DOI: 10.5137/1019-5149.JTN.14559-15.1
Received: 10.03.2015 / Accepted: 16.04.2015
Published Online: 26.04.2016

Original Investigation

The Effect of Ankaferd Blood Stopper® on Epidural Fibrosis After Laminectomy in Rats: An Experimental Study

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ABSTRACT

AIM: Lumbar epidural fibrosis is increasingly recognized as a cause of persistent back pain. The aim of this study was to examine the effect Ankaferd Blood Stopper on epidural fibrosis following laminectomy in rat models.

MATERIAL and METHODS: Twenty Sprague-Dawley male rats were randomly allocated to 2 groups of 10 each. The dura mater and nerve root were exposed after L1 unilateral laminectomy. Close attention was paid not to traumatize the dura, the nerve roots, or the dissected muscles. Immediate muscle and skin closure was made in sham group. In the Ankaferd Blood Stopper group, cotton wool soaked with 1 mL Ankaferd Blood Stopper was applied to the exposure site for 5 minutes, and muscle and skin closure was then made. Histological analysis was performed at four weeks postoperatively.

RESULTS: Epidural fibrosis formation evaluation and fibroblastic activity evaluation revealed that there was a significant difference between the sham and the Ankaferd Blood Stopper treated groups ($p = 0.011$, $p = 0.009$). Severe epidural adhesions were observed in the Ankaferd Blood Stopper group. Dissection of these epidural adhesions was difficult and accompanied by bleeding and disruption of the dura mater.

CONCLUSION: The results of this study showed that there was no positive effect of Ankaferd Blood Stopper on the prevention of epidural fibrosis, which is one of the most significant problems following spinal surgery, and the epidural fibrosis actually increased.

KEYWORDS: Epidural fibrosis, Spinal surgery, Ankaferd Blood Stopper

INTRODUCTION

Lumbar epidural fibrosis is increasingly recognized as a cause of persistent back pain (6). The reported incidence of postoperative epidural fibrosis ranges from 10% to 75% (28). Postlaminectomy epidural fibrosis is a well-known complication of spinal surgery, first mentioned in 1948 (18). Epidural fibrosis (EF) is a pathological process in which normal epidural fat is replaced by scar tissue (21). The epidural space is irregularly filled with scar tissue that can compress or retract the dural sac and/or nerve roots. A relationship between extensive peridural fibrosis and increased low back pain and/or recurrent radicular pain has been reported (22, 27), and the presence of peridural fibrosis has been described in up to 24% of patients with failed back surgery syndrome (5). Since scar excision surgery generally yields poor results, many authors have suggested that prevention of postoperative adhesions is an essential goal in low back surgery.

Some anti-neoplastic agents such as mitomycin C, 5-fluorouracil, cyclosporin and some high molecular weight molecules such as hyaluronan, oxidized regenerated cellulose,

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free fat grafts, steroid solutions, dural adhesion barriers, various chemical agents, and many surgical techniques such as smooth dissection or preservation of ligamentum flavum, and even external beam radiation therapy have been used to reduce or inhibit epidural fibrosis formation (9,12,16,17,30).

Ankaferd Blood Stopper® (ABS) is a herbal product obtained from the mixture of five different plant roots at specific ratios: Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum and Urtica dioica (10). Ankaferd Blood Stopper® (ABS) (Trend Teknoloji İlaç AS, Istanbul, Turkey) has been approved for use in the management of external hemorrhage and dental surgery by the Ministry of Health in Turkey (7).

There are various reports in literature of ABS as a powerful haemostatic agent with the property of preventing peritoneal adhesions and that it has a positive effect in the early period of fracture healing (7,14). Several clinical studies have shown the use of ABS as an effective haemostatic agent in dentistry applications, gastrointestinal and cardiovascular systems (19,29). The anti-inflammatory and antioxidant properties of ABS have also been shown in previous studies (14). ABS is therefore known to have anti-inflammatory and anti-adhesional effects. We chose to evaluate it in epidural fibrosis as its effect on epidural fibrosis had not been studied.

The aim of this study was to histopathologically examine the effect on epidural fibrosis of ABS application following laminectomy in spinal surgery and to investigate the *in vitro* use of ABS in spinal surgery.

