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

Kenneth B. Kirby; B.A., Langford Research Institute: provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; drafted the article, revised it critically for important intellectual content, gave final approval of the version of the article to be published, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Chandan A.S. Alam, M.D.; Langford Research Institute: provided substantial contributions to analysis and interpretation of data; revised the article critically for important intellectual content, gave final approval of the version of the article to be published, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Atholl Johnston, Ph.D.; .Barts & The London NHS: provided substantial contributions to analysis and interpretation of data; revised critically for important intellectual content, gave final approval of the version of the article to be published, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

William Kirsh, D.O,; TD Solutions: provided substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; revised the article critically for important intellectual content, gave final approval of the version of the article to be published, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Gordon von Nehring, Ph.D.; Langford Research Institute : provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; drafted the article, revised it critically for important intellectual content, gave final approval of the version of the article to be published, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosure: Kenneth Kirby and Chandan Alam are shareholders in Transdermal Delivery Solutions Corp.

The authors have no other conflicts of interest to disclose.

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

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 Human Insulin 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? 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 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 measured by comparison to baseline within-subject normal serum glucose as well as post-prandial response. 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.

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.

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.

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 diluted with HDS delivery solution. The

transdermal solution was prepared by diluting to a 50 IU/mL concentration and measured for dose with a 100 IU insulin syringe used to draw and then dispense the insulin delivery solution 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 and adverse reaction. On 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 with the doses shown in Figure 1 based on their weight in Kgs as determined at their physical exam.

