ORIGINAL ARTICLE

Enhancement of oral bioavailability of insulin in humans

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Abstract

OBJECTIVE: The purpose of this study is to investigate oral absorption of 1, 2 and 3 U/kg oral insulin five test products with different particle sizes in comparison with 0.1 U/kg subcutaneous reference formulation. **METHODS:** Twenty five healthy volunteers participated in five studies using a two-phase, two-sequence crossover design with washout period of one day. Mean disposition kinetics was determined by non-compartmental analysis using Kinetica program. Absorption kinetics of insulin products were then determined using SIMCYP simulator utilizing ADAM model. **RESULTS & CONCLUSIONS:** Dimensional analysis results showed the superiority of formula 4: 2 U/kg oral dose with 57 nm particle size over other oral formulations when compared with subcutaneous route. Optimized intestinal permeability coefficients ($\times 10^{-4}$) of insulin best test and reference formulations were 0.084 and 0.179 cm/sec respectively. Total fraction of insulin dose absorbed (Fa) for the test and reference products were 3.0% and 19% respectively. Subcutaneous product exhibited higher absorption rate and extent than oral insulin. Yet that was compensated by the increase in other factors such as Fa*, Peff* and oral dose, leading to similar insulin plasma levels and similar effect on glucose infusion rates. Oral insulin bioavailability was shown promising for the development of oral insulin product.

INTRODUCTION

The oral bioavailability of an agent is affected by many factors including: dissolution, transit time, intestinal permeability, formulation additives and first pass metabolism in the gut and/or liver. Intestinal permeability, a key step in drug absorption, quantitates the fundamental transport property of the intestinal mucosa for a particular compound. The rate of permeation is dependent upon several factors including the structure and integrity of the intestinal membrane, the physiochemical properties of the drug, the specific transport mechanisms involved, and sometimes the inclusion of formulation additives. Intestinal permeability can be determined experimentally by different methods such as single-pass perfusion technique in situ and regional jejunal perfusion technique in vivo (T. Z. Csaky, 1984; K.Ewe *et al*, 1994; H. Lennernäs *et al*, 1992, 1994; R. Modigliani *et al*, 1973; D. C. Taylor *et al*, 1985).

On the other hand, recently with more information available about gastrointestinal tract physiology such as gastric emptying rates and mean residence times in different parts of the gut and with more understanding of the variables affecting them (Diabetes Care, 1998; Diabetes, 1996), Intestinal permeability can be estimated indirectly from drug plasma profiles when drug pharmacokinetic and physicochemical properties are known. This can be achieved with the aid of new computer softwares especially designed for such purposes. In this paper, an experimental application to this approach is presented to investigate about absorption kinetics of different insulin products.

Optimal management of diabetes mellitus (DM) often requires intensive insulin therapy to achieve and maintain good glycemic control and to reduce late microvascular complications. However, multiple daily injections are inconvenient and uncomfortable, making some patients fearful of them and making compliance a concern. To avoid the discomfort of insulin injections, oral formulation is preferred. The goal of oral insulin formulation is to provide a noninvasive and easy-to-use means of meeting prandial insulin requirements (Diabetes Care, 1998; Diabetes, 1996; Lauritzen T, Zoffmann V, 2004).

The objective of this research is to compare the pharmacokinetic, pharmacodynamic and absorption kinetics of several oral insulin formulations with those of subcutaneous formulation in humans.

2. MATERIALS AND METHODS

2.1. Drugs

Five oral test products of insulin nanoemulsion of particle sizes 57 to 220 nm were obtained from Jordan Pharmaceutical Manufacturing Co as shown in Table 1. The novel methodology for preparation of nanoformulations is patented. However, the detailed information about each formulation is beyond the scope of this paper and will be published separately. Reference subcutaneous insulin product was Humulin®R from Lilly USA.

