Comparative Medicine
Copyright 2005
by the American Association for Laboratory Animal Science

Vol 55, No 1
February 2005
Pages 55-60

Gender Differences in Deep Venous Thrombosis in a Rat Model: A Preliminary Study

Leslie A. VanLangevelde,[1,3] Shira E. Anchill,[1,3] Shirley K. Wrobleski,[1] Marisa J. Linn,[1] Thomas W. Wakefield, MD,[1] and Daniel D. Myers, Jr., DVM[1,2]

The purpose of this study was to determine whether gender differences have an effect on inflammation and thrombosis in a rat model of venous thrombosis. A thrombus was created in mature female (n = 12) and male (n = 12) Sprague Dawley rats (*Rattus norvegicus*) by ligating the inferior vena cava (IVC). The IVC containing the thrombus was harvested at 1 and 3 days postligation, weighed, measured, and submitted for immunohistochemical analysis. In addition, hematology was performed at selected time points. There were no statistically significant differences in thrombus mass (mean ± 1 standard deviation) between female and male rats at 1 ($683 \pm 47.7 \times 10^{-4}$ versus $660 \pm 112.0 \times 10^{-4}$ g/cm) or 3 ($683 \pm 83.3 \times 10^{-4}$ versus $580 \pm 86.0 \times 10^{-4}$ g/cm) days post-ligation. Females had significantly more platelets than did males on day 1 (741 ± 37.2 versus 523 ± 55.1 K/μL, $P < 0.01$). Day 3 males showed significant increases in vein wall neutrophils (18.0 ± 2.30 versus 11.2 ± 1.38, $P < 0.05$), ED-1-positive monocytes (54.4 ± 16.0 versus 18.7 ± 5.63, $P < 0.05$), and circulating white blood cells ($15.4 \pm 0.947 \times 10^{3}$ versus $10.9 \pm 0.714 \times 10^{-3}$/μL, $P < 0.01$) at post-thrombosis when compared with females. We conclude that although female rats had greater thrombus mass, the male rats demonstrated more inflammatory cells in circulation and in their vein walls. This finding suggests that inflammation plays a role in thrombus resolution.

Deep venous thrombosis (DVT) accounts for approximately 250,000 cases per year in the United States and continues to be an important health concern in the general adult population (3). Several risk factors may predispose a person to DVT, including age, obesity, immobilization, infectious diseases, pregnancy, the use of oral contraceptives, and hormone replacement therapy (25). It also has been shown that female gender, age, and impaired renal function can be associated with increased venous thromboembolism in patients undergoing hip fracture surgery (14). Patients who develop DVT can have long-term complications including post-thrombotic syndrome, in which pain, swelling, and numbness may occur. A leading complication of DVT is thromboembolism, specifically pulmonary embolism (PE), which has the potential to be fatal. Deep venous thrombosis and pulmonary embolism are associated with approximately 300,000 to 600,000 hospitalizations per year and up to 50,000 deaths per year (2).

Several clinical studies have examined how gender, age, and hormonal differences influence DVT formation in men and women. Exogenous estrogen, for example, has been shown to cause a threefold increase in the incidence of DVT in women when used as an oral contraceptive or as postmenopausal therapy (24). Among patients who experienced DVT or PE during a 25-year period, the incidence of DVT remained constant for males in all age groups but increased in women with age (26). In other retrospective studies, the incidence of DVT (based on positive phlebographies of patients) increases at a younger age in males than in females (12, 21). In addition, men older than 50 years were more likely to experience DVT than were women of the same age (12). According to these studies, DVT in women seems to occur later in life than in men.

Historically, animal models for vascular disease have only included males. Justification for this practice was based on reducing the experimental variability that accompanies fluctuating hormone levels throughout the female estrous cycle (17). Sex steroid hormones previously have been shown to have regulatory effects on circulating leukocytes in females (4). Female sex hormones therefore could affect the coagulation and inflammatory events leading up to thrombus formation and result in differences in thrombus size between males and females. In studies using only male animals, it has been implied that treatments that work in the male could also be applied to females with the same outcome (17). However, endogenous estrogen or other sex-specific factors may alter the pharmacokinetics of a drug so that it is not as effective in the female as the male. Gender studies are important in discovering the differences in the pathogenesis of a disease between the sexes as well as verifying the efficacy of a treatment of those diseases. For example, recent studies have begun to include both male and female animals in order to better understand the role that endogenous sex hormones have in various arterial vascular disorders such as aortic aneurysms, stroke, and arthrosclerosis (1, 17, 23). These studies raise the question of whether the protection provided by endogenous estrogen in females against most cardiovascular diseases could extend beyond the arterial system and into the venous vascular system to prevent venous thrombi.

Therefore, the development of a rat model of DVT that parallels the human condition in both genders would permit further investigations of DVT formation as well as the exploration of

Received: 7/02/04. Revision requested: 9/01/04. Accepted: 9/07/04.
Jobst Vascular Research Laboratories, [1]Section of Vascular Surgery,[2]Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109; [3]College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824.
[*]Corresponding author.

possible prophylactic and treatment options, as is currently being explored in the arterial system. The overall purpose of our study was to determine whether gender differences affect acute inflammation (increased systemic and local inflammatory cells) and venous thrombus formation in the rat model by comparing thrombus mass and histological specimens.

