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The effect of recombinant growth hormone on intestinal anastomotic wound healing in rats with obstructive jaundice

Tıkanma sarılığı oluşturulan ratlarda growth hormon kullanılmasının intestinal anastomoz iyileşmesi üzerine etkisi

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Background/aims: *Several clinical and experimental studies have shown that obstructive jaundice delays wound healing. Growth hormone may prevent delayed wound healing, since it has effects on the release of mediators in jaundice, as well as increasing the protein synthesis.* ***Methods:*** *Forty male Wistar rats were allocated to four groups: Group I (n=10): intestinal anastomosis to normal small bowel, Group II (n=10): intestinal anastomosis to normal small bowel followed by growth hormone therapy (2mg/kg/day, subcutaneously), Group III (n=10): intestinal anastomosis to obstructive jaundice rat's small bowel, Group IV (n=10): intestinal anastomosis to obstructive jaundice rat's small bowel followed by growth hormone therapy at the same dosage The animals were observed for seven days then killed. Intraabdominal adhesions, anastomotic complications and anastomotic bursting pressures were recorded and tissue samples from the anastomotic site were obtained to measure hydroxyproline levels and for histopathologic examination.* ***Results:*** *Growth hormone had a beneficial effect on the healing of intestinal anastomosis in both jaundiced and non-jaundiced rats. This was demonstrated by clinical and mechanical parameters such as a significant increase in anastomotic bursting pressure, hydroxyproline content and histopathalogical scores.* ***Conclusion:*** *Growth hormone reverses the adverse effects of obstructive jaundice on small bowel anastomotic healing. It can be hypothesized that this effect is due to augmentation of insulin-like growth factors, protection of hepatocytes, enhancement of intestinal epithelization, and reversal of the resultant malnutritional state caused by growth hormone in obstructive jaundice.*

Key words: Obstructive jaundice intestinal anastomotic healing, recombinant growth hormone.

Amaç: *Tıkanma ikterinin yara iyileşmesi üzerine olumsuz etkisinin olduğu klinik ve deneysel çalışmalarla gösterilmiştir. Growth hormon uygulamasının yara iyileşmesi üzerine olumlu etkilerinin olduğu bilinmektedir. Ancak tıkanma ikterinde intestinal yara iyileşmesi üzerine etkisi ile ilgili bir bilgi mevcut değildir. Hem protein sentezin artırıcı etkisi hem de tıkanma ikterinde salınan mediatörler üzerine etkisinden dolayı Growth hormon kullanılmasının yara iyileşmesi üzerine olumlu etkisi olabileceği düşünülerek bu çalışma planlanmıştır.* ***Yöntem:*** *40 erkek Wistar rat dört gruba ayrıldı: Grup I (n=10) normal ince barsağa anastomoz. Grup II (n=10) Normal ince barsağa anastomoz ve daha sonra 7 gün süreyle 2 mg/kg GH. Grup III (n=10) koledok ligasyonu ile tıkanma ikteri oluşturulmuş ratlara anastomoz. Grup IV (n=10) tıkanma sarılığı oluşturulmuş ratlara anastomoz ve 7 gün süreyle 2 mg/kg Growth hormon. Yedi gün takip sonunda hayvanlar sakrifiye edildiler. Yara enfeksiyonu, karın içi ve anastomoz bölgesi yapışıklıkları, anastomoz darlığı ve kaçakları, anastomoz patlama basınçları saptandı. Histopatolojik inceleme ve doku hidroksiprolin düzeyi için anastomoz hattından doku örnekleri alındı.* ***Bulgular:*** *Tıkanma sarılığının intestinal yara iyileşmesini olumsuz etkilediği ve bu etkinin growth hormon kullanılması ile önlenebildiği hem klinik hemde mekanik parametrelerle gösterildi.* ***Sonuç:*** *Growth hormon tüm vücutta anabolik etkiye sahiptir. Yara iyileşmesinin temel unsurlarından biri olan protein sentezini artırır. Ayrıca hepatositleri koruyucu etkisi yanısıra intestinal epitelizasyonu artırıcı etkisinin de yara iyileşmesinde rolü olduğu düşünülmektedir.*

Anahtar kelimeler: Growth hormon, tıkanma sarılığı.

