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^{125}I used for labelling of proteins in an absorption model changes the absorption rate of insulin aspart

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Abstract

The aim of this study is to validate the ability of the disappearance model to predict absorption rates of insulin aspart in pigs. The disappearance model is used as a screening tool to estimate absorption rates after subcutaneous injections in humans or pigs especially of insulin and insulin analogues. The disappearance model measures remaining radioactivity at the injection site and therefore radioactive labelling of the insulin analogue is necessary. The labelling is done with ^{125}I. One of the assumptions for the disappearance model to be reliable is that absorption rates of the labelled and non-labelled molecules are comparable. In this study, we compared disappearance data with absorption calculated from plasma samples of insulin aspart. The calculated absorption is based on non-labelled insulin aspart. The absorption rate from the disappearance data was statistical significant ($p = 0.0028$) different from the absorption rate based on plasma samples. A control study was carried out where ^{125}I labelled insulin aspart was compared to ^{127}I (the natural non-radioactive isotope) insulin aspart. In this study, absorption rate from the disappearance data and absorption rate based on plasma samples were similar ($p = 0.63$). *Conclusion:* Iodination of insulin aspart changes the subcutaneous absorption rate.

Keywords: Disappearance; Absorption; Insulin aspart; Iodinated insulin aspart; Deconvolution

1. Introduction

In treatment of insulin-dependent diabetes subcutaneous (s.c.) injections are the prominent route of administration in most patients. The absorption of insulin from the subcutaneous tissue plays a major role in securing sufficient plasma insulin levels and thereby securing the therapeutic efficiency of the insulin treatment. Further, patients with near-normoglycemic blood glucose have a slower progression of disease complications (The Diabetes Control and Complications Trial Research Group, 1993).

During development of new insulin analogues in the pharmaceutical industry subcutaneous absorption and absorption rate are important selection parameters. The absorption profile determines the action profile of the insulin analogues. Early in the developing phase a specific analytical method for measuring the new insulin analogue in plasma is often not available. The absorption rate based on plasma samples can therefore not be obtained. However, the disappearance of radioactively labelled insulin analogues is often used as an alternative method to compare and rank absorption rates between new insulin analogues or between new analogues and marketed insulin products.

For the disappearance model to be a reliable model the following three assumptions should be fulfilled. The labelled insulin molecule should have the same absorption kinetics as the non-labelled insulin molecule, no degradation should occur at the injection site and the externally measured radioactivity should be proportional to the actual amount of drug at the injection site remaining to be absorbed. The disappearance model has for several decades been used for testing insulin and insulin analogues in both humans (Binder, 1969; Brange et al., 1990; Kang et al., 1991; Clauson and Linde, 1995) and pigs (Deckert, 1982; Ribel et al., 1985; Markussen et al., 1996; Clausen et al., 2002).

The use of ^{125}I-insulin to predict insulin absorption has been validated before for human insulin (Deckert, 1982; Ribel et al., 1985) and bovine and porcine insulin (Binder, 1969). All

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validations have found a good correlation between the behaviour of labelled and non-labelled insulins. Two methods have been used. In one method, the tissue containing the injected solution has been excised after a certain time and the insulin has been extracted, and the specific radioactivity was compared to the specific radioactivity of the injected insulin. In the other method, plasma profiles of radioactivity were compared to plasma profiles of insulin concentration after subcutaneous injection.

The actual absorption into the blood can be determined by measuring the insulin plasma concentrations. The absorption profile can be calculated by deconvolution of the plasma concentration profile after s.c. injection with the insulin plasma concentration profile after i.v. injection (Pedersen, 1980; Cobelli et al., 1987; Levitt, 2003).

A preliminary study indicated a discrepancy between the actual absorption rate and the disappearance of radioactively labelled insulin at the injection site. The discrepancy indicates a difference in absorption rates between insulin and iodinated insulin.

The aim of this study is to validate the ability of the disappearance model to predict absorption rates of insulin aspart in pigs.

