Original Article / Özgün Araştırma

Effects of Glutamine, Arginine and Beta Hydroxymethybutyrate on Anastomotic Leakage in Experimental Colon Anastomosis

Deneysel Kolon Anastomozunda Glutamin, Arjinin ve Beta Hidroksibütiratın Anastomoz Kaçağına Etkisi

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Abstract

Objective: The studies on anastomotic leakage which is one of the leading serious complications of colonic anastomoses keep going as well as other surgical researches. This study was designed to investigate effects of hydroxy-ß-methyl butyrate (HMB) on anastomotic healing.

Materials and Methods: Forty rats were randomized into four groups. Group A (n=10) rats received chow food plus arginine+glutamine+HMB rich diet for 7 days before right colonic transection followed by an end-to-end anastomosis. Group B (n=10) rats received chow food plus arginine and glutamine rich diet for 7 days before the same surgical procedure. Group C (n=10) rats underwent the same procedure after a 7-day chow plus glutamin rich diet. Group D (n=10) rats had the surgery after a chow food only diet for seven days. All the subjects were fed accordingly to their groups for 7 days postoperatively. On the 7th day, all rats were sacrificed under anesthesia to measure anastomotic bursting pressure and evaluate hydroxyproline levels as well as histopathological scoring of anastomotic line.

Results: This study revealed significantly increased hydoxyproline level at the 7th day in group A ($p<0.002$) and group B ($p<0.001$) relative to group D. There were no significant differences among the groups for anastomotic bursting pressures and histopathological scores.

Conclusion: Enteral HMB support may result statistically significant increased biochemical anastomotic strength with an insignificant difference in biomechanical force. Although more studies are needed to delineate better efficacy, preoperative enteral HMB support may decrease postoperative morbidity and mortality.

Öz

Amaç: Kolon anastomozu ile ilgili kaçak nedenleri konusunda devam etmekte olan araştırmalar vardır. Bu çalışmada anastomoz dayanıklılığının ameliyat öncesinde beta-hidroksi-metil bütirat (HMB) ile artıp artmayacağı denetlenmiştir.

Gereç ve Yöntemler: Kırk rat 4 gruba randomize edildi. Grup A (n=10) ratları standart rat yemi ile birlikte arjinin+glutamin+HMB'den zengin diyetle 7 gün beslendikten

sonra sağ kolon transeksiyonu ve uç uca anastomoz uygulandı. Grup B (n=10) denekleri rat yemi ile birlikte arjinin ve glutaminden zengin diyet 7 gün beslendikten sonra aynı cerrahi işlem uygulandı. Grup C (n=10) 7 günlük rat yemi ile birlikte glutaminden zengin gıda ile beslendikten sonra cerrahiye alındı. Grup D (n=10) ratları sadece standart yem ile 7 gün beslendikten sonra ameliyata alındı. Tüm denekler postoperatif 7 gün boyunca beslendiler. Yedinci gün tüm ratlar anestezi altında sakrifiye edilerek anastomoz patlama basınçları ölçüldü. Hidroksiprolin değerleri ve histolopatolojik skorlama için anastomoz hattından örnek alındı.
Bulgular: Çalışmamızda A ve B grubunda hidroksiprolin düzeylerinde 7 günde belirgin ölçüde artmış hidroksiprolin düzeyleri saptandı. Gruplar arasında anastomoz patlama basıncı ve histolojik iyileşme parametreleri anlamında fark yoktu.
Sonuç: Enteral HMB desteği anlamlı artmış biyokimyasal anastomoz direnci sağlayabilir, ancak biyokimyasal güçte fark olmayabilir. HMB'nin yararını ortaya koymak için daha fazla çalışma gereksinimi olmasına rağmen preoperatif enteral HMB desteği postoperatif postoperatif mortalite ve morbiditeyi düşürebilir.

