Pharmacology of Intravenous Insulin Administration: Implications for Future Closed-Loop Glycemic Control by the Intravenous/Intravenous Route

Nils K. Skjaervold, M.D.,^{1,2} Oddveig Lyng, M.Sc.,³ Olav Spigset, M.D., Ph.D.,^{4,5} and Petter Aadahl, M.D., Ph.D.^{1,2}

Abstract

Background: Our group is attempting to construct an artificial pancreas based on intravenous glucose monitoring and intravenous insulin delivery. To do so, the pharmacology of intravenous insulin administration must be studied. We used a pig model to determine the inherent lag time in the insulin/blood glucose system. The goal was to suggest a method that reduces the blood glucose level in a rapid and yet predictable manner.

Methods: Six pigs received continuous intravenous insulin infusions at 0.04, 0.08, or 0.4 IU/kg/h for 60 min. Two pigs received short-term intravenous infusions at 0.4 IU/kg/h for 2 min, repeated five times at 60-min intervals. Four animals received five intravenous insulin bolus injections at 60-min intervals, two at 0.01 IU/kg and two 0.02 IU/kg, with a final dose of 0.04 IU/kg. The blood glucose level was measured every 1–5 min.

Results: A high rate of intravenous insulin infusion led to rapid declines in blood glucose levels. The same rapid decline was achieved when the infusion was halted after 2 min. Using the latter method and with intravenous insulin boluses, blood glucose levels started to rise again after approximately 15–20 min. Insulin boluses led to a first detectable decrease in blood glucose level after 2–6 min and to a maximum rate of decrease shortly thereafter.

Conclusions: We found that intravenous bolus injections of insulin lowered blood glucose levels rapidly and predictably. Repetitive small intravenous insulin boluses together with an accurate and fast-responding intravascular continuous glucose monitor should be studied as a method of closed-loop glycemic control.

Introduction

THE DEVELOPMENT OF AN ARTIFICIAL endocrine pancreas has been a major research area in diabetes care for over 50 years. It consists of a continuous glucose monitor, an insulin infusion system, and a control algorithm, assembled as a closed-loop regulatory system with no need for manual adjustments. The Biostator[®] (Miles Laboratories, Elkhart, IN) was constructed in the late 1970s, as a system that combined continuous blood sampling analyzed ex vivo with automated intravenous insulin infusion.¹ The system performed very well but was hampered by its large, bulky size, its invasiveness, and the need to immobilize the patient. In the past 20 years most research has been focused on subcutaneous continuous glucose monitoring and subcutaneous insulin administration.² Despite considerable effort and abundant use of resources on establishing fully automated blood glucose regulation by this route, success has been elusive. At the turn of the century, blood glucose control in the intensive care

setting awakened substantial interest because of studies describing its beneficial role on intensive patient outcome.^{3–5} This led to a renewed interest in more invasive and aggressive methods of glucose control, reopening the intravenous route to both insulin administration as well as glucose monitoring.

We have previously described a novel intravascular continuous glucose monitor ideal for intensive care use.⁶ Our goal is to further develop this technology into a fully automatic closed-loop regulatory system, primarily for improved blood glucose control in intensive care patients and subsequently as a solution for outpatient diabetes care. An obvious advantage of using intravenous monitoring and insulin delivery—as opposed to the subcutaneous route—is to minimize the time lag between glucose monitoring and insulin delivery and between insulin delivery and the time a new blood glucose level (BGL) is reached. The time lag problem is a serious challenge in subcutaneous/subcutaneous closed-loop regulatory systems, calling for complicated mathematical modeling of the insulin control algorithm.⁷ Even so, there is also a time lag issue with

¹Department of Circulation and Medical Imaging, ³Unit of Comparative Medicine, and ⁴Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, Trondheim, Norway.

