Article

Association between Aquaporin 7 Expression and Myocardial Protection with Nicorandil or Del Nido Cardioplegia: An Experimental Study

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Abstract

Background: Aquaporin 7 (AQP7), a member the aquaglyceroporin subgroup of the AQP family, is a water channel that controls transport of glycerol and water in heart tissues. It facilitates the uptake of glycerol, a substrate for cardiac energy production, in cardiomyocytes. St. Thomas' Hospital cardioplegic solution No. 2 has cardio-protective effect even in AQP7-deficient hearts. Here, we aimed to determine whether nicorandil or del Nido cardioplegia (DNC) solution can protect AQP7-deficient hearts. **Methods**: The hearts of male AQP7 knockout (KO) and wild-type (WT) C57/B6N mice (age >15 weeks) were aerobically perfused using the Langendorff technique, and cardiac function was measured as left ventricular diastolic pressure (LVDP) throughout the study. Troponin T was measured as an indicator of myocardial damage after reperfusion for 60 min. We compared WT and KO controls subjected to 25 min of global ischemia as well as WT and KO groups infused with nicorandil (100 μM) for 10 min followed by 25 min of global ischemia. We also compared WT-DNC and KO-DNC hearts administered with DNC for 2 min followed by 23 min of global ischemia (Study 2). **Results**: The final recovery rates of LVDP were $20.8 \pm 7.0\%$, $28.1 \pm 7.6\%$, $40.0 \pm 8.4\%$, and $38.7 \pm 4.7\%$ in the WT control, KO control, WT nicorandil, and KO nicorandil groups, respectively. The LVDP recovered faster in the hearts treated with DNC and reached a significantly higher plateau in the KO than in the WT hearts. Troponin T values were 2144 ± 493 and 1313 ± 717 in the WT and KO groups, respectively ($p = 0.041$). **Conclusion**: The Langendorff perfusion model revealed similar myocardial protective effects of nicorandil in AQP7-deficient mice as in WT mice. AQP7 deficiency did not impair the cardioprotective effects of DNC solution.

Keywords

aquaporin 7; cardioprotection; nicorandil; del Nido cardioplegia; murine heart

Introduction

Aquaporins (AQPs) constitute a major and diverse transmembrane channel family of proteins in most living organisms. They facilitate movement of water, with high selective permeability, along osmotic gradients and were originally called water channels [1]. To date, 13 mammalian AQPs (AQP0–12) encoded by *AQP0–AQP12* genes are known, which have been categorized into AQP1, 2, 4, and 5, which belong to the classical family that selectively transfer water; AQP3, 7, 9, and 10, which are aquaglyceroporins that transfer both water and small molecules; and AQP6, 8, 11, and 12, which are the unorthodox uncategorizable type [2]. The aquaglyceroporins are permeable to glycerol, urea, and some other small solutes. Most studies have targeted AQP function in the brain, kidney, glands, and skeletal muscle. Several AQP subtypes have been detected in cardiac tissues of humans and other animal species using the real-time polymerase chain reaction (RT-PCR) [3–5].

Nicorandil is an adenosine triphosphate-sensitive potassium (K_{ATP}) channel opener that is used to manage coronary artery disease. Intravenous nicorandil combined with coronary angioplasty enhanced functional recovery and clinical outcomes in patients with acute myocardial infarction [6]. A double-blind placebo-controlled large clinical trial demonstrated that nicorandil improved the prognosis of stable angina pectoris [7]. Nicorandil and preconditioning has been shown to exert cardioprotective effects in various experimental animal models of myocardial ischemia-reperfusion [8,9]. Moreover, ischemic and pharmacological preconditioning is involved in the regulation of AQP expression [10–13].

In cardiomyocytes, aquaporin 7 (AQP7) acts as a facilitator of glycerol, which is a key substrate required for cardiac energy production [14]. When wild-type (WT) and knockout (KO) mice were challenged with isoproterenol or operated on for transverse aortic constriction, higher mortality rate was observed in AQP7 KO mice than in WT mice [14]. More people with obesity are at increasing risk for cardiac surgery, and the *AQP7* gene is downregulated in

patients that consume fat-rich diets [15]. This might influence insulin resistance or reduce adenosine triphosphate (ATP) contents in cardiomyocytes [16]. Therefore, the cardioprotective effects conferred by standard hyperkalemic cardioplegia during cardiac surgery is a matter of concern. We previously described the relationship between AQP7 and myocardial injury in normothermic global ischemia and elucidated the effects of hyperkalemic cardioplegia with St Thomas' Hospital cardioplegic solution No. 2 (STH2) on patients of various ages with an AQP7 deficiency [17,18]. Del Nido cardioplegia (DNC) has been extensively used in congenital heart surgery for >20 years [19]. It has an electrolyte composition similar to extracellular fluid and is delivered in patients along with fully oxygenated blood in a 4:1 ratio, providing potassium-based myocyte depolarization with concurrent lidocaine sodium channel blockade. DNC may be a safe and effective alternative to conventional hyperkalemic cardioplegia for routine adult patients, as the clinical outcomes are comparable [20,21]. However, the cardioprotective effect of DNC in AQP7 deficiency remains unknown.

