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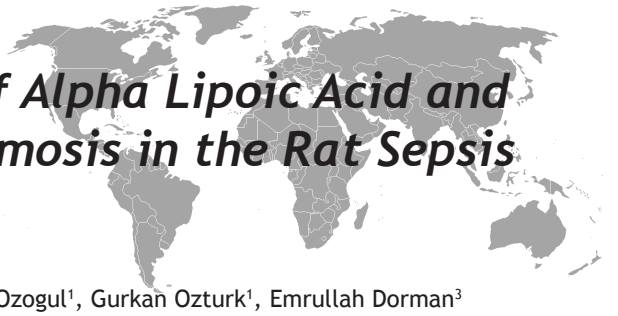
Original Article



Comparison of the Effects of Alpha Lipoic Acid and Vitamin C on Colonic Anastomosis in the Rat Sepsis Model

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ABSTRACT

Peritoneal contamination is considered to be risk for anastomosis. In our study, the effect of vitamin C and lipoic acid combination on anastomosis healing following colonic anastomosis performed in rats with experimentally-induced intraabdominal sepsis has been investigated. The rats in the control group were given only routine enteral feed in the preoperative and postoperative periods. The rats in the vitamin C group were given 100 mg/kg vitamin C IV/S.C, the rats in the lipoic acid group were given 50 mg/kg lipoic acid daily via the peroral route, and the rats in the vitamin C + lipoic acid group were given vitamin C and lipoic acid via the same routes beginning from the operation day. Perforation was induced in the sigmoid colon and intraabdominal sepsis was induced, and this perforation site was resected 24 hours later and an end-to-end colonic anastomosis was performed. The anastomosis site was taken out of the abdomen and the bursting pressure was measured. Biochemical analysis was performed for this site in order to analyze the prolidase enzyme, hydroxyproline and malondialdehyde thereafter. While the mean bursting pressures and hydroxyproline levels of the combination group were found to be statistically significantly higher than those of the other study groups (p<0,001), the prolidase and malondialdehyde levels of the combination group were found to be statistically significantly lower than those of the other study groups. Use of antioxidants vitamin C and lipoic acid in combination provides better anatomosis healing and tension.

Key words: *Rat, intraabdominal sepsis, anastomosis, vitamin C, lipoic acid*

Sıçan Sepsis Modelinde Kolon Anastomoza Alfa Lipoik Asit ve C vitamini Etkilerinin Karşılaştırılması

ÖZET

Periton kontaminasyonun anastomoz için risk olarak kabul edilir. Bizim çalışmamızda, deneysel kaynaklı karın içi sepsis ile sıçanlarda yapılan kolon anastomoz iyileşmesi üzerine C vitamini ve lipoik asit kombinasyonunun etkisi araştırılmıştır.Kontrol grubundaki sıçanlar preoperatif ve postoperatif dönemde sadece rutin enteral besleme verildi.C vitamini grubundaki fareler, 100 mg/kg vit C IV/SC verildi, lipoik asit grubunda farelere oral yoldan her gün 50 mg/kg alfa lipoik asit verildi ve C vitamini+lipoik asit alan grupta farelere operasyon gününden itibaren aynı yol ile C vitamini ve lipoik asit verildi. Perforasyon sigmoid kolonda oluşturularak karın içi sepsis meydanagetirildi. Bu perforasyon sitesi 24 saat sonra rezeke edildi ve uç uca kolon anastomoz yapıldı. Anastomoz yeri karın dışına alındı ve patlama basıncı ölçüldü. Biyokimyasal olarak prolidaz enzimi, hidroksiprolin ve malondialdehit bu site için analiz edildi. Kombinasyon grubunda patlama basıncı ve hidroksiprolin düzeyleri diğer gruplardan istatistiksel olarak anlamlı derecede yüksekti (p <0,001). Prolidaz ve malondialdehit düzeyleri kombinasyon grubunda diğer gruplarına göre istatiksel olarak dfüşük bulundu. Antioksidan olarak vit C ve lipoik asidin kombine kullanımı dah iyi anastomoz iyileşmesi ve gerginlik sağlamaktadır.

