Title: Novel Insulin Product Study in Healthy Volunteers with a Transdermally Delivered Human Insulin

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KEY SEARCH TERMS: Insulin, transdermal insulin, needle-free delivery, reducing insulin injections, first-in-class, healthy volunteers

Abstract

Context: To test a more patient-compliant and convenient needle-free method for delivering insulin transdermally.

Objective: To determine whether Insulin can be supplemented transdermally (TD) on a comparable basis with injectable dose forms.

Design: Open label, multiple dose Phase 0 study comparison of response to 3 increasing doses of as measured by serum glucose levels.

Setting: Analytical and formulation laboratory, within a facility with on-site primary care.

Subjects: 5 (5+2) Healthy volunteers ranging in age from 20 to 66 years old, with average serum glucose below 130 and Protein A1c less than 6.5 were screened. 2 were withdrawn from the study dataset.

Interventions: Doses of from 0.075 IU/Kg body weight to 0.15 IU/Kg body weight or 5-6 IUs to 15-16 IUs Human Insulin dosed transdermally via a novel sprayed-on, liquid medium.

Main Outcome Measure: comparison of insulin response to serum glucose as compared to historic studies of response to sub-cutaneous and IV-injected dosing conducted as predicate to the adoption of recombinant HI as the standard of care.

Results: All doses resulted in glucose modulation. Test day conditions included no fasting and dosing approximately 3 hours before the meal, with dosing for the same meal each day. In addition to no hypoglycemia and no skin reactions of any kind, some flattening of serum glucose after initiation of transdermal dosing was observed.

Conclusion: This current project with Human Insulin, in 5 subjects shows response in healthy volunteers similar to that of published studies conducted in the early 90's. The results are believed significant on several accounts. It is clear that administration of the HypoSpray® insulin product to the skin results in a flux of human insulin of sufficient quantity to down-modulate serum glucose in healthy volunteers.

Introduction

Millions of individuals with Types 1, 2 & 3 Diabetes around the world utilize injected insulin to manage their daily variations in serum glucose in an attempt to achieve Normoglycemia and avoid Hypoglycemia.

Heretofore, monomer human insulin at nearly 6,000 Daltons molecular weight was assumed to be beyond the capabilities of any transdermal system however recent research has demonstrated that delivering insulin transdermally is feasible.[1]

This paper describes outcomes of testing 3 dose formulations on a group of healthy volunteers ages ranging from 30 to 66 years old.

Transdermal Delivery Challenges

The history of transdermal drug delivery is focused on the work of Zaffaroni[2], Maibach[3] and others who in the late 1970's demonstrated the possibility of delivering a limited number of small molecules, effective in small doses and leached in by first-order kinetics from barrier-and-reservoir or monolithic drug-in-adhesive systems across intact skin. Scientists at the Langford Research Institute, the William Harvey Institute at Barts, and the London NHS have demonstrated the ability of this system to effectively deliver large drug doses, including small peptide drugs, across intact skin via dissolving the API or ABI in a solution formulated for the bolus of API/ABI. These formulas model certain parameters of the physical chemistry common to substances able to transmigrate intact skin. [4] [5] [6] [7] This is accomplished without a patch, plaster or other device attached to the skin. Doses are delivered by spraying with a metered pump or applying the liquid system directly to the skin from a unit-dose bladder.

Recombinant Human Insulin

Advances in molecular biology and synthetic nucleotide chemistry in the 1980's led to the Bio-reactor fermentation technology for development of mammalian polypeptide hormones including human

Insulin. Collectively, these new developments have given rise to an era of recombinant DNA technology, wherein specific polypeptides can be produced in bacteria or yeast by inserting the appropriate gene structure into the organism's plasmid DNA. The synthesis of the human insulin A- and B-chains by recombinant DNA methods using Escherichia coli fermentations was reported in the 80's by Goeddel et al. from the Genentech Company and the City of Hope National Medical Center.[8] In 2000- Kjeldson described the optimization of expression of proinsulin in the yeast S. cerevisiae [9] which has become the dominant source base for recombinant Human Insulin and the various basal and short acting analogs. The general strategy for preparing human insulin by this approach has been reviewed recently by several investigators.

Materials and Methods

The assumption in the experimental design phase was that, based on the authors' previous work, the system would be relatively efficient at delivering human insulin across intact skin as it employed a formula based on one of the optimum formulae developed from the previous series of experiments(1).

