Journal of Surgical Research **141,** 192–195 (2007)
doi:10.1016/j.jss.2006.12.544

MF-Tricyclic Inhibits Growth of Experimental Abdominal Aortic Aneurysms

W. Brent Keeling, M.D.,* Amy E. Hackmann, M.D.,* Mary E. Colter, Ph.D.,† Patrick A. Stone, M.D.,* Brad L. Johnson, M.D.,* Martin R. Back, M.D.,* Dennis F. Bandyk, M.D.,* and Murray L. Shames, M.D.*,[1]

**University of South Florida Division of Vascular and Endovascular Surgery; and †H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida*

Submitted for publication June 14, 2006

Background. **Experimental abdominal aortic aneurysm (AAA) development can be pharmacologically suppressed by inhibiting matrix metalloproteinase-9 (MMP-9). Cyclooxygenase-2 (COX-2) inhibitors are potent anti-inflammatory agents that have been demonstrated to inhibit experimental aneurysm development. We hypothesized that treatment with MF-tricyclic, a selective COX-2 inhibitor, incorporated into rodent chow would inhibit aneurysm development in a rat AAA model.**

Methods. **Twelve male Sprague Dawley rats underwent induction of experimental AAA using intra-aortic porcine elastase infusion. Six rats received control feed, and six received MF-tricyclic rodent chow for a period of 14 days. Aortic diameters were measured pre- and postinfusion as well as at harvest. Aortic tissue samples were evaluated by real-time polymerase chain reaction (RT-PCR) for MMP-9, by immunohistochemistry for elastin.**

Results. **Elastase infusion produced AAA in all untreated rats. At 14 days MF-tricyclic-treated rats had significantly reduced aortic diameter (1.9 ± 0.1 mm *versus* 2.4 ± 0.0 mm, $P = 0.00001$). Percent increase in aortic diameter was also significantly less in animals receiving MF-tricyclic (65.7 ± 8.5% *versus* 132.3 ± 7.3%, $P = 0.0001$). RT-PCR demonstrated a decrease in the mean expression of MMP-9 in the treated animals (0.414 ng of RNA *versus* 1.114 ng of RNA) ($P = 0.07$). Sections stained for elastin demonstrated preserved elastin integrity in MF-tricyclic treated aortas.**

Conclusions. **COX-2 inhibition helps to retard the growth of experimental AAAs possibly through inhibition of MMP-9. Experimentally treated animals demonstrated smaller aortic diameters and lower levels of tissue MMP-9 when compared to untreated animals. Selective COX-2 inhibition may offer an additional method to pharmacologically inhibit AAAs.**

Key Words: **hepatic IR injury; *in vivo* EPR; *ex vivo* EPR; CV159; CaM.**

INTRODUCTION

Abdominal aortic aneurysms (AAA) remain a significant cause of mortality among elderly males. Indeed, rupture of AAA is the 13th leading cause of death in the United States [1, 2]. Within the past decade, laboratory and clinical research regarding the pathogenesis of AAA has focused on the etiologic and propagative roles of matrix metalloproteinases (MMP) in the structural degradation of the aortic media. Specifically, MMP-9 has been studied as the primary agent of elastolysis, and it is elevated in both the aortic wall and the serum of patients with AAA [3, 4]. MMP-9 has been inhibited experimentally with a resultant decrease in aortic diameter, and mice deficient in MMP-9 did not form aneurysms [5].

Production of MMP-9 by monocytes and macrophages has been demonstrated to be directly regulated by prostaglandin E_2 (PGE_2) [6]. Elevated levels of PGE_2 have been found in AAAs [7]. Cyclooxygenases (COX), which are known to directly inhibit prostaglandins, exist in two isoforms: COX-1 is constitutively expressed in a variety of cells, and COX-2 is expressed at sites of inflammation [8]. Because of this variable expression, COX-2 inhibitors do not induce many of the deleterious side effects associated with generalized COX inhibition such as gastric ulceration and renal toxicity [9, 10].

[1] To whom correspondence and reprint requests should be addressed at University of South Florida, Vascular and Endovascular Surgery, 4 Columbia Drive, Suite 650, Tampa, FL 33606. E-mail: mshames@hsc.usf.edu.

0022-4804/07 $32.00

Prior experimenters have used this upstream inhibitory pathway involving nonspecific COX inhibitors, and they proved that inhibition of AAA growth in an experimental model treated with indomethacin was successful [7]. This same study demonstrated decreased MMP-9 activity in indomethacin-treated animals. Human studies using generalized COX inhibition have not been undertaken, however, due to prohibitive side effect profiles. Selective COX-2 inhibitors have also been shown to possess side effects as both rofecoxib and celecoxib have been linked with an increase in myocardial events [11]. Our goal in undertaking this research was to use a new-generation COX-2 inhibitor to down-regulate MMP-9 production as a way to retard the expansion of experimental AAA.

