An optimal establishment of an acute hyperglycemia zebrafish model

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Recently, zebrafish has shown the potential to become an important in vivo model for diabetes-related research. In this study, we performed a validation study for the establishment of optimal hyperglycemia involving the following factors: alloxan concentrations of 100, 200, 300, and 400 mg/100 ml; exposure times of 30, 60, 120, and 180 min to an alloxan solution and water glucose solution; and water glucose solution concentrations of 1, 2, and 3%. The results have shown that exposure to water glucose solution following alloxan treatment might increase blood glucose level in zebrafish in a dose- and time-dependent manner. The appropriate hyperglycemia zebrafish model was induced in 300 mg/100 ml alloxan solution for 30 min, 1% water glucose solution for 30 min, and water for 1 h. We suggest that our zebrafish model could be an alternative hyperglycemia animal model in the future.

Key words: Zebrafish, hyperglycemia, alloxan, blood glucose.

INTRODUCTION

Diabetes mellitus (DM) is a type of metabolic disease that is brought about by either insufficient production of insulin or the inability of the body to respond to insulin formed within the system. The disease can be classified into two different categories: type 1 and 2 diabetes. Type 1 DM is caused by the loss of beta cells found in the islets of Langerhans in the pancreas. Type 1 diabetic animal models exist: the non-obese diabetic (NOD) mouse, N-ethyl-N-nitrosourea diabetes mouse, Goto-Kakizaki (GK) rat, Zucker diabetic fatty (ZDF) rat, and KK-ay mouse (Fenner et al., 2011; Galipeau et al., 2011; Gledson et al., 2007; Hempe et al., 2011; Hu et al., 2011; Mikai et al., 2010; Yoshinari and Igarashi, 2011; Zhao et al., 2011). The model is induced when either alloxan or streptozotocin is administered to destroy the beta cells, resulting in hyperglycemia (Gohil et al., 2010; Hemalatha et al., 2010; Idan-Feldman et al., 2011; Lee et al., 2011; Liu et al., 2011; Ojezele and Abatan, 2011). Type 2 DM is generally characterized by the body’s resistance to insulin. This is primarily attributed to the loss of certain insulin receptors in the tissues that normally mediate the entrance of insulin into the body’s cells. Type 2 diabetic animal models exist as db/db and ob/ob mice (Chen et al., 2011; Huang et al., 2011; Tauchii et al., 2011; Tsuruta et al., 2011; Zhao et al., 2011).

Recently, zebrafish models of human disease have been established for a wide range of human pathologies, including genetic disorders and acquired diseases, as many cellular processes are highly conserved throughout vertebrate evolution (Bassett and Currie, 2004; Berghmans et al., 2005; Darland and Dowling, 2001; Lieschke and Currie, 2007; Sun et al., 2004; Van der Sar et al., 2004). The zebrafish offers several advantages that make it an important complement to previous models of disease. The attributes of the zebrafish include its small size, fecundity, and production of optically clear embryos that undergo exceptionally rapid development (Amsterdam and Hopkins, 2006). As a vertebrate, the greatest advantage of the zebrafish is that it has very similar genome structure to that of humans and consequently is used for studies of human genomic function. Additionally, large numbers of genetic studies performed on nematodes and the pomace fly can be performed on zebrafish (Dahme et al., 2009; Ekker, 2008; Kinkel and Prince, 2009; Olsen et al., 2010).

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# These authors contributed equally to this work.
Many laboratories have performed diabetes studies using zebrafish. These studies used an acute hyperglycemia zebrafish model induced with only water glucose or streptozotocin (Gleeson et al., 2007; Kinkel and Prince, 2009; Olsen et al., 2010). The diabetic zebrafish model induced with only water glucose is required for a long time, and the model induced by streptozotocin has not been performed according to various concentrations and exposure times of streptozotocin. In this study, we performed a validation study of the acute hyperglycemia zebrafish model to establish optimal hyperglycemia.

**MATERIALS AND METHODS**

**Materials**

Chemical and materials were obtained from the following sources: alloxan monohydrate and D-(-) glucose, >99.5% from Sigma-Aldrich (St. Louis, MO, U.S.A.); saline solution from JW Pharmaceutical (Hwaseong, Republic of Korea); Tricaine MS-222 (ethyl 3-aminobenzoate methanesulfonate salt) from Sigma-Aldrich (St. Louis, MO, U.S.A.); blood glucose tester and blood glucose test strips from Allmedicus (Anyang, Republic of Korea).