INTRODUCTION

It is well known that obstructive jaundice (OJ) has a deleterious effect on wound healing. Several experimental and clinical studies have shown that the incidence of delayed healing of surgical wounds impaired peritoneal healing, wound dehiscence, and compromised immunological function are high in OJ patients (1-4). The possible causes of high mortality and morbidity in OJ patients are hyperbilirubinemia, malnutrition and intestinal endotoxemia (5-6).

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Recombinant growth hormone (GH, Genotropin“ Gonotropin recombinant 16IU (5.3 mg), Pharmacia & UpJohn AB, Stocholm, Sweden Genotropin), which is produced from E.Coli cultures, has an anabolic effect on the whole body. It increases protein synthesis in ribosomes by increased production of mRNA in the nucleus. It stimulates fibroblastic activity and procollagen synthesis during the initial phase of wound healing (7). Different types of wound healing models in rats have been studied and GH was found to have a beneficial effect on wound healing (8-9). However, there is no available data in the literature regarding the effect of GH on the healing of intestinal anastomosis in OJ. This study was therefore designed to investigate the effect of GH on the healing of intestinal anastomosis in rats with OJ.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing 200-300 g were used. They were allowed free access to water and standard laboratory chow and were kept under controlled environmental conditions. Prior to surgery, the rats were allowed free access to only water for 12 hours. The procedures followed in this study were in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Instıtutes of Health publication no 86-23, revised 1985).

Surgical Procedures

All animals were anesthetized with an intramuscular injection of 40 mg/kg ketamine (Ketalar, Parke Davis and Co.Inc). Abdominal access was achieved through a midline incision 4 cm long, and in order to minimize evaporation from the tissue, the intestines were covered with moist clean gauze pads. Body temperature was maintained at 37°C by use of a heating lamp.

Surgery

A total of 40 rats were used. In group I (n=10), laparotomy was performed as described; these rats served as a control group. After a 1 cm segment had been resected from 10 cm above the cecaum, end-to-end anastomoses were performed using interrupted 6-0 polypropylene sutures. In group II (n=10), all rats underwent the same procedure as in group I. Postoperativelly, these rats received a daily subcutaneous injection of 2 mg/kg/day of GH for seven days.

In group III (obstructive jaundice group n=10), surgery was performed twice: The aim of the first operation was to establish obstructive jaundice whereby the common bile duct was ligated and divided above the duodenum following laparotomy. The abdomen was then closed with interrupted silk sutures. The rats were monitored for five days untill obstructive jaundice occurred and then a second procedure was performed whereby the abdominal wall was opened through the same midline incision. Bowel resection and anastomosis was performed as in Groups I and II. In Group IV (OJ + treatment with GH n=10) surgery was performed twice as in Group III. After the second operation, these animals also received a daily subcutaneous injection of 2 mg/kg/day of GH for seven days. The rats in each group were monitored for seven days then killed.

Treatment

The rats in Group I and III received daily injections of 2 ml of normal saline while the rats in Group II and IV were treated with a daily injection of 2 mg GH per kg of body weight which was administered once daily by subcutaneous injection in the nape of the neck. Treatment was commenced on the day of operation immediately after the skin incision was closed and continued for seven days.

Evaluation parameters

Wound complications, intraperitoneal adhesions, intestinal obstuctions and anastomotic complications (anastomotic stenosis, anastomotic dehiscence) were recorded.

