Neointimal Hyperplasia and Vasoreactivity Are Controlled by Genetic Elements on Rat Chromosome 3

Andrea L. Nestor Kalinoski, Ramona S. Ramdath, Kay M. Langenderfer, Saad Sikanderkhel, Sarah DeRaedt, Marlene Welch, James L. Park, Timothy Pringle, Bina Joe, George T. Cicila, David C. Allison

Abstract—Neointimal hyperplasia (NIH) can lead to restenosis after clinical vascular interventions. NIH results from complex and poorly understood interactions between signaling cascades in the extracellular matrix and the disrupted endothelium, which lead to vessel occlusion. Quantitative trait loci (QTLs) were reported previously on rat chromosomes 3 and 6 through linkage analysis of postinjury NIH in midiliac arterial sections. In the current study, substitution mapping validated the RNO3 NIH QTL but not the RNO6 NIH QTL. The SHR.BN3 congenic strain had a 3-fold increase in the percentage of NIH compared with the parental spontaneously hypertensive rat strain. A double congenic study of RNO3+RNO6 NIH QTL segments suggested less than additive effects of these 2 genomic regions.

To test the hypothesis that changes in vessel dynamics account for the differences in NIH formation, we performed vascular reactivity studies in the Brown Norway (BN), spontaneously hypertensive rat (SHR), SHR.BN3, and SHR.BN6 strains. De-endothelialized left common carotid artery rings of the SHR.BN3 showed an increased vascular responsiveness when treated with serotonin or prostaglandin F2α, with significant differences in EC50 and maximum effect (P<0.01) values compared with the spontaneously hypertensive rat parental strain. Because both vascular reactivity and percentage of NIH formation in the SHR.BN3 strain are significantly higher than the SHR strain, we postulate that these traits may be associated and are controlled by genetic elements on RNO3. In summary, these results confirm that the RNO3 NIH QTL carries the gene(s) contributing to postinjury NIH formation. (Hypertension. 2010;55[part 2]:555-561.)

Key Words: neointimal hyperplasia ■ QTL ■ congenics ■ rat ■ vascular reactivity

Cardiovascular interventions such as arterial grafts, stents, and balloon angioplasties often fail because of recurrent vascular stenosis attributed to the development of neointimal hyperplasia (NIH). NIH adversely affects patient outcome after vascular interventions. For example, restenosis after clinical interventions in the vascular system occurs in upward of 37% of patients after balloon angioplasty for coronary artery disease1-3 and in 20% of patients after vascular interventions. Furthermore, there is a 10% yearly failure rate for grafts in the coronary and peripheral arterial systems,5-7 with NIH being the major contributing factor. Currently, there are no effective medical therapies to prevent or treat NIH. A need for more specific therapies is underscored by the failure of clinical trials evaluating the empirical use of aspirin, heparin, and calcium channel blockers1,8 to prevent restenosis after vascular interventions. Furthermore, the use of drug-eluting stents to prevent NIH is currently being closely examined because of the additional risk of drug toxicity without any observed increase in long-term efficacy compared with normal stents.9-11 The lack of effective therapies to prevent or medically treat NIH after vascular procedures may be attributed, in part, to a poor understanding of the mechanisms underlying vascular remodeling after cardiovascular interventions. Previously, we obtained evidence for genetic factors governing NIH. Differences in NIH-injury response of the left common and external iliac artery were observed among inbred strains of rats2 and a subsequent genome scan of a segregating F2 (spontaneously hypertensive rat [SHR]×Brown Norway [BN]) population phenotyped for postinjury NIH formation and vascular wall differences identified possible NIH-related quantitative trait loci (QTLs) on rat chromosomes 3 and 6 (RNO3 and RNO6).12 The current report constitutes a continuation of this project with the long-term goal to identify the gene(s) controlling vascular remodeling and restenosis after injury. We now confirm the presence of an NIH QTL on RNO3 containing the allele(s) in part responsible for controlling the strain differences in the formation of injury-induced NIH. Unexpectedly, this newly developed RNO3 congenic rat strain also provided evidence for eliciting genetic control of vasoreactivity.
Methods

