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Effect of a honey and arginine-glutamine-hydroxymethylbutyrate mixture on the healing of colon anastomosis in rats immunosuppressed with tacrolimus

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ABSTRACT

We compared the effect of honey and a mixture of arginine-glutamine-hydroxymethylbutyrate (AGHMB) on healing of a descending colon anastomosis in rats that were immunosuppressed with tacrolimus (Tac). Sprague-Dawley rats were divided into four groups: untreated control, Tac, Tac + honey and Tac + AGHMB. Colon resection and anastomosis were performed on day 14 and re-laparotomy was performed on the day 21 of the study. Anastomotic bursting pressure, macroscopic adhesion score, weekly body weight changes, histopathological features and immunohistochemical staining of TGF-β1 were determined for all groups. We found no significant difference in anastomotic bursting pressure among the experimental groups. We found significant weekly increases in body weight for the Tac + honey group. We found no significant difference in the weekly body weight measurements for the Tac + AGHMB group. We found significant increases in TGF-β1 expression in the Tac + honey group compared to the control and Tac groups. No significant differences in inflammatory cell infiltration, fibroblast proliferation or collagen deposition were found between the Tac + honey and Tac + AGHMB groups; however, a significant difference in neovascularization between these groups was found. Neovascularization in the Tac + honey group was significantly greater than for the Tac + AGHMB group. We found that both honey and the AGHMB mixture were beneficial for anastomotic wound healing in rats that were immunosuppressed using Tac.

KEYWORDS

Anastomosis; arginine; bursting pressure; colon; glutamine; honey; hydroxymethylbutyrate; tacrolimus

The major causes of morbidity and mortality following colorectal surgery are complications of anastomosis such as fistula formation and leakage (Raptis et al. 2012). These complications are due to anemia, blood transfusion, chemoradiotherapy, diabetes, hypoalbuminemia, inflammatory bowel disease, malignant disorders, malnutrition, obesity, poor surgical technique, prolonged surgery, smoking and use of immunosuppressive medication (Raptis et al. 2012, 2018; Ekinci et al. 2018). Increased frequency of organ transplantation and incidence of autoimmune disorders have increased the use of immunosuppressive agents including corticosteroids and tacrolimus (Tac, FK506, fujimycin). Tac is a potent immunosuppressive agent that was isolated in 1985 from the fungus, *Streptomyces tsukubaensis* (Raptis et al. 2012, 2018; Zheng et al. 2017). Although Tac delayed wound healing in some clinical and experimental studies, it has been suggested that Tac actually promotes healing by inducing release of growth factors such as transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF), which stimulate collagen synthesis and angiogenesis, respectively (Saber 2010; Raptis et al. 2012).

Dietary supplementation with amino acids has been investigated for accelerating wound healing including arginine, glutamine, β-hydroxyl-β-methyl butyrate (HMB) (Raptis et al. 2012; Yaman et al. 2013; Bozkırlı et al. 2015). A mixture of arginine, glutamine and HMB (AGHMB) has been reported to promote wound healing and prevent apoptosis.

Honey also has been studied for its effect on wound healing (Gollu et al. 2008; Ergul and Ergul 2010; Saber 2010; Gencay et al. 2008). Honey has been used since antiquity for treating wounds and surgical incisions (Gollu et al. 2008; Gencay et al. 2008). The literature contains many reports of local or oral application of honey for accelerating wound healing. Although honey has been reported to promote wound healing in experimental gastrointestinal anastomosis models, we have found no report of the effects of honey in an

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immunosuppressed rat model (Gollu et al. 2008; Gencay et al. 2008). Consequently, we compared the effects of both honey and AGHMB on healing of a descending colon anastomosis in rats that had been immunosuppressed by Tac.

