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Equilibration between Insulin Crystal Dissolution and Insulin Consumption in Human Body

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Abstract

Crystalline insulin is used in anti-diabetic drugs to sustain basal insulin needs. To select individual doses for people with diabetes, it is necessary to consider patient's medical status. Our objective is to estimate how long sets of equally-sized insulin crystals should be able to ensure sufficient drug supply. Considered is the equilibration between insulin supply from dissolving crystals and its consumption in the human body. Assuming diffusion control of both insulin crystal dissolution and elimination, we conclude that the stationary insulin concentration level is lower than insulin crystal solubility. It depends on the ratio of the total surface of all insulin receptors to the total insulin crystals' surface, multiplied by (reverse) ratio of the diffusion layer thicknesses. Valuable clues for personalized diabetes treatment can be inferred from insulin half-life time in man. Supposing that insulin elimination from the body follows first order chemical reaction law, we conclude that sets of equally-sized insulin crystals can ensure adequate insulin supply with low frequency of injection in parenteral controlled release.

Keywords: Diabetes; Dissolution Of Insulin Crystals; Personalized Care; Prolonged Therapeutic Effect;Stationary Insulin Concentration

Introduction

Over the years, much research effort has been put to clarify insulin pharmacokinetics (the plasma levels of insulin over time) and pharmacodynamics (the resulting effects on glucose over time). It is reported [1] that insulin pharmacokinetics is governed by a multitude of factors of importance, such as absorption process, distribution that includes binding to circulating antibodies (if present) and to insulin receptors, and its ultimate degradation and excretion. The duration of absorption naturally determines the time for equilibration between the amounts injected and absorbed. Therefore, special attention has been devoted to insulin absorption [2]. Radiotracer measurements have shown that absorption rate of iodine (I^{125}) labeled insulin, remaining at the injection site, depends on many factors, such as insulin form (dissolved, amorphous or crystalline) and concentration, injection volume, blood flow, and presence or absence of degradation at the injection site. For instance, if the injection is given in the abdominal area it results in the most rapid insulin absorption after subcutaneous administration, and the femoral region in the slowest resorption. The measurements were carried out with dissolved, amorphous and crystalline (Lente, Ultralente) insulins from pig and beef. It was found that external monitoring of the radioactivity remaining at the site of injection provides a reliable measure of the amount of insulin remaining in the tissue [2].

To suppress "Peaks and valleys" in blood concentration some insulin formulations contain micro-crystals, usually of Zn-insulin. Dissolving more slowly than the amorphous drugs, such crystals ensure a basal insulin replacement, and a prolonged therapeutic effect [3-5]; in some cases, up to one day. Preferred must be crystals of narrow size distributions because such crystals dissolve in a nearly parallel way. Otherwise, if differently sized, the smaller crystals dissolve sooner than the larger species, and with their disappearance the drug starts to deplete.

To reduce crystal polydispersity internal seeding of equally-sized crystals was suggested recently [6], its advantage being avoidance of crystal grinding, sieving and any introduction of impurities. Nucleating all crystals quasi-instantaneously (for a suffciently short time) and then abruptly depriving the system of its capability to further produce nuclei impels all seed crystals to

grow from an initial nearly equal size, which is the critical nucleus size (or slightly larger). Then, during a slow uniform process, these crystals overgrow further to form the nearly monodisperse crystalline matter desired.

The long-acting basal insulins are combined with rapid acting insulin analogues, mainly intended for prandial doses. Varied patients and lifestyles necessitate a variety of basal and bolus doses. Therefore, selection of a proper basal and bolus balance must always address variations in an individual's age, weight, gender, activity, live stile, and general physical condition. One of the four processes in personalized medicine strategy is to tailor individual insulin dose for each patient [7]. In view of this requirement, equilibration between insulin supply from dissolving crystals and its disappearance from the subcutaneous depot is considered here. In particular, we study the duration of the balance between insulin supply resulting from crystal dissolution and its consumption. The balance duration is estimated assuming insulin elimination obeying first order chemical reaction law [8]. So, basal insulin doses are calculated from insulin half-life time in human body.

Model Considerations

For establishing the balance between insulin supply (resulting from dissolution of crystals in the anti-diabetic formulations) and its consumption in human body, we consider the dissolution driving force, the so-called undersaturation, (c_e - c), where c is the actual concentration of the substance in bulk solution and c_e is solubility. The concentration, c increases systematically during crystal dissolution, reaching finally c_e, when the dissolution driving force disappears. Then the dissolution process stops, so that the (dissolving) crystals cannot render concentration higher than c_e. Noyes-Whitney equation [9] describes the diffusion-controlled crystal dissolution flux, which is the amount of mass dissolved per unit time and per unit dissolving area:

$$dM/dt = -S_{cryst}(D/\delta_{cryst})(c_e - c) \quad \textbf{(1)}$$

where M is mass of solid remaining at a specific time t and S_{cryst} is the total crystal surface; D is the diffusion coefficient of the solute and δ_{cryst} is the diffusion layer thickness; the latter depends on temperature, solvent viscosity, dissolving surface geometry, and stirring solvent hydrodynamics.

