Effects of the Maternal Hypertension in Renal Development in Offspring of Rats
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Abstract

Background: As nephrogenesis takes place entirely before term birth and many factors may have an impact on kidney development and reduce nephron numbers. The objective of the study was to analyze the effect of hypertension during pregnancy on glomeruli and microvasculature of the kidneys in fetal and neonates.

Methods: Total nine sub-groups allocated from 3 main groups of fetuses (20th d) and newborns (2nd and 15th d) offspring’s from normotensive mothers (C), SHR and L-NAME were performed. Glomerular area and the number of glomeruli per area were determined per animal in 25 random fields of the right kidney. Also, it has assessed the thickness of tunica media of renal microvessels.

Results: Nephrons number was lower in L-NAME (2.18 ± 0.82; 2.18 ± 0.73) group compared to C (2.51 ± 0.83; 2.71 ± 0.79) at 2nd and 15th d, respectively. Glomerular area in hypertensives (L-NAME: 1.80 ± 0.46; 1.91 ± 0.44 and SHR: 1.70 ± 0.47; 1.53 ± 0.42 at 2nd and 15th d, respectively) were smaller than C (1.83 ± 0.62 and 2.17 ± 0.61, at 2nd and 15th d, respectively). Thickening of the media of arterioles was found in hypertensive animals at 2nd and 15th d compared to C.

Conclusion: Maternal hypertension causes impaired renal development which potentially may lead to hypertension in later life.

Keywords: Hypertension; Pregnancy; Nitric oxide; Kidney; Nephrogenesis; Rats

Introduction

Reports derived from animal studied indicate that changes in fetal environment may affect renal development. Maternal conditions as hyperglycemia, anemia, glucocorticoids exposure and low-protein diet throughout pregnancy cause hypertension in the adult offspring rat that may be due, in part, to a deficit in neuron numbers [1-4].

In man and rodents, nephrogenesis is completed during fetal and early post-natal life, respectively [5,6]. There is evidence that fetal growth restriction is associated with impaired nephrogenesis and reduced number of mature nephrons in man and other species [7-10]. It has been proposed that such impairment of renal growth may contribute to increased blood pressure in later life [7,10].

In this study, we promoted fetal growth restriction by administering a nitric oxide synthase (NO) inhibitor, L-NAME (Nω-Nitro-L-Arginine Methyl Esther) during pregnancy of rats to study the role of nitric oxide on the kidney development. Oral L-NAME administration causes hypertension, proteinuria, trombocytopenia and renal damage in the gravid rats [11,12].

Nitric oxide is generated in the human feto-placental circulation, contributing to control of vascular tone [13,14]. Moreover, the nitric oxide was shown to be involved in post developmental vascular remodeling and angiogenesis, as well as in the formation of limbs, atrioventricular septation, lung and brain development [15-18].

The aim of the present study were, therefore, to determine: (1) the effect of maternal hypertension during pregnancy on the renal morphology in the offspring; (2) compare changes renal morphology among fetuses and newborns delivered from normotensive and hypertensive (L-NAME and Spontaneously Hypertensive Rats - SHR) mothers.

Thirty females 14-16-week-old Wistar and fifteen SHRs with a body weight of 200-250 g were mated with male rats. The Wistar dams were randomly assigned to groups control and L-NAME (Nω-Nitro-L-Arginine Methyl Ester). The L-NAME animals received the NO synthase inhibitor (hydrochloride, L-NAME, Sigma, St Louis, MO, lot 7017703) in drinking water (12 mg/day/rat), throughout the pregnancy (21 days). The rat arterial pressure was evaluated by tail cuff plethysmography at the beginning and end of gestation. The fetuses and neonates from control, L-NAME and SHR groups were separated in three age groups of five each: 20 post-conception days (pcd), 2 and 15 post-natal days (pnd). Only one animal randomly selected per litter was used. They were sacrificed under pentobarbital anesthesia. All procedures and experimental protocols were approved by the Ethical Committee of the Botucatu Medical School – UNESP, Brazil.