Introduction

Although anastomoses are an essential part of gastrointestinal (GI) surgery, they have carry a high risk for morbidity and mortality due to potential complications. Despite the current developments in GIS surgery, complications and particularly anastomotic leakage remains a major issue and post-operative anastomotic leakage occurs at a rate of 10-20% (1-3). The Various local and systemic factors influence on anastomotic recovery. Many investigators have tried many techniques and chemicals with regard to supporting anastomotic recovery and reducing post-operative risks (4-6). The one of the factors influencing wound healing and colon anastomosis is diet (5). The cases whose diet is inadequately planned are at higher risk for surgical complications, the wound healing in particular. Ensuring and maintaining appropriate nutritional supplementation are manifested as the important primary aim of perioperative care (7). Various amino acids influence wound healing. Glutamine, which is the most common amino acid, found in the body, accelerate mucosal growth and healing, reduce GIS-derived sepsis by inhibiting bacterial translocation and mediate nitrogen balance (8). Arginine, acts as L-arginine to improve immune system functions, accelerate wound healing and increase resistance to infections (9). A leucine metabolite named β-hydroxy-β-methyl butyrate (HMB) plays a significant role in wound healing and cachexia (10).

The aim of our study is to determine the role of perioperative glutamine, arginine and HMB-rich diet on anastomosichealing after elective colonic anastomosis in rats.

Materials and Methods

This study was conducted with approval of Local Animal Ethics Committee (approval number: 050.04/2011/099). Forty female Wistar Albino rats weighing between 170-220 g were used for the study. The rats were divided equally into four groups.

Group A (arginine+glutamine+HMB): Received 7 days of standard chow food plus arginine+glutamine+HMB rich diet preoperatively followed by right colonic transection and end-to-end anastomosis and received 7 days of standard chow food plus arginine+glutamine+HMB rich diet postoperatively before being sacrificed (n=10).

Group B (arginine+glutamine): Received 7 days of standard chow food plus arginine and glutamine rich diet preoperatively followed by segmental right colonic transection and end-to-end anastomosis and received 7 days of standard chow food plus arginine and glutamine rich diet postoperatively before being sacrificed (n=10).

Group C (glutamine): Received 7 days of standard chow food plus glutamine rich diet preoperatively followed by right colonic transection and end-to-end anastomosis and received 7 days of standard chow food plus glutamine rich diet postoperatively before being sacrificed (n=10).

Group D (control): Received 7 days of standard chow food preoperatively followed by right colonic transection and end-to-end anastomosis and received 7 days of standard chow food plus postoperatively before being sacrificed (n=10).

In addition to standard chow food, group A received 7 days of glutamine 1.44 g/kg/day, arginine 1.44 g/kg/day and HMB 2.6 g/kg/day (Abound Abbott Nutrition, Access Business Group, 19600 6th Street, Lakeview, CA92567-8403, USA) mixed in still tap water and continued at the same dose for 7 more days after the operation until sacrificed.

Group B received 7 days of standard chow food plus glutamine 1.5 g/kg/day and arginine 1.63 g/kg/day (Impact Glutamine, Nestle Health Care Nutrition

Germany) before the operation and continued at the same dose for 7 more days after the operation until sacrificed.

Group C received 7 days of standard chow food plus glutamine 1.3 g/kg/day (Glutamine Resource Nestle Health Care Nutrition Germany) mixed in still tap water and continued at the same dose for further 7 days after operation until sacrificed. Since it is an experimental study on nimals, there is no need for informed consent.

Surgical Procedure

Standard chow food was discontinued in all rats 12 hours before the operation and no colon cleansing was performed. However, there was no limitation for water in the control group and enteral nutritional solution in the other groups. The abdomen was entered by a 3 cm mid-line incision in sterile conditions. Following entering the abdomen, the caecum was found and complete transection of the colon segment 4 cm distal to the ileocecal junction was performed preserving the mesocolon. End-to-end anastomosis was established with 8 single-layer inverting sutures using atraumatic 4/0 polypropylene suture material (Figure 1). Abdominal incision was sutured with 3/0 silk material and operation was completed. The rats were started feeding relative to their groups 6 hours after the procedure.