MATERIAL and METHODS

Approval for the study was obtained from the Ethics Committee for Experimental Animals at Istanbul University Medical Faculty. The study was conducted at the university experimental animal research laboratory (DETAM).

A total of 20 Sprague-Dawley male rats (400-500 g) aged 6-8 weeks were randomly allocated to 2 groups of 10 in each. The rats were acclimatised to a housing facility (25±2°C room temperature, 50% room humidity, 12 hours light-dark cycle) for 1 week. At 30 minutes preoperatively, antibiotic prophylaxis of cefazolin sodium 20 mg/kg was administered intramuscularly and an additional dose was given at 8 hours postoperatively. General anaesthesia was applied to all the subjects by the peritoneal injection of a mixture of 5 mg/kg Xylazin hydrochloride (Rompun; Bayer Healthcare, Leverkusen, Germany) and 6 mg/kg Ketamin HCL (Ketalar, Pfizer, Istanbul, Turkey). Capnography, pulse oximetry, and automated ventilator module were used for an improved assessment of the physiological status of the rats under anesthesia. The temperature of the rats was monitored and regulated during surgery. The experiment was made on the thoraco-lumbar junction of the rats, since the thoraco-lumbar junction is the largest part of the spine in a rat model and has the least motion. The animals were placed on the operating table in the prone position. The hairs around L1 and L2 were shaved, and the exposed skin was sterilized. A 3 cm median skin incision was made on the rat's thoraco-lumbar junction and the paraspinal muscles were separated from the L1-L2 vertebrae. The dura mater of the L1 vertebra was exposed after removing the spinous process and vertebral plate with a rongeur. Unilateral laminectomy was done to expose the nerve root. Bipolar cautery, bone wax, surgical, or other hemostatic materials were not used. When the laminectomy site was free of active haemorrhage, it was irrigated with 10 ml of saline and then dried with gauze. Close attention was paid not to traumatize the dura, the nerve roots, or the dissected muscles. Immediate muscle and skin closure was undertaken using Dexon 3-0 and Nylon 3-0 in sham group. In the ABS group, cotton wool soaked with 1 mL ABS was applied to the exposure site for 5 minutes and then the muscle and skin closure was realized in the same way as for the sham group. All procedures were performed using loupe magnification of ×5. Regular feeding and care of the rats was continued for 4 weeks.

Histological analysis was performed at four weeks postoperatively. After 4 weeks, the rats were sacrificed using intraperitoneal 100 mg/kg sodium thiopental (Pentothal Sodium, Abbott, Istanbul). The whole L1 and L2 vertebral column including the paraspinal muscles and epidural scar tissue was resected en bloc and microdissection was performed on the operation field. The samples were then fixed in 10% phosphate-buffered formaldehyde solution. After decalcification and dehydration with 20% sodium citrate, 50% formic acid solution for 2 days, the samples were embedded in paraffin, and 5 µm axial sections of the laminectomy site were stained with hematoxylin and eosin (H&E). The epidural scar adhesion was evaluated under a light microscope. Epidural fibrosis was graded based on the scheme devised by He et al. (11); Grade 0: the dura is free of scar tissue; Grade 1: only thin fibrous bands are observed between the scar tissue and dura; Grade 2: continuous adherence is observed in less then two-thirds of the laminectomy defect; Grade 3: scar tissue adherence is large, affecting more than two-thirds of the laminectomy defect, or the adherence extends to the nerve roots. Microscopic evaluation was made by a single pathologist blinded to the operative details to minimize bias. To quantify the fibroblast cells in the scar tissue, the cells in three different areas (two borders and the center of the laminectomy defect) were counted and the mean was calculated. The fibroblast densities were graded as follows:

Grade 1: less than 100 fibroblasts per × 400 field;

Grade 2: 100 to 150 fibroblasts per × 400 field;

Grade 3: more than 150 fibroblast cells per × 400 field.

For statistical analysis of the results obtained in the study, the IBM SPSS Statistics 22.0 program was used. In the evaluation of the study data, we used descriptive statistical methods (mean, standard deviation) and in the comparison of quantitative data of parameters not showing a normal distribution we used the Mann Whitney U-test for the comparison between the two groups (epidural fibrosis). In the comparison of qualitative data (fibroblast density), Fisher's Exact test was used. A p value of <0.05 was accepted as statistically significant.

