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ORIGINAL ARTICLE

Evaluation the effects of N-acetyl cysteine on pericardial adhesions in experimental rabbit models

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Abstract Adhesions are one of the main causes of re-sternotomy complications. In this study, we evaluated the potential of N-acetyl cysteine irrigation in reducing or preventing pericardial adhesions after cardiac surgery in rabbits. Thirty rabbits were allocated randomly into two groups. Group C served as the control, and group N was the treatment group. After anaesthesia, sternotomy and opening a pericardial flap, the pericardial cavity was irrigated with 1 ml/kg saline (group C) or with 100 mg/kg of N-acetyl cysteine (group N). After 2 months, the sternum was re-opened and the site of operation evaluated for macroscopic and microscopic changes. Macroscopic evaluation revealed 40 % (n=6) with grade 1, 46 % (n=7) with grade 2, and 13.3 % (n=3) with grade 3 adhesions in group C. In group N, there were 60 % (n=9) with grade 0 (no adhesions) and 40 % (n=6) with grade 1 adhesions. N-acetyl cysteine irrigation appears to significantly reduce macroscopic adhesions, and there was also a trend towards a reduced reaction microscopically; therefore, further studies are warranted.

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Introduction

Most patients with congenital heart defects require repeat surgeries, and in these cases, the re-surgery risks are well known (Macmanus et al. 1975; Dobell and Jain 1984, Jacobs et al. 1996); pericardial and mediastinal adhesion can cause damage to the right ventricle, aorta, right atrium, and any aortocoronary bypass grafts (Siga and Takai 2004).

The incidence of re-sternotomy cardiac surgeries has increased in recent years, and the resulting complications are among the main causes of mortality in these cases. The main causes of re-sternotomy mortality include cardiac rupture during sternal bone opening and excess bleeding during lysis of pericardial adhesions (Siga and Takai 2004). Despite precautions, including preparing arterial and venous access for rapid institution of cardiopulmonary bypass if needed, using a method to open the sternum that provides secure control and careful dissection of adhesions, catastrophic situations can develop.

In an effort to reduce adhesions, it is common to make sure that all points of minor blood loss are addressed and to irrigate the pericardial cavity thoroughly with copious amounts of warm saline before closing. Other methods to reduce adhesions include closing the pericardium, use of special gloves and use of substances (Vander et al. 1988) such as steroids, hyaluronic acid, BioGlue (Yuji et al. 2008) and carboxymethyl chitosan (Kara et al. 2001). The drawback is that some of these substances are expensive and sometimes not available.

N-acetyl cysteine was first used as an antidote for paracetamol intoxication. Its other uses are as mucolytic, in combination therapy for the treatment of cystic fibrosis, aspergillosis, leishmaniosis (Khuri et al. 2010) and HIV infections. It

has also been effective in treating renal failure and especially as prophylaxis in preventing dye-induced nephropathy (Anderson et al. 2011), as well as being used in hepatic failure, myocardial infarction and in cancer adjuvant therapy. The advantages of N-acetyl cysteine are its anti-inflammatory effects, its comparative safety, its safe use as an adjuvant in cardioplegia solution (Sakuma et al. 2005) and the fact that it is a relatively inexpensive, easy to obtain, multipurpose drug.

In this study, we examined the effects of N-acetyl cysteine on ameliorating the effect of pericardial adhesions to the heart after the first sternotomy in experimental surgeries on rabbits.

Material and methods

Thirty male adult white New Zealand rabbits with weight of 1.5–2 kg were selected and, 1 week before the procedure, individually housed in cages in the Science and Research Laboratories at the University and kept under standard conditions—fed standard laboratory rabbit food with water ad libitum and a 12-h light-dark cycle. The study was approved by the Animal Research Ethics Committee of Isfahan University.

