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Research Article

The Incidence of Vasculitis is Increased in Female Stroke-Prone Hypertensive Rats Compared to Males

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ABSTRACT

Background: Vascular changes in hypertension share common characteristics with inflammatory wall injury. Since it is known that chronic inflammatory diseases are frequently more prevalent in females, this study tested the hypothesis that vasculitis would be more evident in female stroke-prone spontaneously hypertensive rats (SHRSP) than in males. **Methods:** Arterial lesions were characterized in the gastrointestinal tract of necropsied SHRSP and normotensive Wistar-Kyoto rats (WKY). Systolic blood pressure was measured using the tail cuff method. **Results:** Vasculitis was present in 54% of SHRSP (n=357). None of the WKY rats had the vascular disease (n=373). Arterial lesions were not evident in young SHRSP (1-1.5 months of age) before the development of high blood pressure. The earliest appearance of vasculitis in SHRSP was at ~8 months of age after full establishment of elevated arterial pressure. Systolic blood pressures during the maintained phase were greater than 200mmHg for SHRSP and less than 130mmHg for WKY rats (ages: 8-18 months). Within SHRSP, lesions were more common in females, in spite of higher mean systolic pressure found in males (both $p<0.05$). Of the female SHRSP, 70% (n=234) had severe micronodular vasculitis whereas 24% of male SHRSP showed lesions (age matched). **Conclusion:** These observations indicate that in SHRSP: 1) inflammatory responses in arteries occur with greater incidence compared to WKY rats; 2) there is a relationship between incidence of vascular lesions and age, but not elevated arterial pressure; and 3) there is a higher incidence of vasculitic lesions in females compared to males.

INTRODUCTION

The malignant phase of elevated arterial pressure in the stroke-prone spontaneously hypertensive rat (SHRSP) is characterized by necrotizing vasculitis classified as polyarteritis nodosa (Saito, et al., 1990, 1991, 1995). This is a segmental inflammation occurring particularly in testicular and mesenteric arteries accompanied by destruction of the vascular wall with polymorphonuclear and eosinophilic leukocyte infiltration during the earlier stages of the disease, and mononuclear leukocyte infiltration dominant in the later stages. In the final stage, fibrosis and scarring of the vascular wall are obvious. The disease causes vascular occlusion, resulting in regional ischemia, hemorrhage and tissue necrosis.

Interestingly, vasculitic lesions do not occur in the Wistar Kyoto (WKY) normotensive rat and it is known that a substantial elevation in systolic pressure is necessary for the development of periarteritis nodosa in the mesenteric vascular bed of SHRSP (Saito et al., 1991).

Furthermore, little is known about sex differences in the clinical presentation and animal models of the disease. In most studies, vasculitis is more prevalent in women but there is great variation with respect to the type or classification of the vasculitis (giant cell, periateritis nodosa, etc.) and co-morbidities (lupus, arthritis, etc.) that complicate the inflammatory

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response (Nir-Paz, et al. 2002; Narvaez, et al. 2002; Sadurska, et al. 2012). There is also variability between different regional vascular beds and variation related to vessel caliber (large, medium, small, arteriolar). It has also been noted that the sex divergence in human studies is most prominent among the oldest age groups. Since it is known that chronic inflammatory diseases are frequently more prevalent in females (Fairweather and Rose, 2004; Ngo et al. (2014), we hypothesized that vasculitis would be more evident in female SHRSP than in males. We characterized arterial lesions in the gastrointestinal tract of necropsied SHRSP and normotensive WKY rats. The range in age of the animals was 4 to 72 weeks and systolic blood pressures were measured by the tail cuff technique.

MATERIALS AND METHODS

Animals

All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Michigan and were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH).

Male and female SHRSP (n=357) and WKY rats (n=373) were obtained from a breeding colony maintained at the University of Michigan which was initially derived from stock procured from the National Institutes of Health. The rats were fed standard rodent chow (Purina) and drinking water *ad libitum* under conditions of constant temperature (22ºC). The rats were maintained on a 12-hour light-dark cycle and housed two per cage. Systolic blood pressure was measured in awake animals by the tail cuff method (pneumatic transducer).

Rats were killed at various ages (4 to 72 weeks) by exsanguination following anesthesia (sodium pentobarbital, 50 mg/kg; i.p.).