Anastomotic bursting pressure

The strength of each anastomosis was assessed by measuring its bursting pressure using a fluid pump, operating at 1 ml/min with a pressure transducer. The fluid pump’s catheter was inserted into the bowel 2 cm above the level of anastomosis and the bowel was then ligated around the catheter. The other line, coming from transducer, was inserted into the bowel 2 cm below the level of anastomosis, and the bowel was then ligated around the catheter. The pressures were recorded in millimeters of mercury on a monitor. The pressure was observed and leakage was identified by a sudden loss of pressure.

Histopathology

Full-thickness sections of the intestinal anastomoses were obtained at necropsy, fixed in 10%

buffered formalin, embedded in paraffin, cut into 4-to-5 mm-thick sections and stained with hematoxylin and eosin. An experienced pathologist examined the tissue sections under light microscopy in a blinded fashion. Epithelization, cellular infiltration, fibroblastic proliferation, collagen deposition and neovascularization were graded from 0-2 (0= absent, 1=mild to moderate, 2=marked) as modified from Greenhalgh et al (10). Scores were then totalled and averaged to give a mean histological score.

Anastomotic hydroxyproline content

Anastomotic hydroxyproline content was measured by *spectrophotometric determination of hydroxyproline method as* described by Bergman (11): all the tissues were washed out with cold saline solutions and dried on filter papers and cut to weigh 40-50 mg on an electronic scale. The tissues were placed in hydrolysis tubes, concentrated HCl equal in volume of 5.0 mM potassium phosphate at a pH of 7.0, followed by hydrolysis for 16 h. The samples' pH was adjusted to 8.0-8.5 by using a dilute solution of NaOH or HCl as necessary. The samples were then mixed in a test tube with chloramine-T solution (0.1 ml). After four minutes, 2 ml of aldehyde/perchloric acid solution was added and the mixture shaken thoroughly and the tubes then immersed into a water bath to keep the temperature at 60°C for 25 minutes. The tubes were then removed from the water bath, cooled under running tap water and the color then read in a spectrophotometer at a wavelength of 560 nm. Results were expressed as mg hydroxyproline/ gram of wet tissue.

Statistical Analysis

The data were reported as the mean ± SE. The differences between the groups were analyzed by the Kruskal-Wallis test followed by Mann-Whitney U test. Probability values $p<0.05$ were considered significant.

RESULTS

There was no mortality in groups I, II and IV. All rats in these groups remained healthy and did not lose weight before they were killed. Four rats in group III, however, died in the postoperative period. The necropsy finding showed that the cause of death was either anastomotic dehiscence or intestinal obstruction.

Table 1. Mean bursting pressures, histologic scores and hydroxyproline levels of groups.

Groups	*Mean bursting pressure (mmHg)±SEM*	*Mean histologic scores±SEM*	*Mean hydroxyproline level (µg/mg-wet tissue) ±SEM*
Group I (n=10)	210.2±15.3	6.3±0.8	1.6±0.6
Group II (n=10)	270±12.6	7.8±1.2	2.17±0.8
Group III (n=10)	190±18.5*	4.5±1.3**	1.2±0.5***
Group IV (n=10)	290±14.6	8±1.2	2.2±0.4

Kruskal-Wallis Test: X2=12.250, DF=3, P=0.01
* p<0.05 between group III and IV, group III and II
** p<0.01 between group III and IV, group III and II
*** p<0.05 between group III and IV, group III and II

Gross findings at necropsy

The rats in Group I and II had minimal intraabdominal adhesions and no intestinal obstruction or anastomotic dehiscence. They also had normal-appearing bowels when they were killed on the seventh post operative day seven. The bowels which had been subjected to OJ then intestinal anastomosis (group III) were pale and inflamed with extensive adhesions and areas of transmural intestinal ischemia. Four of eight rats in-group III had partial anastomotic stenosis and bowel dilatation on the proximal side of the anastomosis. In contrast, rats in group IV had minimal intraabdominal adhesions and minimal anastomotic stenosis. There was no intestinal obstruction or anastomotic dehiscence in these rats.

