Research paper

Subcutaneous glucose monitoring with GlucoDay®: comparison of the results to those obtained with the Endocrine Artificial Pancreas

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ABSTRACT

OBJECTIVE: This study was undertaken to assess the accuracy of GlucoDay® – a portable detector of subcutaneous glucose – by comparing the results to those obtained by Biostator® an established and reliable method for continuous glucose measurement in whole blood. **DESIGN:** Subjects with type 1 diabetes (n:6), subjects with type 2 diabetes (n:6), and six healthy controls were studied for 24 hours; they consumed three main meals. The GlucoDay® was connected to the subjects by inserting a microfibre probe into the periumbilical subcutaneous area, whilst the Biostator® was inserted by a double-lumen catheter into an antecubital vein. A third catheter was inserted into a separate vein for blood withdrawal to measure glucose by the hexokinase method. **RESULTS:** The three methods (GlucoDay®-Biostator®-hexokinase) were equally accurate in measuring glucose levels ($p=0.233$, Kruskal-Wallis test). The glucose measurements performed with GlucoDay® and Biostator® were significantly correlated with those performed with hexokinase ($p<0.001$, $r^2=66.65\%$ and $p<0.001$, $r^2=64.4\%$, respectively, using simple regression analysis). **CONCLUSIONS:** Measurements of glucose fluctuations in the subcutaneous tissue with the GlucoDay® were close to those in blood determined by the Biostator®. GlucoDay® is therefore a reliable method for continuous glucose monitoring and may prove useful for optimizing treatment in patients with type 1 or type 2 diabetes.

Key words: Artificial Pancreas, Diabetes, GlucoDay®, Glucose monitoring, Subcutaneous tissue

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INTRODUCTION

It is well established that strict metabolic control protects against the development of chronic complications of diabetes.^{1,2} Measurements of blood glucose fluctuations are essential for optimization of treatment, but the available methods have limitations (inconvenience and discomfort of finger-pricking, high cost of strips, puncture of veins), which make them unsuitable for frequent estimations of glucose levels. Continuous estimation of blood glucose levels can be performed with the Artificial Endocrine Pancreas (Biostator®),³⁻⁵ but this requires an intravenous line.

Changes in glucose levels in the subcutaneous tissue are similar to those in blood.⁶⁻⁹ Observations showing that changes in glucose levels in the subcutaneous tissue are similar to those in blood⁶⁻⁹ opened up the way for the development of portable devices which provide continuous measurement of glucose in the subcutaneous tissue.¹⁰⁻¹⁴ Over the last few years, this method has attracted wide clinical interest because patients with diabetes can check their metabolic status on a 24-hour basis, including rarely monitored time periods such as postprandial and nocturnal.^{15,16}

The present study was undertaken to assess the results of glucose monitoring using the GlucoDay® – a new portable detector of subcutaneous glucose¹⁷⁻¹⁹ – by comparing them to those obtained by Biostator®, an established method for continuous intravenous glucose monitoring.³⁻⁵ As a reference method for both devices, we used glucose measurements in venous blood with hexokinase. These experiments were carried out in subjects with type 1 and type 2 diabetes and in healthy subjects over a 24 hour period.

MATERIAL AND METHODS

We studied 6 subjects with type 1 diabetes (age 30 ± 4 years, BMI 23 ± 1 kg/m², HbA_{1c} $6.5 \pm 0.6\%$), 6 subjects with type 2 diabetes (age 59 ± 3 years, BMI 27 ± 1 kg/m², HbA_{1c} $6 \pm 0.4\%$) and 6 healthy subjects (age 43 ± 6 years, BMI 26 ± 1 kg/m²). This study was approved by the hospital ethics committee and subjects gave their written informed consent.

On the day of the study, the subjects were admitted to the Artificial Pancreas Unit of our Department at

07:00 and remained supine for 24 hours. During this time, the patients consumed three main meals (breakfast at 09:00, lunch at 14:00 and dinner at 19:00).

Since the objective of the study was to compare glucose levels measured with the three methods, no attention was paid to the optimization of metabolic control and the diabetic subjects followed their usual treatment schedule (diet, insulin injections or oral anti-diabetic agents).

Three catheters were inserted in each patient: one subcutaneously for connecting the GlucoDay®, one in an antecubital vein for connecting the Biostator® and one for drawing blood to measure glucose with hexokinase.