Vasculitic lesions

Vasculitic lesions were grossly examined in the gastrointestinal tract of necropsied animals. The mesentery was dissected from the rats and immersed in a physiological salt solution for examination by an experienced observer. The presence of macroscopic bead-like thickenings was evidence for vasculitis. Figure 1 shows typical samples from an 18-month old female SHRSP and an age-matched female WKY rat.

Arteriogram

In order to provide more detailed morphological information about the vasculitic lesions, arteriograms were made in a subset of SHRSP and WKY rats. The rats (n=6 for each group) were anesthetized with

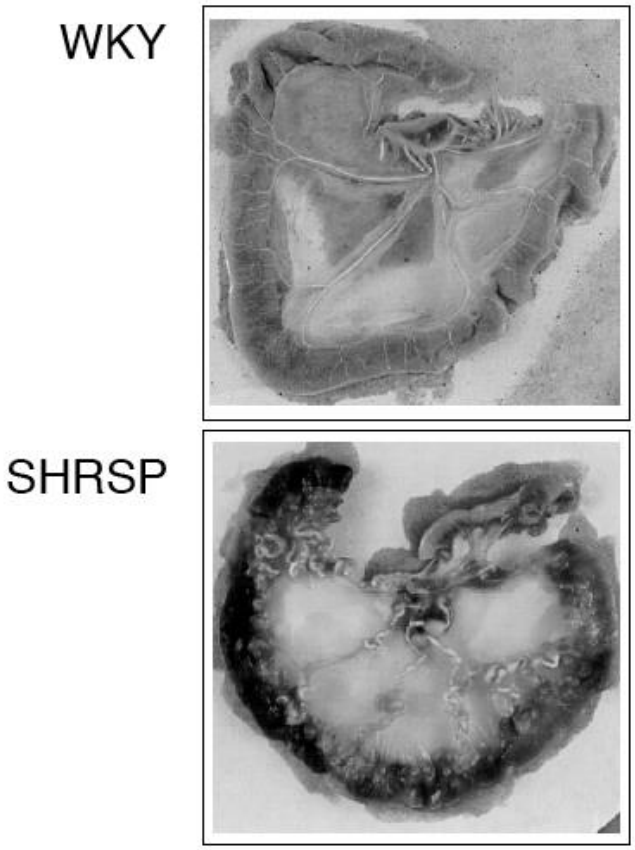

Fig. 1: Vasculitis in intestinal tract of female SHRSP and WKY rat (18 months old). Gross inspection of the entire intestinal tract reveals vasculitic lesions in female SHRSP (bottom panel), whereas none exist in the WKY rat (top panel).

sodium pentobarbital (50 mg/kg, i.p.) and a midline incision was made. Using a surgical magnifier, the renal and ileal arteries were tied to obtain a more effective injection pressure in the mesenteric vascular bed. The colon was lifted from its attachments and a winged infusion needle (1.0 mm) was inserted into the abdominal aorta with the tip placed near origin of the mesenteric artery. The aorta was clamped at the level of the celiac trunk and the vasculature was flushed with 200 ml of saline perfusate. The portal vein was kept open during the first 5 min of perfusion and then tied to promote colonic venous outflow via the inferior vena cava. Approximately 20 ml of 50% barium sulfate in gelatin was then injected through the same needle using an infusion pump. The barium suspension was injected until it became visible in the arterial branches of the intestinal serosa. Finally, the colon and arterial branches were clamped and the tissue specimen was explanted for radiologic investigation.

Qualitative inspection of the mesenteric vascular bed following infusion of barium sulfate-gelatin showed that vasculitic lesions were prominent in SHRSP and absent in WKY rats (Figure 2).