Materials and Methods

Animal model. Female (n = 12; age, > 76 days) and male (n = 12; age, > 61 days) sexually mature Sprague Dawley rats (*Rattus norvegicus,* Harlan Sprague Dawley, Indianapolis, Ind.) weighing 249 to 275 g were selected for this study. According to the vendor, this weight range corresponds to ages that are well beyond sexual maturity in both the males and females (10). They were further divided into 1- and 3-day groups with six animals in each group. Rats were anesthetized with isoflurane gas (1 to 2%) mixed with oxygen (100%) via nose cone during the procedure. A midline laparotomy was performed, the small bowel was retracted slightly to the left of the abdominal midline, and the inferior vena cava (IVC) was approached directly by careful blunt dissection. The IVC side and back branches were isolated carefully and ligated with nonreactive 6-0 prolene (Ethicon, Inc., Somerville, N.J.) ligatures. Finally, a ligature was placed around the IVC just below the level of the renal veins (Fig. 1). The laparotomy site was then closed in two layers with 3-0 coated Vicryl sutures (Ethicon, Inc.), and the animals recovered from anesthesia. Animal groups were euthanized on days 1 and 3 postligation for tissue harvest and data analysis (19).

Thrombus mass (TM). This technique is an indirect measure of thrombus content. At euthanasia, the IVC containing the thrombus was removed, weighed (g), and measured for length (cm). The weight was then normalized to length (weight/length). Previous research from our laboratory has shown that the major component of weight is the thrombus and not the vein wall (7, 11, 18).

Hematology. Hematology (complete blood cell [CBC] and platelet counts) was performed on blood drawn from the ventral tail artery of each rat at the time of ligation (baseline) and at euthanasia. The evaluation of 20 µl of EDTA-treated whole blood samples were performed by an automated Hema VET (CDC Technologies, Inc., Oxford, Conn.). Hematology profiles consisted of red and white blood cell counts (RBC and WBC, respectively), hemoglobin concentration, hematocrit, mean corpuscular hemoglobin concentration, platelet count, and mean percentage volume as well as the percentage and number of neutrophils, lymphocytes, monocytes, eosinophils, basophils, and nucleated red blood cells.

Gross sample analysis. At time of euthanasia, IVC and thrombi samples were preserved in 10% formalin for 24 h and then transferred to 70% reagent-grade ethyl alcohol. The IVC and thrombus then were divided into proximal, middle, and distal segments, photographed, and submitted for histochemistry.

Vein wall morphometrics. (i) Hematoxylin and eosin staining. Standard methods for tissue fixation were used for the analysis. Sections were stained with hematoxylin and eosin from paraffin-embedded tissues. The vein wall surrounding each proximal section of thrombus was examined under high-power (×100) oil-immersion light microscopy. Five representative high-power fields (HPF; one HPF is 210 µm in diameter) of the IVC wall were examined, and the inflammatory cells at points corresponding to positions 12, 2, 5, 7, and 9 on the face of a clock were counted manually. Cells were identified as neutrophils or lymphocytes based on standard histologic criteria (5, 19, 31). Results from the five HPF were added together for each section of vein wall studied, and the mean ± significant error (SE) (SPSS Sigma Stat 2.0, Aspire Software International, Leesburg, Va.) was calculated for each group. The vein wall surrounding the middle and distal section of each thrombus were not examined, but are being retained for future studies.

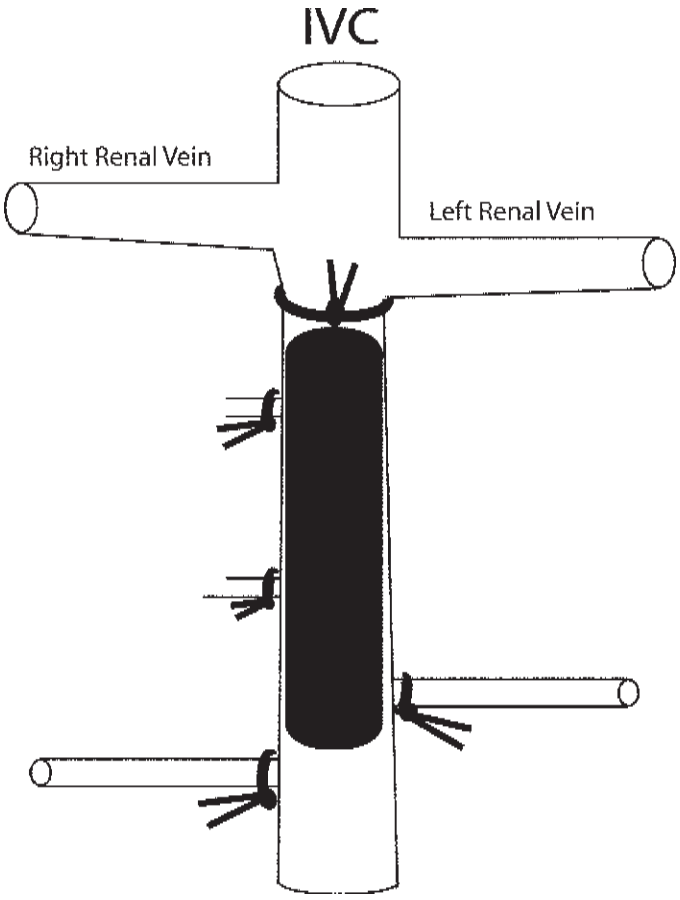

Figure 1. Rat ligation model of deep venous thrombosis.

