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ORIGINAL ARTICLE

Open-label study to assess the safety and pharmacodynamics of five oral insulin formulations in healthy subjects

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Aim: Orally delivered insulin is predicted to bear therapeutic advantages in diabetes management when compared to injectable insulin, because of its ability to mimic the natural route of endogenous insulin secreted by the pancreas into the portal vein and directly to the liver. Oramed Pharmaceuticals is developing an oral insulin product which consists of unmodified recombinant human insulin combined with adjuvants that protect it from enzymatic degradation in the gastrointestinal tract and promote its absorption from the gut. The aim was to determine the optimal adjuvants to insulin ratio which can provide for the best pharmacodynamic profile, while maintaining the safety of the product.

Methods: Eight healthy, male volunteers participated in this open-label study which included five independent visits. During each visit, subjects were administered one of the five encapsulated oral insulin formulations which contained equal amounts of insulin but varying proportions of adjuvants. Parameters measured included safety, C_{max} and T_{max} for insulin and C_{min}, T_{min} and area under the curve (AUC) for glucose and c-peptide. Comparisons were made between formulations and between post-treatment time periods within each visit.

Results: All five oral insulin formulations were well tolerated and no serious adverse events were reported. All formulations resulted in a significant response in the response period (60–300 min) in comparison to baseline (0–60 min); this was captured both in the c-peptide response and the glucose response (all five formulations $p < 0.05$). However, none of the formulations turned out significantly different in response over the other. Formulation 5 showed the most profound reduction in c-peptide when AUC_{0-60} (baseline) was compared to AUC_{60-300} ($p < 0.007$).

Conclusions: All five oral insulin formulations resulted in glucose and c-peptide reductions, where formulation 5 demonstrated the most pronounced effect on c-peptide concentration reduction. This formulation was deemed the lead formulation to be advanced to future clinical studies. This study also reinforces the notion that oral insulin can maintain its biological activity after delivery, suggesting a potential role for this product in management of diabetes.

Keywords: diabetes, glucose, hepatic glucose metabolism, oral absorption

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Introduction

Inadequate control of hyperglycaemia in patients with type 2 diabetes is associated with an increased risk of complications, all of which can be mitigated or prevented by timely intervention [1–4]. Despite the recognition of the importance of glycaemic control by both care givers and patients, this goal has remained elusive for a significant proportion of diabetics. Early in the course of the disease, life style interventions including diet and exercise and/or oral antidiabetic drugs are effective in improving glycaemic control; however, as the disease progresses, these modalities are likely to fail and parenteral insulin therapy is then required [5–7]. Because currently available insulin is administered by injection, it has often been relegated as a last resort therapy, despite the fact that it remains the most effective drug in achieving treatment goals [8]. Replacement of standard injectable insulin by an oral insulin product could potentially provide enormous benefits for people with diabetes. More specifically, oral insulin can provide a basis for earlier intervention and potentially promote patient compliance and adherence, thereby improving treatment outcome. Furthermore, oral insulin may be advantageous over its parenteral counter product in that it mimics the natural route of endogenous pancreatic insulin secretion directly to the portal-hepatic circulation. In this manner, orally administered insulin maintains the physiologic portal–peripheral gradient while minimizing incidence of hyperinsulinaemia. In contrast, parenteral insulin is absorbed directly into the peripheral circulation without initial hepatic extraction, leading to peripheral hyperinsulinaemia relative to the portal circulation.

Oramed Pharmaceuticals has developed an oral insulin dosage form based on its proprietary technology, which

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combines unmodified human recombinant insulin with adjuvants designed to protect insulin from enzymatic degradation in the gastrointestinal tract and to facilitate its absorption through the epithelial lining of the gut. The current study aimed at testing the safety and pharmacodynamics (PD) of five different formulations of oral insulin in healthy volunteers in an effort to identify a lead formulation to be advanced for further clinical application.

Methods

This study was conducted in compliance with the declaration of Helsinki and good clinical practice standards. The study protocol and informed consent were approved by Hadassah Medical Center Internal Review Board.

Study Design

Eight healthy male volunteers (ages 25–31, BMI 19–30) participated in this open-label study which included five independent visits, where each was separated by a 72–96-h wash-out period. The studies were conducted on the morning after an overnight fast. During each visit, subjects were administered one of the five encapsulated oral insulin formulations. All of the formulations contained equal amounts of insulin (8 mg) with varying adjuvant proportions (table 1).