Whole right kidneys from all groups were fixed in 10% buffered formalin and paraffin embedded. Five-micrometer-thick sections were stained. Morphometry was performed with a Pro-Plus (Media Cybernetics, USA) computerized system on histological sections from the kidneys at three different developmental stages. Every glomerulus present in each section was counted, and the total area of each one was measured. The measurements were performed at level ×40 with a unit area of 3.35 mm2. In addition, renal microvasculature was measured in five sections of each kidney. Only renal vessels with external diameter...
less than 25 microns were studied. The following variables for each blood vessel were: total vessel area, vessel wall area, vessel lumen area, wall-to-lumen ratio (the area of the vessel wall divided by the area of the blood vessel lumen), minimum and maximal diameters, and external perimeter. In quantification, nonround vessels resulting from oblique transection or branching were excluded, and only round vessels (minimum diameter/maximum diameter > 0.60) were studied.

Comparisons of the maternal arterial pressure, fetal and neonatal length, body and renal weights and relative renal weight were performed by one-way ANOVA. Comparisons of the wall-to-lumen ratio, total vessel area, vessel wall area, vessel lumen area and, glomeruli area and a number were performed by two-way ANOVA followed by the multiple-comparison tests. A value of P<0.05 was considered significant.

Results

L-NAME and SHR pregnant rats showed higher arterial pressure (p < 0.001) when compared to normotensive dams (Figure 1). It was observed a small fall in blood pressure in the Wistar (98.8 ± 4.6) and SHR (179.0 ± 6.4) dams at the end of the gestation when compared at the beginning of the pregnancy (102.0 ± 5.2 and 179.0 ± 6.4, respectively).

As described in Table 1, fetal (20 days of gestation) and newborn renal weights of L-NAME groups were lower than controls, except for 15 days of age. Kidney weight of the SHR neonates decreased significantly compared with controls at 2 and 15 days of age (p<0.01 and p<0.05, respectively). When expressed as % body weight, the relative kidney weight was significantly greater in SHR fetuses, L-NAME and SHR newborns at 15 post-natal days. SHR and L-NAME fetuses and neonates were significantly smaller and less heavy than their counterparts, except for L-NAME at 15 days of age, which were greater than controls.

The area of glomeruli was different among the groups for all stages of development (Table 2). The kidneys of L-NAME and SHR fetuses presented more quantity of connective tissue in between of renal cortex and medulla, indicating retardation on kidney development.

Nephron number was determined at day 20 gestation, 2 and 15 days of age. Exposure to L-NAME throughout gestation, increased nephron number at day 20, as like as SHR fetuses had a greater number of glomeruli relative to controls. In L-NAME and SHR newborns of 2 and days, the nephron number was reduced relative to controls, but this decrease was statistically different only in L-NAME animals at 2 and 15 days (Table 2).

The fetal and neonatal kidneys of L-NAME and SHR groups exhibited increased apoptosis in the glomeruli. A greater accumulation of collagen in the tubular interstitium, periadventitia of renal cortical vessels and glomeruli was observed in both the groups without a difference in frequency (Figure 2).

A significant difference was found in the wall-to-lumen ratio of arterioles among L-NAME, SHR and control newborns only at 2 days of age (Figure 3). However, there was significant thickening of the media in L-NAME and SHR groups at 2 days and in L-NAME at 15 days (Figure 4).

Discussion

It was demonstrated higher kidney-to-body weight ratio in SHR fetuses at 20 days and in L-NAME and SHR newborns at 15 days than control animals at the same ages. This increase suggests hypertrophy in kidneys, without an increase in glomeruli size and number.

The fetal kidney appears to be extremely vulnerable to the effects of growth retardation [19]. Studies of growth-retarded human infants indicate that the kidneys are disproportionately affected relative to the other organs [20,21].