(ii) Immunohistochemical staining for monocytes (ED-1). Using a previously described protocol (27), depariffinized slides were incubated with a 1:100 dilution of mouse anti-rat ED-1 (Serotec Inc., Raleigh, N.C.), for 30 min at room temperature. Color development was performed with Vector Red substrate (Vector Laboratories, Inc., Burlingame, Calif.) for approximately 20 min; then slides were counterstained with hematoxylin QS (Vector Laboratories, Inc.) (16). ED-1-positive monocytes were counted using the same method as described for the vein wall inflammatory cells.

Statistical evaluation. Statistical analysis included the mean ± standard error of the mean, analysis of variance (ANOVA), and unpaired Student *t* tests for parametric data (SPSS Sigma Stat 2.0, Aspire Software International, Leesburg, Va.). Significance was defined as $P \leq 0.05$. Comparisons between individual groups were run if the overall ANOVA was significant between all groups.

Animal use. All rats (n = 24) were housed three per cage in 136-in^2 (ca. 877 cm^2) polycarbonate rodent caging. Animals were

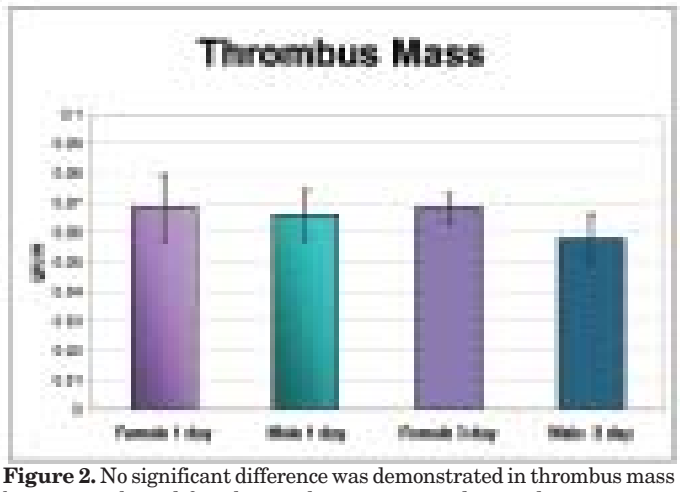

Figure 2. No significant difference was demonstrated in thrombus mass between male and female rats; however, a trend toward greater average thrombus mass (TM) in female animals was shown at day 3 only (female, 0.0683 g/cm; male, 0.0580 g/cm).

kept on a 12:12-h light:dark cycle at an average temperature of 21°C and average relative humidity of 70% and were cared for by the University of Michigan Unit for Laboratory Animal Medicine. Each animal was fed rat chow (Purina Rat Chow 5001, Ralston Purina Canada, Chomedy, Quebec) and water ad libitum. The health status of all animals was monitored monthly by use of a sentinel program, and all animals were free of the following pathogens: Toolan's H-1 virus, Sendai virus, rat coronavirus/sialodacryoadenitis virus, reovirus type 3, Kilham rat virus, *Mycoplasma pulmonis*, rat parvovirus, pneumonia virus of mice, mouse adenovirus, lymphocytic choriomeningitis virus, cilia-associated respiratory bacillus, endoparasites, and ectoparasites. The University of Michigan is an Association for the Assessment and Accreditation of Laboratory Animal Care, International-accredited facility under the direction of a veterinarian according to the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (22) and *Guide for the Care and Use of Laboratory Animals* (20). The University of Michigan Committee on Use and Care of Animals approved this research protocol.

Results

Thrombus mass. There were no statistically significant differences in thrombus mass between female and male rats at either 1 ($683 \pm 47.7 \times 10^{-4}$ versus $660 \pm 112.0 \times 10^{-4}$ g/cm) or 3 ($683 \pm 83.3 \times 10^{-4}$ versus $580 \pm 86.0 \times 10^{-4}$ g/cm) days postligation. In addition, there were no statistically significant differences in thrombus mass within each gender between days 1 and 3. However, female animals showed an overall increase in thrombus mass compared with that in male rats, and day 3 male rats demonstrated a trend toward a decreased thrombus when compared to males at day 1 (Fig. 2 and 3).

Hematology. Hematology evaluation showed a statistically significant increase in platelet counts in female versus male rats at day 1 only ($741 \pm 37.2 \times 10^3$/μl versus $523 \pm 55.1 \times 10^3$/μl, $P < 0.01$). Female rats also had more red blood cells than did male rats on both days 1 ($7.72 \pm 0.158 \times 10^6$/μl versus $6.53 \pm 0.202 \times 10^6$/μl, $P < 0.01$) and 3 ($7.57 \pm 0.178 \times 10^6$/μl versus $6.74 \pm 0.130 \times 10^6$/μl, $P < 0.01$). Day 3 males showed a significant increase in circulating WBC at preligation ($15.3 \pm 0.710 \times 10^3$/μl versus $9.25 \pm 0.560 \times 10^3$/μl, $P < 0.01$) and postthrombosis ($15.4 \pm 0.947 \times 10^3$/μl versus $10.9 \pm 0.714 \times 10^3$/μl, $P < 0.01$) when compared with those in female rats (Fig. 4A and 4B).