Anahtar kelimeler: *Rat, karın içi sepsis, anastomaz, vit C, lipoik asit*

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INTRODUCTION

Leakage of anastomosis is seen at a rate of 0-35% following intestinal anastomosis(1). Peritoneal contamination is accepted to be risky for anastomosis. One of the issues that is not taken into consideration is oxidative damage developing at the colonic ends that are damaged in the contaminated abdomen(2).

Various antioxidants have been used to exert an effect on wound healing mechanisms. Among these, although vitamin C is the most investigated and used antioxidant, lipoic acid has not been investigated very much(3).

Prolidase is an enzyme that degrades iminopeptides, which include proline and hydroxyproline in the C-terminal which comes up in collagen degradation and re-enters collagen production without joining the systemic aminoacid pool. The prolidase enzyme can be analyzed as an indicator of tissue oxidative stress parameters and wound healing in order to determine the effects of antioxidants on wound healing at tissue level(4).

The aim of this study was to determine and compare the effect of vitamin C and lipoic acid on colonic anastomosis healing in an intraabdominal sepsis model, and also to put forth the availability of prolidase enzyme as an indicator in colonic anastomosis healing.

MATERIALS AND METHODS

This study was conducted in the laboratory of the Atatürk University Experimental Research and Training Center (ATADEM) in April 2011. A total of 48 male Sprague Dawley Albino rats weighing between 200-250 grams were included in the study and allocated to 6 group with 8 rats in each. The groups and the nutritional and surgical procedures applied to the groups have been presented in Table 1. A method resembling the Bao method was used in order to produce intestinal fistula in the experimental animals(5).

Afterwards, the rats were sacrificed with high dose ketamin. Having obtained 1 cm of tissue samples so as to include 0.5 cm distal and proximal parts of the anastomosis site, they were fixed in 1 cc of saline solution and stored at -22 oC in order to determine the tissue prolidase enzyme, hydroxiproline and malondialdehyde levels. The team members who made the measurements were blinded to group names during the measurements of bursting pressure and other biochemical parameters.

Measurement of anastomosis bursting pressure

The bowel loops at 1 cm distance distal and proximal were resected and taken out of the abdomen. Then, both the open ends of the intestinal loops were closed so as to make it water-resistant and an 18 G needle was placed into the lumen. This needle was connected to the infusion pump and to the monitor that would measure the intraluminal pressure with a triple tap. The pressure was measured while the diluted methylene blue was administered from the infusion pump at a rate of 2 ml/min. Bursting was accepted to have occurred when

***Table 1**. The nutritional and surgical procedures applied to the groups*

		the first day	*1st postop day*	*5th postop day*
Control group	*routine enteral feed*	*sigmoid resection -anastomosis exploration*	----	*excision of the anastomosis area*
Sham group	*routine enteral feed*		----	*excision of the sigmoid colon*
Sepsis group	*routine enteral feed*	*sigmoid perforation*	*sigmoid resection -anastomosis*	*excision of the anastomosis area*
Vitamin C group	*100 mg/kg/day vitamin C/ I.M*	*sigmoid perforation*	*sigmoid resection -anastomosis*	*excision of the anastomosis area*
Lipoic acid group	*50 mg/kg/day lipoic acid /P.O*	*sigmoid perforation*	*sigmoid resection -anastomosis*	*excision of the anastomosis area*
Combination group	*100 mg/kg/day vitamin C/ I.M + 50 mg/kg/day l ipoic acid /P.O*	*sigmoid perforation*	*sigmoid resection -anastomosis*	*excision of the anastomosis area*

***Table 2**. The mean bursting pressures, the hydroxyproline, prolidase and malondialdehyde levels*

	Bursting pressures (mmHg)	*Hydroxyproline levels (mg/ml)*	*Prolidase levels (mmol/min /gr protein)*	*Malondialdehyde levels (nanomol/gr protein)*
Control group	*54.00±9.65*	*1.14±0.42*	*4.42±0.26*	*1.97±0.48*
Sham group		*1.75±0.88*	*3.47±0.27*	*1.47±0.15*
Sepsis group	*38.83±9.16*	*1.23±0.60*	*3.70±0.27*	*2.22±0.82*
Vitamin C group	*74.33±6.53*	*2.65±0.49*	*6.08±0.49*	*2.32±0.49*
Lipoic acid group	*81.83±8.81*	*1.60±0.43*	*3.67±0.27*	*2.19±0.94*
Combination group	*96.83±10.45*	*3.85±1.03*	*3.54±2.27*	*1.18±0.33*

the pressure abruptly decreased and diluted methylene blue was seen to leak from the repair site.

