EXPERIMENTAL STUDY

Effects of transcutaneous electrical nerve stimulation on rats with post-operative ileus

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ABSTRACT

BACKGROUND: Post-operative ileus (POI) is a type of bowel dismotility causing accumulation of gas and fluid. Transcutaneous electrical nerve stimulation (TENS) has been frequently used for medical applications such as pain treatment and nervous stimulation. In this experimental animal model of POI, our aim is to investigate the effects of TENS on POI, and to demonstrate histopathological changes in rat intestine after TENS application.

METHODS: The present study is an experimental animal model of POI. Sixteen Wistar-Albino male rats in two groups were used and laparotomy was performed. After colorectum and small intestine were manipulated, activated charcoal and Nile red were administered by oral gavage. Electrodes were placed to the abdomen skin of the rats and TENS method was used. Rats in two groups were sacrificed on 24 h. The esophagus, stomach, and all intestines of the rats were resected and a direct X-ray and computerized tomography scan, and "J" images were taken, and the progression of active coals was measured radiologically. Histopathological and microscopic evaluation was performed.

RESULTS: The median of activated charcoal measure was 429 mm (178–594) in TENS group, 203 mm (149–313) in the control group, respectively, and these were statistically significant (p=0.004963). There was a significant difference between the two groups in terms of histopathological necrosis (p=0.041). In addition, the amount of Nil Red (550 nm) in the GI track is increased after 8 h of gavage with sequential applications of TENS.

CONCLUSION: This study demonstrated the protective and therapeutic efficacy of TENS in POI in a rat model by radiologically and histopathologically. In clinical practice, TENS may be examined on POI. Further studies are warranted to validate and generalize our findings, and to assess the impact of TENS for post-operative pain also.

Keywords: *In vivo* 3D optical tomography visualization; motility; post-operative ileus; transcutaneous electrical nerve stimulation.

INTRODUCTION

Post-operative ileus (POI) is a type of bowel dismotility causing accumulation of gas and fluid in the gastrointestinal tract due to decreased bowel movements or delayed passage.[1] POI can be seen after all type of surgeries with general anesthesia. In surgical practice, POI is a common problem with a incidence of 17–24%.[2] The pathophysiology of POI is described as a transient impairment of the intestine with the symptoms of nausea, pain, and emesis. It

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increases the risk of post-operative complications, delayed feeding, prolonged hospitalization, and consequently results in increased medical costs. POI is a related with a multifactorial etiology and the most important risk factors have not been described yet.

Meanwhile, many methods have been described in the treatment of POI. According to the literature, one of the newer modalities with early intestinal or spinal cord stimulation through electroacupuncture and neuromodulation may be useful for the treatment of POI or chronic spasm.[3] At present, transcutaneous electrical nerve stimulation (TENS) is being frequently used in medical applications such as pain treatment and nervous stimulation. In the literature, TENS has ameliorating effects on dysmotility and pain palliation with electroacupuncture autonomic-cytokine mechanism, in experimental models.[4] In addition, prokinetic agents are involved in POI therapy through the mechanism of neuromodulation targeted pathways.[5,6]

In this experimental model of POI, our aim is to investigate the effects of TENS on ileus duration and severity, gastrointestinal motility, and *in vivo* visualization of the gastrointestinal tract. It was planned to demonstrate histopathologic changes in rat intestine through TENS application.

MATERIALS AND METHODS

The present study is an experimental controlled animal model of POI. All experimental protocols were performed according to the guidelines for the ethical use and care of experimental animals, and the study was approved by the Ethics Committee of Ankara University Faculty of Medicine. In our study, 16 Wistar-Albino rats with an average of 3 months old male and 180–260 g in weight were used. The POI model applied in the study was adapted with the “running the bowel technique.[7]” To prevent the effect of diurnal variations in regenerative response, operations were performed during the first half of the day. Rats were randomly allocated in to two groups; TENS and control group (n=8). The rats were deprived of food and water for 6 h preoperatively. Rats were anesthetized with an intraperitoneal injection of 50 mg/kg ketamine HCl (Ketalar®) and 10 mg/kg xylazine (Alfazyne®). Immediately after anesthesia induction, activated charcoal (10 ml/kg) was given to the both group by orogastric way (1.5 g of charcoal in 20 ml of physiological saline). In addition, Nile Red (Invitrogen-N1142) solution (0.2 ml) was given by oral gavage immediately after the administration of activated charcoal to the TENS group, to obtain an “*in vivo* image” of whether intestinal motility is increased after TENS administration and to monitor gastrointestinal motility.