RESULTS

Two rats (1 in each group) died during the follow-up period due to diarrhea. No cases of paralysis, dural tear, or superficial or deep infection were noted after the laminectomy or during follow-up.

Epidural fibrosis formation evaluation revealed that there was a significant difference regarding fibrosis score between the sham and the ABS treated groups (p=0.011) (Table I). Severe epidural adhesions were observed around the laminectomy sites in the ABS group, and were difficult to dissect as the scar adhesions were accompanied by bleeding and disruption of the duramater. In the histological assessment of the rats, it was observed that laminectomy regions were filled with scar tissue, and this tissue adhered to the dural sac by proceeding towards the spinal canal. In these areas, collagen fibres bundled together, and there were numerous oval shaped fibroblasts among these bundles. Moreover, it was observed that capillaries were full of erythrocytes (Figures 1, 2).

When the groups were compared for fibroblastic activity, a significant difference was determined between the sham and ABS treated groups (p=0.009) (Table I). Fibroblast density in the sham group was lower than in the ABS group.

DISCUSSION

This study aimed to investigate the effects of Ankaferd Blood Stopper® on epidural fibrosis. The hypothesis of this study was that Ankaferd Blood Stopper® would reduce the extent of epidural fibrosis. The results obtained from the study do not support this hypothesis.

Epidural fibrosis is one of the most frequently encountered problems after spinal surgery. The defect area which forms after laminectomy fills with scar tissue and this scar tissue adheres to the dura and nerve root, thus causing failed back surgery syndrome (FBSS) and unfavourable clinical outcomes by reducing the mobility of the nerves and the dura (15).

EF has been reported as the cause of pain developing in the lower posterior trunk or lower extremities in as many as 25% of all FBSS cases (8). Diagnosis of epidural fibrosis is made by contrast-enhanced magnetic resonance imaging (26). There is no effective medical treatment for prevention of postoperative epidural fibrosis. However, some surgical methods were described to prevent or minimize formation of postoperative fibrosis. Preserving the ligamentum flavum in lumbar discectomy and fusing unstable segments may help prevention of epidural fibrosis (2,25). Reoperation to excise this fibrous tissue produces a poor surgical result and further scarring. Many different mechanical barriers and chemical agents have been used to reduce or prevent epidural fibrosis. Materials used as mechanical barriers may cause a foreign body reaction, infection or compression of the neural structures (20,24).

Several authors have reported that the physiopathology of epidural fibrosis is multifactorial (postoperative haematoma,

Table I: Comparison of Fibrosis Score and Fibroblast Number Between Sham Group and Ankaferd Blood Stopper® Group

		Sham	ABS	p
Fibrosis Score; mean±SD (median)		1.89±0.33 (2)	2.67±0.5 (3)	**[1]0.011***
Fibroblast number; n(%)	< 100	1 (11.1)	0 (0)	**[2]0.009****
	100-150	8 (88.9)	3 (33.3)	
	> 150	0 (0)	6 (66.7)	

*[1]Mann-Whitney U test, [2]Fisher's Exact test, *p<0.05, **p<0.01.*

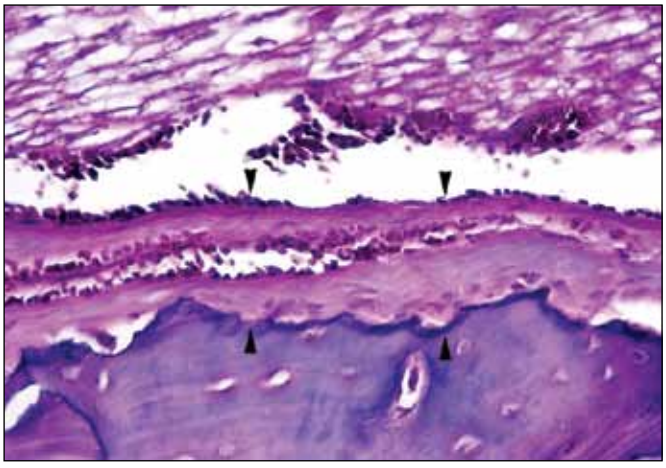

Figure 1: Grade 2 fibrosis and Grade 2 fibroblast density at the laminectomy site in sham group. (H&E, x400).

