Cardiovasc Surg Int 2023;10(2):125-133
http://dx.doi.org/DOI: 10.5606/e-cvsi.2023.1538
www.e-cvsi.org

Cardiovascular Surgery and Interventions

Original Article Open Access

Comparison of the cardioprotective effects of St. Thomas and del Nido cardioplegia

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Received: May 09, 2023 ***Accepted:*** June 16, 2023 ***Published online:*** July 21, 2023

ABSTRACT

Objectives: This study aimed to histopathologically examine the cardioprotective effects of St. Thomas and del Nido (DN) cardioplegia.

Materials and methods: A total of 18 rabbits aged 23 weeks and weighing 2000 g were included in the experimental animal study. The animals were randomized to three groups, with six rabbits in each group. The first group was determined as the control group and no cardioprotective agent was given after ligation of the aorta. The rabbits in the second group received DN cardioplegia solution, and those in the third group received the St. Thomas cardioplegia solution. The groups were histopathologically graded and evaluated with six different scores.

Results: There were statistically significant differences between St. Thomas, DN, and control groups with hematoxylin and eosin, caspase 3 and connexin 43 staining at 30, 60, and 90 min ($p<0.05$). However, the St. Thomas, DN, and control groups showed equal score 2- and score 3-weighted, score 3-weighted, and score 3-weighted distributions with connexin 43 at 90 min, respectively; there was no statistically significant difference between the groups ($p=0.144$).

Conclusion: The most adverse tissue damage observed were localized hemorrhage and localized necrosis areas at the end of 90 min of cellular damage. Both cardioplegia applications significantly reduced tissue loss compared to the control group. However, we believe that DN cardioplegia has a longer application time and has better protection.

Keywords: Cardiac protection, cardiac surgery, cardioplegia, del Nido, St. Thomas.

Cardiovascular diseases are among the leading causes of death. The treatment of some of these diseases has only a surgical option. Open heart surgery was introduced in the 1950s. With the discovery of heparin, surgeries were accelerated, and they became more common with the development of the heart-lung machine. Solutions used to stop the heart during open heart surgery are called cardioplegia solutions. There are many types of cardioplegia solutions based on their content. The content and cardioprotective effect of each solution is different.[1]

The content of del Nido (DN) cardioplegia is 1000 mL liquid containing magnesium chloride $6H_2O$ 0.030%, potassium chloride 0.037%, sodium acetate $3H_2O$ 0.37%, sodium chlorid 0.53%, sodium gluconate 0.5%, potassium phosphate monobasic 0.00082%, sodium phosphate dibasic 0.012%, 20% mannitol 17 mL, 15% magnesium sulfate 14 mL, 8.4% (1 mEq/L) sodium bicarbonate 13 mL, 7.5% (1 mEq/L) potassium chloride 26 mL, and 2% lidocaine 6.5 mL. The dose can be given as 20 mL/kg. The cardioprotective effect of the DN solution is longer (90 to 120 min), and it provides better protection. The content of St. Thomas cardioplegia is 110 mEq/L sodium, 16 mEq/L potassium, 2.4 mEq/L calcium, and magnesium. Its duration of action is shorter, and it should be repeated every 20 to 30 min.[2]

Cardiomyocyte death occurs by necrosis or apoptosis. *In vivo* animal studies revealed that caspase 3 and membrane attack complex are among the earliest markers of cell death.[3] Caspase 3 protease degrades several proteins important for cell death signaling (intrinsic or extrinsic apoptosis).[3]

Functional markers are important for normal cardiomyocyte function or maintenance of cardiac

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Citation:
Çayır MÇ, Kaya AD, Alşalaldeh M. Comparison of the cardioprotective effects of St. Thomas and del Nido cardioplegia. Cardiovasc Surg Int 2023;10(2):125-133. doi: 10.5606/e-cvsi.2023.1538.