Insulin disappears gradually from plasma in man, the liver and the kidneys being major sites of insulin degradation, and about 10% are appearing in the urine. Proteolytic degradation of insulin occurs both at cell surfaces and in the lysosomes. Therefore, equalization should be maintained in the body [8] between insulin supply (due to crystal dissolution) and its disappearance from the subcutaneous depot. The equilibration has another important impact, because it is known [10] that the receptors vary in number inversely with the insulin concentration to which they are exposed. Therefore, keeping constant insulin concentration should ensure a constant number of receptors.

Diffusion-control of insulin elimination is assumed here. Thus, the equalization between the crystal dissolution flux and the insulin elimination flux yields:

$$S_{cryst}(D/\delta_{cryst})(c_e - C) = S_{recept}(D/\delta_{recept})C \qquad (2)$$

where C is the equilibration concentration and $c_{recept} = 0$. Thus:

$$C = c_e/[1 + (S_{recept}\delta_{cryst}/S_{cryst}\delta_{recept})] \qquad (3)$$

This result shows why the size of the crystals should be adjusted to the insulin receptors' capacity. Therefore, personal medical data will be needed to tailor individual insulin dose for diabetes treatment. The ideal case, $S_{cryst} = S_{recep}$, and $\delta_{cryst} = \delta_{recept}$, is shown in Figure 1, where the two concentration gradients (the one from the crystals and the second one towards the receptors) are equal. (In such a special case, $C = c_e/2$.)

Indeed, the blood stream diminishes the thickness of the diffusion layer; but it never vanishes. Insulin diffusion supply to the receptors can be augmented, but so may insulin supply from dissolving crystals be increased. So, the crystals are dissolved only to the extent, which corresponds to the insulin demand. It is known that in disease situation blood rheology is altered, and plasma and whole blood viscosity are markedly higher in diabetes cases.

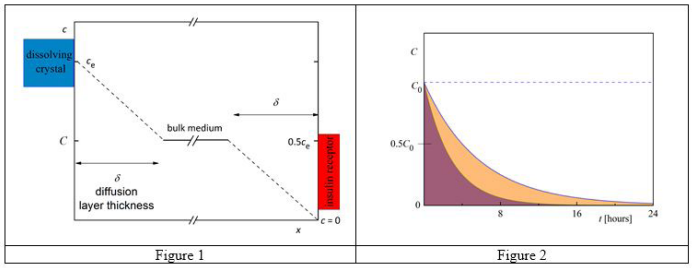

Figure 1 Figure 2

Figure 1: Schematic (not to scale) presentation of the equilibration between insulin crystal dissolution and insulin elimination. (In this ideal case: $S_{cryst} = S_{recep}$, and $\delta_{cryst} = \delta_{recept}$.).

Figure 2: Exponential decay of insulin concentration in injection depots with time, t elapsing after injections; upper curve - subcutaneous, lower curve - intramuscular injection.

Approaching insulin disappears from plasma in man, we accept first order kinetics law (see [8]):

$$c = c_o \exp(-\kappa t) \qquad \textbf{(4)}$$

where κ [s^{-1}] is rate constant. It is known that in this case κ is related to the half-life time $\tau_{1/2}$, $\tau_{1/2} = \ln 2/\kappa$, and that both κ and $\tau_{1/2}$ do not depend on the initial concentration. Indeed, biological half-life depends on the route of exposure. A typical half-life time, $\tau_{1/2} \approx 4$ hours is observed after a subcutaneous injection; after an intramuscular injection $\tau_{1/2}$ is 2 hours [10].

Discussion

Although the site of administration is one place in the subcutaneous tissue, the insulin molecules from the injection depot are absorbed via the capillaries into the blood stream. So, the latter conveys the physiologically active insulin monomers to the insulin receptors that are widely distributed throughout the whole human body. That is why, although highly simplified, our model can reflect adequately the pharmacokinetic and pharmacodynamic situation. The course of subcutaneous depot dissipation (i.e. insulin disappearance from it) is presented in Figure 2. The upper curve shows that about 7% of subcutaneous depot content is remaining after 15 hours (i.e. dissolved crystal volume 93 %). Correspondingly, about 99% of the crystal volume is dissolved during the same time after intramuscular injection (see the lower curve in Figure 2). The time course of insulin consumption presented in Figure 2 explains the direct (radiotracer) measurements of iodine (I^{125}) labeled residual amount of insulin at the site of injection see [1]. The same exponential decay is measured in [1] but with half-life time $\tau_{1/2} = 8$ h. Keeping in mind the considerable injection regional differences [2] and the intrapatient variability in insulin absorption (which may reach 35%, see [1]), the results seem to correlate. Therefore, personalized tailoring of basal insulin doses can be made based on the c vs t plots in Figure 2.