Rat Strains

Inbred SHR (SHR/NHsd) and Brown Norway (BN/SsNHsd) rat strains (obtained from Harlan Sprague-Dawley, Indianapolis, IN) were used to establish colonies that were maintained in the University of Toledo Health Science Campus Division of Laboratory Animal Resources. Hereafter, these inbred strains are referred to as “SHR” and “BN,” respectively. The previous genome scan of an F2 (SHR x BN) population12 and the present subsetting mapping were conducted using the same SHR and BN colonies. Breeding programs were approved by the institutional animal care and use committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All of the rats were housed with the appropriate temperature and humidity controls with 12-hour light/dark cycles and maintained on standard rat chow (Ralston Purina, Diet 5001) with ad libitum access to water.

Congenic strains were developed using a marker-assisted, speed congenic approach13 on the SHR strain as the background and the BN strain as the donor strain. The breeding paradigm was as follows: male F1 rats, bred by crossing male BN rats with female SHR rats, were backcrossed to female SHR rats. In general, male progeny heterozygous for the RNO3 and RNO6 NIH QTL-containing regions (loci genotyped are described below for each congenic strain) and carrying the fewest number of BN-rat alleles at the other loci in the genome were selected for backcrossing to female SHRs. After the first backcross generation, SHR.BN3 and SHR.BN6 congenic strains were developed independently. For each generation, the male heterozygous for all of the markers in the NIH QTL-containing region and carrying the fewest BN-rat alleles in the remainder of the genome was bred with ≤ 5 female SHRs. In 1 generation, a female RNO3 backcross rat was bred with a male SHR, thus the RNO3 and double-congenic strain carry the SHR Y chromosome. However, the RNO6 congenic carries the BN Y chromosome.

Five backcross generations were required to breed the congenic strains. Male and female rats heterozygous for the RNO3 or RNO6 NIH QTL-containing regions and lacking BN-rat alleles in the background were then mated to fix the BN-rat alleles in the congenic regions and SHR-rat alleles outside of the congenic segment and on all of the other chromosomes. Brother-sister mating was subsequently used to maintain congenic rat strains. The double-congenic strain was similarly bred by crossing female SHR.BN6 and male SHR.BN3 congenic strains and selecting rats heterozygous for both the RNO3 and RNO6 QTL-containing regions in the F1 progeny. Male and female rats heterozygous for both congenic regions were then mated to fix BN alleles in the congenic regions, and brother-sister mating was subsequently used to maintain the strain. Hereafter, this congenic strain will be referred to as “SHR.BN” (3+6).

Vascular Injury

Male rats 10 to 12 weeks of age (300 g) underwent a standard balloon vascular injury, as described previously.2 Briefly, rats were sedated with 2% isofluorane by O2 inhalation and anesthetized with ketamine at 80 mg/kg of ketamine and 12 mg/kg of xylazine. A 2-F balloon catheter was placed in the left femoral artery and equilibrated for 15 minutes with washes every 20 minutes. Before the determination of concentration response curves, the rings were subjected to a “wake-up” protocol consisting of 2 consecutive contractions with isotonic high-K+–containing physiological salt solution (in millimoles per liter: NaCl 130.00, KC1 4.70, KHPO4 1.18, MgSO4 1.17, CaCl2 1.60, NaHCO3 14.90, dextrose 5.50, and CaNa2 EDTA 0.03). Carotid artery rings were set at 5 g of passive tension and equilibrated for 1 hour with washes every 20 minutes. Before the determination of concentration response curves, the rings were subjected to a “wake-up” protocol consisting of 2 consecutive contractions with isotonic high-K+–containing physiological salt solution (in millimoles per liter: NaCl 14.70, KC1 100.00, KHPO4 1.18, MgSO4 1.17, CaCl2 1.60, NaHCO3 14.90, dextrose 5.50, and CaNa2 EDTA 0.03), followed by a contraction with phenylephrine (10−7 mol/L). After the wake-up protocol, serotonin (5HT; Sigma Chemical Company; 10−9 to 10−4 mol/L) and prostaglandin F2α (PGF2α; Cayman Chemical; 10−9 to 10−4 mol/L) to 3×10−7 mol/L) concentration responses were performed in the endothelium-denuded rings. Both concentration responses were performed in each of the rings, but the order of the agonists was randomized on each day that the experiments were performed.16

