A Review on The Effectiveness of Oral insulin in Type I and Type II Diabetes Mellitus

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Approval

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Ethics statement

This study does not involve any kind of human trial and animal trial.

Abstract

Diabetes mellitus is now a highly prevalent chronic metabolic disorder that has now encompassed epidemic proportions. With the aim of keeping the blood glucose levels in control, administration of insulin over the years have been done using subcutaneous injections and antidiabetic drugs but some serious disadvantages come along with such therapy like occasional hypoglycemia and patient noncompliance. Thereby leading to alternate methods of insulin delivery such as oral delivery of insulin. An oral form of insulin will not only help in increasing the patient compliance but also help in other effects such as avoidance of peripheral hyperinsulinemia, rapid hepatic insulinization and prevent potential hypoglycemia along with weight gain. In this paper, we would look deeper into the mentioned advantages of oral insulin as well as different formulations used over the years and mainly how it can be compared with subcutaneous delivery of insulin in terms of efficiency and glycemic control.

Keywords: Oral insulin, subcutaneous insulin, Type I diabetes, Type II diabetes, glucose

Dedication

Dedicated to my parents

Acknowledgement

I would like to take this opportunity to thank all those personalities who have helped me for all my educational achievements. I would like to begin by expressing my gratitude towards Almighty Allah for providing me with strength during this whole period. Then, I would thank my parents for their support both mentally and financially throughout my academic career. I would like to express my sincere thanks to my supervisor Faria Tahsin, Lecturer Department of Pharmacy, Brac University for being a constant guiding spirit throughout my study and for being such an encouragement. Lastly, I am indebted and would like to express my sincere gratefulness to Dr. Eva Rahman Kabir, Chairperson, Department of Pharmacy, Brac University for her encouragement, guidance and appreciation throughout my study period.

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List of acronyms

T1DM	Type I diabetes mellitus
T2DM	Type II diabetes mellitus
OHA	Oral hypoglycemic agents
HIM2	Hexyl insulin monoconjugate 2
CSII	Continuous subcutaneous insulin infusion
PPG	Postprandial glucose excursions
TI	Trypsin Inhibitor
BBI	Bowman-Birk inhibitor
AP	Aprotinin
CS	Chymostatin
FBG	Fasting blood glucose

Chapter 1

Introduction

A gradual decline in the functioning of the pancreatic β-cells is the main reason for Type I diabetes mellitus (T1DM) and Type II diabetes mellitus (T2DM). Type I diabetes mellitus is an autoimmune disease that follows a consequent loss of β-cell function; however, Type II diabetes mellitus portrays a slightly different reason, early onset of the disease shows a limited release of insulin along with a deficit in prandial suppression of hepatic glucose production, following elevated postprandial glucose excursions (PPG) and delayed insulin hypersecretion. During the late stage of Type II diabetes mellitus, a notable decrease in the β-cell mass occurs causing very little release of endogenous insulin. Main aims of therapy for T1DM and T2DM are:

i. Terminating any symptoms related to hyperglycemia.
ii. Annihilating or reducing the long term macrovascular and microvascular complications of diabetes mellitus.
iii. Helping the patient accomplish a lifestyle that is as normal as possible.

In order to attains such goals several approaches are needed. So, for an individual suffering from T1DM is insulin therapy but for individuals suffering from T2DM, the approach of modifying their lifestyle, starting a medical nutrition therapy and metformin is mostly taken. A combination therapy consisting of further oral hypoglycemic agents (OHA) or basal insulin is started if the metformin therapy doesn't suffice or fails. If the insulin production is seen to decrease further at an alarming rate, then multiple injection of long acting and short acting insulins are given to control the postprandial glucose excursions (Iyer, Khedkar, & Verma, 2010).

Insulin still remains as the most efficacious and essential drug to control hyperglycemia for diabetic patients (Morishita et al., 2006). Ever since its application clinically in the 1920s, significant progresses have been done in the field of management of diabetes. Thus, it resulted in a striking boost in the wellbeing and prognosis for Type I and Type II diabetic patients (Gordon Still, 2002).

Administration of insulin orally where needles, syringes or pens are not required as like subcutaneous insulin delivery pathway marks this delivery route as the most easy and fascinating approach (Luzio et al., 2010). The pain of injecting insulin might seem insignificant to most patients but to some it is intolerable (Hanas & Ludvigsson, 1997) and thus can considered as one of the reasons to pursue oral administration. Over the years several unsuccessful attempts have been made to deliver insulin through the oral route. If administered orally insulin gets absorbed from the gastrointestinal tract, then directly to the liver through the portal vein, thereby imitating the regular pancreatic insulin release with a low risk of systemic hyperinsulinaemia and subsequently reduced threat of hypoglycemia (Luzio et al., 2010). As opposed to subcutaneous insulin, oral insulin has various feasible advantages. The main advantage being the absence of any form of pain due to needles and as it follows a more physiological route it adds on to being a more convenient method of delivery (Halberg et al., 2019). Thus, such foreknown advantages of oral insulin therapy make it almost impossible to let go. Oral insulin gives us an option for a more realistic execution for insulin therapy (Zijlstra, Heinemann, & Plum-Mörschel, 2014). Hence, availability of oral insulin will not only ease insulin therapy but might also motivate people to use insulin who before were against it due to its method of administration (Hanas & Ludvigsson, 1997; Zijlstra et al., 2014).

Chapter 2

Aim and objective

The main aim of this review paper is:

To efficiently compare oral insulin delivery with subcutaneously injected insulin.

The objective of this review paper is:

To determine the ability of oral insulin in lowering blood glucose levels and enhancing glycemic control in Type I and Type II diabetes mellitus patients.

