The effect of intraperitoneal n-acetylcysteine on postoperative adhesions in rat models

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AIM: *In this study, we researched the effect of local administration of N-acetylcysteine (NAC) on postoperative intra-abdominal adhesion formation in the rat models.*
METHODS: *20 female Wistar Albino rats which were 5-7 months old are used for the study. The rats were divided into two equal groups. Group one was administered saline solution (n=10) while group two was administered NAC (n=10) after caecal abrasion. They were dissected on postoperative tenth day and were examined macroscopically and microscopically for the adhesion formation. Intraperitoneal adhesion formation was scored blinded with Evans model. The most adherent bowel section was excised for histopathologic examination. Mann Whitney U test were used for statistical analysis.*
RESULTS: *In Group one, all rats have had adhesions. None of the rats in Group two had either severe inflammatory cell reaction or dense interstitial fibrosis. Macroscopic adhesion formation and microscopic inflammatory cell reaction and interstitial fibrosis formation after surgery were less at the group two (NAC applied) ($p<0.05$, $p<0.05$, $p<0.05$).*
CONCLUSION: *We believe that the intraperitoneal single dose usage of NAC may be promising for decreasing the postoperative intraabdominal adhesions.*

KEY WORDS: N-acetylcysteine, Postoperative adhesion, Rat, Fibrosis

Introduction

Intraabdominal adhesions are pathological bonds formed between peritoneal surfaces during the recovery of peritoneal surface defects. These bonds may vary from a thin connective tissue band to a highly vascularized, thick and fibrous band or to a direct connection between two organ surfaces [1,2]. Intraabdominal adhesions may arise, to a greater or lesser extent, after each abdominal surgical procedure regardless of the method used. Development of intraabdominal adhesions is an important cause of mortality and morbidity which may lead to rehospitalization and reoperation. The length of stay and the risk of infection to the patients are increased by intraabdominal adhesions [3]. Although the adhesions are often asymptomatic, mortality due to adhesions was reported as 4.3-13% [4]. Although various studies were carried out in order to prevent the development of intraabdominal adhesions, no single method was considered to be sufficient on its own to prevent their development [5]. Since several medications were reported to have been used in the literature, none could be preferred because of their side effects or inaccessibility. Hence, there is an ongoing search for a new drug.

NAC is a thiol compound and a precursor in the formation of L-cysteine and glutathione (GSH), which are used for degradation of reactive oxygen radicals at antioxidant reactions [6]. NAC essentially supplies cysteine for GSH synthesis in the organism and is released into blood circulation upon conversion into glutathione in the liver [7]. Besides, NAC is able to act as an antioxidant directly.

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The studies show that NAC inhibits the free oxygen radical-induced apoptotic process and the redox potential imbalance. This activity of NAC is associated with the antioxidant and nucleophilic properties of thiol within its structure [8]. Thus, NAC can be used in the treatment of the diseases resulting from oxidative stress by its effects on oxidative stress, inflammation and angiogenesis pathways [8]. These diseases cover a wide range from ischemia/reperfusion injury [9,10] to tumorigenesis modulation [11].

Also, as it contains sulfhydryl groups, NAC inhibits construction of the newly formed collagen, formation of disulfide bonds between peptide bonds and between the collagen molecule and extracellular matrix components. In this way, it impairs the collagen's stabilization in the connective tissue [12]. We assumed that both properties of NAC, its antioxidant effect and inhibition of collagen formation, may help us to prevent intraabdominal adhesion formation.

In this study, the sterile ampoule form of N-acetylcysteine (NAC) was used by local administration (intra-peritoneally) to prevent intraabdominal adhesions in rats and we investigated its effects on the development of adhesions.

Methods

Study Setting

This experimental study was carried out in the Faculty of Medicine Experimental Research and Animal Laboratory, Ba kent University, from which the rats that were used for this study were supplied. The histopathological investigations were performed in the Department of Pathology, Faculty of Medicine in the Ba kent University. The study was approved by the Ethics Committee for Experimental Animals, Faculty of Medicine, Ba kent University (Project No.: DA13/17).

Groups and Surgical Procedure

Twenty female Wistar Albino rats aged between 5 and 7 months that weighed 160-300 g were used. All rats were preoperatively anesthetized with intra-peritoneal injection of 75 mg/kg Ketamine HCl and 7 mg/kg XylazineHCl. The subject's abdominal surface was shaved and sterilized with 10% povidone iodine antiseptic solution. The rats were placed in heated beds. The operators opened the abdomen with a 2 cm incision on the median line. The cecum was removed and put on a wet sponge. The serosal petechiae were created on the surface of the cecum by rubbing the cecum with a dry sponge. The rats were divided into two groups. After adhesion formed by cecal abrasion at group 1, physiological saline (PS) (2 cc) was applied intra-peritoneally (n = 10), at group 2 N-acetylcysteine (150 mg/kg, diluted to 2 cc by PS) was given intra-peritoneally (n = 10). Abdominal wall was closed by two continuous lines by 2/0 silk suture.

