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Original Investigation

Efects of Melatonin and Octreotide on Peridural Fibrosis in an Animal Model of Laminectomy

Hayvan Laminektomi Modelinde Melatonin ve Oktreotidin Peridural Fibrozise Etkileri

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ABSTRACT

AIM: To investigate the effects of melatonin and octreotide in the prevention of peridural fibrosis in an experimental rat model.

MATERIAL and METHODS: A total of 36 rats were divided into three groups: Group I was laminectomized and not given any treatment. Group II received an intraperitoneal 30 μg/kg/day dose of octreotide for six weeks after the laminectomy. Group III rats were injected with melatonin 7.5 mg/kg/day for six weeks after the laminectomy. At the end of six weeks, plasma transforming growth factor beta-1 levels and peridural fibrous tissue hydroxyproline concentrations were determined and histopathological examinations was performed.

RESULTS: Serum TGF-ß1 levels of the octreotide and melatonin groups were found to be lower than the control group. The lower levels of TGF-ß1 was statistically significant in both of the groups. Hydroxyproline levels of the octreotide and melatonin groups were found to be lower than that of the control group. The decrease was statistically significant only in the melatonin group. Peridural fibrosis scores of the octreotide and melatonin groups were lower than the control group. This histopathological improvement was statistically significant only in the melatonin group.

CONCLUSION: Melatonin and octreotide prevented TGF-ß1 increase in peridural fibrosis, but only melatonin significantly improved hyroxyproline levels and fibrosis scores as demonstrated.

KEYWORDS: Melatonin, Octreotide, Peridural fibrosis, Transforming growth factor, Hydroxyproline

ÖZ

AMAÇ: Deneysel rat modelinde oktreotid ve melatoninin peridural fibrozisi önleme üzerine etkilerini inceledik.

YÖNTEM ve GEREÇ: 36 adet rat 3 gruba ayrıldı. 1. Grup ratlara laminektomi yapıldı ve tedavi uygulanmadı. 2. gruptaki ratlara laminektomiden sonra 6 hafta boyunca 30 μg/kg/gün oktreotid intraperitoneal yolla uygulandı. 3. gruptaki ratlara laminektomiden sonra 6 hafta boyunca 7.5 mg/kg/gün melatonin intraperitoneal yolla uygulandı. Altıncı hafta sonunda serum TGF-ß1 miktarları, peridural fibröz dokuda hidroksiprolin miktarı ve fibröz dokunun histopatolojik incelemeleri yapıldı.

BULGULAR: Oktreotid ve melatonin verilen gruplar kontrol grubu ile karşılaştırıldıklarında serum TGF-ß1 düzeylerinin düşük olduğu gözlendi. TGF-ß1 deki bu azalma her iki tedavi grubunda da istatistiksel olarak anlamlıydı. Oktreotid ve melatonin verilen gruplar kontrol grubu ile karşılaştırıldıklarında, doku hidroksiprolin düzeylerinin düşük olduğu gözlendi. Hidroksiprolin düzeylerin-

deki bu düşüş sadece melatonin uygulanan grupta istatistiksel olarak anlamlıydı. Oktreotid ve melatonin verilen grup kontrol grubu ile karşılaştırıldıklarında, peridural fibrozis skorlama puanlarının düşük olduğu gözlendi. Histopatolojik düzelme de sadece melatonin grubunda anlamlıydı.

SONUÇ: Peridural fibrozisi önlemede melatonin ve octreotidin TGF-ß1 artışını önlediğini, ancak hidroksiprolin düzeylerini ve fibrozis skorunu sadece melatoninin anlamlı olarak düzelttiğini gösterdi.

ANAHTAR SÖZCÜKLER: Melatonin, Oktreotid, Peridural fibrozis, Transforming growth faktör, Hidroksiprolin

INTRODUCTION

Peridural fibrosis and scar formation is one of the most common and problematic complications encountered after lumbar disc surgery. Various studies have shown the underlying cause of the symptoms to be peridural fibrosis in 3-24% of patients with "failed-back" syndrome that exhibits pain during its course. In those patients, lumbar or radicular pains re-occur after spinal surgery and create clinical problems (12, 15). The operations to be performed in the future due to fibrosis and scar tissue also pose a risk for neural tissue during surgery.

