A Simple Method of Correlating Pharmacodynamic Equivalence with Absolute Bioavailability Following Noninvasive Delivery of Insulin

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INTRODUCTION

The noninvasive delivery of peptides and proteins is of considerable interest to pharmaceutical scientists and formulators. Systemic uptake of these agents across the nasal, pulmonary, rectal, dermal and conjunctival mucosae is in general low and variable, leading to difficulties in accurately assessing absolute bioavailabilities. On the other hand, most of these bioactive protein compounds trigger changes in physiological parameters such as blood glucose, Ca^{2+} level, blood cell count, etc. For these compounds, the measurement of pharmacodynamic response appears to be much easier technically and more reliable therapeutically.

Many previous studies describing insulin absorption with different formulations or by alternative routes did not attempt to measure plasma insulin concentrations directly. Instead, pharmacological availability and relative efficacy have been utilized by these researchers (1–7). However, these pharmacodynamic methods impose some limitations when correlating pharmacological response with absolute plasma bioavailability due to the fact that the basis to mathematically establish those correlations deviated from absolute bioavailability definition.

The purpose of this study has been to establish a strong fundamental relationship between pharmacokinetic and pharmacodynamic parameters following pulmonary administration of insulin. This report points out that the troublesome task of measuring plasma insulin concentrations may indeed be substituted by simple measurement of blood glucose levels. This method may also be applied to other protein drugs in which pharmacological response can be easily and accurately monitored.

MATERIALS AND METHODS

Materials

Crystalline porcine zinc insulin (26.3 IU/mg) was kindly donated by Eli Lilly and Company (Indianapolis, IN). Sodium glycocholate (NaGC) was purchased from Sigma Chemical Co. (St. Louis, MO). Sterile saline solution (Abbott Laboratories, North Chicago, IL) was used to dissolve insulin and to replace the blood volume taken during sampling.

Preparation of Insulin Solution

A minimal volume of 0.1 N HCl solution was added to solubilize the solid zinc insulin powder 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 case of micellar solutions, sodium glycocholate was added to the insulin solution and the mixture was sonicated at room temperature for 2 minutes.

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°C 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 (4). 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-50). Blood samples were withdrawn from the jugular vein at predetermined time intervals. Blood glucose levels were determined by Chemstrip bG® strips in an AccuChek IIm® Blood Glucose Monitor (Boehringer Mannheim Corporation, Indianapolis, IN).

Data Analysis

Three different pharmacodynamic methods have been utilized to get a measure of insulin pharmacodynamic availability. The first two methods have already been proposed by Ritschel and Ritschel (6) and Aungst et al. (1). We are proposing a third method in this report. A comparison of all three methods with regard to predicting insulin plasma availability is described in the results and discussion section.

(a) Pharmacological Availability Method

The area above the blood glucose–time curve and below the 100% line ($AAC_{0-240\ min}$) is estimated by the linear trapezoidal method. Pharmacological availability (f) is calculated by using the following equation proposed by Ritschel and Ritschel (6):

$$f(\%) = \frac{AAC_{0-240\ min\ it}}{AAC_{0-240\ min\ iv}} \times \frac{Dose_{iv}}{Dose_{it}} \times 100 \qquad (1)$$

The subscripts it and iv refer to intratracheal and intravenous administrations, respectively.

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(b) Relative Efficacy Method

The calculation of relative efficacy has been reported by Aungst et al. (1). First, the log(dose)-response curve is constructed following i.v. administration of insulin at different dose levels, as shown in Fig. 2. AAC values following pulmonary administration of insulin is then substituted into the i.v. curve to obtain a $dose_{iv}$ equivalent. Relative efficacy could be calculated by using equation (2).

$$\text{Relative Efficacy (\%)} = \frac{\text{Dose}_{iv}\ \text{Equivalent}}{\text{Actual Non-iv Dose}} \times 100 \quad (2)$$

