The protective effect of epigallocatechin 3-gallate on mouse pancreatic islets via the Nrf2 pathway

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Short Title: protective effect of EGCG on mouse islets

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Abstract

Purpose: Epigallocatechin 3-gallate (EGCG), a green tea polyphenol, has been shown to have anti-oxidant and anti-inflammatory effects *in vitro* and *in vivo*. The aim of this study was to investigate the effects and mechanism of EGCG on isolated pancreatic islets as pre-conditioning for pancreatic islet transplantation.

Methods: The pancreatic islets were divided into two groups: an islet culture medium group (control) and an islet culture medium with EGCG (100 μM) group. We investigated the islet viability, Nrf2 expression, reactive oxygen species (ROS) production, and heme oxygenase-1 (HO-1) mRNA. Five hundred islet equivalents after 12 h of culture for the EGCG 100 μM and control group were transplanted under the kidney capsule of streptozotocin (STZ)-induced diabetic ICR mice.

Results: The cell viability and insulin secretion ability in the EGCG group were preserved, and the nuclear translocation of Nrf2 was increased in the EGCG group ($p<0.01$). While the HO-1 mRNA levels were also higher in the EGCG group than in the control group ($p<0.05$), the ROS production was lower ($p<0.01$). An *in vivo* functional assessment showed that the blood glucose level had decreased in the EGCG group after transplantation ($p<0.01$).

Conclusion: EGCG protects the viability and function of islets by suppressing ROS production via the Nrf2 pathway.

Keywords: Epigallocatechin 3-Gallate (EGCG); nuclear factor erythroid 2-related factor 2 (Nrf2); islet transplantation; reactive oxygen species (ROS) production; pre-conditioning

Introduction

Type 1 diabetes mellitus (DM) is a chronic auto-immune disorder characterized by the destruction of pancreatic β-cells and insulin deficiency. Pancreatic islet transplantation is the treatment of choice for resolving a lifelong dependence on insulin injections and results in reducing the risk of hypoglycemic events and other serious complications. However, it has been reported that isolated islets trigger an instant blood-mediated inflammatory reaction (IBMIR), and 70% of the transplanted islets' insulin-producing β-cells are lost after 24 h [1]. Consequently, transplantation of fresh islets is recommended, and because they are considered to be very fragile, pre-conditioning to preserve the islets is deemed necessary.

Pancreatic islets are proposed from inflammatory cytokines, reactive oxygen species (ROS), and a hypoxic environment [2-4]. Thus, pre-conditioning may be necessary for improving the islet survival and function, as islet loss results from several stresses associated with islet isolation. Epigallocatechin 3-gallate (EGCG) has been used as a green tea, and its safety and low rate of adverse events have already been proven [5-7]. We also reported previously anti-oxidative and anti-inflammatory effects of EGCG concerning liver regeneration [8]. We therefore focused on the protective effect of EGCG on islets.

EGCG is a green tea polyphenol and is well-known as the most beneficial catechin. Polyphenols are pharmacologically safe compounds for humans and are known to exhibit various biological properties, including protection from deoxyribonucleic acid (DNA)

damage and free radical scavenging [9, 10]. In addition to the ability to act as a neutralizing agent for excessive ROS, the anti-oxidative and anti-inflammatory effects of EGCG reduce liver fibrosis [11, 12]. Furthermore, we previously reported that EGCG contributes to liver regeneration and a decrease in liver injury as well as being effective in protecting cells from inflammatory cytokines and ROS [8]. As a tumor suppressor, EGCG inhibits cancer cell growth and induces apoptosis in hepatocellular carcinoma [13].

The nuclear factor-erythroid 2-related factor 2 (Nrf2) signaling pathway, which plays a central role in the cellular anti-oxidant defense system, is activated by the effective mechanism of EGCG [14]. It was reported that nuclear translocation of Nrf2 is increased by EGCG using human mesenchymal stem cells while the phosphorylation of p53 induced by oxidative stress is reduced by EGCG [15]. Oxidative stress induced by nitric oxide synthase in mouse pancreatic islets has been shown to induce Nrf2, thereby increasing the expression of anti-oxidant enzymes and maintaining the size and function of the pancreatic islets [16]. In response to oxidative stress, Nrf2 upregulates the expression of anti-oxidant and detoxifying genes by binding to anti-oxidant response elements (AREs) in the promoter region of the encoding genes [17, 18].