Normal oral feeding was initiated in the postoperative period. In order to achieve postoperative analgesia, 0.02 mg/kg Fentanyl was administered subcutaneously. All the subjects were sacrificed using 150 mg/kg Ketamine HCl on the postoperative Day 10 (1). The sacrificed rats were examined macroscopically and microscopically for the development of adhesions.

Macroscopic Examination

Ten days after the initial surgery, the abdomen was opened using reverse U incision and the intraabdominal adhesions were examined macroscopically according to the Evans model (Table I) [13]. Macroscopic evaluation was performed blindly by another surgeon. After that, the cecum was excised en bloc for histopathological examination.

Histopathological Examination

Cecum samples resected from the subjects were identified in 10% formaldehyde and made into a paraffin block which was then divided into 5 µm parts and stained with hematoxylin and eosin for histopathological examination. The interstitial fibrosis and inflammatory response of the cells were evaluated blindly in the histopathological examination by one pathologist.

Statistical Calculations

Continuous data were expressed as the mean ± standard deviation (SD) with the range. We used the Mann Whitney U test to compare two groups. A p value of <0.05 was considered statistically significant. All analyses were undertaken using SPSS Statistics Version 15.0 (IBM, Armonk, NY, USA).

Results

No rats died due to surgery or anesthesia. No intraabdominal infection findings or other complications such as perforation, necrosis or obstruction were detected in any of the rats. Distribution of EvansScore of groups (Fig. 1), distribution of histopathologic fibrosis of groups (Fig. 2) and distribution of cellular inflammatory response of groups (Fig. 3).

In group one, all rats have had adhesions; in three rats the adhesions were separated easily; in four rats the adhesions were separated by traction, and in three rats the

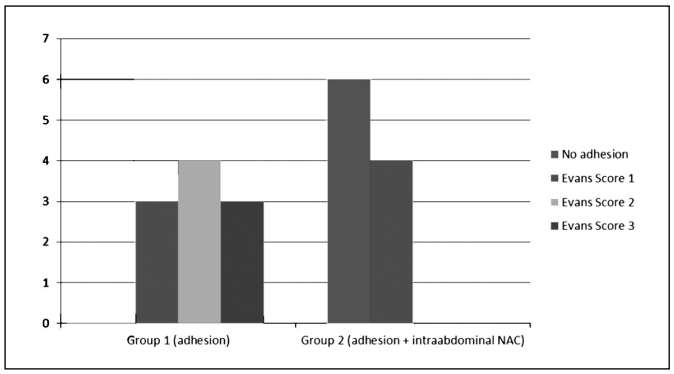

Fig. 1: EvansScores of groups.

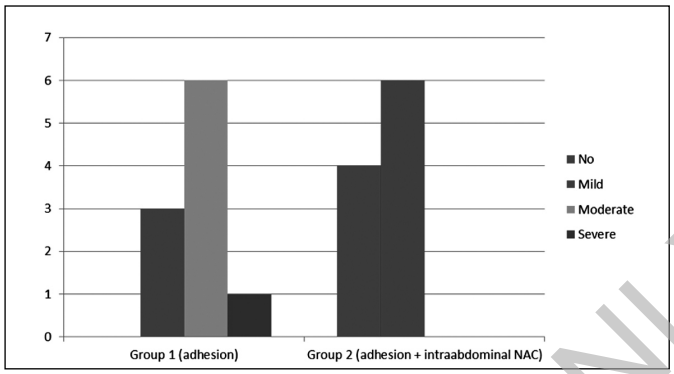

Fig. 2: Histopathologicfibrosis of groups.