Anastomotic Bursting Pressures Measurement

All rats were sacrificed 7 days after the operation by cervical dislocation under ether anesthesia. After the sacrification, the anastomotic segment was found at relaparotomy (Figure 2). Two umbilical 6-Fr catheters were inserted at 2 cm proximal and distal regions of the anastomosis using cut-down technique (Umbilical catheter–Bıçakçılar®). The catheters were fastened to the colon to ensure retention using 2/0 silk sutures. Thus, a 4 cm luminal segment including the anastomosic line in the middle was established (Figure 3). The intraluminal pressure was monitored electronically using a transducer (SS13L pressure transducer) and pressure line (Morton pressure resistant pressure line code: 441-Turkey) between the monitor (Biopac MP30 ultimate system Santa Barbara USA) and the catheter while saline fluid (SF) infusion at steady speed was introduced into the catheter through the distal end of the anastomosis. The highest pressure measured by the monitor just before the pressure reduction due to anastomosic leakage was recorded as the bursting pressure. The bursting pressure values were recorded in mmHg.

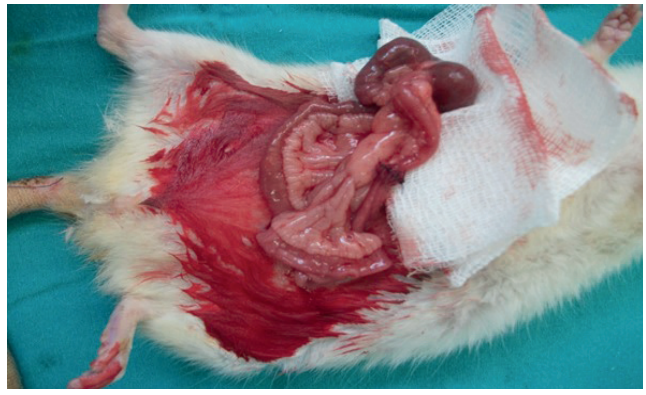

Figure 1. Anastomosis using 4/0 polypropylene suture material

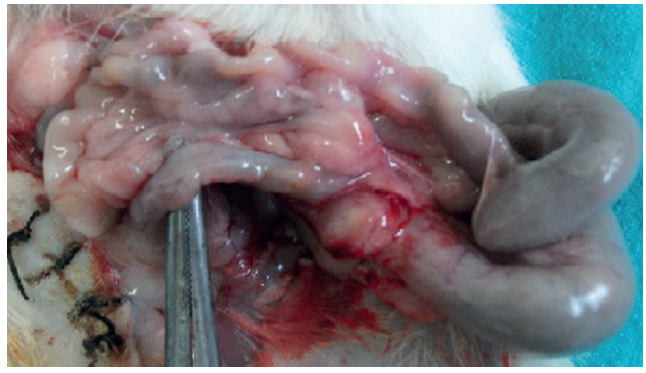

Figure 2. Relaparotomy-the anastomotic segment was found

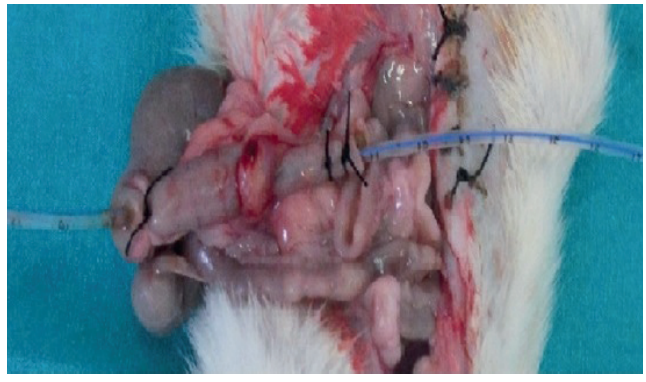

Figure 3. Anastomotic bursting pressure measurement model

Subsequently, tissue samples for histopathological and biochemical examinations were obtained from the anastomosic lines. The tissue hydroxyproline levels were measured. The neutrophilic infiltration scores were used for histopathological scoring (Table 1).