Vascular Reactivity Experiments

Left common carotid arteries were cut into rings (3 mm in length), and the endothelium was removed by perfusing the ring lumen with 100 μL of 0.1 Triton-X 100 in PBS. Carotid rings were mounted in a myograph system (DMT-USA, Inc) and bathed with warmed 37°C, aerated (95% O2/5% CO2) physiological salt solution (in millimoles per liter: NaCl 154.00, KCl 4.70, KHPO4 1.18, MgSO4 1.17, CaCl2 1.60, NaHCO3 14.90, dextrose 5.50, and CaNa2 EDTA 0.03). Carotid artery rings were set at 5 g of passive tension and equilibrated for 1 hour with washes every 20 minutes. Before the determination of concentration response curves, the rings were subjected to a “wake-up” protocol consisting of 2 consecutive contractions with isotonic high-K+–containing physiological salt solution (in millimoles per liter: NaCl 14.70, KC1 100.00, KHPO4 1.18, MgSO4 1.17, CaCl2 1.60, NaHCO3 14.90, dextrose 5.50, and CaNa2 EDTA 0.03), followed by a contraction with phenylephrine (10−7 mol/L). After the wake-up protocol, serotonin (5HT; Sigma Chemical Company; 10−9 to 10−4 mol/L) and prostaglandin F2α (PGF2α; Cayman Chemical; 10−9 to 10−4 mol/L) to 3×10−7 mol/L) concentration responses were performed in the endothelium-denuded rings. Both concentration responses were performed in each of the rings, but the order of the agonists was randomized on each day that the experiments were performed.16

Vasoreactivity Data and Statistical Analysis

Agonist EC50 values were calculated with a nonlinear regression analysis with the algorithm [effect = maximum response/(1 + (EC50/ agonist concentration))] in the computer program GraphPad Prism.
Data are expressed as mean±SEM, and concentration-response data were analyzed using 1-way ANOVA followed by a Bonferroni post hoc test. Differences in EC50 values were evaluated using 1-way ANOVA with a Bonferroni post hoc test (P<0.05).

**Results**

**Extent of the Regions Introgressed in the Congenic SHR.BN3, SHR.BN6, and SHR.BN (3+6) Strains**

Physical maps summarizing the regions of BN RNO3 and RNO6 introgressed into the SHR genetic background are presented in Figure 1. The SHR.BN3 strain carries a 50.6-megabase region of the BN-rat RNO3 q-terminus, between D3Rat159 and D3Rat1 (Figure 1). The SHR.BN6 strain carries a 45.5-megabase region of RNO6, between markers D6Rat40 and D6Rat170 (Figure 1). The double-congenic strain incorporated both regions of RNO3 and RNO6 from the BN strain introgressed into the SHR genetic background and will be referred to as “SHR.BN (3+6).”

