의학박사 학위논문

Serial ultrastructural evaluation of myocardial ischemic injury after infusion of del Nido cardioplegia in the human heart

사람 심장에서 del Nido 심정지액 주입 이후
심근 허혈성 손상의 진행에 대한 초미세구조 관찰

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서울대학교 대학원
의학과 흉부외과학 전공
정 준 철

Ph.D. Dissertation of Doctor of Medicine

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August 2021

The Graduate School
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College of Medicine
Thoracic and Cardiovascular Surgery Major
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부위원장 황 호 영

위 원 정 진 행

위 원 양 지 혁

위 원 최 재 웅

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Examiner Ho Young Hwang

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Joon Chul Jung

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Joon Chul Jung
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Chair	Hae-Young Lee
Vice Chair	Ho Young Hwang
Examiner	Jin-Haeng Chung
Examiner	Ji-Hyuk Yang
Examiner	Jae Woong Choi

Abstract

Serial ultrastructural evaluation of myocardial ischemic injury after infusion of del Nido cardioplegia in the human heart

Joon Chul Jung

Thoracic and Cardiovascular Surgery Major

College of Medicine

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Objectives: The safe ischemic time after a single-dose del Nido cardioplegia (DNC) infusion has not yet been established. This study evaluated the progression of myocardial ischemic injury to establish the safe ischemic time after a single-dose DNC infusion in the human heart using a transmission electron microscope.

Methods: Seven hearts extracted from heart transplant recipients after infusion of 1000 mL single-dose DNC were evaluated. Serial left ventricular myocardial tissue samples were collected every 30 minutes for 180 minutes. Ischemic injuries in the mitochondria and nuclei were scored from 0 to 3 (0 = normal, 0.5 = slight, 1 =

moderate, 2 = severe, and 3 = irreversible).

Results: At the time of extraction, 83.5% of the mitochondria were normal. The proportion of mitochondria with moderate ischemic injury increased gradually from 1.4% at extraction to 52.5% at 180 minutes. From 90 minutes to 180 minutes, the proportion of mitochondria with severe and irreversible injury increased from 0.8% to 4.4% and 0.3% to 1.3%, respectively. A significant linear correlation was identified between the average ischemic injury score of mitochondria and ischemic time ($P < .001$). Most nuclei showed moderate to severe ischemic injury at every time point (61.0%-85.2%). A significant linear correlation was also found between the average ischemic injury score of nuclei and ischemic time ($P < .001$).

Conclusions: Myocardial ischemic injury progresses gradually, and irreversible ischemic injury begins to occur 90 minutes after initial DNC infusion in the adult human heart. Therefore, the adult human myocardium may be safe from ischemic injury until 90 minutes after the single-dose DNC infusion.

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Keyword: del Nido cardioplegia, myocardial ischemic injury, transmission electron microscope

Student Number: 2015-21999

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INTRODUCTION

Del Nido cardioplegia (DNC) was developed in the early 1990s and has been widely used in pediatric cardiac surgery.[1] The advantages of DNC include the convenience of single-dose infusion, a small infusion volume, and the spontaneous restoration of sinus rhythm after the release of the aortic cross-clamp (ACC).[2-4] Because of these advantages, many centers have applied DNC in adult cardiac surgery, and sufficient myocardial protection equivalent to that of conventional blood cardioplegia has been demonstrated.[2-10]

The original delivery protocol suggested that subsequent doses were usually not required within 3 hours after the initial infusion.[11] However, a safety margin after a single-dose infusion of DNC has not yet been established in adult cardiac surgery, and subsequent doses of DNC after 90 minutes of ACC application have been administered based on experts' opinions without any experimental evidence.[12,13]

Therefore, this study was conducted to evaluate the progression of myocardial ischemic injury and to establish a safety margin for ischemic time after a single-dose infusion of DNC in the human heart using a transmission electron microscope.

MATERIALS AND METHODS

Patient Selection

This prospective study was approved by Seoul National University Hospital Institutional Review Board (approval number: H-1806-024-949) and all participating patients signed an informed consent form. Adult patients who were to undergo heart transplantation were considered for study enrollment. Exclusion criteria included patients who had coronary artery disease because there was a possibility of uneven distribution of cardioplegia and patients with previous cardiac surgery because there was a risk of traumatic myocardial injury during adhesiolysis. From June 2018 to January 2020, a total of 17 heart transplantations were performed at our institution and 7 of these patients were enrolled (Figure 1). The mean age at operation was 57.9 ± 8.0 years, and 4 patients (57.1%) were men. Primary diagnoses were dilated cardiomyopathy and restrictive cardiomyopathy in 6 and 1 patient, respectively (Table 1).

Heart Extraction and Tissue Sampling

Heart transplantation was performed via median sternotomy and aorto-bicaval cannulation. Before extracting the heart, the DNC was infused into the recipient's heart after applying the ACC to mimic routine cardiac surgical procedures. The DNC was applied in a 4:1 mixture of crystalloid component with the patient's blood as originally introduced (Table 2). A 1000 mL aliquot of DNC was infused in an antegrade manner under a perfusion pressure of 100 to 130 mmHg and a temperature of 7°C to 8°C. After completion of the cardioplegia infusion, the heart

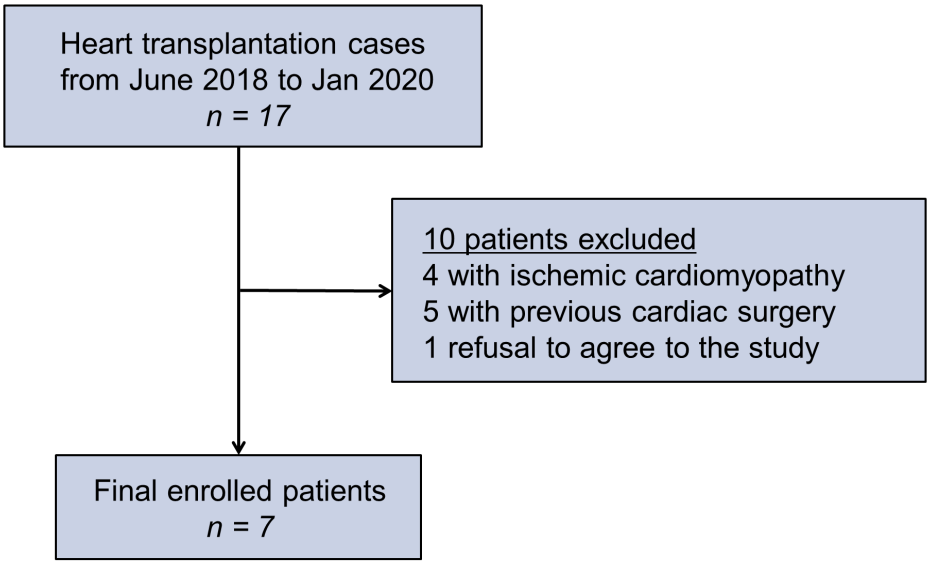

Figure 1. Summary flow chart of study enrollment.

