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Review of current insulin and proposal of an advanced nanotechnology inhaled protaphane-polystyrene as alternative

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Abstract: Objective: Find out the characteristic of an inhaled insulin as a substitute to injectable insulin.

Methods: Gather the pitfall of current inhaled insulin and suggest solution based on current nanotechnology and commonly used insulin.

Findings: Currently available inhaled insulin is expensive and can cause hypoglycemia, bronchospasm, and other adverse effects in subjects with pre-existing respiratory diseases as shown in lung function tests. The particle diameter is 1-5 microns. Bioavailability is fair and the inhaled insulin dose is 3-10 times higher than injectable insulin one since only 10% is absorbed through the alveoli to enter the bloodstream. Insulin induces cell division wherever it is deposited which may lead to aberrant cell growth.

Current subcutaneous insulin therapy regime is based on the normal human physiology. Either metformin plus 2 doses of intermediate-acting insulin daily or 3 doses of short-acting insulin plus one dose of long-acting insulin daily. Nanoparticle passes directly through epithelium to enter the bloodstream. There is less respiratory tract irritation and the bioavailability is good.

Nanoparticle corona (<100 nm) allows hypothetical inhaled protaphane to pass through the lung fluids and its action can be slowed down by polystyrene polymerization. Nanoporous membranes or adding specific nanoreceptors to drug surfaces are other alternatives.

Conclusion: Pure inhaled insulin lowers blood glucose quickly and, being a growth factor, potentially increases aberrant cell growth. Neutral protamine Hagedorn (NPH) insulin is a stable and intermediate-acting insulin. Nanotechnology allows the protaphane nanoparticle to pass through respiratory epithelium and to enter the blood vessels with minimal interaction with pneumocytes.

Key words: Inhaled insulin, protaphane, polystyrene nanoparticles, nanotechnology, Kaplan-Meier estimator.

Introduction

Human pancreatic ß-cells release insulin into bloodstream in response to the carbohydrate load. When glycemic control is unsatisfactory despite dietary control and oral hypoglycemic agents, subcutaneous insulin injection will be offered to mimic the physiological pulsatile release of insulin. Current recommendations for type 2 diabetes patients are metformin plus insulin. For those who cannot tolerate oral hypoglycemic agents, 2 injections of intermediate- acting insulin or 1 injection of long-acting insulin per day will be alternatives. Intense insulin regimen includes at least two injections of insulin (rapid-acting and long-acting) daily, or insulin pump as in type 1 diabetes patients. Different types of insulin are shown in Table 1. All patients require blood glucose monitoring, preferably using test strip and glucose meter. (Figure 1)

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Table 1. Types and pharmacokinetics of subcutaneous insulin (Elizabeth Blair, A.N.P., at Joslin Diabetes Center).

Type	Brand Name	Onset	Peak	Duration
Rapid-acting	Insulin lispro(Humalog), insulin aspart(NovoLog), insulin glulisine(Apidra)	10 - 30 min	30 min - 3 h	3 - 5 h
Short-acting	Insulin regular	30 min -1 h	2 - 5 h	Up to 12 h
Intermediate-acting	Insulin NPH(Neutral Protamine Hagedorn), NPL(neutral protamine lispro)	1.5 - 4 h	4 - 12 h	Up to 24 h
Long-acting	Insulin glargine(Lantus), insulin detemir(Levemur)	0.8 - 4 h	Minimal peak	Up to 24 h
Very long-acting	Insulin degludec	0.5 - 1.5 h	No peak	Up to 48 h

Figure 1. How to check the blood glucose level by glucose meter

Other routes of insulin administration include intravenous (IV), transdermal and inhaled (IH). IV insulin gives fast effect in diabetic ketoacidosis in hospital setting. SC insulin can be given at home by insulin pen with risks of pain and lipodystrophy. Transdermal insulin absorption is affected by skin healthiness. Inhaled insulin, even in the size of few microns, can cause bronchospasm. Moreover, the reliance on the distal respiratory tree for absorption leads to low bioavailability, around 10-20% of the counterpart SC insulin.

When preparing inhaled insulin, the onset time will be shorter but short-acting means that the insulin effect does not last long and frequent dosing is required. The action of inhaled insulin can be then slowed down by non-covalent binding to protamine and addition of zinc salts. The latter also reduces the solubility of insulin [1].