Materials and methods

Experimental protocol

All procedures were in compliance with the National Health Institute's Animal Research Guidelines. Our investigation was approved by the Inonu University Animals Research Ethics Committee (2011/A-110). We used 48 10–11-week-old, 230–310 g male Sprague-Dawley rats obtained from Inonu University Laboratory Animals Production and Research Center. The rats were housed at 21 ± 2 °C, 60 ± 5% humidity with a 12 h light:12 h dark cycle and free access to rat food and water. The animals were divided randomly into four groups of 12. The control group was not subjected to any experimental treatment. The Tac group was treated daily with 0.5 mg/kg/day Tac (Prograf; Astellas Phama Inc., Tokyo, Japan) by gavage from day 1 of the study. The rats in the groups other than the control group were administered a single dose of 0.5 mg/kg/day Tac by gavage to induce immunosuppression using the method of Uysal et al. (2017). The Tac + honey group was given 10 g/kg/day liquid honey (Balparmak, Altiparmak Pazarlama Koll Sti, Istanbul, Turkey) by gavage in addition to standard rat feed from day 1 of Tac treatment. The Tac + AGHMB group was given a mixture containing 308 g/kg glutamine, 308 g/kg arginine and 54 g/kg β-hydroxyl-β-methyl butyrate (Abound; Abbot, Lakeview, CA) at a dose of 0.24 g/350 g by gavage in addition to standard rat feed from the day 1 of Tac treatment.

All rats were weighed at weeks 1, 2 and 3 of the study. On days 14 and 21, the rats were anesthetized for surgical procedures by intraperitoneal (i.p.) administration of 40 mg/kg ketamine hydrochloride (Ketalar; Eczacibasi Warner-Lambert Laboratories, Istanbul, Turkey) and 5 mg/kg xylazine hydrochloride (Rompun, Bayer Laboratories, Istanbul, Turkey). On day 14, all rats were anesthetized and placed in a supine position. After shaving the anterior abdominal wall and cleaning with povidone iodine, a 4 cm abdominal incision was made to expose the abdominal cavity. After resecting a small segment of the descending colon, a colo-colonic anastomosis was created using 6/0 polyglactin 910 (Vicryl; Ethicon, Edinburgh, UK). Abdominal fascia and skin were sutured with 4/0 polyglactin 910 suturing material (Vicryl; Ethicon). On day 21 all rats were re-anesthetized as described above. After another laparotomy, intra-abdominal adhesions were checked macroscopically as described below. After resecting a large colonic segment that included the anastomosis line, all rats were sacrificed using an overdose of ketamine/xylazine.

Anastomotic bursting pressure

Measurement was based on the methods of Ergul and Ergul (2010), Gollu et al. (2008) and Raptis et al. (2012). The distal end of the resected colon segment was bound firmly with a suture material. The tube from a sphygmomanometer was inserted into the lumen from the proximal end of the resected colon segment. The colon segment was submerged in a water-filled container and the blood pressure cuff inflated. The pressure reading (mm Hg) at which air bubbles first emerged through the anastomosis line was recorded as the burst pressure.

Macroscopic scoring of adhesion

Adhesions were scored using the adhesion severity criteria described by Evans et al. (2008). The scoring system was: 0, no adhesion; 1, spontaneously separating adhesion; 2, separation of adhesion with traction; 3, separation of adhesion with sharp dissection. Scoring was conducted by an experienced surgeon who did not participate otherwise in the study.

Histopathology

The colon tissues were fixed in 10% neutral buffered formalin for 48 h. Tissues were washed in running water, then dehydrated through 50, 75, 96 and 100% ethanol. After dehydration, specimens were cleared in xylene and embedded in paraffin wax. Sections were cut at 5 μm, mounted on slides and stained with both hematoxylin and eosin (H & E) and periodic acid-Schiff (PAS)-Alcian blue (Bancroft and Gamble 2008). Stained sections were examined using light microscopy and graded using a scale that was modified from Ehrlich and Hunt's scoring system (Ehrlich et al. 1973). We evaluated collagen deposition, fibroblast proliferation, inflammatory cell infiltration and neovascularization (Ergul and Ergul 2010; Raptis et al. 2012; Ekinci et al. 2018). To do this, each parameter was assessed individually using the following scale: 0, no evidence; 1, occasional evidence; 2, moderate evidence; 3, abundant evidence; 4, confluent cells or fibers. All sections were examined

using a Nikon Eclipse 80i light microscope and Nikon Image Analysis system (Nikon, Tokyo, Japan)