2.2. Subjects

25 healthy male subjects gave written informed consent to participate in 5 studies. Studies were approved by the Institutional Review Board of the study site, Almowasah hospital and also by Jordan Food and Drug Administration. Subjects aged 23–33 years (mean 28.40 ± 3.97), weighed 68–82 kg (74.60 ± 6.07), height 171–177 cm (mean 175 ± 2) and body mass index (BMI), 21.95–26.47 (mean 24.41 ± 1.77). They were judged healthy based on medical history, physical examination, complete blood count and serum chemistry. In addition, all subjects were medication free, including over-the-counter agents, for 7 days prior to the study.

2.3. Clinical Experiments and Assay Procedure

Following a ten-hour overnight fast, blood glucose level was kept constant, at a target of 90 mg/dL (+5 mg/dL), using Euglycemic clamp technique. This technique involves continuous IV insulin infusion to acutely raise the plasma insulin concentration while holding the blood glucose concentration constant at basal levels with a concurrent variable IV glucose infusion throughout the study period, thus reaching a steady-state condition of euglycemia (DeFronzo RA *et al*, 1979).

After achieving euglycemia, oral insulin or SC insulin was administered in a two-phase, two-sequence crossover study with one day washout period. The estimated mean amount of insulin injected was based on the average body weight of a healthy male adult around 70 kg and on data obtained from unpublished animal study conducted by JPM, which indicated that JPM Oral Insulin is equivalent to 30% insulin subcutaneous injection in animals**.** Blood samples were collected at 20 minute intervals from 140 minutes before dose to dosing time; and at 10 minute intervals from 0 minutes before dosing until 360 minutes after dosing. Samples were stored at –20 °C until analyzed for insulin by a sensitive ELISA method. Glucose infusion rate was also measured directly throughout all experiment duration at 5 minute intervals from –140 minutes before dosing to 360 minutes after dosing.

2.4. Data Analysis

2.4.1. Disposition Kinetics

Area under plasma concentrations (AUC), maximum concentration (C_{max}), time to reach maximum concentration (T_{max}) for insulin concentrations and glucose infusion rates were calculated by non-compartmental analysis for all subjects using Kinetica® software V4.11 (Kinetica 2000).

2.4.2. Absorption Kinetics

Insulin physicochemical parameters and human gastrointestinal physiological parameters were obtained from literature and from our pharmacokinetic analysis and were used as baseline values for all simulations. The effective permeability at different parts in the gastrointestinal tract was optimized to match the mean insulin

Table 1: Oral Insulin Formulations

FORMULA TION #	1	2	3	4	5
DOSE (U/KG)	1	2	3	2	3
NanoEMULSION PARTICLE SIZE (nm)	100	100	100	57	220

Table 2: Dimensional Analysis Ratios (SE) of Pharmacokinetic, Pharmacodynamic and Absorption Parameters

		FORMULA 1	FORMULA 2	FORMULA 3	FORMULA 4	FORMULA 5	
	Parameter Ratios	Oral 1 U/kg (100 nm size)	Oral 2 U/kg (100 nm size)	Oral 3 U/kg (100 nm size)	Oral 2 U/kg (57nm size)	Oral 3 U/kg (220 nm size)	P-value
INSULIN	AUC*	0.94 (0.13)	1.25 (0.14)	1.01 (0.14)	0.85 (0.06)	0.93 (0.10)	0.375
INSULIN	Cmax*	0.85 (0.10)	1.27 (0.18)	1.19 (0.14)	0.98 (0.11)	1.19 (0.20)	0.062
INSULIN	T_{max} *	0.52 (0.21)	0.96 (0.07)	2.02 (1.19)	0.84 (0.26)	1.01 (0.43)	0.148
INSULIN	$ Fa *	0.16	0.11	0.05	0.16	0.06	0.406
INSULIN	$ Peff *	0.29	0.29	0.29	0.47	0.37	0.406
INSULIN	$ $F_{relative}$	0.09	0.04	0.03	0.04	0.03	0.406
GIR	Cmax*	0.72 (0.04)	0.85 (0.07)	0.97 (0.11)	0.86 (0.07)	0.94 (0.06)	0.967
GIR	AUC *	0.77 (0.03)	0.89 (0.05)	1.04 (0.12)	0.86 (0.07)	0.92 (0.06)	0.953
GIR	T_{max} *	0.80 (0.14)	1.52 (0.3)	1.52 (0.28)	1.16 (0.11)	0.95 (0.09)	0.058

$ No standard error reported since mean insulin curves were used for in silico simulations.
* Star symbol indicates a dimensionless parameter as detailed in section 2.4.3.

plasma concentrations using SIMCYP® software V8.11 (Simcyp, 2007).