Many surgical techniques as well as various biological and synthetic materials have been tried in a vast number of clinical and experimental studies for the prevention of peridural fibrosis that may occur following laminectomy. These include smaller surgical incision and muscle dissection, limited manipulation, techniques involving metastasis, lavage of epidural space, steroid and non-steroid anti-inflammatory drugs, fatty grafts, and synthetic materials such as dacron, vicryl, and silastic (7,9,12,17). However, all of those have met with limited success. Most of the materials deemed as protective have been tried while considering the barrier effect they could inflict upon the operative field.

Melatonin, which is secreted from glandula pinealis, is known to be a considerably effective free radical scavenger as well as having protective influence on oxidative pathologies and activating the antioxidative enzymes of glutathion reductase, glucose-6-phosphate dehydrogenase, and superoxide dismutase, while inhibiting nitric oxide, which is a pro-oxidative enzyme, along with significantly reducing the effects of lipid peroxidation (14,27). Melatonin has been shown to have positive effects against fibrosis at tissue level in histopathologic, immunohistochemical, and electron microscopic studies (1,11).

Octreotide, a somatostatin analog, bears the structure of a cyclic 14-aminoacid peptide, and inhibits thyrotropin (TSH), prolactin (PRL), and adrenocorticotropin (ACTH) release from the anterior pituitary gland. The antioxidative, antiproliferative, antiedematous, and antiadhesive, properties of octreotide that also happens to be a free radical scavenger, are known to inhibit transforming growth factor-ß1 (TGF-ß1) that plays a role in the process of fibrosis and influence the level of hydroxyproline, one of the most important markers of fibrosis (21).

However, we were unable to reach any data concerning the effects of octreotide or melatonin in experimental peridural fibrosis models. Our aim in this study was to investigate the effects of melatonin and octreotide on the fibrosis score, and levels of serum TGF-ß1 and tissue hydroxyproline in the experimental peridural fibrosis model.

MATERIALS and METHODS

Thirty-six male Wistar rats each weighing between 200 and 250 g were used in this study. All animals received humane care as outlined in the Guide for the Care and Use of Laboratory Animals. (25). The animals, which had been fed rat chow, were deprived of food for 24 hours before surgery, but were allowed free intake of water. Each animal received one intramuscular injection of cefamezine (100 mg/kg) before the operation. After 400 mg/kg of chloral hydrate was administered to induce anesthesia, a 4-cm midline incision was made in the lumbar region. With the aid of the operating microscope, the paravertebral muscles were separated and a T8–L3 total laminectomy was performed in all animals. The laminectomy window was created using a 1-mm Kerrison rongeur. Following the laminectomy, the fascia and subcutaneous layers were closed with 2.0 vicryl sutures.

The rats divided into three groups:

Group I (n:12): was the only laminectomized/sham group; no medication.

Group II (n:12): received a single intraperitoneal (ip) dose of octreotide (Sandostatin, Sandoz, Türkiye) (30 μg/kg) just after the laminectomy and then 30 μg/kg/day divided into three equal doses for six weeks.

Group III (n:12): was ip injected with melatonin (Melatonin, Sigma, MO, USA) 7.5 mg/kg as a single dose just after laminectomy and then 7.5 mg/kg/day divided into three equal doses for six weeks.

All groups were cared for in separate cages and the rats were killed 6 weeks later. All dissections were made by an observer blinded to the treatment group. Observations were noted during careful dissections that extended in a posterior to anterior direction, outside the spinal canal. The specimens, which included the laminectomy window, were carefully removed in 2-cm sections and frozen at -70°C. Any scar tissue attached to the posterior dura mater within the laminectomy window was also removed to be assessed using a biochemical analysis. Simultaneously, 3-ml blood samples were drawn into tubes for measurement of tissue TGF-ß1 levels.

Determination of TGF-ß1

Plasma levels of TGF-ß1 were measured by using the TGF-ß1 ELISA kit (Catolog number; KAC1688, Bio- Source International, Inc., Camarillo, California, USA).

Determination of Hydroxyproline

Hydroxyproline levels were determined spectrophotometrically using Woessner's method (30).