The primary aim of this study was to determine whether the K_{ATP} channel opener, nicorandil, and pharmacological preconditioning are equally effective in AQP7-KO and WT murine hearts. Our secondary aim was to determine whether an AQP7 deficiency influences the myocardial protective effect of DNC.

Materials and Methods

Animals

AQP7-deficient mice (B6;129-Aqp7 <tm1Tfun>) were generated and maintained as previously described [16,18]. Frozen sperm from these mice (RIKEN BioResource Research Center, Tsukuba, Japan; https://knowledge.brc.riken.jp/resource/animal/card?brc_no=RBRC06294&__lang__=en) was restored and used to generate WT and AQP7-KO C57BL/6N mice by intercrossing AQP7 heterozygous (+/–) mice (Jackson Laboratory, Tsukuba Japan). Six-week-old mice were fed with regular chow and maintained at 22 °C under a 12/12-h dark/light cycle (light: 8 a.m.–8 p.m.).

AQP7 genotyping used the following primers: WT allele (Primer set: CTTGGTCTGCTGCTTCAGGTC and CAGAGTCCCTCTCACGTCAC) and AQP7 deficient allele (Primer set: CAGAGTCCCTCTCACGTCAC and GCAATCCATCTTGTTCAATGGCC). The resultant WT and AQP7 deficient allele bands were 1288 bp and 800 bp, respectively. PCR was performed with 30 cycles consisting of 10 seconds at 98 °C, 30 seconds at 60 °C, and 2 minutes at 68 °C. Male and female AQP7-KO and WT littermate control mice were analyzed in the AQP7 genotyping analysis. However, these female mice were not used in the actual experiments (Fig. 1a).

To analyze AQP7 messenger RNA (mRNA) expression, RNA was extracted from the left ventricle of C57BL/6 mice using an RNeasy Fibrous Tissue Mini kit (Qiagen, Hilden, Germany) and reverse transcribed using SuperScript IV VILO Master mix (Thermo Fisher Scientific, Tokyo, Japan) according to the manufacturer's protocol. Quantitative real-time PCR analysis was performed using TaqMan Fast Advanced Master mix (Thermo Fisher Scientific, Tokyo, Japan) by Applied Biosystems 7500 (serial number 275001975, Thermo Fisher Scientific, Tokyo, Japan). In relative quantification, the ratio between the amounts of AQP7 mRNA and Rps18 mRNA, as an internal control, was determined. This ratio was then compared between the WT and AQP7-KO groups (Fig. 1b).

Heart Isolation and Perfusion

The mice were anesthetized with an intraperitoneal injection of 50:50 sodium pentobarbital (100 mg/kg) (MOE1755, Nakalai tesque, Kyoto, Japan) and anticoagulant heparin (1000 IU/kg) (C411, Mochida Pharmaceutical Co., Ltd., Tokyo, Japan). The hearts were excised and immersed in Krebs-Henseleit bicarbonate buffer (KHB) at 4 °C. The aorta was rapidly cannulated and perfused with KHB in the Langendorff (VIDTEC, Fukuoka, Japan) apparatus (Fig. 2a) at a constant pressure of 80 mmHg and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide to attain a pH of 7.4 at 37 °C.

The left atrial appendage was removed, then a fluid-filled balloon catheter attached to a pressure transducer was introduced into the left ventricle via the mitral valve. The balloon was inflated until the left ventricular end-diastolic pressures (LVEDP) reached 4 and 8 mmHg and the balloon volume remained unaltered thereafter. All pressure transducers were connected to a PowerLab system (AD Instruments, Dunedin, New Zealand) to monitor pressure throughout the experiment. All hearts were surrounded by a thermostatically controlled water-jacketed chamber to maintain temperature at 37 ± 0.2 °C throughout the experiment.

The hearts were equilibrated with 20 min of aerobic perfusion, and baseline left ventricular systolic pressure (LVSP, mmHg), LVEDP (mmHg), heart rate (beats/min), and coronary flow (mL/min) were determined. Left ventricular developed pressure (LVDP) was calculated as the difference between the LVSP and LVEDP. At the time of baseline measurement, hearts were excluded if the acceptable ranges of LVDP (>50 mmHg), heart rate (>300 bpm), and coronary flow (<1 mL/min, > 5mL/min) were not achieved.