(c) Pharmacodynamic Equivalence Method

In this article, we are proposing this method to predict absolute plasma bioavailability from response-time profiles. By definition, the absolute bioavailability is calculated by the following equation:

$$F\ (\%) = \frac{AUC_{it}}{AUC_{iv}} \times \frac{Dose_{iv}}{Dose_{it}} \times 100 \quad (3)$$

Since it is assumed that AUC is directly proportional to dose, the direct division of AUC by dose is reasonable. After the AUC has been normalized by dose, the ratio becomes unit-dose AUC_{it} over unit-dose AUC_{iv}. However, in the pharmacological response versus dose data, the response has a linear relationship with the logarithmic dose instead of simple dose, as reported by many researchers (8–9). The relationship between the pharmacological response and dose can be expressed by the following equation:

$$\text{Pharmacological response} = \text{Slope} \times \log(\text{Dose}) + \text{Intercept} \quad (4)$$

According to this equation, at an insulin dose of 1 U/kg, the log(dose) term becomes zero. The intercept is equivalent to pharmacological response at unit dose. Therefore, the ratio of intercepts of non i.v. (in this case intratracheal) route relative to i.v. at the dose level of 1 U/kg can be defined as pharmacodynamic equivalence as expressed by the following equation:

$$\text{Pharmacodynamic equivalence (\%)} = \frac{\text{Intercept}_{it}}{\text{Intercept}_{iv}} \times 100 \quad (5)$$

Since the pharmacodynamic equivalence calculation adopted the same principle as the absolute bioavailability calculation, it is possible that it may have a better correlation with plasma bioavailability values. A comparison of all three pharmacodynamic response calculation methods in predicting the absolute plasma bioavailability of insulin following intratracheal administration is discussed in the following section.

RESULTS AND DISCUSSION

Figure 1(A) displays the observed hypoglycemic effects following intravenous administration of insulin at different doses to rats. Figure 1(B) illustrates blood glucose depression curves following intratracheal instillation of insulin at different doses in the presence of 20 mM NaGC. In all cases, an increase in insulin dose resulted in a significantly elevated hypoglycemic response. $AAC_{0-240\ min}$ values were then calculated using data in Figs. 1 (A) and (B). Figure 2 depicts the relationship between the cumulative pharmacological response ($AAC_{0-240\ min}$) and insulin doses following intravenous administration and intratracheal instillation of insulin in the absence and presence of NaGC. As illustrated in Figure 2, higher intratracheal insulin doses will be needed to achieve equivalent response to that of intravenous administration. The linear regression equations of each linear profile shown in Figure 2 are listed below:

Insulin i.v.: $AAC_{0-240\ min} = 21354 + 13733 \log(\text{dose})$
Insulin + 20 mM NaGC i.t.: $AAC_{0-240\ min} = 17121 + 13599 \log(\text{dose})$
Insulin + 10 mM NaGC i.t.: $AAC_{0-240\ min} = 14075 + 10052 \log(\text{dose})$
Insulin i.t.: $AAC_{0-240\ min} = 1385 + 11457 \log(\text{dose})$

Table I lists some pharmacodynamic parameters in the form of pharmacological availability, relative efficacy, pharmacodynamic equivalence, and absolute bioavailability values. The calculations using the pharmacological availability

