



Asian Journal
of
PHARMACEUTICAL RESEARCH
Journal homepage: - www.ajprjournal.com

TECHNOSPHERE INSULIN INHALER – A REVIEW

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ABSTRACT

Inhaled Insulin is a recent therapeutic advance for the treatment of Diabetes. It offers a convenient, as well as accepted, alternative to injected Insulin. Subcutaneous administration of Insulin results in the pain, inconvenience, and disruption of lifestyle associated with the injection of Insulin, so there has been increasing demand in the development of Aerosolized Insulin. Recent studies shown that Aerosolized Insulin is effectively delivered to the alveolar region of the lung, absorption and glucose levels rates are shown similar to those achieved with SC delivered Insulin. Aerosolized Insulin is well-tolerated and avoids changes in pulmonary function. Due to some limitations in Aerosolized inhalation, there are novel drug delivery systems of different formulations of Insulin, which are under development, to improve the efficiency of Insulin to deliver into the systemic circulation. Among them Techno sphere Insulin Inhaler offers a viable therapeutic option for the treatment of Diabetes.

Key words: Technosphere, Insulin, Diabetes Mellitus, Fumaryl Diketopiperazine, Glycosylated hemoglobin (A1C).

INTRODUCTION

On oral administration, Insulin degrades in the stomach due to the acid levels in stomach. Only the route to administrate the Insulin into the body is subcutaneously. Injection of proteins, peptides and Insulin cause of the pain, inconvenience, disruption of lifestyle. Diabetes patients need to daily multiple injections which lead to noncompliant of patients. To improve treatment Aerosolized Insulin developed to replace subcutaneously (SC) delivered formulations. Recent studies shown Aerosolized Insulin has good absorption and glucose levels rates are similar to those achieved with SC-delivered Insulin [1,2]. Aerosolized Insulin is well-tolerated and avoided changes in pulmonary function. However, new devices and novel drug delivery formulations of Insulin, which are in development, used to improve the efficiency of Insulin. The treatment of Diabetes with Aerosolized Insulin will provide an effective alternative means for controlling plasma glucose levels in diabetic individuals. Technosphere Insulin also will serve as a developmental model for this route of administration for a number of other

therapeutic peptides that are currently administered by injection only [3-5].

Mechanism of Inhaled Insulin absorption

The pulmonary route has been the most non-invasive alternative to subcutaneous administration of polypeptides, proteins and Insulin. It offers the greatest potential for systemic Insulin delivery; since some of the features that make the lung so well suited for gas exchange particularly its huge surface area also make it an ideal organ for absorption of small molecules into the bloodstream. Pulmonary drug absorption can occur by both the transcytotic pathway, in which proteins passes through the cells, and paracellular pathways, in which the drug diffuses through the tight junctions, which is formed by the help of Lactate Dehydrogenase between the cells [6-9].

Alter of pulmonary function by Aerosolized Insulin

Concerns regarding the potential for pulmonary toxicity with chronic use of inhaled Insulin have been raised due to the growth-promoting and immunogenic

properties of Insulin. In order to provide proper lung function new developed Aerosolized Insulin formulations are to be introduced into both Type 1 and Type 2 Diabetes patients and reports have to taken. So then on prolong Aerosolized Insulin the safety profile of inhaled Insulin on pulmonary function has been extensively studied throughout its clinical development. Among them the Technosphere Insulin powder showed safety profile of inhaled Insulin in the clinical development.

Advantages over subcutaneous injection: It is a non invasive technique.

- Rapidly absorbed
- More rapid decrease of endogenous glucose production (EGP) after a meal than the Subcutaneously injected Insulin
- Fast onset of action [10-12].

Classification of Diabètes

Type 1 Diabetes Mellitus : It is characterized by loss of the Insulin-producing beta cells of the Islets of Langerhans in the pancreas leading to Insulin deficiency. This is also called as immune-mediated or idiopathic.

Type 2 Diabetes Mellitus : Type 2 is characterized by Insulin resistance which may be combined with relatively reduced Insulin secretion. The defect is due to less use of Insulin by body tissue Insulin receptors.

Gestational Diabetes Mellitus

(GDM) is same as Type 2 Diabetes in several respects, involving a combination of relatively inadequate Insulin secretion and responsiveness. It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery. Gestational Diabetes is fully treatable but requires careful medical supervision throughout the pregnancy. It causes problems to baby. Increased fetal Insulin may inhibit fetal surfactant production and cause respiratory distress syndrome, decreased placental function.