Anastomotic bursting pressure

Mean anastomotic bursting pressures of all groups are shown in Figure 1. The mean bursting pressure of the anastomosis in group I was lower than group II but it failed to reach statistical significance ($p>0.05$, Table I). The mean bursting pressure of the anastomosis in group III was lower then the other groups; there was a significant difference between group III and II and between group III and IV ($p<0.05$). The GH therapy groups (group II and IV) had similar bursting pressure values.

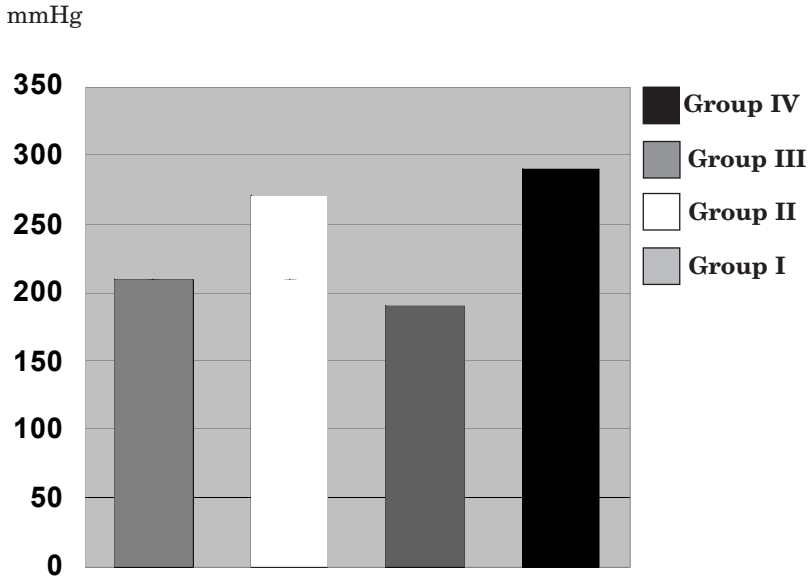

Figure 1. Mean anastomotic bursting pressures of groups (mmHg)

Anastomotic hydroxyproline content

Mean anastomotic hydroxyproline content of group I was lower than group II ($p<0.05$, Table I); it was increased by 24% in rats treated with GH (group II) compared with the control rats. When intestinal anastomosis was performed after OJ (group III), hydroxyproline content was decreased by 20% compared to non-OJ rats (group I), although it was not significant. Treatment with GH for seven days significantly increased (82%) the mean hydroxyproline content of anastomosis in rats with OJ (groupIV) compared to non-treated OJ rats (group III, $p<0.05$). No difference was found in the content of hydroxyproline between GH treatment groups (group II and IV). Mean anastomotic hydroxyproline levels of all groups are shown in Figure 2.

Histologic evaluation

Mean histologic scores of all groups are shown in Figure 3. In Group I rats, microscopic examination of full-thickness biopsies of the small bowel anastomosis site revealed moderately thick granulation tissue, moderate neovascularization and epithelial migration. There was moderate to severe cellular infiltration and fibroblast and collagen deposition. Mean scores were found to be 6.3±0.8 (range 4.2-8.1) in this group.