Table 1. The scoring for mucosal healing and neutrophilic infiltration. The mucosal healing was not prominent in groups, neutrophilic infiltration showed no significant difference (p=0.373)

Score	Mucosal healing	Neutrophilic infiltration	Macrophage, fibroblast and neovascularization
0	No healing	>75%	None
1	Surface epithelial healing	25-75%	Minimum
2	Submucosal healing	25-50%	Intense
3	Muscularis mucosa healing	<25%	-
4	Complete healing	None	-

Table 2. The anastomotic bursting pressures (mmHg). The difference was not significant among groups. The bursting pressure measurements were evenly distributed and there was no statistical difference between groups (p=0.54)

Rats	Group A (HMB)	Group B (glutamine+arginine)	Group C (glutamine)	Group D (control)
1	133.14	233.65	330.3	167.93
2	138.91	210.97	204.63	28.58
3	137.27	193.47	248.85	351.23
4	146.16	209.31	230.22	-
5	237.56	215.78	174.88	254.87
6	13.45	236.87	251.34	201.35
7	238.08	264.92	318.63	302.67
8	271.84	253.98	334.69	206.25
9	-	266.8	250.91	-
10	-	-	244.07	-
Mean value	**164.55±82.6**	**231.75±26.2**	**258.85±53.4**	**216.12±104.1**

HMB: Hydroxy-ß-methyl butyrate

Statistical Analysis

The results of the biochemical tests and the bursting pressure measurements were analyzed using One-Way variance analysis (ANOVA). Tukey's test and Bonferroni test were used as post-hoc tests. Kruskal-Wallis test was used to compare the histopathological scores. The difference was considered significant if p value is less than 0.05.

Results

Two rats from group A and 1 from group D died preoperatively and 1 rat from group D and 1 from group B lost at the postoperative period were not included in statistical analyses. One sacrificed rat from group D found to has already anastomosic leakage was also not included in statistical analyses. The bursting pressures and the group mean values at post-operative day 7 are presented in Table 2.

The bursting pressure measurements were distributed and there was no statistical difference between the groups (p=0.54)

The histopathological score evaluations were as follows; intense inflammatory cell infiltration, fibrin, granulocytes, fibroblasts and foreign body giant cells were detected at the anastomosic line in group A.

Prominent granulation tissue formation and almost chronic inflammation was found in group B. Abundant granulocytes, fibroblasts and foreign body giant cells were seen in group C. Minimal granulocytes, fibroblasts and foreign body giant cells were found in group D. Although mucosal healing was absent in all of the groups, statistical assessment for neutrophilic infiltration showed no significant difference (p=0.373).

The mean hydroxyproline levels as µg/mg wet tissue on day 7 after the operation are presented in Table 3. The difference was significant among the groups (p=0.0006).

Table 3. The tissue hydroxyproline levels (µg/mg wet tissue). The difference was significant among groups (p=0.0006)				
Rats	**Group A (HMB)**	**Group B (glutamine+arginine)**	**Group C (glutamine)**	**Group D (control)**
1	5.701710	6.224519	3.165495	4.307143
2	4.577269	5.996345	5.744869	3.869284
3	4.422086	5.154676	3.060069	3.194277
4	5.768745	4.230429	4.648631	3.207473
5	6.763813	9.149859	4.185815	2.982836
6	7.300578	4.685690	4.873407	3.755051
7	5.971772	6.108625	5.558381	3.304370
8	4.681921	10.757017	4.444135	3.161469
9	-	4.448646	3.798093	-
10	-	-	4.419914	-
Mean value	**5.64±1.0**	**6.30±2.2**	**4.39±0.8**	**3.47±0.4**

The hydroxyproline levels were higher in group A which included HMB administration compared to both the control group and group C which included glutamine administration (p=0.02). On the other hand, the hydroxyproline levels were higher in group B rats which received glutamine+arginine compared to both p and group C which included glutamine administration (p<0.01).

Discussion

Colon resections and anastomoses, particularly distal portion of the left colon, carry higher risk for anastomotic leakage and disintegration. Despite developments in surgical techniques and tools, morbidity and mortality rates related to anastomotic leakage after colon surgery remain high. Consequently, the studies on intestinal anastomosis focus on the colon (11). Thus, we preferred to investigate the effects of HMB on colonic anastomoses.

