Interactive effects of superoxide anion and nitric oxide on blood pressure and renal hemodynamics in transgenic rats with inducible malignant hypertension

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Patterson, Matthew E., Cynthia R. Mouton, John J. Mullins, and Kenneth D. Mitchell. Interactive effects of superoxide anion and nitric oxide on blood pressure and renal hemodynamics in transgenic rats with inducible malignant hypertension. Am J Physiol Renal Physiol 289: F754–F759, 2005. First published May 17, 2005; doi:10.1152/ajprenal.00419.2004.—Superoxide anion contributes to the elevated arterial blood pressure and renal dysfunction in malignant hypertension. However, the influence of superoxide anion on blood pressure and renal hemodynamics in transgenic rats with inducible malignant hypertension [strain name: TGR(Cyp1a1Ren2)]. Malignant hypertension was induced in male Cyp1a1-Ren2 rats (n = 6) through dietary administration of the aryl hydrocarbon, indole-3-carbinol (0.3%) for 7–9 days. Mean arterial pressure (MAP) and renal hemodynamics were measured in pentobarbital sodium-anesthetized Cyp1a1-Ren2 rats before and during intravenous infusion of the superoxide dismutase mimetic tempol (100 μmol/h). Basal MAP and renal vascular resistance (RVR) were elevated in rats induced with indole-3-carbinol compared with noninduced rats (n = 5) (184 ± 4 vs. 127 ± 3 mmHg, P < 0.01, and 29 ± 2 vs. 21 ± 1 mmHg·ml−1·min−1·g−1, P < 0.01, respectively). Hypertensive rats had elevated excretion of urinary 8-isoprostane compared with normotensive rats (41 ± 4 vs. 13 ± 6 pg·min−1·g−1, P < 0.01). There were no differences in renal plasma flow and glomerular filtration rate between groups. Systemic administration of tempol decreased MAP (184 ± 4 to 151 ± 4 mmHg, P < 0.01) and RVR (29 ± 2 to 25 ± 2 mmHg·ml−1·min−1·g−1, P < 0.05) in hypertensive but not in normotensive Cyp1a1-Ren2 rats. In addition, tempol administration decreased urinary excretion of 8-isoprostane (41 ± 4 to 25 ± 4 pg·min−1·g−1, P < 0.05). Renal plasma flow and glomerular filtration rate remained unaltered during tempol administration in both groups. The administration of the nitric oxide synthase inhibitor nitro-L-arginine attenuated the decrease in MAP and RVR in response to tempol. These findings indicate that superoxide anion contributes to the elevated RVR and increased arterial blood pressure, by a mechanism that is at least in part nitric oxide dependent, in Cyp1a1-Ren2 rats with malignant hypertension.

tempol; kidney; renin-angiotensin system; GFR; peptide hormones

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Recent transgenic rat line [strain name TGR(Cyp1a1Ren2)] was created that allowed the induction of various degrees of ANG II-dependent malignant hypertension (9). This transgenic rat line was generated by inserting the mouse Ren2 renin gene, fused to an 11.5-kb fragment of the cytochrome P-450 1a1 (Cyp1a1) promoter, into the genome of the Fischer 344 rat (9). Cyp1a1, which catalyzes the oxidation of a wide range of endogenous lipophilic compounds and xenobiotics (1, 3, 33), is not constitutively expressed. However, Cyp1a1 is highly inducible on exposure to various aryl hydrocarbons such as indole-3-carbinol (I3C) (1, 3, 4, 8, 12, 22, 33). Induction of Cyp1a1 is mediated by the aryl hydrocarbon receptor, which is a basic helix-loop-helix transcription factor that binds to specific DNA elements in the Cyp1a1 gene.
promoter (1, 33). Rats transgenic for the Cyp1a1-Ren2 construct do not constitutively express the Ren2 renin gene. Rather, the Ren2 gene is expressed, primarily in the liver, only after induction of the Cyp1a1 promoter by aryl hydrocarbons such as I3C (9). In essence, induction of the Cyp1a1 promoter by I3C is used to drive hepatic expression of the Ren2 renin gene. In this transgenic rat model, induction of the Cyp1a1 promoter by dietary administration of I3C results in a fixed level of expression of the Ren2 renin gene and in the development of ANG II-dependent hypertension (9). Thus this inducible transgenic rat model allows genetic clamping of renin gene expression and thus of plasma renin levels that are not subject to the normal physiological feedback mechanisms regulating the activity of the renin-angiotensin system. At a dose of 0.3% (wt/wt), dietary I3C induces malignant hypertension, characterized by loss of body weight, polyuria, polydypsia, lethargy, and piloerection. This model, therefore, allows the induction of ANG II-dependent malignant hypertension using a benign and naturally occurring dietary supplement without the need for surgical intervention, dietary salt manipulation, or the administration of steroids.