Determination of the hydroxyproline amount

Biochemical analyses were performed at the laboratory of Atatürk University Biochemistry Department. The hydroxyproline amount was determined using the 'High-performance Liquid Chromatography' method(6). The results were obtained as miligram/mililiter 16 minute after the samples had been introduced to the High-Performance Liquid Chromatography system.

Determination of the prolidase amount

Tissue prolidase activity was measured based on the method described by Myara et al.(7).This method that is based on determination of proline level with the Chinard reactive was used after having made some improvements(8). Protein concentrations were measured in the homogentisate supernatants using the Bradford method and the prolidase activity was expressed as micromol /min/gram protein (U/g protein).

Determination of the malondialdehyde amount

The tissue malondialdehyde amount was measured using the method described by Ohkawa et al.(9). The lipid peroxidase levels were expressed as nanomol/gram protein malondialdehyde.

We investigated the bursting pressure of the anastomosis site, and the hydroxyproline, prolidase and malondialdehyde levels in order to evaluate the anatomosis healing in our study.

Statistical Analysis

The data of our study were statistically analyzed using the SPSS 18.0 (Chicago, Illn, US) software. The data were expressed as mean and standard deviation. Analysis of the inter-group quantitative data was carried out using the ANOVA Test (analysis of variants). A p level of <0.05 was accepted as statistically significant.

RESULTS

A total of 8 rats died (2 rats in the control group died just after ketamine anesthesia and the others died after intestinal repair) and they were excluded from the study. Macroscopic leakage was not observed after anastomosis. The mean bursting pressures, and the hydroxyproline, prolidase and malondialdehyde levels have been presented in Table 2.

Bursting pressures

When the bursting pressures of the combination group were compared with the bursting pressures of the control, the vitamin C and lipoic acid groups, they were found to be statistically significantly higher (p<0.01 for each). The inter-group relations of bursting pressures have been displayed in Table 3.

Hydroxyproline Levels

The hydroxyproline levels of the combination group were found to be statistically significantly higher than those of the control, vitamin C and lipoic acid groups

Table 3. The inter-group relations of bursting pressures

	Control group	**Vitamin C group**	**Lipoic acid group**
Vitamin C group	*p=0.001*	*---*	*p=0.163*
Lipoic acid group	*p<0.001*	*p=0.163*	*---*
Combination group	*p<0.001*	*p<0.001*	*p=0.008*

Table 4. The inter-group relations of the hydroxyproline levels

	Control group	**Vitamin C group**	**Lipoic acid group**
Vitamin C group	*p=0.001*	*---*	*p=0.013*
Lipoic acid group	*p=0.254*	*p=0.013*	*---*
combination group	*p<0.001*	*p=0.005*	*p<0.001*

Table 5. The inter-group relations of the prolidase levels

	Control group	Vitamin C group	Lipoic acid group
Vitamin C group	*p<0.001*	*---*	*p<0.001*
Lipoic acid group	*p<0.001*	*p<0.001*	*---*
Combination group	*p<0.001*	*p<0.001*	*p=0.488*

Table 6. The inter-group relations of the malondialdehyde levels

	Control group	*Vitamin C group*	*Lipoic acid group*
Vitamin C group	*p=0.324*	*---*	*p=0.730*
Lipoic acid group	*p=0.518*	*p=0.730*	*---*
Combination group	*p=0.033*	*p=0.003*	*p=0.007*

(p<0.01 for each). The inter-group relations of the hydroxyproline levels have been presented in Table 4.