Perfusion Medium and Drugs

Fresh KHB (118.5 mmol/L NaCl, 25.0 mmol/L NaHCO$_3$, 4.8 mmol/L KCl, 1.2 mmol/L MgSO$_4$, 1.18 mmol/L KH$_2$PO$_4$, 1.4 mmol/L CaCl$_2$, and 11.0 mmol/L

Figure 1a

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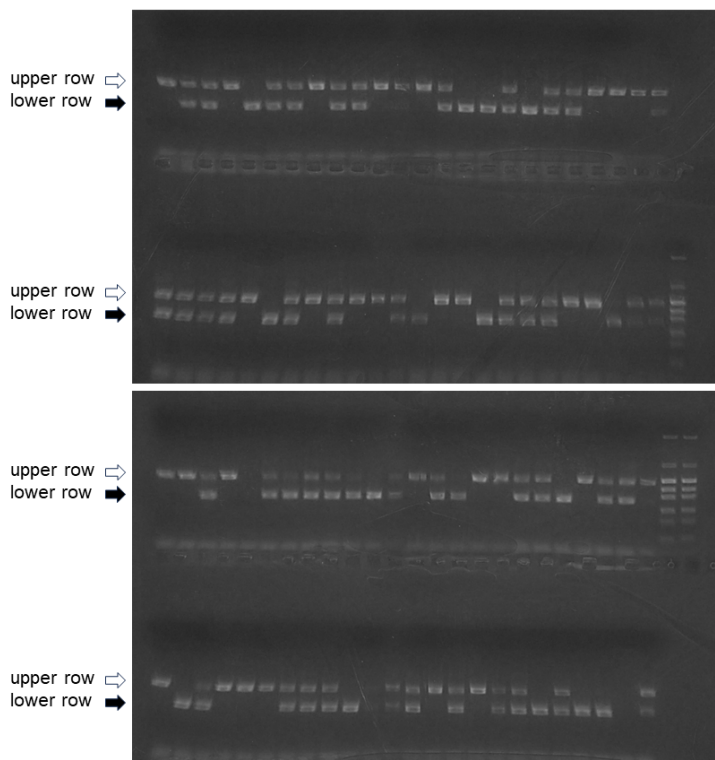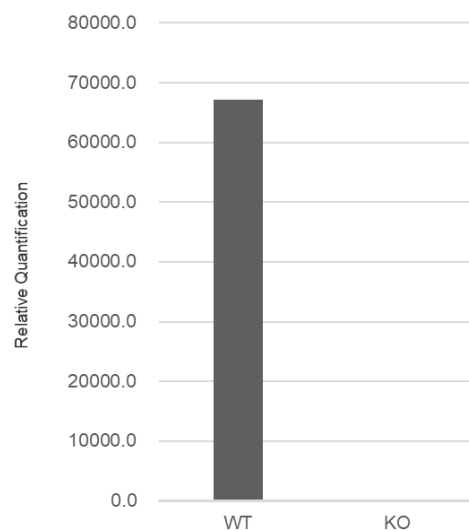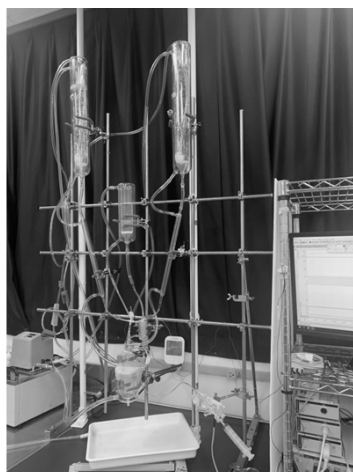lower row ➡

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Figure 1b

Fig. 1. Genotyping and mRNA expression of AQP7. (a) Genotyping. Two bands indicate hetero-type. Wild-type shows only the upper row. AQP7-KO shows only the lower row. (b) AQP7 mRNA expression. In relative quantification, the ratio between the amounts of AQP7 mRNA and Rps18 mRNA, as an internal control, was determined. This ratio was then compared between the WT and AQP7-KO groups. mRNA, messenger RNA; AQP7, aquaporin 7; KO, knockout; WT, wild-type.

Figure 2a

Langendorff apparatus

Figure 2b

(Study 1)

(i) WT-C
or
(ii) KO-C

perfusion | global ischemia | reperfusion
30 | 25 | 60 (min)

(iii) WT-NICO
or
(iv) KO-NICO

20 | 10 | 25 | 60 (min)

▨ 100µM Nicorandil

(Study 2)

(i) WT-DNC

(ii) KO-DNC

perfusion | global ischemia | reperfusion
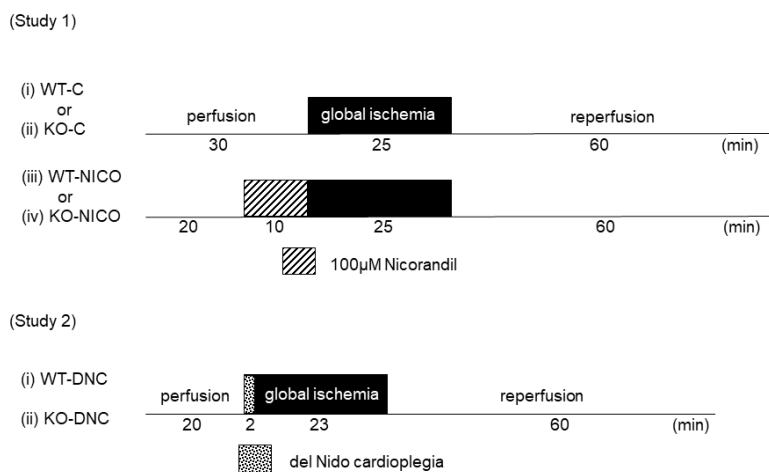20 | 2 | 23 | 60 (min)

