Experimental Diabetes Model in Chick Embryos Treated with Streptozotocin

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The aim of the present study was to investigate whether diabetes model can be made by treatment of streptozotocin (STZ) in chick embryos and this model can be used to predict the effect of drug. When STZ (0.3 mg/egg) was injected into the albumen of fertile eggs on the 14th day of incubation, level of blood glucose significantly increased than that of the control on the 17th day of incubation, and level of serum insulin significantly decreased. In addition, the enhanced level of blood glucose in STZ-treated embryos reduced by injection of human insulin. In conclusion, STZ-treated embryos may be applicable to evaluate human insulin and anti-diabetes drugs as an experimental diabetes model.

Key words chick embryo; streptozotocin; diabetes; blood glucose; serum insulin

In an attempt to reduce the number of mammals used in drug research, we have been examining the use of chick embryos and found that they may be superior for predicting the effects of drugs.1—9) Recently, we reported those thyroid abnormal models in chick embryos treated with thiamazol showed a similar reaction to digoxin, as in human.10) From these experiments, we expected that it might be able to produce an experimental diabetes model by the developing inhibition of the endocrine hormone in the pancreas of chick embryos.

Streptozotocin (STZ) was first reported to have a specific diabetogenetic effect.11) And this agent has been widely used as a model of insulin-dependent diabetes destroying β-cells of pancreas in animal.12) However, it was not known STZ brings about any changes in pancreas of chick embryos at a late stage of incubation.

In the present study, we determined the levels of blood glucose and serum insulin in developing embryos and examined to produce a model of diabetes in chick embryo by treatment of STZ. Furthermore, we evaluated the effects of human insulin, which used for diabetes therapy in insulin-dependent patients, using this diabetes model.

MATERIALS AND METHODS

Fertile eggs of White Leghorn chicks, which were obtained from Ohmiya Poultry Science (Ohmiya, Saitama) were incubated at 37.5±0.2 °C at a relative humidity of about 65%, turned automatically every hour (Showa Incubator Laboratory, Ohmiya, Saitama).

Streptozotocin (STZ, Sigma Chemical C., MO, U.S.A.) was dissolved in physiological saline and sterilized through a membrane filter (DISMIC-25, Advantic Toyo, Toyo Roshi Kaisha, Ltd.). Regular human insulin (humulin R, Eli Lilly Japan K.K., Kobe, Japan) was dissolved to desired concentrations with sterilized physiological saline.

Each ten fertile eggs was used on the 10, 12, 14, 16 or 17th day of incubation. Large vitelline veins of egg were selected and marked by a pencil under the fluorescent lamp (150-lux). Eggshell of a marked range of 10×5 mm was removed by electric drill and a drop of water was filled to project clearly an artery (Fig. 1). Whole blood was collected from a vein of egg by means of a tuberculin syringe with a 0.55×32-mm needle (Termo Co., Ltd., Tokyo, Japan).

Level of glucose in blood was immediately measured by enzyme-electrode method (Arkray Factory, Tokyo, Japan). Remained blood sample was centrifuged at 3000 rpm for 10 min and serum was collected. The serum samples were stored at −20°C until they were analyzed. Insulin level in serum was measured using ELISA Insulin kit (Seikagaku Co., Tokyo, Japan).

STZ (100 mg/egg, 300 mg/egg or 1000 mg/egg) was each injected into the albumen of each ten eggs on the 14th day of incubation and blood was collected on the 17th day of incubation in same methods. The levels of blood glucose and serum insulin were determined in same methods.

Regular human insulin (4 U/egg) was injected into the air sac of STZ-treated ten eggs on the 17th day of incubation. After 5 min of insulin injection, blood was collected and level of blood glucose was measured in same methods.

All the results are given the mean±S.D. Data were analyzed by one way analysis of variance (ANOVA) when the variance was homogeneous. If there was a significant difference among the groups, multiple comparison test was conducted by Dunnett’s test. The fiducial limit of 0.05, two-tails, was used as the criterion for significance.

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RESULTS

Levels of blood glucose and serum insulin in chick embryos increased with developing stages (Fig. 2). While small amount of serum insulin was shown in the 12th day-embryos, thereafter insulin level gradually increased.

Levels of blood glucose and serum insulin were 157±15 mg/dl and 143±18 pg/ml in the 17 day-embryos, respectively.

Blood glucose level in the 17th day-embryos treated with STZ was significantly higher than that in the control. Conversely, serum insulin level was lower than that in the control (Fig. 3).

In addition, the enhanced level of blood glucose in STZ-treated embryos reduced by injection of human insulin (Fig. 4).

DISCUSSION

In the present study, we proposed an experimental animal model with diabetes in chick embryos treated with STZ and the effects of human insulin using this model.

Pancreas of chick embryo lies in duodenum that consists of a long U-shaped loop. It begins to develop rapidly during the 5th day of incubation and to acquire its definitive structure by 12th day of incubation.13)

Because we did not have the data on levels of blood glucose and serum insulin in normal chick embryos, we first experimented to obtain these data from 12 to 17 days-embryos. It is reported that the blood glucose level is about 130 mg/dl in 10 days-embryos and it is from 135 within 150 mg/dl in 17 day-embryos.14) Our data in normal chick embryos was not greatly different from their data.

And it is reported that insulin is secreted by B-cells from the 4th or 5th day of incubation.15) The level of serum insulin in chick embryos increased gradually after the 12th day of incubation. However, attempts to measure the concentration of serum insulin before the 12th day of incubation was unsuccessful, probably because the concentration was below the sensitivity of the current methods.

Based on the levels of blood glucose and serum insulin in the normal chick embryos, STZ was injected into the albumen on the 14th day of incubation. By the injection of STZ on the 14th day of incubation of STZ, the biochemical data in the 17 day-embryos showed the characteristic findings of diabetes, increase of blood glucose level and decrease of serum insulin level, as in rodents and man. A dosage of STZ, 0.3 mg/egg in a volume of 0.2 ml per egg, was adequate for obtaining a increase of blood glucose and decrease of serum insulin levels. Significant low levels of blood glucose and serum insulin in chick embryos that induced by the injection of 1 mg/egg of STZ may be overdose.

Regarding this dose, if the whole weight of a fertile egg is supposed to be about 60 g, 0.3 mg/egg of STZ may be equivalent to 5 mg/kg, which are lower than the usual doses (32 mg/STZ in mice or 50 mg/kg in rats) that are intravenously injected in rodents to induce diabetes. It is suggested that STZ is easily absorbed from the albumen to embryo and it may be maintained for long time.

It is well known that insulin shows the teratogenic effects in the early stage of chick embryos.16,17) However, it is unknown whether human insulin shows any therapeutic effects in STZ treated-chick embryos.

When regular human insulin, which has been high frequency used to therapeutic for diabetics, was injected into the air sac of STZ (0.3 mg/egg)-treated eggs on the 17th day of incubation, 4min after the injection, blood was collected and blood glucose level was measured. Data are the mean±S.D. *p<0.05 to the control.
cause there is various types of insulin, regular, rente types etc., for the treatment of insulin-dependent diabetes.

In conclusion, STZ-treated embryos may be applicable to evaluate human insulin and anti-diabetes drugs as an experimental diabetes model.

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REFERENCES