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

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

Figure 2 Daily Tracking of Blood Glucose S005

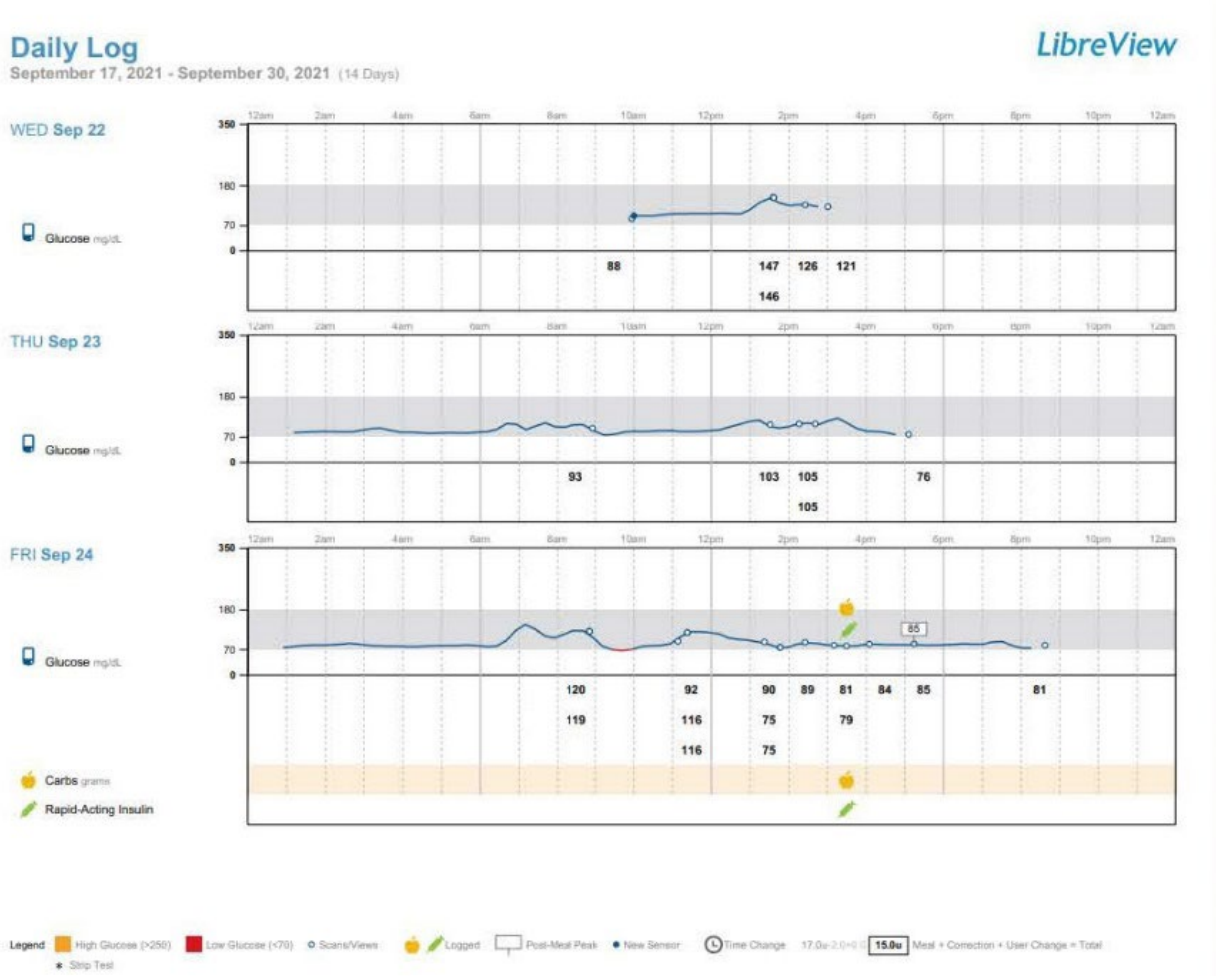

Figure 3 shows pre dose: average Daily Glucose (G Avg), Average Post Prandial Excursion PPE Avg), and post dose: G AVG, and Post Prandial Excursion (PPE)

Figure 3

Subject	Pre Dose Period		Dose Day 1	Day	Dose Day 2	Day	Dose Day 3	Day
	G Avg*	PPE Av**	G Avg	PPE	G Avg	PPE	G Avg	PPE
S001	102	119(+17)	95	91(-3)	95	91(-4)	94	91(-3)
S002	103	133(+30)	98	104(+6)	98	103(-5)	101	110(+9)
S004	88	115(+27)	85	84 (-1)	85	84 (-1)	85	94 (+9)
S005	87	109(+22)	87	111(+21)	93	93 (0)	87	88 (+1)
S006	102	117(+15)	105	101(-4)	103	104(+1)	101	104(+3)

*** This is the average for all glucose readings recorded pre dosing.**

****This is an average of all pre dose PPEs recorded, excluding PPE during 6-hour post dose period**

Statistical Analysis

Two measures were analyzed: Average Daily Serum Glucose, baseline vs. Average Glucose on Insulin Dose Days and Daily Average post-prandial response vs. Baseline. Figures 4 and 5 present the data and the resulting p values and confidence intervals for Daily Average Glucose and Post prandial response.

Figure 4

	Baseline Ave Glucose	Dose Day 1	Dose Day 2	Dose Day 3	3-Day Ave	P Value	Conf. Interval
S1	102	95	95	94	95	0	0.05
S2	103	98	98	101	99	0.000003	0.05
S4	88	85	85	85	85	0.000002	0.05
S5	87	87	93	87	89	0.000035	0.05
S6	105	103	101	101	103	0.000004	0.05

Figure 5 presents the Dose Day daily average post prandial excursion vs baseline. Positive and negative values represent values relative to Dose Day average glucose readings

Figure 5

	Baseline PPE Glucose	Dose Day 1	Dose Day 2	Dose Day 3	3-Day Ave
	+119	-20	-24	-25	-23
P Value		0.000016	0.000016	0	0.000174
CI		0.05	0.05	0.05	0.05

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.

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 dosing
- 5 of 5 Subjects showed a down modulation in average serum glucose as compared with non-dosing days. See Figure 4 Above.
- 4 of the 5 Subjects showed a clear dose response within the 6-hour pharmacodynamic period including the meal. See Figure 3 above. The fifth Volunteer ate an uncharacteristically high-carbohydrate lunch on his 3rd dosing day resulting a slightly higher-than-average, but still lower than his typical 35+ 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
- While obviously not a direct comparison to historical SC administration of insulin to healthy volunteers (Fasting versus not fasting), volunteer response to transdermal dosing can be characterized as of 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® human 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 consistent with clinical experience of healthy volunteers in earlier research. 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.