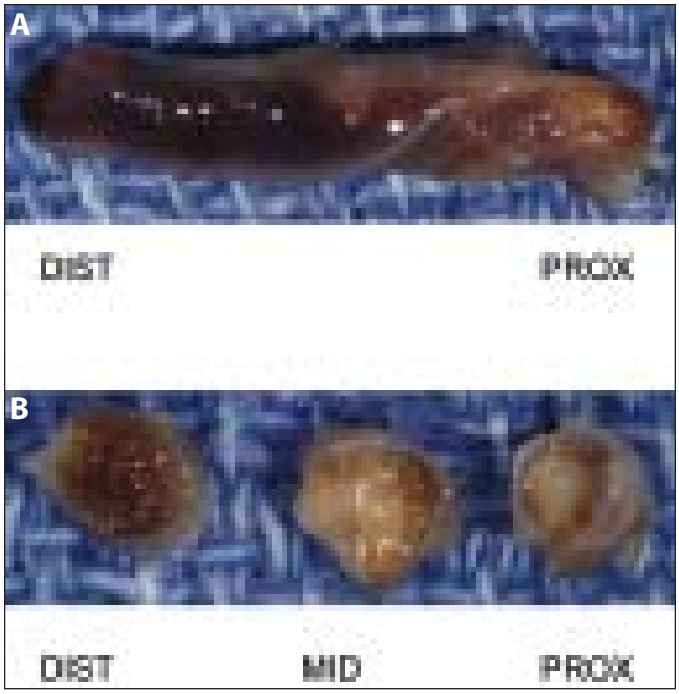

Figure 3. (A) Intact day 3 inferior vena cava thrombus. (B) (left to right): On-end views of the distal (DIST), middle (MID), and proximal (PROX) sections of the thrombus in the inferior vena cava shown in (A).

Vein wall morphometrics. Male rats exhibited an increase in vein wall neutrophils on day 3 as compared with those in female rats (18.0 ± 2.30 cells/5 HPF versus 11.2 ± 1.38 cells/5 HPF, $P < 0.05$; Fig. 5A, 5B, 6). In addition, male animals showed an increase in vein wall ED-1-positive monocytes on day 3 when compared with those in females (54.4 ± 16.0 cells/5 HPF versus 18.7 ± 5.63 cells/5 HPF, $P < 0.05$; Fig. 6). Vein wall neutrophils in male and female rats on day 1 were significantly greater than those in male and female rats respectively on day 3 (male: day 1, 43.2 ± 3.71 cells/5 HPF; day 3, 18.0 ± 2.30 cells/5 HPF; $P < 0.01$; female: day 1, 57.0 ± 15.3 cells/5 HPF; day 3, 11.2 ± 1.38 cells/5 HPF; $P < 0.05$). The ED-1 immunohistochemical staining process allowed us to better differentiate monocytes from other inflammatory cells. The number of ED-1-positive monocytes in the vein wall surrounding the thrombi of male rats on day 3 was significantly greater than that in male rats at 1 day postligation (54.4 ± 16.0 cells/5 HPF versus 9.80 ± 1.72 cells/5 HPF, $P < 0.05$), (18.6 ± 2.09 cells/5 HPF versus 8.20 ± 2.92 cells/5 HPF, $P < 0.05$, respectively).

Discussion

Animal models have been used extensively to explore the role of gender in a number of cardiovascular disorders, but until now the effect that gender has on the formation of venous thrombi had not been explored in an animal model. The purpose of this study was to examine how gender affects the inflammatory process and formation of acute DVT in a rat model.

The male rats in our study demonstrated a significantly greater number of inflammatory cells both in circulation and in their vein walls than did females, yet the female animals formed