It is therefore well-known that EGCG exerts an anti-oxidant effect by activating the Nrf2 signaling pathway, which is involved in the cellular anti-oxidant defense system. It has been reported that Nrf2, on the one hand, increases the levels of several anti-oxidant enzymes,

including superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1) mRNA in pathological conditions [19]. Among these, HO-1 has been reported to be a key enzyme of a powerful cyto-protective protein in several disease states, including that from renal injury [19]. On the other hand, oxidative stress, mediated by ROS, including hydrogen peroxide, superoxide anion radical, hydroxyl radical and peroxide, plays a crucial role in the induction of cyto-toxicity. ROS activate various signaling pathways that result in DNA damage, cellular senescence and apoptosis.

The purpose of this study was to examine the protective effects of EGCG on mouse pancreatic islets focusing on the Nrf2 expression. Furthermore, as a mechanism, Nrf2 was shown to be induced by EGCG, and the viability of islets was shown to be preserved through the induction of the anti-oxidant enzyme HO-1 mRNA and the suppression of ROS production.

Materials and Methods

Animals and experiment design

Eight-week-old male C57BL/6 mice (Charles River Laboratories, Kanagawa, Japan) and ICR mice (Charles River Laboratories, Kanagawa, Japan) were used for the experiments. The mice were allowed free access to water and standard laboratory food and were housed at a temperature of 22 ± 2 °C, relative humidity of 55% ± 5% and a 12 h light: dark cycle with lights. The present study was conducted in compliance with the Division for Animal Research Resources, Graduate School of Biomedical Sciences, Tokushima University (T29-29). The experiments and procedures were approved by the Animal Care and Use Committee of the University of Tokushima and were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Islet isolation

Under isoflurane (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) anesthesia, a midline abdominal incision was performed on each mouse, and the pancreas was exposed. The bile duct at the level of the duodenum was located and clamped. A 32-gauge needle attached to a 5-ml syringe was used to puncture and intubate the common bile duct. The pancreas was slowly distended by intra-ductal injection of pre-cooled Hank's balanced salt solution (HBSS) (Mediatech, Herndon, VA, USA) containing 1.0 mg/ml collagenase type IV

(17104019, Gibco; Thermo Fisher Scientific Inc., Waltham, MA, USA) at a dose of 1–2 ml for each mouse. After distension, the whole pancreas was harvested and incubated in a 50-ml conical tube at 37 °C for 33 min. Subsequently, digestion was stopped by the addition of 50 ml of cold HBSS, and the conical tube was shaken vigorously. The suspension, filtrated through a metallic filter net, was washed with HBSS 3 times and centrifuged (1500×rpm, 30 sec, 4 °C). The islets were then collected and washed in HBSS.

Culture and EGCG treatment

The isolated islets were seeded onto 12-well plastic plates (untreated 60-mm dishes; BD Biosciences, San Jose, CA, USA), and each of 50 islet equivalents (IEQs) were incubated with islet culture medium (RPMI1640 [Gibco, Thermo Fisher Scientific Inc.] + 10% fetal bovine serum [Gibco, Thermo Fisher Scientific Inc.] + 1% glutamine [Gibco, Thermo Fisher Scientific Inc.] + 1% Penicillin/Streptomycin [Gibco, Thermo Fisher Scientific Inc.]). The EGCG was purchased from Bio Verde (Kyoto, Japan). First, both the EGCG-treated group (50, 100, 360 and 500 μM) and the control group were divided. Cell viability and insulin secretion were assessed for each group as a functional test after 24 h. Next, the islets were divided into 2 groups—the islet culture medium group (control group) and the islet culture medium with EGCG 100 μM group (EGCG group)—before being cultured for 0, 6 and 24 h for the assessment of cell viability.

Determination of mouse islet cell viability

As already described, propidium iodide (PI) staining was performed after islet isolation to determine the viability of the mouse islet cells [20]. In brief, 50 islets were incubated with 20 µl of PI (1:10; PI; Dojindo, Kumamoto, Japan) for 10 min and kept in the dark at room temperature. After washing with phosphate-buffered saline (PBS) (Fisher Scientific, Pittsburgh, PA, USA) three times, they were counted under a fluorescence microscope (Keyence, Keyence Corporation, Chicago, IL, USA). Dead cells were identified as red and viable cells as green. The percentage of viable cells in each islet was assessed.