Immunohistochemistry

For immunohistochemical analysis, sections were mounted on polylysine coated slides. After rehydrating, samples were transferred to citrate buffer, pH 7.6, and heated in a microwave oven for 20 min for antigen retrieval. After cooling for 20 min to room temperature, the sections were washed with phosphate-buffered saline (PBS). Sections were placed in 0.3% (v/v) H_2O_2 for 7 min, then washed with PBS. Sections were incubated with anti-rabbit anti-TGF-β1 antibody (sc-146; Santa Cruz Biotechnology, Dallas, TX) for 60 min according to the manufacturer's instructions. Sections then were rinsed in PBS and incubated with biotinylated goat antipolyvalent IgG for 10 min and streptavidin peroxidase for 10 min at room temperature according to the manufacturer's instructions. After 20-fold dilution of the AEC chromogenic substrate with AEC diluent solution, staining was completed with the commercial ready-to-use liquid AEC (3-amino-9-ethylcarbazole) chromogenic substrate solution (sc-24979; Santa Cruz Biotechnology) by incubating 15 min at room temperature. Staining was completed using ready-to-use AEC HRP Red chromogen + substrate (sc-24979; Santa Cruz Biotechnology) for 15 min at 25 °C and slides were counterstained with Mayer's hematoxylin for 1 min, rinsed in tap water, and dehydrated. Staining for anti-TGF-β1 was identified by brown coloration. The relative intensity of TGF-β1 immunostaining was scored as: 0, absent; 1, slight; 2, moderate; 3, strong. All sections were examined using a Nikon Eclipse 80i light microscope and Nikon Image Analysis system (Nikon).

Statistical analysis

Statistical analyses were carried out using the IBM SPSS Statistics v25.0 software package. Body weight and anastomotic burst pressure were recorded as means ± SD, median and minimum–maximum values. The numerical information describing histopathologic and immunohistochemical data were recorded as means ± SD. Adhesion scores were reported as numbers and percent. The Pearson chi-squared test was used to compare qualitative data. The Shapiro-Wilk test was used to test the normality of quantitative data. The Kruskal Wallis test was used to compare non-normally distributed quantitative data. To demonstrate which groups created statistical significance, Kruskal Wallis 1-way ANOVA (k samples) all pairwise multiple comparisons test, or the Mann Whitney-U test, was used. The Friedman test was used for repeated intra-group measurements. The Wilcoxon-signed rank test was used to determine which measurements reached statistical significance between repeated measurements. Values for $p \leq 0.05$ were considered statistically significant.

Results

Adhesion scores

Adhesion scores among the study groups did not differ significantly.

Anastomotic bursting pressure

The median anastomotic bursting pressures of the control, Tac + AGHMB, Tac + honey and Tac groups were 220.5 (189–241) mm Hg, 180 (110–220) mm Hg, 142.5 (84–220) mm Hg and 110 (48–210) mm Hg, respectively ($p = 0.001$). Statistical significance was as follows: control group vs. Tac group ($p < 0.001$) and control group vs. Tac + honey group ($p = 0.005$). None of the rats developed anastomosis dehiscence.

Body weight changes

We found significant differences in body weight among the groups during the first ($p = 0.002$), second ($p = 0.006$) and third weeks ($p = 0.003$). The first week body weight for the Tac group was significantly greater than for the control ($p = 0.01$) and Tac + AGHMB ($p = 0.003$) groups. No significant difference in first week body weight was found between the Tac and Tac+ honey groups. The second week body weights for the Tac group were significantly greater than for the Tac + AGHMB group ($p = 0.006$). The second week body weight for the Tac + honey group was significantly greater than for the Tac + AGHMB group ($p = 0.035$). We found no significant difference in second week body weights between the Tac and Tac + honey groups. Third week body weight of the Tac + honey group was significantly greater than for the Tac + AGHMB group ($p = 0.01$). We found no significant difference in third week body weights among the control, Tac and Tac + honey groups.