Individual blood samples (29 totals) were taken at predefined time points for up to 5 h after dosing to allow for PD analysis. Venous blood samples were collected in EDTA blood collection tubes, centrifuged and the plasma was separated and frozen for insulin and c-peptide radioimmunoanalysis (Linco Research, St. Charles, MO). Concurrent glucose concentration measurements were assessed by glucometer (BREEZE®2 Blood Glucose Monitoring Systems, Bayer) and average results of two glucometer readings at each time point were calculated.

Parameters measured included safety, and C_{max} and T_{max} for insulin and C_{min}, T_{min} and area under the curve (AUC) for glucose and c-peptide. Comparisons were made between formulations and between time periods within each formulation. Paired *t*-tests were used to assess changes in glucose and c-peptide concentrations from baseline. Differences were considered significant at $p < 0.05$. All statistics were calculated with SPSS statistical software and AUC was calculated using a dedicated Excel procedures. AUC_{0-60}, AUC_{60-300} and AUC_{0-300} were calculated for glucose and c-peptide using the trapezoidal rule and are presented as percentages to account for the differences between the baseline (0–60 min) and response (60–300 min) periods. The baseline period was arbitrarily chosen and is based on the fact that Oramed's capsules are pH-sensitive and only disintegrate at compatible pH levels of the small intestines, after a minimal duration of 90 min.

Table 1. Composition of oral insulin formulations

Visit	Carrier (mg)	$Adjuvant_A$ (mg)	$Adjuvant_B$ (mg)	Capsule size (ml)
1	150	125	24	1
2	100	100	24	1
3	100	100	24	0.5
4	150	75	24	1
5	150	75	24	0.5

Results

All five oral insulin formulations were well tolerated by the volunteers, and no serious adverse events were reported. Administration of an oral dosage form of insulin to healthy, fasting volunteers induced a significant reduction in c-peptide levels ranging from 27 to 90% (table 2), where the formulations administered on visits 4 and 5 induced the most prominent reductions with an average percent decrease of 57 and 43%, respectively (figure 1). Blood glucose concentrations dropped by 11–35% (table 2) in the majority of volunteers at all five visits, with the most pronounced effects observed on visit 5 (table 2). Analyses of mean c-peptide AUC_{60-300} demonstrated significantly lower (6.1–14%) ratios for oral insulin-treated subjects in comparison to their AUC_{0-60} responses ($p = 0.001–0.005$) (figure 1). Similarly, post-treatment glucose measurements demonstrated 3.3–5.8% lower mean AUC_{60-300} values when compared the AUC_{0-60} estimations ($p = 0.007–0.042$) (figure 1). On average, minimal glucose and c-peptide concentrations (C_{min}) were attained within 90–300 min of capsule administration (all five formulations $p < 0.05$).

In comparisons between formulations, administration of an oral dosage form of insulin to healthy, fasting volunteers induced a significant reduction in c-peptide levels and plasma glucose concentrations values between baseline (0–60 min) and response periods (60–300 min) ranging from 27 to 90% and 11 to 35%, respectively ($p < 0.05$). However, none of the formulations turned out significantly different in response over the other (table 2). In comparing between time periods within each formulation, the formulations administered on visits 4 and 5 induced the most prominent reduction in c-peptide and glucose concentrations (table 2). In formulation 5, when comparing c-peptide AUC_{60-300} values to c-peptide AUC_{0-60}, a mean reduction of 14 ± 9.5% was observed ($p < 0.005$). On average, minimal glucose and c-peptide concentrations (C_{min}) were observed after 90 min and the average duration of observed effect extended over 70 min, after which levels typically returned to preadministration values within the 300-min monitoring session.

Comparison of insulin between formulations and within formulations did not show a significant difference as is expected for hormones undergoing first-pass hepatic metabolism with up to 80% extraction. However, 11 out of 40 total visits demonstrated insulin peaks characterized by a 1.5–2.4-fold increase from basal readings. Such peaks were observed within approximately 120 min of drug administration and rapidly returned to baseline thereafter.

Discussion

The objective of this study was to evaluate the safety, tolerability and PD of five encapsulated oral insulin formulations. Each

Table 2. Individual glucose and c-peptide responses following treatment of healthy subjects with ORMD-0801