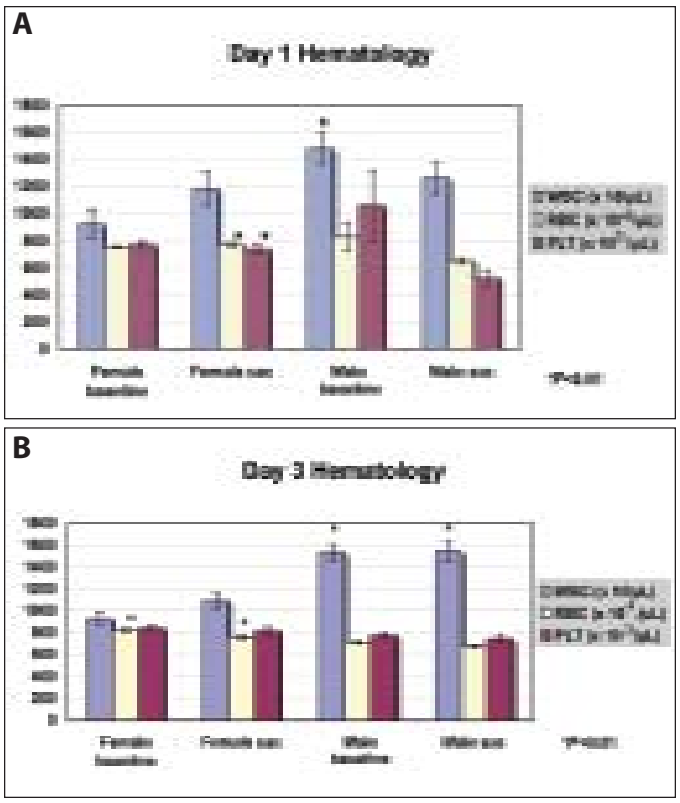

Figure 4. (A) At day 1, baseline male rats showed a significant increase in circulating white blood cells (WBC) when compared with baseline female animals (*, $P < 0.01$). Female rats demonstrated a significant increase in RBC and platelets at euthanasia when compared with male rats at euthanasia (*, $P < 0.01$). (B) At day 3, baseline males showed a significant increase in WBC counts when compared with baseline females (*, $P < 0.01$); however, baseline female rats demonstrated a significant increase in red blood cells (RBC) when compared with baseline male animals (*, $P < 0.01$). Male animals at euthanasia had a significantly greater number of WBC than did female rats at euthanasia (*, $P < 0.01$) but significantly fewer RBC (*, $P < 0.01$).

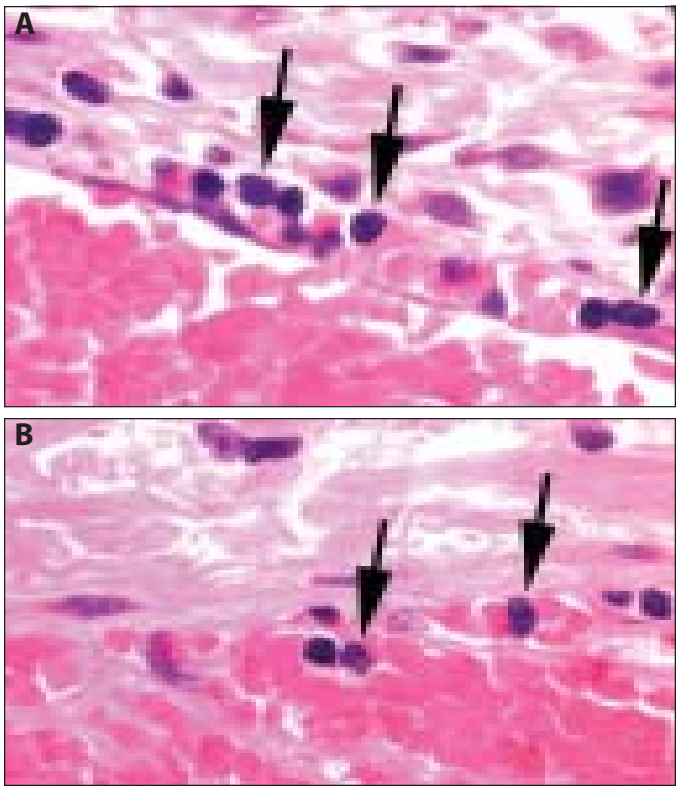

Figure 5. Histological sections (magnification, ×100) of the inferior vena cava (IVC) vein wall–thrombus interface in male and female rats were evaluated 3 days postthrombosis. Representative histology sections showed that vein wall neutrophils (large arrows) were increased in male rats (A) when compared with female rats (B) at the same time point.

larger thrombi than did male rats. Although this difference in thrombus mass was not significant, our study suggests that the variation in the inflammatory responses between male and female rats may have contributed to the differences in the formation and resolution of venous thrombi. Male rats euthanized on day 3 also showed significantly more ED-1-positive monocytes in their vein walls than did male rats on day 1. Previous studies of inflammation and its effect on DVT have indicated that venous thrombosis and inflammation are directly related and as a result, prevention of DVT has often focused on the suppression of inflammation (6, 8, 30, 31). However, because the males had significantly more monocytes in their vein wall at day 3 but developed smaller thrombi compared with those on day 1, our study suggests that inflammatory cells could actually play an important role in thrombus resolution at a more chronic stage in development. The significant difference between vein wall neutrophils and ED-1 monocytes on day 3 in male and female rats but not on day 1 also indicates that a more chronic inflammatory response may be needed for thrombus resolution. Neutrophils and monocytes play an important part in the inflammatory response and are similar in that they are both migratory and phagocytic. However, neutrophils are activated and recruited much earlier in the inflammatory response and do not last as long in tissues as do monocytes (9). One study found that thrombi in rats with induced neutropenia were larger, more fibrotic, and showed greater recanalization than those in controls (29). Although one of the key roles of inflammatory cells is the initiation of thrombus formation, neutrophils also possess cellular mediators, such as plasminogen activator and elastase, which may prove to be thrombolytic after the thrombus has formed (9, 29). Therefore, the abundant influx of neutrophils in male rats on day 1 could have lead to a smaller thrombus on day 3. This study supports our findings that DVT resolution largely depends on the number and type of inflammatory cells present as well as the age of the thrombus.