**Animals**

All experimental procedures were performed in accordance with the Principles of Laboratory Animal Care (NIH publication, 80-23, revised in 1996) and the Animal Care and Use Guidelines of Nambu University specifically approved this study and the use of animal. The strains of zebrafish (*Danio rerio*) used were wild-type (from the Soo jung zebrafishery in Republic of Korea). All zebrafish were acclimated to constant laboratory conditions (14°C ± 0.5°C, 10 h light:10 h dark photoperiod, diet, water, 28°C) for at least one week in stock aquaria before experiments were conducted. Adult zebrafish were maintained in tap water conditioned chlorine. Fish were fed twice daily with a mixture of brine shrimp eggs (0.5 mg/fish/day; tetra bits, Spectrum Brands Company, Germany) and flake food (3.5 mg/fish/day; TOPMEAL, Tabia, Korea). All zebrafish used in these experiments were randomly chosen adults.

**Induction of hyperglycemia**

Three solutions were used for inducing zebrafish hyperglycemia. First, the zebrafish were treated in 100 ml half saline solution of various concentrations of alloxan (100, 200, 300 or 400 mg) for various periods of time (10, 20 or 30 min) at room temperature. The solution was then changed to a 100 ml solution of various concentrations of water glucose (1, 2 or 3%) solution for various time periods (30 min, 1, 2 or 3 h) at room temperature. After two steps with alloxan and water glucose exposures, sequentially, the solution was changed to 100 ml water for various periods of time (1, 2 or 3 h) at room temperature. Each group comprised of 10 zebrafish. We performed the process as detailed in Figure 1.

**Measurement of blood glucose level**

Following induction of hyperglycemia, zebrafish were anaesthetized in 0.04% Tricaine MS-222 (tricaine methane sulphonate). Zebrafish were considered to be anaesthetized when all movement in the water had ceased. Zebrafish were removed from the solution, patted dry with a Kimwipe and was placed on a glass surface. Zebrafish were decapitated using a sharp blade behind the eyes, where the heart is located. After decapitation, blood was collected on a strip directly from the punctured heart, and the blood glucose level was read.

**Statistical analysis**

Data were analyzed using SigmaPlot software (Systat Software, Chicago, IL, USA). All data are expressed as mean ± standard error of the mean (SEM). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) with Tukey's post hoc test in blood glucose levels. P-values less than 0.05 were deemed to indicate statistical significance.

**RESULTS**

Blood glucose level change was evaluated according to the water glucose solution concentration (Figure 2). The blood glucose level of zebrafish exposed to 1% water glucose solution for 30 min was similar to those of the control group. The blood glucose level of zebrafish exposed to the 3% water glucose solution for 30 min increased significantly to an average of 198 mg/dl (*p < 0.05). We selected this solution for our next experiment, because exposure to the 1% water glucose solution for 30 min did not have an effect on blood glucose level. No deaths occurred during the appropriate model determination (data not shown).

The change in blood glucose level according to alloxan solution concentrations is as shown in Figure 3A. Blood glucose levels increased in a dose-dependent manner. The blood glucose level of zebrafish exposed to a 100 ml half saline solution of 100 mg alloxan for 30 min was similar to that of the control group. The blood glucose level of zebrafish exposed to a 100 ml half saline solution of 300 or 400 mg alloxan for 30 min increased significantly, with an average of 244 and 347 mg/dl, respectively (**p < 0.01). However, the death rate was 40% (Figure 3B).

The blood glucose level change depending on alloxan solution concentration and exposure time is as shown in Figure 4A. The blood glucose level of zebrafish exposed to a 100 ml half saline solution of 200 mg alloxan for 10 min was similar to that of the control group. The blood glucose level of zebrafish exposed to a 100 ml half saline solution of 300 mg alloxan for 30 min increased significantly (*p < 0.05). When the zebrafish were exposed to a 100 ml half saline solution of 400 mg alloxan for various times (20 or 30 min), the blood glucose level changes were statistically significant, with an average of 262 and 347 mg/dl, respectively (*p < 0.05, **p < 0.01). However, the death rates were 10 and 40%, respectively (Figure 4B).

After defining an alloxan concentration appropriate for the acute hyperglycemia model, we studied water glucose exposure time (Figure 5). The blood glucose level of zebrafish exposed to a 1% water glucose solution for 1 h increased significantly, with an average of 259 mg/dl
Alloxan treatment

Glucose treatment

Water treatment

Measurement of glucose level

**Figure 1.** Process for induction of hyperglycemia.

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**Figure 2.** Blood glucose level depending on water glucose solution concentration without alloxan solution. The zebrafish were exposed to 100 ml water with various water glucose concentrations (1, 2 or 3%) for 30 min and then exposed to water without water glucose for 1 h. After induction, blood glucose levels were measured, *p < 0.05 vs. 0% water glucose.

(*p< 0.05). No death occurred during the appropriate model determination (data not shown).

