The Rat Femoral Arteriovenous Fistula Model: Increased Expression of Matrix Metalloproteinase–2 and −9 at the Venous Stenosis

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PURPOSE: To determine whether a femoral arteriovenous (AV) fistula model in a rat was feasible and whether there is increased expression of matrix metalloproteinase (MMP)–2 and −9 and the tissue inhibitors of MMPs (TIMPs) at the venous stenosis of the fistula.

MATERIALS AND METHODS: Fifteen male Sprague-Harley rats weighing 353 g ± 26 underwent creation of an AV fistula between the left femoral artery and ipsilateral femoral vein, with the contralateral femoral vessels serving as controls. The animals were euthanized at day 14 ($n = 5$) and day 28 ($n = 10$) after fistula creation. Zymography and Western blot analysis for TIMP-1 and TIMP-2 were performed at the venous stenosis and in control vessels. Hematoxylin and eosin, Verhoeff–van Gieson, Masson trichrome, and α–smooth muscle staining were performed at the stenosis and in controls at day 28 in four animals. The intima/media ratio was determined at day 28.

RESULTS: By day 14, pro–MMP-2 measurements were 8.13 ± 1.06 at the venous stenosis and 4.1 ± 1.33 in controls ($P < .05$). By day 28, they had increased to 18.95 ± 4.8 at the stenosis and 12.11 ± 4.84 in controls ($P < .05$). By day 14, active MMP-2 measurements were 7.38 ± 1.25 at the stenosis and 2.31 ± 1.04 in controls ($P < .05$). By day 28, they had increased to 12.12 ± 3.45 at the stenosis and 9.26 ± 3.97 in controls ($P < .05$). By day 28, pro–MMP-9 measurements were 11.77 ± 4.71 at the stenosis and 7.78 ± 3.49 in controls ($P < .05$), with no difference at day 14. There was no difference in expression of TIMP-1 and TIMP-2. The average intima/media ratio of the stenosis increased by 28% versus controls, and the neointima was composed of primarily α–smooth muscle actin–positive cells.

CONCLUSIONS: A rat femoral AV fistula model was created with venous stenosis formation characterized by thickened neointima composed of α–smooth muscle actin–positive cells compared with controls. At the venous stenosis, there was increased expression of pro–MMP-2 and active MMP-2 by days 14 and 28, with significantly increased expression of pro–MMP-9 by day 28.

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Abbreviations: AV = arteriovenous, MMP = matrix metalloproteinase, PTFE = polytetrafluoroethylene, TIMP = tissue inhibitor of matrix metalloproteinase

MAINTENANCE of vascular access patency is essential to secure optimal delivery of hemodialysis to patients with end-stage renal disease. The arteriovenous (AV) fistula is the preferred vascular access (1). Unfortunately, the patency rate of AV fistulas is estimated to be only 62% at 1 year (2). This high failure rate results primarily from venous stenosis, which is caused by intimal hyperplasia resulting in impairment of blood flow and thrombosis at the AV anastomosis (3,4). Understanding the mechanisms that lead to the formation of intimal hyperplasia may be of critical importance in developing strategies that delay or prevent AV fistula failure.

Intimal hyperplasia is produced by the proliferation of vascular smooth muscle cells together with matrix deposition (3,5). Although various cytokines have been implicated in this process, matrix metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of MMPs (TIMPs) may be im-

