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Evaluation of resorption and biocompatibility of collagen hemostats in the spinal epidural space

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Abstract

BACKGROUND CONTEXT: Collagen hemostats have different characteristics depending on their properties and configuration. In vivo serial evaluation of local reactions because of placement of hemostats in the epidural space has not been reported.

PURPOSE: This study compared the resorption and biocompatibility of two types of collagen hemostats placed in the epidural space.

STUDY DESIGN: This in vivo study used experimental animals to evaluate collagen hemostats that were placed in the epidural space.

METHODS: A ligamentum flavum resection model was created in Japanese white rabbits (n=65). A microfibrillar collagen hemostat (MCH group, n=5), cotton-type collagen hemostat (CCH group, n=5) that was chemically cross-linked, or no hemostat (control group, n=4) was placed in the spinal epidural space. For histologic evaluation, each group was euthanized 1, 2, 4, and 8 weeks postoperatively (PO), and hematoxylin-eosin and immunohistochemical (IHC) staining for inflammatory cytokines (tumor necrosis factor [TNF]-α, interleukin [IL]-6), cyclooxygenase (COX)-2, and macrophages (CD68) was performed. To evaluate exudate accumulation and the degree of inflammation in the epidural space, magnetic resonance imaging at 7.04 T was serially performed in each group (n=3) under anesthesia and sedation.

RESULTS: The collagen hemostats in both groups were reabsorbed at 4 weeks PO. In the MCH group, there was inflammatory cell infiltration and granuloma formation around the hemostat, TNF-α–positive cells were seen up to 1 week, and IL-6–, COX-2–, and CD68-positive cells were seen at all evaluation times. In the CCH group, no inflammatory cell infiltration around the hemostat was observed, and IHC staining showed no positive cells at 4 weeks PO and later. T2*-weighted MR images showed significantly higher mean signal intensity of the epidural space in the MCH group than in the CCH group but only at 1 week PO ($p<.05$).

CONCLUSIONS: Resorption of both hemostats was similar. In the MCH group, there was intense tissue inflammation around the hemostatic material, and MR images showed high signal intensity because of exudate accumulation in the epidural space. This indicated a strong foreign-body

FDA device/drug status: Not applicable.

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reaction to the MCH, thus demonstrating a difference in biocompatibility with the CCH.

Keywords: Collagen hemostat; Spinal epidural space; Biocompatibility; Resorption; Antigenicity; Inflammation

Introduction

Collagen hemostats in spinal surgery are mainly used to control bleeding from the epidural venous plexus. After hemostasis, removal of as much of the collagen hemostat as possible is recommended [1,2], but we have encountered cases in which rebleeding after removal occurs, thus requiring retention of a topical hemostat. However, neurologic deficits because of swelling and granulation of topical hemostats have also been reported as a postoperative (PO) complication [3–8]. In addition, differences in resorption and biocompatibility of hemostatic materials depending on their properties and configuration have been reported [1,2,9–16]. When a hemostatic material is placed in the epidural space, inflammation and cytotoxicity because of the hemostatic material may adversely affect surrounding tissue such as the spinal nerves. Nevertheless, to our knowledge, local reactions that may occur because of the placement of hemostatic materials in the spinal epidural space have not been investigated in vivo.

The purpose of this study is to compare the resorption and biocompatibility of two types of collagen hemostats when placed in the epidural space. We created a ligamentum flavum resection model in Japanese white rabbits, and we placed two different types of collagen hemostats used in spinal surgery in the epidural space. Histologic analysis was performed to evaluate resorption and the biological response to each hemostatic material. In addition, high magnetic field magnetic resonance imaging (MRI) in the experimental animals under anesthesia and sedation was performed for serial imaging to evaluate exudate accumulation and the degree of inflammation in the epidural space.

Materials and methods

Hemostatic materials placed in the spinal epidural space

We used two types of hemostatic materials purified from collagen that was obtained from bovine dermis. One type was the microfibrillar collagen hemostat (MCH) (Avitene; Zeria Pharmaceutical Co. Ltd, Tokyo, Japan). Microfibrillar collagen hemostat, which was developed in the United States in the 1970s, is a hemostatic material developed by purifying collagen so that the natural fibril cross-links are maintained. The other type was the cotton-type collagen hemostat (CCH) (Integran; Nippon Zoki Pharmaceutical Co. Ltd and Koken Co. Ltd, Tokyo, Japan). The raw material was atelocollagen, in which telopeptide, the main antigenic determinant site in collagen, had been removed to reduce antigenicity [12]. CCH was produced by spinning atelocollagen into cotton-like fibers and chemical cross-linking the fibers using a polyepoxy compound. CCH is a type of collagen hemostat developed in Japan in the 1990s to reduce antigenicity and to improve handling and resorption. In this study, to insert the hemostatic material under the vertebral arches, the press sheet type with cotton fibers spread along a plane was used.

Resected ligament flavum model for spinal dura mater exposure

This animal study was approved by the Experimental Animal Committee at our institution. We created a ligamentum flavum resection model using 65 Japanese white rabbits (males, age 13 weeks, 2.5–2.8 kg). The animals were purchased from Oriental Bio Service (Kyoto, Japan).

Surgery was performed under inhalation anesthesia. A 3-cm posterior longitudinal incision was made in the dorsum at the level of L7/S1. The fascia was opened, the spinous processes were spread, and the ligamentum flavum at L7/S1 was exposed. Then, the ligamentum flavum was resected, and the epidural fat was removed to expose the dura mater.

For histologic and immunohistochemical (IHC) evaluation, 0.5 mg of each material was placed on the dura under the first sacral vertebral arch in 56 rabbits. For MRI, 5 mg of each material was inserted on the dura between the L7 and S1 vertebral arches in nine rabbits. The fascia and skin were closed with 3-0 nylon suture.

The experimental model was divided into MCH (placement of MCH), CCH (placement of CCH), and control (resection of ligamentum flavum only, but no placement of a hemostatic material) groups.

The experimental animals were returned each to their individual cages. They were free to move about and eat and drink ad libitum. The surgical wounds were examined, including a check for any lower limb paralysis.

Histologic evaluation of inflammatory cell infiltration to the collagen hemostats and resorption

Histologic analysis was performed to evaluate inflammatory cell responses to the collagen hemostats and their resorption when placed in the epidural space. At 1, 2, 4, and 8 weeks PO, MCH (n=5), CCH (n=5), and control (n=4) group