Materials and Methods

Crystallization of rhombohedral insulin crystals (Figure 3) was studied experimentally using two different insulin sorts, from BioChemika (BioChemika, ≥ 85% (GE), ~24 IU/mg) and from SIGMA, Denmark, Lot # 080M1589V. Identical crystallization conditions were used with insulin concentrations between 4.0 and 8.0 mg/ml. The crystallization solution included also 0.001M HCl, 0.05M citrate buffer at pH = 6.98, 0.005M $ZnCl_2$, 15% (v/v) acetone. The experimental procedure is simple. The weighted amount of insulin was dissolved in highly diluted HCl. Then consecutively citrate buffer (pH = 6.98), $ZnCl_2$ and finally acetone was added. Bi-distilled water from quartz distiller was used.

Insulin crystals are transparent and colorless, and to distinguish them in the water solution (from amorphous precipitates or from the precipitating agent crystals) we apply interference contrasting via differential image splitting. The application of this method required the use of cuvettes of optical quality. Therefore, custom made quasi-2D all-glass cells were used [11]. In such a cell, the crystallization proceeded in the thin solution layer confined in the gap between the two glass plates, the gaps being varied in series of cells, from 0.05 cm (volume 0.35 cm^3) down to 0.002 cm. For cell production two circular glass plates of optical quality were welded in exactly parallel position (the letter being controlled interferometrically by using a Mach-Zehnder scheme). An advantage of the 2D-cell is that it has relatively small inside volume, and is characterized by low solute consumption. Besides, the cell allows perfect cleaning. The most important benefit of the cell is the rapid imposing of supersaturation shifts, via appropriate temperature changes. In view of temperature dependence of insulin solubility, supersaturation was altered using water baths having the desired temperature. Simply, the all-glass cell containing the protein solution was immersed in the water.

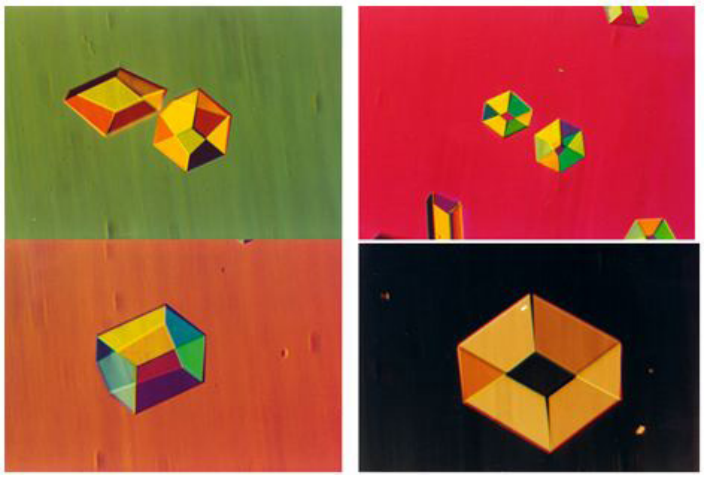

Figure 3: Rhombohedral insulin crystals formed by {101} growth faces. (Crystal sizes are ~ 200 to 400 μm.) The crystals are visualized by interference contrasting via differential image splitting. The latter means displaying two object images at a distance shorter than the resolution limit of the microscope objective lens. The so-called Interphako method, involving a homogeneous microscopic field, is used with a microscope Peraval-Interphako, Carl Zeiss, Jena, Germany. The homogeneous microscopic field is obtained by fine-tuning the two fronts of the white light so as to situate them in a precise parallel position. Thus, the interference fringe of the selected color is expanded to a degree allowing for the entire microscopic field to appear in a single color. In such an image, the protein crystals jutted-out at the single-color background of the optically homogeneous solution.

Indeed, systems with acetone are inappropriate for medical application; the crystallization technology used to produce therapeutic insulin crystals is quite different. This notwithstanding, the principle to steer the crystallization process aimed at reducing crystal polydispersity can find practical application. And finally, dissolution of rhombohedral insulin crystals in human blood plasma was studied in-vitro. Sufficient details allowing replication of the experimental studies are provided in the original paper [12].

Conclusion

Considering the balance between insulin supply from dissolving equally-sized crystals and insulin elimination, we explain the prolonged therapeutic effect achieved with low frequency of injection in parenteral controlled release. The calculation model presented here can help in designing personalized schemes for treatment of people with diabetes mellitus, by differentiating basal and bolus insulin doses. The insulin crystal mass in the drug for-

mulation must be adjusted adequately.

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