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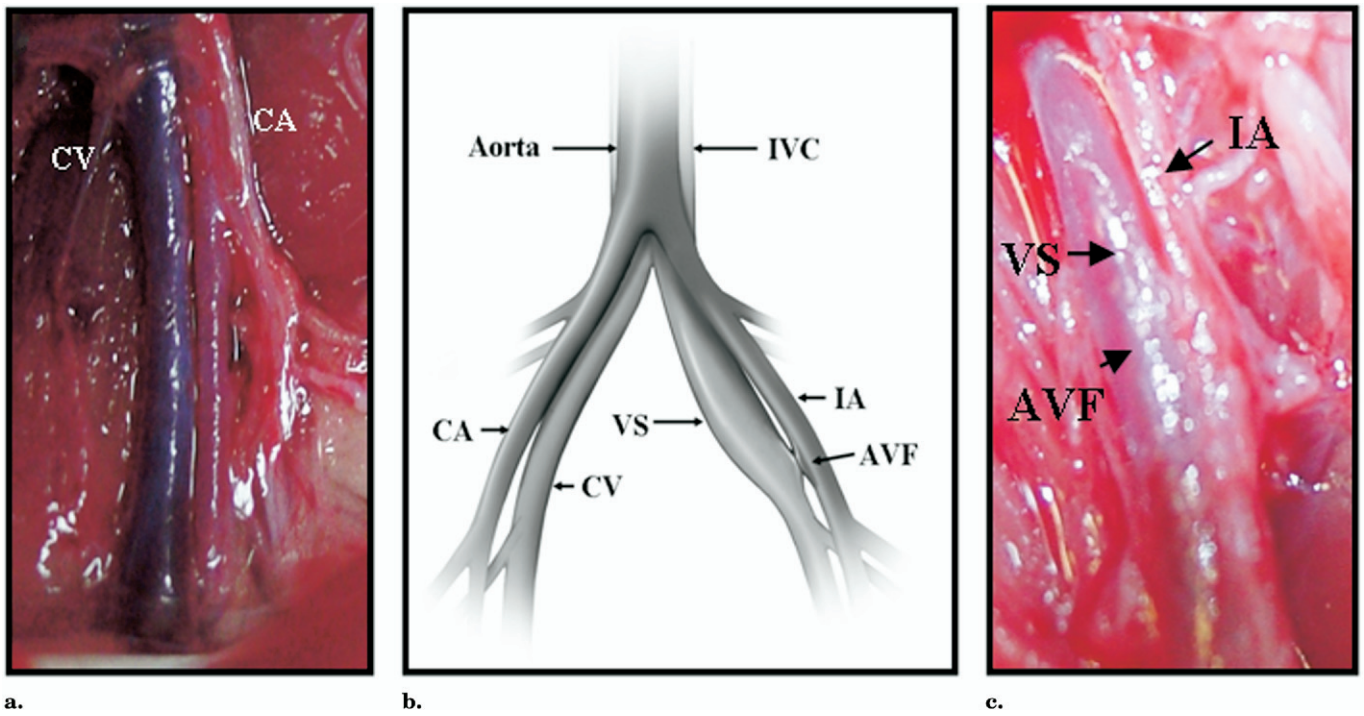

Figure 1. Creation of AV fistula between the femoral artery and femoral vein: intraoperative view of control vessels at day 28 **(a)**. (*CA*, control femoral artery; *CV*, control femoral vein; *AVF*, AV fistula; *VS*, venous stenosis; *IA*, inflow artery.) A schematic shows the creation of the AV fistula **(b)**. Another intraoperative view is shown for day 28 after fistula creation **(c)**. (Available in color online at *www.jvir.org*).

portant (6–8). MMPs are Zn^{2+}-dependent proteinases that participate in tissue remodeling associated with vascular disease (9). The expression of these enzymes is tightly regulated at the transcription (ie, messenger RNA) and translational levels, and by their inhibitors of MMPs (ie, TIMPs) (9,10). They are secreted as proenzymes (eg, insulin) and cleaved to the active form in the extracellular space. There have been several studies that have evaluated the role of MMPs in polytetrafluoroethylene (PTFE) hemodialysis graft failure (7,8,11). Increased expression of MMP-2 and MMP-9 has been observed in experimental models of AV fistula in the rat (12). Recently, a study performed in failed AV fistula specimens removed from patients showed that there is increased expression of pro–MMP-9 in these fistulas compared with control specimens (8). Taken collectively, these studies suggest that MMPs (specifically MMP-2 and MMP-9) may play a prominent role in the pathogenesis of intimal hyperplasia of hemodialysis AV fistulas. However, the role of MMPs and TIMPs (ie, inhibitors of MMP-2 and MMP-9) in AV fistula failure remains unknown.

Animal models of hemodialysis graft failure have been created to understand the mechanisms responsible for intimal hyperplasia. The most commonly used animal model has been the porcine model (5,7,11,13–15). Recently, small animal models including the rat and mouse have been described (12,16,17). In the present article, we describe our experience with a rat femoral AV fistula model. With this model we determined the expression of matrix regulatory proteins (ie, MMP-2 and −9) and their inhibitors (ie, TIMP-1 and −2) after AV fistula creation at 14 days and 28 days.

MATERIALS AND METHODS

Study Design

Institutional animal care and use committee approval was obtained before we performed any procedures on animals. The housing and handling of the animals was performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals revised in 2000. Seventeen male Sprague-Harley rats (Harlan Laboratories, Indianapolis, Indiana) weighing 325–350 g were used. AV fistulas were created from the femoral artery to the ipsilateral femoral vein (as described later), and the contralateral femoral artery and vein served as controls (**Fig 1**). The animals were sacrificed at day 14 ($n = 5$) and day 28 ($n = 10$) after fistula creation. These specimens were snap-frozen in liquid nitrogen and stored at −80°C for Western blotting and zymography. Four specimens removed from animals in the 28-day group were placed in formalin and then embedded in paraffin for immunohistochemical analysis. The outcomes measured included technical success of the surgery, protein measurements (ie, TIMP and MMP), and immunohistochemical analysis at the venous stenosis and control vein. Technical success was defined as the ability to create the fistula and the documentation of dilation of the anastomosed vein with a larger diameter than the control vein (12).

Creation of AV Fistula

Before experimentation, all animals were housed in a room with a 22°C temperature, 41% relative humidity, and 12-hour light/dark cycles in the