Table 1. Baseline characteristics of the study patients

Characteristics	Value
Demographics	
Age, y	57.9 ± 8.0
Male: female	4: 3
Body mass index (kg/m^2)	24.1 ± 1.2
Comorbidities, n (%)	
Smoking	2 (28.6)
Hypertension	3 (42.9)
Diabetes mellitus	3 (42.9)
Dyslipidemia	1 (14.3)
Cerebrovascular disease	1 (14.3)
Atrial fibrillation	3 (42.9)
Peripheral vascular disease	0
Chronic renal failure	0
Chronic obstructive pulmonary disease	0
Diagnosis, n (%)	
Dilated cardiomyopathy	6 (85.7)
Restrictive cardiomyopathy	1 (14.3)
Echocardiography data	
LVEF (%)	23.3 ± 12.2
LVIDd (mm)	74.3 ± 19.7
LVIDs (mm)	64.6 ±20.7

Values are presented as mean ± standard deviation, n:n, or n (%). *LVEF*, left ventricular ejection fraction; *LVIDd,* diastolic left ventricular internal diameter; *LVIDs*, systolic left ventricular internal diameter.

Table 2. The composition of the crystalloid component of del Nido cardioplegia solution

Component	Volume (mL)
Plasma-Lyte A solution (Baxter International Inc, Deerfield, Ill)	1000
Potassium chloride (2 mEq/mL)	13
8.4% Sodium bicarbonate	13
20% Mannitol	16.3
10% Magnesium sulfate	20
1% Lidocaine	13

was extracted from the patient and moved to a double water bath filled with normal saline. The bath was kept in the same air-conditioned operating room near the patient. The myocardial temperature was maintained at approximately 18°C according to a transseptal myocardial temperature probe with intermittent cold saline irrigation.

To obtain the myocardial tissue samples from the same part of the heart, the samples were obtained from the left ventricular wall near the apex between the left anterior descending artery and the diagonal branch. STARCUT 14-gauge needle biopsy guns (TSK Laboratory, Hirayanagi-Cho, Tochigi-Shi Tochigi-Ken, Japan) were inserted from the outside of the heart, and cylindrical-shaped full-thickness myocardial samples were extracted (Figure 2). The first sample was obtained immediately after the heart was extracted. Then, serial samples were obtained at 60, 90, 120, 150, and 180 minutes after the infusion of cardioplegia. After 180 minutes, the myocardial temperature was increased to 36.5°C with warm saline irrigation to accelerate ischemic injury. Care was taken not to overheat the myocardium above 38°C to prevent thermal tissue injury. The last sample was obtained at 240 minutes after the infusion of cardioplegia (Figure 3). A total of 7 samples were obtained from each patient.

Tissue Preparation for Electron Microscopy

The tissue samples were immediately placed in microtubes filled with 2.5% glutaraldehyde 0.1 M phosphate buffer solution and fixed for 24 hours in the refrigerator. Then, the samples were processed according to the standard electron microscope tissue processing protocol.[14] Because it has been known that the rate of

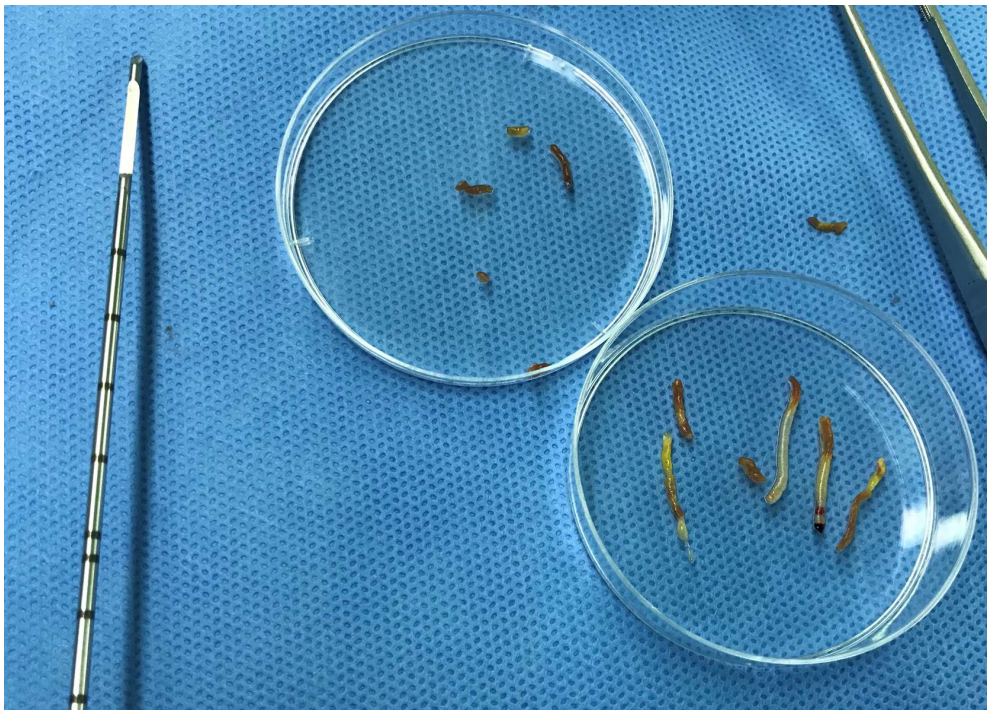

Figure 2. The needle biopsy gun and extracted myocardial samples.

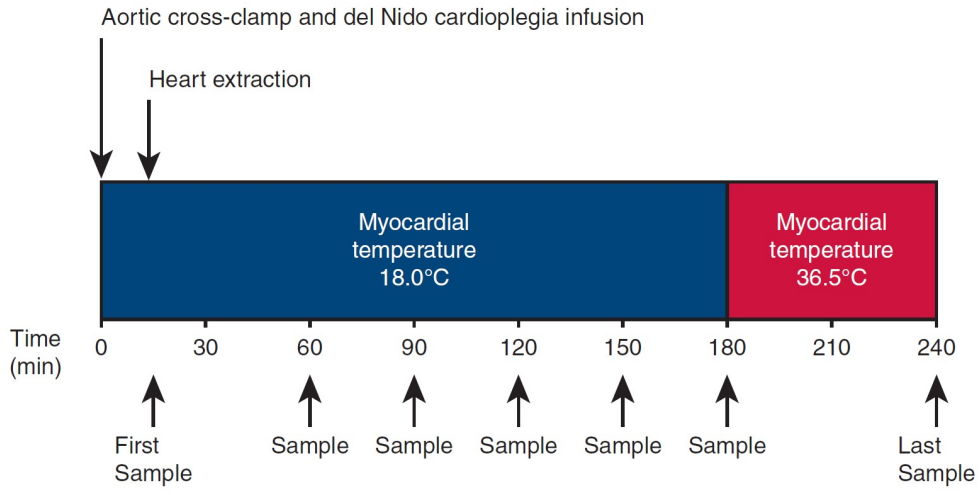

Figure 3. Timeline of the experimental study protocol. The first myocardial tissue sample was obtained immediately after the infusion of del Nido cardioplegia and heart extraction. Then, serial samples were obtained during 180 minutes of ischemia. The last sample was obtained at 240 minutes after rewarming the heart to accelerate ischemic injury.

progression of myocardial ischemic injury is different according to the layer of the myocardium, only the midportion of myocardial samples was selected and processed. The samples were washed in Sorenson's phosphate buffer, postfixed in 2% osmium tetroxide, dehydrated in alcohol at increasing concentrations, treated with propylene oxide, and embedded in Spurr's embedding media. Semithin sections (500 nm) were cut, stained with toluidine blue, and examined by light microscopy. Artifact-free areas were selected. At least 5 ultrathin sections (70 nm) were obtained from each sample, mounted on copper grids, and stained with uranyl acetate and lead citrate.