Chapter 3

Types of insulin

The details given below mainly represents information that can be applied to both Type I and Type II diabetes. Certain structural changes or alterations can be made to the insulin molecule that can either extend its length of activity (as a basal insulin, that follows the normal release of insulin nightlong and between meals) or reduce its activity (can be consumed pre prandially in order to imitate the insulin response to a meal) (Wallia & Molitch, 2014).

Short-acting human insulin:

Recombinant DNA technology was used to create the first insulin product and it was used traditionally as bolus insulin that copies the action of physiological insulin to a meal and is also able to rectify premeal and inter meal hyperglycemia. In low concentrations the human insulin remains as a monomer and gets distributed into the blood stream through subcutaneous injection (Borgoño & Zinman, 2012). Regular insulin has a retarded onset of action of 30 to 60 minutes. Regular insulin is instructed to be injected 20 to 30 minutes prior to meals in order to complement the carbohydrate absorption and insulin availability. It functions almost immediately when injected (DeWitt & Hirsch, 2003).

Intermediate-acting human insulin:

Currently, isophane NPH insulin is the only conventional intermediate acting human at clinical use (Borgoño & Zinman, 2012). Neutral protamine Hagedorn (isophane in insulin; NPH) insulin is steadily absorbed during incorporation of protamine to regular insulin (DeWitt & Hirsch, 2003). The onset of action is seen to 1.5 to 4 hours after injection due a significant delay in its absorption from the subcutaneous tissue thus resulting a duration of up to 24 hours and a striking peak at 4 to 10 hours after administration (Borgoño & Zinman, 2012).

Rapid-Acting Insulin Analogs:

For meal time insulin or as bolus aspart, glulisine and lispro are the three commercially available rapid analogues. Each rapid acting insulin are structured modified at the β chain to hinder the self-aggregation of the insulin particles into multimeric complexes (Borgoño & Zinman, 2012). These rapid acting analogs when administered 0 to 15 minutes prior to meals mimics the endogenous insulin release increase stimulated by food (Wallia & Molitch, 2014).

Long-acting insulin analogues:

Insulin glargine and insulin detemir are the currently licensed long-acting insulin analogs as a substitute for basal insulin and supplementation. Each of the analogue has been distinctly modified in order to extend the absorption subsequently after subcutaneous injection and comparatively a peak less 24 hour time action profile which is way more similar to the biologic basal insulin in comparison to NPH insulin (Borgoño & Zinman, 2012).

Premixed Insulin Preparations:

These insulin preparations are normally given pre-prandially. As two separate insulin types are used, the dosing is such that depicts peaks of both the insulin forms. The dosing being twice daily is the main benefit of premixed insulins. Adjustment of the doses and timing of the meals are a bit difficult for premixed insulins as both the types needs to be adjusted concurrently (Wallia & Molitch, 2014).

Studies have shown that when the clinical efficacy of the premixed insulin analogues and premixed human insulins were compared, the insulin analogues were superior regarding control of PPG with fewer intrasubject variation, still did not exhibit consistent improvements in HbA1c levels (Borgoño & Zinman, 2012).

Chapter 4

Formulation of oral insulin

The oral delivery of insulin is challenging due to the various physiological barriers. One of which is the gastrointestinal tract that contains various different enzymes such as trypsin, pepsin, chymotrypsin, pancreatin and carboxypeptidase that are mainly responsible disintegrating the larger protein molecules into amino acids and smaller peptides. Thus, most of the proteins after arriving the gastrointestinal tract gets disintegrated quickly and its absorption as a whole is normally very small (<1%) (Iyer et al., 2010). Through different pharmaceutical technologies it is possible to increase the bioavailability without the necessity to modify the epithelial cell physiology or proteolytic enzyme activity. Therefore, several pharmaceutical companies have been actively developing several oral delivery systems hoping they might be useful clinically. Even though majority are still in the development stage, quite a lot have advanced past the proof- of concept stage to clinical trials.

4.1 Emerging formulations:

Oral insulin spray:

Oral insulin spray is a novel, non-injectable route of insulin delivery. This system of delivery utilizes an aerosol of uniform shaped droplets consisting of insulin at high speed into the oropharyngeal cavity for local transmucosal absorption. A study was carried on healthy male volunteers aged 21 to 25 years using euglycemic clamp technique (a method for quantifying insulin resistance and secretion) to compare the pharmacokinetic and pharmacodynamic properties of single dose oral insulin versus a subcutaneous injection. The subjects were given single dose oral insulin in one visit and a single dose of subcutaneous injection in the other. This study showed that the oral insulin spray was associated with a shorter t_{max} and a

higher C_{max} and a quicker peak glucose uptake as opposed to subcutaneous insulin injection. It also showed a 120 min duration of action. Thus, the short t_{max} and 120 duration of action indicates that oral insulin spray can be a favorable replacement for meal related insulin needs in individuals with diabetes mellitus (Cernea, Kidron, Wohlgelernter, Modi, & Raz, 2004).

Another study was done using a human recombinant insulin (Humulin R) on seven healthy adult male individuals to determine the dose response relationship of an oral insulin spray. A single-center, blinded, randomized, five-way, cross-over, open-label study seven days apart on five different occasions, were done where subjects were given four different doses of oral spray (insulin spray: 5 puffs, 10 puffs, and 20 puffs; placebo spray: 10 puffs) and one dose if 0.1 unit/kg subcutaneous human regular insulin. The euglycemic clamp technique was used too in this study as well in order to learn about the pharmacokinetic and pharmacodynamic characteristics of the spray. No characteristic effect was seen for the t_{max} over the dosage range studies ($P = 0.63$). The time to maximum concentration was seen to be shorter for oral insulin than subcutaneous. The maximum metabolic effect (GIR max) and the amount of glucose infused (GIR-AUC) increased as the dose of insulin increased. The oral administration of the insulin was seen to have caused a more rapid increase in glucose consumption than for subcutaneous insulin injection (Cernea, Kidron, Wohlgelernter, Modi, & Raz, 2005).