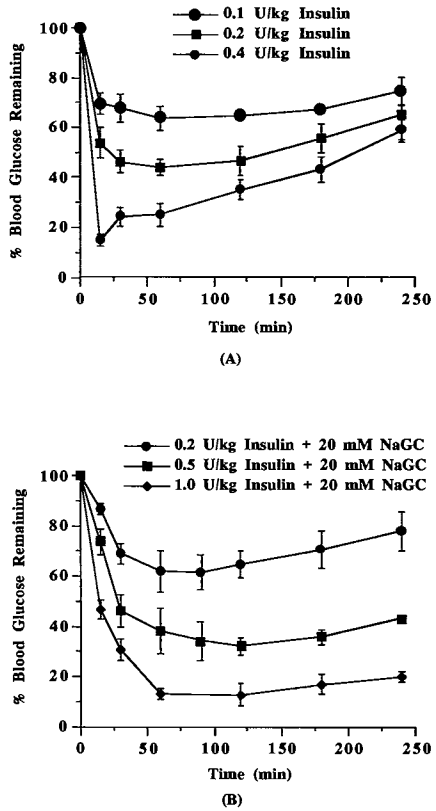

Fig. 1 (A). Changes in blood glucose levels following intravenous administration of insulin at different dose levels. Values represent means ± SE (n = 6). (B). Blood glucose depression following intratracheal instillation of insulin in the presence of 20 mM NaGC. Values represent means ± SE (n = 3–4).

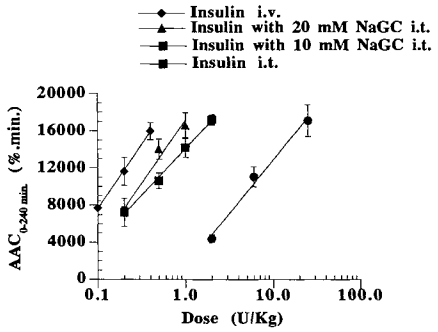

Fig. 2. The relationship between pharmacodynamic response (AAC) and logarithmic insulin dose following intravenous injection and intratracheal instillation of insulin in the absence and presence of NaGC. Values represent means ± SE (n = 3–4).

and relative efficacy method could be made at various doses. For the calculation of pharmacological availability, the intravenous dose of 0.2 U/kg has been utilized as the reference. The measured absolute bioavailability values are on the order of 9.1% and 79% for insulin in the absence and presence of 20 mM NaGC. Pharmacological availability values of 3.8, 3.2 and 1.2% were obtained at insulin dose levels of 2.0, 6.0, and 25.0 U/kg following intratracheal delivery of insulin in the absence of NaGC, respectively. These values appear to decrease with increases in insulin dose while all being smaller in magnitude than the absolute bioavailability of 9.1%. Pharmacological availabilities of 62, 48, and 23% were achieved following intratracheal delivery of insulin in the presence of 20 mM NaGC. These values are significantly lower than the absolute bioavailability of 79%. In addition, the pharmacological availability value appear to be strongly dose-dependent. Relative efficacy calculations generated slightly lower value of 2.9, 3.0, and 2.0% at insulin dose levels of 2.0, 6.0, and 25.0 U/kg respectively for insulin alone. Values of 46, 59, and 45% at insulin dose levels of 0.2, 0.5 and 1.0 U/kg were obtained for insulin with 20 mM NaGC. Theoretically, relative efficacy should not be dose-dependent due to the fact that the calculation has been based on the established i.v. regression line. However, among the three non-i.v. doses, a moderate variation indeed occurred which utilized AAC values resulted from single doses. When pharmacodynamic equivalence method as proposed in this article is utilized, values of 6.5% and 80% were obtained following intratracheal delivery of insulin with and without 20 mM NaGC, which lie much closer to actual bioavailabilities. Therefore, pharmacodynamic equivalence calculation appears to yield better results comparable to absolute bioavailability than any other methods.

The merits of using pharmacodynamic response data with insulin stems from the ease of plasma glucose determination. In addition, this parameter is most relevant to therapeutic effects. Pharmacological availability calculations neglect the fact that the response is related linearly to the logarithm of insulin dose rather than simply the dose. However, technically, it requires the administration of only one i.v. dose and one transmucosal dose. This method has, therefore, been repeatedly used. In the case of relative efficacy, from the bioavailability definition point of view, it is not as well defined as the pharmacodynamic equivalence method. It requires several dosing groups for the i.v. dose-response curve, while only needing one dosing group for transmucosal administration. The pharmacodynamic equivalence method requires several dosing groups for both i.v. and transmucosal routes, which is associated with a disadvantage of performing more experimental work to generate both regression lines.