In the present study, the interactive effects of superoxide anion and nitric oxide on renal hemodynamics and blood pressure were assessed during the development phase (7–9 days) of malignant hypertension, before the occurrence of severe renal morphological changes, which have been shown to exist after more prolonged (14 days) induction of the Cyp1a1-Ren2 transgene (9).

METHODS

The experimental procedures in this study conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of Tulane University Health Sciences Center. Experiments were performed on male Cyp1a1-Ren2 transgenic rats, weighing between 250 and 300 g, bred at Tulane University School of Medicine from stock animals supplied from the University of Edinburgh. In one group, male Cyp1a1-Ren2 rats (n = 6) were fed normal rat food containing the aryl hydrocarbon, I3C (0.3% wt/wt; diet TD 00554, Harlan-Teklad, Madison, WI) for 7–9 days to induce malignant hypertension. In a second group (n = 5), age-matched male Cyp1a1-Ren2 rats fed normal rat food (diet TD 90229, Harlan-Teklad), which did not contain I3C, served as normotensive controls. Renal clearance experiments were performed on all rats. For the clearance experiments, the rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed on a thermostatically controlled surgical table to maintain body temperature at 37°C. A tracheostomy was performed, and the animals were allowed to breathe air-enriched oxygen, which has been shown to improve the stability of arterial blood pressure of pentobarbital sodium-anesthetized rats (17–19). The left femoral artery was cannulated to allow monitoring of arterial blood pressure. Blood pressure was monitored with a Statham pressure transducer (model P23 DC) and recorded with a computerized data acquisition system (MP100 system; BIOPAC Systems, Santa Barbara, CA) with the AcqKnowledge software package (version 3.2.4, BIOPAC Systems). The left external jugular vein was cannulated to allow intravenous infusion of solutions and additional anesthetic. The rats were infused intravenously, at a rate of 1.2 ml/h, with isotonic saline containing 6% albumin (bovine; Calbiochem, San Diego, CA) during the surgery and thereafter with isotonic saline containing 1% albumin, 7.5% polyfructosan (Inutest, Lentz, Austria), and 1.5% para-aminohippuric acid (PAH; Merck, Whitehouse Station, NJ). A suprapubic incision was made, and the bladder was exposed by blunt dissection through the abdominal wall. The bladder was catheterized to allow timed urine collections to be made. The rats were allowed to stabilize for 1 h after completion of the surgery.

The experimental protocol consisted of two 30-min control urine collections, after which the superoxide dismutase mimetic tempol (100 μmol/h; Sigma, St. Louis, MO) was infused intravenously. After a 30-min stabilization period, two additional 30-min urine collections were obtained. Subsequently, the rats were infused intravenously with tempol (100 μmol/h) together with the nitric oxide synthase inhibitor nitro-L-arginine (NLA; 20 μg · 100 g⁻¹ · min⁻¹; Sigma); after a 30-min equilibration period, an additional 30-min urine collection was obtained. At all times, the rate of intravenous fluid administration was constant at 1.2 ml/h. Arterial blood samples (~300 μl) were obtained at the midpoint of control, tempol, and tempol plus NLA infusion periods to allow determination of whole kidney hemodynamics. At the end of each experiment, both kidneys were removed, decapsulated, blotted dry, and weighed.