METHODS

Animal Surgery

Twelve 300-g Sprague Dawley male rats were obtained from Harlan (Indianapolis, IN) and underwent experimental creation of AAA using a known protocol of intra-aortic elastase infusion [5]. Prior to the initiation of the experiment, all protocols were reviewed and approved by the University of South Florida Institutional Animal Care and Use Committee. Anesthesia included intraperitoneal injections of ketamine (60 mg/kg) and xylazine (6 mg/kg). A standard midline celiotomy incision was performed following sterilization of the skin. The infrarenal aorta was dissected, exposed, and isolated, and tourniquets were applied to both common iliac arteries and to the infrarenal aorta immediately inferior to the left renal vein. The aorta was occluded, and a small aortotomy was created at the bifurcation. A PE-10 catheter (VWR Scientific Products, Bridgeport, NJ) was then inserted through the aortotomy, and the infrarenal aorta was perfused with 1 mL porcine pancreatic elastase for 5 min using manual pressure through a tuberculin syringe. This action produced distention in all aortic segments and uniformly produced AAAs after 2 weeks (>100% increase in aortic diameter) in tests conducted prior to the start of the experiment. Following elastase perfusion, the aortotomy was sutured closed, and hemostasis was obtained. Aortic diameters were measured with an ocular caliper under 10× power with gradations of 0.1 mL prior to and following elastase infusion under physiological conditions with pulsatile blood flow. The fascia and skin then were closed with suture, and the rats were allowed to awaken and recover. Rats were housed two to a cage according to their treatment arm. Food intake was monitored but not regulated, and no rodent received food outside of the treatment algorithm. Half of the rats received standard rat chow while the other half received chow laden with drug. All rats were re-explored on postoperative day 14. At that time, aortic diameters were again measured under physiological conditions with pulsatile flow prior to euthanasia, and aortic specimens were harvested.

Drug Information and Aortic Infusion

All rats received aortic infusion with Type 1 porcine pancreatic elastase (E1250; 2.9 mg protein/mL; Sigma, St. Louis, MO) at a volume of 1 mL over 5 min. Rats in the treatment arm received rat chow infused with MF-Tricyclic [3-(3,4-difluorophenyl)-4-(4-(methylsulphonyl)phenyl)-2-(5H)-furnaone] ($C_{17}H_{14}F_2O_2S$) (Merck and Co., Whitehouse Station, NJ), a novel selective COX-2 inhibitor, at a dose of 5 mL/kg. Dosing information was obtained from Merck and was based upon prior work with the compound [12]. All rats had immediate access to chow and water immediately following surgery, and volume of chow was not specifically regulated.

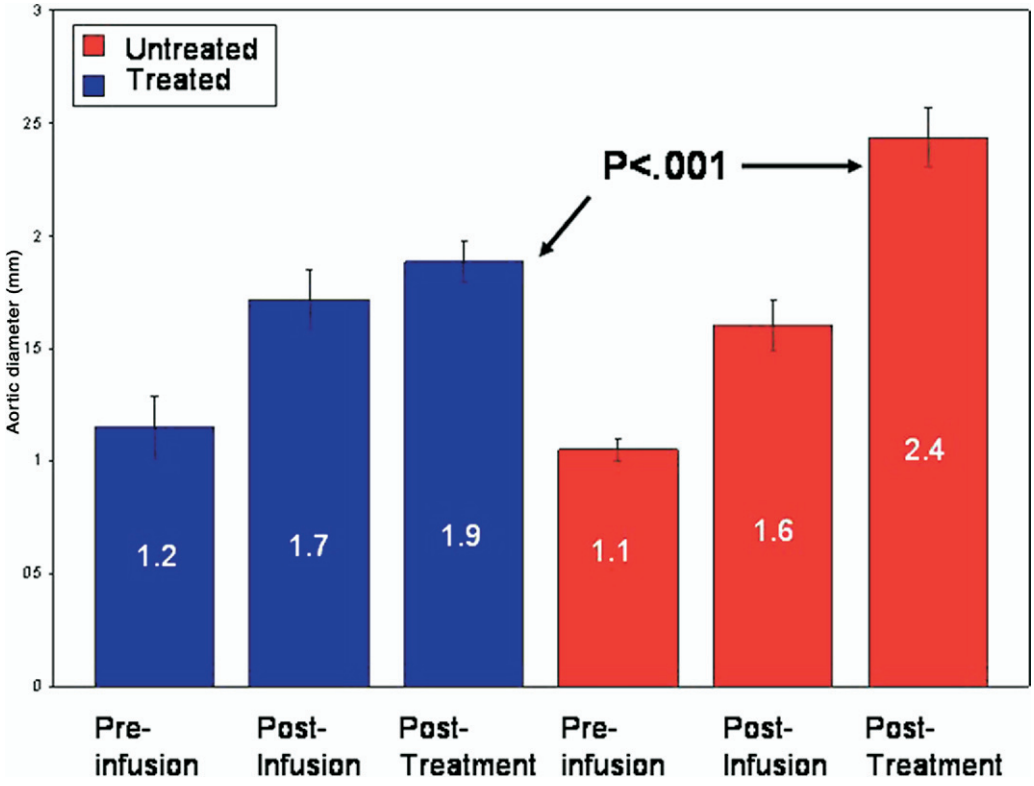

FIG. 1. Graphic representation of mean aortic diameters. (Color version of figure is available online.)