▨ del Nido cardioplegia

Fig. 2. Langendorff apparatus and experimental perfusion protocol. (a) Langendorff apparatus. (b) Experimental perfusion protocol. Hearts were aerobically perfused for 20 minutes using Langendorff technique at constant pressure equivalent to 80 mmHg of stabilization before global ischemia. Study 1: Hearts from mature WT and KO mice were assessed. WT- and KO- C: 25 min of global ischemia, then 60 min of reperfusion; WT- and KO- NICO, 10 min of 100 µM nicorandil infusion, 25 min of global ischemia, then 60 min of reperfusion. Study 2: Hearts were infused with DNC for 2 min then exposed to global ischemia for 23 min, then 60 min of reperfusion. WT, wild-type mice; KO, aquaporin 7 knockout mice; C, control; NICO, nicorandil; DNC, del Nido cardioplegia.

Table 1. Baseline and final recovery values of parameters in Study 1.

		WT-C	KO-C	WT-NICO	KO-NICO	p-value
HR	Baseline (bpm)	336.0 ± 16.0	375.0 ± 48.0	388.0 ± 46.0	385.0 ± 18.0	0.070
	Recovery (%)	103.9 ± 13.5	95.0 ± 5.5	94.8 ± 9.6	92.4 ± 5.5	0.176
LVDP	Baseline (mmHg)	74.8 ± 14.1	71.8 ± 8.8	89.7 ± 10.4	82.0 ± 19.6	0.147
	Recovery (%)	20.8 ± 7.0	28.1 ± 7.6	40.0 ± 8.4*	38.7 ± 4.7*	<0.001
LVEDP	Baseline (mmHg)	5.6 ± 1.7	5.5 ± 1.4	5.7 ± 1.1	5.4 ± 1.1	0.973
	Final value (mmHg)	46.0 ± 11.0	47.3 ± 10.9	42.5 ± 9.0	51.2 ± 7.7	0.503
CF	Baseline (mL)	2.0 ± 0.6	2.8 ± 1.6	2.1 ± 1.0	1.1 ± 0.3	0.067
	Recovery (%)	109.9 ± 46.5	90.6 ± 14.2	92.4 ± 13.8	83.7 ± 29.0	0.470

Data are shown as means $\pm$ SD. *$p < 0.05$ *vs*. WT-C. HR, heart rate; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; CF, coronary flow; WT, wild type; KO, aquaporin 7 knock-out; C, control (no drug); NICO, nicorandil; SD, standard deviation (of the mean).
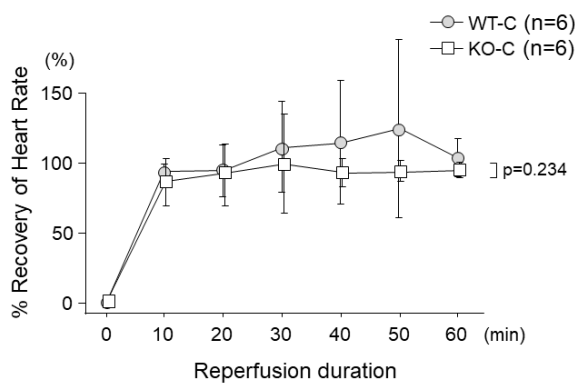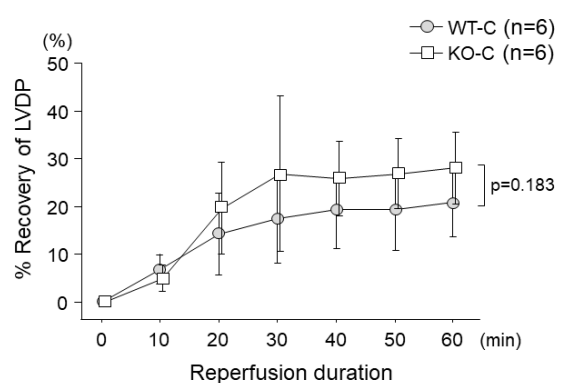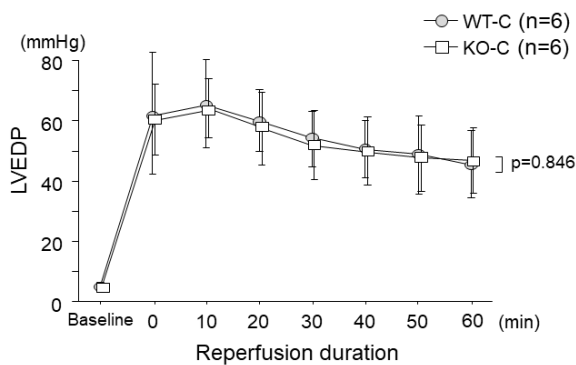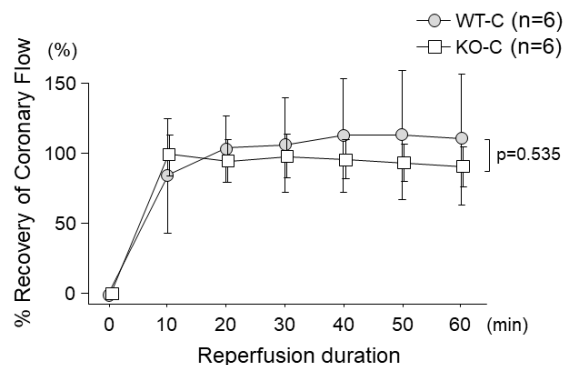