Prolidase enzyme levels

While the prolidase levels of the combination group were found to be statistically significantly lower than those of the control and the vitamin C groups (p<0.01 for each), no statistically significant difference was found between the combination group and the lipoic acid group (p=0.488). The inter-group relations of the prolidase levels have been displayed in Table 5.

Malondialdehyde levels

When the MDA levels of the combination group were compared with those of the control, vitamin C and lipoic acid groups, the levels of the combination group were determined to be statistically significantly lower (p<0.05, p<0.05, p>0.01, respectively). The inter-group relations of the malondialdehyde levels are shown in Table 6.

DISCUSSION

The likelihood of complication is higher in colonic anastomosis compared to other anastomoses of the gastrointestinal system (10). While systemic effects, such as protein catabolism and circulation insufficiency occur in intraabdominal sepsis, it also leads to local inflammation, tissue edema, impairments in collagen metabolism, tissue perfusion and oxygenation (11,12). Hence, the risk increases further in intestinal anastomosis performed in intraabdominal sepsis (13).

In experimental studies, biochemical, mechanic and histopathological methods are used for the assessment of anastomosis healing. As a biochemical parameter, the tissue collagen amount is determined through measurement of the level of the amino acid, hydroxyiproline (14). The bursting pressure, which indirectly shows the tissue collagen accumulation, is used as the mechanic method (15). Prolidase is the enzyme which degrades iminodipeptides involved in collagen production (4). The prolidase level and oxidative parameters obtained from the anastomosis sites may serve as parameters for the assessment of anastomosis healing. Lipid peroxidation negatively affects wound healing, and malondialdehyde, the last compound of lipid peroxidation, is used as the indicator of oxidative status (16).

Vitamin C is the most investigated antioxidant for colonic anastomosis healing. However, we did not encounter many publications about the effect of lipoic acid, another antioxidant, on colonic anastomosis. Vitamin C has effects on collagen synthesis, nitric oxide sysnthesis, cholesterol metabolism, and protects the antioxidants that are soluble at lipid phase from oxidative injury (17,18). Beside the known positive effects, vitamin C is known to strengthen the wound through stabilizing the collagen structure and balance the inflammation phase by exerting an effect at the hydroxylation stage of proline (19). Alpha lipoic acid has been suggested to positively affect wound healing through supressing the negative effects of the oxidative stress on collagen synthesis brought about by the effects of nitric oxide synthase, reducing the tissue free oxygen radical amount, protecting the tissue from ischemia-reperfusion effect and reducing lipid peroxidation (20-22).

In our study, the mean bursting pressures of vitamin C, lipoic acid and combination group were found to be statistically significantly higher than those of the control group. Furthermore, the pressures of the combination group were found to be statistically significantly higher than those of both the vitamin C group and the lipoic acid group. While the bursting pressures of the vitamin C and lipoic acid groups were significantly higher than those of the control group, the significantly higher bursting pressure of the combination group than both groups suggests that these two antioxidants potentiate

the effects of each other and enhance the positive effects on wound healing.

While the mean hydroxyproline levels of the vitamin C group and the combination group were statistically significantly higher than those of the control group, no statistically significant difference was found between the control group and the lipoic acid group. Moreover, while the hydroxyproline levels of the vitamin C group were found to be statistically significantly higher than those of the lipoic acid group, the hydroxyproline levels of the combination group were determined to be statistically significantly higher than those of both the vitamin C group and the lipoic acid group. The increase of hydroxyproline amount in the lipoic acid group not being statistically significant suggests that lipoic acid mainly exerts its effect through reducing the tissue free oxygen radical amount, protecting the tissue from ischemia-reperfusion effect, inhibiting the negative effects of oxidative stress on wound healing, and not through increasing the collagen amount. Furthermore, we saw that lipoic acid increased the effects of vitamin C in the combination group, and the results of the vitamin C group are consistent with the literature. In the study of Kaplan et al. (23) carried out with rabbits, it was seen that the vitamin C and hydroxyproline levels were correlated until the fifth day following wound formation and thereby, vitamin C had positive effects on collagen synthesis, especially in the first days of healing.