**Injured Vessel Analysis**

Table 1 shows the injured vessel parameters measured for the parental SHRs compared with those of the SHR.BN3 and SHR.BN6 congenic rat strains. The injured vessels of the SHR.BN3, which carry high injury-induced NIH QTL allele(s), showed significant increases in the %NIH (P<0.001) and NIH area (P=0.002) compared with the parental SHR strain (Figure 2 and Table 1). Specifically, a 3-fold higher %NIH developed 8 weeks postinjury in the SHR.BN3 congenic strain (28.31%; Table 1 and Figure 2B) as compared with the parental, SHR strain (9.63%; Table 1 and Figure 2A). Although not significant, the increase in NIH area in the injured SHR.BN3 vessels was associated with a decrease in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHR injured</th>
<th>%NIH</th>
<th>NIH Area, mm²</th>
<th>Circular Area, mm²</th>
<th>Media Area, mm²</th>
<th>Media Width, mm²</th>
<th>Lumen Size, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR injured</td>
<td>20</td>
<td>9.63±0.90†</td>
<td>0.0125±0.100†</td>
<td>0.3624±0.17†</td>
<td>0.1020±0.20</td>
<td>0.0438±0.16</td>
<td>0.3699±0.16</td>
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<tr>
<td>SHR.BN3 injured</td>
<td>13</td>
<td>28.31±6.7‡</td>
<td>0.0377±0.77‡</td>
<td>0.3899±3.1‡</td>
<td>0.0846±3.3‡</td>
<td>0.0378±4.3‡</td>
<td>0.3522±3.2</td>
</tr>
<tr>
<td>SHR.BN6 injured</td>
<td>25</td>
<td>12.60±7.2†</td>
<td>0.0172±0.80†</td>
<td>0.4507±17‡</td>
<td>0.1120±18†</td>
<td>0.0446±17‡</td>
<td>0.4335±17‡</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±% coefficient of variation.

*Data show P<0.01 vs SHR by 1-way ANOVA.
†Data show P<0.01 vs SHR.BN3 by 1-way ANOVA.
‡Data show P<0.01 vs SHR.BN6 by 1-way ANOVA.
the media area of these vessels when compared with the injured SHR vessels \( (P \text{ value not significant; Table 1 and Figure 2}) \). There was also a slight increase in circular area compared with injured SHR vessels \( (P \text{ value not significant; Table 1 and Figure 2}) \). Thus, the injured SHR.BN3 vessels did not show a significant postinjury dilation. In contrast, the SHR.BN6 congenic strain, carrying a possible high \%NIH QTL and the QTL(s) associated with low media area and media width in SHR.BN3 congenic strain, did not develop a significant increase in NIH compared with the parental SHR strain \( (P=0.0076) \), media area \( (P=0.0036) \), and media width \( (P=0.0032) \); Table 2). These differences were possibly attributed to the uninjured control vessel QTLs for media area and media width found previously on RNO6 \( ^{12} \) (Table 2).

### Comparison of Uninjured Control Versus Injured Vessel Measurements Within Strains

A comparison of uninjured control and injured vessel parameters for each of the 3 rat strains is shown in Table S1 (available in the online Data Supplement at http://hyper.ahajournals.org). A significant decrease was found in the lumen size of the uninjured control vessels compared with the injured vessels of the parental SHR strain \( (P=0.0045; \text{ Table S1}) \). The results observed for the congenic SHR.BN6 strain \( (n=25) \) were similar to the parental SHR strain, with significantly smaller injured vessel media area \( (P=0.0005) \) and media width \( (P=0.0013) \) compared with those of uninjured control vessels \( (P=0.0005) \). There were no significant differences in the circular area, lumen size, media area, or media width of the uninjured control and injured vessels of the SHR.BN3 strain \( (P=0.0005) \).

