Journal of Pediatric Surgery 50 (2015) 1928–1932

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.elsevier.com/locate/jpedsurg

Histological changes of the unligated vein wall adjacent to the central venous catheter after open cutdown in rats☆

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ARTICLE INFO

Article history:
Received 10 February 2015
Received in revised form 26 March 2015
Accepted 30 April 2015

Key words:
Vein wall
Histological changes
Cutdown
Rat
Jugular vein

ABSTRACT

Background: The sequelae of a central venous cutdown usually include venous deformity causing venous stenosis or stricture. However, the cellular mechanisms causing these deformities have not been elucidated.
Methods: Silicone 2.7-Fr catheters were placed via the right external jugular vein of 16 rats with the cutdown method. After fixation with formalin at scheduled intervals (1 week, 2 weeks, 4 weeks, and 8 weeks; 4 rats in each group), the vein segment with the catheter in situ was harvested. Histological changes in the vein wall were studied and serially compared with light microscopy; standard hematoxylin-eosin staining, Masson's trichrome staining, van Gieson's elastin stain, and immunohistochemical stain against α-actin.
Results: Pericatheter sleeve formation, circumferential smooth muscle cell proliferation and infiltration into the pericatheter sleeve by direct contact were noted in all 4 rats of 1-week model; this indicated the initiation of neointimal hyperplasia. The neointimal hyperplasia was located inside the elastin layer. At 2 weeks, the SMCs stained faintly but the components of the vein wall were largely replaced by collagen. The proliferation and infiltration of SMCs stabilized at 4 weeks and no SMCs were stained around the catheter. At 8 weeks, luminal narrowing was noted and the venous wall was composed mainly of collagen.
Conclusions: Circumferential neointimal hyperplasia occurred after surgical cutdown of the external jugular vein in a rat model and was caused by SMC activation, proliferation, and infiltration into the pericatheter sleeve.

Over the last two decades, the primary choice for central venous catheter (CVC) placement has shifted from the surgical cutdown to the percutaneous technique, and the cutdown method has been reserved for more premature infants who are small enough to require an invasive cutdown procedure [1]. However, serious anatomical deformities causing stenosis or stricture have been documented in veins, which had previously harbored indwelling CVCs [2,3]. In clinical conditions in which CVC change is required with the previously placed catheter remaining in situ, it would not be difficult to employ surgical manipulation that would allow reuse of the same vein [4]. However, when catheter reinsertion is required after removal of the previously placed catheter, the previously accessed veins may or may not remain patent, requiring use of a new access site. This can result in loss of potential venotomy targets [5]. Neonates who need a cutdown procedure, usually ELBW (extremely-low birth weight) infants, are prone to require repeated CVC insertion; therefore, every effort should be made to preserve readily accessible central veins as much as possible. Another major event occurring after CVC placement is pericatheter sleeve formation; the composition and development of such a pericatheter sleeve have been well documented [6]. We hypothesized that structural changes occurring in the venous wall and possible interaction with the pericatheter sleeve would be the cause of the venous deformities, but currently, limited data are available on such changes after a surgical cutdown. Thorough understanding of these changes could lead to the prevention or resolution of such deformities and preservation of central veins could be possible. The aim of this study was to evaluate the serial histological changes and interactions with the pericatheter sleeve occurring at the unligated jugular vein wall at the venotomy site after cutdown in rats.

1. Materials and methods

1.1. Animals

Male Sprague–Dawley rats, aged 8 weeks with an initial weight of 250 g, were used. The animals were purchased from a specific pathogen free laboratory animal company (DBL Co., South Korea). We did not restrict diet; standard rat chow and tap water were supplied before and after the operation. Humane care was applied in compliance with the Guide for the Care and Use of Laboratory Animals (National Institute of Health [NIH], publication no. 85-23, revised 1996). This study was approved by the Kangwon National University Institutional Animal Care and Use Committee (KNU-IACUC, KW-140911-1).

☆ Conflicts of interest: none.

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http://dx.doi.org/10.1016/j.jpedsurg.2015.04.019
0022-3468/

1.2. Catheters

The catheters used in this study were 2.7-Fr single lumen silicone catheters (Broviac®; Bard access system, Utah, USA). The catheters were sterilized before the surgical procedure and flushed with heparin solution (100 IU/mL).

1.3. Surgical procedure

The rats were anesthetized with a single intraperitoneal dose of tiletamine/zolazepam (Zoletil; Virbac Laboratories, Carros, France) 20 mg/kg. A single subcutaneous dose of atropine (Daihan Pharm Co., Seoul, South Korea) 0.04 mg/kg was administered to decrease tracheal secretions and a single intraperitoneal dose of cefmetazole (Daewoong Co., Seoul, South Korea) 50 mg/kg was administered. A right paramedian vertical incision was made over the pulsating jugular vein. The platysma muscle was opened and the external jugular vein was exposed and released from the junction of the facial and maxillary veins to the junction with the subclavian vein. Small side branches were cauterized with a hand-held, portable electrocautery (Bovie®, Bovie Medical Corporation, FL, USA). After applying proximal and distal control with a fine vascular loop, a venipuncture in the anterior wall of the external jugular vein with a 20-gauge needle was made and the beveled-tip Broviac catheter was inserted through the venipuncture opening to a depth of 4 cm length; this enabled the tip to be positioned at the end of the anterior vena cava. Closure of the venipuncture was not required. After a bolus injection of heparin solution (100 IU/mL), the catheter end was closed with a titanium clip (Surgiclip®; Covidien, CT, USA) leaving 1 cm length from the venipuncture site and embedded in the subcutaneous layer. The platysma layers were approximated with 6-0 absorbable suture (Vicryl®, Ethicon, USA) and the skin was approximated with a skin stapler. The catheter was left in situ for the following durations: 1 week ($n = 4$), 2 weeks ($n = 4$), 4 weeks ($n = 4$), and 8 weeks ($n = 4$) postoperatively. Because the goal of this study was to evaluate the histological remodeling of the jugular vein after a cutdown procedure and evaluation on the major determinant for such changes, either surgical manipulation itself or catheter-vein interactions, was not a part of this study, sham operation was not performed.