Inhaled insulin obviates the need for subcutaneous injection. Afrezza technosphere (2-3μm size with peak action in 15-30min) is the only approved inhaled insulin in United States. It is expensive and can induce cough, bronchospasm and decline in lung function. Inhaled insulin in California is $150 for the equivalent of a 10-ml regular insulin vial, which costs $30-50 (Table 2). In addition, the price of inhaled insulin device is expensive $800 per set [2].

Table 2. Price of different types of insulin [3]

Insulin	**Cost of basal insulin (US dollars)**
Protaphane (NPH) SC	$22-50 per 1000-unit vial
Deter/ glargine SC	$70-90 per 1000-unit vial
Deter/ glargine pen SC	$170-200 per pens (1500 units)
Alfrezza inhaled	$278.6 per 600-unit

Nanotechnology allows inhaled insulin to be absorbed everywhere along the whole respiratory tract leading to increased bioavailability. The reduction of the total dose of insulin leads to less aberrant cell growth. Nanoparticle polymerization further prolongs the action of inhaled insulin and thus less frequent dosing is required. Although there are other options of long-acting insulin for nanoparticle production, protaphane HM (Neutral Protamine Hagedorn) is cheaper with favorable cost-effectiveness profile.

Advance in nanotechnology and controlled drug delivery

Nanoparticle production involves substrate supersaturation in solution and nucleation processes in a particular temperature. Nucleation is followed by diffusion limited or reaction limited growth regimes. Separation of different sizes of particles then follows. We can manipulate the process using different building blocks like nanoclusters (≤1 nm size), nanoparticles (1-100nm diameter), filled nanowires

and hollow nanotubes (1-100nm diameter and up to few mm long and beyond) and 1 atom-thick nano-sheet. The size distribution of the nanoparticles can be controlled by changing the degree of supersaturation [4]. The critical radius of nanoparticle for nucleation R is given by the following formula:

$$R^4 = 2\gamma a_v / K_b T \, InS$$

where γ is the surface energy of the material, a_v is the atomic volume, K_bT is the thermal energy, InS is the natural log of supersaturation, that depends on pressure and concentration. For diffusion-limited regime, the reaction rate is based on the transport of reactants through the reaction medium (a solution for nanocrystal growth) whereas, for reaction-limited regime, the reaction rate is controlled by adsorption and reaction rate at the surface.

Nano-particle corona (<100 nm) allows nanoparticle to pass through the lung fluids, translocate across the epithelium and enter the bloodstream [5]. The onset of action of inhaled insulin can be slowed down by the formation of nano-capsules in which the drug is confined to an aqueous or oily core surrounded by a shell like wall. Alternatively, the drug can be covalently attached to the surface or into the matrix. These nanoparticles allow controlled delivery of drugs and prolonged storage. However, polymeric nanoparticles possess limited drug-loading capacity and on repeated administration, toxic metabolites may be formed during the biotransformation of the polymeric carriers [6].

These nano-capsules can be prepared by emulsion polymerization method (monomers are emulsified in a non-solvent phase) or dispersion polymerization method (monomers are dissolved in a solvent for the resulting polymer). Natural hydrophilic polymers can be assembled using proteins (gelatin, albumin, lectins, legumin) or polysaccharides (alginate, dextran, chitosan). Synthetic hydrophobic polymers instead can be prepared using pre-polymerized polymers (poly e-caprolactone, poly lactic acid, polystyrene) or polymerized in process polymers(poly isobutylcyanoacrylates, poly butyl cyanoacrylates). Polymeric micelles are investigational nanocarriers. Examples are Soluplus, Pluronic F68, F108 and F127 [7].

An ideal inhaled insulin should be of low production cost and better bioavailability as compared with subcutaneous insulin. It should be used friendly without significant risk of hypoglycemia, bronchospasm or aberrant cell growth. With nanocarriers, the inhaled insulin degrades less, is less immunogenic and passes easily through the mucosa to enter the cells, then the blood vessels. The mechanisms involved are both trans-cellular (major mechanism) and para-cellular transport. Nanocarriers also allow the controlled release of insulin [1].