Optimization was performed using ADAM (Advanced Dissolution and Metabolism) Optimization model. This is a more sophisticated way than a single compartment absorption model, as it accounts for the interactive time dependencies of absorption, gut metabolism and the pharmacokinetic phenomena. The optimized parameters were then used to calculate the fraction of oral dose absorbed (Fa) from each part of the GIT (Simcyp, 2007).

2.4.3. Dimensional Analysis

Dimensional analysis approach, at individual basis, was used to better monitor and compare pharmacokinetic and pharmacodynamic profiles of the different insulin formulations. This approach is advantageous as it accounts for the intra-individual variabilities in different parameters leading to more accurate and less variable analysis. This was done by the use of the following dimensionless parameters:

$AUC^* = \text{Oral } AUC_{0-360min} / \text{SC } AUC_{0-360min}$
$C_{max}^* = \text{Oral } C_{max} / \text{SC } C_{max}$
$T_{max}^* = \text{Oral } T_{max} / \text{SC } T_{max}$

Fa*= Fraction of oral dose absorbed / Fraction of SC dose absorbed
Peff*=Oral insulin effective membrane permeability/ SC effective membrane permeability

In addition, relative bioavailability was calculated using the equation:

$F_{relative} = (\text{Oral } AUC_{0-360} / \text{SC } AUC_{0-360}) (\text{SC dose} / \text{Oral dose})$

2.4.4 Statistical Analysis

T-test for dependent samples was performed for ccmparison of pharmacokinetic and pharmacodynamic parameters within each formulation after logarithmic transformation. In addition, analysis of variance (nested design) was used for comparison of AUC* and Cmax* between the different formulations after logarithmic transformation. Kruskal-Wallis non parametric test for independent samples was done for comparison of Tmax* between the different formulations. No significant differences were found within each pharmacokinetic and pharmacodynamic parameter, and between the five formulations at 0.05 level of significance.

3. RESULTS AND DISCUSSION

Mean insulin plasma concentrations and mean glucose infusion rates (GIR) after oral and subcutaneous administrations were presented in Figures 1 & 2. Dimensional analysis plot for insulin is presented in Figure 3. Figures 4–6 showed in silico absorption profiles and correlation for best oral formulation. A summary of pharmacokinetic, pharmacodynamic and absorption parameters was presented in Table 2.

As shown in Figures 1 & 2, oral insulin plasma ratio profiles and ratios of glucose infusion rates were close to unity, thus approaching the subcutaneous ones, with more sustainable effect after 270 minutes than subcutaneous dose for 2U/kg oral doses. This suggests continuous insulin release and absorption over time from different parts of GIT as shown in Figures 4 & 5. Dimensional analysis for insulin showed that AUC and Cmax parameter ratios were above 80%. Permeability ratios were similar for 100nm size, but were higher with 57 nm and 220 nm sizes due to either size reduction or formula change respectively. This explains the higher effective permeability ratio (Peff*) in formula 4.