Experiments were performed to determine the rates of 8-isoprostane excretion in Cyp1a1-Ren2 rats before and after intravenous administration of the superoxide mimetic tempol. The experimental protocol used was similar to that described above except that a single 30-min urine collection was obtained from anesthetized Cyp1a1-Ren2 rats with malignant hypertension (n = 7) and in noninduced normotensive Cyp1a1-Ren2 rats (n = 8). In the rats with malignant hypertension, an additional 30-min urine collection was also obtained during intravenous administration of tempol (100 μmol/h) to determine whether acute administration of a superoxide dismutase mimetic could attenuate the elevated urinary 8-isoprostane excretion in Cyp1a1-Ren2 rats with malignant hypertension. Experiments were also performed to determine the rates of 8-isoprostane excretion in hypertensive Cyp1a1-Ren2 rats (n = 6) before and after intravenous administration of the AT1-receptor blocker candesartan (0.1 mg/kg; AstraZeneca, Molndal, Sweden). The experimental procedure was similar to that described above. After a 1-h postsurgical stabilization period, two 30-min urine collections were obtained under control conditions and then after intravenous administration of candesartan. The magnitude of the pressor response to intravenous bolus administration of 50 ng of ANG II (Sigma) was measured before and after administration of candesartan to determine the effectiveness of blockade of AT1 receptor by candesartan. Experiments were also performed to determine the basal urinary excretion rate of nitrate/nitrite (NOx) in both induced hypertensive rats as well as noninduced normotensive rats. In these experiments, urinary NOx excretion was determined from a single 30-min urine collection from pentobarbital sodium-anesthetized induced (n = 9) and noninduced (n = 8) Cyp1a1-Ren2 rats.

Urine volume was determined gravimetrically. Inulin and PAH concentrations in both urine and plasma were measured by standard spectrophotometry. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were estimated from the clearance of inulin and PAH, respectively. Renal blood flow was calculated as RPF/(1 – hematocrit). Renal vascular resistance (RVR) was determined from the quotient of mean arterial pressure (MAP) and calculated renal blood flow. Urinary excretion rates of 8-isoprostane and NOx were determined with the use of enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI).

Statistical analyses were performed using one-way ANOVA and one-way repeated-measures ANOVA followed by Tukey’s test when appropriate. Statistical significance was defined as P < 0.05. All data are expressed as means ± SE.

RESULTS

Chronic administration of 0.3% I3C in Cyp1a1-Ren2 rats (n = 6) for 7–9 days resulted in the development of severe hypertension (184 ± 4 vs. 127 ± 3 mmHg, P < 0.01; Fig. 1). The hypertension was associated with a reduction of body weight from 254 ± 5 to 213 ± 6 g (P < 0.01). In addition, rats...
induced with I3C had elevated hematocrits compared with noninduced rats (55 vs. 48%, $P < 0.05$). The rats also demonstrated polyuria, severe lethargy, piloerection, and adoption of hunched posture, which are manifestations of malignant hypertension in the rat (10, 34, 35). Thus administration of 0.3% I3C induced malignant hypertension in the Cyp1a1-Ren2 transgenic rats. Despite the markedly elevated MAP, the basal values for GFR and RPF averaged 1.16 ± 0.1 and 2.95 ± 0.18 ml·min⁻¹·g⁻¹, respectively, which were similar to values observed in noninduced normotensive rats (see Figs. 3 and 4). This observation that both GFR and RPF were normal even in the face of a markedly elevated blood pressure indicates that there is a marked increase in RVR in Cyp1a1-Ren2 rats with malignant hypertension. Indeed, as shown in Fig. 2, the basal value for RVR in Cyp1a1-Ren2 rats with malignant hypertension averaged 29 ± 2 mmHg·ml⁻¹·min⁻¹·g⁻¹, a value substantially higher ($P < 0.01$) than that observed in noninduced normotensive rats.