### Double-Congenic Vessel Analysis

Table S2 summarizes the vessel parameters measured in a separate experiment comparing the parental SHR strain with the double-congenic strain SHR.BN \( (3+6) \), which carries both the RNO3 and RNO6 QTL-containing regions. The

<table>
<thead>
<tr>
<th>Strain</th>
<th>N</th>
<th>Circular Area, mm(^2)</th>
<th>Media Area, mm(^2)</th>
<th>Media Width, mm(^2)</th>
<th>Body Weight–Adjusted Heart-Weight, mg/g(\text{§})</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR control</td>
<td>17</td>
<td>0.4479±21</td>
<td>0.1218±20</td>
<td>0.0483±14</td>
<td>1272±6</td>
</tr>
<tr>
<td>SHR.BN3 control</td>
<td>13</td>
<td>0.3937±28†</td>
<td>0.1008±46†</td>
<td>0.0418±28†</td>
<td>1402±12</td>
</tr>
<tr>
<td>SHR.BN6 control</td>
<td>25</td>
<td>0.4977±21†</td>
<td>0.1396±22†</td>
<td>0.0524±17†</td>
<td>1303±5</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±% coefficient of variation.

*Data show \( P<0.01 \) vs SHR by 1-way ANOVA.
†Data show \( P<0.01 \) vs SHR.BN3 by 1-way ANOVA.
‡Data show \( P<0.01 \) vs SHR.BN6 by 1-way ANOVA.

Figure 2. Representative images of NIH formation in the left iliac arteries of the (A) SHR, (B) SHR.BN3, (C) SHR.BN6, and (D) SHR.BN \( (3+6) \) strains 8 weeks postinjury. Vessels were formalin-fixed, paraffin-embedded sections stained with Verhoeff-Van Gieson for elastin \( (×40 \text{ magnification}) \). IEL indicates internal elastic lamina.
double-congenic and the SHR.BN6 rats were similar in that the vessels of both strains had significantly lower media area \((P=0.0430)\), media width \((P=0.0163)\), and lumen size \((P=0.0359)\) in injured vessels when compared with uninjured control vessels (Table S2 and Figure 2D). Injured vessel parameters of the SHR and double-congenic rats were, as expected, significantly different in %NIH \((P=0.0269)\), NIH area \((P=0.0223)\), media area \((P=0.0157)\), and media width \((P=0.0125\); Table S2). These results suggest that the two %NIH QTL-containing regions (RNO3 [%NIH: 28.31; Table 1 and Figure 2B] and RNO6 [%NIH: 12.60; Table 1 and Figure 2C]) showed less than additive effects in the double-congenic strain (%NIH: 23.76; Table S2), possibly because of differences in media area and media width.

**Vascular Reactivity Experiments**

Vascular reactivity was studied to further characterize phenotypes potentially linked to the observed NIH response. Figure 3 shows the results of reactivity experiments performed on the parental (BN and SHR) and congenic (SHR.BN3 and SHR.BN6) rat strains with two different agonists. Concentration response curves were generated (Figure 3) in endothelium-denuded carotid artery rings with increasing concentrations of PGF\(_{2\alpha}\) and 5HT. We compared the vasopressor response of the individual congenic strains to both the SHR and BN strains at each concentration. PGF\(_{2\alpha}\) caused a concentration-dependent contraction in the endothelium-denuded rings from each of the rat strains. The SHR.BN3 congenic strain showed significantly higher sensitivity \((E_{50}: 6.54\pm0.14)\) to increasing concentrations of PGF\(_{2\alpha}\) compared with the parental SHR and BN strains \((E_{50}: 5.74\pm0.04 \text{ and } 5.57\pm0.03\text{, respectively})\) in the concentration-response curve (Figure 3). Furthermore, we observed a significant increase in PGF\(_{2\alpha}\) maximum effect \((E_{\text{max}})\) for the SHR, SHR.BN3, and SHR.BN6 strains \((E_{\text{max}}: 152.05, 154.20, \text{ and } 157.04\text{, respectively})\) compared with the BN strain \((E_{\text{max}}: 102.75; P<0.05; \text{ Figure 3A})\).