tissue structural integrity. The best-known functional markers include myoglobin, troponins, creatine kinase, and various cytoskeletal proteins. Connexin 43 (Cx43) is the predominant link in the cardiac cavity junction channels. These channels provide intercellular exchange with ions and small regulatory molecules. Thus, it provides electrical propagation between cardiomyocytes and synchronized heart contractions. Minor Cx43 phosphorylation differences (one of the changes associated with ischemia-induced dissociation) are evident 15 min after the onset of ischemia.[3]

Inflammation is activated to clear the damaged myocardium. Damaged cardiomyocytes diffuse into the extracellular space with the damage-associated molecular pattern, also known as alarm or distress signals. Recent inflammatory markers include S100A8/9, also known as MRP8/14 and calprotectin, interleukin (IL)-6, IL-1β, IL-1α, IL-1 receptor-activated kinase 1, chemokine ligand 2, also known as macrophage inflammatory protein 2, and chemokine ligand 2, also known as MCP-1. Among the earliest *in vivo* inflammatory changes investigated in the human myocardium are S100A8/9, IL-6, and IL-1β.[3]

The histopathological effect of both methods on the heart has not been demonstrated. Hence, this study aimed to histopathologically examine the cardioprotective effects of St. Thomas and DN cardioplegia.

MATERIALS AND METHODS

A total of 18 rabbits aged 23 weeks and weighing 2000 g were included in the experimental animal study. The animals were randomized to three groups, with six rabbits in each group. Standard feed and water were given to the animals that were kept at room temperature (20 to 25°C) with a 12-h dark/light cycle until the experiments.

The first group was determined as the control group and no cardioprotective agent was given after ligation of the aorta. The rabbits in the second group received DN cardioplegia solution, and those in the third group received the St. Thomas cardioplegia solution.

After the protocol, each rabbit was given intraperitoneal anesthesia with a mixture of 10% ketamine 90 mg/kg and 2% xylazine 15 mg/kg. Cold cardioplegia was used in every group (4°C). The dose of cardioplegia was 20 mL/kg for every group. Cardioplegia was applied in 3 to 5 min. In the St. Thomas group, cardioplegia was given every 20 min till the end time (90 min) and once in the DN group. External cold materials were not utilized after applying cardioplegia. After the measurement of body weight, the rabbits were sacrificed by exsanguination. Samples were taken after cardiac arrest since our main goal in this study was to calculate the cardioprotective effect of the cardioplegia solutions. Reperfusion was not evaluated. Heart tissue samples were collected as 30, 60, and 90 min and sent to the histology and embryology laboratory within %10 formaldehyde. Figure 1 demonstrates tissue collection from the animals.

Histological evaluation of heart tissues

Tissue samples collected from all groups were kept in %10 formaldehyde. At the end of 72 h, a histological tissue treatment method was applied to these tissues, and after paraffin blocking, 5 µm serial sections were taken for the histological evaluation of the heart tissue. The sections were stained with hematoxylin and eosin (H&E). The images were examined with the Olympus BX-51 light microscope (Olympus, Tokyo, Japan) and Olympus PP72 Digital Camera (Olympus, Tokyo, Japan) and recorded and scored histologically. The groups were histopathologically graded using the evaluation method of Zhu et al.[4] with six different scores: score 0, natural histopathological appearance; score 1, patchy eosinophilic changes; score 2, localized hemorrhage with localized eosinophilic changes; score 3, localized areas of necrosis; score 4, diffuse hemorrhage with diffuse eosinophilic changes; score 5, diffuse liquefaction necrosis.

Statistical analysis

Data obtained in this study was analyzed using the IBM SPSS version 25.0 (IBM Corp., Armonk, NY, USA) software. The chi-square test was used for the comparison of categorical variables between the groups. A p-value <0.05 was considered statistically significant.