As shown in Figs. 1 and 2, systemic administration of the superoxide dismutase mimetic tempol decreased MAP and RVR in rats with malignant hypertension but had no effect on noninduced normotensive rats. Tempol decreased MAP (184 ± 4 to 151 ± 4 mmHg, $P < 0.01$) and RVR (29 ± 2 to 25 ± 2 mmHg·ml⁻¹·min⁻¹·g⁻¹, $P < 0.05$) in hypertensive Cyp1a1-Ren2 rats. As shown in Figs. 3 and 4, intravenous infusion of tempol did not alter GFR or RPF in either the normotensive or hypertensive rats. Subsequent administration of the nitric oxide synthase inhibitor NLA (20 μg·100 g⁻¹·min⁻¹) attenuated the tempol-induced decrease in MAP and RVR in the hypertensive rats (Figs. 1 and 2). Indeed, coadministration of NLA together with tempol increased MAP and RVR (from 151 ± 4 to 165 ± 4 mmHg and from 25 ± 2 to 38 ± 4 mmHg·ml⁻¹·min⁻¹·g⁻¹, respectively, $P < 0.05$ in both cases) in the hypertensive Cyp1a1-Ren2 rats. Similarly, coadministration of NLA and tempol also increased MAP (from 124 ± 2 to 156 ± 1 mmHg, $P < 0.05$) and RVR (from 19 ± 0.6 to 26 ± 2 mmHg·ml⁻¹·min⁻¹·g⁻¹, $P < 0.05$) in the noninduced normotensive Cyp1a1-Ren2 transgenic rats (Figs. 1 and 2).

As shown in Figs. 3 and 4, administration of tempol and NLA together elicited decreases in GFR (to 0.9 ± 0.06 ml·min⁻¹·g⁻¹, $P < 0.05$) and RPF (to 2.3 ± 0.3 ml·min⁻¹·g⁻¹, $P < 0.05$) in hypertensive Cyp1a1-Ren2 rats but not in noninduced normotensive rats.

As shown in Fig. 5, Cyp1a1-Ren2 transgenic rats with malignant hypertension exhibited significantly higher basal rates of urinary 8-isoprostane excretion compared with noninduced normotensive rats (41 ± 4 vs. 13 ± 6 pg·min⁻¹·g⁻¹, $P < 0.01$). Intravenous administration of the superoxide dismutase mimetic tempol decreased urinary excretion of 8-isoprostane from 41 ± 4 to 25 ± 4 pg·min⁻¹·g⁻¹ ($P < 0.05$) in the hypertensive Cyp1a1-Ren2 rats. The effect of intravenous administration of candesartan (0.1 mg/kg) on urinary 8-isoprostane excretion is shown in Fig. 6. Administration of candesartan caused a marked reduction in 8-isoprostane excretion in hypertensive Cyp1a1-Ren2 rats (42 ± 10 to 7 ± 2 pg·min⁻¹·g⁻¹, $P < 0.05$). In addition, the magnitude of the pressor response to intravenous injection of 50 ng of ANG II

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**Fig. 1.** Effects of tempol (100 μmol/h) and nitro-L-arginine (NLA; 20 μg·100 g⁻¹·min⁻¹) on mean arterial pressure (MAP) of normotensive and hypertensive Cyp1a1-Ren2 rats. *$P < 0.05$ vs. control; #P < 0.05 vs. tempol.

**Fig. 2.** Effects of tempol (100 μmol/h) and NLA (20 μg·100 g⁻¹·min⁻¹) on renal vascular resistance (RVR) of normotensive and hypertensive Cyp1a1-Ren2 rats. *$P < 0.05$ vs. control; #P < 0.05 vs. tempol.

**Fig. 3.** Effects of tempol (100 μmol/h) and NLA (20 μg·100 g⁻¹·min⁻¹) on glomerular filtration rate (GFR) of normotensive and hypertensive Cyp1a1-Ren2 rats. *$P < 0.05$ vs. control; #P < 0.05 vs. tempol.
was markedly reduced from 45 ± 3 to 7 ± 1 mmHg (P < 0.01) 10 min after administration of candesartan. The pressor response to ANG II remained similarly attenuated at the end of the experiment, some 90 min after candesartan administration, and averaged 7 ± 1 mmHg (P < 0.01). These findings indicate that the dose of candesartan used elicited substantial and sustained blockade of AT1 receptors. In addition, Cyp1a1-Ren2 transgenic rats with malignant hypertension (n = 9) exhibited significantly higher basal rates of urinary NOx excretion compared with noninduced normotensive rats (n = 8) (0.130 ± 0.03 vs. 0.013 ± 0.002 μmol·min⁻¹·g⁻¹, P < 0.001).