	Visit 1		Visit 2		Visit 3		Visit 4		Visit 5	
	Glucose‡	c-Peptide‡	Glucose	c-Peptide	Glucose	c-Peptide	Glucose	c-Peptide	Glucose	c-Peptide
	$C_{basal} - C_{min}$, T_{min}‡	$C_{basal} - C_{min}$, T_{min}	$C_{basal} - C_{min}$, T_{min}	$C_{basal} - C_{min}$, T_{min}	$C_{basal} - C_{min}$, T_{min}	$C_{basal} - C_{min}$, T_{min}	$C_{basal} - C_{min}$, T_{min}	$C_{basal} - C_{min}$, T_{min}	$C_{basal} - C_{min}$, T_{min}	$C_{basal} - C_{min}$, T_{min}
Subject 1	$4.6 - 4.0, 190'$	NR*	NR*	NR	$4.8 - 4.2, 120'$	$1.4 - 0.8, 190'$	$4.2 - 2.8, 200'$	$1.1 - 0.1, 230'$	$5.0 - 3.3, 80'$	$4.0 - 2.1, 260'$
Subject 2	NR	$2.8 - 2.2, 270'$	$4.7 - 3.8, 180'$	$3.2 - 2.6, 140'$	$5.2 - 3.9, 150$	$2.5 - 1.2, 260'$	NR	$1.9 - 1.4, 190'$	$5.1 - 3.8, 230'$	$3.5 - 2.3, 300'$
Subject 3	$5.6 - 4.5, 140'$	$2.0 - 1.2, 130'$	$5.6 - 5.1, 90'$	$2.2 - 1.4, 130'$	$5.0 - 4.2, 110'$	$1.6 - 1.1, 160'$	$4.9 - 4.3, 160'$	$2.0 - 1.0, 150'$	$6.0 - 4.9, 80'$	$5.2 - 3.0, 150'$
Subject 4	$6.2 - 4.6, 200'$	$2.3 - 1.1, 200'$	$5.7 - 4.8, 95'$	$2.0 - 1.5, 250'$	$5.8 - 4.9, 220'$	$1.6 - 1.2, 230'$	$4.9 - 4.2, 260'$	$2.8 - 1.7, 220'$	NR	$2.2 - 1.6, 160'$
Subject 5	$5.2 - 4.3, 250'$	NA†	NR	NR	$5.3 - 4.6, 120'$	$1.7 - 1.5, 180'$	NR	NR	NR	$1.5 - 0.9, 200'$
Subject 6	$4.9 - 3.6, 120'$	$1.2 - 0.5, 160'$	$4.6 - 3.7, 140'$	$1.8 - 1.0, 280'$	$4.6 - 3.5, 170'$	$1.6 - 0.8, 170'$	$4.5 - 3.3, 140'$	$1.5 - 0.3, 140'$	$5.1 - 3.4, 180'$	$2.0 - 0.8, 190'$
Subject 7	$4.7 - 4.0, 170'$	$1.6 - 1.2, 220'$	$4.6 - 4.1, 190'$	$1.6 - 1.1, 250'$	NR	$2.2 - 0.8, 300'$	$5.5 - 4.0, 160'$	$1.4 - 0.6, 160'$	NR	$1.8 - 1.4, 170'$
Subject 8	$4.6 - 3.9, 170'$	$1.6 - 1, 210'$	NR	$2.0 - 1.1, 180'$	$4.7 - 3.7, 110'$	$1.3 - 0.8, 180'$	NR	$1.4 - 0.6, 160'$	$4.5 - 3.4, 150'$	$1.3 - 0.4, 170'$
Mean per cent change C_{min}/C_{basal}	19 ± 5.6	39 ± 4.5	15 ± 4.8	33 ± 10.4	18 ± 5.0	39 ± 16.6	23 ± 9.1	57 ± 22.4	27 ± 6.7	43 ± 15.9
Mean $AUC_{60-300} - AUC_{0-60}$ (%)	-5.8 ± 4.8	-9.2 ± 4.5	-3.3 ± 3.8	-6.9 ± 4.8	-4.6 ± 3.5	-6.1 ± 3.8	-3.7 ± 3.3	-8.7 ± 4.1	-3.5 ± 3.8	-14.0 ± 9.5

*NR, no response, set at $C_{min} < 5\%\ C_{basal}$.
†NA, not available.
‡Glucose units, mmol/L; c-peptide units, ng/ml; T_{min} units, min.

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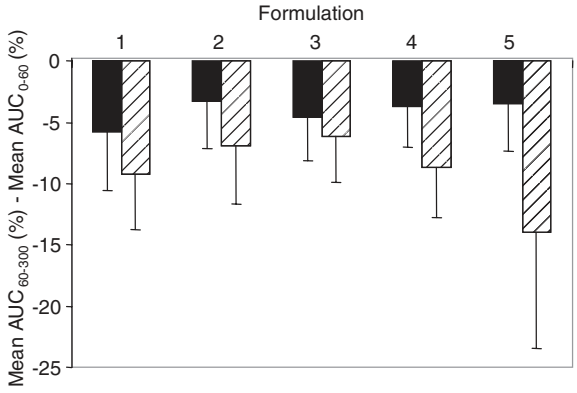