Histological Preparation

Samples were fixed in 10% neutral buffered formalin for 24 hours so that they could be used in the histological studies. Later, they were immersed in decalcification solution containing formic acid, where they remained for 48 hours. Slices were cut horizontally through the spinal cord, providing decalcified tissue samples. After a 13-hour standard follow-up process, the tissue samples were embedded in paraffin blocks and cut into 5-mm-thick sections. Afterward, the tissues were deparaffinized and stained with Masson trichrome.

The extent of fibrosis was graded according to the following classification (16):

Grade 0: the duramater was free of scar tissue.

Grade 1: only thin fibrous band(s) between the scar tissue and duramater were observed.

Grade 2: continuous adherence was observed but for less than two thirds of the laminectomy defect.

Grade 3: scar tissue adherence was large (more than two thirds of the laminectomy defect) and/or extended to the nerve roots.

Statistical analysis

SPSS 10.0 software was used for statistical calculations and graphs. Group data were statistically compared using Kruskal-Wallis variance analysis. If a statistical difference was identified, the groups were compared two by two by Mann-Whitney U test. Probability values <0.05 were considered to indicate a significant difference.

RESULTS

Biochemical Analysis Results:

Serum TGF-ß1 Levels: Serum TGF-ß1 levels of the octreotide and melatonin groups were found to be lower than that of the control group. The lower levels of TGF-ß1 were statistically significant in both the groups ($p<0.05$). No significant difference was determined between the two study groups in terms of the TGF-ß1 level (Figure 1).

Hydroxyproline Levels: The octreotide and melatonin groups were compared with the control

Table I: Comparison of mean hydroxyproline, TGF ß-1 and fibrosis values of the groups. All the levels of the octreotide and melatonin groups were found to be lower than the control group.

	Hydroxyproline(mg/g)	TGF ß-1 (pg/dl)	Fibrosis score
Group1 (Control) (n=12)	4.1±1,3	683±210	2.4±0,7
Group 2 (Octreotide) (n=12)	3.4±1,1	441±216	2.0±0,6
Group 3 (Melatonin) (n=12)	3.0±0,9	486±335	1.0±0,2

(TGF-ß1 :Transforming growth factor beta-1)

group and their hydroxyproline levels were found to be lower than that of the control group. While the decrease in the melatonin group was statistically significant ($p<0.05$), it was not statistically significant in the octreotide group ($p>0.05$). There was no significant difference between the octreotide and melatonin groups regarding hydroxyproline levels (Figure 2).

Histopathological Analysis: Dense fibrosis was found on the laminectomy area in the control group subjects (Figure 3). Peridural fibrosis scores of octreotide and melatonin were lower than the control group (Figure 4). While this histopathological improvement was statistically significant in the melatonin group, the improvement in the octreotide group was not statistically significant. The difference between the two study groups was significant (Figure 5).

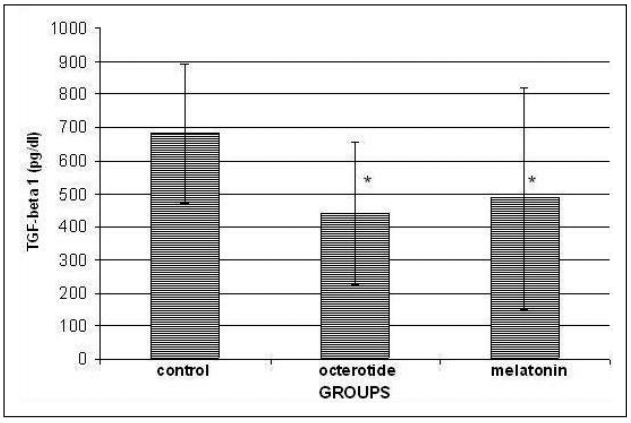

Figure 1: *Comparison of mean TGF-ß1 values of the groups. TGF-ß1 levels of octreotide and melatonin groups were found to be lower than the control group.*
(TGF-ß1 : Transforming growth factor beta-1)
** : P<0.05, versus control group*