Glucose-stimulated insulin secretion (GSIS) test

To determine the *in vitro* potency of isolated mouse islets, the insulin secretory response to glucose was measured using a modified method previously described [21]. In brief, 50 islets were randomly transferred to a cell culture insert with Krebs buffer (115 mM NaCl; 5.0 mM KCl; 2.3 mM $CaCl_2$; 1.0 mM MgCl; 1.2 mM KH_2PO_4; 25 mM $NaHCO_3$; pH 7.4), 25 mM HEPES (Gibco, Thermo Fisher Scientific Inc.) and 0.1% BSA fraction V (Sigma-Aldrich, St. Louis, MO, USA) containing 2.8 mM glucose and incubated at 37 °C for 1 h as pre-incubation. Thereafter, they were suspended 3 times for 1 h at 37 °C in Krebs buffer with the addition of various glucose concentrations (basal I: 2.8 mM, stimulation: 22 mM,

respectively). The supernatants were collected and stored at -20 °C. The insulin level was measured using a mouse Insulin Enzyme-Linked Immunosorbent Assay Kit (AKRIN 011T; Wako Shibayagi Corporation, Gunma, Japan).

Immunofluorescence and confocal microscopy

The mouse islets in culture medium were fixed with iP gel® (Nippon Genetics Corporation, Tokyo, Japan) and 10% formalin (Gibco, Thermo Fisher Scientific Inc.) and embedded in optical cutting temperature compound and frozen, after which 5-μm-thick sections were cut, rinsed in PBS and 5% BSA, and incubated with Universal Blocker Reagent for 30 min in a humidified chamber at room temperature. Thereafter, sections were incubated with primary antibodies, anti-Nrf2 (1:500 dilution; Ab62352, Abcam Inc., Cambridge, UK) and anti-insulin (1:100 dilution; 4590, Cell Signaling Technology, Tokyo, Japan) overnight at 4 °C in a humidified chamber. After being washed with PBS and 4′, 6-diamidino-2-phenylindole (DAPI; Invitrogen Corp., Carlsbad, CA, USA), the sections were applied to the slides and incubated at room temperature for 2 h in the dark. The cellular composition was determined by manually counting the stained cells using a fluorescent microscope (Keyence Corp., Chicago, IL, USA). The β-Cell content was determined by taking the number of positively stained β-cells and dividing by the total number of stained cells. Intra-nuclei Nrf2-positive cells were also counted in the isolated islets.

RT-PCR analyses

The total ribonucleic acid (RNA) was isolated using a RNeasy Kit (Qiagen, Venlo, the Netherlands) from 50 IEQs and was converted to cDNAs using Moloney murine leukemia virus reverse transcriptase (Invitrogen Corp.). The resulting cDNAs were used as templates for PCR amplification with specific primer pairs. Total RNA (0.5 μg) was used for cDNA synthesis. Mouse HO-1 mRNA (Mm00516005_m1; Applied Biosystems, Foster City, CA, USA) and GAPDH (4352339E; Applied Biosystems) were amplified with their specific primers.

ROS detection

The Cellular Reactive Oxygen Species Detection Assay Kit (Abcam, Tokyo, Japan) was used to measure the formation of the intra-cellular ROS. The cells were seeded into a 96-well dish and incubated for 24 h. The cells were incubated with 2',7'-Dichlorofluorescin diacetate (H2DCFDA 5 μM; Sigma) for 30 min in the dark at 37 °C. After the cells were washed with phosphate-buffered saline (PBS), the fluorescence (Ex/Em=488/535 nm) was measured with the plate reader (SpectraMax i3; Molecular Devices, Tokyo, Japan).

Islet transplantation

To induce diabetes, 200 mg/kg streptozotocin (STZ; Sigma-Aldrich) was administered to the ICR mice intra-peritoneal as a single injection. Animals were classified as diabetic after 2 consecutive measurements of blood glucose above 350 mg/dl or a single measurement of blood glucose above 400 mg/dl approximately with a glucometer (Terumo Corporation, Tokyo, Japan).

Then, 500 IEQs were carefully hand-picked and after 12 h cultured the EGCG 100 μM group and the control group were transplanted under the kidney capsule of each STZ-induced diabetic ICR mouse. The non-fasted glucose levels of all the mice were measured from the tail vein of each animal every two days after surgery. The graft-bearing kidneys were then removed from some animals to be investigated histologically. We conducted nephrectomy on one transplanted mouse and monitored its blood glucose level.