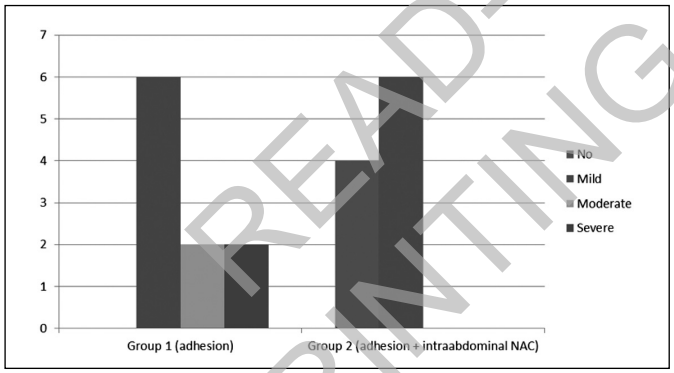

Fig. 3: Cellular inflammatoryresponse of groups (histopathologicexamination).

adhesions were needed dissection for separation. In group two, six rats had no adhesions; in four rats the adhesions were separated easily, and no rats have had heavy adhesions that needed traction or dissection for separation. In histopathologic examination, all rats in group one had interstitial fibrosis and inflammatory cell reaction. Three rats had minimal, six rats had moderate, and only one rat had dense fibrosis.In this group, six rats had mild, two rats had moderate and two rats had severe inflammatory cell reaction.

In group two, four rats had neither interstitial fibrosis nor inflammatory cell reaction. Six rats had mild inflammatory cell reaction and interstitial fibrosis. None of theratsin group two had either severeinflammatorycellreactionordenseinterstitialfibrosis.Macroscopic adhesion score, microscopic interstitial fibrosis and cellular inflammatory response were significantly lower in N-acetylcysteine group (group 2) ($p< 0.05$, $p< 0.05$, $p< 0.05$).

Discussion

Intraabdominal adhesions are very important causes of mortality and morbidity in patients undergoing abdominal surgeries [3]. Although various studies have been carried out to have an understanding of their pathogenesis and prevention, a single medication or method to be used on its own to prevent adhesion development is yet to be found today. Various studies have shown that the increase in inflammatory response and the decrease in tissue oxygenation secondary to surgery play an important role in the increment of the development of adhesions [1,8,9,12-14]. When inflammation increases, oxidative stress also increases, which interferes with mesothelial cell functions, decreases its fibrinolytic activity and cause s intraabdominal adhesions [3,9,10,12,14,15].

It was shown that the increase in hypo-oxygenation and oxygen radicals lead to an increase in postoperative intraabdominal adhesions [3]. In this case, free radicals rapidly appear following endothelial injury, react with oxygen and thus cause damage at peritoneal surface, which heals with extensive fibrosis leading to adhesions [16]. NAC is a precursor of L-cysteine and glutathione (GSH), which are used at degradation of reactive oxygen radicals at antioxidant reactions. Also, NAC significantly reduces peritoneal oxidative stress by directly affecting the membrane-associated oxidases, such as nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidase or glucose 22 oxidase [3]. Additionally, it reduces oxidative stress and inflammation by reducing oxidative burst and myeloperoxidase activity in neutrophils [3], such that NAC can directly act as an antioxidant [3,7,17]. Chu et al. in their study showed that glutathione increases with the antioxidant effect of NAC and this effect reduces the adhesions [3]. It w as also reported that in addition to antioxidants, anti-inflammatory and cellular protective effects, NAC also increases microvascular blood flow and provides endothelial protection [18]. Based on these properties, various studies were evaluated which showed the efficacy of NAC in reducing postoperative adhesions [3,13,17].

There are also data indicating that peritoneal fibrinolytic activity is closely associated with the development of adhesions [19]. Fibrin development is balanced with the fibrinolytic activity, such that when there is a lack of fibrinolytic activity, it causes stable fibrins and that may lead to adhesion. Thus, there are experiments and trials

in the literature about using fibrinolytic drugs to prevent the formation of adhesions as fibrinolytic activity decreases the adhesion formation. Animal studies have shown that these drugs succeed in reducing adhesions [20-22]. But, it is also known that they have several side effects such as bruising, bleeding and impaired wound healing [17]. Moreover, the majority of these drugs are not suitable for clinical use as they are expensive.

The enteral form of NAC increases tPA and PAI-1 mRNA levels in adhesion tissue, thus enhancingfibrinolytic activity and reducing the formation of adhesions [3]. NAC does not only increase fibrinolytic activity but also reduces fibroblast activity by inhibiting the expression of inducible nitric oxide synthase (iNOS), which results in a decrease in the formation of adhesions [12]. NAC also causes fibroblasts to stay in G1 phase and thus keep superoxide radical anions at a constant level and reduced cyclinD1 protein levels [23] so that as NAC reduces fibroblast activity and increases fibrinolytic activity, it is an ideal drug to be used to prevent the formation of adhesions. Also, NAC could be more suitable for clinical use as it is cheaper and also has less side effects.

