Indian Journal of Experimental Biology
Vol. 58, January 2020, pp. 39-44

Renovascular hypertension in rats: Temporal antioxidant adaptation

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Received 31 August 2018; *revised 22 April 2019*

Renovascular hypertension is a form of secondary hypertension, and reactive oxygen species play an important role in the pathophysiology of hypertension. Here, we tried to evaluate temporal changes in lipid peroxidation and antioxidant enzymes activity, in the course of renovascular hypertension. To induce renovascular hypertension, adult male Wistar rats were submitted to Goldblatt 2K1C surgery or sham-operated (sham). The blood pressure was directly assessed after 7, 14, 21 and 28 days. Lipid peroxidation, superoxide dismutase, catalase, glutathione peroxidase (GPx), and glutathione-S-transferase activities were evaluated in heart and kidneys. Mean blood pressure (mmHg) was higher by 20, 14, 23 and 22% (P <0.05), respectively in hypertensive groups 7, 14, 21 and 28 days, than in the control groups (108±7 in 7 days, 101±4 in 14 days, 109±7 in 21 days and 104±7 in 28 days in sham group; and 130±12 in 7 days, 116±4 in 14 days, 135±16 in 21 days and 127±6 in 28 days in hypertensive group). Lipid peroxidation, superoxide dismutase, catalase and glutathione S-transferase showed no significant changes. The GPx activity (nmoles.min^{-1}/mg protein) was 47% higher in the hearts (90.12±17.63 in sham group and 132.53±12.43 in hypertensive group), 98% in right kidney (66.13±15.10 in sham group and 131.23±28.32 in hypertensive group), 98% in left kidney of the hypertensive group 7 days in relation to sham group 7 days (67.05±17.87 in sham group and 132.87±35.31 in hypertensive group, P <0.05). The main adaptive change promoted by hypertension includes an induction of GPx during hypertensive status development.

Key words: Blood pressure, Goldblatt hypertension, Free radicals, Oxidative stress, ROS

Hypertension is a common chronic clinical condition[1], and from 1999 to 2016, the estimated absolute burden of hypertension among US adults increased[2]. Patients with hypertension generally have no clear etiology and are classified as having primary hypertension. However, some of them may present secondary hypertension, which may be caused by renal parenchymal disease, hypothyroidism, coarctation of the aorta, renal failure, and atherosclerotic renal artery stenosis[3].

Human renovascular hypertension, a form of secondary hypertension, accounts for 1-2% of all cases of hypertension in the general population and 5.8% of secondary hypertension[4]. Furthermore, although reduction of blood flow to the kidneys represent less than 10% of the cases of clinical hypertension, target-organ injury develops in a pattern similar to primary hypertension, and models of secondary hypertension might be suitable for assessing target-organ injury[5].

To study renovascular hypertension, the experimental Goldblatt model through partial constriction of the renal artery (two-kidney/one clip-2K1C) is used which increases blood pressure levels moderately. In this model, hypertension is developed in one week, and blood pressure is persistently elevated by several weeks[6]. Interestingly, some proteins are transiently increased, like phospho-extracellular signal-regulated kinase, cell cycle inhibitors p21 and p27, and transforming growth factor-β, which return to baseline levels after five weeks[7]. In this context, it is possible that many factors present the same transient profile, such as oxidative stress markers.

It has been indicated that reactive oxygen species (ROS) play an important role in the pathophysiology of hypertension, deriving from metabolic processes, which include mitochondrial enzymes, uncoupled nitric oxide (NO) synthase (NOS)

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and NADPH oxidase activity in the vascular endothelium[8]. In this sense, redox reactions can modulate vascular tone and structure, since NO could react with superoxide anion ($O_2^{\cdot-}$) to form peroxynitrite, which itself may cause vasoconstriction and increase total peripheral resistance[9]. Such vascular alteration can predispose the establishment of hypertension, which is associated with increased production of superoxide[10] and hydrogen peroxide[11], while nitric oxide synthesis is decreased[12]. On the order hand, the antioxidant defense system plays an important role in maintaining blood pressure. As highlight antioxidant, the glutathione metabolism seems to be relevant and transiently changed on hypertension, since newly diagnosed hypertensive patients show overexpression of glutathione peroxidase (GPx), as a counter regulatory response to the vascular stress[13], but in elderly patients, who often experience hypertension for longer, no difference in GPx was observed in relation to control group[14].

