive indicator of protein integrity. When a 10 mg/ml suspension of lysozyme (lyophilized from pH 6.0) was nebulized for 10 min, the collected condensate looked identical to the original suspension and had a protein concentration of 14 mg/ml. The latter fact and the observation that the total suspension volume (i.e., that of the condensate plus that remaining in the nebulizer) declined upon nebulization indicate that some ethanol evaporates in the process. We also demonstrated the feasibility of nebulizing a much more concentrated lysozyme suspension, 90 mg/ml, but ethanol losses were greater still.

To ascertain whether the lysozyme molecule undergoes irreversible damage during the nebulization, we measured the specific enzymatic activity of its original ethanol suspension (equated here to 100%) and that after a 10-min nebulization. Neither the specific activity of lysozyme in the condensate nor that in the remaining suspension (94 ± 8% and 94 ± 10%, respectively) differed significantly from the original value (in all instances, the ethanol suspensions were diluted with an aqueous buffer by at least 1,000-fold and assayed). Thus the nebulization of lysozyme from ethanol suspensions causes no detectable loss of enzymatic potency. Given that the latter is highly sensitive to the protein structure (35), we conclude that the integrity of the lysozyme molecule is not compromised (at least not irreversibly) by the nebulization.

Next, we investigated the storage stability of lysozyme in ethanol. A 10 mg/ml suspension was incubated at room temperature; periodically, aliquots were withdrawn after a thorough mixing and assayed for enzymatic activity. After 4 days, no appreciable change in the lysozyme activity was observed. Previous work on the thermal stability of enzymes in anhydrous solvents revealed that even small amounts of water could markedly destabilize enzymes (15, 36). Therefore, to avoid such destabilization effects, we decided to investigate lysozyme's long-term room temperature stability in ethanol that had been extensively dried by shaking with molecular sieves before the addition of enzyme; furthermore, fresh molecular sieve granules were added to the dry solvent along with the lyophilized enzyme powder. After this the container was sealed. Under such anhydrous conditions, even after 3 weeks, the enzyme still retained 101 ± 12% of its initial activity.

Similar results were obtained with another, unrelated enzyme, horseradish peroxidase. Peroxidase suspended (10 mg/ml) in commercial ethanol retained its full catalytic activity after 4 days. In the anhydrous solvent, the enzyme retained 105 ± 4% of its

Table 1. Analysis of the lung lavage fluid of rats before and after their exposure to ethanol aerosols

Parameter measured	Before exposure	Hours after exposure		
		2	6	24
Total number of cells, $10^5 \times$ cell/ml	3.3 ± 0.8	2.9 ± 0.2	2.8 ± 0.4	3.4 ± 0.6
Cell type				
Macrophages, %	98.5 ± 2.3	97.3 ± 2.4	98.6 ± 0.8	99.0 ± 0.7
Neutrophils, %	1.0 ± 1.7	1.0 ± 1.7	0.5 ± 0.7	0.5 ± 0.4
Eosinophils, %	0.5 ± 0.7	1.7 ± 1.4	0.9 ± 0.7	0.5 ± 0.4
Lactate dehydrogenase, mIU/ml*	31 ± 10	31 ± 11	25 ± 19	39 ± 20
β-Glucuronidase, U/ml*	4.7 ± 1.1	4.7 ± 0.6	3.8 ± 1.5	4.2 ± 1.0
Protein, mg/ml	0.16 ± 0.15	0.15 ± 0.07	0.04 ± 0.03	0.67 ± 0.68

Insulin suspensions in ethanol (10 mg/ml) were nebulized, and the resultant aerosols were inhaled by rats for 10 min. Then the rats were sacrificed, their lungs and airways were lavaged, and the resultant fluids were analyzed. Each of the four animal groups used consisted of four or five rats. The data presented in the table are the average values with the standard deviations indicated. For other experimental conditions, see *Materials and Methods.*

*mIU stands for international milliunits of the lactate dehydrogenase activity; in the case of β-glucuronidase, U stands for the Sigma Chemical Co. units of activity.

activity after 3 weeks under conditions that were otherwise the same. These observations show that enzymes can be extremely stable in their suspensions in ethanol, especially if the solvent is anhydrous.

Encouraged by the foregoing nebulization and stability data with model enzymes, we switched to the inhalation delivery of the therapeutic protein insulin, widely used for the treatment of diabetes (16). At the outset, we addressed the issue of the insulin particle size needed for the effective nebulization from ethanol suspensions.

Aerosol particles of some 1 to 3 μm in aerodynamic diameter are required for maximal deep-lung delivery; larger particles tend to deposit in the upper airways, and smaller ones are exhaled (11). To fit into 1- to 3-μm aerosol droplets of ethanol, the suspended protein particles must optimally be submicron. However, our direct microscopic examination showed that when the commercial bovine Zn^{2+}-insulin powder was suspended (1 mg/ml) in ethanol, sonicated, and vigorously stirred, most of the protein particles were substantially larger than 1 μm. The same unacceptable result was obtained with insulin lyophilized by us from its 5 mg/ml suspension in an aqueous buffer at pH 7.4 (insulin did not completely dissolve under these conditions). However, when that same aqueous suspension of insulin was first acidified with concentrated H_3PO_4 to pH 3.3, at which the protein completely dissolved, followed by rapid adjustment of the pH with concentrated NaOH back to pH 7.4, at which the protein remained dissolved (presumably forming a supersaturated solution) and *then* lyophilized, suspended in ethanol, sonicated, and stirred, all of the resultant insulin particles were of submicron size. Thus insulin lyophilizates obtained from an aqueous solution, unlike those obtained from an aqueous suspension, afford a finely dispersed suspension in ethanol, with the particle size in the aforementioned desired range (<1 μm). This procedure was used in all subsequent experiments.