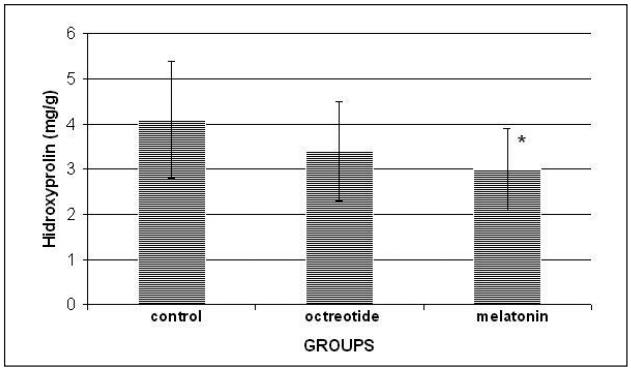

Figure 2: *Comparison of mean hydroxyproline values of the groups. Hydroxyproline levels of octreotide and melatonin groups found to be lower than that of the control group.*
** : P<0.05, versus control group*

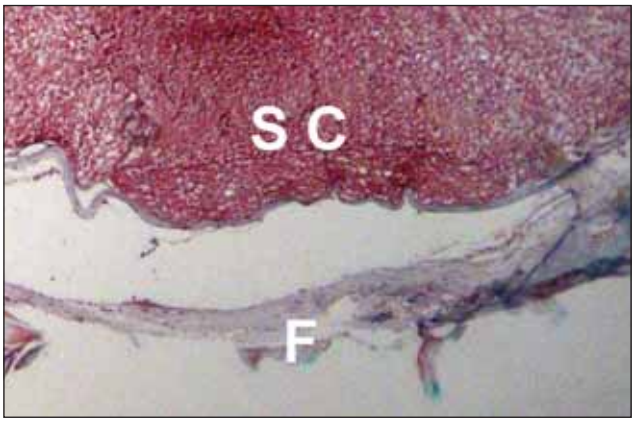

Figure 3: *Photomicrograph showing a specimen from the control laminectomy group. Severe fibrosis (F) and spinal cord (SC) retraction are delineated. Masson trichrome, 100X.*

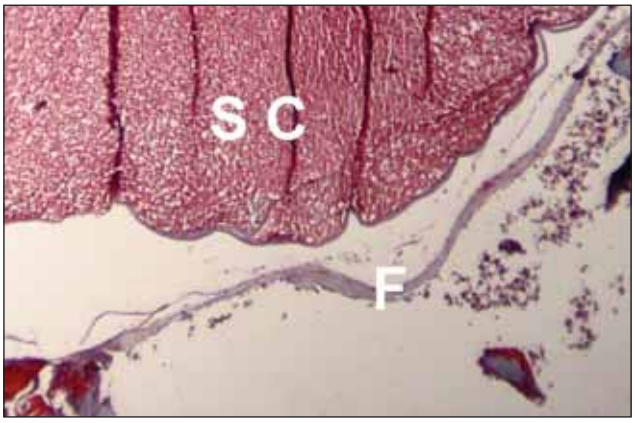

Figure 4: *The photomicrographs demonstrating specimens from the melatonin group. Minimal fibrosis (F), no dural or spinal cord (SC) adhesion and no spinal cord retraction are observed. Masson trichrome, 100X.*

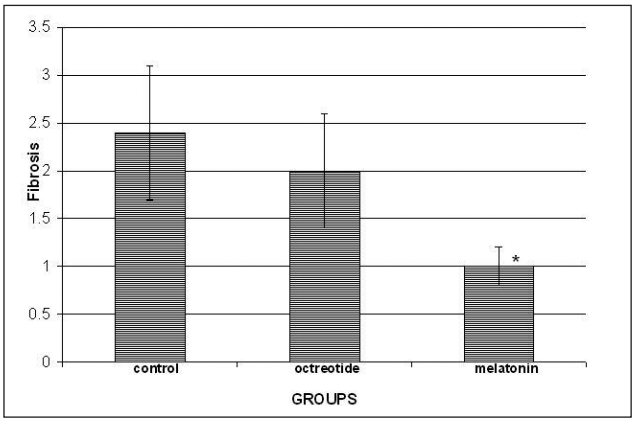

Figure 5: *Comparison of mean histopathologic scores of the groups.*
** : P<0.05, versus control group.*

DISCUSSION

Peridural fibrosis, known to be one of the most common complications of lumbar spinal surgery

postoperatively, is the fibroblastic invasion of peridural nerve roots due to the widened peridural space (6,12,26). The formation of peridural fibrosis is a part of the natural healing process. Opening of canalis vertebralis by surgery and resection of ligamentum flavum causes migration of fibroblasts towards epidural space. Fibrosis and adhesion of epidural space therefore take place as a result of the natural reaction of the healing muscle adjacent to the osseous components of the spine. This fibrosis formation causes lower back pain or pseudoradicular pain (12,15,17).