Moderate oxidative stress may lead to adaptive responses of antioxidant defenses, in order to establish appropriate redox environment in an injury situation[15], but there is a lack of information whether a sustained increase in afterload imposed by hypertension could lead to renal and cardiac adaptations of antioxidant system. According to our knowledge, this is the first report in literature showing a profile of adaptation in antioxidant enzymes, in the course of 2K1C hypertension. Therefore, in this study, we evaluated the temporal changes in lipid peroxidation and antioxidants enzymes activity, in the course of renovascular hypertension.

Materials and Methods

Animals and surgical procedures

All experimental procedures described in this study were conducted according to Brazilian National Council for the Control of Animal Experimentation (CONCEA) and were approved by the Universidade Federal do Rio Grande do Sul (No. 4382000).

Healthy adult male Wistar rats (250-320 g) were purchased from the Central Animal House of the Universidade Federal do Rio Grande do Sul (Porto Alegre, Brazil). Animals were maintained under standard conditions, housed in plastic cages (five animals each) and having free access to water and pelleted food. They were maintained under standard laboratory conditions (controlled temperature of 21ºC, 12 h light/dark cycle).

To induce renovascular hypertension, the classical technique of Goldblatt was followed. Rats were anesthetized with ketamine hydrochloride (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.). Renovascular hypertension (2K1C) was induced by a silver clip with a 300 μm gap width placed around the left renal artery. Sham-operated rats (sham) underwent the same surgical intervention, without clipping[16].

Eighty animals were included in the study, and according to the surgical treatment, animals were previously randomly divided in eight experimental groups: 7, 14, 21 and 28 days after the surgery: 1) sham 7 days (n=10); 2) hypertensive 7 days (n=10); 3) sham 14 days (n=10); 4) hypertensive 14 days (n=10); 5) sham 21 days (n=10); 6) hypertensive 21 days (n=10); 7) sham 28 days (n=10); 8) hypertensive 28 days (n=10). However, the mortality rate, consequent from procedures of renovascular hypertension induction and arterial catheterization, was 25%. Therefore, the remaining sixty animals were as follows: 1) sham 7 days (n=7); 2) hypertensive 7 days (n=9); 3) sham 14 days (n=6); 4) hypertensive 14 days (n=7); 5) sham 21 days (n=6); 6) hypertensive 21 days (n=9); 7) sham 28 days (n=7); 8) hypertensive 28 days (n=9).

Hemodynamic measurements

After 7, 14, 21 and 28 days, and 24 hours before the blood pressure measurement, one catheter filled with 0.06 mL saline was implanted, under ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia, into the carotid artery (PE-50) for direct measurement of blood pressure. The arterial catheter was connected to a strain gauge transducer (P23Db, Gould-Statham, Oxford, CA, USA), and blood pressure signals were recorded during a 15 min period equipped with an analog-to-digital converter board connected to a microcomputer (CODAS, 2 kHz sampling frequency, Dataq Instruments, Inc). The recorded data were analyzed on a beat-to-beat basis to obtain averaged blood pressure and heart rate[17].

Tissue preparation

After hemodynamic measurements, animals were killed by cervical dislocation, their kidneys and hearts were excised and homogenized in 1.15% KCl at 0-4ºC, in an Ultraturrax tissue blender. The suspension was centrifuged at 1000 ×g for 10 min at 0-4ºC to remove the nuclei and cell debris[18]. The supernatant was collected to determine antioxidant enzyme activities and lipid peroxidation measurement.