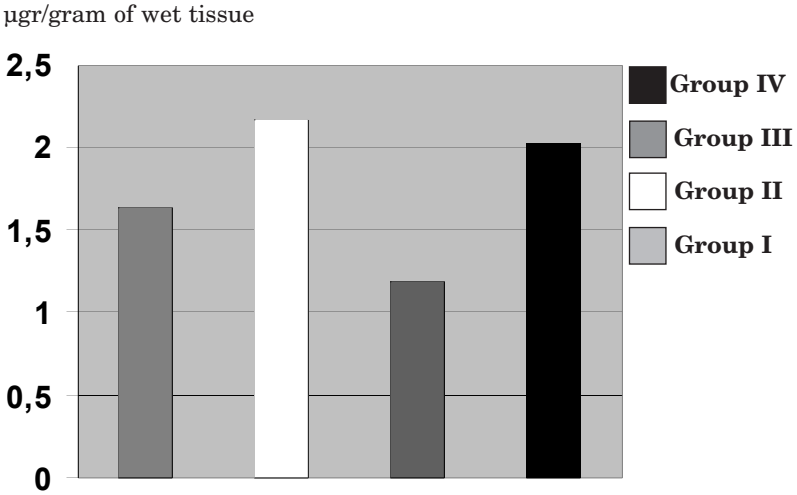

Figure 2. Mean anastomotic hydroxyproline content of groups (µgr/gr of wet tissue).

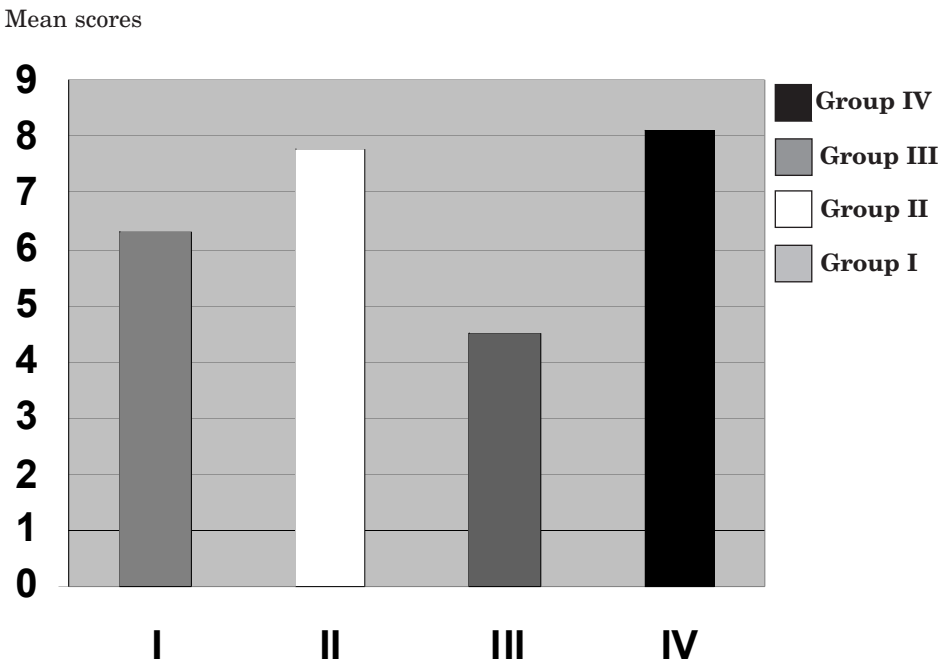

Figure 3. Mean histologic scores of groups.

Fibroblast and collagen deposition was more severe in Group II rats when compared to Group I. They also had extensive neovascularization, moderate to severe epithelial migration and cellular infiltration. Most of them had almost complete wound healing. With these findings, mean histologic scores of Group II animals (7.8±1.2) were significantly higher than Group I ($p<0.01$).

Group III rats had the least intestinal anastomotic healing, histologically, compared to other groups. Histologic examinations of most of the intestinal anastomosis in this group revealed minimal epithelization, cellular infiltrations and neovascularization which the important indicators of wound healing. Fibroblast and collagen deposition was also weak in this group. Most of the animals had poor intestinal healing and mean scores were 4.5±1.3 (range 2.8-5.5).

The rats in group IV had almost the same histologic findings as group II, with a mean score of 8.0 (range 6.6-9.2).

Mean bursting pressures, histologic scores and hydroxyproline content of all groups are shown in Table 1.

