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Comparison of the acute effects of hemostatic agents on neural tissues in spine surgery: Histologic analysis in rat models

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ABSTRACT

Objective: The most frightening and likely complication of chemical hemostatic agents is neurologic deficit. The histological basis of this potential complication is still unknown. The aim of this study is to observe the acute histologic effects of routinely used hemostatic agents on neural tissues in a rat model.

Methods: Eighteen Winstar Albino rats were operated and same-level laminectomies were performed. The rats were divided into three groups. In group 1 (control group), surgical layers were sutured in routine manner after laminectomy. In group 2 (gelatin sponge group), dura mater was covered with gelatin sponge after laminectomy, while oxidized cellulose was used for coverage in group 3 (oxidized cellulose group). Neurologic evaluations were made for all test subjects. Forty-eight hours after the operation, rats were sacrificed and lumber spines were excised with all surrounding tissues for evaluation by light microscopy of the acute effects of agents on neural tissues. Neurologic scores and histologic findings were compared with double-blind evaluation.

Results: There were no statistically significant differences between the three groups in the histologic findings and clinical evaluations. However, the inflammatory reaction was more severe in the oxidized cellulose group.

Conclusion: Both gelatin sponge and oxidized cellulose did not increase the cellular necrosis of neural tissues. However, oxidized cellulose may lead to an increased local inflammatory reaction.

Key words: *Oxidized cellulose, absorbable gelatin sponge, hemostasis, spine surgery, rat model*

Introduction

Epidural bleeding and hematoma are important and devastating complications in spinal surgery [1, 2]. Control of bleeding during the surgery can be problematic for the surgeons. Electrocoagulation, hemostatic agents and pressurization methods are available to control blood loss during the spinal surgeries. Oxidized cellulose and bone wax gelatin sponge are used as local hemostatic agents [3, 4]. Local hemostatic agents are widely used to control blood loss [3-5]. Hemostatic effects of local hemostatic agents generally bind the blood cells and facilitate the formation of fibrin clot [6].

In the literature many complications have been reported about the local hemostatic agents [7]. Neurological deficit is the one of most serious and frighten-

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ing complication of local hemostatic agents [7-9]. The mechanisms of these complications were not fully elucidated [10-12]. Local pressure effects, and histological necrotizing effects were blamed for these complications [8, 9, 12]. However, it is not clear which factors lead to neurologic deficits after application of the hemostatic agents. The aim of our study was to compare histological effects of the commonly used hemostatic agents gelatin sponge and oxidized cellulose on neural tissues in a rat model.

Materials and Methods

Eighteen Winstar Albino rats, which were 230-250 grams in weight, were included into the study. Ethical commission approval (6th of November, protocol no: 62/2009) was obtained from the local ethical committee. Preoperative and postoperative neurologic status of animals were evaluated with the Basso-Beattie-Bresnahan (BBB) scoring system [13]. In this scoring system, the range of values varies between zero points, given for the animals if there is no movement in rear extremities (total paraplegia), and twenty-one points, which indicates the healthy neurological status of the animals.

The rats were anesthetized with intraperitoneal 35 mg/gr Ketamin (Ketalar, Parke Davis) and 5 mg/kg Xylazin (Rompun, Bayer). After incubation, subjects were placed in prone position and all hairs on the lumbar area were shaved. The incision site was prepared with Polyvidoniyod (Batticon, Adeka). The interscapular line was determined for reference point to find the exact level of the lumbar vertebrae. After midline skin incision, the lumbar fascia and paravertebral muscles were dissected from the spinous processes and posterior surfaces of the laminae. Muscles were retracted with small-automated retractor. Total laminectomy was applied with 1 mm Kerrison rongeur between L2-L3 laminas. All laminectomies were performed in the same levels. Dura mater was protected during this process (Figure 1).

The selected rats were randomized and then divided into three groups. Each group had 6 rats. In the first group, defined as the control group, after laminectomy wounds were closed without any hemostatic agents. In the second group, the laminectomy defect was covered with gelatin sponge (spongostan) over a 10x5mm area (Figure 2). In the third group, oxidized cellulose

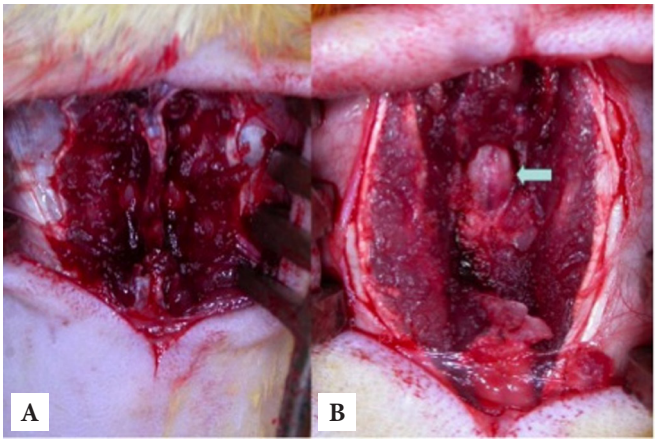

Figure 1. Laminectomy of lumbar spine in rats. **A:** Subperiosteal stripping of paravertebral muscles was performed with fine dissectors. **B:** Posterior surface of dura mater (white arrow) can be seen after total laminectomy.