Protein measurement

Protein was measured following Lowry *et al.*[19] with bovine serum albumin as standard.

Lipid peroxidation assay

Lipid peroxidation was measured trough the thiobarbituric acid reactive substances assay, according the method described by Buege & Aust[20]. Commercially available malondialdehyde was used as a standard. Results were expressed as μmoles/mg protein.

Antioxidant enzyme activities

Superoxide dismutase (SOD) activity, expressed as units/mg protein, was based on the inhibition of superoxide radical reaction with pyrogallol[21]. Catalase activity was determined by following the decrease in 240 nm absorption of hydrogen peroxide (H_2O_2). The results were expressed in nmoles of hydrogen peroxide reduced.min^{-1}/mg protein[22]. Glutathione peroxidase (GPx) activity was measured following NADPH oxidation at 340 nm. The activity of glutathione peroxidase was expressed in nmoles. min^{-1}/mg protein[23]. Glutathione- S-transferase (GST) activity was measured following the formation of dinitro-phenil-glutathione, at 340 nm. The activity of glutathione transferase was expressed in nmoles. min^{-1}/mg protein[24].

Statistical analysis

Data were analyzed using GraphPad Prism 5.0, and reported as mean ± standard error, after Kolmogorov–Smirnov test for normality. As demanded in each case, data were analyzed by one way ANOVA with Student-Newmann-Keuls post hoc test. P values of at least 0.05 were considered significant.

Results

No significant changes in heart rate values (bpm) were apparent among groups [364±52 (7 days), 341±35 (14 days), 375±51 (21 days) and 354±38 (28 days) in sham group and 340±38 (7 days), 327±23 (14 days), 373±42 (21 days) and 345±45 (28 days) in hypertensive group].

Mean blood pressure measured in hypertensive group was 20% higher than in sham group 7 days, 14% than in sham group 14 days, 23% than in sham group 21 days, and 22% higher than sham group 28 days (P <0.05) [absolute values: 108±7 (7 days), 101±4 (14 days), 109±7 (21 days) and 104±7 (28 days) in sham group and 130±12 (7 days), 116±4 (14 days), 135±16 (21 days) and 127±6 (28 days) in hypertensive group] (Fig. 1).

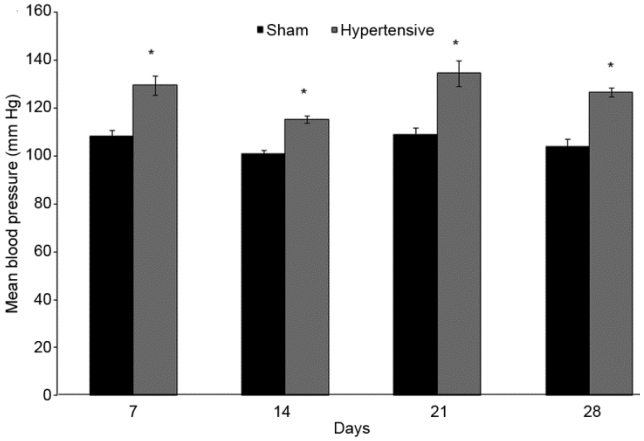

Fig. 1 — Mean blood pressure. [Data expressed as mean ± standard error of mean. * Represents significant difference compared to respective control group at the same time point (P <0.05)]

Content of TBA-RS, SOD, GST and catalase activity showed no significant changes in the homogenates of hearts, right kidneys and left kidneys among groups. Activity of GPx (nmoles/minute per miligrama of protein) was 47% higher in hearts (90.12±17.63 in sham group and 132.53±12.43 in hypertensive group), 98% in right kidney (66.13±15.10 in sham group and 131.23±28.32 in hypertensive group), 98% in left kidney of the hypertensive group 7 days in relation to sham group 7 days (67.05±17.87 in sham group and 132.87±35.31 in hypertensive group, P <0.05) (Table 1).

