



The Effect of Nanohydrogel Oral Insulin Therapy on Serum Glucose and Insulin Level in Patients with Type 2 Diabetes: A Pilot Study

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ABSTRACT

Background: This pilot study was designed to investigate the effect of orally delivered nanohydrogel insulin on serum glucose and insulin levels in patients with type 2 diabetes (T2DM).

Methods: In this pilot before-after study, 8 T2DM patients received 300 IU insulin loaded nanohydrogel orally and the serum concentration of glucose and insulin were measured before treatment and consecutively during treatment. The area under the curve (AUC) was calculated for serum glucose and insulin and repeated measures of ANOVA, paired t-test were used for statistical analysis.

Results: The changes in serum glucose level was not significant pretreatment ($p=0.10$) and during oral insulin treatment ($p=0.71$). Compared with pretreatment value, the serum glucose level was significantly lower in 7 A.M. (immediately after insulin therapy) and 8.30 A.M. during oral insulin treatment. The maximum reduction was observed 1.5 hours after insulin therapy and its effect lasted for more than 6 hours. The mean AUC of glucose was insignificantly decreased ($p=0.16$). The pretreatment ($p=0.10$) and during treatment ($p=0.30$) changes in serum insulin level were not statistically significant. The serum insulin level was increased significantly immediately after oral insulin therapy compared with pretreatment values. The mean AUC of insulin increased marginally significant ($p=0.056$).

Conclusion: The results of the present pilot study showed that 300IU nanohydrogel oral insulin was effective in rapid lowering of serum glucose and its glucose lowering effect lasting for more than 6 hours. However, for any precise conclusions further studies with longer duration are needed.

Introduction

Diabetes mellitus is a common problem nowadays and its prevalence is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. Diabetes is caused by decreased production of insulin or by decreased ability to use insulin and as a results, the blood glucose level increases^{1,2}.

The most effective drug for diabetic patients to control their blood glucose levels is insulin. The usual route for insulin delivery is subcutaneous injections. However, due to the inconvenience of the subcutaneous administration of insulin injection, various attempts have been made by biotechnologist to develop an efficient and

noninvasive method of insulin delivery. Oral administration of insulin is one of the interesting methods that enter insulin in the blood through the gastrointestinal tract. According to inactivation of insulin by proteolytic enzymes in the gastrointestinal tract and low permeability through the intestinal membrane, scientist use different carriers for insulin. Hydrogels have been shown to be potential candidates for such a system^{3,4}. The pH responsive hydrogels have been studied as drug carriers for the protection of insulin from the acidic environment of the stomach, before releasing in the small intestine⁵.

In an animal study, insulin was administered orally to healthy and also diabetic rats through pH

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responsive hydrogels. Within 2 hours of administration of oral insulin, strong hypoglycemic effects were observed in both healthy and diabetic rats. These effects lasted up to 8 hours after administration⁵.

In another study, the ability of the insulin loaded hydrogels to decrease the blood glucose levels of the diabetic rats was investigated. The blood glucose level has been decreased in rats that received the insulin loaded hydrogel. The glucose lowering effect of the insulin loaded hydrogel lasted for 8 to 10 hours⁶.

Insulin loaded Nanohydrogel was developed by merging the advantages of two methods (nanoencapsulation and temperature sensitive property of smart hydrogels), to obtain a nano system for passing the stomach and release insulin in intestine for oral delivery of insulin⁷. In the previous study, the pH sensitive nanohydrogels containing human insulin was developed and the amount and rate of insulin releasing from nanohydrogels in different pH environments were assessed in order to condense in the gastric pH for insulin protection and decondense in intestine pH⁷. However, there is no human study which assesses the effectiveness of insulin loaded nanohydrogel in diabetic patients. In this regard, this pilot study was designed to investigate the impact of orally delivered nanohydrogel insulin on serum glucose and insulin level in patients with type 2 diabetes.