All surgical procedures were performed by the same experienced surgeon. After operation site cleaning with povidine iodine, a 2.5 cm-long midline laparotomy performed to both groups. Colorectum and small intestine were manipulated for 1 min by free cotton. The small intestine and colorectum of both groups were removed from the abdomen and then replaced back. The abdominal incisions were closed with non-absorbable 2/0 prolactin suture material. Two rats who developed asphyxia during orogastric lavage were excluded from the study and new rats were included in the group to maintain the number of rats in each group. Electrodes were placed on the abdominal skin for 10 min at 0^{th}, 4^{th}, 8^{th}, and 24^{th} h, Frequency: 80 Hz, Intensity: 20 MA TENS applied to the TENS group, while the other group was only observed. TENS unit was a dual-channel stimulator with variable frequency and pulse duration settings. The current applied during TENS was in diphasic, rectangular and symmetric pulse to obtain either sensorial or motor responses depending on the current amplitudes.[8] During TENS, the animals were housed in rat-restraining cages. A pair of self-adhesive electrodes was used to deliver the electrical stimulation and they were placed 2 cm near the laparotomy incision. In addition after 8 h (after having two TENS application), the intensity of Nile Red in GI track was measured with ImageJ software in TENS group (Figs. 1 and 2). Rats in two groups were sacrificed on 24 h after ketamine HCl anesthesia (50 mg/kg).

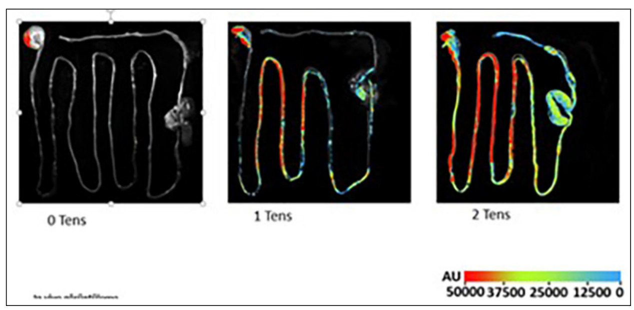

Figure 1. The intensity of Nile Red in GI track with *in vivo* imaging (imagej).

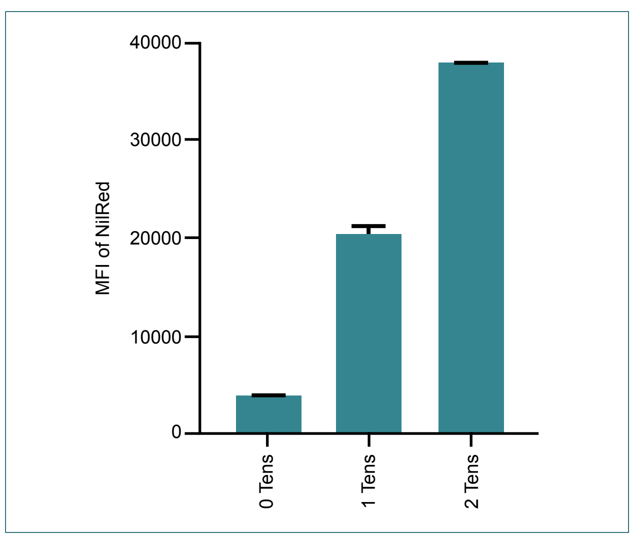

Figure 2. The diagram of Mean Fluorescence Intensity with the increasing number of TENS application.

