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AN EXPERIMENTAL MODEL FOR EVALUATION OF GLUCOSE TOLERANCE IN RABBIT

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Summary

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This study was conducted to develop an experimental model for evaluation of glucose tolerance in rabbits based on the determination of glucose kinetic parameters. Six clinically healthy New Zealand White male rabbits, 3–3.5 months of age, weighing between 2.4 and 3 kg were used. The following kinetic parameters of glucose after intraperitoneal glucose tolerance test (IPGTT) were calculated – half-life for blood glucose disappearance ($t_{½\beta}$, min), glucose elimination rate constant (K_{el}, min^{-1}), area under the blood glucose concentration versus time curve ($AUC_{0–150\ min}$, mmol.min/L), mean residence time for blood glucose (MRT, min), maximum blood glucose concentration (C_{max}, mmol/L) and the time needed to reach C_{max} (T_{max}, min). Blood glucose concentrations markedly increased after the intraperitoneal injection of glucose at a dose of 2 g/kg, reaching peak values (C_{max} = 17.1 ± 3.29 mmol/L) at 47.5 ± 14.75 min (T_{max}), then gradually declined and returned to baseline levels at 150 min. The mean values of $t_{½\beta}$, K_{el}, $AUC_{0–150\ min}$ and MRT were 78.3 ± 10.5 min, 0.0090 ± 0.0012 min^{-1}, 1822.6 ± 297.4 mmol.min/L and 127.3 ± 18.4 min, respectively. The results of the present study indicate that the evaluation of kinetic parameters of glucose provides more detailed information on glucose tolerance status than the determination of blood glucose concentration alone and could be used as a basis for further elaboration of criteria to distinguish rabbits with normal and impaired glucose tolerance.

Key words: glucose kinetic parameters, glucose tolerance, rabbits

INTRODUCTION

Diabetes mellitus type 2 (DMT_2) is a common endocrine disease in humans, and among domestic animals, cats are most often affected (Nelson *et al.*, 1990; Panciera *et al.*, 1990; Leonardi *et al.*, 2003; Rand *et al.*, 2004). In herbivores, diabetes is rarely encountered, even though there have been reports of sporadic cases with rabbits (Conaway *et al.*, 1981; Wilson *et al.*, 1982).

The impaired glucose tolerance (IGT), which is considered as a consequence of

the induction of insulin resistance in tissues and an early stage of DMT_2, is observed in humans, carnivores (Cavaghan *et al.*, 2000; Rand *et al.*, 2004), as well as in several rabbit breeds (Sarov *et al.*, 2004; Gonzales *et al.*, 2005). In the early stages of IGT, the fasting blood concentration of glucose is usually approaching the upper limits of the reference values (O'Brien *et al.* 1985; Nelson *et al.*, 1990; Weyer *et al.*, 1999), which impedes the timely diagnosing of the condition.

It has been determined that rabbits are very suitable as test animals for chronic experiments upon glucose homeostasis and the disturbances of its controlling mechanisms (Sarov *et al.*, 2004; Gonzales *et al.*, 2005) and for both testing of the effect of newly synthesized medical products on the blood glucose concentrations and the secretion of insulin (Al-Hader *et al.*, 1994; Dastmalchi *et al.*, 2005; Gupta *et al.*, 2005). At the same time, there are no objective criteria for the differentiation of rabbits with normal and those with impaired glucose tolerance. In order to examine the glucose tolerance status (GTS), glucose tolerance tests are used (GTT). The oral GTT is most commonly used in humans (Sturmvoll *et al.*, 2000), and intravenous GTT (Nelson *et al.*, 1990; Pechereau *et al.*, 2001; Thiess *et al.*, 2004), as well as intraperitoneal GTT (Al-Hader *et al.*, 1994; Ogawa *et al.*, 2004; Toye *et al.*, 2005) – in animals. The determination of the serum insulin, which is usually done along with the measurement of blood glucose after application of GTT in humans, is difficult to perform in animals because it requires the usage of species-specific antibodies, the production and availability of which are limited and expensive. Therefore, it is necessary to look for and utilize other methods allowing a simple and precise examination of GTS of animals.

That is why, the current study aimed to develop an experimental model of glucose tolerance evaluation in rabbits based on the determination of certain kinetic parameters of glucose in blood after intraperitoneal glucose tolerance test (IPGTT).

MATERIALS AND METHODS

Test animals

In the current experiment, 6 male clinically healthy New Zealand white rabbits, 3–3.5 months of age and weighing 2.3–3 kg were used. The trials were performed in May and June. For the duration of their adaptation and experimental periods, the recommendations of caring for and treatment of rabbits reared for experimental purposes were followed (Boers *et al.*, 2002). The animals were kept in individual metal cages with dimensions 80/60/40 cm, located in a room where a constant temperature of 20–22 ^{o}C was kept. The light/dark regime corresponded to circadian cycle. They were fed with a standard pelleted diet and straw, and had free access to water. The rabbits were kept in those conditions for a 7-day period of adaptation prior to the start of the experiment. Their health condition during both adaptation and experimental periods was determined through daily monitoring of their behaviour, food and water intakes, and the consistency of their excrements.