In our study, it was also investigated whether prolidase enzyme could be used as a wound healing parameter or not. The mean prolidase levels of the lipoic acid group and the combination group were found to be statistically significantly lower than those of the control group. In addition, the prolidase levels of both the lipoic acid group and the combination group were found to be statistically significantly lower than those of the vitamin C group. With these findings displaying the prolidase level not decreasing in the vitamin C group and significantly decreasing in the lipoic acid and the combination group, suggests that lipoic acid has a positive effect on wound healing through decreasing the prolidase level through inhibiting nitric oxide, and that prolidase can be used as a wound healing parameter. This is consistent with the statement in the literature that nitric oxide is one of the regulators of prolidase enzyme, which is also a free radical, and nitric oxide is reported to stimulate prolidase activity(24). It has been demonstrated that the nitric acid synthase enzyme is also activated during ischemia-reperfusion injury and abundant nitric oxide leads to bacterial translocation through impairing the barrier function of the intestine (25). Lipoic acid has been shown to inhibit the nitric oxide synthase enzyme in various studies (20,26). Prolidase has been shown to be the most important factor for limiting the collagen synthesis in studies investigating the inhibition of prolidase enzyme (27). Oono et al. (28) reported in chronic wound healing that the level of prolidase enzyme was seen to have increased in fluid samples obtained from the wound.

In our study, while it was seen that there was no statistically significant difference between the mean malondialdehyde levels of the sepsis group, vitamin C group, lipoic acid group and the control group, the malondialdehyde levels of the combination group were found to be statistically significantly lower. Furthermore, the malondialdehyde levels of the combination group were found to be statistically significantly lower than those of both the vitamin C and the lipoic acid groups. As a result of these findings, while the effects of lipoic acid and free radicals on oxidative stress have been shown in the literature, the malondialdehyde levels not having decreased compared to the control group, particularly in the lipoic acid group in our study, was not consistent with the literature; however, the malondialdehyde levels in the combination group made us think that the combination of vitamin C and lipoic acid contributed to wound healing through reducing the effects of oxidative stress and hindering lipid peroxidation. In the literature, in a study carried out with the rat sepsis model, lipoic acid was shown to be able to prevent neutrophil accumulation through reducing the oxidative stress, after which lipid peroxidation increased as a result of oxidative stress, and that lipoic acid reduced the lipid peroxidation in the lungs(29). In three different studies carried out parallely, lipoic acid was reported to significantly reduce the increased lipid peroxidation(30-32). In another study, the cerebral free radical amount and the effects of reperfusion were shown to decrease through lipoic acid administration to the animals which had been exposed to ischemic reperfusion induced through cerabral artery clamping (21). In three different experimental rat studies carried out by Arivazhagan et al.(33) the lipid peroxidation levels that increase with aging were evaluated in different organs, and lipoic acid was put forth to have reduced the malondialdehyde levels in plasma, liver, kidney and different brain areas, and to

prevent lipid peroxidation and protein oxidation.

When we evaluated the anastomosis assessment parameters as a whole, finding significantly higher bursting pressures and hydroxyproline levels and finding significatly lower prolidase and malondialdehyde levels in the combination group compared to both the control group and the vitamin C group, made us think that both the antioxidant molecules exerted a strong effect by potentiating each other's effects and that of the other antioxidant molecules. Lipoic acid is known to increase the effects of antioxidants such as vitamin C, vitamin E and glutathion. In our study, the values in the combination group being much more significant than the values in the vitamin C group suggests that lipoic acid contributes to wound healing through increasing the effects of vitamin C on increasing the collagen amount, fixing the collagen structure, balancing the inflammation phase of wound healing, reducing nitric oxide synthesis, and increasing the antioxidant parameters.

In conclusion, our study indicated that although the single use of vitamin C or lipoic acid has been found to be effective in reducing the leakages from the anastomosis which increase due to intraabdominal sepsis, and in order to make the anastomosis safer, the use of these two antioxidants in combination acts more effectively on reducing the tension of the anastomosis. Clinical use of these two agents in combination in patients undergoing risky intestinal anastomosis may be beneficial for reducing the risk of leakages from the anastomosis. The prolidase levels and the oxidative parameters obtianed from the anastomosis tissue may serve as parameters for assessment of anastomosis healing.

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