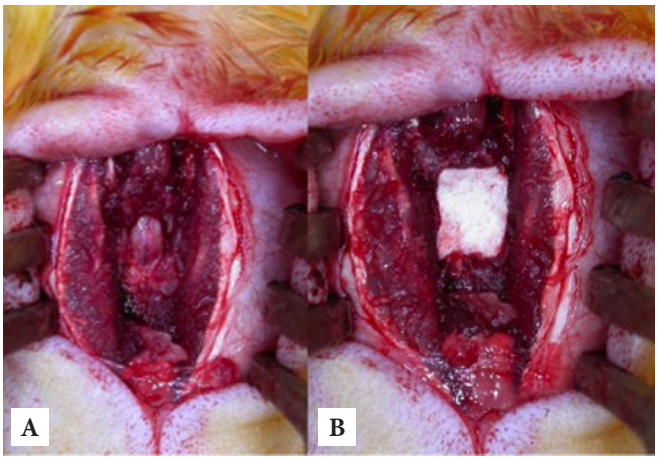

Figure 2. Hemostatic absorbable gelatin sponge application on the dura mater after laminectomy. Dura mater was rather widely exposed with laminectomy **(A)** and posterior surface of dura mater was covered with hemostatic sponge **(B)**.

was spread on the dura mater over a 10x5mm area. The whole posterior surface of dura mater was covered with hemostatic agents in all subjects of groups 2 and 3. Wounds were sutured in routine manner with 3/0 prolene suture. Postoperative neurological assessment was made at 24th and 48th hours. Forty-eight hours after operation, the subjects were anesthetized, and then sacrificed with an intracardiac KCl injection. Four adjacent vertebrae in which the center area was the laminectomy site were excised with spinal cord and surrounding soft tissues. The samples were transferred to the histology department in formaldehyde solution.

The preparations were examined with a light microscope (Olympus BX 50) and nonparametric changes with edema, inflammatory reaction and necrosis caused by local hemostatic agents on the neural tissues were recorded.

Inflammatory reactions were graded as follows:

0: Normal tissue and cells

1: Low edema and low polymorphonuclear leucocytes (PNL)

2: Edema and intense PNL

3: Edema and intense PNL and necrosis

Histological and neurological findings were evaluated by chi-square test. Data were analyzed using SPSS (Version 13.0; SPSS Inc, Chicago, IL).

Results

All subjects were in healthy condition 48 hours after the surgery. Neurological details of the subjects at 24 hours and 48 hours are given in Table 1. All groups were compared statistically with each other and no statistically significant differences were found (p=0,588).

There were low PNL accumulations between the dura mater and muscle tissue in most of the subjects of the control group (Figure 3). Histologic features of the hemostatic sponge group were also similar to the control group (Figure 4). However, intense PNL accumulation was observed in the oxidized cellulose group (Figure 5). The histologic findings are summarized in Table 2. Histological findings were analyzed and no statistically significant differences were found (p=0,694). According to the Mann-Whitney U test, no statistically significant differences were found between three groups.

Discussion

Local mass effect, compression of hematoma, neural tissue ischemia and granulomatous reaction developed by inflammatory responses have been identified as mechanisms for neurologic deficits [6, 14, 15]. The most serious complication that can occur after the use of local hemostatic agents is neurologic deficit [7-9, 16]. However, the mechanism of neurologic damage caused by hemostatic agents is not clear [10-12]. The association between the neurologic deficits due to the local hemostatic agent application is not well understood. Neural tissue changes have been attributed to the acidic agents excreting from the dissolving oxidized cellulose. Dissolved oxidized cellulose turns into organic acids and decreases the pH of the tissue. The agents forming acidic pH such as oxidized cellulose may affect neural cells. In our study, the PNL activity in the oxidized cellulose group was much higher than in