Experimental design

For the purpose of adapting the animals to the experimental procedure and to avoid any stress reactions during the intraperitoneal glucose tolerance test (IPGTT), the rabbits were subjected to preliminary immobilization three consecutive days for 5

min, followed by measurement of blood glucose concentration immediately and by the 5th min, before the experimentation period began. In order to be immobilized and prepared for injection, the rabbits were placed on a small mobile table, were fixed very carefully, by being firmly held with one hand in the cervical region, while the other hand held the animal's waist. The rear side of the table was slightly elevated at an angle of 30–40º so that proper conditions for the safe administration of the glucose could be achieved.

Before the start of the experiment, the animals were deprived of food but not of water for 12 h during the night. The GTT was performed on the next morning, through intraperitoneal injection of 2 g/kg glucose using a 22 G needle. The injections were carried out at an angle of 30–45º in the lower left abdominal quadrant. Prior to injection, syringes were checked for lack of situation that may enable aspiration of fluid, and only after that, glucose was administered.

Blood samples were obtained from the ear vein (*v. auricularis externa*) using sterile lancets (Vitrex Medical Aps, Denmark) before (0 min) and on min 15, 30, 45, 75, 120 and 150 after glucose injection. The blood concentrations of glucose were measured immediately after blood collection by a glucometer (Home Diagnostics, Inc., USA), based on the glucose oxidase method, using one drop of whole blood.

Determination of the kinetic parameters of glucose

For determination of blood glucose kinetic parameters, we took into consideration the presence of endogenous glucose, but the baseline individual values were not subtracted from the corresponding post treatment levels.

Using a non-compartmental analysis, based on the statistical moment theory (Gibaldi, 1984; Martinez, 1998) of the individual data readings of glucose after IPGTT, selected model-independent kinetic parameters, indicative for the "fate" of glucose within the test rabbits' organisms were calculated. To this end, a specialized software, WinNonlin Professional 4.0.1 (Pharsight Corporation, 800 West El Camino Real, Mountain View, CA, USA) was used (Riviere, 1999). The values of maximum concentrations (C_{max}, mmol/L) and the time for which they were reached (T_{max}, min) were directly obtained from test results. The area under the concentration-time curve ($AUC_{0–150\ min}$, mmol.min/L) was calculated by the trapezoidal rule. The following kinetic parameters were also calculated: biological half-life (elimination half-life) of blood glucose ($t_{½\beta'}$, min), the elimination rate constant of blood glucose (K_{el}, min^{-1}) and the mean residence time of injected glucose in the bloodstream (MRT, min).

Statistical analysis

The statistical processing of data was performed by ANOVA (Statistica for Windows, StatSoft Inc., USA, 1993). All data are presented as mean values ± standard deviation (mean ± SD). The statistical significance of differences in the values of glucose before and after injection were determined by the LSD test at a level of significance $P < 0.05$. The data were preliminarily analyzed for normal distribution through a Shapiro-Wilk test (Statistica for Windows, StatSoft Inc., USA, 1993).

RESULTS

For the duration of the adaptation and experimental periods, the rabbits did not exhibit any deviations regarding their

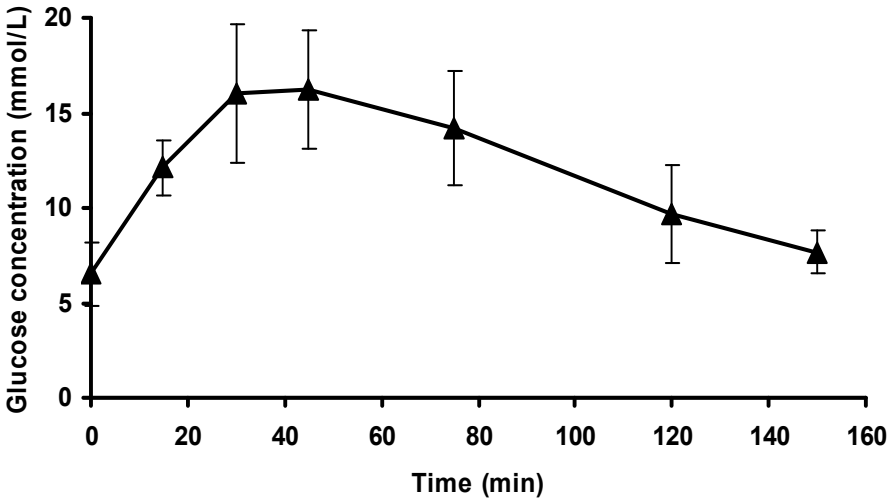

Fig. 1. Glucose concentrations (mmol/L; mean ± SD) in blood after its intraperitoneal injection at a dose of 2 g/kg after glucose tolerance test in rabbits (n=6).

common behaviour, appetite, water intake, or excrement consistency.