1.4. Fixation, tissue harvest, and staining

After the scheduled duration had elapsed, the animals were sacrificed. Fixation methods were modified from previously published protocols [5]. In brief, the animals were anesthetized using the same protocol described above. Through a long midline incision, from the chin to the xiphoid process, the right carotid artery and left external jugular vein were exposed and cannulated with a 26-gauge catheter. After heparinization (1000 IU/kg) was administered to the left external jugular vein, and a bolus of 1000 mg/kg anesthetic solution was injected into the left external jugular vein for euthanasia. A 10% buffered formalin solution, 100 cc, was infused at physiologic pressure of 100 mmHg through the right carotid cannula (inflow) and the left external jugular cannula was used as drainage (outflow). After in situ fixation, the veins were infused with 10 cc of 10% gelatin solution (Sigma–Aldrich, USA), heated to 36 °C, via the left external jugular vein to prevent luminal collapse. The rats were then cooled for 40 minutes at 4 °C in a refrigerator. After cooling, the entire length of right external jugular vein, anterior vena cava, and heart was excised en bloc including the surrounding tissues through a median sternotomy and immersed in formalin overnight. After immersion fixation, a 0.5 cm-thick segment of the external jugular vein, centered at the catheter entry site and the junction with the subclavian vein, was obtained and embedded in a cassette. All tissues were stained with hematoxylin and eosin (H&E). Masson's trichrome staining was used to delineate collagenous tissue. With immunohistochemical staining, smooth muscle cell (SMC)-specific anti α-actin was stained to confirm the presence of SMCs at the medial layer. van Gieson's elastin stain was used to delineate the internal elastin layer in some sections.

2. Results

There were no perioperative death or clinical evidence of infection, and no animal died during the observation period. Figs. 1–4 show the typical, representative sections at each time point, according to each staining method (A, H&E stain; B, Masson's trichrome stain; C, SMC stain; D, van Gieson's elastin stain). All of the pictures were taken at ×40 magnification (Insets, ×400 magnification).

2.1. 1 weeks ($n = 4$)

On H&E staining, concentric, circumferential proliferation of the SMCs at the media (medial layer hyperplasia) as well as the formation of a pericatheter sleeve was the initial events noted in all 4 rats of 1-week model. The proliferating SMCs were noted to have infiltrated into the sleeve circumferentially on immunohistochemistry; thus, initiating neointimal hyperplasia (Fig. 1 A–C). The neointimal hyperplasia was located inside the elastin layer (Fig. 1 D).

2.2. 2 weeks ($n = 4$)

Progressive neointimal hyperplasia was seen with mixed cellular and extracellular components. At this time point, pericatheter sleeve was incorporated circumferentially into the true vein wall, thereby forming a common vein wall. SMCs stained faintly, but the components of the vein wall were largely replaced by collagen (Fig. 2 A–C).

2.3. 4 weeks ($n = 4$)

The proliferation and infiltration of SMCs stabilized by 4 weeks; cellular components were near-totally replaced by an extracellular matrix. No SMCs were stained around the catheter, and the vein wall was primarily composed of collagen in the 4-week models (Fig. 3 A–C).

2.4. 8 weeks ($n = 4$)

In the 8-week models, a faint, thin layer of SMCs reappeared, but the major component of the venous wall was collagen. At this time, the lumen of the vein was narrowed to the catheter diameter (Fig. 4 A–C).

3. Discussion

The aim of this study was to evaluate the serial anatomical changes of the venous wall adjacent to the CVC and its interactions with pericatheter sleeve after surgical cutdown in a rat model. Vascular wall damage causing structural or functional abnormalities after surgical manipulation, either in an artery or a vein, has widely been recognized and most surgeons perform vascular surgery with minimally invasive techniques and less traumatic instruments. However, even with the most delicate performance and fine instruments, such damage cannot be avoided and the exact cellular mechanisms causing such damage remain unclear. Forauer et al. provided some insight into the histological changes in the vein wall adjacent to the CVC in humans [7]. They noted focal areas of endothelial injury with or without thrombi in patients with short-term catheter placement, and in patients with long-term placement, they noted the vein wall thickening caused by the increased number of SMCs. However, this observation was the result of an autopsy study with a small sample size ($n = 5$), and the methods of catheterization, either percutaneous or cutdown, were not described. Moreover, the SMC proliferation observed in that study did not involve the entire circumference of the vein along the catheter course, and this might imply a somewhat different mechanism of cellular changes, which would be inappropriate to apply to our results.