Other types

Pre-Diabetes which has been termed "America's largest healthcare epidemic, Latent autoimmune Diabetes in which Type1 Diabetes develops in adults [13].

Technosphere Insulin

Proteins, Peptides and Insulin are critical for treatment of many diseases and represents for future therapeutics [14].e.g., Human growth hormone for treatment of Turners syndrome, Salmon calcitone for treatment of osteoporosis [15].

The instability of proteins, peptides and Insulin made difficult to develop new formulation other than injection [16-21] in that way pulmonary administration is safe and efficient delivery system [22-25].

Advantages

Directly enters systemic circulation [26-28]. Avoid first pass metabolism [29, 30]. Avoid digestive enzymes which cause poor bioavailability [31-33].

Pulmonary absorption of drug takes place by both paracellular and transcytotic pathway. Technosphere Insulin is a new inhalation powder formulation used for the treatment of Diabetes Mellitus [34].Technosphere particle formed by self-assembly of crystals of Fumaryl Diketopiperazine which is advantages of this particle [35]. Average diameter of this particle is 2-2.5 μm which is shown in (Figure 1) [36].

Advantages of Fumaryl Diketopiperazine as carrier

High internal porosity, High surface area for the adsorption of many drugs and proteins. It adsorbs Insulin powder appropriate in the average size range which delivers the Insulin into the deep lungs. It increase Insulin concentration in blood stream than the Insulin deliver by the subcutaneous injection and it also show pharmacokinetic profile of Insulin as same as shown by the Insulin released by pancreas (Figure 2) [37]. This novel drug delivery system also helps other protein and hormones to administer in this route. Naturally rapid absorption of Insulin occurring in pulmonary system by Technosphere Insulin. So it is important to find whether Technosphere is a permeation enhancer or not. If permeation enhancer then it causes damage or pulmonary problems [38].

Pulmonary safety by Technosphere Insulin was founded by various studies

Permeability Studies, Cytotoxicity Studies. For doing following tests or studies cell culture resembling the lung cell culture is needed [39].

Cell Culture

In this Calu-3 cells used as cell culture. Mainly absorption site for drug in lung is alveolar cells (Type 1 Pneumocytes), covers nearly 90% of lung surface area. They help in exchange of gases, drugs and Insulin through which consists of thin monolayer cells. Calu-3 cells are bronchial cells resembling the Type 1 cells, structure which has tight junctions with the thin monolayers. Calu-3 cells cultures which represents the pulmonary absorption mechanism [40-43].

Dosing solutions

The PH of solution adjusted to 6.2 or 6.9 and 7.7 or 8.2 by using HCL or NaOH respectively. I10: Insulin solution prepared at concentration 10 U/ml by dissolving Insulin powder in buffer. TI10: Technosphere Insulin 10 was prepared by concentration of Insulin 10U/ml dissolved in 3.3mg of Technosphere powder per millilitre of solution. TI25 and TI50: Prepared by dissolving 25 or

50 U/ml respectively in Technosphere powder per millilitre of solution [44-46].

Permeability studies

Permeability experiments or studies were done by using Alexa Fluor Labelling, Enzyme Linked Immunosorbent Assay.

Alexa fluor labeling

The various Insulin concentrations, permeation enhancers, and Technosphere Insulin concentrations were labelled with Alexa Fluor 488 protein labelling kit and introduced into the 14-day Calu-3 cell cultures. The unbound label was founded by dialysis. The labelling was confirmed by using Reverse Phase High Pressure Liquid Chromatography by absorption at 495 and 220 nm [47, 48].

Observation

Permeability experiments with Insulin as control, permeation enhancers, and Technosphere Insulin concentrations were compared. It shown that Alexa Fluor 488 protein labelling is not shown in Technosphere Insulin and Insulin concentrations which shown in permeation enhancer .Which was calculated by apparent permeability defined as amount of substance across the membrane. Apparent permeability was calculated by $P_{app} = \frac{DQ}{dt AC}$.

DQ/dt = Steady state rate of Insulin accumulation in cell culture, A = Cell culture surface area, C = Insulin concentration which is introduced into the cell culture. This study given that no little Insulin transported across the membrane that is no labelled Insulin is seen in Technosphere Insulin and Insulin (as control) but seen in permeation enhancers (Figure 3C).

Cytotoxicity assays

Done by following assays like Lactate Dehydrogenase Assay, MTS Assay.

Lactate Dehydrogenase assay

Lactate Dehydrogenase helps in the body for cell integrity maintenance. LDH leakage found by cytotox kit [49-51].

MTS assay

MTS assay used for determining the proliferation of cells. MTS means 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, innersalt) [52, 53].