Theoretically, the relative efficacy method measures the difference in doses (e.g., transmucosal and i.v.) required to elicit an equal pharmacological response. This is similar to the fashion pharmacologists often estimate bioavailability by comparing ED_{50} values. An advantage is that a single transmucosal dose can be used, which may not be at the ED_{50} as long as it is within the linear portion of the response-log(dose) curve. The pharmacodynamic equivalence method measures the differences in response at a given dose (1 U/kg insulin), rather than the difference in doses for an equivalent response.

The pharmacodynamic equivalence method is not just applicable to intratracheal delivery of insulin; it may also be utilized in other noninvasive delivery routes or even other protein drugs in which the pharmacological response could be easily monitored. Pharmacodynamic equivalence may not necessarily be equivalent to absolute plasma bioavailability, nor should it substitute for the need to determine plasma drug profiles. Nevertheless, it provides value in accurately predicting the bioavailability by making maximal use of available pharmacological response data.

Table I. A Comparison of Several Pharmacodynamic Response Parameters with Absolute Plasma Availability for Insulin Delivered Intratracheally in the Absence and Presence of NaGC

	Insulin only			Insulin + 20 mM NaGC		
	Dose (U/kg)			Dose (U/kg)		
Parameters	2.0	6.0	25.0	0.2	0.5	1.0
Pharmacological availability (%)	3.8	3.2	1.2	62	48	23
Relative efficacy (%)	2.9	3.0	2.0	46	59	45
Pharmacodynamic equivalence (%)		6.5			80	
Absolute bioavailability (%)		9.1[a]			79[a]	

[a] From Ref. 4.

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REFERENCES

1. B. J. Aungst, N. J. Rogers, and E. Shefter. Comparison of nasal, rectal, buccal, sublingual and intramuscular insulin efficacy and the effects of a bile salt absorption promoter. *J. Pharmacol. Exp. Ther.* 244:23–27 (1988).
2. B. J. Aungst and N. J. Rogers. Site dependence of absorption-promoting actions of laureth-9, Na salicylate, Na_2EDTA, and aprotinin on rectal, nasal, and buccal insulin delivery. *Pharm. Res.* 5:305–308 (1988).
3. I. Morishita, M. Morishita, K. Takayama, Y. Machida, and T. Nagai. Hypoglycemic effect of novel oral microspheres of insulin with protease inhibitor in normal and diabetic rats. *Int. J. Pharm.* 78:9–16 (1992).
4. Y. Li, Z. Shao, and A. K. Mitra. Effect of a conjugated bile salt on the pulmonary absorption of insulin in rats. *Eur. J. Pharm. Biopharm.* 39:216–221 (1993).
5. Z. Shao, Y. Li, R. Krishnamoorthy, T. Chermak, and A. K. Mitra. Differential effects of anionic, cationic, nonionic, and physiologic surfactants on the dissociation, α-chymotryptic degradation, and enteral absorption of insulin hexamers. *Pharm. Res.* 10:243–251 (1993).
6. W. A. Ritschel and G. B. Ritschel. Rectal administration of insulin. In B. Glas and C. J. de Blaey (eds.), Rectal Therapy, J. R. Prous Publishers, Spain, 1984, pp. 67–84.
7. F.-Y. Liu, Z. Shao, D. O. Kildsig, and A. K. Mitra. Pulmonary delivery of free and liposomal insulin. *Pharm. Res.* 10:228–232 (1993).
8. S. Hirai, T. Yashiki, and H. Mima. Effect of surfactants on the nasal absorption of insulin in rats. *Int. J. Pharm.* 9:165–172 (1981).
9. S. Hirai, T. Ikenaga, and T. Metsuzawa. Nasal absorption of insulin in dogs. *Diabetes,* 27:296–299 (1978).