Ultrastructural Evaluation and Ischemic Injury Scoring

The samples were evaluated using a JEM-1400 Flash transmission electron microscope (JEOL USA, Inc., Peabody, MA, USA). The samples were placed in random order by an electron microscopist, and the researcher was blinded to the order. To ensure the representativeness of the entire sample, at least 5 sections per sample were examined, and at least 3 fields of micrographs were randomly taken for each section. The micrographs were reviewed by one researcher (J. C. Jung), and the degree of ischemic injury was scored. Another researcher (S.-I. Kim) repeated this process at a different time point. The number of mitochondria and nuclei to be evaluated in each sample were planned to be 200 and 20, respectively, based on previous studies[15,16] in which 150 to 300 mitochondria and 20 nuclei were examined. The degree of ischemic injury of each mitochondrion was scored according to the following criteria: 0 = normal, 0.5 = slight, 1 = moderate, 2 = severe, and 3 = irreversible (Table 3 and Figure 4). The ischemic injury of each

nucleus was scored according to the following criteria: 0 = normal or slight, 1 = moderate to severe, and 3 = irreversible (Table 3 and Figure 5).[17-19]

Table 3. The criteria for ischemic injury scoring

Score	Degree of injury	Mitochondrion				Nucleus	
		Normal matrix granule	Amorphous matrix densities	Clearing of matrix	Fragmentation of cristae	Clearing of matrix	Clumping and margination of chromatin
0	Normal	+	-	-	-	-	-
0.5	Slight	-	-	+	-	-	-
1	Moderate	-	-	++	+ or ++	+	+
2	Severe	-	-	+++	+++	+	+
3	Irreversible	-	+	+++	+++	++	++

-, absent; +, slight; ++, moderate; +++, severe.

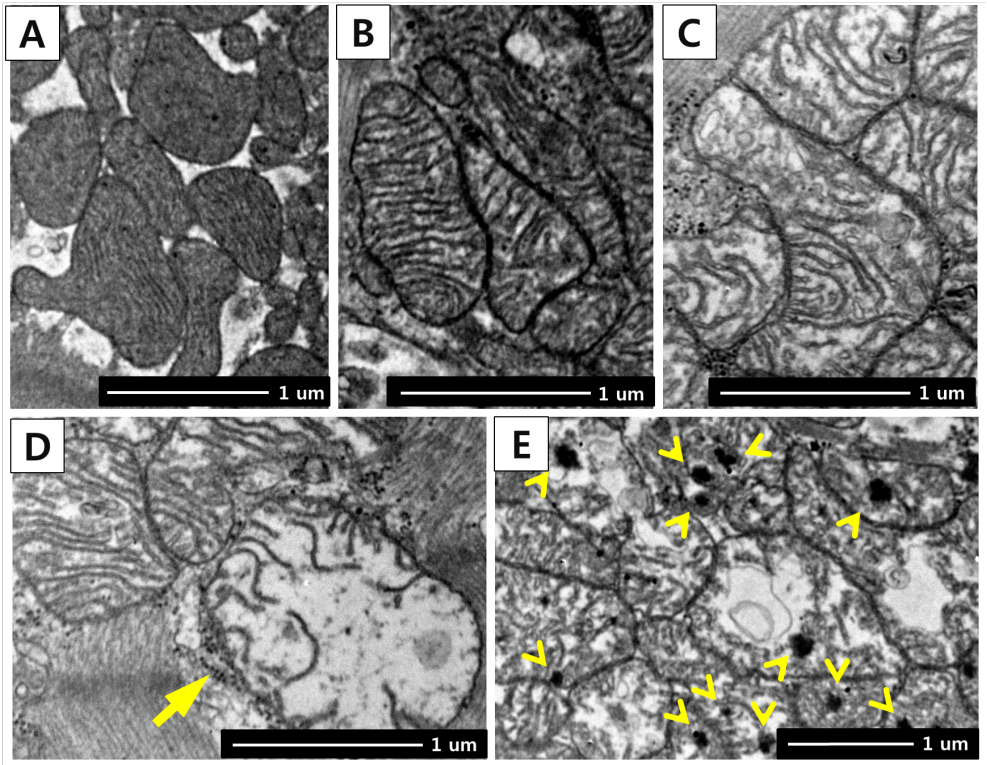

Figure 4. Transmission electron micrographic findings of mitochondria. A, Score 0: Regular cristae and a dense matrix with normal matrix granules (25000×). B, Score 0.5: Slight clearing of the matrix with intact cristae (30000×). C, Score 1: Moderate clearing of the matrix with partial fragmentation of cristae (30000×). D, Score 2 (*arrow*): Nearly complete clearing of the matrix with mostly absent cristae (30000×). E, Score 3: Typically observed large amorphous matrix densities (*arrowhead*s) (20000×).

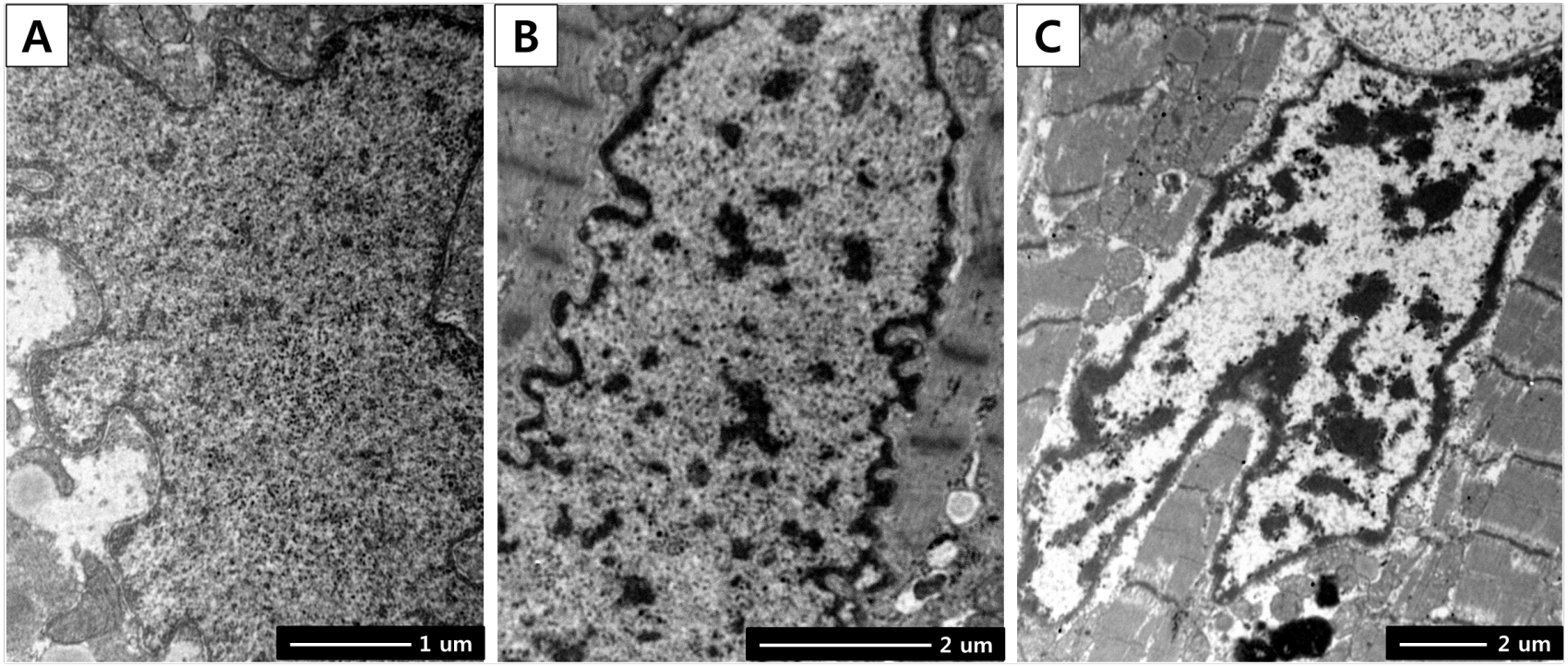

Figure 5. Transmission electron micrographic findings of nuclei. A, Score 0: Finely dispersed chromatin (25000×). B, Score 1: Slight clumping and margination of chromatin. (12000×). C, Score 3: Nearly complete clearing of the matrix with severe clumping and margination of chromatin (8000×).