GlucoDay®

The technical characteristics of GlucoDay® have been previously described.¹⁷⁻¹⁹ The microfibre was inserted subcutaneously in the periumbilical region by using an 18-gauge Teflon catheter as a guide, and was then connected to the portable unit worn by the subjects with a belt. It was perfused with Dulbecco's buffer containing sodium-benzoate as preservative at a rate of 10 µl/min. The system makes a glucose measurement every second and stores an average value every 3 minutes, for a total of 480 measurements per day; glucose is measured by glucose oxidase.¹⁷⁻¹⁹ The GlucoDay® was calibrated by using a glucose level in venous blood 120 min after the insertion of the microfibre.

In one patient, the GlucoDay® was interrupted after a few hours due to breakage of microfibre and malfunctioning of the device and therefore the number of subjects who completed the study was 17.

Endocrine Artificial Pancreas (Biostator®)

The Biostator® performs blood glucose measurements 6 times per minute (8640 times per 24 hours) via the glucose oxidase method. It was connected to the patients by inserting a double-lumen 18-gauge catheter into an antecubital vein, as previously described.⁵

Hexokinase

A third catheter was inserted into a separate vein for measurements of glucose in blood samples by hexokinase; this was used as a reference method for both devices. Eight blood samples were withdrawn

during the experiment as follows: 120min after the microfibre implantation (this value was used for the calibration of the GlucoDay®), before lunch, 60min after lunch, 120min after lunch, before dinner, 60 min after dinner, 120 min after dinner and immediately before the end of the experiment (at 08:00 the next morning). The specimens were stored in fluoride tubes for glucose determination.

Statistical analysis

The Kolmogorov-Smirnov test was used to examine the normality of data. The Kruskal-Wallis test was performed to test the “null hypothesis” that the medians within each the 3 methods are the same. Correlations were calculated according to the Spearman Rank Correlation coefficient.

We performed simple regression analysis to estimate the slopes of the regression lines. A comparison of alternative models based on the highest r^2 value was made in order to find the best fitted between several curvilinear models (i.e. multiplicative, exponential, double reciprocal, logarithmic-X, s-curve, square root-Y, linear, etc.).

Specifically, by performing simple regression analysis to describe the relationship between hexokinase and GlucoDay® and applying the comparison of alternative models, the “double reciprocal model” yielded the highest r^2 value. By applying simple regression analysis to describe the relationship between Biostator® and hexokinase and by performing the comparison of alternative models, the “multiplicative model” yielded the highest r^2 value. The three methods were then compared with “Multiple Box and Whisker plots”.

Elaboration of data was accomplished by the Stat-graphics Statistical Package (Manugistics, Inc., Rockville, MD). A “p” value less than 5% was considered statistically significant.

RESULTS

No adverse events were observed at the site of GlucoDay® implantation. The device was well tolerated by all patients, even during the overnight recording.

We examined the correlations between each pair of methods (GlucoDay® vs Hexokinase and Biostator®

vs Hexokinase). It turned out that GlucoDay® and hexokinase were significantly correlated ($r=0.772$, $p<0.001$). Similar results were obtained with Biostator® and hexokinase ($r=0.775$, $p<0.001$).

We then compared the median values of the three methods (GlucoDay®-Biostator®-hexokinase) and we found no statistically significant difference amongst the median values ($p\text{-value}=0.233$).

Performing simple regression analysis to describe the relationship between hexokinase and GlucoDay®, the equation of the fitted model was: $\text{Glucose}_{\text{hexokinase}} = 1/(0.002 + 0.863/\text{Glucose}_{\text{GlucoDay}})$, $r=0.82$, $r^2=66.65\%$, $p<0.001$ (Figure 1).

Applying simple regression analysis to describe the relationship between Biostator® and hexokinase, the equation of the fitted model was: $\text{Glucose}_{\text{hexokinase}} = (2.032) \times (\text{Glucose}_{\text{Biostator}})^{0.846}$, $r=0.80$, $r^2=64.36\%$, $p<0.001$ (Figure 2).

Figure 1. (A) Simple regression analysis of glucose levels (mg/dl)* measured with the GlucoDay® and hexokinase [$Y=1/(0.002+0.863/X)$, $r=0.82$, $r^2=64.36\%$, $p<0.001$]. (B) Comparison of glucose levels measured with GlucoDay® and those predicted by the “double reciprocal model”.