Discussion

This is the first study to characterize the oxidative profile of renovascular hypertension in rat's kidneys and hearts. Besides no changes in catalase, SOD and GST, GPx shows a transient elevation, suggesting an adaptation in antioxidant status.

There are a lot of models to study hypertension, such as excessive salt intake, genetic predisposition, as observed in spontaneously hypertensive rat, and drug induction. Meanwhile, renovascular hypertension model is extremely interesting, in which renin-angiotensin-aldosterone (RAS) system plays a pivotal role[6]. Angiotensin II represents the major vasoactive peptide derived by a RAS activation which might be involved in the production of mitochondrial ROS[25]. In this model, seven days after surgery resulted in increased blood pressure, and the plateau was sustained in the following weeks. Rizzi *et al.*[26] showed high pressure in 15 days, with an increasing in 30 and 75 days. Furthermore, about 20% of the

Table 1 — Oxidative stress biomarkers: lipoperoxidation and antioxidant enzymes

	Sham				Hypertensive			
	7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days
Heart								
TBARS	0.62±0.21	0.74±0.12	1.61±0.46	0.68±0.14	1.2±0.35	0.62±0.34	1.43±0.38	0.58±0.11
CAT activity	4.46±0.43	3.60±0.26	3.28±0.38	3.17±0.28	4.26±0.62	3.30±0.18	4.74±0.89	2.64±0.16
GPx activity	90.12±17.63	49.65±1.58	90.57±21.32	60.76±4.86	132.53±12.43*	53.16±2.51	87.63±24.89	61.53±9.90
GST activity	1.62±0.42	0.75±0.16	1.52±0.22	1.09±0.10	1.31±0.13	0.78±0.12	1.60±0.19	1.03±0.16
SOD activity	14.41±2.54	12.32±1.41	12.08±0.84	16.66±0.92	14.09±1.38	11.69±1.80	15.83±1.51	11.50±1.13
Right kidney								
TBARS	0.82±0.26	2.13±0.29	1.65±0.73	1.44±0.11	0.96±0.23	1.06±0.12	1.66±0.37	1.95±0.16
CAT activity	9.66±0.76	7.80±0.73	8.32±2.27	8.54±0.44	9.93±0.72	7.62±0.51	11.90±1.29	7.99±0.48
GPx activity	66.13±15.10	36.72±4.62	74.16±15.68	39.47±3.02	131.23±28.32*	44.66±6.12	73.20±18.66	42,57±3.96
GST activity	2.66±0.26	1.87±0.26	3.07±0.98	3.22±0.28	3.04±0.53	2.41±0.11	3.37±0.32	2.61±0.44
SOD activity	12.16±0.80	11.41±0.66	9.09±2.08	11.97±0.28	9.78±0.66	10.03±1.11	13.21±0.99	10.12±0.30
Left kidney								
TBARS	1.05±0.34	1.29±0.21	1.4±0.62	1.31±0.11	1.04±0.23	0.53±0.12	1.42±0.30	1.31±0.26
CAT activity	10.50±1.10	8.68±0.65	9.28±2.29	8.23±0.75	9.31±0.58	9.32±0.78	10.15±0.26	6.85±0.85
GPx activity	67.05±17.87	38.99±4.51	82.24±16.76	61.32±20.92	132.87±35.31*	36.26±2.38	60.91±7.76	33.58±4.26
GST activity	2.49±0.36	2.13±0.27	3.01±0.81	3.02±0.33	2.70±0.44	2.04±0.39	2.59±0.13	2.46±0.60
SOD activity	11.34±0.71	10.44±0.96	8.08±0.87	11.64±0.51	10.6±0.87	8.84±1.50	12.74±1.32	12.18±1.67

[TBARS: thiobarbituric acid reactive substances (μmol/mg prot); CAT: Catalase (nmol/mg prot); GPx: glutathione peroxidase (nmol/min/mg prot); GST: glutathione transferase (nmol/min/mg prot); SOD: superoxide dismutase (U SOD/mg prot). Data expressed as mean ± standard error. *$P \leq 0.05$]

animals did not develop hypertension, results that are in agreement with literature data[27]. Animals that did not develop hypertension were with normal-appearance left kidney. They did not reach high pressure values probably due to small renal artery stenosis, which was not sufficient to trigger hypertension, due to contralateral compensation. Some animals, about 5%, presented left kidney atrophied. However, these animals had hypertension, showing that blood flow to the kidney was not blocked, only decreased.