Fig. 3. Postischemic cardiac function during reperfusion. (a) Recovery of heart rate. (b) Recovery of left ventricular developed pressure. (c) Actual value of left ventricular end-diastolic pressure. (d) Recovery of coronary flow. Results are shown as ratios of baseline values or as means $\pm$ SD of six hearts per group. Light gray circles and white squares, WT and KO mice in control groups, respectively. WT, wild-type mice; KO, aquaporin 7 knockout mice; C, control; LVDP, left ventricular diastolic pressure; LVEDP, left ventricular end-diastolic pressure.

glucose) (NaCl: DLP6649, Fujifilm Wako Pure Chemical Corporation, Chuo, Tokyo, Japan; NaHCO3: SKE6367, Fujifilm Wako Pure Chemical Corporation, Chuo, Tokyo, Japan; KCl: CAF6822, Fujifilm Wako Pure Chemical Corporation, Chuo, Tokyo, Japan; MgSO4: HPN7742, Fujifilm Wako Pure Chemical Corporation, Chuo, Tokyo, Japan; KH2PO4: SKP4049, Fujifilm Wako Pure Chemical Corporation, Chuo, Tokyo, Japan; CaCl2: lCAP4648Fujifilm Wako Pure Chemical Corporation, Chuo, Tokyo, Japan; glucose: SKJ2468, Fujifilm Wako Pure Chemical Corporation, Chuo, Tokyo, Japan) was prepared daily and filtered through a 5-µm cellulose nitrate filter.
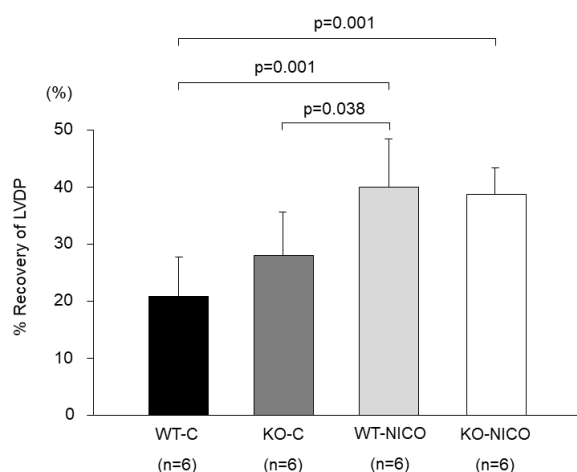
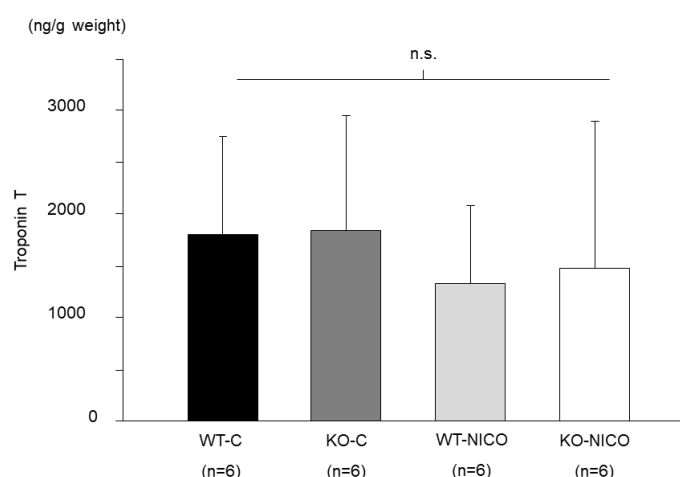Figure 4a   Figure 4b

Fig. 4. Nicorandil treatment (Study 1): Post-ischemic recovery of LVDP and troponin T levels. (a) Final recovery of left ventricular developed pressure (expressed as %). (b) Total troponin T leakage (expressed as ng/g wet weight) during 60 min of reperfusion. LVDP, left ventricular developed pressure; WT, wild-type mice; KO, aquaporin 7 knockout mice; C, control; NICO, nicorandil; n.s., not significant.

Table 2. Baseline and final recovery values of all parameters in Study 2.