Radiological Evaluation and Measurement

The esophagus, stomach, and all intestines of the sacrificed rats were resected and a direct X-ray and computerized tomography scan images were taken. The last level reached by activated charcoal was determined and the length was measured according to the starting point and finally enrolled. From these images, the headway of activated charcoal was measured radiologically. Segments were opened to measure the farthest active carbon (Fig. 3).

In Vivo Imaging

Nile red (Invitrogen-N1142) solution was used to examine the gastrointestinal tract movement. The Nile red solution prepared for this purpose was administered by oral gavage to healthy rats in TENS group who remained in a hungry state overnight, immediately after anesthesia induction. The gastrointestinal tract of surgically sacrificed rats was removed as a whole and the segments of the organ were placed to be displayed in the Newton 7.0® (Live Imaging System) (Vilber, France) system. The channel movement was quantitated according to the effect of TENS by looking at the density of the dye solution along the gastrointestinal tract (Figs. 1 and 2).

Histopatological Analyses

After formalin fixation, 3–4 mm thick sections were prepared from paraffin blocks prepared by full-layer tissue sampling in such a way that all intestinal layers can be seen. All sections were stained with Hematoxylin Eosin and then examined by light microscop. Microscopic evaluation was performed on three parameters which were inflammation, edema, and necrosis, and each parameter was semi-quantitatively scored in itself as described below. The total score was obtained with the sum of the obtained scores on a case-by-case basis.

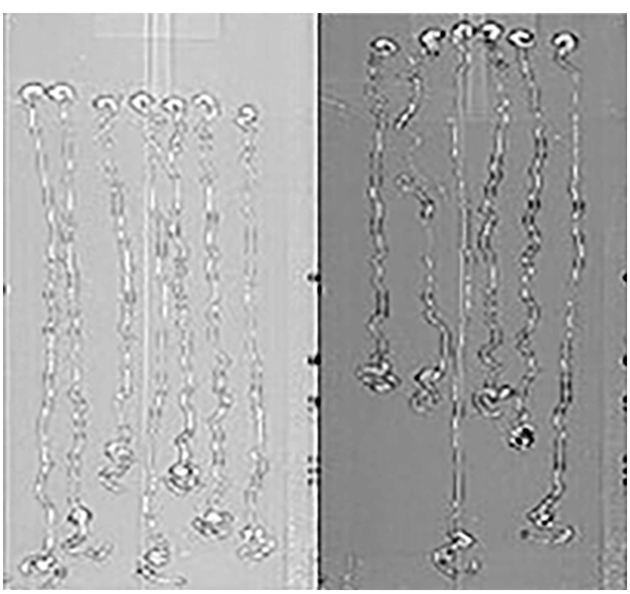

Figure 3. Views of the CT of the Control and TENS group.

Inflammation: no (0), mild (1), moderate (2), and severe (3) (Fig. 4).

Edema: no/light (0), moderate (1), and severe (2) (Figs. 5 and 6).

Necrosis: no (0), mucosal (1), and transmural (2) (Figs. 7 and 8).

Statistical Analysis

Continuous variables were expressed as mean±standard deviation and median (min-max), whereas categorical variables were denoted as numbers or percentages where appropriate. Kolmogorov–Smirnov Goodness of Fit test was used to test the distribution of data. Since the distributions were not normal, Mann–Whitney U test was used to compare these variables. Collected data were analyzed by Statistical Package for the Social Sciences version 22.0 (SPSS Inc., Chicago, IL, USA). Two-tailed $p<0.05$ was accepted to be statistically significant.

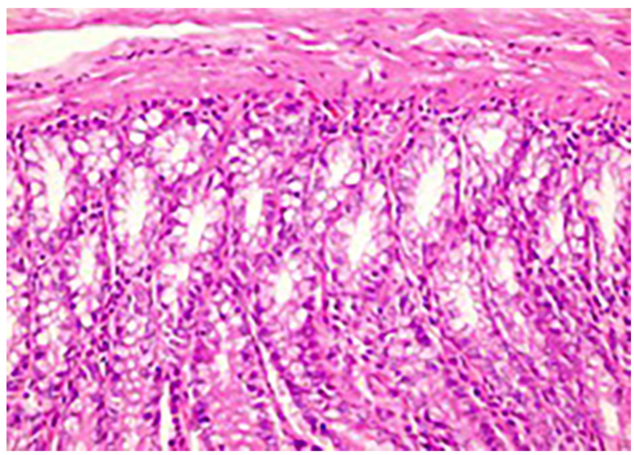

Figure 4. Light inflammation of lamina propria in the TENS group (HE ×400).