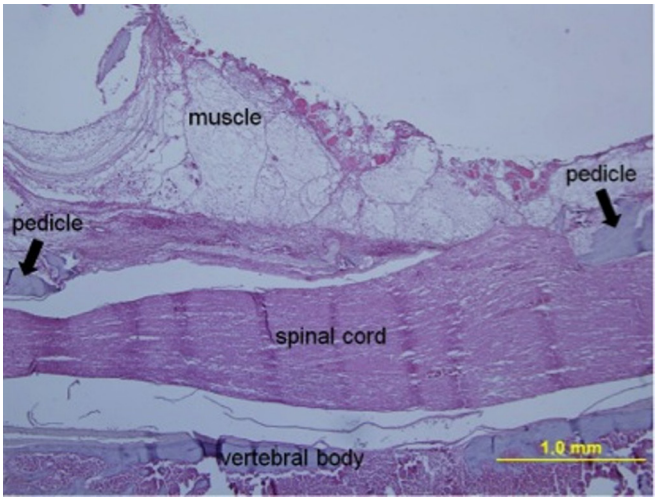

Figure 3. An example of sagittal histologic section of control group. The midline section shows the correlation of paravertebral muscles, medulla spinalis and durameter. Black arrows indicate the upper and lower pedicles (10x magnifier).

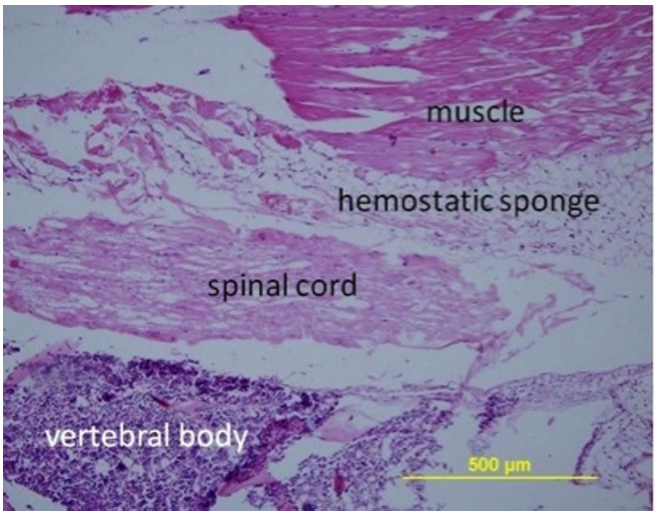

Figure 4. Histologic sections of hemostatic sponge group were quite similar control group. PNL accumulation was equal to the control group in hemostatic sponge. Only limited amount of PNL can be seen on dura mater (20X magnifier).

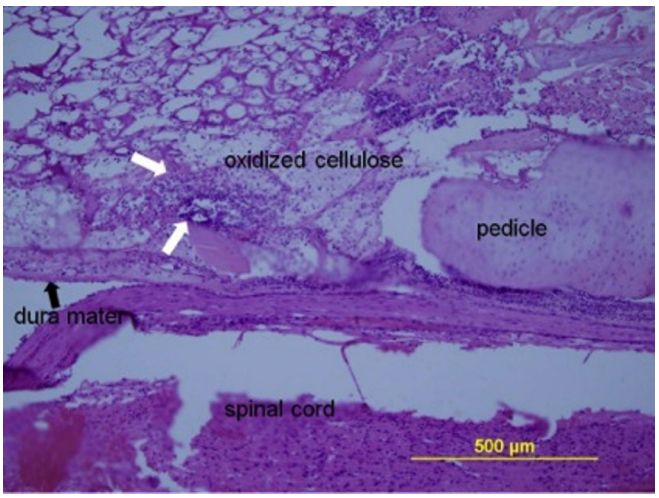

Figure 5. In the oxidized cellulose group, marked PNL accumulation can be seen into the oxidized cellulose particles and indicated by white arrows. Increased PNL and inflammatory reaction lays on the dura mater (20x magnifier).

Table 1. Postoperative neurologic status of the subjects at 24 and 48 hours.

Time	Neurological status	Control n (%)	Oxidized Cellulose n (%)	Hemostatic Sponge n (%)	P value
24th hours	Two joint full and third joint slightly movement (6 points)	1 (16,7%)	1 (16,7%)	None	0,570
	Full motion (7 points)	5 (83,3%)	5 (83,3%)	6 (100%)	
48th hours	All joints full range of motion but the body unstable (20 points)	1 (16,7%)	None	None	0,347
	All joints full range of motion and the body stable (21 points)	5 (83,3%)	6 (100%)	6 (100%)	

Table 2. The distribution of histologic findings in rats.

Inflammatory response	Control n (%)	Oxidized Cellulose n (%)	Hemostatic Sponge n (%)
Normal (0)	1 (16,7)	1 (16,7)	0
Low PNL (1)	4 (66,7)	2 (33,3)	5 (83,3)
Intense PNL (2)	1 (16,7)	3 (50)	1 (16,7)

n:number, PNL:polymorphonuclear leucocytes

the hemostatic sponge and control groups. These findings may be related with the acidic effect of the oxidized cellulose.