The significant decrease in circulating white blood cells and vein wall inflammatory cells in the female could be due to the effects estrogen has on the female immune system. Endogenous estrogen comes in different forms, but the main type of estrogen in circulation is 17β-estrodiol, and it is probably the greatest variable separating the two sexes (23). The estrogen produced naturally by the body is different than that given to menopausal women in hormone therapy. Human hormone therapy usually consists of a combination of estrogen of equine origin along with a synthetic form of progesterone (Prempro) (28, 33). Therefore, replacement therapy and oral contraceptives consist of multiple types of hormones that are not identical to the naturally occurring endogenous hormones of women, and this difference could be the reason why women taking exogenous steroids have a higher incidence of DVT than do premenopausal women that have circulating endogenous estrogens (13). 17β-estrodiol has

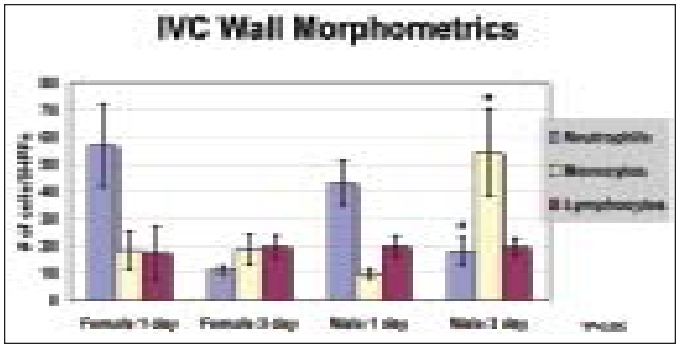

Figure 6. Thrombi at the day 3 time point in male rats showed a significant increase in both neutrophils and ED-1-positive monocytes when compared with those in female animals (*, $P < 0.05$).

been used in gender studies to determine the effect of sex hormones on the immune and cardiovascular system. Endogenous estrogen has been shown to influence inflammatory processes through its inverse relationship to neutrophil accumulation and antioxidant enzyme activities (17). Estrogen's inhibitory effect on neutrophils has been used to explain the fact that pre-menopausal women have lower rates of stroke than male and post-menopausal women (17). Estrogen also stimulates endothelial regeneration after cell injury and inhibits leukocyte adhesion, both of which help to delay the onset of arthrosclerosis in women (23).

In a study by Ailawadi and colleagues, it was determined that gender differences exist in the formation of abdominal aortic aneurysms (AAA). Fourteen days post-perfusion of elastase, intact female rats presented with a lower incidence compared with males because of the protective properties of the female hormones. Consequently, the incidence of AAA in the female rats increased once their ovaries were removed (1). This evidence supports the theory that estrogen serves as a protective mechanism against arterial vascular diseases. Because inflammation contributes to most of these vascular conditions, we hypothesized that the inhibition of inflammatory cells and adhesion molecules by estrogen would also prevent or ameliorate cardiovascular disease in the venous system. However, according to our data, this is not the case. The mature female rats in our study had significantly fewer inflammatory cells yet demonstrated a trend toward slightly larger thrombi than those in male rats. Therefore, we suggest that because of estrogen's inhibitory effect on leukocytes, fewer inflammatory cells, namely neutrophils, were available to aid in thrombus resolution, which then resulted in female rats demonstrating a slightly greater thrombus mass. In contrast, male thrombus mass decreased over a 2-day period in the presence of an increased inflammatory response. Therefore, the anti-inflammatory effects of estrogen do not seem to attenuate DVT in our animal model as occurs in arterial vascular disease, in light of the fact that DVT appears to diminish from the thrombolytic effects of inflammatory cells. In order to confirm whether the anti-inflammatory protection endogenous estrogen provides could extend beyond the arterial system and prevent venous thrombi can only be found after more in-depth studies are performed.

Platelets serve many functions in different areas of the body. They are not only key effectors in the coagulation cascade, but they are also active mediators of the inflammatory process. The fact that female rats showed a significantly greater number of circulating RBC and platelets when compared with males is important because there would be more platelets and RBC available to adhere to the vascular endothelium and therefore contribute to the coagulation cascade and thrombus growth. This significant increase in platelets and RBC could have been due to the influence of sex hormones, but this association should be verified through future studies that compare estrogen levels with platelet counts. One study that compared the effects of platelets and estrogen on femoral veins found that ovarian hormones regulate the release of vasoactive substances from platelets so that high concentrations of platelets in intact female pigs caused more venous contraction than that in ovariectomized females (16). The decrease in diameter as a result of venous contraction could cause a change in blood flow dynamics which would then predispose an animal to the formation of venous thrombi. Vessel diameter was not measured in this study. In addition to causing dynamic changes in the vessels, platelets also play a role in the inflammatory cascade by releasing proinflammatory mediators and interacting with leukocytes (32). Although platelets are proinflammatory, the platelets in the female rats were significantly elevated only during the early phases of inflammation in this study. Future studies should investigate the role of circulating platelets and vein wall inflammatory cells (neutrophils and monocytes) in thrombus formation. These properties make platelets key effectors in thrombosis, and therefore the thrombocytosis seen in our female rats could have predisposed them to thrombosis.