Thiolated chitosan-insulin tablets:

This is an oral insulin delivery system that utilizes the enzyme inhibiting properties of a covalently associated enzyme inhibitors with the permeation augmenting and mucoadhesive properties of a Thiolated polymer**.** These tablets are composed of chitosan-TBA (chitosan 4 thoibutylamidine) as carrier matrix, chitosan-BBI-conjugate and chitosan-elastatinal-

conjugate as the enzyme inhibitors along with reduced glutathione was developed as permeation mediator. Due to the strong mucoadhesive property of chitosan it helps in enhancing the efficacy for oral administration of insulin. These tablets were given to non-diabetic rats where it was seen that they notably decreased the blood glucose level as compared to non-coated liposomes. The mucoadhesive property of the chitosan TBA insulin tablets can help in forming an intimate contact to the mucosa that can avert presystemic metabolism of insulin during absorption on the membrane (Krauland, Guggi, & Bernkop-Schnürch, 2004).

Nanospheres:

Nanospheres are devised as such to accommodate the biologically active insulin and after being administered orally, it helps in protecting the protein from any enzymatic degradation in the intestinal tract and also encourages systemic uptake of insulin through gastrointestinal tract (Carino, Jacob, & Mathiowitz, 2000). They were created using a cyanoacrylic monomer by interfacial emulsion polymerization. The monomer was placed in an oily phase (Miglyol 812) and the insulin along with a surfactive agent (poloxamer 188) was mixed to an aqueous dispersion medium. When these nanospheres loaded with insulin were given to diabetic rats through oral administration in a medium containing surfactive agents, they were able to reduce the blood sugar of the rats. The duration of the glycemic effect was seen to be dependent on the ratio of insulin to polymer used. A lower insulin/polymer ratio gave a longer hypoglycemic effect. The addition of the polymer also aided in protecting the insulin protein from degradation from any proteolytic enzymes in the gastrointestinal tract. When these nanospheres were incubated in vitro along with pepsin, chymotrypsin, and trypsin, they were not damaged and was well preserved but any free insulin got degraded (Damgé, Vranckx, Balschmidt, & Couvreur, 1997).

Another study on nanospheres formed of poly anhydride poly(fumaric-co-sebacic) anhydride [P(FA:SA)] and the poly(lactide-co-glycolide) (PLGA) were seen to cross the intestinal epithelium within 1 to 6hrs of oral administration. 1.6% of zinc can cross the intestinal epithelium within 1 to 6 h of being administered orally. This certain formulation of 1.6% of zinc insulin in FAO:P LGA (1:2), 10% Fe_3O_4 was seen to be able to lower the glucose levels in normal fasted rats and also in rats that were given glucose through subcutaneous injection. It was seen to be about 11.4% as effective as intraperitoneal injections of zinc insulin thus proving that the above formulation can be used for oral administration of insulin due to its promising results. The FeO and poly-anhydride in the formulation might be improving the bioadhesive properties of the spheres enabling for a pronounced interaction between the nanosphere and the gastrointestinal epithelium leading to a greater uptake of nanosphere (Carino et al., 2000).

Liposomes coated insulin particles:

Liposomes are phospholipid vesicles that has the ability to encapsulate both hydrophilic and hydrophobic drugs and are non-toxic and biodegradable in vivo. The hypoglycemic effect was seen to be dependent on the lipid composition, surface charge and the physical state of the of the phospholipid bilayer. The liposomes were made by adding phosphatidylinositol (‘fluid’, 1:1 w/w) and phosphatidylcholine or dipalmitoyl phosphatidylethanol (‘solid’, 1:1 w/w) and dipalmitoyl phosphatidylcholine or the latter supplemented with palmitoyl–stearoyl sucrose (1:1:0.2 w/w/w) in chloroform. Multilamellar liposomes are yielded when the insulin is dissolved in 10 mM Tris–HCl buffer (pH 8.0) having 1 mM EDTA was added to the dried lipid, then the mixture was disseminated with a shaker under an argon atmosphere at 50°C (25°C for ‘fluid’ liposomes). Results have shown that when liposomes were encapsulated accordingly, they were able to become an active therapeutic agent when administered orally. These liposomes when were given to rats through oral administration,

they were seen to notably decrease the blood glucose level. Through the microcalorimetric data, it was seen that these liposomes were solid at 37°C. The liposomes having negatively charged phosphatidylinositol and phosphatidylcholine had the ability to entrap copious amounts of insulin and then releasing them into the bloodstream from the gastrointestinal tract (Kisel et al., 2001).

In a different study the sugar chain section of mucin and polyethyleneglycol (PEG) were used for coating the surface of the liposome. Here the liposomes showed a slower delivery of insulin in the acidic solutions of pH 2.0. But in the presence of bile salts such as sodium glycocholate and taurocholate a greater delivery of insulin was seen from noncoated liposomes. Particularly in the presence of sodium taurocholate, the delivery was exceptionally quick. Thus, showing that bile salts might be interacting with the liposomes and causing them to burst by withdrawing lipids from the liposomal membrane. The following surface coating has shown to be effective against bile salts (Iwanaga et al., 1997).