Experimentation involving injecting insulin into healthy volunteers must be approached with due diligence and care and consequently it was determined to dose from the low end of sensitivity.

Early experiments on the bioequivalence of the recombinant insulin describe that Healthy Volunteers can tolerate up to 0.5 IUs per Kg body weight ("KBW") without suffering any deleterious effects. This is roughly double the limit for diabetics. Consequently, early experiments in healthy volunteers started incremental dosing trials at 0.05 to 0.06 IUs KBW. We determined to dose at a level beginning at 0.075 per KBW on day one, 0.1 per KBW on Day 2 and 0.15 per KBW on Day 3.

Transdermal test materials were prepared by Langford Research Institute from a commercially available Human Insulin 500 IU/mL product. The transdermal solution was built to a 50 IU/mL concentration and measured for dose with a 100 IU insulin syringe used to draw and then squirt the insulin onto the skin. Volunteers underwent a screening physical examination and blood analysis using complete metabolic panels which revealed serum glucose and protein A1c. On study days the study nurse took vital signs and digital photographs of the inner aspect of the forearms to which the Insulin product was applied before and at set periods after dosing. The nurse monitored the patients for 45 minutes to 1 hour after dosing to monitor for an adverse reaction. One week after the final dose, the nurse checked vital signs, examined, and photographed the skin to which the transdermal insulin was applied.

Experimental

The study of physiological response to insulin therapy in healthy volunteers is complicated by the multi-variate factors affecting glucose metabolism including diet, exercise, dose schedules and other factors.

The authors determined that we must endeavor to gather both baseline data for comparison before and after dose days and to monitor serum glucose hourly throughout the course of the study days and attempt to control for as many variables as possible in a study of this nature. As Human Insulin has a pharmacodynamic window of 6 hours with peak activity at 3 hours, it was determined to dose at target meal minus 3 hours so if lunch was observed, dosing at 9-10 am would result in maximum effect at 12 to 1pm.

Subjects were dosed on 3 successive days with the increasing doses shown in Figure 1 based on their weight in Kgs as determined at their physical exam.

The charts following depict the potency of insulin and dosing of the various doses for the Subjects.

Figure 1 – Individual Subject doses

Subject	Low dose	Medium Dose	High Dose
S001	8 IUs	11 IUs	16 IUs
S002	5.6 IUs	7.5 IUs	11.2 IUs
S004	6.1 IUs	8.1 IUs	12.2 IUs
S005	7.6 IUs	10.2 IUs	15.3 IUs
S006	6 IUs	8 IUs	12 IUs

A commercially available wearable continuous blood glucose monitor system (FreeStyle Libre 14-Day) was used to monitor blood glucose. 5 of 7 subjects completed the study with sufficient data to include. We also endeavored to maintain a consistent

meal schedule and a standard meal each day although there was some variability in this.

Research Ethics – This research was conducted as Phase 0, Physician-initiated research on an active natural substance. The research was conducted under a fully informed consent status with each Volunteer and under the medical supervision of Dr. William Kirsh, D.O. with the necessary patient safety measures in place. The Study was reviewed and approved by the Langford Institute Institutional Review Board (IORG0006005). The potential risks were reviewed in detail with each subject and it was determined that, given that the doses of insulin (5-16 IUs) were relatively low, the potential benefits achievable outweighed any risk which was agreed to be minimal. Subjects were allowed to withdraw at any time.

Results

Figure 2 is an extract from the FreeStyle Libre 14-Day monitoring software For S005 showing the 3 Dose days. Notations and symbols for "Carbs" and "Rapid-Acting Insulin" are artefactual.