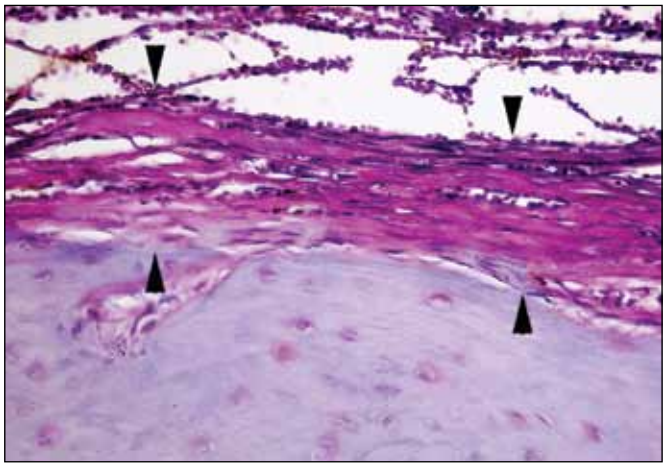

Figure 2: Grade 3 fibrosis and Grade 3 fibroblast density at the laminectomy site in ABS group. (H&E, x400).

laminectomy technique, amount of bone removed) (16). According to several previous studies, although the mechanism of adhesion formation is not fully understood, fibroblast activity and inflammatory responses are believed to be important in the pathogenesis of adhesion formation (23). The most significant histopathological finding observed in the surgical field is excessive fibroblast migration, which causes scarring and fibrosis (11,16). In the current study, excessive fibroblast clustering and migration was observed in the surgical field. These findings were seen to be consistent with those of He et al. (11) and Kasimcan et al. (16). Increased vascular permeability and angiogenesis has since been shown in the early phases of wound repair, theoretically allowing deposition of the fibrin-rich matrix necessary for cellular migration and proliferation, and new granulation tissue is thus formed (3).

Ankaferd Blood Stopper is obtained from a mixture of plant essences and has been shown to be haemostatic and anti-inflammatory, to prevent adhesions, and to have a positive effect on fracture healing in both *in vivo* and *in vitro* studies. In a study by Comert et al. ABS was shown to prevent peritoneal adhesions in an experimental rat model (7). Conversely, Behcet et al. showed that ABS was not efficient in reducing postoperative intra-abdominal adhesions, with the degree of fibrosis and fibroblast amount increasing with the dosage of ABS (1). Nazli et al. showed that topical application of Ankaferd could increase pericardial adhesion after abrasive injury of the epicardial surface in an experimental rabbit model (23).

In the current experimental study, ABS was observed not to have prevented the formation of epidural fibrosis and, in comparison with the sham group, epidural fibrosis had increased. As reported in previous studies, haematoma has been shown to have an effect on the formation of scarring and fibrosis. Our hypothesis that scarring and fibrosis would be prevented due to the haemostatic property of ABS in preventing haematoma and bleeding at the laminectomy site, which has been shown in several clinical and experimental studies, was seen to collapse. As shown in another study, the increased fibroblast intensity in the fibrosis area explains the etiopathogenesis of scarring and epidural fibrosis. However, immunohistochemical analysis is required to explain how ABS causes an increase in epidural fibrosis. The peridural mechanism of peridural fibrosis is fibroblast migration into the surgical area and it seems to be a key factor affecting scar and peridural fibrosis formation (16). Increased VEGF protein in granulation tissue has been shown to be effective on fibrosis (4,13). There is a need for further detailed *in vitro* studies to be made on immunohistochemical analysis (VEGF protein) to investigate the cause of the negative effect of ABS on epidural fibrosis.

Limitations of this study are the low number of samples and the lack of immunohistochemical analysis (IL-6, TGF-B1, VEGF protein, hydroxyproline).

CONCLUSION

The results of this study showed that there was no positive effect of ABS on the prevention of epidural fibrosis, which is one of the most significant problems following spinal surgery, and the epidural fibrosis actually increased. Further experimental and clinical studies are needed to investigate the effect of this plant extract on epidural fibrosis.

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