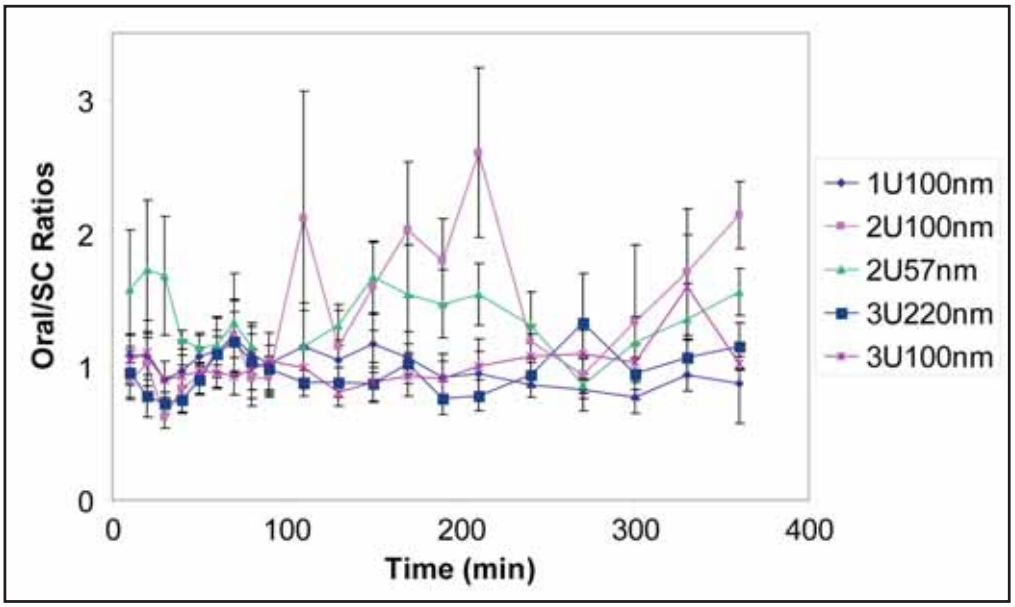

Figure 1. Mean (SE) Oral/ SC Ratios for Insulin plasma levels of the tested formulations specified by dose and particle size.

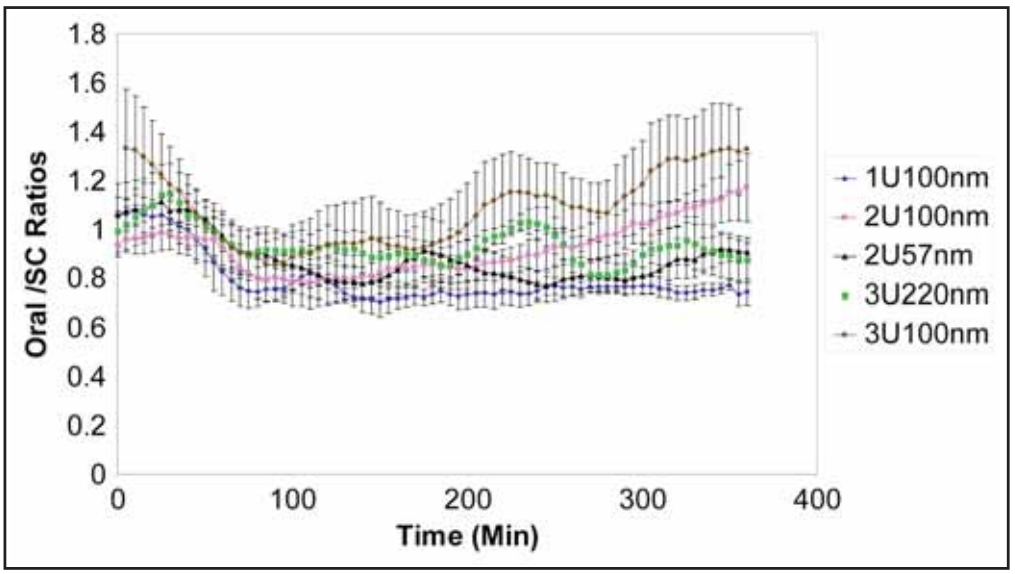

Figure 2. Mean (SE) Oral/ SC Ratios for Glucose Infusion Rate of the tested formulations specified by dose and particle size.