Two male rats, one from each time point, had to be eliminated from this study because of inadequate hematology data. Therefore, these two rats were excluded from all other analyses to ensure consistency. We do not expect this exclusion to be a discriminatory factor because both rats were male and came from each of the time points. According to the vendor, all the animals were approximately the same age and sexually mature (11); however it should be noted that the phase of estrous cycle was not identified in the female rats. Therefore, depending on what stage in the cycle the animal was in when we sampled them, the number of inflammatory cells could change due to estrogen's effect on inflammation. For example, during proestrus, there are higher levels of estrogen compared to metestrus. Therefore, if the majority of females in our study were in proestrus on day 3, this could explain why they had fewer circulating WBC and vein wall neutrophils and ED-1-positive monocytes than did the males at the same time point.

Future studies evaluating gender and DVT should include a larger sample size, a more diverse age range of animals, hormone supplementation, chronic time points, monitoring of the female estrous cycle, performing ovarioectomies, analyzing different sections of the vein wall and its associated thrombi, and uncovering genetic markers of vascular disease in both males and females.

In conclusion, this preliminary study has shown that gender is an important factor in venous thrombus formation. The significant increase in vein wall inflammatory cells noted in male rats during early thrombosis when compared with females appears to have modulated thrombus formation and resolution. Although the anti-inflammatory effects of endogenous estrogen seems to provide protection against arterial vascular diseases, our data show this benefit did not exist in our animal model of venous thrombosis. Whether there are different mechanisms of inflammation contributing to diseases seen in arteries versus veins is unclear. However, it appears in our study that estrogen had a

negative effect on DVT because of the suppression of inflammatory cells that would have eventually contributed to thrombus resolution. Future mechanistic studies will determine how these sex differences might be utilized in order to provide gender specific treatment of DVT.

Acknowledgments

We would like to thank Howard G. Rush (Unit for Laboratory Animal Medicine, University of Michigan Medical Center, Ann Arbor, Mich.) and John C. Baker (College of Veterinary Medicine, Michigan State University, East Lansing, Mich.) for their support in making this research possible.

