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The effect of alpha-lipoic acid in the prevention of peritoneal adhesions

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Background/aims: *Peritoneal adhesions, which occur most frequently after abdominal and pelvic operations, may lead to serious complications such as small intestine obstruction. In various studies, it has been shown that oxidative stress may play a role in the development of peritoneal adhesions, and studies carried out with antioxidants reported positive results. In the present study, the probable preventive role of alpha-lipoic acid, a strong antioxidant, in the development of peritoneal adhesions was investigated.* ***Methods:*** *Sixteen Sprague-Dawley male rats weighing 200-250 grams were employed. Under ketamine+xylazine anesthesia, on the antimesenteric aspect of the cecum, an adhesion model was formed with an incision, and half of the experimental animals were administered a daily single dose 100 mg/kg alpha-lipoic acid through orogastric gavage, and the other half formed the control group. Abdomens were opened 15 days later, and after adhesions were scored macroscopically, tissue samples were taken for evaluation of biochemical parameters.* ***Results:*** *In both adhesion scoring methods, a statistically significant decrease was found in the alpha-lipoic acid group compared to the control group ($p<0.05$). The decrease in adhesions was also confirmed by the significantly lower hydroxyproline levels in the alpha-lipoic acid group ($p<0.05$). In addition, alpha-lipoic acid decreased malondialdehyde levels in the adhesion region and prevented the increase in superoxide dismutase and glutathione peroxidase activities significantly ($p<0.05$).* ***Conclusions:*** *It can be concluded from the findings of our study that alpha-lipoic acid decreased the development of adhesions in a peritoneal adhesion model and increased the quality of healing. These findings suggest that alpha-lipoic acid, already long used in various indications, may be tried clinically in patients about to undergo abdominal operations.*

Key words: Alpha-lipoic acid, peritoneal adhesion, antioxidant, oxidative stress

Peritoneal yapışıklık oluşumunun engellenmesinde alfa-lipoik asitin etkisi

Amaç: *En sık olarak abdominal ve pelvik operasyonlardan sonra ortaya çıktığını gördüğümüz batın içi yapışıklıklar (peritoneal adezyon) ince bağırsak tıkanması gibi ciddi komplikasyonlara yol açabilmektedir. Yapılan araştırmalar, peritoneal adezyon oluşumunda, oksidatif stresin rolünün olabileceğini göstermiştir. Bu çalışmada, güçlü bir antioksidan olan alfa-lipoik asitin peritoneal yapışıklık oluşumundaki olası önleyici rolü araştırılmıştır.* ***Yöntem:*** *Çalışmada 16 adet 200–250 g ağırlığındaki Sprague-Dawley cinsi erkek sıçan kullanılmıştır. Ketamin+ksilazin anestezisi altında, çekumun antimezenterik yüzünde bir kesi ile adezyon modeli oluşturulan deney hayvanlarının yarısına orogastrik gavaj yoluyla günde tek doz 100 mg/kg alfa-lipoik asit verildi, diğer yarısı kontrol grubu olarak değerlendirildi. On beş gün sonra, karınları açılan deney hayvanlarında adezyon bölgeleri makroskobik olarak skorlandıktan sonra biyokimyasal parametreler için doku örnekleri alındı.* ***Bulgular:*** *Her iki adezyon skorlamasında da alfa-lipoik asit grubunda kontrol grubuna göre istatistiksel olarak anlamlı azalma görüldü ($p<0.05$). Yapışıklığın azalması, alfa-lipoik asit grubundaki anlamlı düşük hidroksiprolin düzeyleri ile de doğrulandı ($p<0.05$). Ayrıca alfa-lipoik asitin, adezyon bölgesindeki malondialdehit düzeylerini anlamlı olarak azaltırken ($p<0.05$), süperoksit dismutaz ve glutatyon peroksidaz aktivitelerindeki artışı da engellemiş olduğu görüldü.* ***Sonuç:*** *Çalışmamız verilerinden, uyguladığımız alfa-lipoik asitinin peritoneal adezyon modelinde yapışıklık oluşumunu azalttığı ve iyileşme kalitesini arttırdığı sonucu çıkarılabilir. Bu bulgular, uzun zamandır çeşitli endikasyonlarda zaten kullanılan, alfa-lipoik asitinin batın operasyonuna alınacak hastalarda klinik olarak da denenmesinin faydalı olabileceğini işaret etmektedir.*