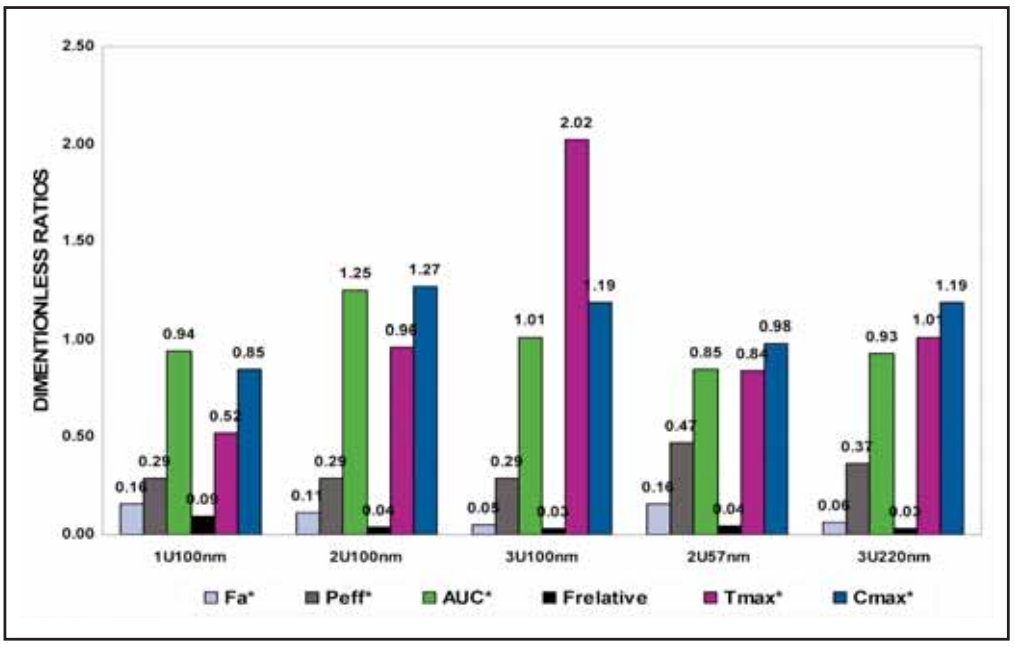

Figure 3: Dimensionless Parameter Ratios Plot for insulin plasma levels of the tested formulations specified by dose and particle size.

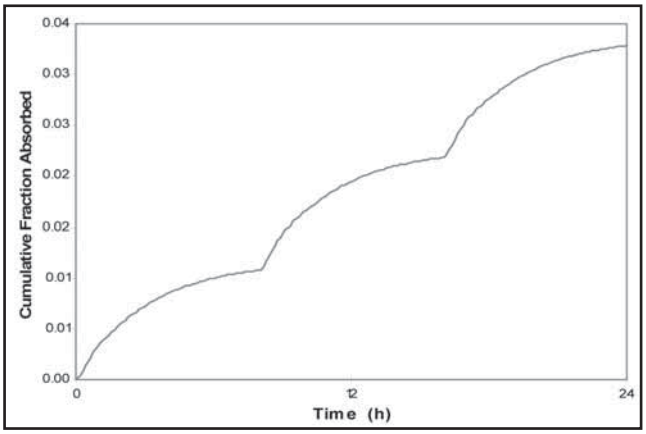

Figure 4: Cumulative Fraction Absorbed of Oral Insulin for a representative Formula: 2U57nm.

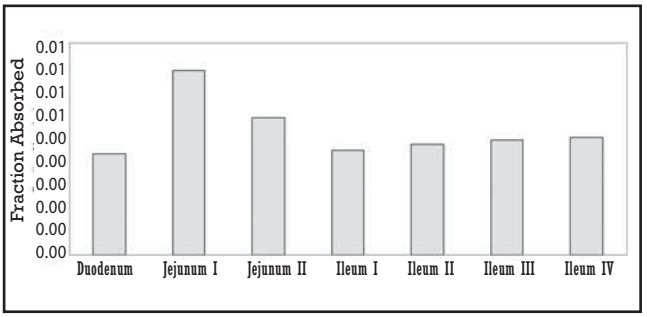

Figure 5: Regional Distribution of the Fraction of Insulin Oral Dose Absorbed for a representative Formula: 2U57nm.