Inhaled protaphane nanoparticle with polystyrene polymerization

Protaphane is the most commonly used intermediate-acting insulin and is prepared from crystalline insulin with pH 6.9 - 7.5. For each 100 units of insulin, there are 300 - 600 g protamine sulphate, less than 40 g zinc, a suitable bactericide, and sodium phosphate buffer. Polystyrene nanoparticles, by activating the ion channels in respiratory epithelial cells, are a good vehicle for drug delivery for the treatment of lung diseases [8]. Action duration of the proposed protaphane nanoparticles is shown in Table 3.

Table 3. Action duration of the proposed protaphane nanoparticles.

Subcutaneous Insulin	Corresponding inhaled protaphane HM preparation
Short-acting insulin	Pure protaphane HM nanoparticle
Intermediate/ long acting insulin	Protaphane HM in polymeric matrix or polymeric membrane wall structure containing the oily or aqueous core of protaphane HM

Just like other new drug development, the proposed inhaled protaphane nano-particles needs to pass four phases. **Phase I trials in healthy volunteers** determine the drug safety and dosing. **Phase II trials are multidose and parallel-design study,** that determine the drug efficacy and safety in small samples (usually 10 to 40 patients per group and 3 to 4 groups for study). The pharmacodynamics and pharmacokinetics can be evaluated by blood glucose concentration at various time-points following inhalation. Kaplan-Meier curve analysis is performed to assess the therapeutic effect of the nanoparticle. The peak serum drug concentration is reflected by the lowest serum glucose concentration. The duration that takes for the serum glucose concentration to get back to pre-experimental level reflects how long-lasting the protaphane nanoparticle is. This in-turn determines the dosing frequency of the inhaled nanoparticle [9]. **Phase III trials** are similar to phase II but with a bigger sample size. **Phase IV trials** are post-Food and Drug Aministration approval studies or post-marketing surveillance studies.

Manufacture, sterilization and delivery of protaphane nanoparticles:

Insulin nanoparticles are produced by emulsification followed by freeze-drying. Purified nanoparticles are suspended in hydrofluoroalkane, using essential oils as suspension stabilizers to form pressurized metered dose inhaler (pMDI) formulations. The metering valve delivers propellant and metered dose of insulin nanoparticles in colloid or solution. On actuation of the device, the dose is released on atmospheric depressurization. The propellant evaporates leaving the protaphane nanoparticles behind. Insulin integrity

is checked by high performance liquid chromatography, size exclusion chromatography, circular dichroism and fluorescence spectroscopy [10].

Many technology companies provide this product in a sterile packing. If sterilization is a concern, gamma irradiation and irradiation with accelerated electrons at a dose of 15 kGy is useful without affecting the chemical structure of the nanoparticles [11].

Metabolism and excretion of polystyrene nanoparticle.

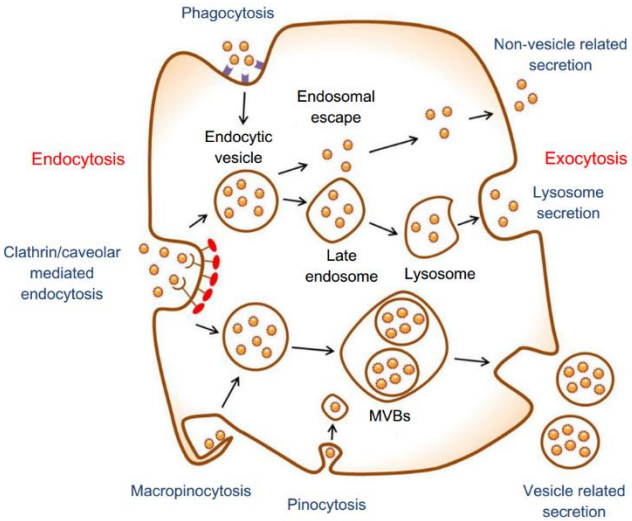

Figure 2. Phagocytosis and pinocytosis of nanoparticles (Nuri Oh. endocytosis and exocytosis of nanoparticles in mammalian cells. International Journal of Nanomedicine)

After the inhalation of the protaphane nanoparticles, the aerosol is deposited in respiratory tract. In the blood, macrophages phagocytize the debris and inside the phagosomes formed inside fuse with lysosomes. Enzymes (in acid medium) and reactive oxygen species then degrade the debris. The metabolism of nanoparticles can be traced in-vivo by scintigraphy. Animal studies and clinical trials allow us to study the pharmacokinetic. The biochemical and physiological effects of the nanoparticles can be shown in pharmacodynamics study in human [12]. In rat model, polystyrene nanoparticle was excreted through the bile into the bowel. After parenteral administration of 50nm FITC fluorescence polystyrene microspheres, 36% of the dose is excreted in bile and only 30% remains in the blood after 24 hours [13]. In first order elimination kinetics, the drop concentration in the nanoparticles can be described by the following formula:

$$C = C(0) * e^{-\lambda * t}$$

(where C = drug concentration at time t, C(0) = extrapolated initial drug concentration, λ = elimination rate constant and t = time).