Intra-group body weight changes also were evaluated. Significant increases were found between the weekly body weight measures for the control group (1st week vs. 2nd week, $p = 0.012$; 1st week vs. 3rd week, $p = 0.012$). We found significant decreases between the weekly body weight measures for the Tac group (1st week vs. 3rd week, $p = 0.002$; 2nd week vs. 3rd week, $p = 0.002$). We also found significant

increases between the weekly body weights for the Tac + honey group (1st week vs. 2nd week, $p = 0.004$; 1st week vs. 3rd week, $p = 0.004$; 2nd week vs. 3rd week, $p = 0.015$). We found no significant difference between the weekly body weight changes for the Tac + AGHMB group ($p > 0.05$ for 1st week vs. 2nd week, 1st week vs. 3rd week and 2nd week vs. 3rd week). Details for body weights for all groups are presented in Table 1.

Histopathology

The control group exhibited normal histological structure (Figure 1a). In the Tac group, the submucosa exhibited numerous dilated blood vessels, the glands were dilated at the site of anastomosis and inflammatory cell infiltration in the lamina propria and submucosa were evident (Figure 1b–d). Histopathological differences between the control and Tac groups were statistically significant ($p < 0.05$). In the Tac + honey and Tac + AGHMB groups, the colon tissue appeared nearly normal (Figure 1e,f). The amount of inflammatory cell infiltration in the Tac ($p < 0.05$), Tac + honey ($p < 0.005$) and Tac + AGHMB ($p < 0.005$) groups was significantly greater than the control group. Collagen deposition in the Tac ($p < 0.01$), Tac + honey ($p < 0.001$) and Tac + AGHMB ($p < 0.001$) groups was significantly greater than the control group. We found no significant difference in the amount of inflammatory cell infiltration or collagen deposition among groups. Neovascularization in the Tac ($p < 0.05$), Tac + honey ($p < 0.001$) and Tac + AGHMB ($p < 0.001$) groups was significantly greater than the control group. Neovascularization in the Tac + honey ($p < 0.01$) and Tac + AGHMB ($p < 0.01$) groups was significantly greater than for the Tac group. Neovascularization in the Tac + honey group was significantly greater than for the Tac + AGHMB group ($p < 0.05$). Fibroblast proliferation in the Tac ($p < 0.01$), Tac + honey ($p < 0.001$) and Tac + AGHMB ($p < 0.005$) groups was significantly greater than for the control group. Fibroblast proliferation in Tac + honey ($p < 0.05$) and Tac + AGHMB ($p < 0.05$) groups was significantly greater than for the Tac group. We found no significant difference in infiltration, fibroblast proliferation and collagen deposition between the Tac + honey and Tac + AGHMB groups. Neovascularization in Tac + honey group was significantly greater than for the Tac + AGHMB ($p < 0.05$). The histopathological evaluation score for each group is presented in Table 2.

Immunohistochemistry

TGF-β1 immunostaining in the control group showed weakly stained normal cells. In the Tac group, TGF-β1 immunostaining was evident in connective tissue. In the same areas in Tac + honey group, the intensity of TGF-β1 immunostaining increased compared to the Tac group ($p < 0.05$) (Figure 2). Slightly increased intensity of TGF-β1 immunostaining in the Tac + AGHMB group was not statistically significant compared to the Tac group. The TGF-β1 immunostaining scores are presented in Table 3.

Discussion

Our investigation addresses a common clinical problem. More than 200 liver transplantation procedures are performed annually at our center, and

Table 1. Comparison of experimental groups in terms of body weight, anastomotic bursting pressure and adhesion score.

Parameters	Control	Tac	Tac + honey	Tac + AGHMB
Weight at 1 week				
Mean ± SD	261.6 ± 20.2	304 ± 28.6	271.2 ± 25.9	257.7 ± 24.4
Median	262.5	307.5	274.5	263
Min.-max.	231–288	270–370	217–309	208–293
Weight at 2 weeks				
Mean ± SD	277.5 ± 22.3	291.2 ± 21.7	283.2 ± 27.6	258.2 ± 15.5
Median	274.5	288.5	284.5	263
Min.-max.	243–309	256–326	230–330	238–287
Weight at 3 weeks				
Mean ± SD	289.2 ± 8.5	274 ± 17.1	294.3 ± 19.4	266.4 ± 19.5
Median	288.5	268.5	295.5	265
Min.-max.	282–296.3	263–284.8	282–306.6	244–303
Bursting pressure				
Mean ± SD	217.6 ± 20.7	116.3 ± 43.8	144 ± 38.4	168.3 ± 33.2
Median	220.5	110	142.5	180
Min.-max.	189–241	48–210	84–220	110–220
Adhesion score				
1 point	4	5	9	3
2 point	4	6	3	8
3 point	0	1	0	0