Statistical Analysis

Statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) and MedCalc version 19.3 (MedCalc Software, Mariakerke, Belgium). Because of the unique design of the present study, the number of study subjects was not determined through sample size calculation; we simply planned to enroll at least 6 patients during the given study period considering the nature of the exploratory investigation and the usual number of heart transplantations at our institution.

Continuous variables were presented as the means ± standard deviations. The ischemic injury score at each time point was presented with the standard error estimated by a generalized estimating equation accounting for multiple observations.

For the ordinal ischemic injury score, the proportional odds assumption was assessed by plotting time against the empirical cumulative logits, and parallel cumulative logits indicated that the assumptions held. The correlation between ordinal ischemic injury scores and ischemic time was assessed with a cumulative logit link in a generalized linear mixed-effects model with time as a fixed effect and subject as a random effect to account for multiple measurements per subject. The influence of time on the proportions of mitochondria and nuclei with more than moderate ischemic injury was evaluated with a logit link in the generalized linear mixed-effects model. The odds ratios and 95% confidence intervals (CIs) were presented to compare the proportions at each time point with those at extraction. The correlation between average ischemic injury score and ischemic time was evaluated using a linear mixed-effects model with time as a fixed effect

and subject as a random effect.

Inter-observer variability was assessed with Bland-Altman plots, with 95% limits of agreement (the mean difference ± 1.96 standard deviations of the difference) constructed by considering multiple observations per individual.

RESULTS

Electron Microscopic Findings of Mitochondria

All samples had sufficient mitochondria, and 200 mitochondria were evaluated in each sample. The representative transmission electron micrographic findings of mitochondria at each time point were presented in Figure 6. At the time of extraction, 83.5% and 15.1% of the mitochondria were found to be normal (score 0) and slightly injured (score 0.5), respectively. Severe (score 2) or irreversible (score 3) ischemic injury was not found in any of the mitochondria. The proportion of mitochondria with moderate ischemic injury (score 1) increased gradually from 1.4% at extraction to 52.5% at 180 minutes. From 90 minutes to 180 minutes, although the proportions were low, the proportion of mitochondria with severe and irreversible injury also increased from 0.8% to 4.4% and 0.3% to 1.3%, respectively. At 240 minutes, only 0.2% of the mitochondria were found to be normal, and irreversible ischemic injury was observed in 22.9% of the mitochondria (Table 4, Figure 7A). The odds of having a higher ischemic injury score significantly increased by 2.46 (95% CI, 2.41-2.51) per 30 minutes ($P < .001$). There was a significant difference among the ischemic times in the proportions of mitochondria with more than moderate ischemic injury (score 1) ($P < .001$). The proportions at 60, 90, 120, 150, and 180 minutes were significantly increased, with odds ratios of 7.5, 24.9, 58.3, 88.7, and 159.0, respectively, compared with that at extraction ($P < .001$ each) (Table 5). A significant linear correlation was identified between the average ischemic injury score of mitochondria and ischemic time, with an increase of 0.139 points every 30 minutes ($P < .001$) (Figure 8A) according to the linear mixed-effects model; the mean values of the ischemic injury scores were

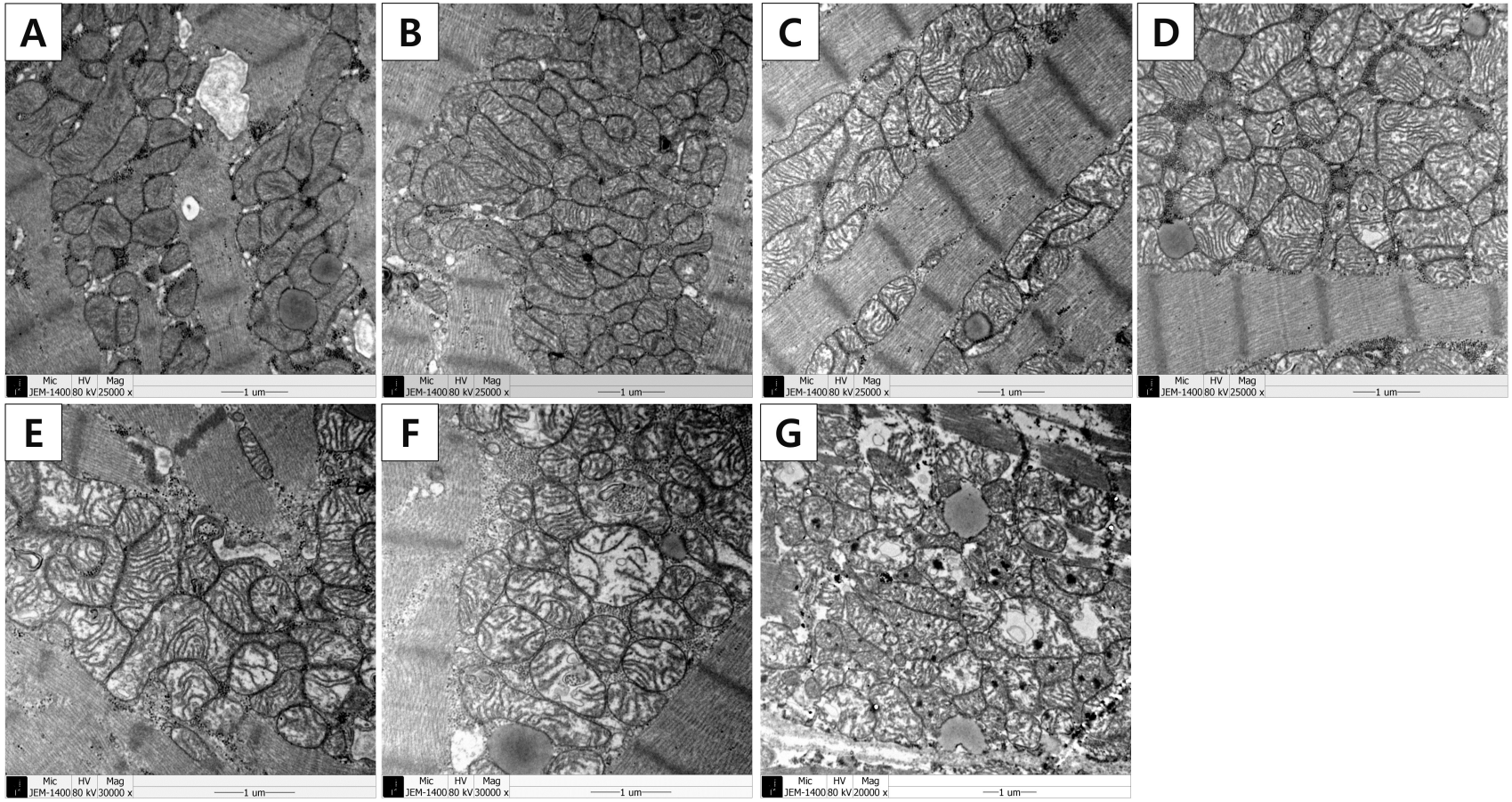
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Figure 6. The representative transmission electron micrographic findings of mitochondria. A, At the time of extraction. All mitochondria show normal morphology (score 0) with dense matrix and regular cristae. B, 60 minutes after the infusion of del Nido cardioplegia. Most mitochondria show slight ischemic injury (score 0.5) with a slight clearing of the matrix, but still regular cristae. C, 90 minutes. Some mitochondria show moderate ischemic injury (score 1) with partial fragmentation of cristae. D, 120 minutes. E, 150 minutes. Some mitochondria show severe ischemic injury (score 2) with nearly complete clearing of the matrix and mostly absent cristae. F, 180 minutes. G, 240 minutes. Many mitochondria show irreversible ischemic injury (score) with large amorphous matrix densities.