Statistical analyses

The data analysis was performed with statistical software (JMP software, version 11; SAS Campus Drive, Cary, NC, USA). Comparisons between the two groups were performed by Mann-Whitney *U* test or Student *t* test and the comparisons between more the three groups were calculated using a one-way analysis of variance (ANOVA) with Tukey-Kramer's test. The one-way ANOVA with Tukey-Kramer's test was used to compare cell number, cell viability, insulin secretion, and *in vivo* tests. In the figures, the median values (75th and 25th

percentile) and the median ± standard deviation (SD) are given, respectively. A value of $p<0.05$ was considered to indicate statistical significance.

Results

Effects of EGCG and proper concentration test

The percentage of cell viability in the EGCG 100 μM group was significantly higher than the other EGCG-treated groups ($p<0.05$, one-way ANOVA with Tukey-Kramer's test, Fig. 1a). The insulin secretion capacity as a glucose stimulation test was also significantly higher in the EGCG 100 μM groups than in the control groups ($p<0.05$, one-way ANOVA with Tukey-Kramer's test, Fig. 1b). Thus, the additional EGCG cyto-protective effects and mechanism were assessed using EGCG 100 μM.

Cell protection and functional test

An islet functional test was used to assess the cell viability transition for the 0-, 6- and 24-h cultures and insulin secretion. Islets were divided into two groups: the islet culture medium group (control group) and the islet culture medium with EGCG 100 μM group (EGCG group).

The cell number was counted using Dithizone staining (Fig. 2a). The number of islet cells were counted under a fluorescence microscope and the EGCG protected islet cells compared with the control group ($p<0.05$, one-way ANOVA with Tukey-Kramer's test, Fig. 2b). Cell viability was examined using PI staining (Fig. 2c). Cell viability was preserved in the EGCG group compared with the control group ($p<0.01$, one-way ANOVA with

Tukey-Kramer's test, Fig. 2d). Regarding the functional test, EGCG preserved insulin secretion in the EGCG group compared with the control group ($p<0.01$, Mann-Whitney U test, Fig. 2e). Insulin secretion was examined using immune-fluorescence staining (Fig. 2f), and complete strong staining was observed in the EGCG group.

Nrf2 and the downstream pathway

The ROS production and HO-1 mRNA expression, generally well-recognized as anti-oxidant enzymes derived from the Nrf2 pathway, were examined in order to investigate the cyto-protective effect of EGCG. Nrf2 is a transcriptional factor that promotes the transcription of anti-oxidant enzymes by translocation in the nucleus, so to gauge its expression, the Nrf2-positive cells in nuclear translocation were assessed by fluorescent staining. The proportion of Nrf2-positive cells in nuclear translocation was higher ($p<0.01$, Mann-Whitney U test, Fig. 3a) in the EGCG group than in the control group (Fig. 3b). The HO-1 mRNA levels were also higher in the EGCG group than in the control group ($p<0.05$, Mann-Whitney U test, Fig. 3c). ROS production after 24 h was lower in the EGCG group than in the control group ($p<0.01$, Mann-Whitney U test, Fig. 4a and 4b).

In vivo *functional assessment of islets*

The STZ-induced diabetic ICR mice were classified as diabetic after 2 consecutive

measurements of blood glucose above 350 mg/dl or a single measurement of blood glucose above 400 mg/dl. Five hundred IEQs were cultured for 12 h in the EGCG 100 μM group and the control group and transplanted under the kidney capsule. The blood glucose levels of the EGCG group and the control group are shown in Figure 5. In the control group, the blood glucose level was maintained at ≥300 mg/dl throughout the experiment. In contrast, the blood glucose level decreased gradually in the EGCG group until day 5 after transplantation and stayed around 200 mg/dl. The blood glucose continued to be maintained at a low level compared with the control group. There was a significant difference between the 2 groups ($p<0.01$, one-way ANOVA with Tukey-Kramer's test, Fig. 5). We then conducted nephrectomy in one transplanted mouse and proved blood glucose level reversal (Fig. 5, dot line).