Weights are in grams. Bursting pressure is in mm Hg. n = 12 for each group. Weight at 1 week: Tac vs. Tac + AGHMB ($p = 0.003$), Tac vs. control ($p = 0.01$). Weight at 2 weeks: Tac + AGHMB vs. Tac + honey ($p = 0.035$), Tac + AGHMB vs. Tac ($p = 0.006$). Weight at 3 weeks: Tac + AGHMB vs. Tac + honey ($p = 0.01$). Bursting pressure: control vs. Tac ($p < 0.01$); control vs. Tac + honey ($p = 0.005$).

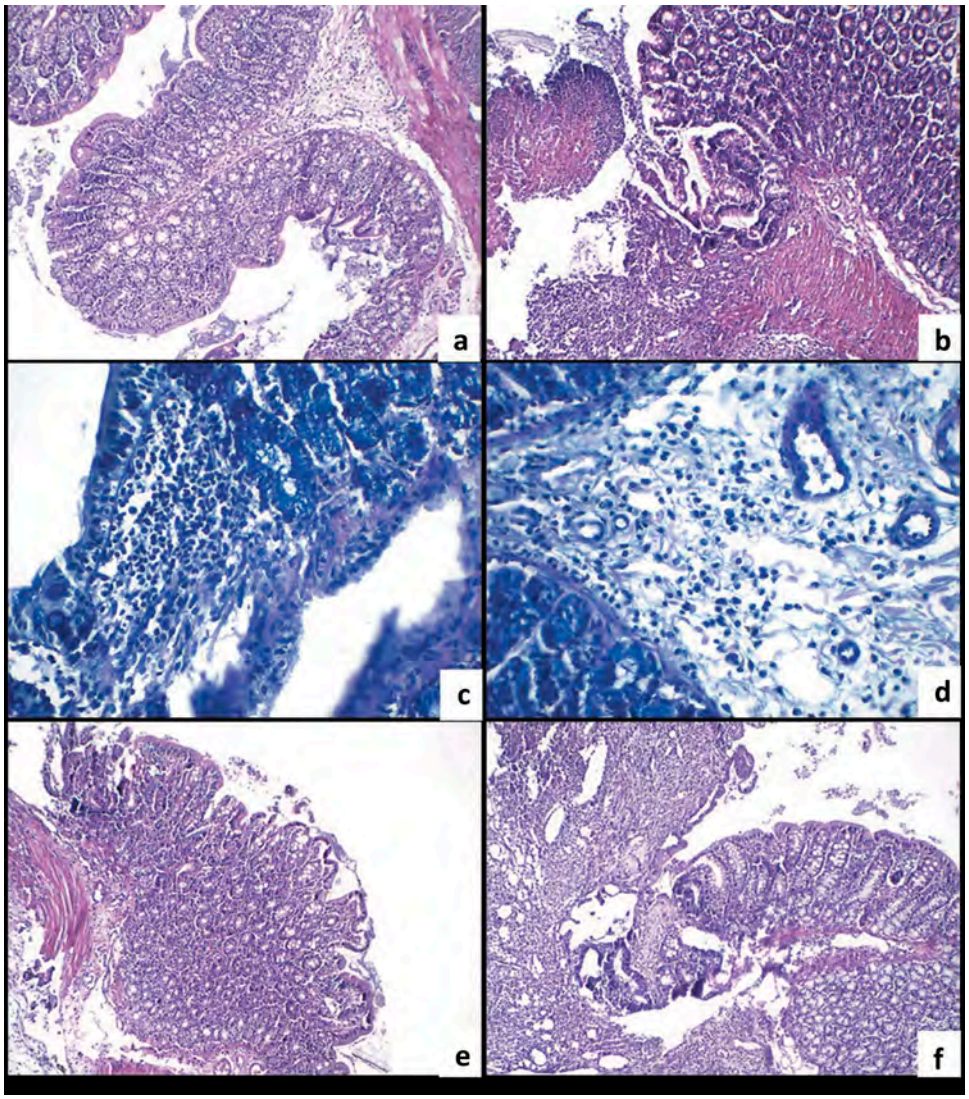

Figure 1. Histological appearance of colon tissues. a) Control group showing normal histological appearance. H & E staining. 10 x objective lens. b) TAC group showing increased fibroblasts and collagen fibers in the submucosal layer. H & E staining. 10 x objective lens. c,d) Tac group showing inflammatory cell infiltration in the lamina propria and submucosa. PAS-Alcian blue staining. 40 x objective lens. e) TAC + honey group showing fibroblast proliferation and synthesis of new collagen in the submucosal layer. H & E staining. 10 x objective lens. f) TAC + AGHMB group showing histological findings similar to TAC + honey group. H & E staining. 10 x objective lens.

Table 2. Histopathological evaluation scores of experimental groups.