Table 4. Temporal changes in proportions of ischemic injury scores of mitochondria and nuclei after the infusion of single-dose del Nido cardioplegia.

Time	Mitochondria (%)					Nuclei (%)		
	Normal	Slight	Moderate	Severe	Irreversible	Normal or slight	Moderate to severe	Irreversible
Extraction	83.5 ± 3.8	15.1 ± 3.2	1.4 ± 1.0	0	0	39.0 ± 6.0	61.0 ± 6.0	0
60 min	40.7 ± 7.7	50.6 ± 5.8	8.5 ± 2.6	0.1 ± 0.1	0	20.4 ± 5.9	79.6 ± 5.9	0
90 min	17.7 ± 4.1	60.7 ± 4.6	20.5 ± 6.2	0.8 ± 0.4	0.3 ± 0.2	26.6 ± 14.4	68.8 ± 11.8	4.7 ± 2.9
120 min	7.1 ± 2.9	56.3 ± 5.2	33.9 ± 6.4	1.7 ± 0.7	0.9 ± 0.4	21.2 ± 9.9	75.0 ± 8.6	3.8 ± 2.2
150 min	5.1 ± 1.6	49.5 ± 6.6	41.9 ± 7.5	2.0 ± 0.5	1.5 ± 0.6	15.1 ± 4.2	83.7 ± 4.0	1.2 ± 1.0
180 min	2.6 ± 0.9	39.2 ± 7.3	52.5 ± 6.9	4.4 ± 1.6	1.3 ± 0.3	7.4 ± 3.7	85.2 ± 5.6	7.4 ± 5.2
240 min	0.2 ± 0.1	15.2 ± 3.9	48.8 ± 6.0	13.0 ± 3.7	22.9 ± 5.3	0	6.1 ± 5.3	93.9 ± 5.3

Values are presented as the mean ± standard error unless the proportion is 0%.

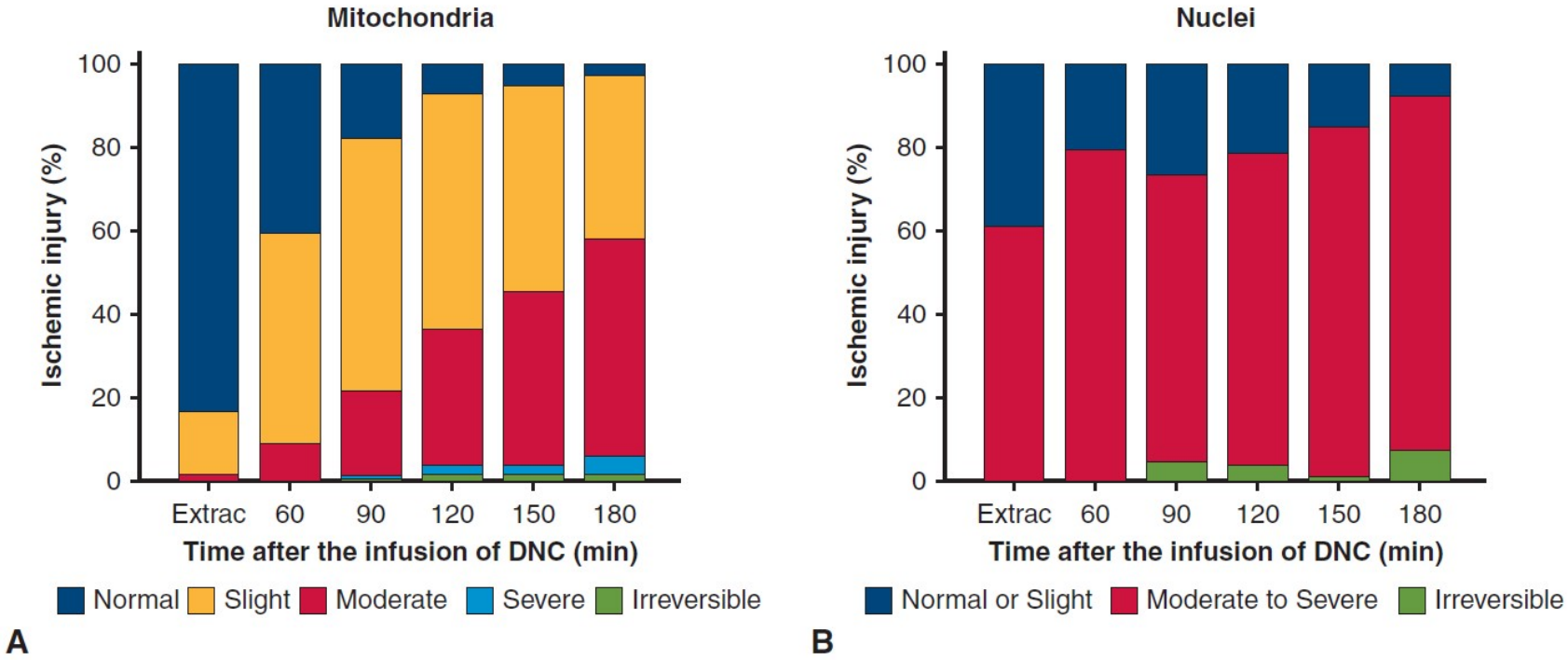

Figure 7. Temporal changes in the proportions of ischemic injury scores of (A) mitochondria and (B) nuclei after the infusion of single-dose del Nido cardioplegia (DNC). The proportions of mitochondria with moderate, severe, or irreversible ischemic injury increased gradually over time, whereas most nuclei showed moderate to severe ischemic injury at all time points.

Table 5. Temporal changes in the proportions of mitochondria and nuclei with more than moderate ischemic injury (score 1) and odds ratios estimated by generalized linear mixed-effects models.

	Time	n (Score≥1)/n (Total)	% (Score≥1/Total)	OR (95% CI)	*P* value
Mitochondria					<.001*
	Extraction	38/2800	1.4	1	
	60	243/2800	8.7	7.5 (5.3-10.7)	<.001
	90	606/2800	21.6	24.9 (17.7-34.9)	<.001
	120	1024/2800	36.6	58.3 (41.6-81.5)	<.001
	150	1271/2800	45.4	88.7 (63.4-124.2)	<.001
	180	1629/2800	58.2	159.0 (113.6-222.5)	<.001
Nuclei					<.001*
	Extraction	64/105	61.0	1	
	60	74/93	79.6	2.3 (1.2-4.6)	0.014
	90	47/64	73.4	1.9 (0.9-3.9)	0.105
	120	82/104	78.8	3.1 (1.6-6.1)	0.001
	150	73/86	84.9	4.3 (2.0-9.1)	<.001
	180	100/108	92.6	12.2 (4.9-30.4)	<.001

OR, odds ratio; *CI*, confidence interval. * *P* value for the difference between groups.