Various surgical techniques and treatment methods have been tried to prevent peridural fibrosis, an important complication of spinal surgery. Minimal invasive surgery is known to reduce peridural fibrosis due to smaller incision and muscle dissection. Limited manipulation and effective hemostasis have also been reported to prevent peridural fibrosis. Fatty grafts have been first used for the prevention of peridural fibrosis after lumbar discectomy (22,23). In order to prevent the postoperative formation of fibrous tissue, Experimental models have been developed in similar studies and materials such as dacron, vicryl mesh, polyethylene oxide/polybutylene terephthalate, polylactic acid, silastic, seprafilm, Gore-Tex, sodium hyaluronate, and various membranes have been used; however, these studies did not produce positive results and none of them showed positive clinical effects (26,29). Steroid and non-steroid anti-inflammatory drugs (9,17), and CO2 laser (10) with systematic usage have also been reported to prevent peridural fibrosis.

Octreotide, whose antioxidant, antiproliferative, antiedematous, antiadhesive, and free radical scavenger effects have been shown in many studies, is known to inhibit the TGF-ß1, that plays a role during development of fibrosis (4), and also to influence the hydroxyproline level, recognized as one of the most important markers of fibrosis (18,19). However, we were unable to find any study where the effects of octreotide were investigated by establishing experimental peridural fibrosis models.

TGF-ß1, which is known to be a polypeptide cytokine, has a stimulatory and mitogenic activity on macrophages and fibroblasts, and stimulates various extracellular matrix components through fibroblasts. Excessive release of TGF-ß1 has been shown to play a role in the pathogenesis of many fibrotic diseases such as peritoneal adhesion formation, pulmonary and intestinal fibrosis, liver cirrhosis, glomerulonephritis, and cutaneous scars (20,30). In the present study, determination of high serum TGF-ß1 levels in the control group that had not been subjected to any medication after the laminectomy was supported by these results.

Several studies have shown that TGF-ß1 levels increase with fibrosis and that fibrous tissue formation can be prevented by suppressing TGF-ß1 (20). In the present study, TGF-ß1 levels were significantly lower in the octreotide group than that of control group.

Similar studies have underscored the antifibrosis influence of octreotide over liver pathologies and intestinal diseases. Obtained data point out that the inhibitory effect of octreotide on growth factors can be effective at the collagen formation or organization stage, known to be a late period during wound healing. Somatostatin and octreotide have antiproliferative effects with the following mechanisms: suppression of DNA synthesis and cell proliferation, angiogenesis, and neovascularization. Wound healing and adhesion formation occur via similar pathways and the functions of those growth factors can therefore have an effect over adhesion formation. Theoretically, octreotide can inhibit tumor growth by those functions just as it can disrupt and delay wound healing (31).

Somatostatin is known to have anti-inflammatory effects (21). Bjorlin (5) showed that somatostatins exercise an anti-inflammatory effect in bladder infections by decreasing the release of histamine and prostaglandins. Octreotide reduces the elevation of leukotrienes of rat gastric mucosa by suppressing 5-lipoxygenase. Moreover, somatostatin influences the lipoxygenase-dependent arachidonic acid metabolism by the same mechanism. This anti-inflammatory effect may be playing an important role in the antifibrosis effect of octreotide. The antiischemic and antioxidant effect of somatostatin over various tissues is also well known (4). These regulatory functions assist the process of fibrosis prevention.