The blood glucose level changes depending on water exposure time are as shown in Figure 6. The blood glucose level of zebrafish exposed to water for 0 h in the alloxan treatment group was similar to that of the no-alloxan treatment group. The blood glucose level of zebrafish exposed to water for 1 h in the with-alloxan treatment group was higher than that of the without-alloxan treatment group, and this difference was statistically significant (***p < 0.001). No death occurred during the appropriate model determination (data not shown).

**DISCUSSION**

This study reports the preparation of an acute hyperglycemia zebrafish model. First, through the blood glucose level dependent on water glucose solution concentration without treatment of an alloxan solution, we fixed the water glucose solution concentration. At that time, the concentration of water glucose solution was set not to cause diabetes, because zebrafish should be induced with diabetes by alloxan only. Then, alloxan solution concentration and the exposure time were determined. When zebrafish were induced with a 100 ml half saline solution of 400 mg alloxan for 20 or 30 min, the blood glucose levels of the zebrafish had statistical significance. However, since some deaths occurred during the appropriate model determination, we selected a model where zebrafish were induced with a 100 ml half saline solution of 300 mg alloxan for 30 min as the exposure time and concentration. At the final stage, water was used due to glucose metabolism. The appropriate hyperglycemia zebrafish model was determined to be that in which the blood glucose level was induced with a 100 ml half saline solution of 300 mg alloxan solution for 30 min, exposed to 1% water glucose solution for 30 min, and then finally exposed to water for 1 h.

Animal models of type 1 diabetes mellitus, caused by
Figure 3. Blood glucose levels (A) and survival ratio (B) depending on alloxan solution concentration. The zebrafish were exposed to various alloxan concentrations in half saline solution; 100 mg/100 ml (100 mg), 200 mg/100 ml (200 mg), 300 mg/100 ml (300 mg), or 400 mg/100 ml (400 mg) for 30 min, moved to a 1% water glucose solution in water for 30 min, and then exposed to water for 1 h. After induction, blood glucose levels were measured; *p < 0.05, **p < 0.01, ***p < 0.001 vs. control group.

The loss of pancreatic β-cells resulting in reduced insulin production, have been reported (Kinkel and Prince, 2009). They are induced by the administration of several chemicals, principally alloxan or streptozotocin. An alloxan-induced acute hyperglycemia zebrafish model of type 1 diabetes that results in hyperglycemia with a destroyed pancreas was generated. This model is a mild acute hyperglycemia model for short-term experimentation, and further research is needed to develop a permanent hyperglycemia zebrafish model (Lee et al., 2010; Lieschke and Currie, 2007). Some zebrafish with a 100 ml half saline solution of 400 mg alloxan were dead in our results. Recently study reported that the toxicity of alloxan may lead to liver damage and this may be the
reason for the death of the fish (Orsolic et al., 2012).

Many laboratories have performed diabetes studies using zebrafish. These studies used an acute hyperglycemia zebrafish model induced with only water glucose or streptozotocin (Gleeson et al., 2007; Kinkel and Prince, 2009; Moss et al., 2009). The diabetic zebrafish model induced with only water glucose is required for a long time, and the model induced by alloxan has not been performed according to exposure times and concentrations. In this study, we performed a validation study of the acute hyperglycemia zebrafish model by water glucose and alloxan to establish optimal hyperglycemia. Hyperglycemia is a condition in which an excessive amount of glucose circulates in the blood plasma (Sommerfield
Figure 5. Blood glucose levels depending on water glucose solution exposure time after alloxan treatment. The zebrafish were exposed to a 100 ml half saline solution with 300 mg alloxan for 30 min, moved to a 1% water glucose solution in water for 30 min, 1, 2 or 3 h, respectively, then were exposed to water for 1 h. After induction, blood glucose levels were measured; *p < 0.05 vs. control group.

Figure 6. Blood glucose levels depending on water exposure time. The zebrafish were exposed to a 100 ml half saline solution of 300 mg alloxan for 30 min, moved to a 1% water glucose solution in water for 30 min, and then exposed to water for 0, 1, 2, or 3 h. After induction, blood glucose levels were measured; *p < 0.05, **p < 0.01, ***p < 0.001 vs. control group.

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced (Lawrence et al., 2008). We suggest that our zebrafish model is acute hyperglycemia model, because it could not show the diabetic symptoms in our short time experiment. The rest is that zebrafish were chosen as the model system due to the extensive use of zebrafish to study visual development and visual impairments similar to those seen in humans, such as night blindness (Alvarez et al., 2010; Gleeson et al., 2007; Li and Dowling, 2000).

Overall, these data indicate that zebrafish can be induced to develop mild and stable hyperglycemia, and this model is appropriate for experiments, which call for a short-term and mild model. We intend to further experiment with diabetic complications using this alloxan-
induced acute hyperglycemia zebrafish model.

ACKNOWLEDGEMENT

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REFERENCES