Protease inhibitor:

Insulin gets easily inactivated and degraded by proteolytic enzymes such as trypsin, chymotrypsin and pepsin. Thus, for this reason many studies were not able to administer free insulin alone to reduce the blood glucose level. Simultaneous addition of inhibitors of proteolytic enzymes enhanced the absorption of insulin. In order to keep the insulin unscathed in the intestinal lumen the first step should obviously be to protection from proteolysis. In a study an oral dosage form was created to carry the insulin into the intestine in the presence of protease inhibitor. Protease inhibitors [Trypsin inhibitor (TI), Bowman-Birk inhibitor (BBI), Aprotinin (AP), Chymostatin (CS)] and insulin (crystalline bovine insulin) were integrated into a polymerized drug carrier in the shape of microspheres. 20 mg insulin microspheres were incubated in the presence of trypsin, α-chymotrypsin or pepsin in

order to assess its protective effect. Free insulin was fully deteriorated while being incubated in pepsin. Whereas for the other preparations, loss of insulin was barely observed for microspheres present pepsin solution. The quantity of insulin prevailing on the surface of the microspheres was very low due to coating of the particles with a pH-dependent copolymer (Eudragit L100). This maybe be the reason for the significant quantity of insulin left behind after the treatment with acid irrespective of the presence of pepsin. In general, it was seen that with increasing enzyme concentration, the protective efficacy of the preparations towards α-chymotrypsinic and trypsinic degradation reduced.

a) In the incubation medium at the lowest trypsin concentration, insulin microspheres containing trypsin inhibitor showed the highest degree of protective efficacy. For BBI and AP containing microspheres, their protective efficiencies were similar but rather lower than the insulin microspheres containing TI.
b) For the α- chymotrypsinic degradation study, insulin microspheres with CS showed the greatest insulin protective efficacy for all enzyme concentrations, On the other hand AP or BBI containing insulin microspheres showed a lower resistance towards α-chymotrypsinic digestion.

For both the enzymatic degradation studies, insulin microspheres with AP were moderately more efficient at protecting the insulin in comparison to microspheres with BBI. Thus, it can be concluded that these microspheres might be able to protect insulin from enzymatic degradation under controlled experimental conditions (Morishita, Morishita, Takayama, Machida, & Nagai, 1992).

4.2 Formulations under trial:

Biocon limited IN-105:

IN-105 is an innovative and novel insulin analogue that is now undergoing development for oral administration of insulin. It is an altered form of the human insulin where a single short-chain amphiphilic oligomer is covalently attached by a non-hydrolysable amide bond to the free amino acid group on the Lys-β29 residue of recombinant insulin (Khedkar et al., 2010). As compared to insulin, IN-105 has better absorption; reduced immunogenicity and reduced mitogenicity. IN-105 shows a comparable pharmacological activity to insulin and maintains a good clearance profile and safety profile in contrast to insulin. Pharmacodynamic and pharmacokinetic studies have shown that IN-105 is absorbed promptly and causes a drop in blood glucose (Sonia & Sharma, 2014). In an open label, sequential ascending dose, multicentric study, a linear dose relationship of glucose at 2-h postmeal was seen for IN-105 as compared to a placebo (Khedkar et al., 2010).

Generex Biotechnology Oral-lyn:

Generex Oral-lyn™ is a regular recombinant human insulin that is made into a liquid aerosol mist formulation administered to the buccal mucosa utilizing a delivery system (RapidMist™ Diabetes Management System, Generex Biotechnology, Toronto, Ontario, Canada) (Pozzilli, Raskin, & Parkin, 2010).

a) In a study Oralin was administered to Type II diabetes mellitus subjects instead of regular mealtime insulin injection, it was seen that the postprandial glucose excursions were equivalent to that of an insulin injection group.

b) When Oralin was given to Type II diabetic patients, it was seen that Oralin was absorbed rapidly as compared to subcutaneous insulin injection and was also successful in postprandial glucose control in contrast to placebo treatment.

Consequently, it can be safely concluded that Oralin can easily control postprandial hyperglycemia and replace mealtime insulin injection with no hypoglycemic episodes as compared to regular human insulin injections (Modi, Mihic, & Lewin, 2002).

Hepatic-directed vesicle (HDV):

A company originating in the United States, Diasome is utilizing a hepatic-directed vesicle (HDV) for insulin treatment (Arbit & Kidron, 2009). The Hepatic-directed vesicle insulin (HDV-I) is an innovative investigational (<150 nm diameter) insulin delivery system. Studies on insulin-binding with HDV has created a formulation where 1IU regular recombinant insulin is strongly attached to 1mg HDV. The HDV-I is made into a dry mix by utilizing a proprietary method resulting in a 5-unit size 2 capsule HDV-I dosage form for oral delivery. This was then administered to diabetic dogs peripherally; it was seen that HDV was able to efficiently deliver insulin to the liver and encouraged hepatic glucose uptake with a potency that is about 100-fold more pronounced than that of the same dose of human recombinant insulin or regular porcine. This specific ability of HDV-I to target the liver redeems it as a crucial metabolic modulator of glucose metabolism (Geho, Geho, Lau, & Gana, 2009).

Oramed ORMD 0801:

ORMD 0801 is an oral insulin capsule. Oramed Pharmaceuticals' Protein Oral DeliveryTM (POD) technology utilizes an enteric coated capsule that incorporates insulin with protease inhibitors and absorption enhancers that assists its delivery in the small intestine (Easa, Alany, Carew, & Vangala, 2019). An open label single center study was done using ORMD-0801 capsules containing 8 mg of insulin to 8 Type I diabetic subjects 3 times daily. These drugs was not only well tolerated it was seen that these oral insulin capsules caused a synergistic reduction in blood glucose concentrations when given with subcutaneous insulin injections during the early evening hours (Eldor, Arbit, Corcos, & Kidron, 2013).