Observations

LDH leakage of Technosphere Insulin compared with Triton X-100 (permeation enhancer, non ionic detergent). It shows that no LDH accumulation observed for Insulin and Technosphere Insulin but observed in

Triton X-100. This shows that LDH in Insulin and Technosphere used for cell integrity in cell culture but Triton X-100 damaged the cell integrity there by leakage of LDH which produced rapid absorption of Insulin not as Technosphere (Figure 3A).

MTS Assay does not show any inhibition on cell proliferation of cell culture after treated with Insulin (as control) and Technosphere Insulin but Capric acid; Triton X-100 shown inhibition of proliferation of cells in cell culture (Figure 3B).

Results

This shows that the Technosphere not affect Cell integrity of cells in lungs, Cell proliferation of cells in lungs, not damage the tight junctions of monolayer's of cells in lungs, not cause any harm to the pulmonary system. So Technosphere is not a permeation enhancer but it acts as substrate or particle which delivers Insulin and other proteins into the blood stream from deep lungs by high solubility, dissolution there by increases concentration of Insulin and proteins at PH of lungs. Large surface area of lungs facilitates the rapid increase of Insulin flux into blood stream by concentration gradient mechanism [54, 55]. Applications: Technosphere Insulin used in the treatment of Diabetes by Aerosolized Insulin therapy, it also used in the treatment of cystic fibrosis by Aerosolized antibiotic therapy e.g., Aerosolized tobramycin solution and also in gene therapy as Aerosolized Gene Delivery.

Pharmacokinetics

Technosphere Insulin dissolve in lung PH after inhalation and then the Insulin released is absorbed by lungs [56]. Bioavailability of Technosphere Insulin 21% to 25% than subcutaneous regular human Insulin, with half life 45min [57].

Clinical trials:

Efficiency

Technosphere Insulin decreases the glycosylated hemoglobin (A1C) level in blood in dose dependent manner and doesn't show any change in lung function. (Table 1)

Adverse events

cough is the side effect other than body weight gain and hypoglycemia, but cough shown only for short term i.e. 6 weeks. But long term adverse events should be still to be proved. (Table 2) [58-60]

Aerosolized Insulin marketed products

Exubera (Pfizer) 2006-2007 then they get off from market due to poor acceptance and providers, another one like AERx (Novo Nordisk) [61].

Failure of Aerosolized Insulin to come into the market

As they consist of poor providers due to poor market acceptance. Previously they are available at short acting human Insulin at minimum doses. Technosphere Insulin technology will overcome above failures but it

needed only long term clinical trials to show there is no adverse effect on long term usage and waiting for FDA approval [61].

Figure 1.

(A) Shape of Technosphere particle by Scanning electron microscopy and
(B) Chemical structure of Fumaryl Diketopiperazine [62]

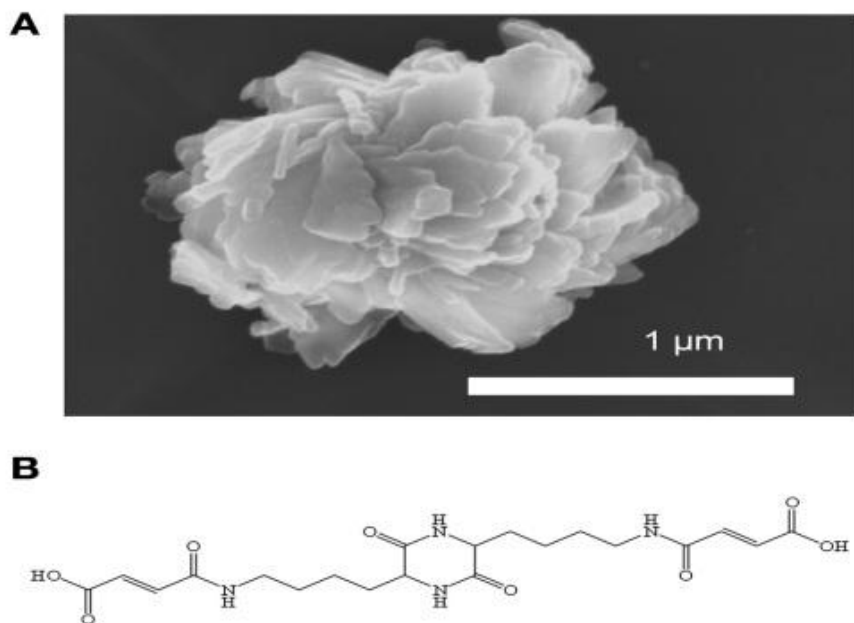


Figure 2. Pharmacokinetic absorption profile of Technosphere and subcutaneous regular human Insulin (RHI) [63]