A 10 mg/ml submicron suspension of insulin in ethanol (insulin/EtOH) was nebulized (importantly, in contrast to aqueous systems, there was no foaming), and the aerosol particle size distribution was determined with a TSI Aerosizer. The mass median aerodynamic diameter (37) of the resultant ethanol aerosol particles was found to be 1.5 μm, with a geometric standard deviation of 1.3. Thus the generated aerosol droplets of insulin suspended in ethanol have dimensions conducive to maximal alveolar deposition (38). The aerosolized insulin was used for inhalation delivery to rats. These *in vivo* studies focused on three critical issues: pharmacokinetics, pharmacodynamics, and ethanol toxicity.

The insulin/EtOH aerosols outlined in the preceding paragraph were directed to a chamber with head-only plethysmography tubes, each containing a rat. After a 40-min exposure, which had been calculated (see *Materials and Methods*) to deliver an ≈100-μg deposited dose of insulin (contained in about 1 ml of ethanol) to each rat, rats were anesthetized, and blood samples were analyzed for glucose with a commercial glucose meter (25) and for insulin with an RIA (26).

Fig. 1*A* depicts the time course of the change in the blood glucose level of rats exposed to the insulin/EtOH aerosols (curve a); curve b corresponds to the control animals, i.e., those who breathed room air instead. One can see that in control rats the glucose level expectedly remained at the normal level (110–160 mg/dl). In contrast, in animals exposed to the insulin/EtOH aerosols, the blood glucose concentration dropped to one-half after an hour following the exposure and stayed at that hypoglycemic level for the next 4 h (Fig. 1*A*, curve a).

Fig. 1*B* shows a dramatic burst in the serum insulin concentration of the rats that inhaled the insulin/EtOH aerosols (curve a). Three hours after the exposure, their insulin level in the serum returned to the initial negligible level, which the control animals maintained all along (Fig. 1*B*, curve b). Thus Fig. 1 illustrates that when rats inhale the insulin/EtOH aerosols, their blood glucose levels drastically decline and insulin levels markedly rise, suggesting the *in vivo* feasibility of the inhalation delivery of insulin from its suspensions in ethanol.

To assess the bioavailability of insulin delivered as described above, we s.c. injected the same equivalent amount (100 μg) of insulin into rats. Curve a in Fig. 2 shows the pharmacokinetic profile for the s.c. injected suspension of the hormone in ethanol; curve b depicts the same time dependence for the insulin/EtOH aerosols. By integrating the areas under the curves and comparing the values with each other (39), we found the bioavailability of insulin (based on estimated deposited lung dose) delivered by inhalation of ethanol aerosols relative to that injected s.c. to be 33%. This value is comparable to those observed when insulin was administered to rats via an aerosol of its aqueous solution (40) or of its dry powder (41).

Ethanol has a history of use in various parenteral therapeutic products (42, 43). With respect to inhalation, it is one of the least toxic organic solvents (44). If the delivery method described herein were to be used by patients, practical considerations dictate that the inhalation times should be no longer than several

minutes; e.g., for Pulmozyme it is 10 min (10). The available data on the inhalation toxicity of ethanol refer to much longer exposures.** Therefore, we undertook our own *in vivo* investigation of acute toxicity of ethanol upon inhalation.

Although a 10-min human exposure corresponds to a 5-min exposure in rats (45), we conservatively selected a 10-min exposure of laboratory rats to ethanol aerosols under conditions that were otherwise the same as in the pharmacokinetic/bioavailability experiments reflected in Fig. 1. The rats were killed 2, 6, or 24 h after the exposure, and a bronchoalveolar lavage (washing the lungs and airways with a physiological saline solution) (46) was performed. The fluids collected were centrifuged; the precipitates were analyzed for the relative contents of various cell types, and the supernatants were analyzed for protein contents and enzymatic activities. These parameters combined should be indicative of damage to the lungs and airways (46) caused by a 10-min inhalation of ethanol aerosols.

Inspection of Table 1 reveals that there is no detectable change in the total cell count of the lavage fluid for up to 24 h after the exposure to ethanol aerosols compared with the nonexposed rats. Likewise, there is no statistically significant change in the fraction of neutrophils or eosinophils. Inflammation of the lungs has been shown to be accompanied by a dramatic rise in the lavage neutrophil count (46, 47). An allergic response in the lungs is known to result in a marked increase in the lavage eosinophil count (46, 47). For example, exposing hamsters to an aerosolized aqueous solution of $CdCl_2$ leads to a 12-fold increase in neutrophils and a 5-fold increase in eosinophils in the lavage fluid (48). Therefore, the cell count data in Table 1 indicate that a 10-min exposure of rats to ethanol aerosols causes no detectable inflammatory or allergic response.

One can also see from Table 1 that the inhalation of ethanol aerosols does not result in statistically significant changes in the protein content or enzymatic activities of lactate dehydrogenase and β-glucuronidase in the lavage fluid. These findings indicate no damage to the alveolar/capillary barrier, no lysed and/or damaged cells, and no lysed macrophages (46, 47).

Thus the present study demonstrates, both *in vitro* and *in vivo*, the initial feasibility of inhalation delivery of proteins from their suspensions in ethanol. Inhalation of relevant quantities of aerosolized ethanol by itself caused no appreciable acute toxicity. In rats, the bioavailability of insulin delivered via this approach was comparable to those observed in the inhalation delivery of insulin aqueous solutions or conventional dry powders. Inhalation delivery of proteins from ethanol suspensions is free of many inherent drawbacks of the aqueous and dry powder approaches. It is amenable to the use of standard nebulizers and should be applicable to the delivery of other macromolecular therapeutics, e.g., nucleic acids and polysaccharides.

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