4.3 Potential formulations:

Erythrocytes as delivery of oral insulin:

An investigational study was done on different forms of the human red blood cells as oral transporter systems for human insulin in vivo. Oral administration of Hins (Human insulin) free or complexed to erythrocyte carrier systems were given to male wistar rats that were made diabetic by a single intraperitoneal injection of streptozocin. The rats were given any of the following: erythrocyte-vesicles, erythrocyte-ghosts, liposomes-ghosts and liposomes-vesicles, carriers of insulin suspension. The initial average blood glucose concentration was about two-fold higher than that of a normal male wistar rat. The average initial blood glucose concentration for these treated rats were over twofold higher than that for a normal male wistar rat. It was seen that after the administration of Hins solution a notably high decease in blood glucose level occurred as compared to a normal saline group. For erythrocyte vesicles or erythrocytes ghost insulins, they also showed a drastic decrease in blood glucose level as compared to the respective control groups. For liposomes-ghosts-insulin and the free-carrier liposomes ghosts, no significant difference was found between them and the control group. Thus, it can be concluded that different erythrocytes carriers of human insulin resulted in a characteristic hypoglycemic effect in diabetic rats (Al-Achi & Greenwood, 1998).

Nano cubicle:

In a study, a distinct homogenous liquid formula was developed that had varying physical properties than that of pre-existing formulas. The formulation was such that after vortexing it with a phosphate buffered saline (PBS pH 7.4) or excess water, nano sized particles are created ranging from 200 to 500nm. The encapsulation efficiency of insulin inside these particles were 70 to 100%. This can be created without any physical force or heat and can be purified easily through filtration. These sub-microns sized lipid particles forming

spontaneously in water are known as nano cubicles. These nano cubicles were tested on three groups of streptozotocin-induced fasted diabetic rats. The rats were either given orally, particles without insulin, particles encapsulating insulin, or soluble insulin in water. A control group was created by injecting a group of rats soluble insulin in phosphate buffered saline. No hypoglycemic effect was seen for the empty particles and insulin solution. But a hypoglycemic effect was noticed for particles encapsulating insulin in 1 hr and it lasted for 6hr that was the duration of the experiment, as compared to the untreated rats the glucose concentration was controlled at 1.7 to 6.7 mmol/l whereas for the untreated rats were 16.7-22.2mmol/l. The hypoglycemic effect was comparatively very rapid considering the mechanism of absorption of oral administration. The serum insulin concentration did not differ from the basal insulin concentration for 6hr for insulin solution or empty nanocubicles. The group of rats feed with nanocubicles encapsulating insulin showed a significant ($p<0.05$) increase in insulin concentration from its normal value. Therefore, this novel development may prove to be revolutionary for the treatment of diabetes mellitus (Chung, Kim, Um, Kwon, & Jeong, 2002).

Microemulsion:

Microemulsions are composed of surfactant molecules that are stable isotropic transparent colloidal systems with their hydrophilic head facing inwards establishing an aqueous core and their hydrophobic tails facing outwards solubilized in organic phase thus creating the corona. These systems are spontaneous and needs to be formed above the critical micellar concentration (CMC) of the surfactant during which the surfactant forms distinctive molecular structures depending on the contents of the organic phase, aqueous phase and surfactant used. A study was done discussing the formation and development of insulin loaded microemulsions for oral administration and evaluating the glucose reducing ability in streptozocin induced diabetic rats. Pharmacokinetic studies were done using healthy male

rats. The animals were split up into 6 groups. 3 groups were given insulin microemulsions orally using gavage needle and three other groups were given the same dose of insulin solution in phosphate buffer orally. Pharmacodynamic studies have shown that the group that was given insulin loaded microemulsion orally exhibit a plasma glucose level around 3.0 mg/ml at 6 h, which represented to 37.5% depletion in plasma glucose level and went back to the hyperglycemic level within 72hrs. The group that was given insulin solution did not reveal any change in their blood glucose level. Another group was given insulin loaded microemulsions through subcutaneous injection and it was seen that the plasma glucose level decreased by 52.3% that is about 2.24mg/ml at 2hr. This indicates that the insulin loaded microemulsions are able to reduce the blood glucose levels through both subcutaneous and oral route (Sharma et al., 2010).

In another study oil-in-water (o/w) emulsions were made utilizing light liquid paraffin as the oily phase and snail mucin powder and numerous combinations of Tween® 80. A vortex was used to mix calculated amount of Tween® 80 and 1ml of 100 IU/mL of insulin. Then the volume was increased up to 100 ml using double distilled water and adding the final solution to the oil phase as the aqueous phase. 30% liquid paraffin was present in the final emulsion. These microemulsions loaded with insulin were then given to overnight fasted albino wistar diabetic rats orally. This caused a reduced rate of blood glucose reduction but its effect was seen to sustain for an extended period of time as compared to subcutaneous injection. For oral administration of insulin solution no noteworthy hypoglycemic effect was seen thereby showing the poor oral absorption of insulin solution in gastrointestinal system (Momoh et al., 2020).

Chapter 5

Mechanism of action

From the pancreatic β cells insulin is discharged into the portal vein which is then carried onto the liver. During this path through the liver 80% of the discharged insulin is extracted and binds to the insulin receptors. This substantial extraction gives way to the "portal signal" and to the portal-peripheral gradient, a more pronounced (2.5- to 3-fold) greater insulin concentration in the portal vein in comparison to that in the systemic circulation (Arbit & Kidron, 2009). Oral insulin follows this exact physiologic route as it is gets absorbed from the gastrointestinal tract into the portal vein (unlike parenteral insulin that is absorbed into the systemic circulation) and eventually might have metabolic outcomes as it directly goes to the liver and then continuation of its role in glucose metabolism (Satake et al., 2002). Oral insulin can ease the β-cells of the pancreas by letting them rest, (Wajchenberg, 2007) and can aid in diabetes prevention through induction of 'oral tolerance' or immunomodulation (Isabelle Bergerot et al., 1999; Skyler, 2005).

As the oral insulin is delivered from the gastrointestinal tract to the liver, it is able to achieve a high porto-systemic gradient. This lowers the systemic insulin exposure and can prevent the excessive weight gain that sometimes occurs with subcutaneous injection. Oral insulin may also be able to rectify the blunting of the first phase release of insulin which is hard with conventional subcutaneous insulins (Stratton et al., 2000).