Materials and Method

Study design

A single-center, open label study was used to determine the glucose lowering properties of a nanohydrogel oral insulin (OI) formulation. The protocol was approved by an ethics committee of Tabriz University of medical sciences, and the study was performed in accordance with the Helsinki Declaration. A total of 8 adult patients (2 females and 6 males) diagnosed with type 2 diabetes (T2DM) for over a year and without insulin therapy were screened after providing written informed consent. The male and female type 2 diabetes patients aged 30-65 years, regularly treated with metformin and sulfonylurea and otherwise healthy were included in the present study.

T2DM had been diagnosed earlier by each patient's physician, by means of clinical characteristics, namely, complete insulin dependence and brittle glucose profiles. Each patient received appropriate dietary and physical activity recommendation before the study and they were asked to follow these recommendations throughout the study. Patient demographics and baseline diabetes-related profiles are summarized in Table 1.

The pH sensitive human insulin loaded nanohydrogel was prepared in medical

nanotechnology department, faculty of advanced medical sciences, Tabriz University of medical sciences. The details of drug preparation and also the amount and rate of insulin releasing in different pH conditions were presented previously⁷.

Table 1. Demographic characteristics of participants (n=8).

Variables	Mean±SD
Age (years)	54.33±10.12
BMI (kg/m ²)	28.66±3
Total Cholesterol (mg/dl)	200.3±39.87
Triglyceride (mg/dl)	156±59.8
LDL-C (mg/dl)	123±25.93
HDL-C (mg/dl)	38±7.05
HbA1C (%)	9.27±2.12
Systolic Blood Pressure (mmHg)	117±8.3
Diastolic Blood Pressure (mmHg)	76±4.8

BMI: Body Mass Index; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; HbA1C: Hemoglobin A1c

Briefly NIPAAm–MAA–HEMA copolymer was synthesized by radical chain reaction with 80:8:12 ratios respectively. Reactions were carried out in condition including 1, 4–Dioxan solutions under Nitrogen gas–flow. For preparation of poly NIPAAm –MAA–HEMA nanohydrogel, N, N–MBAAm was used as cross linker. Benzoyl peroxide was added as initiator of polymerization.

At first 2 days of the study, the baseline levels of glucose and insulin was detected and patients were asked to eat a dietitian defined breakfast at 7 A.M., defined lunch at 1 P.M. and defined dinner at 7 P.M.. At the third day the patients were asked to eat defined breakfast, lunch and dinner with the same pattern. Additionally, they all received the 300 IU insulin loaded nanohydrogel at 6.45 am before eating breakfast. The blood samples were drawn for glucose and insulin measurements at 6.45 am (as a baseline value), 7 A.M., 8.30 A.M., 10 A.M., 11.30 A.M., 1 P.M., 7 P.M., 12 P.M.. At time 0 (6.45 A.M.) baseline blood samples were drawn for HbA1c, cholesterol, triglyceride, glucose, and insulin measurement. For this, after a 12-hour overnight fast, 5 ml blood sample was obtained. The serum was separated from whole blood by centrifugation at 2000 rpm for 10 min at room temperature. Lipid profiles were measured on the day of sampling. The levels of serum Total Cholesterol (TC), high-density lipoprotein (HDL-C) and triglyceride (TG) were measured by enzymatic colorimetric methods. The commercially available kit (Pars Azmone, Tehran, Iran) on an automatic analyzer (Abbott, model Alcyon 300, USA) was used in this regard. Serum LDL-C was calculated by Friedewald equation. Blood glucose and insulin levels were determined by glucometric and chemoluminescence methods (DiaSorin, Liaison, Italy) respectively.

Statistical analysis

Area under the curve for serum insulin concentration was calculated by the trapezoidal rule. Insulin and glucose values were corrected for the baseline values by subtracting the mean insulin or glucose concentrations in the last hour before drug administration from all post administered values. SPSS v.18 statistical computer software was used for all statistical analysis. The mean \pm SE was used for descriptive analysis. The Kolmogorov smirnov test was used to normality analysis. The repeated measures ANOVA was used to evaluate the trend of serum glucose and insulin concentration. The paired sample t-test was used to compare the serum glucose and insulin level pretreatment and during treatment of oral insulin. $P < 0.05$ was considered to be statistically significant.