Clearance (C_L) or the elimination rate of nanoparticles is given as follows:
C_L = Elimination rate / serum drug concentration = volume of distribution x ($\ln 2/t_{1/2}$). If bile duct is obstructed by artificial ligation or gall stone disease, the nanoparticle will be excreted through the intestinal goblet cells into the bowel [14].

Toxicity of protaphane-polystyrene nanoparticles

Zinc is involved in many biochemical processes important for immune, sexual function, cognition and vision. It is a component of many proteins and metalloenzymes. Normal cellular Zn^{2+} is 10 ng/L. Apoptosis occurs when cellular zinc level is < 0.06 ng/L. Toxicity ensures when cellular zinc level > 60 ng/L[15]. Protamine is a relatively nontoxic heparin neutralizer [16].

Polystyrene nanoparticles are commonly used in medicine and industry [17]. In human, polystyrene nanoparticles can activate RBC aggregation and adhesion to epithelial cells [18]. Microscopically, polystyrene nanoparticles only temporarily accumulated in recycling endocytic vesicles and therefore showed limited intracellular accumulation in treating ovarian cancer cells [19]. The potential toxicity of non-biodegradable polystyrene nanoparticles on immune cells is not supported by in-vivo studies. Polystyrene is a good carrier for inhaled medication by activating ionic pores in respiratory epithelium [20]. Polystyrene-NH_2 particles are found to terminate the growth of acute monocytic leukaemic cell line. This effect can be reduced by conjugation of the cationic groups to shield the positive charge, or by replacing the amine groups with amphiphilic head [21]. Most of the produced pulmonary toxicity of nanoparticles is due to the environmental or occupational exposure to nanomaterials.

Different inhaled protaphane nanoparticles preparation

By adjusting the proportion of protaphane HM and polystyrene nanoparticle (say 1:0.1, 1:0.5, 1: 0.8) in the supersaturated solution at a suitable temperature, we can obtain several mixtures of insulin with different duration of action. The test dose of inhaled protaphane nanoparticle starts from 1000 pmol, assuming the nanoparticles enter the bloodstream instantaneously with 100% bioavailability and minimal metabolism. In normal people, serum insulin rises up to 250 pmol/L during meal time as shown in Figure 3. Since nanoparticle can penetrate all compartments, blood volume is used for calculation.

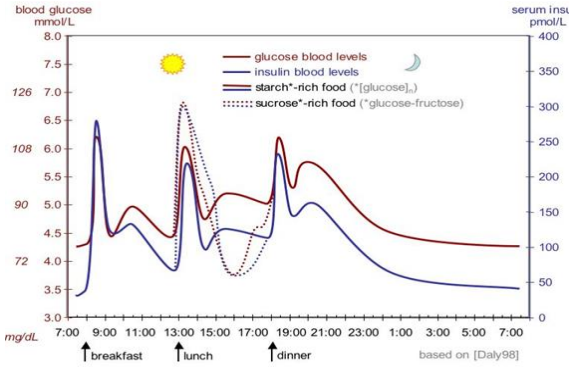

Figure 3. Low blood sugar and Insulin reactions for diabetic subjects

A 70-kg man have a blood volume of 5 L (7% of body weight). Normal resting insulin level of 50 pmol/L (~7μIU/mL) will rise up to 250 pmol/L (~36μIU/mL) soon after we eat. For insulin unit conversion, 1μIU/mL equals to 0.144 pmol/L. By multiplying the insulin level to the blood volume, the corresponding dose of inhaled protaphane nanoparticles ranges from 250 to 1250pmol. Starting the experiment with 1000 pmol is pretty safe.

