The Effects of Tamoxifen on the Wound Healing in Rats

TAMOKSİFENİN RATLARDA YARA İYİLEŞMESİ ÜZERİNE ETKİLERİ

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Summary

Forty-two adult female rats divided into two groups were used in this study, which were planned in order to ascertain the effects of tamoxifen on the wound healing. Treatment group received tamoxifen-admixed diet for 14 clays, while control group received standard mouse chow. Ventral and dorsal incisions, both having 2.5 cm length, were performed on the fifteenth day of this feeding protocol. Following surgery, skin samples from dorsal incisions were obtained for histopathological examination from the third (n-6), sixth (n=7) and tenth (n—7) day subgroups. All of the rats were sacrificed on the tenth day; ventral incisions were resected with adjacent intact skin having 1 cm width, breaking strength was measured using a 0.8 cm broad strip of this skin, and wound hydroxyproline content was determined in the remnant tissue.

Fibroblast count, collagen density, vascularity and epithelization degree were found to be significantly increased in the treatment group ($p < 0.05$). Although hydroxyproline level in the treatment and breaking strength in the control group were found to be increased, it was documented that these parameters did not significantly differ among groups ($p > 0.05$). In the light of these findings, we concluded that tamoxifen does not significantly effect wound healing.

Key **Words:** Tamoxifen, Wound healing

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Özet

Tamoksifenin yara iyileşmesi üzerine olan etkilerini belirlemek amacı ile planlanan bu deneysel çalışmada, 42 erişkin dişi rai kontrol ve tedavi grubu olarak ikiye ayrıldı. Kontrol grubu standart fare yemi ile beslenirken, tedavi grubuna tamoksifen içeren diyet 14 gün süreyle verildi. Bu beslemenin 15. gününde rotların karın ve sırt ciltlerinde 2.5 cm uzunluğunda kesiler yapıldı. Cerrahiyi izleyen 3. gün her gruptan 6'şaı; 6. ve 10. gün ise 7'şer farenin sırtlarında oluşturulan insizyonlardan histolojik inceleme için örnekler alındı. Tüm raflar 10. gün sakrifiye edildi; karın insizyonları 1 cm genişliğinde sağlam cilt ile birlikte çıkarıldı, 0.8 cm eninde bir parça ayrılarak kopma kuvveti ölçüldü, artan dokuda hidroksiprolin düzeyi belirlendi.

Histolojik incelemede; fibroblast sayısı ile kollajenizasyon, vaskülarite ve epitelizasyonun tedavi grubunda anlamlı olarak arttığı bulundu ($p <0.05$). Her ne kadar hidroksiprolin düzeyinin tedavi grubunda, kopma kuvvetinin ise kontrol grubunda daha yüksek olduğu bulunsa da; her iki değişken açısından iki grup arasında anlamlı bir farklılık olmadığı saptandı ($p > 0.05$). Bu bulgulara göre, tamoksifen'in yara iyileşmesini anlamlı derecede etkilemediği düşünüldü.

Anahtar Kelimeler: Tamoksifen, Yara iyileşmesi

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Tamoxifen have been used for various pathologies such as breast cancer, retroperitoneal fibrosis, desmoid tumours and some types of pancreatic neoplasms. Although there are no data for Turkey, about 6 million individuals per year use tamoxifen for various conditions in United States (1-3). It is clear that various urgent and elective surgical interventions may be required in individuals using already tamoxifen.

To our knowledge, there is no study in the literature, which hivestigated the effects of tamoxifen on the wound healing. To shed some light on this issue, considering that it may be clinically important, this experimental animal study was undertaken to determine the effects of tamoxifen on wound healing.

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Materials and Methods

Forty-two adult female Wistar albino rats weighing 180-220 gr were used in this study. Animals were randomly divided into two groups: twenty-one rats received tamoxifen-admixcd diet (0.5 mgr/kg diet) for 14 days (treatment group), while the remaining twenty-one were fed with standard diet (control group) in addition to ad libitum water.