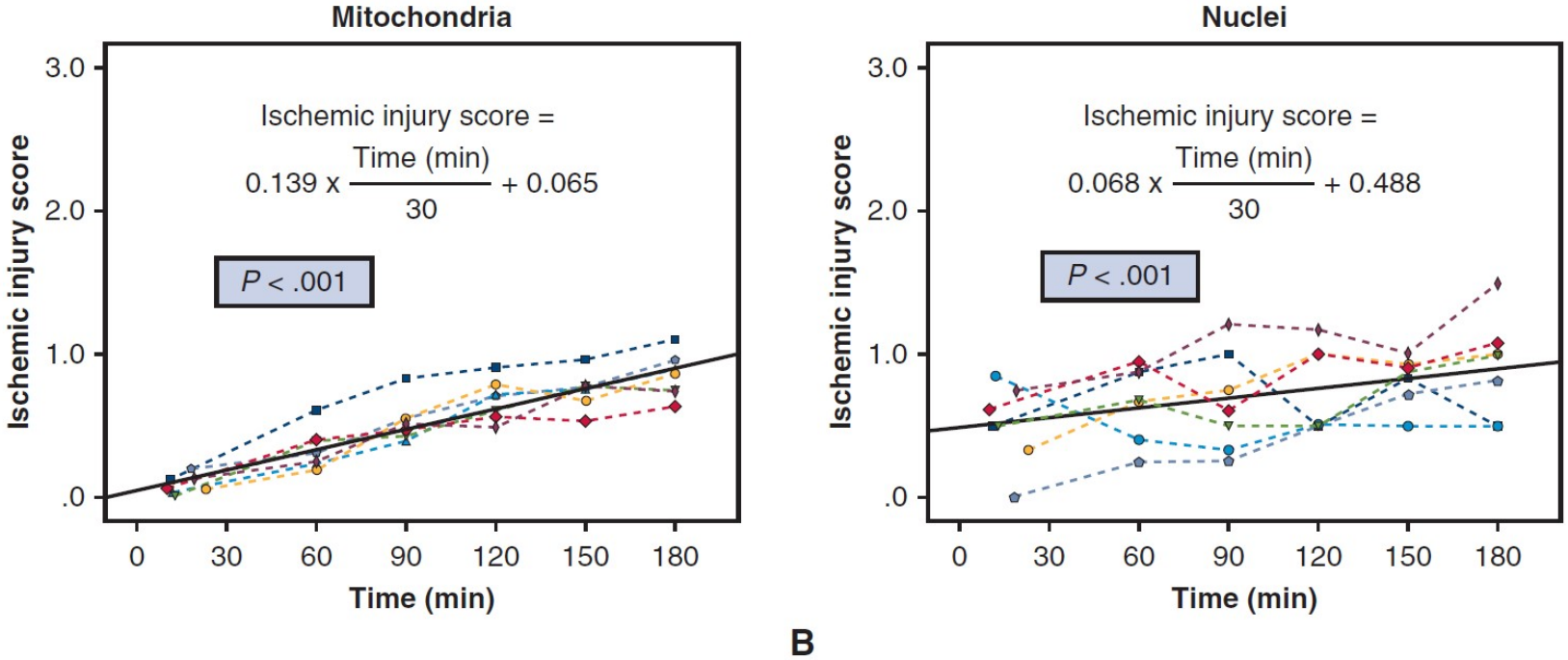

Figure 8. Temporal changes in the average ischemic injury score of (A) mitochondria and (B) nuclei in each of the 7 hearts after the infusion of single-dose del Nido cardioplegia. The *solid black line* represents the average ischemic injury score estimated by the linear mixed-effects model. Significant linear correlations were identified between the average ischemic injury scores of both mitochondria and nuclei and ischemic time.

0.48 at 90 minutes and 0.90 at 180 minutes, respectively.

Electron Microscopic Findings of Nuclei

Sample sections had fewer nuclei than the number that we planned to evaluate. The median number of observed nuclei per sample was 5.5 (interquartile range, 2.75-10.0). The representative transmission electron micrographic findings of nuclei at each time point were presented in Figure 9. Most nuclei showed moderate to severe ischemic injury (score 1) at all time points (61.0%-85.2%). From 90 minutes to 180 minutes, the proportion of nuclei with irreversible injury (score 3) increased from 4.7% to 7.4%. At 240 minutes, no nuclei were found to be normal, and irreversible ischemic injury was observed in 93.9% of the nuclei (Table 4, Figure 7B). The odds of having a higher ischemic injury score significantly increased by 1.48 (95% CI, 1.32-1.67) per 30 minutes ($P < .001$). A significant difference among the ischemic times was observed in the proportions of nuclei with more than moderate ischemic injury (score 1) ($P < .001$). The proportions at 60, 120, 150, and 180 minutes were significantly increased, with odds ratios of 2.3, 3.1, 4.3, and 12.2, respectively, compared with that at extraction ($P = .014$, .001, < .001, and < .001, respectively) (Table 5). A significant linear correlation was also found between the average ischemic injury score of nuclei and ischemic time, with an increase of 0.068 points every 30 minutes ($P < .001$) (Figure 8B).

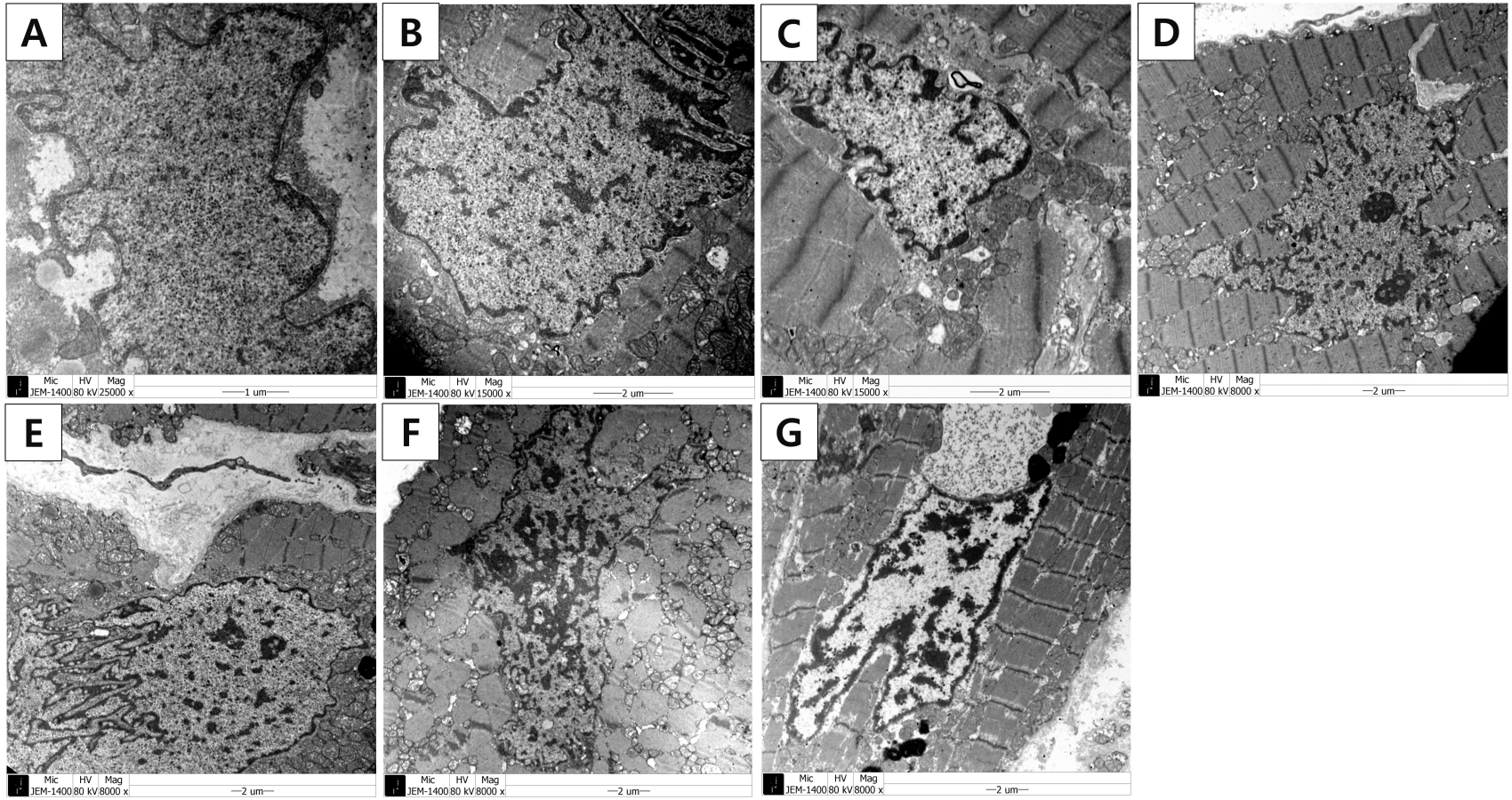
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Figure 9. The representative transmission electron micrographic findings of nuclei. A, At the time of extraction. The nucleus shows normal morphology (score 0) with finely dispersed chromatin. B, 60 minutes after the infusion of del Nico cardioplegia. The nucleus shows moderate to severe ischemic injury (score 1) with slight clumping and margination of chromatin. C, 90 minutes. D, 120 minutes. E, 150 minutes. F, 180 minutes. G, 240 minutes. The nucleus shows irreversible ischemic injury (score 3) with nearly complete clearing of the matrix and severe clumping and margination of chromatin.