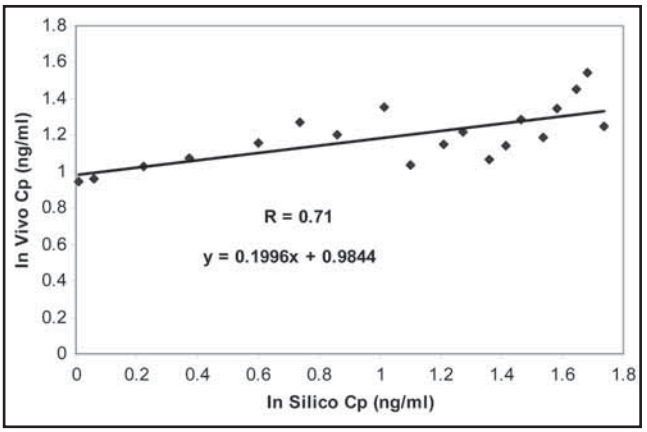

Figure 6: In Vivo – In Silico Insulin Plasma Levels Correlation for a representative Formula: 2U57nm.

The relative bioavailability ratio was highest in formula 1 and decreased by increasing the oral dose suggesting non-linear kinetics. However and for formula 4, this decrease in bioavailability was compensated with the increase in other factors such as Fa*, Peff* and oral dose.

Our simulation, using Simcyp® ADAM model, was shown to produce a good in silico-in vivo correlation (R=0.71) as shown in Figure 6 for formula 4. This supports the determination of absorption parameters obtained by simulation.

Dimensional analysis of the glucose infusion rate as a pharmacodynamic effect showed similar ratio profiles among the tested insulin formulations except formula 1 that exhibited lower ratios which suggested less sustainable effect of formula 1. Formula 4 again exhibited high ratios, and hence support the above findings. More pilot studies are needed to fine tune a final acceptable insulin formulation. Planned studies include a study to test buccal absorption of such formulations, and a mix of some formulations to optimize oral bioavailability.

4. CONCLUSIONS

Subcutaneous product exhibited higher absorption rate and extent than oral insulin, but both show similar insulin plasma levels and similar effect on glucose infusion rates due to the increase in other factors such as Fa*, Peff* and oral dose. Oral insulin formulation bioavailability and action was shown promising for the development of oral insulin product. More pilot studies are needed to fine tune a final acceptable insulin formulation.

5. ACKNOWLAGEMENTS

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REFERENCES

1 American Diabetes Association (1998). Implications of the United Kingdom Prospective Diabetes Study. Diabetes Care. **21**: 2180–2184.
2 Taylor DC, Pownal RI and Burke W (1985). The absorption of -adrenoceptor antagonists in rat in-situ small intestine; the effect of lipophilicity. J. Pharm. Pharmacol. **37**: 280–283.
3 DeFronzo RA, Tobin JD, Andres R (1979). Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol. **237**: E214–E223.
4 Lennernäs H, Ahrenstedt Ö and Ungell AL (1994). Intestinal drug absorption during induced net water absorption in man; a mechanistic study using antipyrine, atenolol and enalaprilat. Br. J. clin. Pharmac. **37**: 589–596.
5 Lennernäs H, Ahrenstedt Ö, Hällgren R, Knutson L, Ryde M and Paalzow LK (1992). Regional jejunal perfusion, a new in vivo approach to study oral drug absorption in man. Pharmaceutical Research. **9**: 1243–1251.
6 Ewe K, Wanitschke R, Staritz M (1994). Intestinal permeability studies in humans. In. T. Z. Csaky (eds.), Pharmacology of Intestinal Permeation II, Springer-Verlag, New York. 535–571.
7 Kinetica® manual, Version 4.1, Innaphase Co., France, 2000.
8 Lauritzen T, Zoffmann V (2004). Understanding the psychological barriers to effective diabetes therapy. Diabetes Voice. **49**: 16–18.
9 Modigliani R, Rambaud JC and Bernier JJ (1973). The method of intraluminal perfusion of the human small intestine. I. Principle and technique. Digestio. 9: 176–192
10 SIMCYP® manual (2007), Version 7.11, SIMCYP Ltd, UK.
11 The absence of a glycemic threshold for the development of long-term complications: The perspective of the Diabetes Control and Complications Trial. Diabetes. (1996) **45**: 1289–1298.
12 Csaky TZ (1984). Intestinal permeation and permeability: an overview. In. Csaky TZ (eds.), Pharmacology of Intestinal Permeation I, Springer-Verlag, New York. 51–59.