On the 15th day of this feeding protocol, general anaesthesia was provided by intramuscular injection of 25 mgr/kg ketamine (Ketalar flacon 50 mgr/ml, Eczacibasi) and 3 mgr/kg xylazine (Rhompun flacon 20 mgr/ml, Bayer). After shaving and cleaning with 10% povidone iodine, all of the rats were subjected to both a ventral and a dorsal incision, which were down to the fascial layers and had a length of approximately 2.5 cm. The incisions were closed with 4/0 silk as interrupted sutures 0.5 cm apart. Animals received their diet as mentioned above thereafter.

The main two groups were further divided into three subgroups, each of which included seven rats. These subgroups were respectively anaesthetised on the postwounded 3th, 6th and 10th days, and samples from dorsal incisions (including a part of intact skin) were harvested. These samples were fixed in 10% formalin and embedded in paraffin, sections were stained with hemotoxylene-eosin, Manson trichrom or van Gissen to examine with light microscope. A single pathologist performed histopathological examinations in a blinded fashion. Polimorphonuclear lymphocyte, histiocyte and fibroblast count with collagen density, vascularity, epithelization and oedema degree were evaluated and graded on a scale from 0 to 3 (poor, moderate, good, excellent) (4). Mean values of subgroups were expressed as arithmetical means of histopathologic scores with their standard deviations.

A11 of the rats, including the 3rd and 6th day subgroups, were sacrificed by decapitation on the 10th day. Ventral incisions were resected with adjacent skin having 0.5 cm width on the both side of the incision. A 1 cm long and 0.8 cm broad strip, which was centered by a segment of the incision line, was prepared from ventral skin of each rat. Incision breaking strength (expressed as Newton) was immediately measured by an uniaxial tensiometer (Hounsfield Test Equipment, Croydan-England) using this strip. Wound hydroxyproline content (expressed as ug/mgr wet tissue) was determined in the remnant ventral skin tissue by the method of Stegemann and Stalder (5).

Mann-Whitney U test was used to compare histopathological data, while incision breaking strength and wound hydroxyproline content were compared among groups with Student t-test. A "p value" less than 0.05 was considered as statistically significant.

Results

Of the 42 animals used, one in each group died within the first two postwounded days. So, it remained 6 rats in each of the 3rd day subgroups.

Results of the histopathological examination are shown in Table 1 as comparing the subgroups. Inflammatory cell infiltration was significantly more prominent in the control group ($p<0.05$). Conversely; fibroblast count, collagen density, vascularity and epithelization degree were found to be significantly increased in the treatment group ($p<0.05$).

Mean values of incision breaking strength and wound hydroxyproline content were shown in Table 2. Although hydroxyproline level in the treatment and breaking strength in the control group were found to be increased when compared with their counterparts, it was documented that differences between groups did not reach statistical significance ($p>0.05$).

Discussion

Tamoxifen is an oestrogen agonist/antagonist often associated with antioestrogenic effects and its affinity to oestrogen receptors is 20 fold weaker than that of oestrogen itself, and tamoxifen competes oestrogen to bind to these receptors (3,6). In the English literature of the last 20 years, we did not find a study, which evaluated wound healing in relation to tamoxifen. There are few studies on the wound healing and oestrogen, results of which are controversial. It was recently documented that oe-

Table 1. Comparison of wound histopathological findings in relation to groups and subgroups (mean±SD)

	3rd day (n=6)			6th day (n=7)			10th day (n=7)		
	Cnt	Tmx	p	Cnt	Tmx	p	Cnt	Tmx	p
PNL	3.0±0.0	2.16±0.37	0.007 S	2.28±0.45	2.0±0.53	0.333 NS	1.71±0.45	1.0±0.83	0.032 S
Lymphocyte	1.66±0.47	2.16±0.37	0.029 S	2.0±0.53	1.28±0.45	0.032 S	1.71 ±0.45	1.14±0.34	0.037 S
Histiocyte	0.66±0.47	1.16±0.37	0.090 NS	1.71 ±0.45	1.14±0.34	0.037 S	1.42±0.49	0.14±0.34	0.002 S
Fibroblast	1.16±0.37	1.83±0.37	0.122 NS	3.0±0.0	3.0±0.0	1.000 NS	1.14±0.34	2.71±0.45	0.002 S
Collagen	0.83±0.37	1.66±0.47	0.006 S	1.71±0.45	2.57±0.49	0.016 S	2.28±0.45	2.85±0.34	0.122 NS
Vascularity	1.16±0.37	1.83±0.37	0.122 NS	2.28±0.45	2.71±0.45	0.122 NS	2.28±0.45	2.85±0.34	0.037 S
Epithelization	0.66±0.47	1.66±0.47	0.006 S	1.71 ±0.45	2.71±0.45	0.002 S	2.28±0.45	2.85±0.34	0.037 S
Oedema	2.66±0.47	2.16±0.37	0.122 NS	1.14±0.34	1.14±0.34	1.000 NS	1.28±0.45	0.85±0.34	0.370 NS