Discussion

Islet transplantation is potentially a curative treatment for people with type 1 DM. However, it currently requires multiple donors in order to secure enough islets to transplant into one recipient to effect a successful outcome. This need for multiple donors may be due to the inefficiency of currently available methods of isolating pancreatic islets. The isolation yield is affected by various factors, all of which increase ROS generation, such as the enzyme concentration, digestion time, temperature, status of the pancreas, mechanical disruption, chemical effects of collagenase and warm and cold ischemic injury. In addition, exposure to severe environments, such as various cytokines or the shear stress of transplanted islets, might cause islet dysfunction or a low rate of islet engraftment [22-24]. Oxidative stress has been shown to play a major role in cell injury, β-cell dysfunction and death during islet isolation and transplantation [25]. Therefore, focus has recently shifted to managing oxidative stress in order to resolve the donor shortage.

The nuclear factor erythroid 2-related factor 2-Kelch-like ECH-associated protein 1 (Nrf2-Keap1) pathway is one of the most critical endogenous defense systems and regulates the anti-oxidant and anti-inflammatory cellular responses in the body. Under a normal state, Nrf2 is retained by Keap1 in the cytoplasm. When oxidative stress or other covalent modifications of thiols occur in some of these cysteine residues, Nrf2 dissociates from Keap1 and translocates to the nucleus, where it binds to the anti-oxidant response elements. HO-1

mRNA, an inducible isozyme, is activated by heme, oxidants, cytokines, glycated albumin and other stressors and may be part of the protective response in many diseases [26, 27], including acute lung injury [28], lipopolysaccharide-induced acute liver failure [29], neurodegenerative disorders [30] and acute kidney injury [19, 31, 32]. Nrf2 plays an important role in the cellular anti-oxidant defense system by activating the expression of anti-oxidant and detoxifying genes, such as superoxide dismutase, HO-1 mRNA and glutathione S-transferases. These genes have been shown to protect cells against oxidative stress caused by ROS by restoring redox homeostasis and inhibiting oxidative damage [18].

Regarding the Nrf2 activator, the effect of treatments such as dimethyl fumarate (DMF), hyperbaric oxygen therapy (HBO), herbal medicine inchinkoto, fluvastatin and others have been previously reported [33-40]. It has been reported that high-pressure HBO induced the generation of HO-1 mRNA, an Nrf2 target gene in the huge hepatectomy model, and improved the hepatic cyto-protective effect and survival rate [34]. Likewise, the herbal medicine inchinkoto was reported to encourage liver regeneration and reduce liver damage by inducing the production of HO-1 mRNA [35]. Furthermore, it has been confirmed that Nrf2 is translocated to the nucleus and activated concentration-dependently by the administration of DMF and that it exerts a cyto-protective effect through an anti-oxidant response to nephron-toxicity caused by cyclosporine [33].

In the present study, we focused on the effect of EGCG as an Nrf2 activator because

of its usefulness in a clinical setting. EGCG has been used as a green tea, and its safety and low rate of adverse events has already been proven [5-7]. EGCG has demonstrated a cyto-protective effect and suppression of oxidative stress in recent studies. We also previously reported the anti-oxidative and anti-inflammatory effects of EGCG in liver regeneration [8]. A study showed that EGCG suppressed H_2O_2-mediated oxidative stress in hMSCs (human Mesenchymal Stem Cell) [41]. Previous studies have shown the beneficial effects of EGCG, such as its anti-oxidative and anti-inflammatory effects. However, other complex elements that have not been previously reported also warrant mention, such as the cell protective effects of EGCG on mouse islets through the Nrf2 pathway.

The present study showed that the nuclear translocation of Nrf2 and HO-1 mRNA was increased while the ROS production was reduced in the EGCG group compared with the control group. The viability and function of islets treated with EGCG 500 μM were significantly lower than those of islets treated with EGCG 100 μM. High-dose EGCG may reduce the cell viability and function due to toxicity. Indeed, it was previously reported that high-dose EGCG induced apoptosis and autophagy [42]. Therefore, in the present study, we considered 100 μM EGCG to be the most suitable concentration for the islet cell survival. The results of our *in vivo* functional study showed that the islet grafts had a significantly better function in the EGCG group than in the control group.

One limitation of this study is not to explore the possibility of other signaling

concerning about the protecting effect of EGCG. It was reported that EGCG also reduced the CCl4-induced expression of pro-inflammatory mediators, such as TNF-α, COX-2, and iNOS, in addition to oxidative stress [11]. However, the Keap1-Nrf2 pathway, on which we focused in the present study, is considered to play the most central role in anti-oxidative effects.