Results

The demographic characteristics of participants were shown in table 1. The mean age of participants was 54 years. All subjects were

overweight and obese and all of them had tolerated the intensive insulin treatment well with no hypoglycemic reactions.

The glucose and insulin excursions are illustrated in Fig. 1 and 2. The basal serum glucose level was not significantly different between pretreatment and during oral insulin treatment.

The results of repeated measures of ANOVA verified that the pretreatment ($P_{\text{trend}}=0.10$) and during treatment ($P_{\text{trend}}=0.71$) changes in serum glucose level was not statistically significant. Compared with pretreatment value, the serum glucose level was significantly lower in 7 am (immediately after insulin therapy) [(253.75 \pm 31.42 vs. 198.75 \pm 18.72 mg/dl) ($p=0.04$)] and 8.30 am [293.25 \pm 33.19 vs. 199.75 \pm 21.71 mg/dl ($p=0.03$)] during oral insulin treatment. The maximum reduction was observed 1.5 hours after insulin therapy and its effect lasted for more than 6 hours. The mean AUC of glucose was insignificantly decreased from 6204.40 \pm 2024.20 mg/dl/17h (pretreatment) to 5556.04 \pm 1838 mg/dl/17h (during treatment) ($p=0.16$)

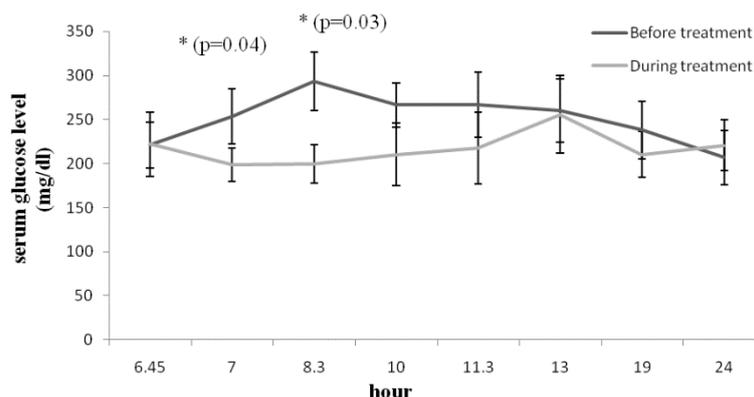


Figure 1. Serum glucose concentration pretreatment and during treatment of nanohydrogel oral insulin (n=8)
*: indicator of significant differences ($p < 0.05$) between pre-treatment and during treatment values.
Values were mean \pm SE
 P_{trend} of before treatment: 0.10
 P_{trend} of during treatment: 0.71

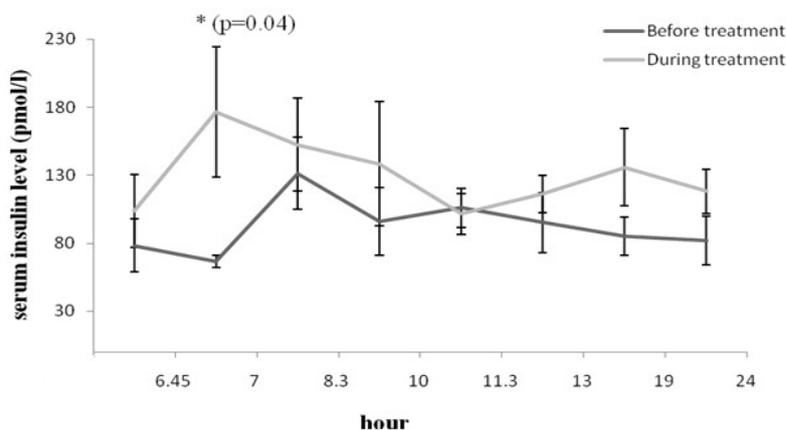


Figure 2. Serum insulin concentration pretreatment and during treatment of nanohydrogel oral insulin (n=8)
*: indicator of significant differences ($p < 0.05$) between pre-treatment and during treatment values.
Values were mean \pm SE
 P_{trend} of before treatment: 0.10
 P_{trend} of during treatment: 0.30