Anahtar kelimeler: Alfa-lipoik asit, peritoneal yapışıklık, antioksidanlar, oksidatif stres

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INTRODUCTION

The term peritoneal adhesion refers to the fibrotic bands emerging between the surfaces in the peritoneal cavity. The most important stimulant of the formation of adhesions is trauma on the peritoneum. Adhesions usually arise due to intraabdominal surgery. In almost all of the intraabdominal interventions, adhesions occur with varying severity (1). Peritoneal adhesions appearing after abdominal and pelvic operations may lead to further pathological states, such as chronic abdominal pain and infertility (2).

The visceral peritoneum is composed of a single layer of mesothelial cells placed on the basement membrane. Basement membranes are the layer in which mesothelial cells are attached to the tissue below with loose connective tissue. Due to its thin and delicate structure, the peritoneum is quite susceptible to trauma (3). In peritoneal damage occurring due to various causes, primarily surgery, mesothelial cells are harmed and shed. In the region where these cells are shed, the inflammation process is initiated and serous exudate is formed. In some cases, as a consequence of the organization of fibrinogen found in exudate, fibrin bands are formed between the damaged mesothelial layers (4). For adhesion development, the occurrence of mesothelial cell damage on a single peritoneal membrane surface is necessary.

Alpha-lipoic acid (a-LA) is an antioxidant substance originally isolated from the bovine liver in 1950 (5). Both a-LA and its metabolites possess antioxidant characteristics due to the special chemical structure of this molecule. During its first contact with free radicals, the a-LA molecule is oxidized and dihydrolipoic acid (DHLA) is formed. Due to its strong antioxidant nature, a-LA has been reported to exert favorable effects in various pathological states, such as diabetes, arteriosclerosis, neuron degeneration, multiple sclerosis, and joint diseases (6,7).

In the development of peritoneal adhesions, circulatory failure and increase in oxidative stress are important factors. Previous studies reported that antioxidants such as methylene blue, melatonin and vitamin E were able to decrease the development of peritoneal adhesions (8 10). The aim of the present study was to investigate the effect of a-LA on the development of intraabdominal adhesions.

MATERIALS AND METHODS

The present study was initiated after the approval of our institutional Ethics Committee for Animal Experiments. A total of 16 adult male Sprague-Dawley rats bred in our laboratory were used. Rats were 12 weeks of age and weighed 200–250 g. Experimental animals were kept under the same laboratory conditions throughout the study period and fed with commercial rat food and tap water. They were allocated into control and treatment groups.

In the first stage of the study, all rats were anesthetized with intramuscular ketamine and xylazine (85+12.5 mg/kg) combination and laid in the supine position. A 3-cm region on the abdominal midline was shaved and cleaned with antiseptic solution (Polyod, Drogsan, Ankara). With midline incision, the cecum was reached and an approximately 1-cm incision was made on its antimesenteric aspect. This incision was sutured with 4/0 atraumatic silk (Dogsan Corporation, Trabzon). Finally, the abdomens of the animals were closed with 3/0 atraumatic silk (Dogsan Corporation, Trabzon) suture (11). Immediately after the operation, 8 of the rats were administered a daily single dose of 100 mg/kg a-LA through orogastric gavage for 15 days.

Tissue Preparation

Fifteen days after the adhesion model operation, the abdomens of the rats were opened again under anesthesia, and macroscopic scoring of the peritoneal adhesions was carried out with the methods described by Mazuji (12) and Moreno (13). The details of scoring methods are shown in Tables 1 and 2.