RESULTS

When the scoring of H&E staining according to time was examined, it was observed that the St. Thomas, DN, and control groups showed score

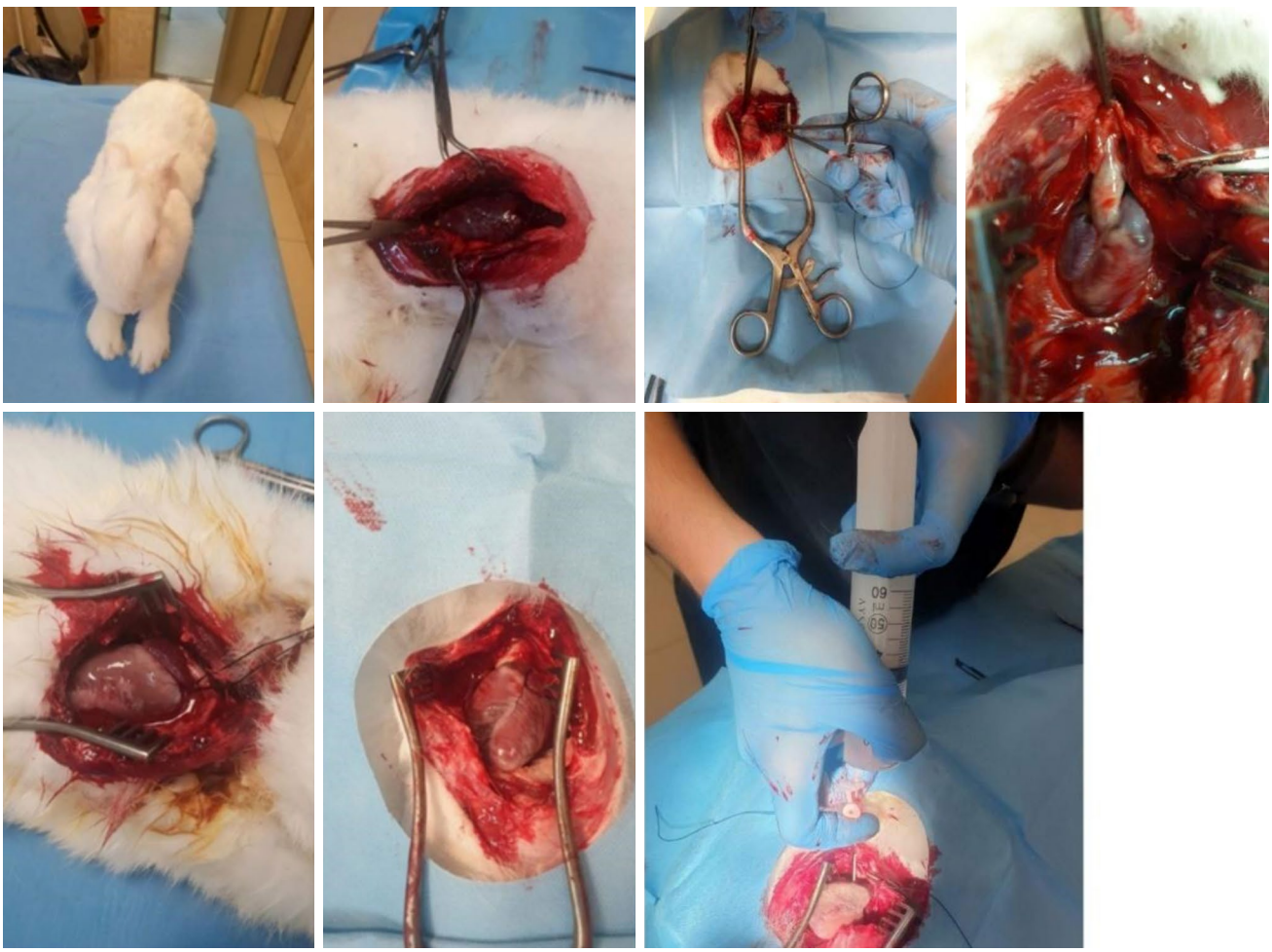

Figure 1. Sample collection from the experimental animals.