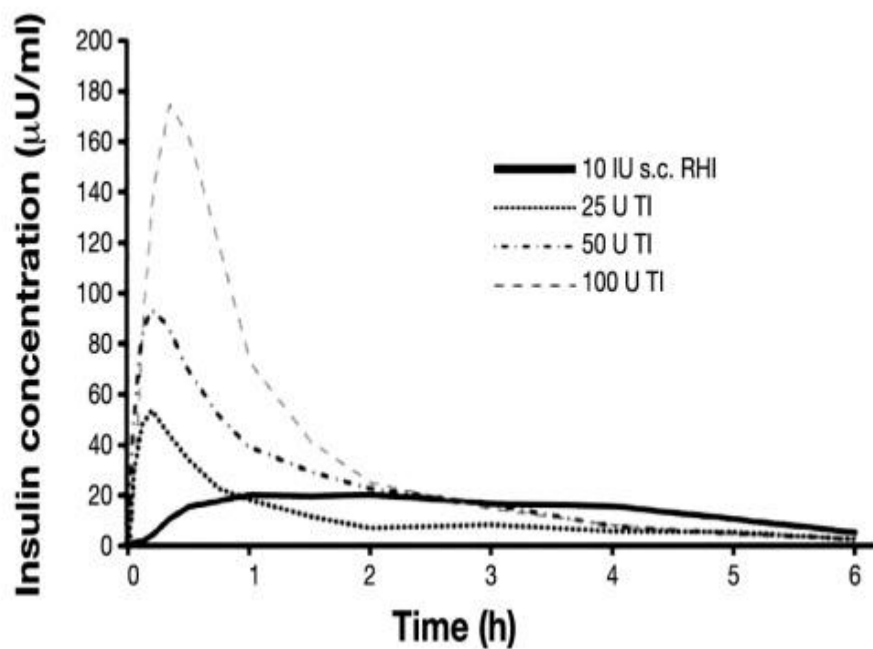


Figure 3. Effect of Technosphere on plasma membrane integrity and cytotoxicity on Fourteen-day Calu-3 cell monolayers were treated for 2 hrs [64]

(A) With Lactate Dehydrogenase leakage comparison with the presence of Triton X-100 0.4 mM, 10 U/ml *Insulin* (I10), or 10 U/ml TI (TI10) (mean \pm SD, n=3).

(B) MTS assay treated by introducing with Triton X-1000 of 4 mM, capric acid of 2 mM, 10 U/ml TI (mean \pm SD, n = 12).

(C) With 10 U/ml TI, 25 U/ml (saturated solution) TI, Triton X-100 of 0.4mM or in the presence of propidium iodide. Fluorescence microscopy used for imaging the cells.