In practice, NAC is mostly used as a mucolytic agent. Being an antioxidant and having rare side effects, it has different areas of use as well. Enteral and parenteral forms of NAC are available in the market. When administered orally and intravenously, its bioavailability in the peritoneum could not be clearly evaluated. For oral administration, peritoneal absorption of NAC was shown to be insufficient to prevent the development of adhesion [3]. It was shown that oral intake of NAC was ineffective inside the peritoneum even in the highest dose (1200 mg/kg) [3]. Chu et al. explained this result with NAC's metabolism in the liver [3]. Therefore, we thought that local administration of NAC would increase its effect on preventing adhesions. In order to achieve high peritoneal level and reduce the systemic side effects as well, the ampoule form of NAC in a single dose inside the abdomen was administered at the end of the operation. As a similar procedure, applying a sponge soaked with NAC to pericardium was shown to reduce pericardial adhesions [17]. Moreover, it is known that various antineoplastic agents are being administered intraperitoneally in order to achieve high intraperitoneal activity [24]. In the previous animal studies, 150 mg/kg NAC was injected intramuscularly for 3-14 days (12) and percutaneous intraperitoneally twice a day for 3 days [3], and it was shown to be effective. Considering the future human studies, we thought that it would be more effective to administer the parenteral form of NAC intraperitoneally at the end of the operation. By this way, NAC achieves a high peritoneal concentration directly without the pain of the intramuscular or intraperitoneal injection route. The parenteral form of NAC was preferred for this route, as it was sterile and homogenous.

The NAC was diluted with PS to standardize the volume and help the spread of drug in abdomen. As the PS was used for diluting NAC, the same volume of PS was given to a sham group such that both groups were faced with the effect of PS on peritoneal adhesions equally. The difference between the groups was due to the chemical effect of NAC only.

Adhesion models vary in the literatureforming adhesions by creating ischemic areas [3], by causing cecal abrasion, by creating parietal peritoneum defect [12] or by opening the pericardium and causing abrasion with a dry sponge [17]. Even though the methods may differ, NAC reduced adhesions at all these studies. As it is a common and easy-to-use method, we formed an adhesion model by cecal abrasion in our study.

Pata et al. in their study histopathologically evaluated the interstitial fibrosis and cellular inflammatory response to evaluate the degree of adhesions [12]. Also, Çolak et al. microscopically evaluated fibrosis and cellular inflammatory response to compare adhesions at their groups [17]. In this study, fibrosis and the number of inflammatory cells playing an active role in adhesion formation were measured and evaluated. A more significant decrease in fibrosis and inflammatory cell migration in NAC group wasalso detected than in the control group which was correlated with low levels of adhesions.

We think that NAC's effect on anastomotic healing and the systemic side effects of peritoneal administration should be investigated as they affect fibrosis and collagen formation. The effects on adhesions at the 30th day or further can be investigated for long time effects. Studies for determining the ideal dose should be carried out before initiating clinical use in humans. We believe that our route of administration could be suitable for clinical use after further studies.

Conclusions

In this study, we observed that local intraperitoneal administration of the parenteral form of NAC in single dose prevents adhesion formation in rats at 10 days. NAC is a safe, cheap, easy-to-get and easy-to-use medication, which has been in clinical use for years, with fairly less side effects and drug interactions than other agents.

Riassunto

La finalità di questo studio è quello di indagare l'effetto della somministrazione locale di N-acetylcysteine (NAC) sulla formazione di aderenze intra-addominali postoperatorie in un modello sperimentale su ratti.

Sono stati impiegati 20 ratti Wistar albini di sesso femminile dell'età di 5-7 mesi, divisi in due gruppi uguali. Nel primo gruppo si è introdotta una soluzione salina mentre nel secondo gruppo è stata introdotta NAC dopo abrasione del cieco. Il riscontro è stato effettuato nel

decimo giorno postoperatorio con esame macroscopico e microscopico per verificare la presenza di aderenze.
Il riscontro di aderenze intraperitoneali è stato valutato con il metodo cieco del modello Evans. Il tratto intestinale maggiormente sede di aderenze è stato sottoposto ad esame istopatologico, usando il test Mann Whitney U per l'analisi statistica.
Nel gruppo 1 tutti i ratti presentavano aderenze, mentre nei ratti del gruppo 2 nessuno ha presentato né grave reazione cellulare infiammatoria o fibrosi interstiziale densa.
La formazione di aderenze macroscopiche e una reazione cellulare infiammatoria e la formazione di fibrosi interstiziale individuabile istologicamente dopo l'intervento chirurgico è risultata minore nel gruppo due (NAC), rispettivamente ($p<0.05$, $p<0.05$, $p<0.05$).
Si conclude che una singola dose intraperitoneale di NAC può determinare una prevedibile minore formazione di aderenze intraddominali postoperatorie.

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