Score	Control	Tac	Tac + honey	Tac + AGHMB
Inflammatory cell infiltration	0.00 ± 0.00	0.50 ± 0.32[a]	0.87 ± 0.22[b]	0.62 ± 0.26[b]
Fibroblast proliferation	0.00 ± 0.00	0.75 ± 0.25[c]	1.25 ± 0.25[d, e]	1.12 ± 0.29[b, d]
Neovascularization	0.00 ± 0.00	0.50 ± 0.18[a]	1.62 ± 0.18[e, f]	1.12 ± 0.12[e– g]
Collagen deposition	0.00 ± 0.00	0.75 ± 0.30[c]	1.50 ± 0.18[e]	1.50 ± 0.18[e]

Data are means ± SD. [a]$p < 0.05$ vs. control, [b]$p < 0.005$ vs. control, [c]$p < 0.01$ vs. control,[d]$p < 0.05$ vs. Tac [e]$p < 0.001$ vs. control, [f]$p < 0.01$ vs. Tac, [g]$p < 0.05$ vs. Tac + honey

Tac is the main immunosuppressive agent. Gastrointestinal complications including hollow organ perforation, anastomotic dehiscence and anastomotic leakage may develop during the early or late postoperative periods in these patients. Most of these problems require gastrointestinal anastomosis or anastomosis repair. It has been suggested that to achieve optimal anastomotic healing in transplant patients with colon anastomosis, the dose of immunosuppressive agent should be reduced or stopped at certain stages (Uysal et al. 2017); however, this increases the risk of rejection of the liver graft.

