Response to paper by Singh et al. “Hyperinsulinemia adversely affects lung structure and function”

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TO THE EDITOR: We have serious concerns regarding the recently published paper by Singh et al. (16), which states, “Our work has important general implications that should hopefully lead to questioning of many current trends or practices including use of inhaled insulin formulations in diabetes.” This conclusion is not supported as evidenced by 1) the technical limitations in the experiments described in the paper and 2) the omission of abundant pertinent data that is neither referenced nor discussed. The pulmonary effects of inhaled insulin in both patients and animals have been well described in the literature and show minimal effects. These peer-reviewed papers are absent in the Singh paper. In summary, this paper applies poorly designed experiments in mice to humans, ignoring decades of relevant research. Below, we detail the major deficiencies. Importantly, we believe it is irresponsible to use the data in the Singh et al. paper as the foundation for giving advice to physicians and their patients.

Technical Limitations

The focus of this paper is the possible pulmonary effects of insulin delivered to the lungs. However, the method used in this paper is an inefficient and imprecise way of achieving pulmonary dosing. They do not use inhalation, and they don’t even use intratracheal instillation (IT). Many labs, including that of one of us [Brain et al. (1)] routinely employ intratracheal instillations in 20-g mice. With nasal instillation as is used in the experiments reported by Singh et al. (16), little of the instilled material may get into the lungs when volumes are small. As the volume of the instilled solution increases, an increasing but variable fraction is aspirated into the lungs (17). In this paper, no information is given that tells us the fraction of the intranasal dose that actually reaches the lungs. They also fail to tell us the instilled volume. The fact that the insulin dose was very high and the animals did not die of hypoglycemia suggests that much of the “delivered dose” was probably swallowed and digested.

The anatomic distribution of particles in the lungs differs following instillation vs. inhalation. Brain et al. (1) and Foster et al. (4) have clearly shown that delivery methods to the lungs of rodents, other than inhalation, give rise to considerably different deposition results. The methods used by Singh et al. for intranasal administration are not described, and they should be presented. Southam et al. (17) have shown large variations in lung deposition depending on intranasal technique.

It is important to recognize that the liquid solution used to deliver the insulin was an acid, pH 2 (0.01 N HCl). Not only is insulin unstable at this pH but the major acidic degradation products, covalent dimers, are the most immunogenic degradation products from insulin. More importantly, acid aerosols of pH 2 have been clearly shown to have adverse effects in both humans (8) and animals (14). Respiratory complications of gastroesophageal reflux are well known and may have influenced results. Use of this inappropriate vehicle may have influenced the results. In addition, 0.01 N HCl is hypotonic. Hypotonic solutions can cause damage and bursting of epithelial cells. Instillations should always be isotonic.

Essential controls are missing from the experimental design. The “controls” describe animals that were given the vehicle, 0.01 normal hydrochloric acid (pH 2). We believe that 11 daily sequential anesthesias and instillations of acid into the nose, pharynx, and lungs had effects on both of the two groups studied. Conspicuous by its absence is a necessary third group, a true control. We regard this omission of animals that received neither of these two treatments as a serious flaw. They compare the acid vehicle only with the vehicle plus insulin. We suspect that these repeated nasal instillations affected the entire physiology of the mice. For example, what were the changes in body weight over the study period? We predict that this harsh treatment would result in loss of body weight, but we don’t know since there is no true control.

The histopathology as presented in the paper is very difficult to interpret and the interpretations are of concern based on the following comments.

- The MATERIALS and METHODS do not provide adequate description of the methods used for fixation of the lungs, which is critical when trying to make statements about changes in lung structure. We had to go back through two references to any mention on the protocols used for fixation and even then the description is minimal. When defining changes to lung structure it would be hoped that the authors would follow the guidelines set out by the ATS for fixation of lung tissue [Hsia CC et al., ATS/ERS Joint Task Force on Quantitative Assessment of Lung Structure (5)].
- There is not a quantitative nor semiquantitative evaluation of the lesions presented. With the advances in digital imaging and imaging software one would expect that some type of quantitative analysis be performed to remove bias from the data present. There are concerns over...
the data presented due to the following observations of
work presented in this manuscript:

1) The photos are of such a low power that they cannot be
evaluated from the publication and descriptions or observations
are vaguely described. For instance a PAS stain is provided to
show goblet cells, however, Alcian blue—should also have been
used.

2) There is a claim of peribronchial collagen production, but
appearance of true collagen in 12 days seems unrealistic.

3) The presence of smooth muscle in the normal mouse lung
is to be expected and depends on what areas of the lung are
sampled (18). Also nonuniform distribution of insulin from the
instillation procedure could have influenced results. Therefore,
the authors would need to perform uniform random sampling
of the lungs and images acquired to accurately interpret find-
ings from these studies. The importance of performing ade-
quate sampling of airways for inhaled toxicants is provided by
Dallas Hyde et al. (6).

4) Tissue quantitation can be influenced heavily by what
parts of the lung are examined since if too much of the upper
airway or mediastinum is included native collagen levels can
skew measurements.