(Cnl —> Control group; Tmx —> Tamoxifen-treated group
NS —> statistically not significant; S —> significant)

Table 2. Wound hydroxyproline content (yg/mg wet tissue) and incision breaking strength (Newton) on the lfjih day (mean±SD)

	Hydroxyproline		Breaking strength	
Control group (n=20)	1.43 ± 0.59	} p>0.05	6.55 ± 1.87	} p>0.05
Treatment group (n=20)	1.66 ± 0.62		5.55 ± 1.90	

strogen leads to impaired formation of connective tissue, probably by affecting the production of chemotactic factors (7). On the other hand, it has been shown that oestrogen given exogenously increased the production of laminin, fibronectin, collagen type-I and -IV, stimulated cell proliferation and had a promoter effect on the neovascularisation (8). These confroversial results are probably due to differences between methods. In the present study, it seems that tamoxifen may stimulate some events in the wound healing process such as epithelization, collagen synthesis and angiogenesis. On the other hand, we found that tamoxifen did not significantly affect wound hydroxyproline content and incision breaking strength. However; since hydroxyproline content and breaking strength were measured only on the 10th day, we were not able to show whether tamoxifen has a significant effect on both of these parameters during the earlier phases of wound healing process, during which collagen density was

found to be significantly increased in the treatment group.

There are some studies, which have demonstrated the effect of tamoxifen on various intrinsic factors affecting wound healing. It has been reported that tamoxifen inhibited the activities of both calmodulin and protein kinase C, which are known to improve wound healing (1,9-11). On the other hand, the activity of TGF-p, (transforming growth factor-pi) was found to be 50 fold increased in the patients receiving tamoxifen treatment (12). TGF-(3, is both chemotactic for macrophages and mitogenic for fibroblasts, it also stimulates collagen synthesis and angiogenesis (13,14). It was also suggested that TGF-B1 may improve wound healing (15,16). In addition, it was shown that tamoxifen decreased the serum level of IGF-1 (insulin-like growth factor-1), probably by increasing its binding to IGFBP-1 (IGF-1 binding protein) (17,18). It was documented that combined administration of IGF-1 and IGFBP-1 increases the incision breaking strength by 33% and wound hydroxyproline content by 67%> (19). Another experimental study demonstrated that depletion of IGF-1 decreased wound protein, DNA and hydroxyproline content of wounds by 50%, while IGF-1 replacement provided partial improvement in these variables (20). According to these results, effects of tamoxifen on wound healing are debatable; one might expect that tamoxifen impairs wound healing depending to its effects on calmodulin, proteinkinase-C and IGF-1, while it is also possible that tamoxifen enhances wound healing by its effect on TGF-p,. Unfortunately, we were not able to assess these factors.

We could not suggest a mechanism, by which tamoxifen treatment results in histopathological differences seen in the present study. Probably, a well-defined balance may exist between the factors of this complex system mentioned partially above. In conclusion; although tamoxifen treatment resulted in significant differences with respect to some histopathological parameters, this study demonstrated clearly that tamoxifen (0.5 mgr/kg diet) does not significantly alter wound hydroxyproline content and incision breaking strength (on the 10th postwounded day), which are main indicators of wound healing.

