

## ARAŞTIRMA

Mevlut Pehlivan<sup>1</sup>  
Sami Doğan<sup>1</sup>  
Mine Kıvrak<sup>1</sup>  
Aysel Güven<sup>2</sup>  
Omer Ozcan<sup>3</sup>  
Meryem Cam<sup>2</sup>  
Mustafa Gultepe<sup>3</sup>

<sup>1</sup>Duzce University Duzce School of Medicine Department of General Surgery; Konuralp-Duzce /Turkey

<sup>2</sup>Duzce University Duzce School of Medicine Department of Histology; Konuralp-Duzce /Turkey

<sup>3</sup>GATA Haydarpaşa Military Hospital Department of Biochemistry; Istanbul/Turkey

### Yazışma Adresi:

Dr. Sami Doğan

Duzce University Duzce School of Medicine Department of General Surgery; Konuralp-Duzce /Turkey  
Email: samidogan@hotmail.com

### Konuralp Tıp Dergisi

e-ISSN1309-3878

konuralptipdergi@duzce.edu.tr

konuralpgeneltip@gmail.com

www.konuralptipdergi.duzce.edu.tr

## The Effect of L-Thyroxine on Colonic Anastomosis Healing By Means of Interactions with Insulin Like Growth Factor-I

### ABSTRACT

**Backgrounds:** Thyroid hormones play a critical developmental and regulatory role on the morphology and biochemistry of gastrointestinal mucosal cells and wound healing. The aim of this study is to determine the effect of L-Thyroxin on colonic anastomosis healing.

**Method:** Thirty male Wistar Albino rats were equally divided (n=10) in to three groups. Group 1 was the sham group whereas groups 2 and 3 were made hypothyroid by administration of propylthiouracil 10mg/kg/day per oral for four weeks. Hypothyroidism was confirmed with measurement of free-T3 and free T4 levels. All groups were subjected to left colonic transection and anastomosis. Group 3 rats have taken L-thyroxin therapy intraperitoneally 5µg in 1cc saline daily for seven days. Anastomosis bursting pressure, hydroxyproline and IGF-I Ab-1 measurements were done on postoperative 7th day.

**Results:** The anastomosis bursting pressure levels of group 3 were significantly higher than both group1 (p=0,03) and group2 (p=0,002). Hydroxyproline levels were significantly higher in group 3 than both group1 and group 2 (Group3 versus group1 p=0,003 and group 3 versus group 2 p=0,002). The IGF-1 Ab-1 staining integrity were higher in group3 versus group1 (P=0,008) and group2 (P=0,005) respectively.

**Conclusions:** Thyroid hormones improve anastomosis healing as documented increased hydroxyproline levels and anastomotic bursting pressure.

**Key words:** Hypothyroid, L-Thyroxin, Colon Anastomosis.

## L-Tiroksinin Insulin Like Growth Factor-I ile Etkileşimi ve Kolon Anastomozu İyileşmesine Üzerindeki Etkisi

### ÖZET

**Amaç:** Tiroid hormonları yara iyileşmesinde ve gastrointestinal mukozal hücrelerinin biyokimya ve morfolojisinde düzenleyici ve gelişimsel bir rol oynarlar. Bu çalışmanın amacı L-Tiroksinin kolon anastomozu iyileşmesine etkisini belirlemektir.

**Yöntem:** Otuz adet erkek Wistar-Albino sıçan eşit olarak üç gruba ayrıldı. Grup-1 sham grubu iken grup-2 ve grup-3, 10mg/kg/gün propiltiyoürasil verilerek hipotiroid yapılmış hayvanlardan oluşmakta idi. Hipotiroidi serbest T3 ve T4 ölçülerek kesinleştirildi. Bütün gruplara sol kolonik transeksiyon ve anastomoz yapıldı. Grup-3'teki ratlara L-tiroksin intraperitoneal olarak 5 µg, 1 cc izotonik içinde yedi gün verildi. Postoperatif yedinci günde anastomoz patlama basınçları, hidroksipirolin düzeyleri, IGF-I Ab-1 ölçümleri yapıldı.