DISCUSSION

Postoperative morbidity and mortality in patients with OJ is still high despite recent advances in perioperative care (12-13). One of the problems is poor wound healing and a number of clinical studies have found an increased incidence of wound dehiscence and incisional hernias are in jaundiced patients (2-3). Bayer and Ellis also reported that OJ in rats resulted in delayed wound fibroplasia and angiogenesis, and a reduction in the mechanical strength of the healing abdominal incision (4).

Extensive systemic effects of jaundice such as upregulations of cytokines, alterations in immune response and hepatic disfunction due to inhibition of Kupffer cell activity may explain the impaired wound healing seen in OJ. It has been shown that hepatic production of Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6), which are considered to be the main mediators in initiating and sustaining the inflammatory cascade, are increased in OJ (14-15). Also, TNF-α stimulates collagenase from fibroblasts, which decreases collagen transcription and procollagen level (16). TNF-a may inhibit protein synthesis by impairing the signalling tranduction pathway involved in stimulation of protein synthesis (17). Fan et al. showed that TNF-α is an important regulator of the GH-insulin-like growth factor axis in vitro and that TNF-α infusion substantially reduced plasma levels of GH and IGF-1 (18). These adverse effects of obstructive jaundice-related cytokines

and especially TNF-α, may contribute to the impaired anastomotic bursting pressure, decreased hydroxyproline levels and impaired healing of the intestinal mucosa in rats with OJ in our study.

GH decreases type I acute-phase protein a1-acid glycoprotein and the pro-inflammatory cytokine IL-1β. This decrease has been associated with increased total hepatic protein concentration and constitutive hepatic protein synthesis (19). GH activates IGF-1 expression and improves wound healing in children with large cutaneous burns by stimulating skin IGF-1 protein and skin IGF-1 receptor concentration (20). IGFs are also known to stimulate the replication of human fibroblasts in vitro, which in turn might positively affect anastomotic healing in the small intestine.

Major surgery is characterized by a hypermetabolic state with increased oxygen consumption, negative nitrogen balance and weight loss. Endogenous GH, which normally counteracts these derangements, decreases following trauma or an operation and lack of anabolic effect of GH jeopardizes the wound healing during the early postoperative days where the strength of the anastomosis is at its lowest point (7,21). Administration of GH after trauma has also been shown to attenuate injury-induced catabolism, enhance immune function, improve wound healing and diminish the hypermetabolic response (22). Furthermore, GH reduces nitrogen loss in malnourished patients undergoing gastrointestinal operation and in fasting volunteers (23-25). It also enhances skin wound healing in malnourished rats, attenuates TNF-α in burned children and has a beneficial effect on the acute-phase response (26-27).

In this study, significant improvement in small intestinal anastomotic healing was demonstrated following GH treatment (2 mg/kg daily, for seven days postoperatively) despite the established adverse features of jaundice on wound healing (4). The dose of GH administered in this experiment (2 mg/kg/day) was obtained from previous studies by Christensen where the author established dose-response curves (8,28). Enhancement of intestinal anastomotic strength was associated with a marked increase in the hydroxiproline content of the anastomosis, which is a direct reflection of collagen accumulation in the wound. Moderate to complete anastomotic healing was observed histologically in rats which received GH, whereas poor anastomotic healing was noted in rats with OJ which had not received GH.

A previous report has shown that GH increases bursting strength in left colonic anastomosis in rats (8,28). Silver also demonstrated that GH increases the breaking strength of colonic anastomosis and stimulates the collagen deposition rate of the anastomotic segment while having no effect on hydroxyproline concentration (29). Silver later reported that GH causes a significant increase in connective tissue thickness of bowel anastomosis (9).

In conclusion, the data in this study demonstrates that OJ impairs intestinal anastomotic healing and that GH can increase the breaking strength and hydroxyproline content of the small intestinal anastomosis in jaundiced rats. Further studies are needed to explain the effect of GH on anastomotic wound healing. Optimal dosage and careful patient selection as a focus of future clinical studies in which cost and toxicity are the primary concerns would be appropriate.

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