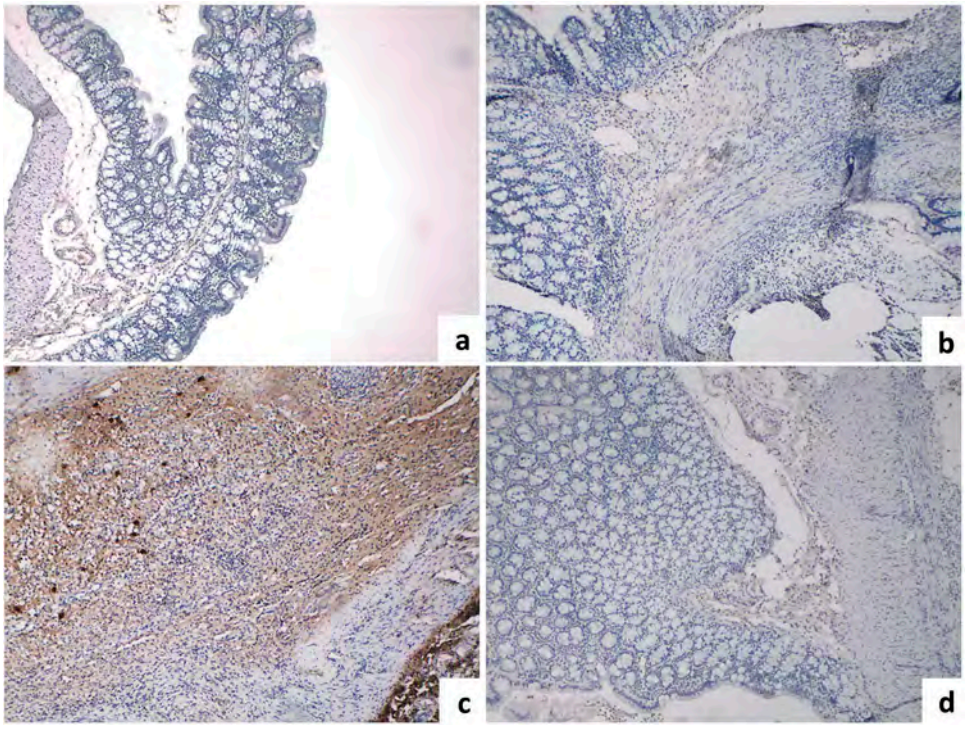

Figure 2. Immunohistochemical staining of TGF-β1. a) Control group showing weakly stained normal cells. TGF-β1 staining. 10 x objective lens. b) Tac group showing TGF-β1 immunostaining in connective tissue. TGF-β1 staining. 10 x objective lens. c) Tac + honey group showing intensity of TGF-β1 immunostaining greater than the TAC group. TGF-β1 staining. 10 x objective lens. d) TAC + AGHMB group showing less intensity of TGF-β1 immunostaining than for the Tac + honey group. TGF-β1 staining. 10 x objective lens.

Table 3. TGF-β1 immunostaining score of all experimental groups.

Score	Control	TAC	TAC + honey	TAC + AGHMB
TGF-β1 immunostaining	0.25 ± 0.16	0.62 ± 0.18	1.37 ± 0.18[a,b]	0.87 ± 0.22

Data are means± SD. [a]$p < 0.005$ vs. control, [b]$p < 0.05$ vs. Tac

Colorectal anastomosis leaks are the leading cause of postoperative morbidity and mortality. Rates of anastomosis leaks between 3 and 6% generally are regarded as acceptable; the actual rates reported in the literature range from 0.5 to 30% (Raptis et al. 2012, 2018; Yaman et al. 2013). Several factors influence leaks; nutritional status is a major factor that affects wound healing (Bozkırlı et al. 2015).

Arginine is an amino acid that is required for patients who undergo major surgical procedures or trauma (Yaman et al. 2013; Bozkırlı et al. 2015). Dietary intake of arginine creates a positive nitrogen balance, increases the tissue hydroxyproline level, promotes collagen accumulation at the bowel anastomosis line, exerts an antioxidant effect and promotes anastomotic healing (Yaman et al. 2013). Arginine also participates in the inflammatory phase of anastomotic healing.

Glutamine is an amino acid that serves as an energy source for gastrointestinal epithelial and immune system cells (Yaman et al. 2013; Bozkırlı et al. 2015). Despite its abundance, its level is reduced by trauma, infection and major surgical procedures (Yaman et al. 2013; Bozkırlı et al. 2015). Glutamine also participates in nucleotide synthesis in fibroblasts and macrophages, and it also is a strong immunomodulatory agent and therefore hastens wound healing (Yaman et al. 2013; Bozkırlı et al. 2015).

HMB is formed during the metabolism of leucine; it participates in regulating protein synthesis and maintaining nitrogen balance (Yaman et al. 2013; Bozkırlı et al. 2015). The AGHMB mixture of arginine, glutamine and HMB is widely used clinically for tumor cachexia, exercise-induced muscular injury and trauma (Seker et al. 2013; Yaman et al. 2013; Bozkırlı et al. 2015; Kusabbi et al. 2015). We have found only three reports, however, concerning the effects of AGHMB on wound healing using an experimental anastomosis model; none of these used an immunosuppressed rat model (Seker et al. 2013; Yaman et al. 2013; Kusabbi et al. 2015).