**Bulgular:** Grup-3'ün anastomoz patlama basınçları grup-1 (p=0,03) ve grup-2'den (p=0,002) belirgin olarak yüksek idi. Hidroksiprolin düzeyleri grup-3'de grup-1 ve grup-2'den anlamlı yüksek izlendi (Grup-3 ile Grup-1 p=0,003; grup-3 ile grup-2 p=0,002). IGF-1 Ab-1 boyanma oranı grup-1 (p=0,008) ve grup-2'ye (p=0,005) göre grup 3'te daha yüksek bulundu

**Sonuç:** Yüksek anastomoz patlama basınçları ve hidroksipirolin düzeyleri ile gösterildiği üzere tiroid hormonu verilmesi kolon anastomozunun iyileşmesini olumlu etkilemektedir

**Anahtar kelimeler:** Hipotiroidi, L-Tiroksin, Kolon Anastomoz.

## INTRODUCTION

The problems related to colonic anastomosis healing have been one of the main concerns for surgeons recently. Although there are advances in nutritional support, bowel preparation, pre and post operative care, there is still a risk for the dehiscence of colonic anastomosis resulting with high mortality and morbidity. Surgery for colonic pathology is often performed under less than ideal conditions such as emergency, malnutrition, and sepsis, or in the setting of chemotherapy or radiation treatment (1). Studies revealed the dehiscence incidence ranging from 5 to 50 percent due to several contributing factors (2).

The pathological changes, factors that related to wound healing such as collagen content, growth factors and the strength of colonic anastomosis (tensile strength or bursting pressure) have been the main determinants of colonic anastomosis healing in experimental models in this field. Thyroid hormones exert a critical developmental and regulatory role on the morphology and biochemistry of gastrointestinal mucosal cells. The effects of thyroid hormones on wound healing (stress ulcer healing, healing of deep dermal burns) have been studied in several experimental models (2-4).

The aim of this study is to determine the effect of L-thyroxin on colonic anastomosis healing on the basis of hydroxyproline level, bursting anastomotic pressure and Insulin like growth factor level.

## MATERIAL AND METHODS

Thirty male Wistar Albino rats weighting 160-250g were obtained from the Duzce School of Medicine Experimental Study Laboratories and equally divided (n=10) into three groups. Each randomly assigned rat was kept in separate wire-bottom cages to prevent coprophagia and to obtain ideal therapeutic drug installation. All conditions and procedures were approved by the Duzce School of Medicine Ethical Committee. All rats were treated according to criteria of "Guide for the care and use of the laboratory animals".

**Study design:** This study consisted with three groups each containing ten randomly assigned rats.

**Operative procedure (Group1, 2 and 3):** After 12 hours fasting period rats in all groups were anaesthetized with intraperitoneal ketamine (30mg/kg) and the operative procedure was undertaken through a midline laparotomy. The distal colon was transected 3cm proximal to pelvic brim. A single-layer, inverting, end-to-end anastomosis was constructed with eight 6-0 interrupted prolene sutures (Ethicon, Inc., Sarnerville, NJ-USA). The laparotomy was closed, and rats were allowed to awaken and returned to their cages. Postoperatively rats were fed in standard fashion.

**Drug administration:** Group 1 (Control group) 1ml/day intraperitoneal saline administrations were

applied postoperatively. Group 2 (hypothyroidism group) received 10mg/kg/day Prophylthiouracil (Solvay-Dr.F.Frink) for four weeks. Group 3 (hypothyroidism + L-Tiroxin replacement group) received 10mg/kg/day Prophylthiouracil (PTU) (Solvay-Dr.F.Frink) for four weeks. Hormonal status was confirmed in both group 2 and 3 with measurement of Free T3 and Free T4 levels from blood samples obtained from tail veins of subjects. While group 2 rats were receiving PTU with the same dose postoperatively; PTU has been stopped and L-Thyroxin 5µg in 1cc saline administered intraperitoneally daily for seven days in group 3 postoperatively.

**Reoperation:** Rats were re-operated on post-operative 7<sup>th</sup> day under intraperitoneal ketamine anaesthesia. No mortality or morbidity has been observed until the second operation. Following laparotomy the colonic anastomosis region was explored for a major anastomosis dehiscence. The colonic segment 3 cm from each side of anastomosis was resected without any attempt to release anastomosis from adhesions around.

**Serum Hormone levels:** Following colonic resection, two milliliter of blood sample was drawn for FT3 and FT4 measurements.