The basal serum insulin level was not significantly different between pretreatment and during treatment of oral insulin. The results of repeated measures of ANOVA showed that the pretreatment ($P_{\text{trend}}=0.10$) and during treatment ($P_{\text{trend}}=0.30$) changes in serum insulin level was not statistically significant. The serum insulin level was significantly increased immediately after oral insulin therapy compared with pretreatment values [(66.39±4.36 vs. 176.61±47.92 pmol/l) ($p=0.04$)]. The mean AUC insulin increased marginally significant from 2301±1000.8 (pretreatment) pmol/dl/17h to 3241.87±1243.15 pmol/dl/17h (during treatment) ($p=0.056$).

Discussion

The results of this pilot study showed that 300IU nanohydrogel oral insulin was effective in rapid lowering of serum glucose and its glucose lowering effect lasting for more than 6 hours. Previous clinical studies have been demonstrated the toxicity of high serum glucose level. Specific gene alterations in the β -cell after exposure to hyperglycemia⁸ have been demonstrated in animal studies. Defects in insulin secretion and action are directly related to hyperglycemia and are correctable with the establishment of euglycemia^{9, 10}. Although in the present study, the euglycemia could not be achieved with one dose of nanohydrogel oral insulin, the mean level of serum glucose was significantly decreased immediately after administration of oral insulin therapy and its effect lasted for more than 6 hours. In the present study, the PH sensitive nanohydrogel was used as a vehicle of human insulin. The immediate increase of insulin level after oral insulin therapy in comparison with pretreatment, demonstrated that the absorption of OI is feasible by loading human insulin in nanohydrogel. Previous studies have shown that nanohydrogel bonded insulin has lowest release in the acidic situation such as stomach environment due to formation of intermolecular polymeric complex and this complex is decomposed in the neutral and alkaline intestinal environment and then the released insulin could reach the liver in high concentration through the portal vein after absorption, which resulting in a more physiological and stronger effect on hepatic glucose production compared with subcutaneous insulin preparation¹¹.

Very few human studies were found in the literature about the effect of oral insulin therapy in diabetic (type 1 and type 2) patients. In a pilot before-after study in type 1 diabetic patients, Eldor et al (2013) demonstrated that treatment with ORMD-0801- a newly developed oral insulin preparation containing 8 mg insulin- in conjunction with subcutaneous insulin injections was associated with a significant reduction in the frequencies of

glucose readings and the mean glucose area under the curve (AUC)¹².

In another clinical study, Kapitza and colleges (2010) studied the effect of 300IU oral insulin in comparison with subcutaneous regular human insulin in ten type 2 diabetic patients. Using the glucose clamp procedure, they demonstrated that the maximum insulin concentration was greater and onset of action was faster with oral insulin in comparison with regular insulin¹¹. In the present study, we used nanohydrogel as human insulin carrier. Among the carriers, nanohydrogels have attracted much attention. These carriers are made from biodegradable natural polymer, responding to chemical and physical changes such as temperature and pH and adding special ions and electric fields¹³⁻¹⁵. The advantages of these polymers over other carriers is the potential of these carriers to be used in a variety of drug-loading and release formats, and also their release characteristics which can be tailored to a range of target environments¹⁶. Moreover, nanohydrogels are materials that are made from biodegradable natural polymers and no in-vitro and in-vivo cytotoxicity were reported⁷. However, for other insulin carriers such as monosodium N-(4-chlorosalicyloyl)-4-aminobutyrate (4-CNAB) some adverse effects such as local inflammation and gastrointestinal infections are reported¹⁷. Additionally, nanohydrogels, because of their small size, can overcome anatomic barriers¹⁴ (the size of the particles in the present study were about 50 nm and after insulin loading they were changed to be 80–200 nm).

Conclusion

In conclusion, this pilot study just provides a proof-of-concept for nanohydrogel oral insulin in type 2 diabetes. There is a need for future long term trials with a larger sample size to measure the impact of nanohydrogel oral insulin on HbA1c, prolonged glycemic control and diabetes complication in type 1 and type 2 diabetes.

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Conflict of interests

The authors claim that there is no conflict of interest.

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