		WT	KO	*p*-value
HR	Baseline (bpm)	315.0 ± 28.0	322.0 ± 19.0	0.634
	Recovery (%)	98.6 ± 2.6	100.8 ± 8.9	0.568
LVDP	Baseline (mmHg)	83.7 ± 17.5	71.8 ± 19.0	0.286
	Recovery (%)	53.0 ± 5.9	$69.0 \pm 8.4^*$	0.004
LVEDP	Baseline (mmHg)	5.2 ± 1.2	5.7 ± 1.6	0.562
	Final value (mmHg)	39.2 ± 10.6	29.6 ± 5.9	0.080
CF	Baseline (mL)	2.4 ± 0.9	2.3 ± 0.3	0.709
	Recovery (%)	121.3 ± 20.5	101.3 ± 8.2	0.050

Data are presented as means $\pm$ standard deviation. $^*p < 0.05$ *vs.* WT. HR, heart rate; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; CF, coronary flow; WT, wild type; KO, aquaporin 7 knockout.

Nicorandil (Sigmart®; Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) 100 µM was dissolved in KHB [8,9].

A modified DNC solution [19] (Plasma-Lyte A, 500 mL; 20% D-mannitol, 6.67 mL; $MgSO_4$, 6.7 mL; 1 mEq/mL KCl, 10.8 mL; 2% lidocaine, 3.25 mL; 8.4% $NaHCO_3$, 5.41 mL; pH 7.8 at 37 °C) (Plasma-Lyte A: Y361187, Baxter International Inc., Deerfield, Illinois, USA; D-mannitol: OE018, Yoshindo Inc., Toyama, Japan; MgSO4: MOK78, Otsuka Pharmaceutical Factory, Tokushima, Japan; KCl: MOJ78, Otsuka Pharmaceutical Factory, Tokushima, Japan; lidocaine: 8Z0101, Maruishi Pharmaceutical Co., Ltd., Toyama, Japan; NaHCO3: KIA77, Otsuka Pharmaceutical Factory, Tokushima, Japan) was also prepared daily and filtered through a 5-µm cellulose nitrate filter.

Perfusion Protocol

We investigated the influence of nicorandil on global ischemia (Study 1) and subsequently assessed the effects of modified DNC in AQP7-deficient male mature mice (Study 2) (Fig. 2b). All mice used in this experiment were ≥15 weeks old, which is considered mature.

Effects of Nicorandil on Ischemia-Reperfusion Injury (Study 1)

The hearts obtained from male mature WT and KO mice were perfused for 20 min with aerobic KHB at 37 °C to attain equilibration in all protocols. Thereafter, the hearts were randomly assigned to groups (n = 6 each) that underwent 25 min of global ischemia, or 10 min of 100 µM nicorandil infusion and 25 min of global ischemia, followed by 60 min of reperfusion with aerobic KHB. The final recovery
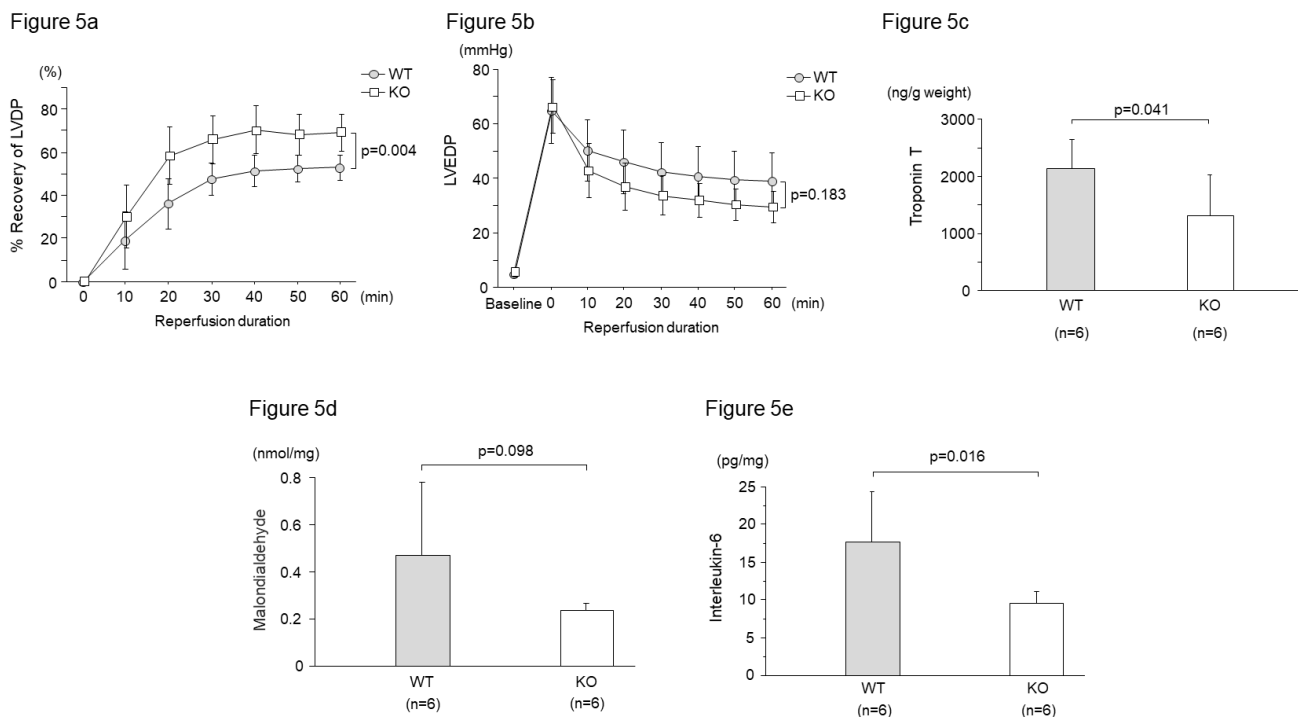
Fig. 5. DNC treatment (Study 2): Post-ischemic recovery of LVDP and LVEDP, troponin T, malondialdehyde and interleukin-6 levels. (a) Recovery of left ventricular developed pressure. (b) Changes in left ventricular end-diastolic pressure. (c) Total troponin T leakage (ng/g wet weight) during 60 min of reperfusion. (d) Levels of malondialdehyde. (e) Levels of interleukin-6. Results are shown as ratios of baseline values or as means ± SD of six hearts per group (a,b). Light gray circles and white squares, WT and KO mice, respectively. WT, wild-type mice; KO, aquaporin 7 knockout mice; LVDP, left ventricular diastolic pressure; LVEDP, left ventricular end-diastolic pressure.