Figure 2 Daily Tracking of Blood Glucose S005

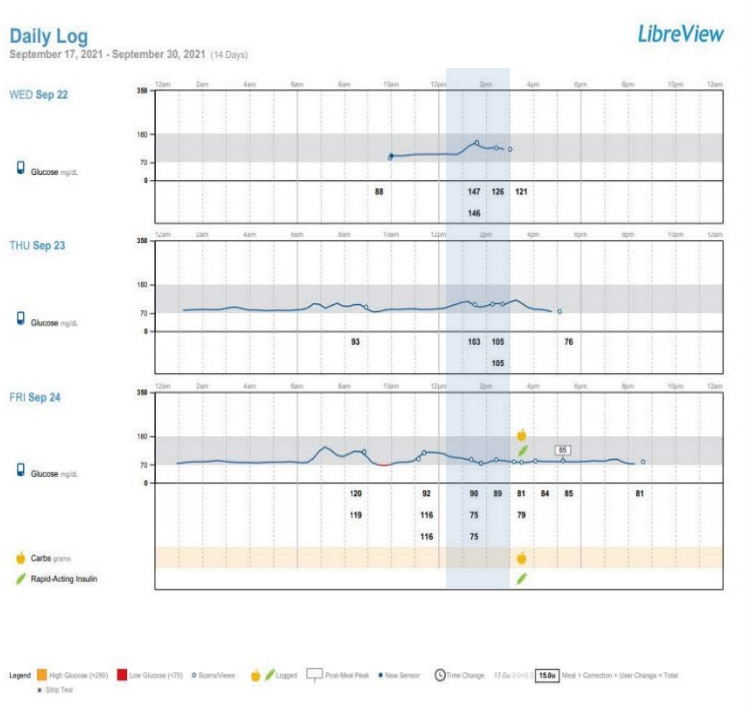

Figure 3 shows performance after dosing against average Daily Glucose and Average PPE

Figure 3

Subject	Average Glucose*	Average PPE**	Dose Day 1	Day	Dose Day 2	Day	Dose Day 3	Day
			Ave G	PPE	Ave G	PPE	Ave G	PPE
S001	102	119	95	91(-3)	95	91(-4)	94	91(-3)
S002	103	133	98	104(+6)	98	103(-5)	101	110(+9)
S004	88	115	85	84 (-1)	85	84 (-1)	85	94 (+9)
S005	87	109	87	111(+21)	93	93 (0)	87	88 (+1)
S006	102	117	105	101(-4)	103	104(+1)	101	104(+3)

*** This is the average for <u>all</u> readings recorded not just the averages shown.**

****This is an average of all available PPEs recorded excluding the 6-hour post dose period**

Subject 3 encountered challenges with the use if the CGM due to his active lifestyle and outdoor work. Consequently his data collection had 8-to-16-hour gaps during his active hours. This was not discovered until after he returned his data reader.

Subject 7 similarly had challenges with his sensor. He dislodged it prematurely on 2 occasions and had 8-to-16-hour gaps due to travel schedules where he left his reader at home.

The Daily Tracking of one of the subjects in Figure 2 shows that

Conclusions

The following summarizes key metrics and findings for the studies.

- All Subjects showed a down modulation of the post-prandial excursion 3-4 hours following initial dosing. Given the known window of efficacy of Human Insulin, data is consistent with a maximum effect at 3-4 hours.
- 5 of 5 Subjects showed a down modulation in average serum glucose as compared with non-dosing days. See Figure 3 above.
- 4 of the 5 Subjects showed a clear response to dose within the 6-hour pharmacodynamic period including the meal as measured by comparing the baseline PPE to the experimental PPE. See Figure 3 above. The fourth Volunteer ate an uncharacteristically high carbohydrate lunch on his 3rd dosing day resulting a slightly higher-than-average, but still lower than his typical 30+ mg PPE.
- There were no incidences of hypoglycemia.
- There were no incidents during or after dosing of skin erythema or other irritation – photographic records were taken pre-dosing, each dose day before and 30 minutes after then at discharge at least one week later.

Discussion

Research conducted in 1979 and 1980 in the U.K.[10] dosed fasted healthy volunteers comparing response to porcine insulin to that arising from dosing recombinant human insulin under Euglycemic clamp conditions. The change in serum glucose varied over 4 hours from -39mg/dL to -20mg/dL at a low dose of 4.8 IUs and from -33mg/dL to -25mg/dL at the higher dose of 9.6 IUs. While obviously not a direct comparison, (Fasting versus not fasting) volunteer response to transdermal dosing can be characterized as in the same order of magnitude.

Summary Conclusion

This current project with insulin, while limited to only five subjects, is believed significant on several accounts. Administration of the HypoSpray® insulin product to the skin results in a flux of human insulin in sufficient quantity to down-modulate serum glucose attributable to post-prandial excursion. Further, the subjects generally had a flattened post prandial excursion when historic data would expect a 30 to 75 mg/dL excursion. Further the system apparently achieves insulin sensitivity equal to responses from injectable products in other studies.

While far from a definitive well-controlled in-patient study, the ability to modulate glucose, including the delivery of commercially prepared products with this system, is believed significant and worthy of further investigation.

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