This study shows that maternal hypertension during pregnancy results in reduced birth weight and a decreased area and number of glomeruli. We hypothesized that factors in the perinatal environment that suppress the nitric oxide synthesis and/or RAS (renin-angiotensin system) in the developing rat fetus/newborn lead to impaired renal development and fewer glomeruli in the offspring, in turn leading to adult hypertension. This could provide a link between maternal environmental factors, particularly nitric oxide inhibition, and the development of hypertension in adulthood [22-25].

The results of the present study may have some important implications for the origin of human hypertension. Some evidences
have demonstrated convincingly an inverse relationship between early growth patterns and risk for adult disease, particularly cardiovascular disease and hypertension [26,27]. This indicates that factors in the maternal environment during pregnancy and development may increase the cardiovascular risk of offspring. Several animal models investigating this phenomenon are currently being studied, including maternal dietary protein or global food restriction, impairment of the uterine or placental circulation, perinatal blockade of the Renin-Angiotensin System (RAS), and increased exposure to maternal glucocorticoids, all of them leading to hypertension in the offspring [1,28,29]. Results from our study suggest that factors that determine an individual's size at birth, particularly maternal environmental factors, may also worsen the prognosis of the hypertension and related cardiovascular disease of offspring, at least in part, through determination of the number of nephrons in which the individual is endowed. Furthermore, the reduced nephrons number and size may also predispose the individual to the development of progressive renal disease.

### Table 2: Number and area of glomeruli present in the kidney of fetuses and newborns at 2 and 15 days of the control, L-NAME and SHR groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Nº of Glomeruli/3.35 mm²</th>
<th>Area of Glomeruli, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.55 ± 0.71*</td>
<td>2.09 ± 0.70</td>
</tr>
<tr>
<td>L-NAME</td>
<td>1.88 ± 0.91*</td>
<td>2.48 ± 0.60</td>
</tr>
<tr>
<td>SHR</td>
<td>1.78 ± 0.80*</td>
<td>2.49 ± 0.59</td>
</tr>
<tr>
<td>2-day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.51 ± 0.83</td>
<td>2.83 ± 0.62</td>
</tr>
<tr>
<td>L-NAME</td>
<td>2.28 ± 0.82*</td>
<td>2.88 ± 0.64*</td>
</tr>
<tr>
<td>SHR</td>
<td>2.34 ± 0.81#</td>
<td>2.70 ± 0.47*</td>
</tr>
<tr>
<td>15-day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.71 ± 0.79</td>
<td>2.17 ± 0.61</td>
</tr>
<tr>
<td>L-NAME</td>
<td>2.18 ± 0.73#</td>
<td>1.91 ± 0.44</td>
</tr>
<tr>
<td>SHR</td>
<td>2.53 ± 0.80</td>
<td>1.53 ± 0.42#</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 5 animals per group.

Mean values of the number of glomeruli were significantly different. *P<0.05 vs. C2, #P<0.05 vs. C15

Mean values of the area of glomeruli were significantly different. *P<0.05 vs. C2, #P<0.05 vs. C15

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Figure 2: Representative photomicrographs of the renal cortex from animals from the group control, L-NAME and SHR at 15 days of age. Tissues shown in A, B, C, D, E and F were stained with picrosirius red. There was greater collagen accumulation in the glomeruli of L-NAME (B) and SHR (C) at 15 days of age than control group (A). Excess collagen (arrow) can be seen in the tubular interstitium of SHR neonates (C) and the periadventitia of cortical vessels in L-NAME (D) and SHR (E) animals. Magnification, ×400.

Figure 3: Bar graph of the wall-to-lumen ratio of renal arteries with external diameters < 25 µm. *P<0.05 vs. C2, #P<0.05 vs. by two-way ANOVA followed by multiple-comparison tests. C2, L2 and S2: control (C), L-NAME (L) and SHR (S) newborns at 2 post-natal days. C15, L15 and S15: control (C), L-NAME (L) and SHR (S) newborns at 15 post-natal days. Values are means ± SEM; n = 5 animals per group.