1-weighted, score 0-weighted, and score 2-weighted distributions at 30 min, respectively; there was a statistically significant difference between the groups (p<0.001). The St. Thomas, DN, and control groups showed score 1-weighted, score 0- and score 1-weighted, and score 2-weighted distributions at 60 min, respectively; there was a statistically significant difference between the groups (p=0.005). The St. Thomas, DN, and control groups showed score 2-weighted, score 1-weighted, and equal score 2- and score 3-weighted distribution at 90 min, respectively; there was a statistically significant difference between the groups (p=0.009, Table 1). Figure 2 displays time-related score changes according to H&E staining.

When the scoring of caspase 3 staining according to time was examined, it was observed that the St. Thomas, DN, and control groups showed equal score 1-and score 2-weighted, score 1-weighted, and score 3-weighted distributions at 30 min, respectively; there was a statistically significant difference between the groups (p=0.001). The St. Thomas, DN, and control groups showed score 3-weighted, equal score 2-and score 3-weighted, and score 3-weighted distributions at 60 min, respectively; there was a statistically significant difference between the groups (p<0.001). The St. Thomas, DN, and control groups showed score 3-weighted, score 2-weighted, and score 3-weighted distributions at 90 min, respectively; there was a statistically significant difference between the groups (p=0.010, Table 2). Figure 3 demonstrates time-related score changes according to caspase 3 staining.

When the scoring of Cx43 staining according to time was examined, it was observed that the

Table 1
Scoring of H&E staining by time

	St. Thomas	Del Nido	Control	*p*
30th min.				
Score 0	1	6	0	<0.001
Score 1	5	0	1	
Score 2	0	0	5	
Score 3	0	0	0	
60th min.				
Score 0	0	3	0	0.005
Score 1	3	3	1	
Score 2	3	0	4	
Score 3	0	0	0	
90th min.				
Score 0	0	1	0	0.009
Score 1	1	4	0	
Score 2	3	1	3	
Score 3	2	0	3	