Honey is a nutrient that has been widely studied using experimental wound healing models. Honey is made by bees from plant nectars and therefore exhibits seasonal and geographical variation. Honey exhibits a wide range of clinically useful effects (Basbug et al. 2011; Gollu et al. 2008; Gencay et al. 2008), although it is not entirely clear how it accelerates wound healing (Hadagali and Chua 2014). Nevertheless, the value of systemic or topical application of honey for healing gastrointestinal anastomosis has been reported for several experimental models (Gollu et al. 2008; Ergul and Ergul 2010; Saber 2010).

Problem-free wound healing is important for gastrointestinal anastomoses (Raptis et al. 2012). During the inflammatory phase of healing, neutrophils and macrophages migrate into the anastomosis line; optimal numbers are reached by about 48 h (Raptis et al. 2012). During the proliferative phase, neovascularization, fibroblast proliferation and migration, collagen synthesis, and crosslinking between collagen fibers occur (Raptis et al. 2012). Fibroblasts contribute to collagen synthesis and construction of extracellular matrix (Uysal et al. 2017). Fibroblasts are vital for forming a strong and durable anastomosis.

VGEF is important for regulation of neovascularization, while TGF-β is important factor for fibrogenic activity (Raptis et al. 2012). TGF-β1 is the main cytokine that stimulates VGEF synthesis. Changes in wound healing phases can be demonstrated by histopathological and immunohistochemical analysis. We found that the greatest TGF-β1 expression at the anastomosis line was in Tac + honey and Tac + AGHMB groups (Table 3).

Tac exerts immunosuppressive effects by inhibiting IL-2 gene expression and disrupting apoptosis and degranulation of leukocytes. Tac also exhibits an anti-inflammatory effect by reducing transcription of proinflammatory cytokines, TNFα, IL-1, IL-3, IL-4, IL-5, IL-6, and IL-8 (Raptis et al. 2012, 2018). Although it has been reported that Tac affects healing of dermal wounds negatively by reducing TGF-β expression and increasing TNFα level (Schaffer et al. 2005), but exhibited no negative effect on colon anastomosis, other reports indicate that Tac does not exhibit tissue-specific effects (Saber 2010; Uysal et al. 2017). Tac has been reported to increase TGF-β1 and VEGF release, however, which induces collagen synthesis and neovascularization (Raptis et al. 2012, 2018). Our findings are consistent with the latter effect. Our comparison of the Tac and control groups suggests that Tac induces collagen synthesis and neovascularization.

Development of postoperative adhesions is an important clinical issue; incidence has been reported to be up to 93% (Raptis et al. 2012). A variety of factors affect adhesion development. It has been reported that TGF-β reduced development postoperative adhesion (Raptis et al. 2012). We found no significant difference in total adhesion scores among the experimental groups. Spontaneously separating adhesions (score 1) were detected in 75% of the rats in the Tac + honey group, however, and separation of adhesion with traction (score 2) was detected in 66.6% of the rats in Tac + AGHMB group, which is consistent with TGF-β1 expression at the anastomosis line (Tables 1 and 2).

Anastomosis burst pressure is an important indicator of anastomotic healing and strength (Raptis et al. 2012). Burst pressure is determined by the amount of collagen and the crosslinks between collagen fibers at the anastomosis line (Raptis et al. 2012). We found that burst pressure was slightly lower in the Tac + honey group than for the Tac + AGHMB group, but the difference was not statistically significant. Both AGHMB and honey supplementation effected a marked improvement in anastomosis burst pressure.

Severe weight loss is a common problem following major surgical procedures (Raptis et al. 2012). Good nutrition is critically important for optimal healing of anastomosis. Inadequate nourishment decreases tissue collagen, which in turn may delay anastomosis healing (Raptis et al. 2012). We found that body weight increased in the Tac + honey group throughout our study, but remained stable in the Tac + AGHMB group. The difference in body weight for the two groups was consistent with our histopathological and immunohistochemical findings.

A limitation of our study was the lack of information concerning tissue hydroxyproline levels at the anastomosis line. Hydroxyproline is a major component of collagen; therefore, tissue hydroxyproline is a marker of collagen synthesis.

We found that both honey and AGHMB exhibited positive effects on anastomotic wound healing in rats that were immune suppressed by Tac.

Disclosure statement

No potential conflict of interest was reported by the authors.

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