Departments of ²Anesthesiology and Emergency Medicine and ⁵Clinical Pharmacology, Trondheim University Hospital, Trondheim, Norway.

the intravenous route. We have observed—both clinically and in animal models—that continuous intravenous insulin infusions reduce the BGL in a rather slow and unpredictable way. It is difficult notably to rapidly lower the BGL without drifting into hypoglycemia. The elimination half-life of insulin is reported to be approximately 6 min,⁸ however, there are no reports on the lag time from intravenous insulin administration until a decrease in BGL is observed.

To take full advantage of the intravenous/intravenous route of blood glucose control, the pharmacology of intravenous insulin administration must be studied. The aims of this study were to use a pig model to determine the inherent lag time in the insulin/blood glucose system and to suggest a method of insulin administration that reduces BGLs in a rapid and yet predictable manner.

Materials and Methods

Animals

The study was approved by the Norwegian State Commission for Animal Experimentation. Twelve pigs (weighing 24–32 kg) were acclimatized and treated in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The pigs were premedicated with intramuscular diazepam (0.4 mg/kg) (Stesolid®, Dumex-Alpha, Copenhagen, Denmark) and azaperon (12 mg/kg) (Stresnil®, Janssen-Cilag, Vienna, Austria). Anesthesia was induced with intravenous atropine (0.04 mg/kg) (Nycomed Pharma AS, Oslo, Norway), ketamine HCl (10 mg/kg) (Parke-Davis, Solna, Sweden), and thiopental sodium (5 mg/kg) (Pentothal®, Abbott Scandinavia AB, Solna, Sweden).

The animals were tracheotomized through a midline surgical cut-down and mechanically ventilated and monitored on an anesthesia machine (Aisys, GE Healthcare Technologies, Oslo). The fraction of inspired oxygen was kept at 0.3, the tidal volume was kept at 10 mL/kg, and minute ventilation was adjusted to maintain an arterial partial pressure of CO₂ of 4.5–5.5 kPa. Anesthesia was maintained by isoflurane (0.5–1.0%) (Forene®, Abbott Scandinavia AB) and an infusion of intravenous fentanyl (at 7 µg/kg/h) (Pharmalink, Spanga, Sweden). Fluid balance was achieved using a continuous infusion of heated (37°C) Ringer's acetate at 5 mL/kg/h. The insulin used in the study was recombinant human insulin (Actrapid®, Novo Nordisk, Bagsværd, Denmark). The animals were euthanized with an overdose of pentobarbital (pentobarbital NAF, Apotek, Lørenskog, Norway) at the end of the study.

An intra-arterial line was placed in the right carotid artery via the tracheotomy wound for monitoring and blood sampling. An intravenous line was placed in the right internal jugular vein through a separate surgical cut-down. The bladder was exposed through a small laparotomy incision for the insertion of a bladder catheter. Glucose was measured in whole arterial blood with a blood gas analyzer (ABL 725, Radiometer, Brønshøj, Denmark). All animals were allowed to rest for at least 60 min to stabilize their BGL before the administration of insulin.

Study protocol

Continuous intravenous insulin infusions. Six pigs were assigned to receive a continuous intravenous insulin infusion of 0.04, 0.08 (“clinical” doses), or 0.4 (a high dose) IU/kg/h

(two pigs in each group) for 1 h. Glucose measurements were performed every 5 min throughout the 1-h study period.

Short-term intravenous insulin infusion. After a preliminary analysis of the continuous insulin infusion group, two animals were assigned to receive a continuous intravenous insulin infusion of 0.4 IU/kg/h insulin for 2 min (a total of 0.013 IU/kg). This infusion was repeated five times at 60-min intervals. Glucose measurements were performed every 2–5 min throughout the 5-h study period.

Intravenous insulin boluses. After preliminary analyses of the continuous and short-term intravenous insulin infusion groups, four animals were assigned to receive repetitive intravenous insulin bolus injections. Based on findings from the studies with insulin infusions and pilot animals, we chose 0.01, 0.02, and 0.04 IU/kg in alternate doses with 60-min intervals. Five intravenous insulin boluses were given to each animal, two at 0.01 IU/kg and two at 0.02 IU/kg in a randomized order and then a final dose of 0.04 IU/kg. Glucose measurements were performed every 1–5 min throughout the 5-h study period, with the most frequent sampling during the first 10 min after each bolus.