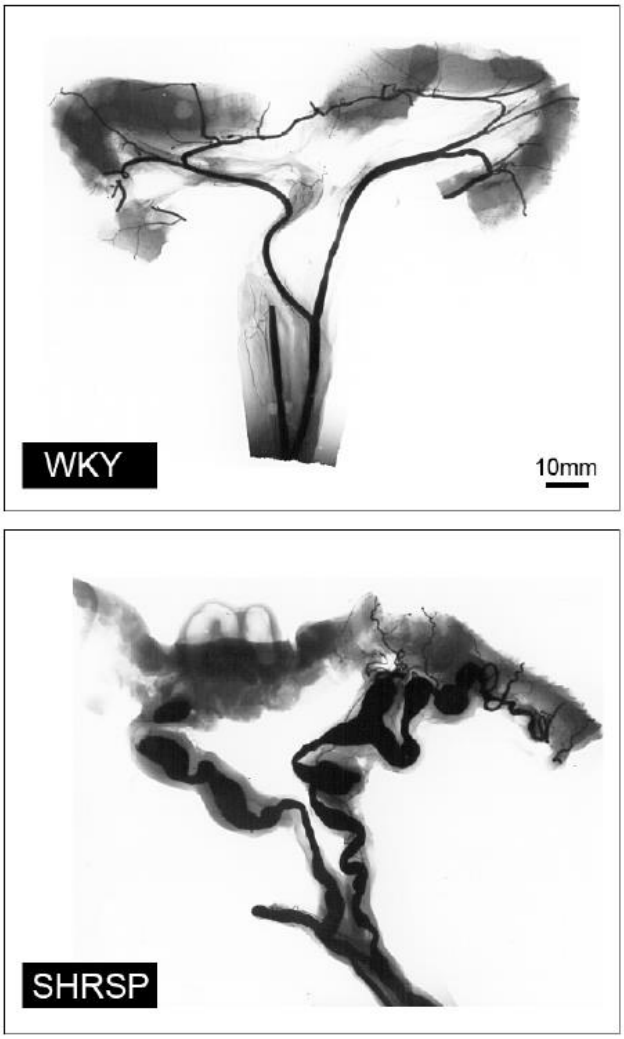

Fig. 2: Mesenteric arteriograms of female SHRSP and WKY rat (18 months old). Qualitative inspection of the mesenteric vascular bed following infusion of barium sulfate-gelatin showed that vasculitic lesions were prominent in SHRSP (bottom panel). Vasculitic lesions were not present in the mesenteric vasculature of WKY (top panel). Each arteriogram shows a single arterial arcade from the jejunum of an 18 month old female rat (magnification: 18x).

Data analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistical differences between and within groups were determined by two-way analysis of variance for blood pressures and chi-square test for lesion incidence with $p<0.05$ considered to be significant. The Bonferroni correction was used to counteract multiple comparisons.

RESULTS

Macroscopic, bead-like lesions were found exclusively in the mesenteric vascular bed of SHRSP. The incidence of occurrence (Figure 3) was 54% for all SHRSP (n=357). None of the WKY rats had the vascular disease (n=373). Arterial lesions were not evident in young SHRSP (1-1.5 months of age) before the development of high blood pressure (Figure 4). The earliest appearance of vasculitis in SHRSP was at ~8 months of age after full establishment of elevated arterial pressure (Figure 4, top). Systolic blood pressures during the maintained phase were greater than 200mmHg for SHRSP and less than 130mmHg for WKY rats (ages: 4.4-8 months; Figure 4, bottom). Within SHRSP, lesions were more common in females, in spite of higher mean systolic pressure found in males. Of the female SHRSP, 70% (n=234) had severe micronodular vasculitis whereas 24% of male SHRSP showed lesions (age matched; Figure 4, top, $p<0.05$). At 16-18 months of age, systolic blood pressures in female SHRSP converged with measures of systolic blood pressures in male SHRSP.

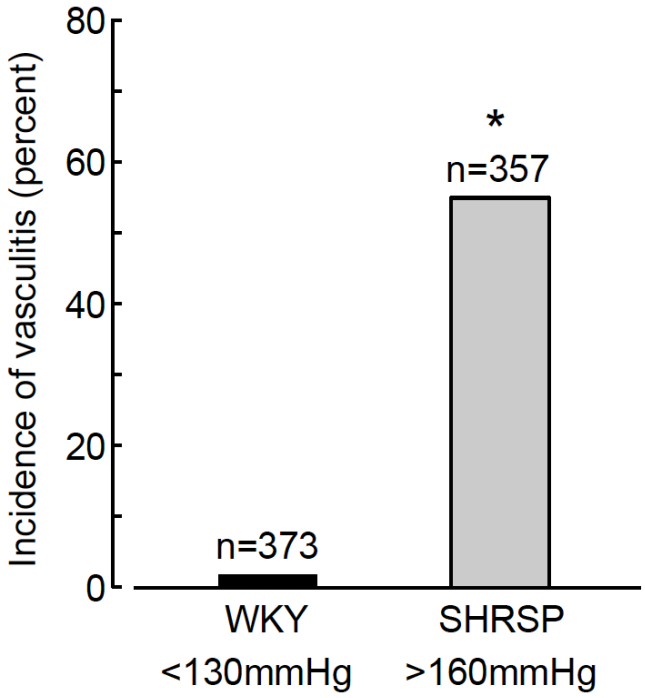

Fig. 3: Systolic blood pressure and incidence of vasculitis in SHRSP and WKY rats. Systolic blood pressures in WKY rats (>9 months of age) were significantly lower than those in SHRSP. The incidence of vasculitis in the intestinal tract was greater in SHRSP; WKY rats did not develop vascular lesions in the intestinal tract. The asterisk indicates a statistically significant difference between SHRSP and WKY rats ($p<0.05$).