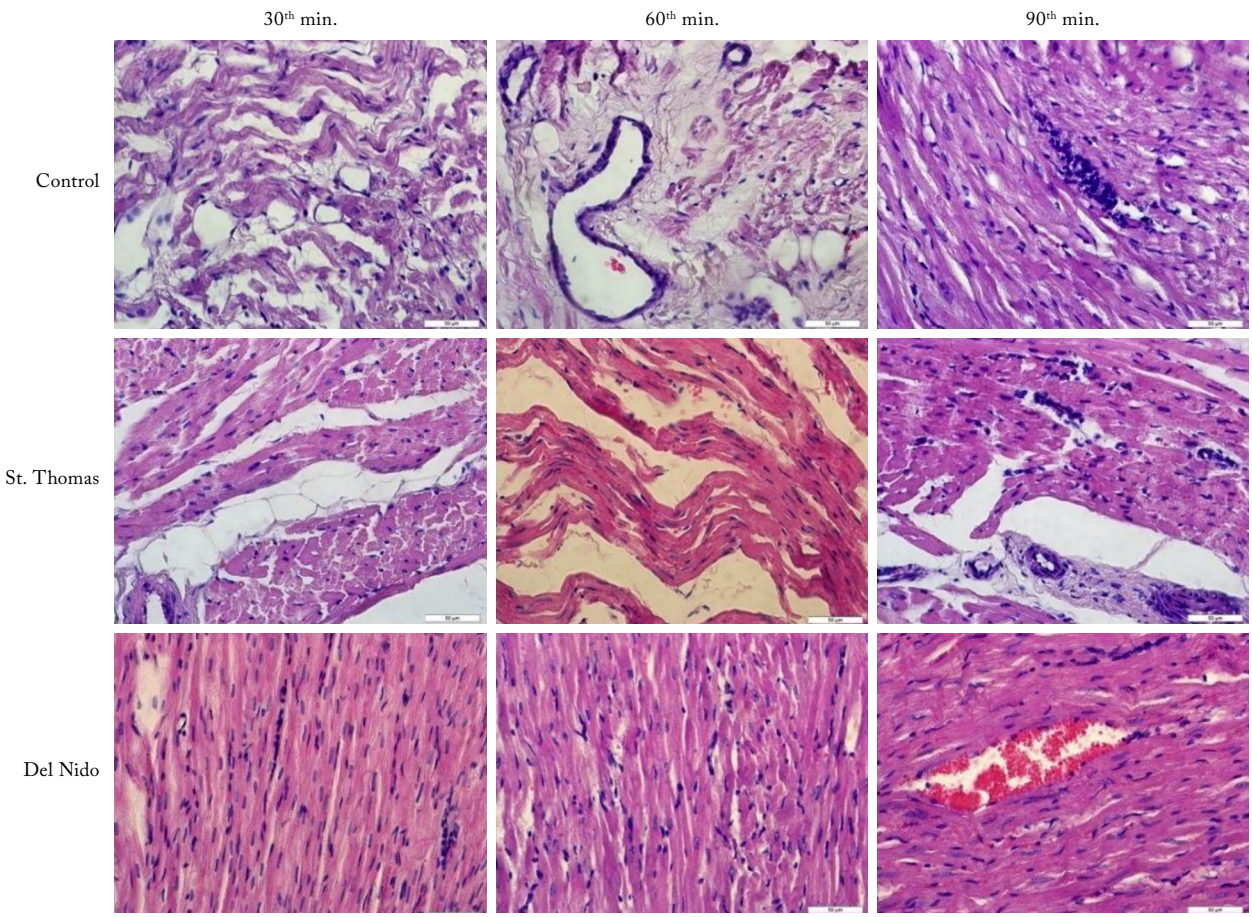

Figure 2. Time-related score changes according to H&E staining (50 μm).

Table 2
Scoring of caspase 3 staining by time

	St. Thomas	Del Nido	Control	*p*
30th min.				
Score 1	3	4	0	
Score 2	3	2	0	0.001
Score 3	0	0	6	
60th min.				
Score 1	0	3	0	
Score 2	2	3	0	<0.001
Score 3	4	0	6	
90th min.				
Score 1	0	1	0	
Score 2	2	5	0	0.010
Score 3	4	0	6	