The SHR.BN3 congenic strain also showed a concentration-dependent response to 5HT (Figure 3B) that was similar to that observed for PGF\(_{2\alpha}\) (Figure 3A), although with a slightly higher \(E_{50}\) (6.66\pm0.05) and a slightly decreased \(E_{\text{max}}\) (139.10\pm0.03; Figure 3). The SHR.BN3 rats showed a significantly different concentration response profile to 5HT compared with both the SHR and BN strains (Figure 3B; \(P<0.05\)). Most notably, the \(E_{50}\) for the SHR.BN3 5HT concentration-response curve showed a leftward shift compared with those of the SHR.BN6, SHR, and BN strains (Figure 3B). Interestingly, the parental BN strain was the least sensitive strain at comparable \(E_{50}\) and \(E_{\text{max}}\) values for both PGF\(_{2\alpha}\) and 5HT (Figure 3). The SHR.BN3 congenic strain was clearly the most sensitive to both agonists and showed a concentration-dependent vasoreactivity within the endothelium-denuded aortic rings, confirming an increased vasoreactivity of SHR.BN3 congenic rats when compared with the parental SHR and BN strains.

**Discussion**

The primary goal of this study was to corroborate the putative NIH QTLs identified in the initial F\(_2\) (SHR×BN) genome scan\(^2\) and to delimit the chromosomal region containing the gene(s) responsible for postinjury NIH formation. Congenic strains were generated to introgress the NIH QTLs identified on RNO3 and RNO6. The phenotypic data shown in Table 1 from these newly developed congenic strains validate the existence of an NIH QTL on RNO3. The identification of the RNO3 interval associated with differences in NIH constitutes an important step in the genetic dissection of the NIH QTL, with the SHR.BN3 congenic strain serving as a substrate for further substitution mapping.

**Figure 3.** Vascular reactivity experiments of endothelium denuded carotid artery rings of the SHR and BN parental strains and the SHR.BN3 and SHR.BN6 congenic strains. Concentration response curves for the agonists (A) PGF\(_{2\alpha}\), and (B) 5HT. C, Potency of agonists \((-\log E_{50}\) in carotid artery rings for each strain.
The second goal of our study was to optimize the morphological parameters to be measured by which the genetic determinants of NIH should be followed by substitution mapping. There were no significant differences between the SHR parental strain and either of the congenic strains in any of the measured, uninjured control vessel parameters (Table 2). This finding leads us to conclude that future mapping studies should be conducted exclusively after vessel injury, because this is the only condition wherein QTL effects are observable among SHR, SHR.BN3, and SHR.BN (3+6) strains (Table 1).

The third objective of the study was to identify associated phenotypes of NIH that may help us gain insight into the underlying mechanisms involved in intimal thickening. Intriguingly, on catheter insertion, we observed marked differences in vascular resistance between the individual parental and congenic strains (data not shown), suggesting that differences in vascular reactivity and the media elastin layers may contribute to the observed differences in NIH between the two strains (Table 1). The finding of elevated vasoconstriction in the SHR vessels compared with BN vessels in response to 5HT and higher concentrations of PGF2α (Figure 3) lends support to the idea that alterations in vessel wall dynamics may contribute to NIH formation in response to injury. It is interesting that the RNO3 alleles from the BN rat introgressed into the SHR.BN3 congenic strain led to a rapid and sustained vasoconstriction when compared with that observed in the parental SHR vessels (Figure 3), and this vasoconstrictive response segregated with the marked increase in %NIH (Table 1 and Figure 2). Thus, the SHR.BN3 congenic strain should provide a foundation to generate newer iterations of congenic substrains to test for possible relationships between these NIH and vasoconstrictive phenotypes.