of myocardial function and coronary flow were measured and compared between the groups. The effects of nicorandil on myocardial injury were assessed by measuring troponin T (expressed as ng/g heart wet weight: ng/g wet weight) in coronary effluents using an electrochemiluminescence immunoassay (Roche Diagnostics K.K., Tokyo, Japan) [17].

Effects of DNC on Recovery of Cardiac Function and Myocardial Injury (Study 2)

Male mature WT and KO murine hearts were equilibrated for 20 min, with aerobic KHB at 37 °C to attain equilibration, infused with DNC for 2 min, exposed to 23 min of global ischemia, then reperfused for 60 min with aerobic KHB. The final recovery of myocardial function and coronary flow were measured and compared between the groups. The effects on myocardial injury were assessed by measuring troponin T in coronary effluents using an electrochemiluminescence immunoassay (Roche Diagnostics K.K) [17]. At the end of the experiment, the hearts were harvested and maintained at –80 °C for subsequent analysis. The frozen ventricles were ground to powder using a liquid nitrogen-cooled tissue grinder. Weighed amounts of frozen tissue were homogenized in the appropriate buffer using a microcentrifuge homogenizer for tissue analysis.

The levels of malondialdehyde (MDA) were spectrophotometrically analyzed through ELISA (BioVision, Milpitas, CA, USA) according to the manufacturer's instructions [22]. The levels of interleukin-6 (IL-6) were spectrophotometrically analyzed through ELISA (R&D Systems, Inc. Minneapolis, MN, USA) according to the manufacturer's instructions [22].

Statistical Analyses

Post-ischemic recovery of the LVDP, heart rate, and coronary flow are expressed as proportions (%) of their respective baseline values, whereas LVEDP (mmHg) and troponin T (ng/g wet weight) are expressed as absolute values. All data are expressed as means ± standard deviation (SD). Continuous variables were compared using Student t-test, analysis of variance (ANOVA), or two-way repeated-measures ANOVA, as appropriate. If significance was established, multiple comparisons were analyzed using post hoc Tukey tests. All statistical tests were two-tailed, and values with $p < 0.05$ were considered statistically significant. All data were statistically analyzed using JMP v. 10.0 (SAS Inc., Cary, NC, USA).

Results

Effects of Nicorandil on Ischemia-Reperfusion Injury (Study 1)

Baseline parameters did not significantly differ among the groups. Table 1 shows the baseline and final recovery of all parameters. Our data demonstrated that AQP7 deficiency did not affect postischemic cardiac function during reperfusion in a Langendorff apparatus (Fig. 3a–d). Post-ischemic recovery of LVDP in the nicorandil treatment-naïve KO and WT controls groups was low (plateau at 20–25% of baseline), whereas gradual post-ischemic recovery of was approximately 40% in the hearts that received nicorandil before global ischemia (Fig. 4a). The troponin T levels did not significantly differ between the nicorandil treatment-naïve WT and KO groups (1801 ± 945 and 1842 ± 1114 ng/g wet weight), respectively, or between the WT and KO groups treated with nicorandil (1326 ± 759 and 1484 ± 1419 ng/g wet weight), respectively (Fig. 4b).