Conclusions

This study investigated the effects and mechanism of EGCG on isolated pancreatic islets as pre-conditioning for pancreatic islet transplantation. EGCG treatment of mouse islets was shown to protect the viability and function of islets through Nrf2 expression, resulting in the expression of anti-oxidative enzymes and suppression of ROS production.

Compliance with ethical standards

Conflict of interest All authors have no conflicts of interest.

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Figure legends

Fig. 1. The evaluation of proper EGCG concentrations. Four groups of mice were divided according to the EGCG concentration (50, 100, 360 and 500 μM) and compared with the control group. The cell viability and stimulation index of the EGCG group were examined after 24-h culture and compared with the control group. **a)** The percentage of cell viability was significantly higher in the EGCG 100 μM group than in the control group ($p<0.05$, n=3). **b)** A glucose stimulation test showed that the insulin secretion capacity was significantly higher in the EGCG 100 μM group than in the control group ($p<0.05$, n=3). All data represent the means ± SD, and significance was tested using a one-way ANOVA with Tukey-Kramer's test, where *$p<0.05$ vs. control 24 h.

Fig. 2. Cell number, cell viability and insulin secretion. An islet functional test was used to examine the cell viability after 0-, 6- and 24-h culture and insulin secretion after 24-h culture. The islets were divided into the EGCG 100 μM group or the control group. **a)** Dithizone staining was performed to identify islet cells. The picture shows representative islet cells stained with Dithizone in the EGCG 100 μM group. **b)** The number of islet cells was counted under a fluorescence microscope, and EGCG protected islet cells compared with the control group after 24 h ($p<0.05$, n=3). **c)** PI staining was performed to measure the effect of cell protection. The cells were counted under a fluorescence microscope, and the percentage of

viable cells was assessed. PI staining: alive cells (green); dead cells (red). upper: control, lower: EGCG 100 μM. **d)** EGCG protected cell viability compared with the control group ($p<0.01$, n=5). **e)** Regarding the functional test, EGCG preserved insulin secretion compared with the control group ($p<0.01$, n=5). **f)** Insulin secretion was examined using IF staining. IF staining: insulin (green); nucleus (blue). upper: control, lower: EGCG 100 μM. All data represent means ± SD, and significance was tested using a one-way ANOVA with Tukey-Kramer's test (cell number and viability) and the Mann-Whitney *U* test (insulin secretion), where **$p<0.01$, *$p<0.05$ vs. control.

Fig. 3. Nrf2 translocation and the HO-1 mRNA expression. Nrf2-positive cells in nuclear translocation and HO-1 mRNA expression were examined. **a)** Nrf2-positive cells in nuclear translocation were confirmed using IF staining. IF staining: Nrf2 (red); nucleus (blue). upper: control, lower: EGCG 100 μM. **b)** The proportion of Nrf2-positive cells in nuclear translocation was higher using IF staining than control ($p<0.01$, n=5). **c)** The HO-1 mRNA levels were also higher in the EGCG group than in the control group ($p<0.05$, n=5). All data represent means ± SD, and significance was tested using the Mann-Whitney *U* test, where **$p<0.01$, *$p<0.05$ vs. control.

Fig. 4. Intensity of ROS generation. ROS production in the EGCG 100 μM group was

examined after 24-h culture and compared with the control group. **a)** The intensity of ROS generation was confirmed using IF staining. IF staining: ROS (green). upper: control, lower: EGCG 100 μM. **b)** ROS production was lower in the EGCG group than in the control group ($p<0.01$, $n=5$). All data represent means ± SD, and significance was tested using the Mann-Whitney *U* test, where **$p<0.01$ vs. control.

Fig. 5. *In vivo* functional test. Five hundred IEQs in the EGCG 100 μM group and the control group were cultured for 12 h and transplanted under the kidney capsule of STZ-induced diabetic ICR mice. In the control group, the blood glucose level remained at a high level. However, in the EGCG 100 μM group, the blood glucose level decreased gradually after transplantation ($p<0.01$, $n=3$). We also performed nephrectomy in one transplanted mouse (Fig. 5, dot line). All data represent means ± SD, and significance was tested using a one-way ANOVA with Tukey-Kramer's test, where **$p<0.01$ vs. control.