The results from the three-day adaptation period showed that there were no statistically significant ($P > 0.05$) variations in glucose values measured immediately after immobilization (6.23 ± 0.39 mmol/L) and 5 min after it (7.09 ± 0.38 mmol/L). In addition, no signs of disturbance during the adaptation and experimental periods, during the glucose injections, or blood sampling were observed.

The concentration of blood glucose (Fig. 1) rose quickly after the intraperitoneal administration of glucose and reached maximum values (C_{max} = 17.1 ± 3.29 mmol/L) between the 30th and 75th min (T_{max} = 47.5 ± 14.75 min). Then, it decreased gradually. The values of glucose on min 120 were within the physiological range and returned to the initial levels on the 150th min.

The average half-life of glucose in blood, measured by $t_{½β}$, was 78.3 ± 10.5 min, with individual variations from 66 to 94 min. K_{el}, reflecting the glucose elimination rate from the central compartment, ranged between 0.0074 min^{-1} – 0.0105 min^{-1}, or 0.0090 ± 0.0012 min^{-1} average for the group. The average values of $AUC_{0–150\ min}$ were 1822.6 ± 297.4 mmol.min/L and varied from 1464.0 to 2213.8 mmol.min/L. The mean residence time of injected glucose in the bloodstream (MRT) was 127 ± 18.4 min, varying from 113 to 157 min.

DISCUSSION

The values of glucose and the lack of significant differences between them immediately, and by the 5th min after the immobilization in the three consecutive days before the IPGTT, showed that the

method of immobilization and blood sampling did not cause any tension in test animals. Therefore, it could be assumed that the observed variations in the glucose concentrations after IPGTT gave an accurate information on both the secretion of insulin and its impact on peripheral tissues, without the intervention of stress hormones – catecholamines and glucocorticosteroids, which are antagonists to insulin in regard to blood glucose (Rizza *et al.*, 1980; Rizza *et al.*, 1982). That is confirmed by the fact that the rabbits did not exhibit any signs of anxiety or discomfort, during the injection of glucose or blood samplings.

Glucose tolerance depends on the interaction between the rate of insulin secretion from the pancreatic β-cells after a glucose load, the delivery of the insulin to peripheral tissues, and the insulin sensitivity of the target cells. With the GTT, the secretion of insulin as well as its hypoglycemic effect in peripheral tissues (Link *et al.*, 1997; Hoenig *et al.*, 2002) could be determined. The information obtained solely from the observation of glucose dynamics at certain intervals following its exogenous application however, is not sufficient to identify the early stages of impaired glucose tolerance, when the secretion ability of ß-cells is still intact, and the changes are localized primarily in the insulin sensitivity of target cells (Jones *et al.*, 1999; Cavaghan *et al.*, 2000; Poitout & Robertson, 2002; Saltiel, 2003; Bouche *et al.*, 2004). The current study showed that the determination of some of the kinetic parameters of glucose after IPGTT can provide objective and more precise information about the time and rates of decreasing of the concentration of blood glucose as a result of its absorption and utilization by the target cells (Nelson *et al.*, 1990; Link *et al.*, 1997; Hoenig *et al.* 2002; Thiess *et al.*, 2004). Therefore, such an approach in the GTT could assist the diagnostic process.

There is neither standardization in testing procedures nor available reference values for glucose kinetic parameters in rabbits. This makes the comparisons of our results extremely difficult. Therefore, further investigations are needed to establish a reference range for the relevant GTT indices in this species. A standard procedure for the glucose tolerance indices is being studied in cats. However, the values of glucose kinetic parameters in this species vary considerably from study to study (Link *et al.*, 1997; Hoenig *et al.*, 2002; Thiess *et al.*, 2004). The mean value of $t_{½\beta'}$ (78.3 ± 10.5 min) and K_{el} (0.0090 ± 0.0012 min^{-1}) in the present study fall witnin the ranges reported for i.v. administration in cats by Link *et al.*, (1997): $t_{½\beta'}$ – 33.2–82.8 min and K_{el} – 0.0084 ± 0.0209 min^{-1}).

When the pharmacokinetics of endogenous substances is studied, one of the two methods is applied – with or without subtraction of physiological levels (Marzo, 1999). It is considered that the studies with endogenous substances should be performed primarily on a large sample of animal population if a subtraction of their physiological ("background") values from those obtained after exogenous administration is envisaged. That is why, in the present study for determination of blood glucose kinetic parameters, the "without subtracting the normal values" approach was adopted.

In conclusion, the results obtained in this study show that the determination of the kinetic parameters of glucose could give more detailed information on the status of glucose tolerance in rabbits, than solely following the dynamics of glucose, and that the kinetic parameters of glucose

could serve as a basis for the development of additional criteria for the differentiation of rabbits with normal from rabbits with impaired glucose tolerance.

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