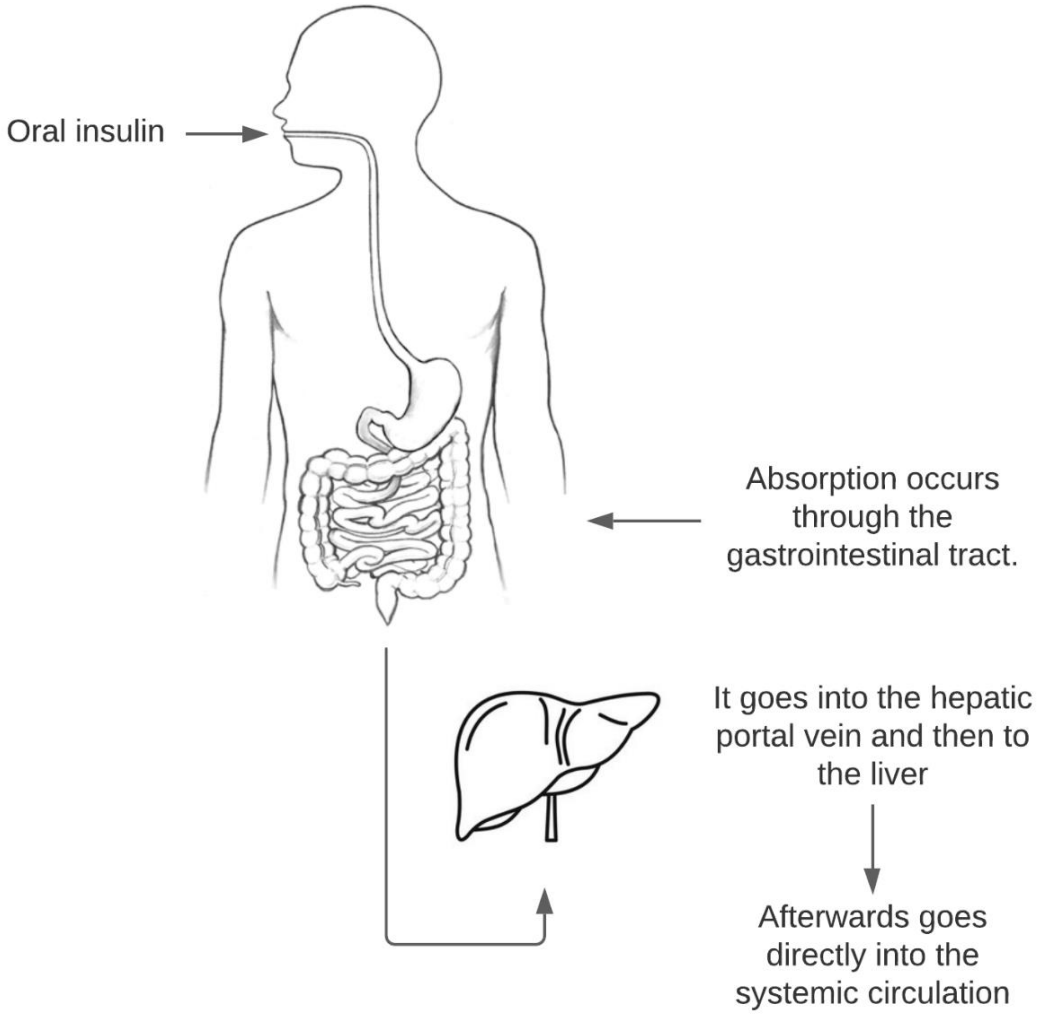

Figure 1: The pathway followed by an oral insulin product (Adapted from Arbit & Kidron, 2009)

Chapter 6

Study selection

Table 1: Characteristics of the studies using oral insulin

Study	Method and duration	Population	Products used		Assessment	Results
			Oral	Subcutaneous/placebo		
(Cernea, Kidron, Wohlgelernter, Modi, & Raz, 2005)	Single-center, blinded, randomized, five-way, cross-over, open label study-7 days apart	7 adult healthy male subjects.	Oral insulin spray with human recombinant insulin (Humulin R).	Subcutaneous human regular insulin.	C_{max}, GIR_{max}, GIR-AUC, C-peptide values.	The time to maximum insulin concentration (T_{max}) was faster as opposed to subcutaneous injection indicating a more rapid glucose consumption. The maximum serum insulin level (C_{max}) along with AUC (area under the curve) displayed a dose response relationship. Reduction in C-peptide values were seen.
(Kapitza et al., 2010)	Single-center, open label, randomized, two-period cross-over, isoglycemic glucose clamp study	14 male patients with T2DM	Drug-carrier molecule monosodium N-(4-chlorosalicyloyl)-4-aminobutyrate (4-CNAB).	Subcutaneous regular human insulin (RHI).	AUC_{INS}, AUC_{GIR}, GIR	Glucose clamp study exhibited that oral insulin gets absorbed faster under fasting conditions and demonstrates quicker enhanced pharmacodynamic and pharmacokinetic responses.