Local hemostatic agents generally bind the blood cells and facilitate the formation of fibrin clot. Hemostatic sponges are commonly used in spinal surgery practice in order to fill up the cavity and stop the bleeding from epidural vessels. Many neurologic complications have been reported about the hemostatic sponges utilization following laminectomy. These include cauda equina syndrome, spinal stenosis, meningitidis, impotence and toxic shock syndrome [3, 7]. Such complications are thought to be associated with the local mass effect of the hemostatic sponge, hematoma and the inflammatory response against the hemostatic sponge [4, 5, 15]. Awwad et al. reported dural compression in two patients who had undergone oxidized cellulose application following partial laminectomy. The symptoms of the patients were resolved with conservative treatment [11]. Henry et al. reported a patient who had thoracic neuroblastoma surgery and neurologic loss 55 hours after surgery. They revised the surgical site and the neurological status improved after removing of oxidized cellulose. They concluded that the oxidized cellulose may become bulky and compress the neurological structures [9]. Neurological complications, which are thought to be related to local hemostatic agents, usually happen in early stage of the post operative period[8, 9]. Thus, we organized the study to evaluate the effect of the local hemostatic agents during the early stages. In our study, huge hematoma formation was not detected in histologic evaluation and there were no distinct clinical neurologic findings. However, rat spine is anatomically small in size and it is not easy to detect hematoma formations.

Neuronal ischemia and necrosis were identified as additional mechanisms of neuronal damage [12, 15]. Nagamatsu et al. investigated the effect of oxidized cellulose on rat sciatic nerve blood flow. They have not found any difference in the blood flow with oxidized cellulose application [12]. However, early axonal degeneration and segmental demyelination has been observed under electron microscopy in the oxidized cellulose group. In our study, neuronal damage was not investigated, but there was no neurologic deficit clinically. The increased inflammatory reactions in the oxidized cellulose group were our major histologic finding, but the importance of these reactions was not proved clinically in our study.

Oxidized cellulose is an organic acid and catalyzes the hemostasis by turning the blood into a gelatinous mass form. As the tissue pH lowers and becomes acidic, the immune system is activated. The degree of the inflammatory response against oxidized cellulose is associated with the amount of the oxidized cellulose, tissue pH and the presence of pathogenic microorganisms [3]. Neural damage may occur following the direct application of acidic pH-creating oxidized cellulose on dura mater. In a study on cultured neural and glial cells by Nedergaard et al., functional decrease of the neuroglial cells following the pH level below 6.8 and cellular death following the prolonged low pH levels was reported [17]. In our study, the PNL activity

in the oxidized cellulose group was much higher than in the hemostatic sponge and control groups. These results may be related to the low pH effect of the oxidized cellulose.

In spinal surgery, many surgeons apply local hemostatic agents on dura mater order to prevent neural tissues from bleeding and fibrosis. After the epidural bleeding, the fibrin clot at the laminectomy spot, heals as a granulation tissue and turn into a dense fibrous tissue [18]. Epidural fibrosis is formed by mononuclear cell activation, and increases in the vascularization and fibroblast activity. Epidural fibrosis associated neural root compression and dural compression may result in neurologic symptoms and failed back surgery syndrome. The fatty tissue and local hemostatic agent applied to the laminectomy spot in order to prevent fibrosis formation may also result in the fibrosis formation [18]. Nevertheless, the fibrosis associated with the local hemostatic agent utilization, setting at the 6-8 weeks postoperatively, is not capable of explaining the acutely arising neurologic deficits. In our study, the etiology of acute neurologic deficits following local hemostatic agent was investigated.

Our study has several limitations. Our groups had small numbers of animals, which may lead to no significant differences between the groups in means of PNL accumulation and neurologic findings. Furthermore, using a microscope to detect and observe the effects of local hemostatic agents on neural tissues may be insufficient. Electron microscopy studies in large sample groups may show more reliable results. A longer follow-up could give us the effects of inflammation in neuronal tissues. An additional limitation of our study was our non-validated histological grading system. This grading system was not originally designed for neural tissue investigation, but same experienced histologists who designed this grading system examined all histologic samples to avoid inappropriate histological results.

Hemostatic agents are beneficial in spine surgery. In our study, both gelatin sponge and oxidized cellulose did not cause cellular necrosis of neural tissues. However, oxidized cellulose led to an increased local inflammatory reaction. The surgeon who plans to use a local hemostatic agent on neural tissue should think about the local effects of these agents. To understand the local effect of the hemostatic agents, studies with the large cohorts and with the longer follow-up should be done.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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