Blood glucose analysis

To analyze BGL we used arterial blood samples on a Radiometer ABL 725 blood gas analyzer. In this instrument, glucose is transported across the outer membrane of a multilayer glucose electrode. The glucose oxidase that is immobilized between the inner and outer membrane layers converts glucose to hydrogen peroxide (glucose + O₂ → gluconic acid + H₂O₂), which crosses the inner membrane toward the electrode's anode. The oxidation of hydrogen peroxide creates an electric current that is proportional to the amount of hydrogen peroxide available and hence is proportional to the amount of glucose in the sample. In addition, the analyzer also uses different electrodes with similar techniques to measure partial pressure of O₂, partial pressure of CO₂, pH, and lactate.⁹

Calculations and statistics

Glucose measurements from the continuous and short-term insulin infusion groups were collected and plotted against time. No further statistical analyses were performed on these data. Six variables were derived from the bolus injection part of the study (Fig. 1). The time until the first detectable decrease in BGL (T_{decr}) was defined as the time between the intravenous insulin bolus administration and a reduction in BGL of ≥ 0.1 mmol/L that was not followed by a rise in BGL above this value within the next 5 min. The maximum rate of decrease (MRD) was calculated using linear regression as follows: individual slopes were constructed using all possible sets of five consecutive data points in the time series, and then the steepest line was chosen and reported as MRD. The time until MRD (T_{decrmax}) was defined as the time between intravenous insulin bolus administration and the first data point used when calculating MRD. The maximum decrease in BGL (ΔC_{max}) was defined as the difference in BGL between baseline and the lowest BGL measured within the next 55 min. The time until the lowest measured BGL (T_{min}) was defined as the time from the intravenous insulin bolus administration until ΔC_{max} was achieved.

FIG. 1. The six variables derived from intravenous insulin bolus injections at time=0. $\Delta C_{\max}$, maximum decrease in blood glucose level; MRD, maximum rate of decrease (linear regression using five consecutive observations [black circles] for all possible combinations and choosing the steepest regression line); T_{decr} , time until the first detectable decrease in blood glucose level; T_{decrmax} , time until the maximum rate of decrease; T_{min} , time until the lowest measured blood glucose level. Gray shading marks the area of the drop in the blood glucose curve for the first 30 min after insulin bolus injection (AOC_{0-30}).

As an overall measure of the effect of insulin on BGL we also calculated the area corresponding to the drop in the BGL curve for the first 30 min after the intravenous insulin bolus administration (area over the curve from 0 to 30 min [AOC_{0-30}]) using the trapezoidal rule (gray shading in Fig. 1). Thirty minutes was chosen as the time frame because the insulin effect seemed to fade after this period, an observation that is in accordance with the previously reported elimination half-life of insulin of approximately 6 min.⁸ Thus, further changes in BGL (increases or decreases) after 30 min would be expected to be a result of the general oscillations of the BGL curve rather than a result of the previous insulin dose.

The statistical analysis was conducted using R software, version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria). We compared the effect of the three dose levels of insulin as fixed factors on the six defined outcome variables using linear mixed-effect models (the *lme* function from the *nlme* package), thereby adjusting for nested sampling from four different animals.

Results

Continuous intravenous insulin infusion

Intravenous insulin infusion led to a dose-dependent sigmoid-shaped decrease of the BGL (Fig. 2). The BGLs decreased throughout the study time of 60 min.

Short-term intravenous insulin infusions

Short-term intravenous insulin infusions produced a rapid BGL decline similar to that caused by high-rate continuous

intravenous insulin infusions. The BGLs reached a nadir at approximately 15–20 min before returning to their baseline value (Fig. 3).