Animals were allocated randomly into two experimental groups. Group C served as the control, and group N received treatment with N-acetyl cysteine. All protocols, including the Helsinki Accords, for humane treatment of animals were followed. The ears were tagged C1 to C15 or N1 to N15, and cages were labelled to correspond to group and protocol number.

Intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg) was given for the induction of anaesthesia. Animals were then intubated and anaesthesia maintained using isoflurane (1 ppm), and prior to surgery, ceftriaxone (15 mg/kg) was infused.

The thorax and abdomen were shaved, prepped and draped, and a skin incision was made with a no. 15 blade. The sternum was opened with sharp straight scissors. In all animals, a 0.5× 0.5-cm flap of the pericardium was opened and the surface of the pericardium and underlying epicardium was abraded five times with a piece of gauze. In the control group, 1 ml/kg of 0.9 % saline was used to irrigate the pericardial cavity. In group N, 100 mg/kg N-acetyl cysteine (Sandoz Company, 200 mg/ml, 10 ml ampoules) was used to irrigate the pericardial cavity. The pericardium was then closed with three 6/0 Prolene (Ethicon Co., NJ) simple sutures.

The sternum, subcutaneous tissue and skin were closed with 2/0 and 3/0 nylon mattress sutures. Rabbits were then transferred back to their cages after regaining full consciousness. Ceftriaxone (15 mg/kg) was injected twice a day for 48 h post-operatively.

After 60 days, the animals were prepared in the same manner as the first procedures and re-sternotomy was performed. The initial surgery site was then evaluated for adhesions of the pericardial flap to the epicardium.

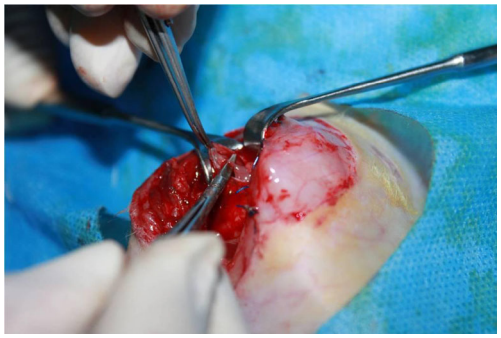

Fig. 1 Macroscopic adhesion of the pericardium to the heart. There is a loose adhesion between the heart and pericardium which lyses easily

Macroscopic adhesions of the pericardium to the heart were classified from grade 0 to grade 4 as follows: grade 0—no adhesions and no fibrin on the surface of the heart; grade 1—no adhesions but some fibrin on the epicardium; grade 2—loose adhesions between the heart and pericardium which are easily lysed; grade 3—adhesions between the heart and pericardium which need careful dissection with a sharp instrument; and grade 4—strong adhesions between the heart and pericardium which cannot be dissected.

A piece of pericardium, from each animal, was sent to an animal pathologist for histopathological evaluation to assess cellular changes at the site of pericardial flap opening. Microscopic changes were graded I to IV as follows: grade I, the inflammatory phase, inflammation plus neutrophils and macrophages; grade II, proliferative phase, granulation tissue, fibrin casts plus collagen formation; grade III, organization phase, fibroblast proliferation plus collagen formation and fibrosis; and grade IV, scar formation, complete fibrosis and collagen formation.

The Mann-Whitney test was used for statistical analysis, and in all cases, a P value equal to or less than 0.05 was considered significant.

Results

Two rabbits died in the first operation during induction of anaesthesia and were replaced. No animals died following the

Table 1 Results of macroscopic difference in two groups

	Gross finding				Total
	Grade 0	Grade 1	Grade 2	Grade 3	
Group NAC	9	6	0	0	15
	60 %	40 %	0 %	0 %	100 %
Control	0	6	7	2	15
	0 %	40 %	46.7 %	13.3 %	100 %
Total	9	12	7	2	30
	30 %	40 %	23.3 %	6.7 %	100 %