Study	Method and duration	Population	Products used		Assessment	Results
			Oral	Subcutaneous/placebo		
(Kipnes, Dandona, Tripathy, Still, & Kosutic, 2003)	Randomized, placebo- and regular insulin–controlled, three-way crossover, dose-escalation study-3 days	36 to 65 years of age with T2DM	Hexyl-insulin monoconjugate 2 (HIM2) in gelatin capsule	Subcutaneous regular insulin (Humulin R)	Maximum plasma glucose concentration, insulin Cmax, insulin Tmax	Oral HIM2 was able to reduce the post-prandial glucose level as opposed to placebo treatment and had similar results compared to subcutaneous injection at lower peripheral insulin concentration. It was also able to suppress hepatic glucose output.
(Cernea, Kidron, Wohlgelernter, & Raz, 2005)	Single-center, randomized, single-blind, open-label, 5-way crossover study	Patients with T1DM	Buccal spray (Oralin)	Subcutaneous human regular insulin	Glucose infusion rate, Tmax	Rapid onset and reduced duration of action as opposed to subcutaneous insulin and after administration it was seen to be effective the first 2 hr.
(Halberg et al., 2019)	Randomized, double-blind, double-dummy, active-controlled, parallel trial- 8 weeks of once-daily treatment	Adult patients with T2DM	Oral insulin 338 (I338) basal insulin analogue	Subcutaneous insulin glargine (IGlar)	Fasting plasma glucose, HbA1c,	The mean plasma glucose concentration at the end of the treatment did not differ significantly from that of IGlar. The approximated difference in the mean plasma concentration between I338 group and IGlar was 0·7 mmol/L.

Study	Method and duration	Population	Products used		Assessment	Results
			Oral	Subcutaneous/placebo		
(Luzio et al., 2010)	Randomized, open, single-center, two-way crossover study- 11 days.	8 T2DM subjects	Oral enteric-coated insulin capsule (150 and 300U)	Actrapid subcutaneous regular human insulin (12U)	Plasma glucose, C-peptide, plasma insulin	Similar glucose infusion rate for both doses. The duration of action of the capsulin was seen to extend the 6hr clamp period due to the significantly high glucose infusion rate till the study period. Subsequent subcutaneous Actrapid infusion resulted in peak plasma concentration within 150-220 min whereas for capsulin presence of insulin barely seen in the systemic circulation.
(Clement, Dandona, Still, & Kosutic, 2004)	Open-label, 2-center, dose-escalation, nonrandomized study- 2 days	T1DM patients receiving basal CSII	Oral hexyl-insulin monoconjugate 2 (HIM2)	Continuous subcutaneous insulin infusion (CSII) therapy.	Baseline plasma glucose, AUC for plasma glucose	It was seen that when the patients received only CSII postprandial plasma glucose concentrations were higher as opposed to combination of HIM2 and CSII. A decline in the glucodynamic variables were seen for HIM2 dosing from 13% to 26%.
(Pozzilli et al., 2005)	Single dose, two-way, crossover-3 days	18 T1DM patients	Buccal spray insulin	Subcutaneous regular insulin	Plasma glucose level, C-peptide levels	Shorter hypoglycemic action seen for buccal spray as opposed to subcutaneous. This route produces target postprandial glucose levels in patients with type 1 diabetes.

Study	Method and duration	Population	Products used		Assessment	Results
			Oral	Subcutaneous/placebo		
(Khedkar et al., 2010)	Open label, sequential ascending dose, multicentric study- 5 consecutive periods.	20 T2DM subjects	IN-105 oral insulin analogue 4 doses (10mg, 15mg, 20mg, 30mg).	Placebo	Glucose level, C-peptide values, Plasma Insulin levels	Drop in plasma glucose levels increased and C-peptide values decreased with increasing dose and average change in glucose at 2h post-meal showed a linear dose–response relationship. Pharmacokinetic studies displayed notable difference between different doses of IN-105 and placebo treatment.
(Guevara-Aguirre, Guevara, Saavedra, Mihic, & Modi, 2004)	Open-label, two-way, crossover, randomized study	21 T2DM patients	Buccal spray (Oralin) + metformin + glyburide	Placebo + metformin + glyburide	HbA1c, Cell antibodies, Plasma glucose, insulin, C-peptide.	Adding Oralin to the existing treatment caused lowering of the glucose levels after a standard meal. Faster onset of action and significant rise in serum insulin as opposed to endogenous insulin.
(Guevara-Aguirre, Guevara-Aguirre, Saavedra, Bernstein, & Rosenbloom, 2007)	Two-way, crossover-12 days	10 T1DM hispanic subjects	Oral insulin spray (Oral-lyn)	Insulin glargine	Plasma glucose levels, HbA1c	Similar glucodynamic responses were observed. It was rapidly absorbed within 5-10 mins and eliminated

Studies using hexyl insulin monoconjugate 2 (HIM2):

Hexyl-insulin monoconjugate 2 is a compound where a single amphiphilic oligomer is covalently linked to the free amino group on the Lys-29 residue of recombinant human insulin via an amide bond. HIM2 has modification in physio-chemical characteristics that facilitates absorption and resists degradation by enzymes as opposed to normal insulin(unmodified). In a study this HIM2 was formulated into a hard gelatin capsule as an oral semi solid formulation. As the oral insulin product HIM2 follows the same physiologic pathway as that of endogenous insulin thereby it suppresses hepatic glucose output. HIM2 were given to Type II diabetes patients and it was seen that it was able to reduce the post-prandial glucose level as opposed to placebo treatment and had similar results compared to subcutaneous injection at lower peripheral insulin concentration (Kipnes et al., 2003). The pharmacokinetic and glucodynamic variables have shown that no noteworthy difference was seen when two doses of HIM2 was used on diabetic patients thereby referring that HIM2 is not dose dependent. The prime action of HIM2 is at hepatic level after its absorption and transport through the portal channels thus this approach of delivery has the possibility to give a complete liver insulinization and to decrease the risk of hypoglycemia that comes with subcutaneous insulin injection (Clement et al., 2004). A study on glucose stabilization effects of HIM2 utilized three increasing doses of the oral insulin product hexyl-insulin monoconjugate 2 (HIM2) on Type I diabetes patients where the first and second dose were administered with a 2-hour difference. The start of glucose lowering or stabilization began in about 20–30 min of initial administration of HIM2. Then later a state of decline or stability was seen for within 2 hours after the primary dose of HIM2. Plasma glucose levels of phase I and phase II results were close thus demonstrating that the glucose stabilizing effects of HIM2 was reproducible (Clement, Still, Kosutic, & McAllister, 2002). During the first pass through the liver, the hepatic extraction of insulin from the venous circulation is around 50%

and the release of insulin from the pancreas to the venous circulation transpires in a pulsatile manner that is not immediately seen in the peripheral blood levels (Song et al., 2000). No remaining secretion of endogenous insulin was seen thus showing that HIM2 definitively affected the glucose control (Clement et al., 2002).