Following this initial evaluation, tissue samples were obtained from the suture area in the cecum for the measurement of hydroxyproline (OH-p), as an indicator of collagen formation; malondialdehy-

Table 1. Mazuji scoring for peritoneal adhesion evaluation (11)

Score	Definition
0	No adhesion
1	Fine adhesions that can be separated with blunt dissection
2	Adhesions that require less than 50% sharp dissection for separation
3	Adhesions that require more than 50% sharp dissection for separation
4	Serosal injury during separation
5	Full-thickness injury during separation

Table 2. Moreno scoring for the evaluation of peritoneal adhesion (13)

Adhesion region	Score
Thickness	
<3 mm	1
3–5 mm	2
>5 mm	3
Types	
Type I (simple, does not require dissection)	1
Type II (requires dissection)	2
Type III (requires sharp dissection)	3
Vascularization	
Avascular	0
Vascular	1

de (MDA), which is the end product of lipid peroxidation resulting from oxidative stress; and activities of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Tissues removed were placed into tubes and rapidly frozen in liquid nitrogen. Frozen tissues were homogenized in phosphate buffer (Heidolph Diax 900 homogenizer, Kelhaim, Germany). Homogenates were divided into 2 or 3 small tubes and kept at –80°C until the day of measurement.

Biochemical Analysis

All chemicals were supplied by Sigma-Aldrich (Germany) and all organic solvents by Merck (Germany). All reactives were prepared on the day of the measurements and refrigerated at +4°C until use.

First, the protein content of the tissues was measured according to the method of Lowry et al. (14) with bovine serum albumin as the standard. Lipid peroxidation level was measured with the method described by Ohkawa et al. (15). In this method, color change occurring as a result of the reaction of MDA with thiobarbituric acid at 535 nm is measured by spectrophotometric method. MDA levels measured were expressed in mmol/g-protein units. SOD activity was determined by the method described by Sun et al. (16), which is based on the reduction of nitroblue tetrazolium at 560 nm into blue-colored formazan. The activity of GSH-Px enzyme was determined according to the method of Paglia and Valentine (17). In this method, NADPH oxidation was measured at 340 nm spectrophotometrically, and GSH-Px activity is calculated by NADH oxidation curve. The activities of both enzymes were expressed in U/g-protein. The quantity of OH-p was measured using the method described by Reddy and Enwemeka (18). In this method, OH-p absorbencies were measured spectrophotometrically at 560 nm calculated as µg/g-protein.

Statistical Analysis

All statistical analysis was carried out using SPSS (version 11.0) program. The Mann-Whitney U test was used to compare the differences among groups; p values <0.05 were considered statistically significant.

RESULTS

Macroscopic Evaluation

When the abdomens of the experimental animals were opened, it was observed that experimental animals administered a-LA were markedly superior to others in terms of adhesion. The macroscopic adhesion scores of the control lesions were estimated as 4 (2-4) [median (minimum-maximum)] by the Mazuji method and 2.5 (2-3) by the Moreno method compared to 2 (2-3) and 1 (1-2) in the a-LA-treated animals, respectively ($p<0.05$ for both Mazuji and Moreno methods).

Biochemical Evaluation

Hydroxyproline (OH-p) levels were significantly decreased with a-LA treatment ($p<0.05$). The OH-p levels of 340.4 µg/g-protein (209.8-476) in the control group were found to be reduced to 96 µg/g-protein (79.6-122) in the a-LA group.

Malondialdehyde (MDA) levels as well as antioxidant enzymes activities were also decreased significantly in the a-LA-administered animals ($p<0.05$ for all). The MDA values of the control animals were found to be 1.7 mmol/g-protein (1.1–1.9), whereas in the a-LA-treated group, these values were 1 mmol/g-protein (0.94–1.3). The SOD activity decreased from 2209.6 (1475.3–2624) to 1212 U/g-protein (9773–1981.3) and the GSH-Px activity from 29.7 (26.9–35.3) to 22.3 U/g-protein (16.5–28.4) with a-LA treatment.