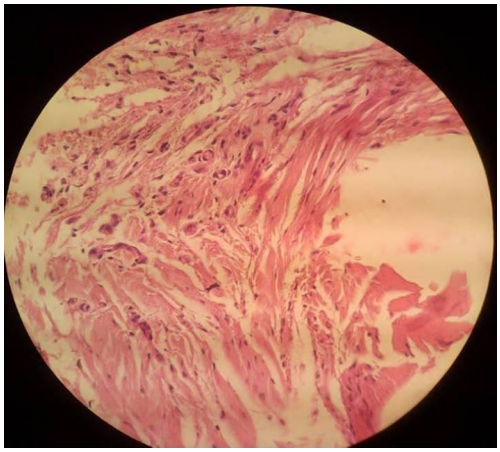

Fig. 2 Photomicrograph under light microscopy. Tissue showing grade III with fibroblast proliferation plus collagen formation and fibrosis (magnification of 4×10, H&E staining)

operative procedure. Two months following the initial procedure, macroscopic analysis of the control group showed no rabbits with grade 0 adhesions, six rabbits (40 %) with grade 1, seven (46 %) with grade 2, and two (13.3 %) with grade 3. Grade 4 adhesions were not detected (Fig. 1).

At the same time point, in group N, there were nine rabbits with grade 0 macroscopic adhesions and six (40 %) with grade 1. Grades 2, 3 and 4 were not detected in group N (Table 1).

To conduct histopathological studies, a piece of the pericardial flap from each animal was resected and sent to the pathologist for grading of pathological changes. In the control group, three rabbits (20 %) were grade I, five (33.3 %) grade II, four (26.6 %) grade III, and three (20 %) grade IV (Fig. 2).

In group N, five rabbits (33.3 %) were grade I, eight (53.3 %) were grade II, and two (13.3 %) were grade III (Table 2). The two groups were compared for macroscopic and histopathological changes by the Mann-Whitney test.

The macroscopic evaluation *P* value was 0.0001 and shows there is a very significant statistical difference between two groups. The *P* value for histopathological examination was less significant, at 0.075.

Table 2 Results of microscopic difference in two groups

	Pathologic finding				Total
	Grade I	Grade II	Grade III	Grade IV	
Groups NAC	5	8	2	0	15
	33.3 %	53.3 %	13.3 %	0 %	100 %
Control	3	5	4	3	15
	20 %	33.3 %	26.7 %	20 %	100 %
Total	8	13	6	3	30
	26.7 %	43.3 %	20 %	10 %	100 %

Discussion

Adhesions after open heart surgery is the greatest factor in causing longer operative and perfusion times, bleeding and injury to the heart or great vessels (Sakuma et al. 2005).

Many experimental and clinical attempts have been made to solve this problem by using different types of pericardial substitutes. The materials for these substitutes have included silicon rubber (Kara et al. 2001), polyurethane (Khuri et al. 2010), E-PTFE sheets (Sakuma et al. 2005; Laks et al. 1981; Revuelta et al. 1985), Dacron (Youmans et al. 1968; Mazuji and Lett 1963) and dura mater (Bonnabeau et al. 1973).

N-acetyl cysteine is a safe, relatively inexpensive, readily available medication (Chu et al. 2011) especially compared to other substances such as hyaluronic acid and carboxymethyl chitosan.

The statistical differences between the control and N-acetyl cysteine group for macroscopic findings following the second operation are significant. This indicates that N-acetyl cysteine can reduce macroscopic pericardial adhesions in cardiac surgeries, and therefore, re-operation on these patients would be safer, due to fewer adhesions which should result in reduced mortality and morbidity in re-sternotomy surgeries. The differences between the two groups microscopically were not significant ($P=0.075$) although there was a trend towards a reduced reaction in group N, indicating that further studies are warranted.

Drug strength, volume and dilution may be important, and electron microscopy may prove valuable. Methods to reduce the complications of re-sternotomy require further research, and it is an important approach to reducing morbidity and mortality. N-acetyl cysteine and other anti-inflammatory agents require further study.

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