DISCUSSION

This study demonstrates that macroscopic, bead-like lesions occur frequently in the mesenteric vasculature of SHRSP, but not WKY rats. There is a relationship between severity of vascular lesions and age, but not elevated arterial pressure. Finally, there is a sex associated increase in susceptibility for vasculitic lesions in females. Studies by other

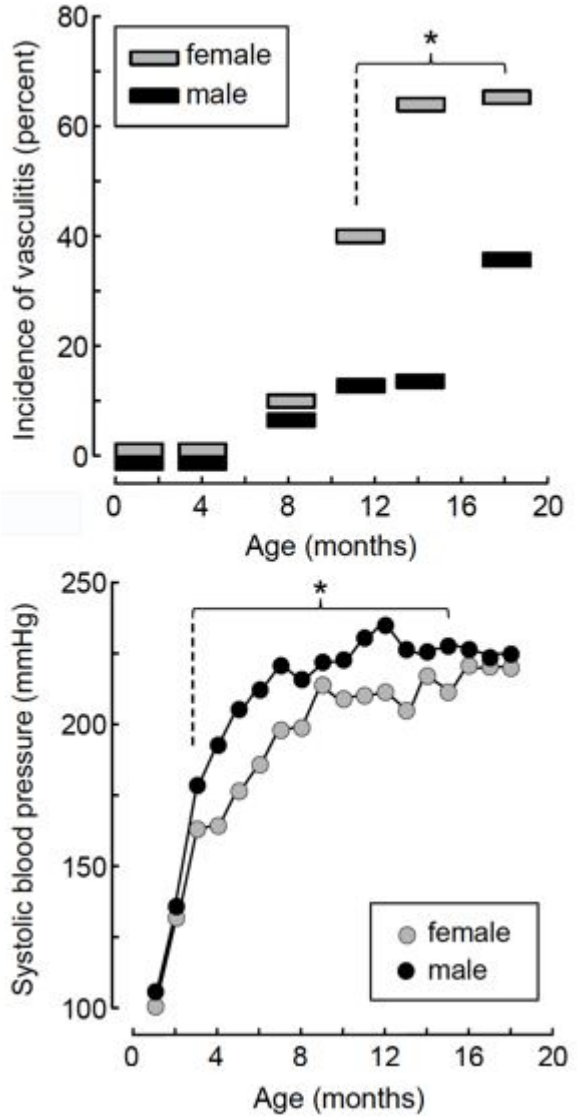

Fig. 4: Development of elevated systolic blood pressure and incidence of vasculitis in male and female SHRSP. The incidence of vasculitis in the intestinal tract was not evident until SHRSP reached an age of 6-9 months (top panel). After this age, the incidence of vasculitis in female SHRSP was greater than in male SHRSP. Systolic blood pressures were significantly lower in female SHRSP (bottom panel) and vasculitis appeared to be associated with the maintained phase of elevated blood pressure (indicated by asterisks, $p<0.05$).

investigators have demonstrated that these lesions can be categorized as polyarteritis nodosa (Saito, et al., 1990, 1991, 1995).

In humans, polyarteritis nodosa is a multi-system, necrotizing vaculitis in medium-sized arteries, occurring usually at vessel bifucations (Stanson, et al. 2001). It commonly affects skin, joints, peripheral nerves, testicles, the gut and the kidney. Polyarteritis nodosa is uncommon in the pulmonary circulation. In more than 30% of the patients with polyarteritis nodosa, hypertension is observed (Sadurska, et al, 2012).

In rats, necrotizing vasculitis has been observed following treatment with various vasoactive compounds such as theophylline (Collins, 1988), endothelin receptor antagonists (Gonzalez-Fernandez and Garcia, 2007), fenoldopam (Yukas, et al., 1985) and selective phosphodiesterase inhibitors (PDE3 and PDE4; Sandasky and Means, 1987; Mecklenburg, et al., 2006; Zhang, 2008). These compounds can induce systemic hypotension and it has been proposed that this may lead to decreased oxygen delivery, necrosis, enhanced endothelial permeability, protein exudation and immigration of inflammatory cells. Considerable evidence suggests that the renin-angiotensin system may be involved in animal models of polyartertis nodosa and angiotensin-converting enzyme inhibitors prevent lesion formation (Peters, 2010).