**Bursting pressure:** After obtaining the samples, the rats were euthanized by ether overdose. The colonic lumen was cleared with isotonic saline off the colonic contents. A closed loop system was created by indwelling and tying two separate catheters through each end of the resected colonic segment. One catheter was used for the air insufflations and the other for the pressure measurement. The colonic segment was then sunk in an isotonic saline solution filled tub. Upon insufflations of air through one of these catheters at a rate of 5ml/minute, a water column attached to the other catheter began to increase. The peak level of water column immediately before a sudden fall or the highest level before air bubbles came out was considered as bursting pressure as cm H<sub>2</sub>O. Three same amount of separate strip shaped 1cm long colonic tissue samples, including and 0,5cm proximal and distal anastomotic sites were taken for measurement of tissue hydroxyproline and insulin like growth factor levels.

**Hydroxyproline content measurement:** Tissue samples were homogenised in ultrasonic homogenisator (Ultrasonic Processor GEX 600) with addition of 70 mg/ml isotonic saline and hydroxyproline levels were measured by using the spectrophotometric method described by Reddy & Enwemeka and normalized with protein levels determined by Lowry's protein measurement method (6).

**Immunohistochemical examination:** The tissue specimens obtained from the colonic anastomotic segment were fixed with 10% buffered formalin and embedded in paraffin. Subsequently, serial

cross-sections of 5µm thick were prepared and examined for the determination of anastomotic regions and mounted on a slide coated with poly-L-lysine. After deparaffinization, sections were stained immunohistochemically with an indirect method using were applied with IGF-I Streptavidin-Biotin-Peroxidase method using IGF-I Ab-1 mouse monoclonal antibody (Insulin like growth factor-I.) (Clone M23). Negative controlled staining with PBS (Phosphate-buffered-saline) instead of primary anti-body was applied in all groups. The staining intensity with IGF-I Ab-1 were scored as grade 0 (none), 1 (weak), 2 (moderate), 3 (strong), 4 (very strong).

**Statistical Analysis:** All the statistical analysis were done with SPSS (Statistical Package for Social Sciences) for Windows 12.0 software. In addition to the descriptive statistics (i.e.: mean, standard deviation), Mann Whitney U Test and Kruskal Wallis analysis were methods of choice for parametric values in groups without normal distribution due to low sample size. Results were evaluated with 95% confidence interval and  $p < 0.05$  was considered significant.

## RESULTS

Day of final surgical procedure (postoperative 7<sup>th</sup> day after colonic anastomosis) both FT3 and FT4

results of group 1 and 3 were significantly higher than group 2 ( $p < 0.01$ ) (Table 1).

Hydroxyproline levels were significantly different between groups ( $p < 0, 01$ ) (Table2). Hydroxyproline levels were significantly higher in group 3 than both group1 and group 2 (Group3 versus group1 Mann Whitney  $U=8, 5$ ;  $P=0,003$  and group 3 versus group 2 Mann Whitney  $U=4$ ;  $P=0,002$ ) (Table 2).

Anastomosis bursting pressures were significantly different between groups (Kruskal Wallis=10, 6;  $P=0,005$ ). The anastomosis bursting pressure levels of group 3 were significantly higher than both group1 (Mann Whitney  $U=18, 5$ ;  $P=0, 03$ ) and group2 (Mann Whitney  $U=4, 5$ ;  $P=0,002$ ). Although there was a trend for an increased levels in group1, the anastomosis bursting pressure levels were not different between group 1 and group 2 (Mann Whitney  $U=24, 5$ ;  $P=0,166$ ) (Table 2).

The findings of immunohistochemical investigations with IGF-1 Ab-1 are summarized in table 2. The staining integrity differences between three groups of samples were significant (Kruskal Wallis=11, 28;  $P=0,004$ ). The IGF-1 Ab-1 staining integrity were higher in group 3 versus group 1 (Mann Whitney  $U=14, 5$ ;  $P=0,008$ ) and group 2 (Mann Whitney  $U=9, 5$ ;  $P=0,005$ ) respectively.