REFERENCES

1. Tonietto G, Agresta F, Libera DD, et al. Treatment of idiopathic retroperitoneal fibrosis by tamoxifen. Eur J Surg 1997; 163 : 231-5.
2. Alican F. Pankreas kanserinin tedavisi. In: Cerrahi Dersleri 3. Kitap: İstanbul: Dünya Tıp Kitabevi Ltd Şti, 1996: 398-405.
3. Jordan VC. Tamoxifen: toxicities and drug resistance during the treatment and prevention of breast cancer. Annu Res Pharmacol Toxicol 1995; 35: 195-211.
4. Turan M, Yıldırır C, Gökgöz MŞ, et al. Yara iyileşmesine difenilhidantoin'in olumlu etkileri. Ulusal Cerrahi Dergisi 1997; 13 (1): 19-27.
5. Stegemann H, Stalder KH. Determination of hydroxyproline. Clin Chim Acta 1967; 18 : 267-73.
6. Morgan MA, Gincherman Y, Mikuta JJ. Endometriosis and tamoxifen therapy. Int J Gynaecol Obstet 1994; 45 (1): 55-7.
7. Frazier Jessen MR, Mott FJ, Witte PL, et al. Estrogen supression of connective tissue deposition in a murine model of peritoneal adhesion formation. J Immunol 1996; 156 (8): 3036-42.
8. Morelcs DE, Mc Kovan KA, ct al. Estrogen promotes angiogenic activity in human umbilical vein endothelial cells in vitro and in a murine model. Circulation 1995; 91 (3): 755-63.
9. Gundimeda U, Chen ZH, Gopalakrishna R. Tamoxifen modulates protein kinase C via oxidative stress in estrogen receptor-negative breast cancer cells. J Biol Chem 1996; 271 (23): 13504-14.
10. Wilson Y, GoberdhanN, Dawson RA, et al. Investigation of the presence and role of calmodulin and other mitogens in human bum blister fluid. J Bum Care Rehabil 1994; 15 (4): 303-14.
11. Lin N, Bazan HE. Protein kinase C substrates in corneal epithelium during wound healing: the phosphorylation of growth associated protein-43 (GAP-43). Exp Eye Res 1995; 61 (4) : 451-9.
12. Butta A, MacLennan K, Flanders KC, et al. Induction of transforming growth factor beta 1 in human breast cancer in vivo following tamoxifen treatment. Cancer Res 1992; 52 (15) : 4261-4.
13. Cohen IK, Dicgelmann RF, Crossland MC. Wound care and wound healing. In: Schwartz SI, Shires GT, Spencer FC (cds). Principles of Surgery: New York : Mc draw Hill Inc, 1994: 279-303.
14. Roberts AB, Sporn MB, Assoian RK, et al. Transforming growth factor type ß: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sei USA 1986; 83: 4167-71.
15. Shah M, Foreman DM, Ferguson MW. Neutralisation of TGF-ßl and TGF-B2 or exogenous addition of TGF-B3 to cutaneous rat wounds reduces scarring. J Cell Sei 1995; 108 (Pt3): 985-1002.

lô. Lin RY, Sullivan K M, Argenta PA, et al. Exogenous transforming growth factor-beta amplifies its own expression and induces scar formation in a model of human fetal skin repair. Ann Surg 1995; 222 (2): 146-54.

17. Reed MJ, Christodoulides A, Koistinen R, et al. The effect of endocrine therapy with medroxyprogesterone acetate, 4-hidroxyandrostenedione or tamoxifen on plasma concentrations of insulin like growth factor IGF-1, IGF-II and IGF-BP-1 in women with advanced breast cancer. Int J Cancer 1992; 52 (2): 208-12.

18. Lonning PE, Hall K, Aakvaag A, ct al. Influence of tamoxifen on plasma levels of insulin-like growth factor I and insulin like growth factor binding protein I in breast cancer patients. Cancer Res 1992; 52 (17): 4719-23.

19 Jyung RW, Mustae JA, Busby WH, et al. Increased wound breaking strength induced by insulin like growth factor 1 in combination with insulin like growth factor binding protein 1. Surgery 1994; 115(2): 233-9.

20. Mueller RV, Hunt TK, Tokunaga A, et al. The effect of insukin like growth factor 1 on wound healing variables and macrophages in rats. Arch Surg 1994; 129 (3): 262-5.