Kaplan-Meier estimator allows us to find the most suitable protaphane-polystyrene nanoparticles combination, so that 50% patients achieve normoglycemia within 2-3 hours after inhalation. Kaplan-Meier curve is a series of steps with Y-axis being the percentage of patients still having hyperglycemia and X-axis being the time during the assessment.

If we take 50% patients still have hyperglycemia on the Y-axis, the corresponding value on the X-axis will be the average time for 50% patients to achieve normoglycemia-median drug response time. Say, 50% of the patients with fasting blood glucose level of 10-16mmol/l after inhaled 1000 pmol (~144μIU) of protaphane nanoparticles after 3 hours in average. If we want 75% of the patients to achieve normoglycemia during the time period specified, we put a line 25% patients still having hyperglycemia on Y-axis, the corresponding value on the X-axis will be the average time required for 75% patients to achieve normoglycemia.

If the peak of action of protaphane nanoparticle occurs too quickly, the percentage of patients having hyperglycemia drops quickly so a longer acting preparation should be used. If a significant proportion of patients develops hypoglycemia, then the dosage of inhaled insulin should decrease.

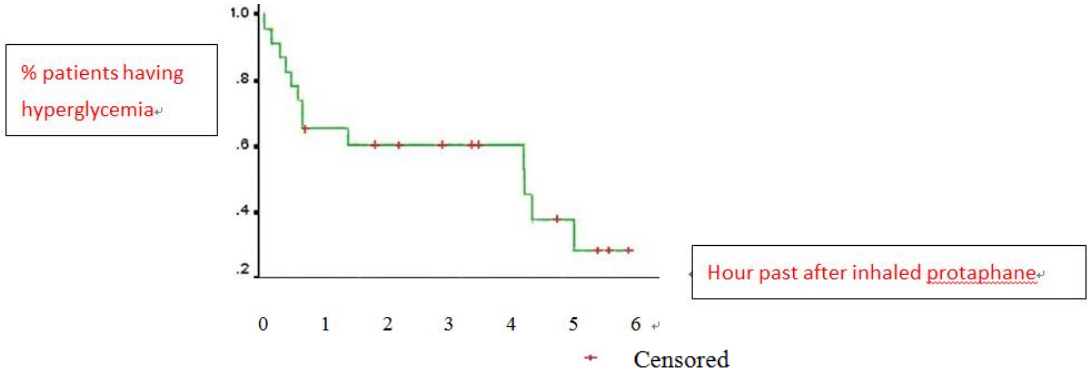

Figure 4. Percentage of patients having hyperglycemia as a function of time after inhaling protaphane nanoparticle.

Proposed experimental protocol

Patient selection: Diabetes patients with fasting blood glucose level 10-16 mmol/l. Other diabetic medications are withheld during the test.

Sample size: 40 diabetes patients or more. This is a Phase II trial as it works on the diabetes patients. It determines the drug efficacy and safety in small samples (usually 10 to 40 patients per group and 3 to 4 groups for study), using multidose and parallel-design study.

- Heparin block is inserted into the vein the day before to avoid the stress response to needle puncture during the test. Heparin-saline is given to prevent the blood clotting in angiocath.

● Patients are allowed to have 1500-1800 kcal breakfast in the morning. 1000 pmol (~144μIU) of inhaled protaphane nanoparticles is inhaled after breakfast (Subcutaneous insulin is injected 30 minutes before breakfast but it is better to inhale the protaphane just after breakfast due to its faster onset of action).

● Check fasting blood glucose using the drop of blood taken from the heparin block after removal of 1ml of blood (dead space in the angiocath). After blood taking, flush the heparin block with heparin-saline again. After inhaling the protaphane nanoparticles, check the blood glucose half hourly up to 6 hours.

● The choice of which preparation to use depends on the shape of Kaplan-Meier curve. If it is widened, we use shorter-acting preparation and if it is too narrowed, we use longer-acting preparation. Setting 6 hours on the X axis is due to the fact that the interval between meal times is around 6 hours. It can be seen in the following graph that the data we obtain can approximate the actual curve by shortening the time interval of blood taking, say every 15 minutes.

Statistical meaning of Kaplan-Meier estimator

Kaplan-Meier estimator is applicable to any composite endpoint including normoglycemia (blood glucose level of 4.4-6.1 mmol/L) [22]. It estimates the fraction of patients having hyperglycemia at the start and achieving normoglycemia after inhaling the protaphane nanoparticles.