* To convert to SI units multiply by 7.175

Figure 2. (A) Simple regression analysis of glucose levels (mg/dl)* measured with Biostator® and hexokinase ($Y = 2.032 * X^{0.846}$, $r = 0.80$, $r^2 = 64.36\%$, $p < 0.001$). (B) Comparison of glucose levels measured with the Biostator® and those predicted by the “multiplicative model”.

* To convert to SI units multiply by 7.175

Figure 3. Multiple “Box and Whisker plots” comparing the three methods. The width of the boxes representing the interquartile range ($Q_3 - Q_1$) is quite similar in all three compared methods.

The three methods were then compared with “Multiple Box and Whisker plots”; the width of the boxes representing the interquartile range ($Q_3 - Q_1$) was quite similar in all three methods (Figure 3).

DISCUSSION

Adequate monitoring of blood glucose is fundamental for good metabolic control in subjects with type 1 or type 2 diabetes.^{1,2} For example, information as regards glucose fluctuations in the postprandial period or during sleep is necessary for the improvement of glycaemic control and the prevention of hypoglycaemia. However, self-monitoring of blood glucose cannot provide sufficient information to detect daily variations in glucose profiles, even when performed several times a day.

The possibility of continuous glucose monitoring in diabetic subjects has generated wide clinical interest and has been made possible by the use of the Biostator®.^{3,4} Indeed, in our study, the Biostator® was accurate in measuring glucose levels, the p-value (with respect to the F distribution test) being less than 0.001, suggesting that there was a significant relationship between glucose levels determined in blood by hexokinase and by the Biostator® at the 99% confidence level (Figure 2). The latter device, however, has serious limitations that restrict its clinical application: it requires an intravenous line and, because of its size, it hampers mobility.

In the last few years, a number of reports have suggested that the changes of glucose levels in the subcutaneous tissue are close to those in peripheral circulation; these observations opened up the way for the development of sensors for continuous subcutaneous glucose monitoring.¹⁰⁻¹⁴

In the present study, we assessed the accuracy of the GlucoDay®, a portable detector of glucose in the subcutaneous tissue,¹⁷⁻¹⁹ by comparing the results to those obtained by Biostator®, the standard method for continuous glucose determination in blood.^{3,4} As a reference method for both devices, we used glucose measurements in blood by hexokinase. In a previous study in healthy and diabetic subjects monitored for 24 hours, the GlucoDay® was shown to be reliable and provided measurements of glucose levels that closely agreed with those made in venous blood.¹⁹

In our study, the concentrations of glucose in the subcutaneous tissue measured by GlucoDay® were in close agreement with those measured in blood with hexokinase over a wide range of values: the p-value

(with respect to the F distribution test) was less than 0.001, suggesting that there was a significant relationship between the two methods at the 99% confidence level (Figure 1). The correlation of GlucoDay® with hexokinase was actually slightly stronger than that of the Biostator® by hexokinase (Figure 2). Since the Biostator® is an established method for continuous glucose monitoring,⁴ the comparison suggests that the GlucoDay® is a reliable and accurate device for this purpose.

GlucoDay® measures glucose in the interstitial fluid and not in blood. Under physiological conditions, there is a free and rapid exchange of glucose molecules between plasma and interstitial fluid. Therefore, changes in blood glucose and interstitial fluid glucose are expected to correlate well. Nevertheless, changes of glucose concentrations in interstitial fluid do not occur at the same time as those in blood; they lag behind, especially when glycaemia is changing rapidly, for example, after a meal.^{20,21} This time lag can explain the variation that is apparent between the GlucoDay® and hexokinase measurements as well as the interesting result of non-linearity between the blood and the subcutaneous fluid glucose values (Figure 1).

Despite this limitation, this study compares for the first time results obtained by GlucoDay® with those of Biostator®, showing that continuous glucose measurements in the subcutaneous tissue with GlucoDay® are close to those in blood determined with a standard continuously glucose measuring method (Biostator®).

In conclusion, GlucoDay® is reliable for continuous glucose monitoring in the subcutaneous tissue. This information may prove useful for optimizing treatment in subjects with type 1 or type 2 diabetes mellitus.

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