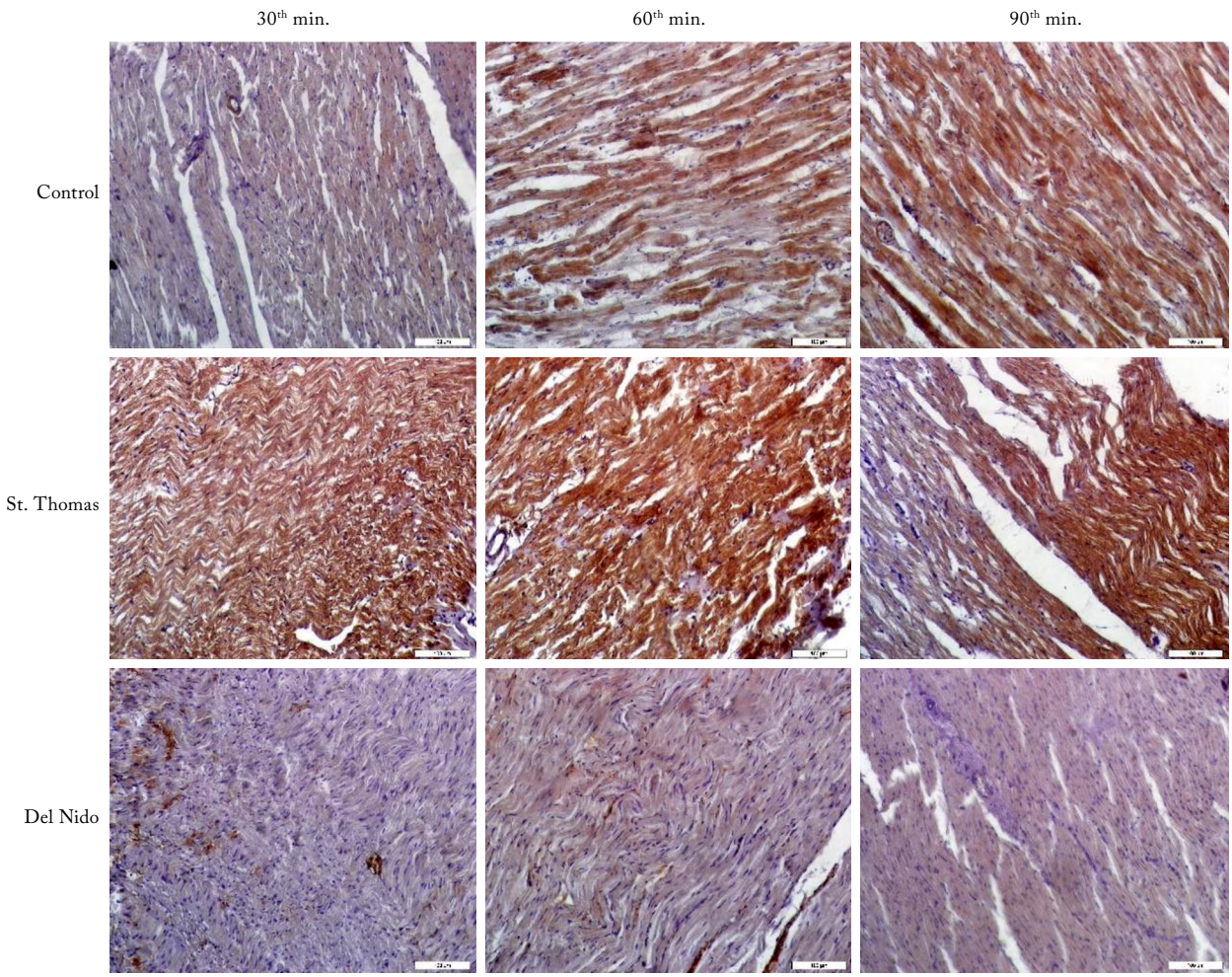

Figure 3. Time-related score changes according to caspase 3 staining.

Table 3
Scoring of C×43 staining by time

	St. Thomas	Del Nido	Control	*p*
30th min.				
Score 1	2	5	0	
Score 2	4	1	6	0.024
Score 3	0	0	0	
60th min.				
Score 1	1	1	0	
Score 2	4	5	2	0.033
Score 3	1	0	4	
90th min.				
Score 1	0	0	0	
Score 2	1	2	0	0.144
Score 3	5	4	6	