DISCUSSION

Peritoneal adhesions occurring after operations influence the quality of life in millions of people adversely. Though adhesions occur after almost all intraabdominal operations, a method of preventing them has yet to be developed (1).

The repair process after the disruption of the integrity of tissue for any reason consists of the stages of inflammation, endothelium and fibroblast proliferation and subsequently maturation cha-

racterized by the synthesis of connective tissue and matrix (19). The healing process resulting from damage in the peritoneum proceeds similarly, and as a consequence of an injury, exudate leaking into the peritoneum occurs in accompaniment to inflammation (20). In a physiologically well-oxygenated tissue without circulation problems, plasminogen activators, which are present in the leaking exudate, form the fibrin-degrading enzyme plasmin. In some cases, incomplete degradation of fibrin clot gives rise to complicated healing in the peritoneum, which is termed as peritoneal adhesion (21).

During peritoneal healing, increase in the level of oxidative stress increases at a degree depending upon biological conditions. In the study of Ten Raa et al. (22), it was demonstrated that during peritoneal healing, oxidative stress increases, and there is a positive correlation between the level of oxidative stress and the severity of adhesion. In another study, Ara et al. (10) showed that the severity of adhesion increases with oxidative stress. In their study, it was also reported that MDA and nitrite/nitrate values increasing with peritoneal adhesions decreased with melatonin, an antioxidant substance, and hence adhesion decreased as well.

In the studies discussed above, MDA measurements have been made in order to measure oxidative stress. MDA is a lipid peroxidation end product, which is frequently used to demonstrate the extension of radical reactions leading to biological injury (23). In the present study, MDA levels were observed to be decreased significantly in the a LA-administered group. This may have been caused by the strong antioxidant capability of a LA.

It is known that in the presence of oxidative stress, antioxidant enzyme activities increase in response (24). The activities of the antioxidant enzymes SOD and GSH-Px in the present study demonstrated a clear rise in the untreated adhesion-created (control) group, proving the existence of oxidative stress. These increased levels were sufficiently lowered by a-LA administrations.

Moreover, both OH-p levels and the severity of the adhesion lesions were found to be significantly higher in the untreated control group than in the a-LA-administered animals in the present work. Thus, a-LA not only exerted an antioxidant effect but also contributed to healing, especially to the quality of healing. In a clinical study by Alleva et al. (25), positive effect of a-LA on wound healing was shown in various parameters. The same study reported that a-LA beneficially supports angiogenesis as well. Measuring OH-p levels, which is an abundant molecule in collagen and is only sparsely found in the structure of other proteins, is a frequently used method in order to determine the amount of collagen (26). In a study of Baykal et al. (27), the severity of peritoneal adhesions was evaluated by measuring OH-p in the tissue obtained from the suture region. That study also demonstrated that the amount of OH-p increased in conjunction with the severity of peritoneal adhesions. Similarly, in the present study, tissue OH-p levels of the a-LA-administered animals were lower compared to the control group, indicating that a-LA prevents leaking exudate from being organized. Macroscopic adhesion scores were in parallel to OH-p levels, supporting the protective effect of a-LA. These findings are also consistent with previous adhesion studies carried out with antioxidants (8,9).

When our data are evaluated as a whole, it can be suggested that in the present peritoneal adhesion model in rats, a-La administration was effective in decreasing the development of adhesions. Parameters measured in the present study indicate that the capacity of a-LA to limit oxidative stress was an important cause of this action. The present results suggest that the prophylactic use of a-LA during surgical interventions may result in serious improvements in terms of patient comfort, duration of healing and cost of treatment. However, in order to describe the mechanism more thoroughly, further studies investigating various cytokines and growth and transcription factors are necessary. As a very cheap and safe molecule with a high dose range and few, if any, side effects, a-LA should be investigated in more detail in experimental as well as clinical studies.

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