**DISCUSSION**

The present study examined the effects of superoxide anion on blood pressure and renal function in Cyp1a1-Ren2 transgenic rats with ANG II-dependent malignant hypertension. Malignant hypertension is a form of severe hypertension characterized by fibrinoid necrosis of arterioles and vascular damage in many tissues, including the kidney (10, 34, 35). The Cyp1a1-Ren2 transgenic rat line allows the induction of ANG II-dependent malignant hypertension (9). This transgenic rat line was generated by inserting a mouse Ren2 renin gene into the genome of the Fischer 344 rat (9). Extrarenal Ren2 renin gene expression is induced by the administration of the aryl hydrocarbon I3C, resulting in the development of ANG II-dependent malignant hypertension (9). Such induction of Ren2 renin gene expression using a benign and naturally occurring dietary supplement leads to the development of ANG II-dependent hypertension as a result of increased renin gene expression and plasma renin levels, which are not subject to normal physiological feedback control mechanisms. In the present study, induction of the Ren2 renin gene by dietary administration of 0.3% I3C for 7–9 days resulted in the development of severe hypertension. The hypertension was associated with a marked decrease in body weight, which was likely due, at least in part, to extracellular fluid volume depletion, as evidenced by the increase in hematocrit. Despite the markedly increased MAP, hypertensive Cyp1a1-Ren2 transgenic rats exhibited normal values of GFR and RPF. This observation that both GFR and RPF were maintained in the normal range indicates that preglomerular vascular resistance is markedly elevated in Cyp1a1-Ren2 transgenic rats with malignant hypertension. The present data do not, however, allow determination of the relative contribution of the direct preglomerular vasoconstrictor actions of ANG II and the autoregulatory response to the increase in arterial blood pressure, which allows maintenance of normal renal hemodynamic function in these rats. Regardless of the mechanism, it is clear that Cyp1a1-Ren2 rats with malignant hypertension exhibit markedly increased RVR and that the ability of the preglomerular vasculature to prevent the transmission of the systemic hypertension to the glomerular capillaries is intact at this stage of the pathogenesis of malignant hypertension.

In the kidney, the vascular lesions of malignant hypertension generally consist of myointimal proliferation and fibrinoid necrosis (10, 34, 35). In this regard, it has been shown that Cyp1a1-Ren2 rats induced with 0.3% I3C for 14 days exhibited malignant vascular injury with fibrinoid necrosis and endarteritis obliterans of interlobular arterioles and afferent arterioles.
arterioles (9). In contrast, no afferent fibrinoid necrosis or endarteritis was observed in kidneys of Cyp1a1-Ren2 rats induced with 0.3% I3C for 7 days (9). However, medial thickening of the vessel walls of interlobular and arcuate arteries was found by day 7 of induction (9). Consistent with these findings, our group (5) recently observed that Cyp1a1-Ren2 rats induced with 0.3% I3C for 7–9 days did not exhibit fibrinoid necrosis and endarteritis of the renal vasculature. Rather, the renal pathological changes observed included myointimal hyperplasia and tubular dilatation, glomerulosclerosis, and tubulointerstitial inflammation and proliferation, particularly in the perivascular areas. These recent findings demonstrate that the renal pathological changes that occur 7–9 days after induction of malignant hypertension in Cyp1a1-Ren2 rats primarily consists of inflammation and cellular proliferation in cortical vessels and tubulointerstitium (5). Such morphological changes together with preglomerular vasoconstriction may act to protect against transmission of the elevated arterial pressure to the glomeruli, thereby contributing to the maintenance of relatively normal values for GFR and RPF observed at this stage of the hypertension. Presumably, continued exposure to the markedly elevated arterial pressure would result in the progression of more severe hypertensive vascular damage characteristic of malignant hypertension. One would predict that this would result in marked decline in renal hemodynamic function, although further studies are required to address this issue.