Table 1. Day of final surgery FT3 and FT4 levels

Groups	Free T3 (pg/ml) mean±SD	p	Free T4 (ng/dl) mean±SD	p
Group 1	3.69±1.2	0.001*	1.21±0.55	0.0001**
Group 2	0.89±0.36		0.16±0.38	
Group 3	4.16±0.66		1.82±0.75	

\*Kruskall Wallis test=16.3, \*\* Kruskall Wallis test= 17.01

Table 2. Results of mean hydroxyproline levels, anastomosis bursting pressure and IGF-1 Ab-1 staining integrity

Groups mean±SD	hydroxyproline/protein mg/g	Anastomosis bursting pressure (cm H2O)	IGF-I Ab-1 staining integrity
Group 1	76,30±36,07	199,00±38,13	1,30±0,48
Group 2	75,70±20,19	176,87±38,63	1,12±0,35
Group 3	137,44±33,10	240,00±23,97	2,66±1,12
p	<b>0,002*</b>	<b>0,005**</b>	<b>0,004***</b>
Group 1 vs Group 2	<b>U=35;p=0,657</b>	<b>U:24,50; p=0,166</b>	<b>U:33,0; p=0,388</b>
Group 1 vs Group 3	<b>U=8,5;p=0,003</b>	<b>U:18,50; p=0,03</b>	<b>U:14,50; p=0,008</b>
Group 2 vs Group 3	<b>U=4,0;p=0,002</b>	<b>U: 4,50; p=0,002</b>	<b>U: 9,50; p=0,005</b>

\*Kruskal Wallis test =12,46, \*\*Kruskal Wallis test =10,60, \*\*\*Kruskal Wallis test =11,28

## DISCUSSION

Despite the advances surgical care and techniques, there is still a relatively considerable risk of anastomotic leakage. Several experimental models have been studied the effects of thyroid hormones

on wound healing (3,4). Data acquired from this trials has drawn a question that whether the exogenous thyroid hormone supplementation preceding colonic surgery improves colonic

anastomosis healing and the risk of dehiscence? For this particular purpose, we investigated the effect of L-Thyroxin on colonic anastomosis healing in an experimental rat model which has propylthiouracil induced hypothyroidism. The FT3 and FT4 levels on the last surgical procedure day were higher in both group 1 and 3 than group 2, which in turn resembles the efficacy of intraperitoneal L-Thyroxin replacement

Anastomosis bursting pressure is one of the determinants of anastomosis healing in many experimental studies (2,7,8). It has been mentioned in a previous study that any differences due to any type of factor related to anastomosis tensile strength should be investigated after postoperative day 6 (9). Jiborn et al has shown that bursting pressure levels were not different in postoperative 7, 10, 14 and 28<sup>th</sup> days and the bursting pressure level of an anastomosis reaches the intact colonic bursting pressure level in postoperative day 7 (10). Thus we evaluated colonic anastomosis healing parameters on post-operative day 7. Our study revealed that L-Thyroxin replacement in hypothyroid subjects improves bursting pressure levels when compared with hypothyroid subjects. It is also conspicuous that bursting pressure levels of L-Thyroxin replacement group were higher than normal subjects.

Although hydroxiprolin does not reflect the anastomosis strength alone it has been shown that there is an interaction between hydroxiprolin content and anastomosis healing (11). L-Thyroxin replacement in hypothyroid subjects resulted with higher hydroxiprolin levels in anastomotic area. However hydroxiprolin levels were not different between hypothyroid and control groups which is in fact predictive that exogen L-Thyroxin replacement is the main cause of increased hydroxiprolin levels thus improved healing in the anastomotic region.

In vivo as in vitro, thyroid hormones play an important physiologic role in regulation of pituitary growth hormone (GH) secretion (12,13). Insulin-like growth factor (IGF-1), the major somatomedine, mediator of peripheral growth-promoting effects of GH has a negative feed-back role in the control of GH release as well as mediating tissue effects of GH (14,15). Thyroid

hormones play a permissive role in these actions, since pituitary cells from hypothyroid animals in culture are less sensitive than cells from euthyroid animals to the inhibitory effects of IGF-1 on GH secretion (16). The direct effect of thyroid hormones on IGF-1 production is additive rather than synergistic and thyroid appears to act independently from GH on the release of IGF-1 by the liver. L-Thyroxin replacement revealed a significantly increased staining intensity for IGF-1 by indirect immuno-histochemical staining with IGF-1 Streptavidine-Biotin-Peroxydase method using IGF-1 Ab-1 (Insulin like growth factor mouse monoclonal anti-body: clone M23). Thyroid hormones, L-Thyroxin in this current study, resulted with increased IGF-1 immunoreactivity in colonic anastomotic region when compared with hypothyroid or euthyroid subjects. Thyroid hormone replacement in hypothyroid subjects improves colonic anastomosis healing through increased IGF-1 production as a mediator for tissue effects of GH. Although there was a trend for a decreased IGF-1 immunoreactivity in hypothyroidism group when compared with control (group 1) this was not significant.