Inter-observer variability

Bland-Altman plots of average ischemic injury scores showed that the mean differences and 95% limits of agreements were -0.06 (95% limits of agreement, -0.48 to 0.35) and 0.25 (95% limits of agreement, -0.67 to 1.16) for mitochondria and nuclei, respectively (Figure 10).

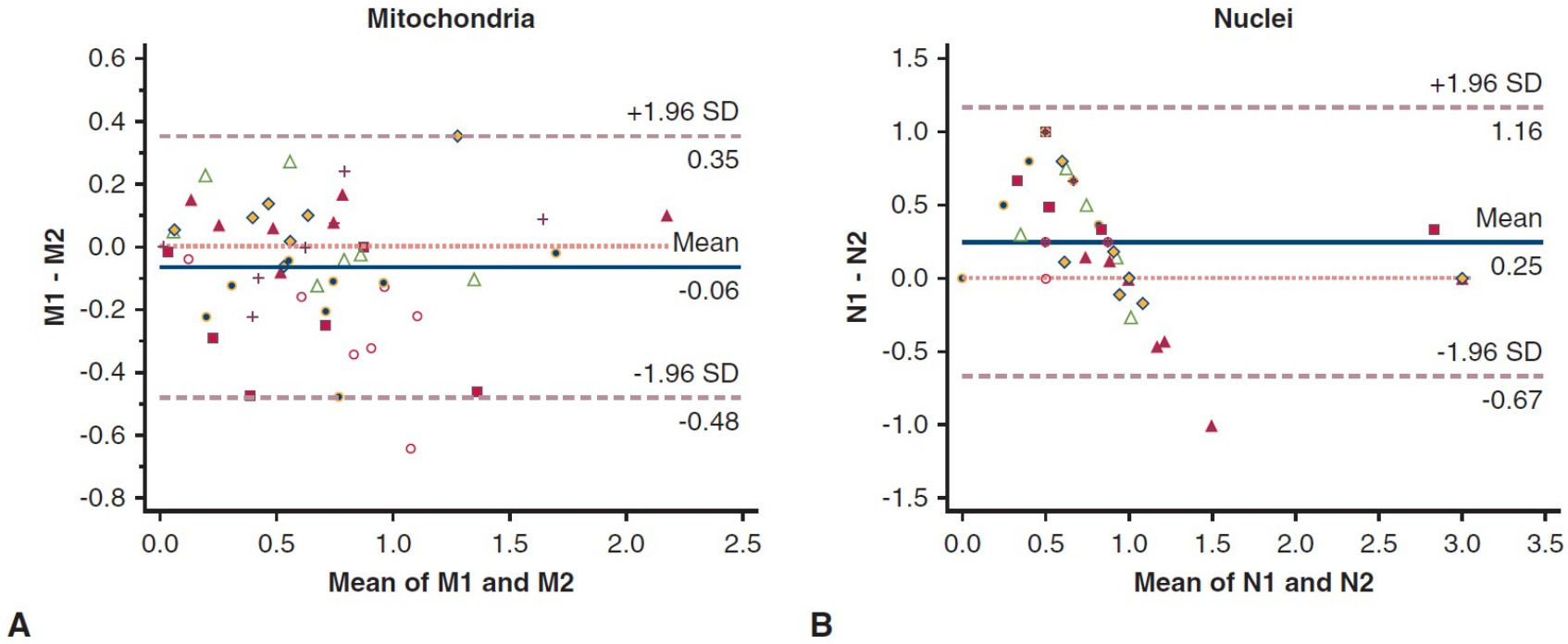

Figure 10. Bland-Altman plots of 2 average ischemic injury scores of (A) mitochondria and (B) nuclei evaluated by 2 researchers in the same samples. *M1*, ischemic injury score of mitochondria observed by researcher 1; *M2*, ischemic injury score of mitochondria observed by researcher 2; *SD*, standard deviation; *N1*, ischemic injury score of nuclei observed by researcher 1; *N2*, ischemic injury score of nuclei observed by researcher 2.

DISCUSSION

The present study demonstrated that ischemic injury progressed gradually over time and that irreversible ischemic injury began to occur 90 minutes after the single-dose infusion of DNC in the human heart.

DNC is classified as modified depolarizing cardioplegia in which lidocaine and magnesium prevent the influx of sodium and calcium ions into the myocytes by blocking sodium and calcium membrane channels during depolarized arrest.[20] It provides reduced inducible electrical activity and reduced calcium accumulation during ischemia compared with standard depolarizing cardioplegia.[21] The use of lidocaine in DNC is unique because the half-life of lidocaine is longer than that of procaine, which is used in other cardioplegia solutions. This is the theoretical background for the theory that a single-dose infusion of DNC is acceptable, even with prolonged ischemic time, compared with other cardioplegias.[22] The original delivery protocol described that subsequent doses might not be required until 3 hours after the initial infusion.[11] A previous study involving congenital heart surgery presented favorable results of a single-dose DNC with ACC times >2 hours.[23] Another study showed superior myocardial protection with a single-dose DNC strategy compared with a multidose DNC strategy in an aged rat heart model in terms of cardiac functional recovery after reperfusion.[24] However, these studies had limitations, such as small numbers of patients with only clinical outcomes[23] and <90 minutes of ischemic time.[24] In contrast, a recent randomized study enrolling 59 pediatric patients suggested that DNC should be infused at <90 minutes intervals because the use of DNC was associated with a worse anti-inflammatory cytokine response after surgery compared with the use of St Thomas

Hospital cardioplegia solution.[25]

Recently, many centers have applied DNC in adult cardiac surgery, and redosing of DNC is usually performed at 90 minutes after the initial infusion following experts' opinions.[12,13] However, studies evaluating myocardial ischemic injury beyond 90 minutes of ischemic time after a single-dose infusion of DNC in the adult human heart may be difficult to conduct due to ethical issues. Therefore, this study was designed to evaluate extracted human hearts from heart transplant recipients.