Wilson et al. (12) mentioned that the studied agent HMB used in our study shows its effect on anti-catabolic and protective mechanisms, thus enhancing protein synthesis. Manzano et al. (13) stated that HMB activates protein synthesis by activating the intracellular protein called mammalian target of rapamycin (mTOR). The mTOR signaling pathway is activated primarily when sufficient amount of intracellular glucose, amino acid and lipoproteins are available and enhances protein and amino acid synthesis by stimulating ribosomes. Insulin like growth factor-1 is the primary stimulus that activates mTOR in muscle cells while increasing HMB effect on mTOR (13,14).

The factors that have influence on wound healing can be classified under two main topics. The factors related to reduced collagen synthesis such as chronic nutritional disorders, diabetes, uremia, trauma, radiation injury, advanced age and the surgical factors such as tissue injury, infection of the tissue, poor vascularization or circulation of the tissue may influence wound healing (15).

The healing of intestinal anastomoses can be evaluated according to mechanical, biochemical or histopathological aspects. Mechanical evaluation includes assessment of bursting and breaking forces while biochemical evaluation includes rate, amount and structure of collagen synthesis at the anastomotic line (16). Croinin et al. (17) reported that the anastomotic bursting pressures gradually increased from the third day on and reached to maximum levels on day 10 while the hydroxyproline levels at the anastomotic area decreased by 40% within the first three days, approached to normal levels by approximately at the fifth day and raised above normal by days 10-14. In our study, the bursting pressure measurements were evenly distributed and there was no significant statistical difference between four groups (p>0.54).

Yıldız et al. (18) investigated the effects of cyanoacrylate on colonic anastomoses and measured bursting pressures and tissue hydroxyproline levels. The bursting pressures were found to be significantly

different between groups while there was no significant difference for the tissue hydroxyproline levels. Madden and Peacock (19) reported that in order to explain the role of collagen in the biology of wound healing, the collagen synthesis and lysis ratio should be considered as well as the available collagen amount. The investigators reported that collagen synthesis and disposition can be determined by applying labeled proline periodically and measuring hydroxyproline levels of the wound and they conducted a study for this purpose on rats. In our study, the hydroxyproline levels at the anastomotic line were significantly increased on day 7 in group A (HMB) compared to group D (control) ($p<0.002$). Similarly, hydroxyproline levels at the anastomotic line were significantly increased on day 7 in group B (glutamine+arginine) compared to group D (control).

Considering that the sutures maintain anastomotic endurance during the early period of reduced collagen concentration (20), the pressure resistance and suturing techniques appear to be correlated.

The tensile strength of the suture material is required for bridging between the newly formed collagen tissue and restoring the original tension of the intestinal wall. Therefore, improved collagen synthesis plays the primary role in wound healing and inhibition of regulation influences anastomotic tension. Additionally, fibrillary quality besides collagen mass determines tension and collagen type and stability of crosslinks are important for tension. The tensile strength depends on mechanical stability of the collagen fibrils and the formation of intermolecular crosslinks (16). In our study, although the mucosal healing was not observed in any of the groups, the statistical analysis for neutrophilic infiltration showed no significant difference.

Conclusion

In our study, the findings regarding tissue hydroxyproline levels were significant only while the anastomotic bursting pressures and the tissue histopathological results were insignificant. Although we believe this may be related with the late effects of HMB on tissue histopathology and tissue biochemistry, we suggest the preoperative enteral HMB supplementation could reduce the postoperative complications by improving anastomotic endurance.

Ethics

Ethical Committee Approval: Adnan Menderes University (ADU, Aydın, Turkey), Animal Ethical Committee (050.04/2011/099).

Informed Consent: Since it is an experiment on animals, there is no need for informed consent.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.K., H.E., A.S., Concept: H.E., E.K. Design: E.K., H.E., Data Collection or Processing: E.K, Ç.Y, İ.M., Analysis or Interpretation: H.E., E.K., Literature Search: E.K., E.M.Y., Writing: E.K., E.M.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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