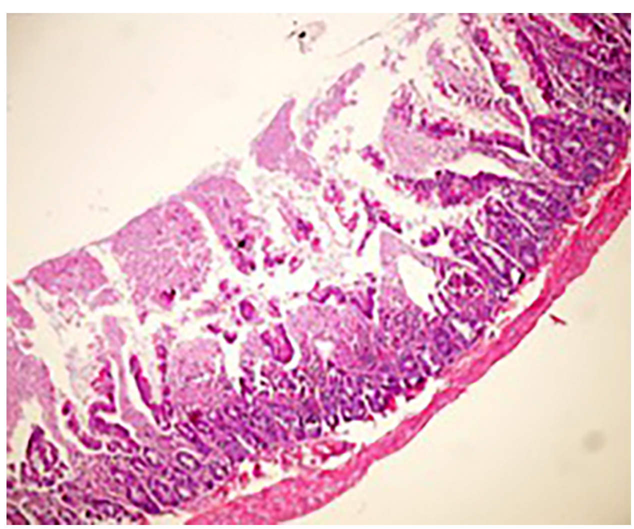

Figure 5. Mucosal necrosis and moderate edema in the TENS group (HE ×100).

Figure 6. Mucosal necrosis in the control group (HE ×40).

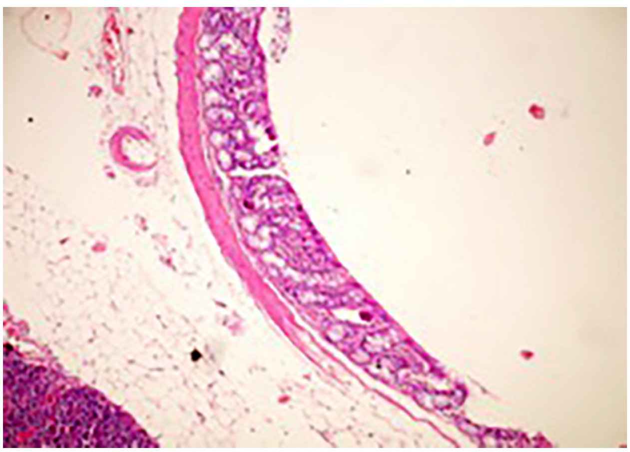

Figure 7. Intestinal tissue with necrosis and mild edema in the TENS group (HE ×400).

RESULTS

Esophagus, stomach, and intestines of all rats in both groups were removed after sacrification. The active coal was 396.5 mm (286.8–466.8) in the TENS group according to direct X-ray and computed tomography images, it was 272.0 mm (194.0–313.0) in the control group (Fig. 3). After the imaging procedures, all the digestive systems of the rats were evaluated and the last level reached by activated charcoal was determined and the length was measured according to the starting point and finally enrolled. The median was 429 mm (178–594) in TENS group, 203 mm (149–313) in the control group, respectively, and it was statistically significant (p=0.004963), (Table 1).

Pathological assessment with hematoxylin-eosin after formalin fixation; in terms of inflammation, TENS group 1.5 (0.8–2.0), control group 2 (1.5–2.3); edema, TENS group 1.5 (0.8–2.0) group 2 (1.8–3.0), and necrosis, TENS group 0.0