The administered dose for the prevention of fibrosis is reflected as 10–20 μg/kg/day in various studies (2,4,19). In the present study, we used a dose of 30 μg/kg/day. Our findings indicated that octreotide contributes to prevention of peridural fibrosis. However, this limiting effect was not statistically

significant (p>0.05). Inadequate blockage of early period mediators of adhesion formation and fibrinolysis might have played a role in this result.

In addition to studies exhibiting the antioxidant effects of melatonin, there are other studies using histopathologic, immunohistochemical, and electron microscopy methods that support its positive influence against fibrosis at the tissue level. Its protective role against oxidant damage in the lungs, neural tissue, kidney, heart, gastrointestinal system, and eye has been shown by experimental studies (13,16,27,31).

Exogenous melatonin is known to prevent nephropathy development by inhibiting lipid peroxidation in renal tissue and inhibition of TGF-ß1 is known to contribute to that limiting influence against fibrosis (1,16). On the other hand, melatonin deficiency causes fibrous tissue development in many locations. Methysergide, a serotonin antagonist, has been shown to disrupt pineal functions and cause retroperitoneal fibrosis (11), whereas fibrous tissue formation has been shown to increase in the abdominal cavity following pinealectomy. Similarly, exogenous injection has been shown to be able to correct the increase in the collagen amount induced by pinealectomy (13). Additionally, the cause of fibrous tissue increase and pseudoarthroses occurring as a result of TGF-ß1 elevation due to melatonin deficiency has been explained (1). The serum TGF-ß1 levels of the melatonin group were found to be significantly decreased in the present study, similar to the hydroxyproline levels, compared to that of rats in the control group. Fibrous tissue formation was also significantly prevented in the melatonin group, supporting the results obtained from other similar studies (p<0.05).

The mechanism by which melatonin limits fibrosis is not currently clear. However, the underlying mechanism probably involves reduction of tissue damage and prevention of fibrosis during the early period. Melatonin also causes limitation of fibrosis by preventing the increase of free oxygen radicals, reducing the inflammatory reaction started by those radicals, influencing antioxidant enzymes in the tissue, and decreasing the tissue malondialdehyde levels (14,28,31). The fibrosis prevention of melatonin has been explained in another study with a different mechanism by which it decreases nitrite and nitrate levels in a fibrosis model induced in lungs (28).

A review of studies in which melatonin has been employed for preventing fibrosis on similar experimental models in various tissues reveals that the administered dose was 4–25 mg/kg/day (18,31). In the present study, the dosage we used was 7.5 mg/kg/day. Our melatonin dose was sufficient to prevent peridural fibrosis at a significant level.

Collagen plays an important role in every stage of wound healing and displays a regulatory and stabilizing function on the forming tissue (8). During wound healing, hydroxyproline forms immediately within the collagen and increases rapidly. Its amount shows a negative correlation with adhesion severity (3,4,29). In the present study, we investigated the collagen synthesis and the tissue hydroxyproline level which is known to be a significant parameter of wound healing. Baykal (4) showed that octreotide prevents postoperative peritoneal fibrosis and that hydroxyproline levels decrease. While the hydroxyproline level was lower in the octreotide group compared to that of control group in the present study, there was no statistically significant difference on the 5th and 14th days. Colak et al. (10) employed the CO2 laser to prevent peridural fibrosis in experimental models and found a decrease in hydroxyproline levels in the forming fibrous tissue. The hydroxyproline levels of the octreotide group were low at the end of the 6th week in the present study. However, as with the histopathologic score of fibrosis, this decrease was not statistically significant (p>0.05). Melatonin has been shown to prevent the increase of hydroxyproline levels in fibrous tissue (31). The hydroxyproline amounts in fibrous tissue decreased in the melatonin group in the present study and this decrease was statistically significant (p<0.05).

In conclusion; melatonin and octreotide used for the prevention of peridural fibrosis developing after laminectomy showed a positive influence. Both reduced the levels of TGF-ß1, which is an effective factor for fibrous tissue formation, in a statistically significant fashion; the evaluation of histopathologic scores and hydroxyproline levels at the end of the 6–week period indicated that only the limiting effect of melatonin was at a significant level. Octreotide lowered the hydroxyproline levels and fibrosis amount but those effects were not statistically significant. Melatonin has been found to be superior to octreotide in preventing peridural fibrosis in our experimental model.

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