Intravenous insulin boluses

The BGL curves of each of the four animals are shown in Figure 4. All insulin doses led to a detectable fall in BGL. The BGL of one animal fell to hypoglycemic values after the fourth bolus dose, making the animal unsuitable for further study. This exclusion left us with the following data: eight boluses in four animals of 0.01 IU/kg, seven boluses in four animals of 0.02 IU/kg, and three boluses in three animals of 0.04 IU/kg.

Results for the six outcome variables are presented in Table 1. T_{decr} appeared within 5 min for all but one of the boluses, for which the change was apparent after 6 min. There was no significant difference in T_{decr} between the various doses. MRD was high at all doses and was also significantly dose-dependent. T_{decrmax} was not found to be dose-dependent. $\Delta C_{\max}$ was found to be highly and significantly dose-dependent, whereas there was no significant difference between the different doses for T_{min} . AOC_{0-30} was highly and significantly dose-dependent.

To provide a visual comparison of the various bolus and continuous infusion doses, the values were converted to ratios by dividing each BGL value with its respective baseline BGL value (Fig. 5).

Discussion

The principal finding of this study is that intravenous insulin infusion at “clinical” rates (i.e., 0.04–0.08 IU/kg/h) led

FIG. 2. Blood glucose levels after continuous intravenous insulin infusion in six pigs. Each of the six animals is depicted with a separate curve. Two animals were given a dose of 0.04 IU/kg/h (open circles), two animals were given 0.08 IU/kg/h (open triangles), and two animals were given 0.4 IU/kg/h (closed squares).

FIG. 3. Repetitive short-term intravenous insulin infusions in two pigs. Each animal received 0.4 IU/kg/h for 2 min (a total of 0.013 IU/kg) six times with 60-min intervals between each infusion. The periods of insulin infusions are marked with gray shading.

FIG. 4. Blood glucose level curves in four pigs receiving repetitive bolus doses of intravenous insulin. The timing of the insulin boluses is indicated with arrows. The insulin doses were given in the following sequence: (0.01 + 0.02 + 0.01 + 0.02 + 0.04) IU/kg in two animals (open circles) and (0.02 + 0.01 + 0.02 + 0.01 + 0.04) IU/kg in two animals (closed squares).

BGLs to decline slowly. To achieve more rapid changes, the infusion rate had to be increased to very high levels, which led to a sustained depression of the BGL. By halting the high rate infusions after 2 min we achieved the same rapid decline, with BGL reaching a nadir within 15–20 min, and then a rise in the BGL toward baseline values without drifting into hypoglycemia. We hypothesized that a 2-min infusion would not be very different from a bolus injection, and so we continued working with boluses. This has the advantage that one only needs to manipulate one variable (the dose), compared with short-term infusions where there are two (the dose and the infusion time).

Intravenous insulin bolus administration led to a detectable decrease in BGL after a time interval of 2–6 min and to a maximum rate of decrease shortly thereafter. Neither the T_{decr}

nor the T_{decrmax} was found to be dose-dependent. However, this finding could be due to a type II error caused by the low number of animals in the study, and both Table 1 and Figure 5 indicate a trend toward a shorter time until the maximum rate of decrease for the highest bolus dose. Intravenous insulin boluses resulted in a dose-dependent reduction in BGLs with regard to both the maximum decrease and the area of the decline under the BGL curve. Figure 5 shows the effect of an intravenous bolus injection of insulin on the relative changes in BGL compared with that of intravenous insulin infusion. The rapid onset and the predictable and short-lasting effect of the boluses at the doses chosen are clearly evident. In particular, the difference between administering 0.04 IU/kg as a bolus (closed squares) and as a 1-h infusion (upper margin of the gray shading) is striking.