In relation to oxidative stress, there were no changes in lipid peroxidation of hypertensive animals compared to controls. Some studies showed increased ROS production in contralateral kidney[28], such as increased lipid peroxidation in plasma of Goldblatt 2K1C hypertensive rats[29] and hypertensive animal organs treated with L-NAME, an inhibitor of nitric oxide synthesis[30]. These different results could be due to the moderate blood pressure levels found in the present work. The values of blood pressure found in hypertensive animals in this study are similar to those found in previous data from our group, in which hypertensive 2K1C females did not change lipid peroxidation[17]. Similar results were also found in stress-sensitive hypertensive ISIAH rats[31]. In addition, when hypertension is induced with L-NAME, it may cause myocardial ischemic injury and especially necrotic foci, which can be related to free radicals[32]. It has been reported that Goldblatt model induces development of left ventricular hypertrophy, but do not ischemic lesions[26]. Since the lesions may be related to free radicals, and this model does not show these lesions, the results are in accordance.

In relation to antioxidant enzyme activity, there was a pronounced increase in GPx activity with seven days of hypertension. Increased activity of GPx was observed in rat hearts using the model of hypertension by inhibiting synthesis of nitric oxide[30] and in human plasma of essential hypertension[33]. Glutathione (GSH) levels are reduced after 7 days[34] and 9 days[35] of 2K1C hypertension in rats, with is in accordance to the increase in GPx activity, since this enzyme expend GSH. However, six weeks after clipping there is no difference in the erythrocyte GSH levels among control and hypertensive groups[36]. These results suggest an increased production of lipid peroxides before seven days of hypertension, the main substrate for this enzyme. This elevated enzyme activity could contribute to the maintenance of lipid peroxidation products in similar levels to control at this stage. The remarkable increase in GPx activity against a variation in blood pressure could also prepare the tissues to sustained high pressure in the subsequent phases (14, 21 and 28 days).

The enzymes SOD, catalase and GST did not change significantly at different time points. In a previous study, human serum SOD level showed no difference[37], but catalase activity was decreased in a study in a hypertension model by aortic stenosis, in

which the pressure levels were higher (162 mm Hg) than we found[38], and was also decreased in cardiac tissue after 12 weeks in rats underwent a renovascular surgery, when compared to control rats[39]. Studies showed that renovascular hypertension enhanced superoxide anion generation in the thoracic aortas[40] and in carotid arteries[41] from 2K1C rats after 8 weeks of surgery, which could increase SOD activity. It is possible that in our study the early increase of GPx was enough to protect tissues against oxidative stress.

The major limitation of our study is that we only accessed the periods of 7, 14, 21 and 28 days, and the transient changes could be seen in different time points. In addition, we carried out measurements of activity of antioxidant enzymes, not expression. Otherwise, clinical insights offered by a study performed with animals are limited.

Conclusion

The observations in this study suggest that main adaptive changes promoted by hypertension include an induction of GPx during hypertensive status development. Temporal change evaluation of oxidative stress, related to the increased blood pressure, is relevant in order to understand the best ways to preventing and treating this risk factor for cardiovascular diseases.

Acknowledgment

We are grateful to Tânia Regina Gattelli for technical assistance. This work was supported by Brazilian Federal Agency for Support and Evaluation of Postgraduate Education (CAPES) and National Council for Scientific and Technological Development (CNPq).

Conflict of interest

The authors report no conflict of interest.

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