Age

Our observations in male SHRSP are very similar to those that have been reported in other studies (Saito, et al., 1990, 1991, 1995). Vasculitic lesions occur in approximately a third of all male SHRSP and the occurrence is age-related with onset at around nine months. This same age dependency characterizes the incidence of vasculitis in female SHRSP, yet the occurrence is much higher with two-thirds developing the lesions in rats 16 months and older. In humans, vasculitic diseases occur in the elderly with an average onset of 72 years of age (Sadurska, et al., 2012).

Blood pressure

Studies by Saito, et al. (1990, 1991, 1995) indicated that vasculitic lesions are associated with elevated arterial pressure in male SHRSP. They subdivided aged, male SHRSP (12-19.5 months) into two groups according to the presence and absence of vasculitis. Male SHRSP with vasculitic lesions had higher systolic blood pressures than SHRSP with no lesions. We made a similar subdivision in our rats, but did not see this association (data not shown). Further, female SHRSP had lower systolic blood pressures than males, yet the females had a greater incidence of vasculitis. Thus, our study does not support the notion that vasculitic lesions are worse in animals that have the highest systolic blood pressures. Interestingly, vascuitis is not associated with elevated arterial pressure in the human population (Leenhardt et al, 1984). It is known that the development of vasculitic lesions in renal arteries causes renal ischemia leading to renal injury and hypertension in humans (Sadurska, et al, 2012).

Sex differences

In our studies on SHRSP, there is a sex associated increase in the incidence of vasculitic lesions in females. This has not been reported in the spontaneously hypertensive rat (SHR) where male rats had an incidence of 83% and females had an incidence of only 17% [paired for blood pressure level (>180 mmHg) (Suzuki, et al. 1979)]. The reason for this difference is unclear but we speculate that it may be related to salt sensitivity. The SHR is relatively salt resistant, whereas the SHRSP is markedly susceptible (Griffin, et al. 2001). With respect to sex differences, in female F2 progeny of SHRSP and WKY rats, blood pressure is strongly linked with the MITR244 locus on chromosome 3 after salt loading and there is no association in male F2 progeny (Matsumoto, et al. 1995).

Vasculitc lesions in experimental rodent models of renal mineralocorticoid hypertension occur at greater frequency when the rats are placed on a high sodium diet (Kempner et al., 1955; Race and Peschel, 1954). Although the rats in this study were not placed on a high sodium diet, commercial rodent diets contain more sodium than the rats need (Martus, et al., 2005). Dietary sodium does induce immune activation and target organ damage in hypertensive humans independent of any blood pressure effect (Yilmaz, et al. 2012). In cultured human endothelial cells, elevation of extracellular sodium within the physiological range is accompanied by vascular changes that facilitate development of cardiovascular disease (Dmitrieva et al. 2015). Finally, dietary salt drives autoimmune disease by activation of pathogenic immune cells (Kleinewietfeld, et al. 2013; Wu, et al. 2013; Yosef, et al. 2013).

Female sex is a factor in 80% to 90% of cases of Takayasu arteritis, with age at onset usually between 10 and 40 years. Takayasu arteritis differs from polyarteritis nodosa in that it affects large arteries (aorta) with massive intimal fibrosis and affects young to middle age women. It is chronic and of unknown cause. Temporal arteritis associated with polymyalgia rheumatica has been reported to be of greater incidence in women and required a longer duration of treatment (Narvaez et al. 2002).

The increased incidence among female SHRSP implies a relationship with sex hormones or reflects the general finding that inflammatory diseases occur with greater incidence in females (Fairweater and Rose, 2004; Ngo 2014). In humans, the disorder is more likely to be present in men between the ages of 40 and 50 years (Stanson, et al. 2001).

SUMMARY AND CONCLUSION

This study demonstrates the occurrence of vasculitis (polyarteritis nodosa) in the mesenteric vasculature of SHRSP, but not WKY rats. There is a relationship between incidence of vascular lesions and age, but not elevated arterial pressure. Although the exact cause of polyarteritis nodosa is unknown, there is a sex associated increase in incidence for vasculitic lesions in females that we speculate that it might be linked to salt-sensitivity.

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