Studies using oral insulin to delay onset of diabetes mellitus:

Certain studies used oral insulin to determine whether the onset of diabetes can be delayed. In a study relative of patients with Type I diabetes mellitus were screened for islet cell antibodies (ICA) and insulin autoantibody (IAA) and first-phase insulin response (FPIR) were evaluated to determine the projected 5-year risk of diabetes. Oral insulin was given in three studies to test the idea of utilizing oral antigen administration as a way to protect islet-cell function in newly diagnosed Type I diabetic patients. But it was seen that oral insulin was not able to retard or stop the development of diabetes (Skyler, 2005). Studies done on mouse models however have shown that oral insulin therapy was able to avert Type I diabetes onset although it was seen to be dose dependent (I. Bergerot, Fabien, Maguer, & Thivolet, 1994; Muir, Schatz, & Maclaren, 1993; Ploix et al., 1998). Another study was done on patients that participated in the early intervention of oral insulin to retard or prevent Type I diabetes mellitus (1994–2003). It was seen that Insulin Autoantibody (IAA) titer was linked with oral insulin. The subjects were given either oral insulin or placebo once a day. Individuals that had confirmed IAA levels greater than or equal to 80nU/mL, after their oral insulin was stopped, the rate of developing diabetes was close to that of the placebo group. Thus, inferring that individuals with an IAA level greater than or equal to 80 nU/mL who got oral insulin was seen to benefit overall by significantly retarding the onset of Type I diabetes even after stopping the treatment. On the other hand, subjects with IAA levels less than 80nU/mL, the complete opposite was seen (Vehik et al., 2011). Therefore, it can be

concluded that there is a slight chance of retarding the onset of diabetes by utilizing oral insulin beforehand.

Chapter 7

Conclusion

One of the standard management conventions for diabetes treatment is the everyday utilization of abdominal insulin injection. Nevertheless, only patient compliance is the downside as the injection sites gets inflamed overtime and become painful. Therefore, looking into other non-invasive methods of insulin delivery is a given.

Throughout the different studies done on oral insulin, no major difference was seen between subcutaneous and oral formulations in terms of controlling HbA1c, FBG, 1 h after meal blood glucose, 2 h after meal blood glucose, glucose infusion rate (GIR), and producing C_{max} and AUC of insulin (Akbari et al., 2016). After the administration of oral insulin formulation, the maximum concentration of insulin in the blood was not significantly different from that of subcutaneous insulin but when oral insulin spray was given to healthy individuals it was seen that the time to maximum insulin concentration (T_{max}) was faster as opposed to subcutaneous injection thus indicating a more rapid glucose consumption (Cernea, Kidron, Wohlgelernter, Modi, et al., 2005). T_{max} for subcutaneous insulin was seen to be 3.5 fold longer compared to oral insulin spray (Cernea et al., 2004). The reason for the shorter T_{max} might be because of the large mucosal surface area of the oral cavity covered by a thin highly vascularized membrane providing efficient access to circulation. Thus, if natural absorption is artificially enhanced then fast-moving fine particle aerosol can easily transverse this thin membrane. After this is done successfully, the insulin molecules with the help of absorption enhancers gets absorbed into the bloodstream and appears in the systemic circulation (Guevara-Aguirre et al., 2007). Therefore, explaining the rapid rate of absorption beyond the oropharyngeal mucosa with relatively large and permeable area.

Oral insulin products have shown to provide insulin in a more physiologic manner causing a consequent decrease in peripheral insulin concentration that would sufficiently “insulinize” the liver (Gwinup, Elias, & Domurat, 1991; Hoffman & Ziv, 1997).

In order for all of this to be a reality oral insulin formulation should have a long-term safety profile and efficacy that should be demonstrated through sufficient studies and should also be able to reproduce the physiologic insulin excursions.

Oral insulin could potentially be regarded as that alternative non-invasive substitute for premeal insulin administration with equivalent efficacy to subcutaneous insulin and greater patient acceptability and providing the benefit of replicating the natural route of endogenous insulin absorption and secretion, an oral insulin product might be the best instrument to encourage early intervention and to foster adherence and compliance among diabetics, thereby refining glycaemic control.

Chapter 8

Future expectations

Whilst the different strategies that were taken until now for oral insulin delivery have shown effects in either increasing the absorption or protecting the insulin from degradation in the gastrointestinal tract, none of the strategies that were put forward or were still being developed have not been recognized to be valid enough for clinical applications. Although a number of the approaches have demonstrated developments in case of oral bioavailability, but in terms of inter- or intra- individual variations, post- and pre- meal effects or reproducibility after repeated dosing no sort of improvements were demonstrated.

Since the discovery of insulin in 1922 we have made strides in many ways yet oral delivery of insulin in the treatment of diabetes mellitus seems like a tough goal. Insulin therapy regimens require multiple daily subcutaneous injections thus making advances in insulin will help in relieving the burden of compliance on patients. Thus, development of effective and safe delivery of insulin should be the focus of research and development. In order for oral insulin to be useful clinically, correct amount of insulin should be able to maintain glucose levels in blood and it should be reproducible every time oral insulin is administered.

Nonetheless oral insulin has no probable side effects, it should be checked in case the other entities biological or chemical attached to the insulin to improve its uptake has any safety issues, especially if taken over long periods of time (Sonia & Sharma, 2014).

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