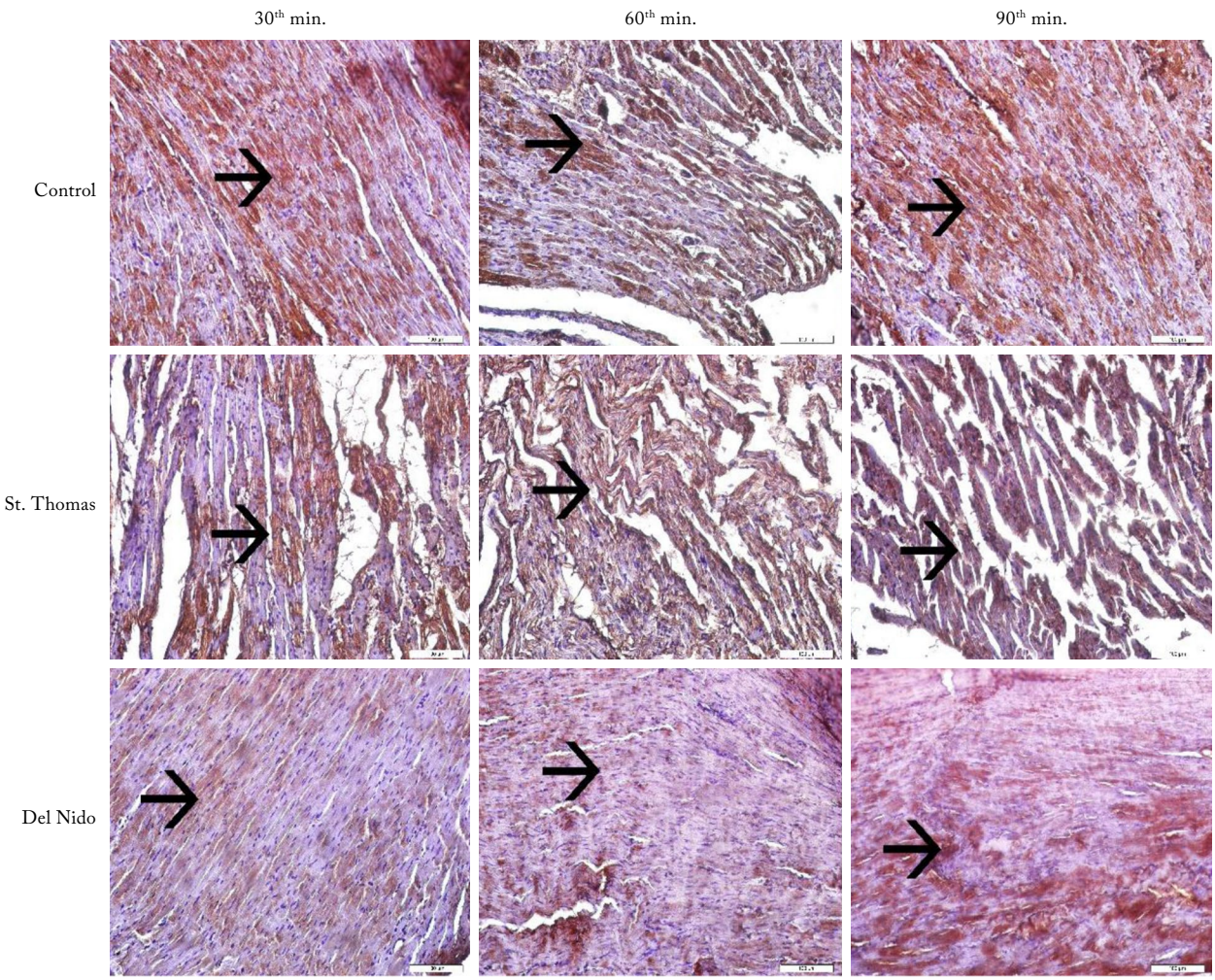

Figure 4. Time-related score changes according to C×43 staining.

St. Thomas, DN, and control groups showed score 2-weighted, score 1-weighted, and score 2-weighted distributions at 30 min, respectively; there was a statistically significant difference between the groups (p=0.024). The St. Thomas, DN, and control groups showed score 2-weighted, score 2-weighted, and score 3-weighted distributions at 60 min, respectively; there was a statistically significant difference between the groups (p=0.033). The St. Thomas, DN, and control groups showed equal score 2-and score 3-weighted, score 3-weighted, and score 3-weighted distributions at 30 min, respectively; there was no statistically significant difference between the groups (p=0.144, Table 3). Figure 4 exhibits time-related score changes according to Cx43 staining.

DISCUSSION

In this study, the cardioprotective effects of St. Thomas and DN cardioplegia, two different cardioplegia solutions used in cardiac surgery, were compared by a histological examination performed on tissues taken from rabbits to observe cellular damage. The DN cardioplegia solution, often used with blood in the research of cardioplegia solutions, was developed in the 1980s to obtain an effective single dose cardioplegia in pediatric cardiac surgery.[1,5] The effect of DN cardioplegia, which has recently been used in adult surgery, on cellular processes has not been experimentally determined.[1,5] In this study, we evaluated St. Thomas and DN cardioplegia on a cellular basis with caspase 3, Cx43, and H&E staining, which measures the pH value change due to infiltration in the cell cytoplasm, and evaluated their cellular protection and tissue damage in cardiac surgery in a 90-min period.