TABLE 1. THE SIX OUTCOME VARIABLES FROM THE INTRAVENOUS INSULIN BOLUSES

	Insulin dose (IU/kg)			P value
	0.01	0.02	0.04	
T_{decr} (min)	3.88 (0.64)	4.00 (1.41)	3.33 (0.58)	0.32
MRD (mmol/L/min)	0.098 (0.010)	0.10 (0.030)	0.14 (0.017)	0.027
T_{decrmax} (min)	4.50 (1.41)	4.57 (1.33)	3.67 (1.15)	0.74
ΔC_{max} (mmol/L)	0.73 (0.14)	1.10 (0.37)	1.57 (0.058)	<0.001
T_{min} (min)	16.1 (4.6)	18.6 (5.6)	21.7 (7.6)	0.46
AOC _{0–30} (mmol/L/min)	9.42 (3.63)	18.51 (6.47)	27.82 (2.48)	<0.001

All data are mean (SD) values.

P values are from comparisons using linear mixed-effect models.

AOC_{0–30}, area over the curve from 0 to 30 min; ΔC_{max} , maximum decrease in blood glucose level; MRD, maximum rate of decrease; T_{decr} , time until the first detectable decrease in blood glucose level; T_{decrmax} , time until maximum rate of decrease; T_{min} , time until the lowest measured blood glucose level.

FIG. 5. Effect of intravenous insulin boluses on blood glucose level (BGL) after repeated injections in four pigs. The doses were 0.01 IU/kg (open circles), 0.02 IU/kg (open triangles), and 0.04 IU/kg (closed squares). The effect of continuous intravenous insulin infusions is shown as a gray area, with a dose of 0.04 IU/kg/h at the upper margin and a dose of 0.4 IU/kg/h at the lower margin. The boluses were plotted as the mean of each dose, with error bars depicting their respective SD.

The pancreas does not secrete insulin into the portal bloodstream continuously, but rather in pulses at 5-min intervals. The amount of insulin secreted in each pulse is primarily regulated by changes in the blood glucose concentration; when the BGL is rising, more insulin is secreted with each pulse, and when the BGL is falling, less insulin is secreted with each pulse.^{10,11} This principle causes tiny fluctuations of the normal blood glucose curve.¹² If a closed-loop system were to resemble nature as closely as possible, it would therefore secrete insulin in pulses at 5-min intervals, as described above and as proposed in a recent article.¹³ With the current knowledge, we still find this to be too complex. However, we do believe it should be possible to construct a closed-loop system using repetitive small boluses of intravenous insulin together with an accurate and fast-responding intravascular continuous glucose monitor. The timing of the bolus injections would be determined by the BGL curve, and the dose of the intravenous insulin boluses would be calculated based on the history of the previous boluses and their effect on the BGL curve, thereby allowing tiny oscillations in BGL, ideally with amplitudes of approximately 0.5 mmol/L.

The strength of this study lies in the use of a model with large animals that resemble humans, which allowed us to obtain numerous consecutive blood samples from each animal and to manipulate BGLs without fear of the implications of hypoglycemia. The major weaknesses include our use of a pig model and the fact that the animals were healthy and did not have diabetes. However, we believe that the fundamental principles from our findings will be applicable to humans

with diabetes, although this issue merits further examination. Another limitation was the choice to give the largest bolus dose only at the end of each experimental course. This procedure was chosen because we were uncertain whether such a high dose would influence the effect of subsequent insulin doses. Finally, the number of pigs examined was low, leading to a risk of type II errors when comparing the different doses. Nevertheless, we believe that this study provides sufficient data to form the basis for performing carefully controlled studies with bolus doses in humans.

In conclusion, we found that continuous intravenous infusion of insulin at normal “clinical” doses leads to slow changes in the BGLs due to the inherent lag time in the insulin/blood glucose system. In contrast, bolus injections of insulin lowered BGLs rapidly and predictably. In the future, closed-loop glycemic control might be achieved by using repetitive small boluses of intravenous insulin together with an accurate and fast-responding intravascular continuous glucose monitor.

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Author Disclosure Statement

All authors declare that they do not have any competing financial interests.

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Address correspondence to:

Nils K. Skjaervold, M.D.

Department of Circulation and Medical Imaging

Norwegian University of Science and Technology

P.B. 8905, MTFS

7491 Trondheim, Norway

E-mail: nils.k.skjaervold@ntnu.no