Hypothyroidism is the commonest endocrine thyroidal disease with 2-10 % prevalence. Especially it is an important endocrine disease in elderly population with its higher incidence and morbidity (17). Bergeron et al mentioned that hypothyroidism is associated with high morbidity and mortality after acute colonic surgical procedures in patients whose hypothyroidism were not documented preoperatively (18).

Lacking of this study can be the weight curve of the animals and serum albumin levels .We think it has to be need for further investigations.

In conclusion, thyroid hormones improve anastomosis healing mainly due to the increased biosynthesis of IGF-1 resembled by increased hydroxiprolin levels and anastomotic bursting pressure. Especially in elderly patients functional status of thyroid gland should be evaluated preoperatively, and if hypothyroidism ensues, should be corrected before colonic surgical procedures.

## REFERENCES

1. Moertel CG, Fleming TR, Macdonald JS, et all. Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: A final report. *Ann Intern Med* 1995;122(5):321-6.
2. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomosis. I. Bursting strength of the colon after left colon resection and anastomosis. *Am J Surg* 1978;136(5):587-94.
3. Hernandez DE, Walker CH, Mason GA. Influence of thyroid states on stress gastric ulcer formation. *Life Sci* 1988; 42(18):1757-64.
4. Lennox J, Johnston ID. The effect of thyroid status on nitrogen balance and the rate of wound healing after injury in rats. *Br J Surg* 1973; 60(4):309.
5. Verhofstad MHJ, Langr WP, van der Laak JAWM, Verhofstad AAJ, Hendriks T. Microscopic analysis of anastomotic healing in the intestine of normal and diabetic rats. *Dis Col Rec* 2001; 44(3):423-31.
6. Reddy GK, Enwemeka CS. A simplified method for the analysis of hydroxyproline in biological tissues. *Clin Biochem* 1996; 29(3):225-9.
7. Morgenstren L, Yamakawa T, Ben-Shoshan M, Lippman H. Anastomotic leakage after low colonic anastomosis. *Am J Surg* 1972; 123(1):104-9.
8. Demirer S, Sengul N, Inan A, Eroglu A, Bumin C, Kuterdem E. Effect of recombinant human granulocyte/macrophage colony-stimulating factor on the healing of colonic anastomosis in rats. *J Invest Surg* 2001; 14(4):221-5.
9. Fukuchi SG, Seeburger JL, Parquet G, Rolandelli RH. Influence of 5-Fluorouracil on Colonic Healing and Expression of Transforming Growth Factor-Beta 1. *J Surg Res* 1999; 84(2):121-6.
10. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomosis. III. Collagen metabolism in the colon after left colon resection. *Am J Surg* 1980; 139(3):398-405.
11. Zuidewijn DBW de Roy van, Hendriks T, Wobbes T, de Boer HHM. Cytostatics and anastomotic healing in the intestine: an experimental study on the effect of parenteral nutrition. *Eur Surg Res* 1992;24(2):103-11.
12. Hervas FG, Morreale de Escobar F, Escobar del Rey. Rapid effects of single small dose of L-Throxine and triiodo-L-thyroxine on growth hormone as studied in the rat by radioimmunoassay. *Endocrinology* 1975: 97(1):91-101.
13. Coiro V, Braverman LE, Christianson D, Fang SL, Goodman HM. Effect of hypothyroidism and thyroxine replacement on growth in the rat. *Endocrinology* 1979; 105(3):641-6.
14. Froesch ER, Schmid C, Schwander J, Zapf J. Action of insulin-like growth factors. *Annu Rev Physiol* 1985;47:433-67.
15. Dieguez C, Page MD, Scanlon MF. Growth hormone neuroregulation and its alterations in disease states. *Clin Endoc* 1988; 28(1):109-43.
16. Melmed S, Yamashita S. Insulin-like growth factor I action on hypothyroid rat and pituitary cells; suppression of triiodothyronine-induced growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology* 1986; 118(4):1483-90.
17. Tarkkinen P, Hagren V, Palenius T, Lovgren T. One-step quantitative thyrotropin assay for the detection of hypothyroidism in point-of-care conditions. *Clin Biochem* 2003;36(2):121-8.
18. Bergeron E, Mitchell A, Heyen F, Dube S. Acute colonic surgery and unrecognized hypothyroidism: a warning. Report of six cases. *Dis Col Rec* 1997: 40(7):859-61.