The RNO6 NIH QTL identified in our previous F2 genome scan12 was not confirmed by substitution mapping. This was perhaps to be expected, because the RNO6 NIH QTL reached only suggestive logarithm of odds scores for all of the parameters measured (ie, %NIH, media area, and media width) in the previous linkage analysis.12 This also means that the net effects of BN-rat RNO6 alleles substituted do not account for differences in NIH observed at this locus in the F2 width) in the previous linkage analysis.12 This also means that the cause-effect relationships between vessel occlusion and vascular reactivity remain unexplored. In this context, our study is unique, because isolation of the RNO3 interval associated with differences in NIH and vascular reactivity in the SHR.BN3 congenic strain can now be used to assess relationships between NIH and vascular reactivity, discern whether these are independent or associated phenotypes, and design and develop new therapies to prevent vascular restenosis following invasive vascular procedures.

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Disclosures
None.

References


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NEOINTIMAL HYPERPLASIA AND VASOREACTIVITY ARE CONTROLLED BY GENETIC ELEMENTS ON RAT CHROMOSOME 3

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Short Title: NIH and Vasoreactivity in a Rat Model

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Table S1. Comparison of the uninjured contralateral control and injured vessel parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Circular Area, mm²</th>
<th>Media Area, mm²</th>
<th>Media Width, mm²</th>
<th>Lumen Size, mm²</th>
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<tr>
<td>SHR Con.</td>
<td>17</td>
<td>0.4479 ± 21</td>
<td>0.1218 ± 20</td>
<td>0.0483 ± 14</td>
<td>0.4479 ± 21</td>
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<td>SHR Inj.</td>
<td>20</td>
<td>0.3824 ± 17</td>
<td>0.1020 ± 20</td>
<td>0.0438 ± 16</td>
<td>0.3699 ± 16</td>
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<td>0.3937 ± 28</td>
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<td>SHR.BN3 Inj.</td>
<td>13</td>
<td>0.3899 ± 31</td>
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</table>

Data expressed as the mean ± %CV.

\[ p < 0.01 \] significant by one-way ANOVA.

N.S. = not significant.
Table S2. Comparison of injured and uninjured contralateral control vessel parameters measured for the SHR and double congenic SHR.BN (3+6) strain.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>%NIH</th>
<th>NIH Area, (\text{mm}^2)</th>
<th>Circular Area, (\text{mm}^2)</th>
<th>Media Area, (\text{mm}^2)</th>
<th>Media Width, (\text{mm}^2)</th>
<th>Lumen Size, (\text{mm}^2)</th>
<th>Body-weight adj Heart-weight, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR Con.</td>
<td>14</td>
<td>N/A</td>
<td>N/A</td>
<td>0.5389 ± 24</td>
<td>0.1364 ± 17</td>
<td>0.0496 ± 10</td>
<td>0.5389 ± 24</td>
<td>1770 ± 11</td>
</tr>
<tr>
<td>SHR.BN (3+6) Con.</td>
<td>17</td>
<td>N/A</td>
<td>N/A</td>
<td>0.5150 ± 16</td>
<td>0.1435 ± 14</td>
<td>0.0530 ± 10</td>
<td>0.5150 ± 16</td>
<td>1686 ± 11</td>
</tr>
<tr>
<td>p</td>
<td>-</td>
<td>-</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>SHR.BN (3+6) Con.</td>
<td>17</td>
<td>N/A</td>
<td>N/A</td>
<td>0.5150 ± 16</td>
<td>0.1435 ± 14</td>
<td>0.0530 ± 10</td>
<td>0.5150 ± 16</td>
<td>N/A</td>
</tr>
<tr>
<td>SHR.BN (3+6) Inj.</td>
<td>17</td>
<td>23.76 ± 44</td>
<td>0.0386 ± 50</td>
<td>0.5150 ± 16</td>
<td>0.1435 ± 14</td>
<td>0.0530 ± 10</td>
<td>0.5150 ± 16</td>
<td>N/A</td>
</tr>
<tr>
<td>p</td>
<td>0.0269</td>
<td>0.0223</td>
<td>N.S.</td>
<td>0.0430</td>
<td>0.0163</td>
<td>0.0359</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± %CV.

\(p < 0.05\) significant by one-way ANOVA.

N/A = not applicable.
NS= not significant.