Enzymes in the extracellular matrix are activated by oxidative mediators and participate in cell damage processes. Therefore, it is important to evaluate these processes for the tissue to be transplanted.[6,7] In this study, scoring was made semiquantitatively based on the prevalence (0: 0-25%; 1: 26-50%; 2: 51-75%; 3: 76-100%) and severity (0: none; 1: mild; 2: moderate; 3: severe) of staining immunoreactivity. The total staining score was obtained by calculating the severity in prevalence.[8]

In a study using DN cardioplegia, both mitochondrial damage and foreign cell formation were evaluated using H&E staining in tissues after ischemia/reperfusion, and it was reported that cold ischemia can be safely performed using DN cardioplegia.[9] According to the analysis of H&E staining in our study, bacterial, parasitic, or fungal tissue formation in the tissues treated with cardioplegia was more prominent in the control group, while it was at a very low level in the DN group. In addition to intracellular protection, the protection of the St. Thomas group was at an acceptable level in the formation of extracellular bacteria, parasites, or fungal tissue.

Caspase immunoreactivity observed in the cardiomyocyte cytoplasm was observed to be intense in the control group. On the other hand, DN application was found to decrease caspase 3 immunoreactivity in cardiomyocytes. In the St. Thomas group, although caspase 3 immunoreactivity in cardiomyocytes was significantly lower than in the control group, it was quite intense compared to DN application. In the examination of caspase 3 activity, we found that the cardioplegia used in both groups was protective on a cellular basis compared to the control group, but the best protection was achieved with the DN group. According to the evaluation criteria of caspase 3, we believe that the protective feature between cardioplegia may be more prominent in longer measurements. However, our results in this study were similar to those in the literature.[10-12]

In our study, when the heart tissue at 30 min in the DN group was evaluated, it was found that the structure of the cells was preserved, and the muscle fibers had a normal appearance. Although local lymphocyte infiltration was observed, the cardiac muscle appeared normal when all groups were evaluated. In the heart tissue at 60 min, leukocyte infiltration was observed around the vessel in places, and although some areas appeared edematous, the myocardium preserved its normal histological appearance. In the 90th min, leukocyte infiltration around the vessel in the myocardial tissue, areas of hemorrhage, albeit very rarely, and areas with necrosis were observed in the DN group. Interstitial edema and local eosinophilic changes have been detected in some rabbits. In our study, caspase 3 and Cx43 half-life and the possibility of ischemia started to become evident at 60 min, and our results were longer than the reported half-life in previously published studies with adult patients.[13,14]

Ota et al.[15] have found a single dose of cardioplegia was administered to 70% of the patients in the

DN group, and the rates reported in adult studies conducted in different centers[16] range from 40 to 84%. Less frequent dosing ensures uninterrupted surgeon operation and reduces the risk of contamination. These advantages of DN may facilitate myocardial protection during adult cardiac surgery.

In this study, it was observed that serum and tissue caspase 3 activities increased in ischemic tissue in the 90th min following myocardial reperfusion injury as an indicator of increased apoptosis. However, we believe that this protection is likely to decrease in cases where the reperfusion time is longer.

The limitation of the study was that the hearts of the rabbits could not be reperfused since there was no tube set for the rabbits.

In conclusion, the most adverse tissue damage observed were localized hemorrhage and localized necrosis areas at the end of 90 min of cellular damage. Both cardioplegia applications significantly reduced tissue loss compared to the control group. Nonetheless, we believe that DN cardioplegia has a longer application time and better protection, and our study supported that by showing histopathological markers. Hence, DN cardioplegia is a safe method for adult cardiac surgery.

Ethics Committee Approval: The study protocol was approved by the Pamukkale University Ethics Committee (date: 23.06.2022, no: PAUHDEK-2021/52). The study was conducted in the Pamukkale University Experimental Animals Laboratory in accordance with the relevant ethical principles of the Declaration of Helsinki.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: All authors contributed equally to the article.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

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