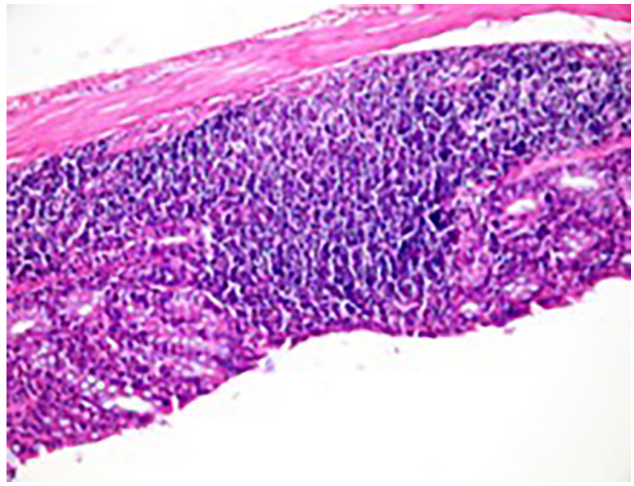

Figure 8. Severe inflammation and formation of gland destruction in the control group (HE ×200).

Table 1. Analysis of measures of the active charcoal

Group 1 (TENS) (mm) Mean: 429 (178–594) Median: 413.28	Group 2 (Control) (mm) Mean: 203 (149–313) Median: 227.85	p
323	310	
364	313	
178	154	
431	272	0.004963
429	203	
574	149	
594	194	
323	310	

TENS: Transcutaneous Electrical Nerve Stimulation.

(0.0–1.0) and control group 1.5 (0.8–2.0). There was a significant difference between the two groups in terms of necrosis (p=0.041).

The channel movement was quantified by looking at the density of the dye solution (Nile Red) along the gastrointestinal tract in proportion to the TENS applied by evaluation with *in vivo* imaging. Gastrointestinal motility was significantly increased with TENS application (Figs. 1 and 2).

DISCUSSION

POI after any type of abdominal surgery is admittedly a foregone conclusion, and accepted as natural for 2–4 days after conventional surgery and for ≤2 days after laparoscopic surgery.[9] The absence of bowel sounds and food intolerance is viewed as physiological in the first 24 h after surgery under normal circumstances, but if these clinic situations remain 3 days and more , it is accepted as prolonged or paralytic ileus.[10] On the other hand, there are also reports that it can barely be meaningful when it lasts more than 6 days.[11]

Since POI is an important problem after surgical procedures, several treatments are proposed.[12] Articles based on neuromodulation and also stimulation of the gastrointestinal tract have been especially considering the inflammatory nature of the POI mechanism. Chapelle et al.[13] reported an animal study with rats that inflammation and POI were reduced notably by decreasing intraperitoneal inflammatory cells and increasing gastrointestinal functions through visceral massage. "Electrical stimulation effect" on gastrointestinal system is provided by vagal and sympathetic pathways.[14] Costes et al.[15] showed that activation of the vagal stimulation pathways due to intestinal inflammation has a reducing effect on the local immunological response being caused by intestinal manipulation. Since the mechanism of electroacupuncture applications has not been clearly understood, a large number of animal and human studies have been conducted for the treatment of POI with electrical stimulation techniques. Neuromodulation method is proven to be a minimally invasive procedure and having low side effects, and being shown to increase gastric and intestinal motility, especially in controlled animal experiments.[16] This effect in question is thought to be mediated through the autonomic nervous system by regulating gastrointestinal functions through sympathetic and parasympathetic efferent pathways.[17] Murakami et al.[4] described a healing effect on the dysmotility and post-operative pain through autonomic-cytokine mechanisms. Contrary to the results of animal studies, human studies show contradictory findings. Although there are many publications reporting neuromodulation has no protective or therapeutic effect on POI, there also are many others showing that it reduces POI duration and analgesic requirement.[3,16]

To date, neuromodulation practices have been clinically involved in the treatment of chronic constipation, fecal incontinence, and urinary incontinence. It has been shown that in patients with slow transit constipation, percutaneous tibial nerve stimulation increases the frequency of vestibular stimulation, reduces the frequency of laxative use, and increases quality of life.[18] Although TENS applications have been used in clinical practice, the exact mechanisms are not understood clearly. The stimulation of large mechanosensitive nerve fibers results in inhibition of sympathetic tonus and vasodilation and the release of vasodilating neuropeptides such as substance-p, calcitonin gene-related peptide, and vasoactive intestinal peptide.[4] TENS applications were modified from electroacupuncture applications. Non-invasive transcutaneous applications for instance needle application have been tried on account of the side effects, such as stress and discomfort, and have been shown to be effective in chronic constipation through possible autonomic mechanisms. Transcutaneous neuromodulation has been considered to shorten transit time by reducing sympathetic nerve activation, increasing parasympathetic activation, and increasing colonic propagating sequences.[19] TENS mediated neuromodulation practices affect the current neurotransmission process by differentiating neurostimulation from direct electrical stimulation of the nerves and muscles.[20] Sacral nerve neuromodulation provided by electrical stimulation has been shown to result in clinical improvement by providing physiological stimulation of the lower bowel, anal sphincter and pelvic floor.[21]