Bibliography

[1] Kirby K.B., Alam CA., Johnston, A., Kirsh, W., von Nehring, G; Novel Insulin Replacement Therapy with a Transdermally Delivered Human Insulin Product, 2019 Publication Pending

[2] Zaffaroni, Alejandro; Applications Of Polymers In Rate-Controlled Drug Delivery.; In: Gebelein C.G., Koblitz F.F. (eds) Biomedical and Dental Applications of Polymers. Polymer Science and Technology, vol 14. Springer, Boston, MA.

3 Feldmann RJ, Maibach HI. Regional variation in percutaneous penetration of 14C cortisol in man.; J Invest Dermatol. 1967 Feb;48(2):181-3

4 Chik Z., Johnston A., Tucker A. T, Chew, S., Alam C.A.,L., Michaels L., "Pharmacokinetics of a new testosterone transdermal delivery system, TDS®-testosterone in healthy males" Brit J of Clin Pharmacol, DOI:10.1111/j.1365-2125.2005.02542.x

5 Tucker A. T., Chik Z., Michaels L., Kirby K., Seed M. P., Johnston A Alam C.A.,.; "Study of a combined percutaneous local anaesthetic and the TDS system for venepuncture" Anaesthesia, Anaesthesia, 2006, 61, pages 123–126

6 Chik Z., Johnston A., Kirby K., Tucker A.T. and "Correcting endogenous concentrations of testosterone influences bioequivalence and shows the superiority of TDS®-testosterone to Androgel®" Int J Clin Pharmacol Ther. 2009 Apr;47(4):262-8

[7] Chik, Z BSc MPhil Johnston A., Tucker A. T., Chew S. L., Michaels L. Pharmacokinetic studies for the development of transdermal drug delivery systems. Thesis is submitted for the degree of Doctor of Philosophy of the University of London

[8] Chance, Ronald E.; Kroeff, Eugene P.; Hoffmann, James A., & Frank, Bruce H.; Chemical, Physical, and Biologic Properties of Biosynthetic Human Insulin; Diabetes Care, Vol. 4 No. 2, March-April 1981

[9] Kjeldsen T. Yeast secretory expression of insulin precursors. Appl Microbiol Biotechnol. 2000 Sep;54(3):277-86. doi: 10.1007/s002530000402. PMID: 11030562

[10] Keen, Harry; Pickup, J. C.; Bilous, R. W.; Glynne, A.; Viberti G. C.; Jarrett, R. J.; Marsden, R.; Human Insulin Produced By Recombinant DNA Technology: Safety And Hypoglycemic Potency In Healthy Men, The Lancet, August 23rd, 1980.

CME Exam

1. **A fundamental goal of insulin replacement therapy is to:**
   A. Avoid peaks and troughs of blood glucose
   B. Restore normal physiological insulin secretion patterns
   C. Avoid Hypoglycemia
   D. All of the above

2. **Human insulin is assumed:**
   A. To be effective at various peptide lengths
   B. To be equally effective delivered via auto injector or insulin syringe
   C. To be too large to deliver transdermally
   D. All of the above

3. **According to experiments reported in this article:**
   A. Human Insulin and fast-acting Novolog® have been effective delivered in a patch
   B. Human Insulin and Humulin R 500 have been effective delivered across intact skin in a solution sprayed onto the skin
   C. Human Insulin and fast-acting Novolog® have been effective delivered by SC injection
   D. Human Insulin has been effective at blunting pot-prandial excursions in healthy volunteers when delivered in a solution sprayed onto the skin.

4. **It is clear that administration of the HypoSpray® insulin product to the skin results in:**
   A. No flux of human insulin in sufficient quantity to down-modulate serum glucose
   B. No achievement of insulin sensitivity equal to injectable products
   C. A. & B.

D. None of the above

Answers:

1. D., All of the above
2. D., All of the above
3. D., Human Insulin has been effective at blunting pot-prandial excursions in healthy volunteers when delivered in a solution sprayed onto the skin
4. D., None of the above