Some patients may still be hyperglycemic at the end of 6 hours, others may drop out from the study early. For these subjects with partial information, we know that the event of achieving normoglycemia occurs sometime after 6 hours. These subjects should not be ignored and are right-censored because they also provide some information about the time to achieve normoglycemia. These "censored observations" are noted by tick marks on the Kaplan-Meier curve. Censoring is based on the assumption that at any time patients who are censored have the same degree of blood glucose drop as those who continue to be followed, and the probabilities of achieving normoglycemia are the same for subjects recruited early and late in the study.

The curve estimates the number of patients achieving normoglycemia endpoint in the presence of censored observations. We compute the probabilities of achieving normoglycemia within each time interval and multiplying these successive probabilities by any earlier computed probabilities to get the final estimate. Time to achieve normoglycemia is variable among different subjects. In such case, the number of normoglycemia per unit duration of time can be assessed by Kaplan-Meier estimator.

The Kaplan-Meier curve describes the probability of having drug response to protaphane nanoparticles in many small time intervals. Group analysis of the normoglycemic response allow us to assess the onset time and the duration of action of inhaled protaphane nanoparticles. This "product limit estimator" computes the probabilities of occurrence of an event at a certain point of time. We multiply these successive probabilities by any earlier computed probabilities to get the final estimate. The probability of hyperglycemia at particular time (t) is as follows: S_t =1 - (number of patients achieving normoglycemia / number of patients at the start)

For each time interval, survival probability is calculated as the number of subjects surviving divided by the number of patients at risk. Subjects who have achieved normoglycemia, dropped out, or move out are not counted as "at risk" i.e. and subjects who are lost are considered "censored" and are not counted in the denominator.

Total probability of achieving normoglycemia till 6 hours is calculated by applying law of multiplication of probability to calculate cumulative probability.

For example, the probability of a patient achieving normoglycemia 2 hours after inhaling protaphane can be considered as the probability of achieving normoglycemia at the 1st hour multiplied by the probability of achieving normoglycemia at the 2nd hour given that the patient has hyperglycemia at the 1st hour. This second probability is a conditional probability. Most subjects can achieve normoglycemia within 6 hours but some are still hyperglycemic at the end of the trial. Even in these conditions we can calculate the Kaplan-Meier estimates as follows.

Table 4. How to calculate the Kaplan-Meier estimate.

Time of achieving normoglycemia (t)	No. of patients achieving normoglycemia (n)	No. of patients still having hyperglycemia at the start of any particular hour (h)	Probability of normoglycemia (n/h)	Probability of hyperglycemia (1-n/h)	Probability of hyperglycemia at the end of time (L)
0/ 0.5/ 1/ 1.5/ 2/ 2.5/ 3/3.5/ 4/ 4.5/5/ 5.5/ 6 h					

The graph plotted between estimated hyperglycemia probabilities/estimated percentages of patients having hyperglycemia (on *Y* axis) and the time past after entry into the study (on *X* axis) consists of horizontal and vertical lines. The Kaplan-Meier curve is a step function: the proportion having

hyperglycemia remains unchanged between the events, even if there are some intermediate censored observations. It is incorrect to join the calculated points by sloping lines.

We can compare the efficacy of two types of insulin therapy, say inhaled protaphane nanoparticles versus subcutaneous protaphane, by comparing the 2 Kaplan-Meier curves. A vertical gap at a specific time point means one group has a greater fraction of subjects having hyperglycemia. A horizontal gap means that it takes longer for one group to experience a certain fraction of patients achieving normoglycemia.