The effects of TENS on pain treatment might be from the factors that influence the POI outcomes in our study. The analgesic mechanism of TENS is mainly explained by the door-control theory.[22] Other mechanisms in question include inhibition of abnormal stimulated nerves, stimulation of endogenous opioid secretion in the brain, sympathetic blockade, and local dilation of blood vessels in injured tissues.[22] It is considered that TENS stimulates the A-beta fibers using low-frequency electrical stimulation, reaching the brain, stimulating the inhibitor pathway through the spinal dorsal horn and thus reducing pain stimulation.[23] We figure that one of the possible routes that could affect the outcome of our study is the pain palliation provided by the possibly intermittent TENS. However, this result cannot be determined as a measurable value in our animal experimental model.

Each part of the gastrointestinal tract has different myoelectric, contractile, and transit responses to the POI which can also be defined as a dynamic ileus. The motility times of stomach, small intestine, and colon are different. It is considered that TENS is effective on POI through its effects on the autonomic nervous system. In the literature; interestingly enteric neurotransmitters such as calcitonin gen-related peptide, nitric oxide, vasoactive intestinal peptide, and substance P are known to be inhibitory to gastrointestinal muscles and probably contribute to ileus.[24] Neutralization of calcitonin gene-related peptides has been reported to improve POI. Inhibitors of nitric oxide and antagonists of vasoactive intestinal peptide have been observed to have a similar effects in rats.[25] It is interesting that, when these treatments are combined, the improvement in ileus is lost. Thus, it seems that some form of gastronintestinal inhibitory activity is needed for POI to be resolved.[26] Türler et al.[26] showed that the dysfunction in the smooth muscle tissue of the muscularis layer of the colon contributed to the POI associated with surgery-related local inflammatory cuffs. The prokinetic effect is important in increasing motility of TENS mediated neuromodulation. TENS application is reliable, cheap, and easy technique with low side effects. Maher et al.[3] have demonstrated gastric emptying and restoration of transit time in the upper gastrointestinal tract by electrical stimulation after electrode implantation to the spinal cord due to sympathetic downregulation and prokinetic activation.

The most valuable aspect of our study is that evaluating the prokinetic and sympathetic system inhibition and possible analgesic effects of TENS on POI radiologically and histopathologically. In addition, the progress of the activated carbon has been evaluated TENS applied rats, radiologically and clinically. Progression of activated charcoal in the TENS group compared to the control group is significant and this progress may be the cornerstone for further clinical studies. TENS applications are simple, inexpensive, and easy to apply in different areas of the clinic. The fact that TENS applications do not carry the risk of complications for the patient in the clinic may also facilitate their use and may be useful in early recovery.

The results are interesting according to the pathological data of our study. Significant results may be expected in a mechanical ileus; however, even in the case of a short-term and transient obstruction, having significant pathological results suggests that POI is a risky clinical entity. Despite the short-term effect of POI on the intestines, it was interesting to find statistically high but non-significant results in terms of inflammation and edema by histopathological evaluation, but a statistically significant difference between two groups in terms of necrosis. It has been shown that histopathologic changes can occur in the intestines even during the reversible POI period.

One of the most important limitations of our work is that the number of rats in groups was determined to be able to obtain the ethics committee permissions during the planning stage of our work and designed under this possession of statistical evaluations and analyzes in the department of Medical Statistics. The animals were either excluded from the study due to complications of surgery or oral lavage. Thus, for histopathological evaluations and investigations, groups should be kept larger.