Figure 1. Response vs. baseline glucose and c-peptide levels. AUC_{0-60} and AUC_{60-300} were calculated for glucose and c-peptide readings as recorded after treatment with a given formulation. Per cent AUC_{0-60} and AUC_{60-300} of the maximal AUC_{0-300} was computed and the differences between mean AUC_{60-300} and AUC_{0-60} were plotted for glucose (black bars) and c-peptide (striped bars) for each formulation (± s.d.). Negative differences indicate response to treatment after 60 min.

formulation contained equal amounts of insulin prepared with varying proportions of antiproteolytic adjuvants [9]. Specifically, the intent of these trials was to identify the modifications in adjuvant ratios to yield maximal safety and efficacy. In the context of this study, all five formulations were well tolerated by all subjects with no serious adverse events including severe hypoglycaemia. However, the formulation of visit 5 outperformed the other tested formulations by inducing significant declines in c-peptide levels, and was consequently chosen as the lead formulation to be further advanced in future clinical studies.

Hepatic uptake of insulin is highly variable and can exceed 80% of the portal delivered insulin leaving minimal fractions for entry into the systemic insulin pool [10]. Thus, plasma insulin levels reflect poorly on the insulin absorption from oral dosage forms because of the hepatic first-pass effect. Direct sampling of the portal vein, a both invasive and impractical technique, would offer the most accurate method of estimating insulin absorption from the gut. Thus, rather than focusing on insulin absorption and bioavailabilty, an emphasis was placed on insulin pharmacodynamic effects by monitoring glucose and c-peptide plasma concentrations. In contrast to insulin, c-peptide, while co-secreted in equimolar concentrations to insulin, is not metabolized by the liver and provides for an indirect, but relatively reliable estimate of endogenous insulin secretion and exogenous insulin absorption [11–13]. Indeed, in the present study, oral administration of insulin led to a significant reduction in c-peptide levels and a concomitant glucose concentration reduction (figure 1). Because of the considerable hepatic insulin extraction from the portal vein on first pass, almost all of the ingested insulin will affect endogenous hepatic glucose production and have only a small effect on glucose disposal in the periphery, which account for the modest glucose reductions seen in this study.

The oral route of drug administration remains the safest and most practical, and one which promotes the highest level of compliance among patients. The oral–portal insulin delivery has the additional benefit of replicating the physiological pathway of endogenous insulin secretion while avoiding peripheral hyperinsulinaemia and hypoglycaemia as observed in this study [14–16]. The latter advantage may be the result of improved portal insulin to glucagon ratios and/or preservation of counter-regulatory responses to hypoglycaemia. Similarly, a decreased risk of hypoglycaemia has also been observed in numerous studies where insulin was either administered directly to the portal vein or indirectly by way of peritoneal insulin administration or through peritoneal dialysates. Avoidance of hypoglycaemia is also regularly reported after islet cell and pancreas transplants, whereby the insulin is secreted directly into the portal circulation [17–19].

Rapid enzymatic degradation of orally administered proteins in the stomach or their digestion and inactivation by proteolytic enzymes in the intestinal lumen have stood as the most significant impediment to their oral delivery. Novel drug delivery technologies that protect proteins from degradation and promote their absorption across the gastrointestinal lining are being advanced [2,20,21]. Oramed's technology offers a technology platform whereby the antiproteolytic and absorption enhancement are provided by inert and safe pharmacopoeial adjuvants in contrast to the new chemical entities used by other companies.

The encouraging results of the current study have provided the impetus to advance formulation 5 to future studies that will determine oral insulin's effect in diabetic patients, the effect of food on its oral absorption and the proximity at which it can be given in relation to a meal.

Conclusions

In addition to offering the advantage of replicating the natural route of insulin secretion and absorption, an oral insulin product may be the best instrument to encourage early intervention and to foster adherence and compliance among diabetics, thereby improving glycaemic control. In this study, five different oral insulin formulations were assessed for their effect on insulin absorption and glucose and c-peptide level reductions. All tested formulations resulted in glucose and c-peptide reduction, where formulation 5 demonstrated an advantage over the others in terms of its effect on c-peptide reduction. This formulation was deemed the lead formulation to be advanced into future clinical studies.

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Conflict of Interest

Dr. M. Kidron is the Chief Scientific Officer for Oramed Pharmaceuticals and a share holder in the company. Dr. Arbit is the Director of Medical Research at Oramed Pharamcauticals. Dr. R. Eldor is Medical Advisor to Oramed Pharmaceuticals, a staff physician at the Hadassah Medical Center and the Principal Clinical Investigator for this study.

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