Effects of DNC on Recovery of Cardiac Function and Myocardial Injury (Study 2)

Table 2 shows baseline heart rates, LVDP, coronary flow, and final LVEDP values. The mature WT and KO murine hearts did not significantly differ at baseline. The hearts administered DNC recovered rapidly, reaching a 50–60% higher plateau after 30 min of reperfusion. The final recovery of LVDP was significantly higher in the KO, than in the WT group (Fig. 5a), and the LVEDP recovery mirrored it (Fig. 5b). The total leakage of troponin T during reperfusion was significantly supressed in the KO compared with the WT group (Fig. 5c). The levels of MDA (nmol/mg) in the WT group were higher than in the KO group but did not reach significance (Fig. 5d). The levels of IL-6 (pg/mg) in the WT group were significantly higher than those in the KO group (Fig. 5e).

Discussion

We investigated the relationship between AQP7, which regulates water and glycerol permeability, and myocardial injury caused by normothermic global ischemia. Additionally, we elucidated the effects of nicorandil and DNC in mature murine hearts with an AQP7 deficiency. We found that DNC conferred cardioprotective effects, preserving cardiac function and reducing myocardial injury in isolated AQP7-KO more than that in WT murine hearts. These findings indicated that an AQP7 deficiency does not hamper the myocardial protective effect provided by DNC.

Aquaporins are involved in the regulation of cardiovascular function and development of associated diseases such as cerebral ischemia, congestive heart failure, hyper-

tension, and angiogenesis. Therefore, further studies are needed to elucidate the mechanism underlying the association between AQPs and vascular function-related diseases to develop novel approaches for their prevention and treatment [23]. Aquaporin regulation under some level of preconditioning has been identified in various types of organs and cells. Some sub-populations of neurons and astroglia express AQP1 after preconditioning induced by 3-nitropropionic acid (3NP) [12]. Conversely, 3NP preconditioning significantly reduces AQP4 levels in the cortex and striatum, resulting in the amelioration of post-ischemic brain edema [10]. Bradykinin preconditioning 20 min before ischemia induces changes in AQP4 protein that are accompanied by a reduction in spinal cord edema at 72 h following reperfusion [24]. Remote ischemic preconditioning attenuates the reduction in AQP2 expression after renal ischemia-reperfusion [12]. Relative AQP7 expression is significantly lower in patients treated with the K_{ATP} channel opener, diazoxide, during coronary artery bypass surgery via cardiopulmonary bypass and blood cardioplegia compared with controls. The present findings found similar post-ischemic LVDP recovery between the control and AQP7-KO groups that received nicorandil. This suggests that the expression of AQP channels other than AQP7 are involved in the recovery of cardiac function induced by nicorandil preconditioning. This requires further investigation.

Crystalloid or blood-based cardioplegic solutions contain various chemical compounds and drugs. DNC has been used in pediatric cardiac surgery for >20 years, and its safety and efficacy are well established. However, DNC has only recently been associated with safety and effectiveness in adults during surgical procedures [20,21]. Myocardial AQP4 levels were markedly lower after ischemic exposure for >90 min in a DNC subgroup compared with histidine–tryptophan–ketoglutarate cardioplegia [25]. The present findings showed significantly better LVDP recovery after cardioplegic arrest and less troponin T leakage in the AQP7-KO compared with that in the WT group. Paradoxically, AQP7 is expressed in cardiac tissue at a level of 83.3% in patients undergoing coronary artery bypass surgery with cardiopulmonary bypass and blood cardioplegia, which might promote myocardial edema and reduce cardiac function [10]. Although an AQP7 deficiency increases the size of myocardial infarcts and causes apoptosis in response to ischemia [26], the functional role of AQP7 in the heart remains unclear.

This study had a few limitations. The hearts were perfused with a standard KHB solution that included glucose but not glycerol or palmitic acid. Therefore, further studies should assess metabolic adaptation and nutrition-related conditions such as specific diets or starvation. Moreover, myocardial ischemic disease is a multifactorial process with a spectrum of damage that affects the method of myocardial protection. The hearts used in this study were obtained from

healthy WT and AQP7-KO mice under normal feeding conditions, and it is likely that any protective effect of nicorandil or DNC would be different in hearts jeopardized by ischemic damage, hypertrophy, or aging. In addition, such hearts are likely to require prolonged periods of ischemia to correct lesions, whereas the ischemic duration adopted in this experimental study was relatively short.

Conclusion

Langendorff perfusion model revealed myocardial protective effects of nicorandil in AQP7-deficient mice similar to that in WT mice. AQP7 deficiency did not impair the cardioprotective effects of DNC.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

YK: The acquisition of data; Drafting the work; Final approval of the version to be published. MF: The conception or design of the work; Interpretation of data for the work; Reviewing it critically for important intellectual content; Final approval of the version to be published. RB: Interpretation of data for the work; Reviewing it critically for important intellectual content; Final approval of the version to be published. YI: Interpretation of data for the work; Reviewing it critically for important intellectual content; Final approval of the version to be published. All authors contributed to editorial changes in the manuscript. And agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

All animals received humane treatment in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH) (NIH publication number: 85-23, revised 1996). The Animal Ethics Committee of Nippon Medical School approved the study (Approval No: 2020-103, 2021-17).

Conflict of Interest

The authors declare no conflict of interest.

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