2. Materials and methods

In this study, s.c. absorption of iodinated insulin aspart is compared to s.c. absorption of insulin aspart. This is done by comparing the disappearance of insulin aspart by external γ-counting from the subcutaneous injection site with the absorption of insulin aspart into the blood. Disappearance is measured with a γ-counter, where only the γ-emitting ^{125}I labelled insulin aspart is measured. A small part of the insulin aspart molecules is labelled with ^{125}I. In the plasma samples both iodinated insulin aspart and insulin aspart are measured but the non-labelled insulin aspart molecules out numbers the ^{125}I-insulin aspart molecules. Therefore, if ^{125}I-insulin aspart had a different absorption profile, it could not be seen from the plasma samples. Actually, it is the disappearance of ^{125}I-insulin aspart and the absorption of insulin aspart into the blood which are compared.

To further investigate if the labelling with iodine influences absorption, an additional experiment was carried out. Instead of using ^{125}I-insulin aspart mixed with non-iodinated insulin aspart, all the insulin aspart molecules were labelled. To avoid very high emission of γ-radiation non-radioactive iodine (^{127}I) were used; the non-radioactive iodine was added the usual tracer amount of ^{125}I needed for measuring disappearance.

Insulin aspart is used in the study present due to the existence of a specific assay for measurement of insulin aspart concentration in pig plasma. Insulin aspart has the same amino acid sequence as human insulin except for the substitution of proline with aspartic acid in position B28. However it would be desirable to perform the experiment with human insulin, but at present no assay at present which can distinguish between human and porcine insulin.

2.1. Materials

^{125}I-insulin aspart labelled in the tyrosine in the A14 position was provided by Isotope chemistry at Novo Nordisk Måløv. Non-radioactive I-insulin aspart was synthesised from insulin aspart by LOPD (lactoperoxidase) plus KI, H_2O_2 (Bayse et al., 1972; Magnusson et al., 1984) and purified by HPLC. The A14 iodinated insulin aspart was separated by HPLC from insulin aspart iodinated in one of the other three tyrosine's and diiodinated insulin aspart. The HPLC system consisted of a c-18 column, 10 mm in diameter and particle size of 15 μm. The mobile phase was 36.75% ethanol, 0.05 M phosphoric acid and 0.1 M tris in water. The flow was 2.5 mL/min the oven adjusted to 40 °C and UV detection at 276 nm.

The flow fluid was collected with an auto sampler. The fractions 1.6 min before to 2.4 min after the A14 labelled top were kept. To reduce volume and remove phosphor acid and tris(2-amino-2-(hydroxymethyl)propane-1,3-diol, the amount of ethanol was diluted to 20% loaded on the HPLC column (diameter 4.6 mm) and washed out with 50% acetonitril (ACN), 0.1% trifluoroacetic acid (TFA) in water. The latter eluent was freeze dried.

2.2. Formulations

Insulin aspart was formulated as the marketed product NovoRapid® (containing: glycerol, phenol, *meta*-cresol, zinc chloride, dibasic sodium phosphate dihydrate and sodium chloride) with addition of tracer amounts of ^{125}I-insulin aspart. Two types of insulin aspart were tested: iodinated insulin aspart and non-iodinated insulin aspart. Iodinated insulin aspart is non-radioactive I-insulin aspart and a tracer amount of ^{125}I-insulin aspart. Non-iodinated insulin aspart is insulin aspart and a tracer amount of ^{125}I-insulin aspart. The formulations were 100 IU/mL or 600 nmol/mL for iodinated insulin aspart and 100 and 200 IU/mL for non-iodinated insulin aspart. The concentrations of radiation were between 1 and 2 μCi/mL.

2.3. Dose administration

Insulin aspart formulation was injected subcutaneously with a NovoPen® adjusted to 5 mm injection depth. The s.c. injections were made on the side of the neck approximately 7 cm behind the ear and 9 cm from the middle of the neck. The i.v. dose was administered trough a catheter leading to vena jugularis followed by 10 mL physiological saline. The dose of iodinated insulin aspart was 8 IU/animal or 48 nmol/animal i.v. and 60 nmol/animal s.c. The dose of non-iodinated insulin aspart was 60 nmol/animal i.v. and 120 nmol/animal s.c.

2.4. Animals

Female crossbred pigs (LYD; Land race, Yorkshire, Duroc) weighing 73–93 kg were obtained from Gundsøgård (Roskilde, Denmark). They were handled in accordance with The Danish Laboratory Animal Act (Order on Act no. 726 of 9 September 1993 of Ministry of Justice). They were housed individually