References

1. **Ailawadi, G., J. L. Eliason, I. Sinha, J. W. Ford, K. J. Roelofs, K. K. Hannawa, P. K. Henke, J. C. Stanley, S. J. Weiss, and G. R. Upchurch, Jr.** 2004. Gender differences in experimental aortic aneurysm formation. Submitted for publication.
2. **Anderson, F. A., Jr., H. B. Wheeler, R. J. Goldberg, D. W. Hosmer, N. A. Patwardhan, B. Jovanovic, A. Forcier, and J. E. Dalen.** 1991. A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism. The Worcester DVT study. Arch. Intern. Med. **151:**933-938.
3. **Coon, W. W.** 1977. Epidemiology of venous thromboembolism. Ann. Surg. **186:**149-164.
4. **Doeing, D. C., J. L. Borowicz, and E. T. Crockett.** 2003. Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. [Online]. BMC Clin. Pathol. Available at http://www.biomedcentral.com/1472-6890/3/3. Accessed 8/07/04.
5. **Downing, L. J., R. M. Strieter, A. M. Kadell, C. A. Wilke, J. C. Austin, B. D. Hare, M. D. Burdick, L. J. Greenfield, and T. W. Wakefield.** 1998. Interleukin-10 regulates thrombus-induced vein wall inflammation and thrombosis. J. Immunol. **161:**1471-1476.
6. **Downing, L. J., R. M. Strieter, A. M. Kadell, C. A. Wilke, S. L. Brown, S. K. Wrobleski, M. D. Burdick, M. S. Hulin, J. B. Fowlkes, L. J. Greenfield, and T. W. Wakefield.** 1996. Neutrophils are the initial cell type identified in deep venous thrombosis induced vein wall inflammation. ASAIO J. **42(5):**M677-M682.
7. **Downing, L. J., R. M. Strieter, A. M. Kadell, C. A. Wilke, L. J. Greenfield, and T. W. Wakefield.** 1998. Low-Dose low molecular weight heparin is anti-inflammatory during venous thrombosis. J. Vasc. Surg. **28(5):**848-854.
8. **Downing L. J., T. W. Wakefield, R. M. Strieter, M. R. Prince, F. J. Londy, J. B. Fowlkes, M. S. Hulin, A. M. Kadell, C. A. Wilke, S. L. Brown, S. K. Wrobleski, M. D. Burdick, D.C. Anderson, and L. J. Greenfield.** 1997. Anti-P-selectin antibody decreases inflammation and thrombus formation in venous thrombosis. J. Vasc. Surg. **25(5):**816-827.
9. **Granelli-Piperno, A., J-D. Vassalli, and E. Reich**. 1977. Secretion of plasminogen activator by human polymorphonuclear leukocytes: modulation by glucocorticoids and other effectors. J. Exp. Med. **146:**1693-1706.
10. **Harlan, Inc.** 2003. Harlan product guide, p. 2. Harlan, Inc., Indianapolis, Ind.
11. **Henke, P. K., L.A. Debrunye, R. M. Strieter, J. S. Bromberg, M. Prince, A. M. Kadell, M. Sarkar, F. Londy, and T. W. Wakefield.** 2000. Viral IL-10 gene transfer decreases inflammation and cell adhesion molecule expression in a rat model of venous thrombosis. J. Immunol. **164:**2131-2141.
12. **Kierkegaard, A.** 1980. Incidence of acute deep vein thrombosis in two districts. A phlebographic study. Acta. Chir. Scand. **146(4):**267-269.
13. **Krupa, D.** 2001. Selected estrogen compounds modify risks of vein clotting, a response to hormone replacement therapy. [Online]. Science Blog. Available at http://www.scienceblog.com/community/older/2001/A/200110818.html. Accessed 8/04/04.
14. **Lassen, M. R. and V. I. Eriksson.** 2003. Efficacy of fondaparinux (Arixtra) in extended thromboprophylaxis in hip fracture surgery is irrespective of patient and surgical characteristics: subgroup analyses of the Penthrifa-Plus study. XIX Congress of the International Society on Thrombosis and Hemostasis, July 2003, Birmingham, U.K.
15. **Lewis, D. A., M. P. Bracamonte, K. S. Rud, and V. M. Miller.** 2001. Selected contribution: effects of sex and ovariectomy on responses to platelets in porcine femoral veins. J. Appl. Physiol. **91(6):**2823-2830.
16. **Matsushima, K. and J. J. Oppenheim.** 1989. Interleukin 8 and MCAF: novel inflammatory cytokines inducible by IL-1 and TNF. Cytokine **1:**2-13.
17. **Murphy, S. J., L. D. McCullough, and J. M. Smith.** 2004. Stroke in the female: role of biological sex and estrogen. ILAR J. **45(2):**147-159.
18. **Myers, D. D., P. K. Henke, S. K. Wrobleski, A. E. Hawley, D. M. Farris, A. M. Chapman, B. S. Knipp, P. Thanaporn, R. G. Schaub, L. J. Greenfield, and T. W. Wakefield**. 2002. P-selectin inhibition enhances thrombus resolution and decreases vein wall fibrosis in a rat model. J. Vasc. Surg. **36:**928-938.
19. **Myers, D. D., Jr., S. K. Wrobleski, P. K. Henke, and T. W. Wakefield**. 2001. Coagulation biology. *In* W. Souba and D. Wilmore (ed.), Surgical research. Academic Press, San Diego, Calif.
20. **National Research Council.** 1996. Guide for the care and use of laboratory animals. National Academy Press, Washington, D.C.
21. **Nordstrom, M., B. Lindblad, D. Bergqvist, and T. Kjellstrom**. 1992. A prospective study of the incidence of deep-vein thrombosis within a defined urban population. J. Intern. Med. **232(2):**155-160.
22. **Office of Laboratory Animal Welfare.** 1996. Public health service policy on humane care and use of laboratory animals. Office of Laboratory Animal Welfare, National Institutes of Health, Department of Health and Human Services, Bethesda, Md.
23. **Pradhan, S. and B. E. Sumpio.** 2004. Do estrogen effects on blood vessels translate into clinically significant atheroprotection? J. Am. Coll. Surg. **198(3):**462-474.
24. **Rosendaal, F. R., F. M. Helmerhorst, and J. P. Vandenbroucke**. 2002. Female hormones and thrombosis. Arterioscler. Thromb. Vasc. Biol. **22:**201-210.
25. **Samama, M. M.** 2000. An epidemiologic study of risk factors for deep vein thrombosis in medical outpatients. Arch. Intern. Med. **160:**3415-3420.
26. **Silverstein, M. D., J. A. Heit, D. N. Mohr, T. M. Petterson, W. M. O'Fallon, and L. J. Melton III.** 1998. Trends in the incidence of deep vein thrombosis and pulmonary embolism. Arch. Intern. Med. **158:**585-593.
27. **Thanaporn, P., D. D. Myers, S. K. Wrobleski, A. E. Hawley, D. M. Farris, T. W. Wakefield, and P. K. Henke.** 2003. P-selectin inhibition decreases post-thrombotic vein wall fibrosis in a rat model. Surgery **134:**365-371.
28. **U. S. Food and Drug Administration.** 2004. Menopause and hormones. [Online] Available at http://www.fda.gov/womens/menopause/. Accessed 8/09/04.
29. **Varma, M. R., A. J. Varga, B. S. Knipp, P. Sukheepod, G. R. Upchurch, S. L. Kunkel, T. W. Wakefield, and P. K. Henke.** 2003. Neutropenia impairs venous thrombosis resolution in the rat. J. Vasc. Surg. **38(5):**1090-1098.
30. **Wakefield, T. W., R. M. Strieter, R. Schaub , D. D. Myers, M. R. Prince, S. K. Wrobleski, F. J. Londy, A. M. Kadell, S. L. Brown, P. K. Henke, and L. J. Greenfield.** 2000. Venous thrombosis prophylaxis by inflammatory inhibition without anticoagulation therapy. J. Vasc. Surg. **31(2):**309-324.
31. **Wakefield, T. W., R. M. Strieter, C. A. Wilke, A. M. Kadell, S. K. Wrobleski, M. D. Burdick, R. Schmidt, S. L. Kunkel, and L. J. Greenfield.** 1995. Venous thrombosis-associated inflammation and attenuation with neutralizing antibodies to cytokines and adhesion molecules. Arterioscler. Thromb. Vasc. Biol. **15:**258-268.
32. **Weyrich, A. S., S. Lindemann, and G. A. Zimmerman.** 2003. The evolving role of platelets in inflammation. J. Thromb. Haemost. **1(9):**1897-1905.
33. **Women's Health Initiative.** 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women. JAMA **288(3):**321-33.