Conclusion

This study demonstrated the protective and therapeutic efficacy of TENS on POI in a rat model radiologically, with in-vivo imaging, and histopathologically. In clinical practice, TENS applications used for chronic anal fissure treatment, chronic cramping, and anal incontinence treatments currently, should be examined for POI, too. This study is a preliminary animal study and further clinical studies are warranted to validate and generalize our findings. Further studies are warranted to validate and generalize our findings, and to assess the impact of TENS for post-operative pain also.

Ethics Committee Approval: This study was approved by the Ankara University Animal Experiment Ethics Committee (Date: 27.10.2015, Decision No: 2015/18/203).

Peer-review: Internally peer-reviewed.

Authorship Contributions: Concept: Ş.B., K.B.Y., S.T.; Design: Ş.B., K.B.Y., S.T.; Supervision: M.A., H.Y., H.K. Resource: M.A., Ş.B.; Materials: E.Ö., İ.G.T., H.K.; Data: H.Y., H.K.; Analysis: İ.G.T., E.Ö., A.İ.; Literature search: Ş.B., K.B.Y., S.T., M.S.; Writing: Ş.B., K.B.Y., M.S.; Critical revision: M.A., H.K., MS.

Conflict of Interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZ*

Transkutanöz elektriksel sinir stimülasyonunun (TENS) postoperatif ileuslu sıçanlar üzerindeki etkileri

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AMAÇ: Postoperatif İleus (POI), bağırsak lümeninde gaz ve sıvı birikimine neden olan bir tür bağırsak dismotilitesidir. Transkutanöz elektriksel sinir stimülasyonu (TENS), ağrı tedavisi ve sinir stimülasyonu gibi tıbbi uygulamalarda sıklıkla kullanılmaktadır. Bu deneysel hayvan çalışmasında amacımız, TENS'in POI üzerindeki etkilerini araştırmak ve TENS uygulamasından sonra sıçan bağırsağındaki histopatolojik değişiklikleri göstermektir.
GEREÇ VE YÖNTEM: Bu çalışma, deneysel bir hayvan çalışmasıdır. İki grupta 16 adet Wistar-Albino erkek sıçan kullanıldı ve laparotomi yapıldı. Kolorektum ve ince bağırsak manipüle edildikten sonra, aktif kömür ve Nil kırmızısı oral gavaj ile uygulandı. Sıçanların karın derisine elektrotlar yerleştirildi ve TENS yöntemi kullanıldı. İki gruptaki sıçanlar 24. saatte öldürüldü. Sıçanların özefagusu, midesi ve tüm bağırsakları rezeke edilerek direkt röntgen ve bilgisayarlı tomografi taraması ile "J" görüntüleri alınarak aktif kömürlerin ilerlemesi radyolojik olarak ölçüldü. Histopatolojik ve mikroskobik değerlendirme yapıldı.
BULGULAR: Aktif kömür ilerlemesinin ölçüm ortancası TENS grubunda 429 mm (178–594), kontrol grubunda 203 mm (149–313) olarak bulundu ve bunlar istatistiksel olarak anlamlıydı (p=0.004963). Histopatolojik nekroz açısından iki grup arasında anlamlı fark vardı (p=0.041). Ek olarak, GI yolundaki Nil Red (550 nm) miktarı, TENS'in ardışık uygulamaları ile sekiz saatlik gavajdan sonra artmıştı.
TARTIŞMA: Bu çalışma, bir deneysel sıçan modelinde TENS'in POI üzerindeki koruyucu ve terapötik etkinliğini radyolojik ve histo-patolojik olarak göstermiştir. Klinik pratikte, TENS POI üzerinde incelenebilir. Bulgularımızı doğrulamak ve genelleştirmek ve ayrıca TENS'in postoperatif ağrı üzerindeki etkisini değerlendirmek için daha ileri çalışmalara ihtiyaç vardır.

Anahtar sözcükler: *In vivo* 3D optik tomografi görselleştirme; motilite; postoperatif ileus; transkutanöz elektriksel sinir stimülasyonu.

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