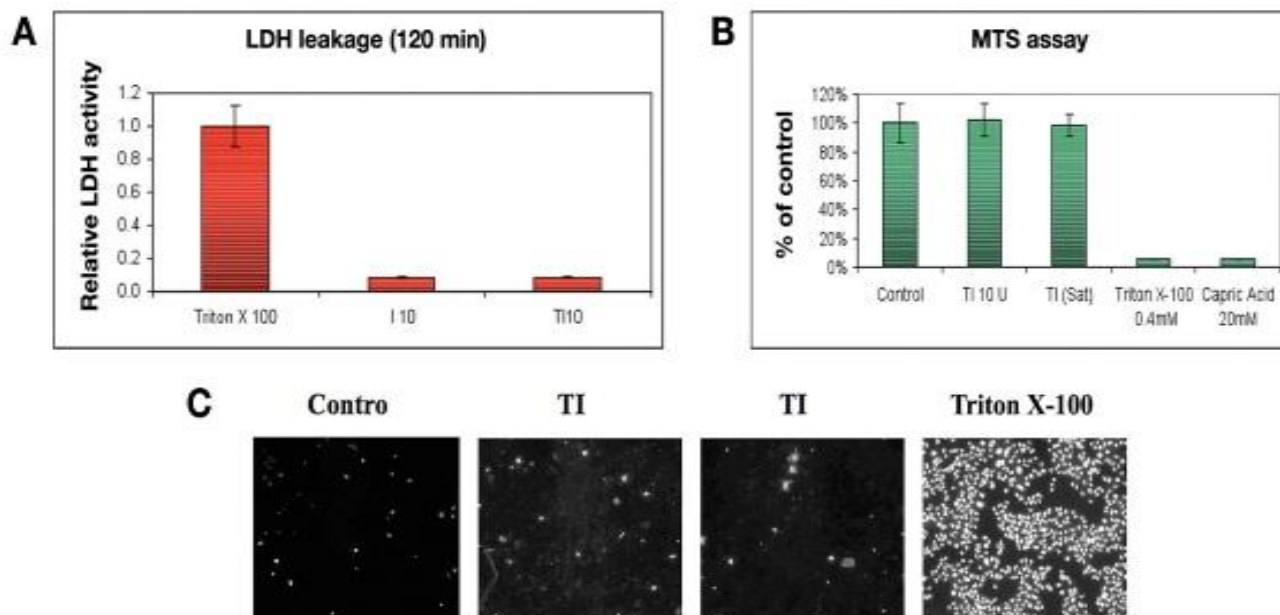


Table 1

Clinical efficacy of Technosphere insulin in randomized controlled trials in patients with type 2 diabetes

Study (n randomized)	Duration	Groups	A1c change, % mean (SD)	Patients achieving A1c \leq 7%, n/N (%)
Rosenstock 2008 N=126	12 weeks	Technosphere insulin (n=61)	-0.72 (NR)	NR
		Technosphere placebo powder (n=62)	-0.30 (NR)	NR
Tack 2008 N=227	11 weeks	Technosphere insulin 14 units (n=43)	-0.4 (1.2)	NR
		Technosphere insulin 28 units (n=43)	-0.5 (1.2)	NR
		Technosphere insulin 42 units (n=41)	-0.5 (0.9)	NR
		Technosphere insulin 56 units (n=42)	-0.6 (1.1)	NR
		Technosphere placebo powder (n=41)	0.2 (0.9)	NR
Rosenstock 2010 N=677	52 weeks	Technosphere insulin plus insulin glargine (n=211)	-0.68 (1.12)	47/213 (22%)
		Biaspart insulin (n=237)	-0.76 (1.09)	65/243 (27%)

Abbreviation: NR=not reported; SD=standard deviation

Formulary/Source: Refs 10-12

Table 2

Adverse events of Technosphere insulin in randomized controlled trials in patients with type 2 diabetes

Study (n randomized)	Duration	Groups (n)	Overall hypoglycemia, n (%)	Mild to moderate hypoglycemia, n (%)	Severe hypoglycemia, n (%)	Weight gain, kg mean (SD)	Cough, n (%)
Rosenstock 2008 N=126	12 weeks	Technosphere insulin (n=61)	26 (43%)	NR	NR	-0.1 (2.0)	18 (30%)
		Technosphere placebo powder (n=62)	22 (36%)	NR	NR	-0.9 (2.9)	17 (27%)
Tack 2008 N=227	11 weeks	Technosphere insulin 14 units (n=45)	11 (24%)	NR	NR	-0.3 (2.2)	10 (22%)
		Technosphere insulin 28 units (n=45)	10 (22%)	NR	NR	1.0 (5.7)	12 (27%)
		Technosphere insulin 42 units (n=44)	13 (30%)	NR	NR	0.7 (2.7)	4 (9%)
		Technosphere insulin 56 units (n=44)	15 (34%)	NR	NR	0.6 (2.5)	6 (14%)
		Technosphere placebo powder (n=42)	9 (21%)	NR	NR	0.2 (3.1)	10 (24%)
Rosenstock 2010 N=677	52 weeks	Technosphere insulin plus insulin glargine (n=323)	NR	155 (48%)	14 (4%)	0.9 (0.3)	103 (32%)
		Biaspart insulin (n=331)	NR	228 (69%)	33 (10%)	2.5 (0.3)	14 (4%)

Abbreviations: NR=not reported; SD=standard deviation

Formulary/Source: Refs 10-12

CONCLUSION

Inhaled Insulin is a recent novel drug delivery therapy used for the treatment of Diabetes but long term usage cause problems like irritation, cough, and some chronic pulmonary obstructions which lead to discontinuation of inhaled therapy occurred. It also decreased the relaunch of inhaled Insulin by companies due its poor acceptance of patients.

But by using the Technosphere Insulin technology, its evidences and results proved as it doesn't alter the lung function, pulmonary cell integrity, cell layer and not affect the cell proliferation of the lung cells. By proving the long term clinical trials, it's usage doesn't give adverse effects and getting FDA approval relaunch of Insulin inhalation, a novel drug delivery system which increase the patients compliance due its invasive character not only for Insulin but also for proteins and peptides which degrade at stomach PH can come into market.

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