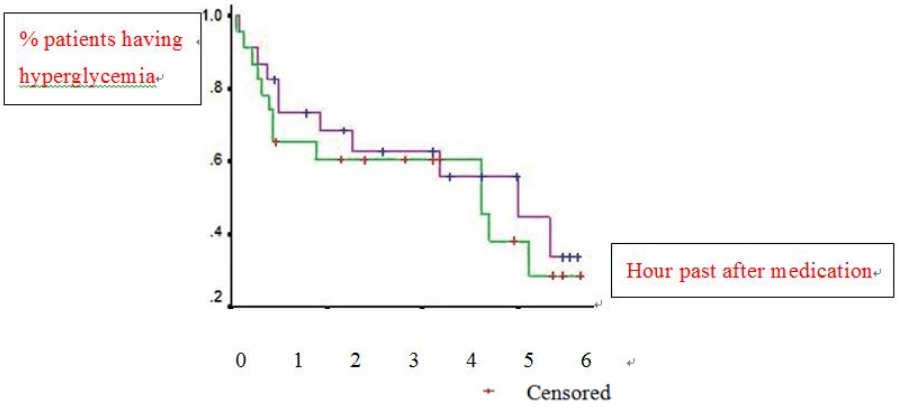

Figure 5. Percentage of patients having hyperglycemia as a function of time in 2 groups of patients (Purple line: inhaled and green line: subcutaneous)

The total number of patients expected to achieve normoglycemia in a group (e.g. E_2) at any specific time is the sum of the number of patients expected to achieve normoglycemia in both groups of patients at any specific time.

At any specific time, the number of patients expected to achieve normoglycemia is the product of risk of achieving normoglycemia with the total number of patients having hyperglycemia at the start of the experiment.

The total number of patients expected to achieve normoglycemia in group 2 is the sum of the expected events calculated at different time.

The total number of patients expected to achieve normoglycemia in group 1 (i.e. E_1) is calculated by subtracting the total number of expected events in group 2 i.e. E_2, from the total of observed events in both groups i.e. $O_1 + O_2$. The log-rank test statistic for the two groups of patients is given by:

$$\text{Log-rank test statistic} = \frac{(0_1 - E_1)^2}{E_1} + \frac{(0_2 - E_2)^2}{E_2}$$

Table 5. Log-rank statistic for the 2 groups of patients.

Time of achieving normoglycemia (t)	Total no. of patients achieving normoglycemia in both gps (N)	No of patients having normoglycemia in gp 2 (O_2)	No. of patients still having hyperglycemia at the start of any particular hour (H)	No. of patients still having hyperglycemia at the start of any particular hour in gp 2 (h_2)	Probability of hyperglycemia at the end of time (L)	Expected probability of normoglycemia in gp 2 (E_2)	Expected probability of normoglycemia in gp 1 (E_1)
0/ 0.5/ 1/ 1.5/ 2/ 2.5/ 3/ 3.5/ 4/ 4.5/ 5/ 5.5/ 6 h							

When comparing the calculated value with the critical value (using chi-square Table) for one degree of freedom, if the test statistic value is lower than the critical value, there is no significant difference between the two groups regarding the time to achieve normoglycemia.

Cox proportion hazard model tests the effect of other independent variables, like body mass index, on the time to achieve normoglycemia in different groups of patients. The principle is just like the multiple regression model. Hazard is the probability of having normoglycemia at a given time assuming that the

patients have hyperglycemia up to that given time. Hazard ratio is the risk of hazard occurring at any given time in one group compared to another group at that time moment. Both log-rank test and Cox proportion hazard tests assume the hazard ratio is constant over time.

Results and Discussion

Adjustment of subcutaneous insulin dosage in diabetes patients is based on 'drug titration' according to the blood glucose and HbA1c level. This also applies to the inhaled insulin nanoparticles. The result just gives a rough estimate. As the diabetes mellitus may get worse over time, actual drug dosage still need titration according to the clinical response.

The result will be expressed in terms of the dosage of inhaled protaphane-polystyrene nanoparticles and the dosing frequency for an average 70kg adult, say 1 puff three times daily, immediately after each meal. Dosage adjustment based on the body weight is preferred. The side effects should be stated, like bad smell, respiratory tract irritation, bronchospasm symptoms and blood tests abnormalities.

Patients excrete a certain fraction of polystyrene in serum into feces. In equilibrium state, the level of fecal excretion of polystyrene particles will be the same each day, provided that patients have inhaled the insulin regularly. Patients are encouraged to report any long term side effects in order to make improvement.

Discussion

Diabetes patients have insulin deficiency or insulin resistance. It is logical to offer them insulin therapy rather than oral hypoglycemia agents (OHA). The latter can cause nausea and vomiting, and should be used cautiously in patients with liver or renal impairment. Most patients defer insulin therapy due to the inconvenience associated with the daily needle puncture. As a result, more damage will occur to the eyes, kidneys, coronary vessels and cerebral vessels. Inhaled protaphane nanoparticles hopefully will improve patients' acceptance to insulin therapy.

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