Previous investigations have shown elevated superoxide anion levels in various models of hypertension (13, 14, 30, 31). ANG II stimulates superoxide anion products via activation of the NAD/NADPH oxidase system (6), and ANG II-mediated elevation of superoxide levels contributes to the pathogenesis of various forms of ANG II-dependent hypertension (11, 21). However, less is known about the effects of superoxide anion on blood pressure and renal function in ANG II-dependent malignant hypertension. 8-Isoprostane is generated by free radical-induced peroxidation of arachidonic acid, and elevated urinary excretion of 8-isoprostane indicates an increase in oxidative stress (20, 23, 31). In the present study, Cyp1a1-Ren2 transgenic rats with malignant hypertension had markedly increased basal rates of urinary excretion of 8-isoprostane compared with the normotensive rats, indicating oxidative stress in this model of ANG II-dependent malignant hypertension. The administration of the AT1-receptor blocker candesartan significantly decreased the urinary excretion of 8-isoprostane, indicating that ANG II stimulates the production of superoxide anion in Cyp1a1-Ren2 rats with malignant hypertension. To further demonstrate the role of oxidative stress in this model of malignant hypertension, the effects of the superoxide dismutase mimetic tempol were compared in normotensive and hypertensive rats. Systemic administration of tempol decreased MAP and RVR in rats with malignant hypertension but had no effect on normotensive rats. This indicates that superoxide anion contributes to the elevated RVR and increased arterial blood pressure in Cyp1a1-Ren2 transgenic rats with malignant hypertension. These results are consistent with previous studies that have shown tempol to have an antihypertensive and renal vasodilatory effect in various hypertensive states (29–32). This observation indicates that anesthetized normotensive rats surgically prepared for renal clearance measurements do not exhibit oxidative stress. With regard to the effects of tempol on arterial pressure and RVR, it should be recognized that tempol has been shown to inhibit sympathetic tone (37). Therefore, it is possible that the reductions in MAP and RVR could have occurred, at least in part, as a result of a tempol-mediated reduction in sympathetic tone.

Increased levels of superoxide anion can reduce the bioactivity of nitric oxide (7, 29, 36), which may contribute to elevated arterial pressures and RVR, leading to the development of malignant hypertension. In the present study, blockade of nitric oxide synthase with NLA attenuated the decrease in MAP induced by tempol in hypertensive rats. This indicates that the elevated arterial blood pressure in Cyp1a1-Ren2 transgenic rats with malignant hypertension is due in part to a superoxide anion-mediated reduction in nitric oxide bioavailability. In addition, NLA administration elicited pronounced decreases in GFR and RPF in the tempol-treated hypertensive rats but not in the normotensive rats. Although these NLA-mediated decreases in renal hemodynamics may have reflected the very high RVR in the hypertensive rats, our observation that urinary NOx excretion was elevated in hypertensive rats indicates that endogenous nitric oxide activity is increased in these hypertensive rats. It is worth emphasizing, however, that single spot measurements of urinary NOx excretion should be interpreted with caution because they reflect total body nitric oxide production and do not provide a specific measure of renal nitric oxide production. In addition, because NOx is reabsorbed by the proximal tubule, it is possible that an impairment of proximal tubular function in Cyp1a1-Ren2 rats with malignant hypertension may also have contributed to the elevated urinary NOx excretion observed in the present study. Nevertheless, the finding that urinary NOx excretion was increased in the hypertensive rats, together with the observation that nitric oxide synthase blockade with NLA elicited pronounced decreases in RPF and GFR, is indicative of increased or maintained nitric oxide activity in Cyp1a1-Ren2 rats with malignant hypertension. Thus our findings do not indicate that the superoxide anion-mediated decrease in nitric oxide bioavailability contributes importantly to the increased RVR in this form of ANG II-dependent malignant hypertension. Rather, the results of the present study indicate that the renoprotective influence of nitric oxide on renal hemodynamics is enhanced or at least maintained in these hypertensive rats.

In summary, the present findings demonstrate that Cyp1a1-Ren2 transgenic rats with malignant hypertension have increased oxidative stress and that elevated superoxide anion levels contribute to the increased RVR and arterial blood pressure. The elevated arterial blood pressure in Cyp1a1-Ren2 transgenic rats with malignant hypertension is produced at least in part by a decrease in nitric oxide bioavailability by superoxide anion. Furthermore, nitric oxide exerts a pronounced renal vasodilator influence in Cyp1a1-Ren2 transgenic rats with malignant hypertension. Such maintained renoprotective effects of nitric oxide would act to prevent excessive renal vasoconstriction and thus contribute to the maintenance of renal hemodynamics after induction of malignant hypertension in Cyp1a1-Ren2 transgenic rats.

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