A previous study reported functional, ultrastructural, and biochemical criteria for the severity of myocardial ischemic injury using a canine heart model[17]; the canine myocardium showed slight, moderate, severe, and irreversible myocardial dysfunction after 15, 30, 60, and 90 minutes of global ischemia. The authors observed the ultrastructure of the myocardium at each degree of ischemic injury and confirmed a correlation between the degree of myocardial dysfunction and the characteristic morphologies of the mitochondria and nuclei. Another study[19] also proposed similar criteria for irreversible lethal myocardial ischemic injury; This grading method using an electron microscope has been used to compare the effectiveness of various cardioplegia solutions in several studies.[15,16,26-28] The present study also evaluated the grade of ischemic injury following these studies. This ultrastructural evaluation is the most objective and reproducible method to evaluate ischemic injury of the myocardium because the changes in the level of myocyte organelles precede the changes in the level of light microscopy and gross inspection.[29]

Although no studies have reported a correlation between ultrastructural changes and the global function of the human myocardium, 1 previous study[17] demonstrated

correlations between ischemic injury scores and ventricular pressure recovery after 50 minutes of reperfusion in the canine myocardium. Canine myocardium with slight ischemic injury had 79% of normal ventricular pressure after reperfusion. However, the canine myocardium showing moderate ischemic injury recovered only 58% of normal ventricular pressure after reperfusion. Because the present study showed an average ischemic injury score of 0.48 at 90 minutes and irreversible ischemic injury started to occur 90 minutes after infusion of DNC, the myocardium may be safe from ischemic injury until 90 minutes after the initial infusion.

The results of the present study showed that the ischemic injury score of mitochondria correlated well with ischemic time. Most mitochondria were normal at the time of extraction, and ischemic injury progressed gradually over time. The inter-observer variability for mitochondria demonstrated fair agreement because the mean difference was only 0.06, and the 95% limits of agreement were <0.5. In contrast, nuclei showed a relatively weaker correlation with ischemic time. Most nuclei showed moderate to severe ischemic injury even at the time of extraction. The mean difference and 95% limits of agreement in the inter-observer variability analysis for nuclei were also greater than those for mitochondria. Some possible explanations are as follows. First, in contrast to the distinct stepwise criteria for mitochondria, including matrix clearing with slight injury, cristae fragmentation with moderate injury, and amorphous matrix densities with irreversible injury, changes in nuclei are continuous and ambiguous. This vague criterion may have resulted in a more advanced ischemic injury estimate at the time of extraction and greater variation of the ischemic injury score for nuclei than for mitochondria between the 2 observers. Second, whereas mitochondria are abundant in

myocardial cells, nuclei are rare. Only a median of 5.5 nuclei could be observed in each sample in the present study. Such a small number may have led to bias and overestimation of the ischemic injury early after extraction.

Study Limitations

Several limitations should be noted in the present study. First, the pathophysiology of diseased hearts in end-stage heart failure might be different from that of diseased hearts requiring corrective cardiac surgery. Although the previous electron microscopy study revealed the decrease of myofilament, the increase of fibrosis, and alterations of the cytoskeleton in the heart with end-stage dilated cardiomyopathy[30], the changes of mitochondria and nuclei in the ischemic injury of the heart with dilated cardiomyopathy have not been reported. At least, most of the mitochondria and nuclei in the present study showed normal morphology at the time of extraction. Second, the influence of reperfusion after releasing the ACC could not be evaluated due to the design of the present study. Third, the number of study subjects could not be determined from power calculation as described previously. Fourth, comparative analyses of the results in the present study with results for explanted hearts that do not receive DNC or receive a second dose of DNC after 45 minutes or 90 minutes of ischemic time might be needed to draw a definitive conclusion on this issue. Fifth, the myocardial temperature was maintained evenly at 18℃ with a normal saline bath. This is lower than that maintained in previous clinical and animal studies, where the myocardial temperature was maintained around 23°C to 25°C and 21.7°C, respectively.[31, 32] Rewarming of myocardium could occur in the clinical setting due

to various heat sources such as the light of the operating room, venous return, and the adjacent organs. Therefore, there is a possibility that the progression of ischemic injury in actual cardiac surgery could be more rapid than the results in the present study. Sixth, although previous studies showed a correlation between the degree of mitochondrial injury and irreversible myocardial dysfunction, this relationship was not evaluated in the present study.

CONCLUSIONS

Myocardial ischemic injury progresses gradually, and irreversible ischemic injury begins to occur 90 minutes after the initial infusion of DNC in the adult human heart. Therefore, the adult human myocardium may be safe from ischemic injury until 90 minutes after the single-dose DNC infusion.

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국 문 초 록

사람 심장에서 del Nido 심정지액 주입 이후 심근 허혈성 손상의 진행에 대한 초미세구조 관찰

정 준 철

의학과 흉부외과학 전공

서울대학교 대학원

목적: 성인 심장 수술에서 del Nido 심정지액의 1회 용량 주입 후 추가 용량 주입 없이 안전하게 심장이 회복될 수 있는 허혈시간에 대해서는 아직 명확히 확립된 바가 없다. 이 연구에서는 투과전자현미경을 이용하여 성인 심장에서 del Nido 심정지액의 1회 용량 주입 후 허혈성 심근 손상의 진행 정도를 평가함으로써 안전한 허혈시간을 확립하고자 한다.

방법: 심장 이식을 받은 성인 환자 7명의 심장을 적출하기 전에 del Nido 심정지액 1회 용량을 주입한 후 심장을 적출하여 분석하였다.

좌심실 심근 조직 표본을 30분 간격으로 180분간 채취하였다. 미토콘드리아와 세포핵의 허혈성 손상을 전자현미경으로 관찰하여 0점에서 3점으로 표현하였다 (0 = 정상, 0.5 = 경미한, 1 = 중간의, 2 = 심한, 3 = 비가역적).

결과: 심장 적출 직후 시점에는 83.5%의 미토콘드리아가 정상이었다. 중간 손상의 미토콘드리아의 비율은 적출 직후 1.4%에서 180분에 52.5%로 점진적으로 증가하였다. 90분부터 180분까지 심한 손상과 비가역적 손상의 미토콘드리아의 비율은 각각 0.8%에서 4.4%, 0.3%에서 1.3%로 증가하였다. 미토콘드리아의 평균 허혈성 손상 점수와 허혈시간 사이에는 유의미한 선형적 관계가 확인되었다 ($P < .001$). 대부분의 세포핵은 모든 시점에서 중간 혹은 심한 허혈성 손상을 보였다 (61.0%-85.2%). 세포핵의 평균 허혈성 손상 점수와 허혈시간 사이에서도 역시 유의미한 선형적 관계가 확인되었다 ($P < .001$).

결론: 심근의 허혈성 손상은 점진적으로 진행하며, 비가역적 허혈성 손상은 del Nido 심정지액 주입 후 90분 후부터 발생하기 시작하였다. 그러므로, 성인 심장 수술에서 del Nido 심정지액의 1회 주입 후 90분까지는 추가 용량 주입 없이 심근 허혈성 손상으로부터 안전하다고 판단된다.

주요어 : del Nido 심정지액, 허혈성 심근 손상, 투과전자현미경

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