Figure 4: Bar graph of the wall area of renal arteries with external diameters < 25 µm. *P<0.05 vs. C2. #P<0.05 vs. C15 by two-way ANOVA followed by multiple-comparison tests. C2, L2 and S2: control (C), L-NAME (L) and SHR (S) newborns at 2 post-natal days. C15, L15 and S15: control (C), L-NAME (L) and SHR (S) newborns at 15 post-natal days.
Kidneys with lower nephron numbers maintain their haemodynamic and excretory functions through an increase in local vascular resistance and glomerular pressure. Increased glomerular pressure within nephrons may trigger the cascade lead to a progressive deterioration and loss of nephrons [30].

Nitric Oxide (NO) is produced within the kidney and plays an important role in the control of many intrarenal processes that regulate the renal response to changes in perfusion pressure and, thus, help to determine systemic vascular volume and blood pressure [31]. Studies have shown that certain animal models of genetic hypertension and forms of human hypertension are associated with a decrease in NO synthesis [32,33].

Our results demonstrated that chronic inhibition NO synthesis with L-NAME during pregnancy was associated with neonatal structural changes of renal microvessels (thickening of the media). The pathogenesis of microvascular remodeling in our model involves at least two possibilities: (1) adaptive responses to maternal arterial hypertension and (2) increased production of mitogen- or growth-promoting factors due to decreased NO synthesis.

NO may inhibit vascular smooth muscle proliferation in vivo and in vitro [34]. Inhibition of NO synthesis upregulates the synthesis of peptide growth factors, such as, platelet-derived growth factor [35]. It is likely, therefore, that the effects of L-NAME on microvascular remodeling were due to its inhibition of the antiproliferative action of NO. Chronic administration of L-NAME might increase sympathetic nerve activity, which may contribute to vascular remodeling [36]. Sakuma et al. showed that renal sympathetic nerve activity increased after administration of L-NAME [37]. Renal vascular remodeling due to inhibition of NO synthesis also may occur by activation of local/systemic RAS [36].

Reductions in NO synthesis reduce renal sodium excretory function, not only through direct action on the renal vasculature, but through modulation of other vasoconstrictor processes and through direct and indirect alterations in tubular sodium transport [38]. Spontaneously Hypertensive Rats (SHR) at 2 days of age also shown an increase in the wall area and in the relation media/lumen because of medial hypertrophy/hyperplasia. Hypertrophy and polyplody are found preferentially in conduit arterioles, whereas hyperplasia and remodeling are found mainly in small arteries and arterioles [39].

In the present study, we demonstrated an increase of thickening media layer in L-NAME and SHR at 2 and 15 days. However, the wall-to-lumen ratio increased significantly in the L-NAME and SHR groups only at 2 days. Probably, the similarity in wall-to-lumen ratio of renal arterioles among groups at 15 days may be temporary, which not occurs in the heart arterioles in this age.

Pups SHR have significantly higher concentrations of renin than Wistar-Kyoto pups from birth until the beginning of the third postnatal week, as well as increased expression of angiotensinogen mRNA [40,41]. The consequence of this up-regulation of renal RAS activity in the SHR pup may be gross changes in renal haemodynamics. The elevated renal renin concentration of the SHR is linked to increased renal vascular resistance and thus to a reduced renal blood flow and glomerular filtration rate [42]. Also, it appears that sustained activity of the renin-angiotensin system may be required for exaggerated vascular growth responses in SHR [43].

Intrauterine growth restriction by nitric oxide inhibition during pregnancy is associated with a decrease in the number and size glomeruli and microvascular remodeling. This study demonstrated that the nitric oxide inhibition during pregnancy promoted structural changes in the kidneys of offspring. However, further studies are needed to know whether these structural changes in the pups of hypertensive mothers may lead to hypertension in adulthood these individuals.

References