- Selective histological examination can compromise out-
comes and the lack of an appropriate control group can
make interpretation uncertain. The β-catenin knockdown
data are also difficult to interpret. Singh et al. (16) do not
describe the carrier system used to deliver the siRNA.
This is problematic since most of these carriers (typically
lipids) are associated with enhancing siRNA-mediated
innate inflammatory, which can confound findings and
must be appropriately controlled (10).

The dose of insulin used, 50 μg, is high and of limited
relevance to human clinical use. The 50-μg dose in a mouse
of typical body weight of 25 g is 2 μg/g, or 2 mg/kg. This dose
is approximately threefold higher than the deposited lung dose
of 0.6 mg/kg insulin that was found to be without effects on
lung histopathology following 6 mo of inhalation exposure in
rats (2). It is also 13-fold higher than the 0.15 mg/kg insulin
lung dose estimated for mean human use for Exubera (2). The
0.15 mg/kg dose is the nominal capsule dose delivered to
humans, while the lung deposited dose is ~0.05–0.07 mg/kg or
30–40-fold less than the mouse lung dose. Thus the Singh
experiments are at doses that would not be achieved in human
use. Rodents metabolize insulin more quickly than humans and
so much higher lung doses can be achieved in rodents com-
pared with humans. We also note the absence of any informa-
tion about the grade of the Sigma human recombinant insulin
used.

Pertinent Animal and Human Data

The paper by Singh et al. (16) omits reference to the
considerable body of information relating to the pulmonary
safety of inhaled insulin in patients and animals. Extensive
clinical data with inhaled insulin provides the most relevant
information. Pfizer’s inhaled insulin product (Exubera) was
approved by both the FDA and the EU. This was based on
more than a decade of experiments and studies of thousands of
patients. Bronchopulmonary lavage after 3 mo of inhaled
prandial insulin showed no inflammatory markers (7) in the
face of measured higher levels of insulin (9). Eli Lilly and
Novo Nordisk also have extensive clinical experience similar
to that of Exubera (15). Finally, more recently, Mannkind’s
inhaled insulin (Afrezza) was approved by the FDA and it is
currently being marketed. For both approved drugs large
amounts of data were submitted, reviewed, and formed the
basis of a positive FDA decision.

The review paper by Siekmeier and Scheuch (15) lists
results from 20 clinical trials that studied the safety of inhaled
insulin. In none of the studies were there any findings of
clinical significance. In one of the largest trials, a 2-year study
of Exubera (13), it was stated that “Small, clinically non-
meaningful treatment group differences in the change in FEV1
during the first 3 mo of treatment were found. Most notably,
the between-group differences did not increase after 3 mo for
up to 2 years, and pulmonary function declined at similar rates
in both groups during months 3 to 24.” Additional clinical
information has also become available since the Siekmeier and
Scheuch (15) review primarily on Afrezza with similar results
(11). The only clinically relevant negative pulmonary finding
in patients has been bronchospasm in individuals with asthma/
COPD with Afrezza (3), which appears to be related to the
fumaryl diketopiperazine excipient specific to the Afrezza
formulation. Also, Singh et al. (16) reported data suggesting an
increase in airway hyperresponsiveness in rats, whereas clini-
cal data with another inhaled growth factor promoter, human
growth hormone, showed no change in airway hyperrespon-
siveness in individuals with asthma (10).

Singh et al. (16) also failed to refer to the information from
long-term 6-mo toxicity studies of inhaled insulin in rats and
monkeys (2) and in dogs (19) at lung deposited doses of 0.6
mg/kg, 0.15 mg/kg, and 0.18 mg/kg, the maximum tolerated
doses in these respective species. All of these studies con-
ducted using the appropriate administration method of inhala-
delivery showed no effects on pulmonary function or lung
histopathology.

The study reported by Singh et al. has many technical
limitations and was conducted at doses not relevant to human
inhalation delivery. These facts should be considered as well as
the overwhelming clinical evidence at relevant doses and the
appropriate inhalation delivery route that shows inhaled insulin
poses minimal risks to patients with diabetes while offering the
possibility that patient preference for inhalation translates to
better compliance and improved outcomes.

DISCLOSURES

R. K. Wolff worked many years on the development of inhaled insulin,
consultant for Dance Biopharm (see below). J. D. Brain worked for many years
as consultant for companies developing inhaled insulin including Inhal/-
Nektar, Mankind and Dance. J. S. Patton declares 25 years working on
development of inhaled insulin at Inhal Therapeutics/Nektar Therapeutics
(Cofounder, CSO) and Dance Biopharma Holdings Inc. (Founder, CEO). D.
Liggitt declares no conflicts.

AUTHOR CONTRIBUTIONS

interpreted results of experiments; R.W., J.D.B., J.P., and D.L. drafted man-
uscript; R.W., J.D.B., J.P., and D.L. edited and revised manuscript; R.W.,
J.D.B., J.P., and D.L. approved final version of manuscript.

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of particles given by intratracheal instillation or by aerosol inhalation.


