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Urinary total antioxidant capacity after unilateral nephrectomy in spontaneously hypertensive rats

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Hypertension is the most important risk factor contributing to cardiovascular disease while oxidative stress is one of the key players in its pathogenesis. The nephrectomy is a surgical manipulation in which one kidney or a part of it is removed. Total antioxidant capacity (TAC) of body fluids has been used for evaluation of disease progression, as well as for overall defense status against oxidative stress. The aim of the current study was to investigate the urinary TAC, determined by ABTS assay, after unilateral nephrectomy (UN) in the animal model of essential hypertension - spontaneously hypertensive rats (SHR). Experiments were performed on conscious, male, normotensive Wistar rats, (n=7) and SHR (n=7) before andfor after UN. The urine samples were collected in metabolic cages for 6 hours between 8.00 – 14.00 h AM. Urine flow rate was determined gravimetrically. Creatinine and urea concentrations were determined spectrophotometrically. Urinary TAC was determined by ABTS assay and expressed as μmol Trolox equivalent per μl. The excretion of creatinine and urea did not differ between Wistar and SHR and did not change during UN. We found that TAC in SHR was lower $p<0.01$ in comparison with normotensive rats and did not change in SHR after UN, unlike Wistar in which TAC was reduced in the first three days after the removal of the kidney. We suggest that the lack of response of urinary TAC to unilateral nephrectomy in SHR could be attributed to the reduced baseline total antioxidant activity, as well as to the altered antioxidant systems characteristics in hypertensive states.

Keywords: unilateral nephrectomy, antioxidant capacity, ABTS assay, SHR

INTRODUCTION

In the living organisms there is an established balance between the reactive oxygen/nitrogen species (ROS/RNS) generated due to exogenous or endogenous sources and the existing complex antioxidant system comprising enzymatic and non-enzymatic molecules. The antioxidant system possesses the capability to neutralize the generated ROS/RNS and to maintain their concentration in optimal ranges. Under certain conditions, when the antioxidant defense system is over-limited due to systemic exceed production of free radicals an abnormal condition of oxidative stress is established [1, 2]. The observed balance or imbalance between the generated ROS/RNS in the organism and its antioxidant defense system is defined as "antioxidant status" of the organism [3]. During the years many studies have been done in order to attempt to estimate the cumulative effect of all the antioxidants present in different types of biological samples. Several authors have used total antioxidant capacity (TAC) of body fluids and tissue homogenate as a parameter in their investigations in order to seek for correlation between patient state, disease progression, outcome and the overall defense status against oxidative stress [4].

Hypertension is a multifactorial disease in which different dynamic interactions between physiological, genetic and environmental factors are involved. The pathophysiological mechanism of hypertension includes activation of the sympathetic nervous system [5], abnormal G protein-coupled receptor signaling [6], immune factors [7] and upregulation of the renin-angiotensin-aldosterone system [8]. G protein-coupled receptors (GPCRs) represent the largest family of membrane receptors and are responsible for regulating a wide variety of physiological processes. G protein-coupled receptor kinases (GRKs), in concert with β-arrestins, classically desensitize receptor signal transduction, thus preventing hyperactivation of GPCR second-messenger cascades. Changes in GRK expression have featured prominently in many cardiovascular pathologies, including heart failure, myocardial infarction, hypertension, and cardiac hypertrophy. Available experimental data indicated that immune factors, altered mainly T-cells function, have a role for development of hypertension [7]. Oxidative stress is one of the important elements, common in all of these processes, which is a result from excess of ROS generation, decreased nitric oxide levels and reduced antioxidant capacity in the brain, vessels, heart and kidneys [9, 10].

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The increased production of ROS that have an important role in the homeostasis of the vascular wall, established in hypertension, is a result of action of different mechanisms: activation of enzymes, such as NADPH oxidase, xanthine oxidase, uncoupling eNOS, mitochondrial dysfunction as well as increased mechanical forces. Available experimental data indicated that activity of NADPH oxidase is under control of hormones such as angiotensin II, endothelin-1 and urotensin. Increased vascular superoxide as a result of activity of NADPH oxidase induced vasoconstriction and increased blood pressure [11]. In addition to control of homeostasis of the vascular wall the role of ROS has been established in the control of cardiac, renal function, as well as in hypertrophy, apoptosis, angiogenesis, hypertrophy, proliferation all of which are important processes involved in the development and establishment of hypertension.

Nephrectomy is the surgical removal of a kidney. The procedure is done to treat kidney cancer, as well as other kidney diseases and injuries. Nephrectomy is also done to remove a healthy kidney from a donor for transplantation. Despite the view that nephrectomy has minimal adverse effects on the overall health status, available data indicate that it provokes a complex physiological response, associated with increased oxidative stress, activation of different types of endocrine and immunologic mediators [12]. It was also established that nephrectomy caused an increased risk of developing hypertension [13]. Having the above mentioned in mind, it is essential to pay particular attention to changes of important blood pressure regulatory factors in patients with hypertension after nephrectomy.

Determination of total antioxidant capacity in various body fluids provides an opportunity to evaluate the antioxidant defense systems in patients with hypertension, acute and chronic kidney disease, and others. In comparison with other biological fluids, urine has a variable chemical composition which reflects the varying environment in the individual organism [14]. It is considered that the urinary antioxidant capacity reflects both renal and systemic antioxidant status [15]. In this regard the determination of TAC is a challenge. Nevertheless, experimental results show that ABTS assay provides a convenient marker for the antioxidant content in urine.

The aim of the present study was to estimate the urinary TAC after unilateral nephrectomy (UN) in the widely used animal model of essential hypertension - spontaneously hypertensive rats (SHR). In this investigation we followed up the changes in urinary total antioxidant capacity in SHR before and for 7 days after unilateral nephrectomy.

MATERIAL AND METHODS

Experimental animals

Experiments were performed on conscious, male normotensive Wistar rats, (n=7) and spontaneously hypertensive rats SHR (n=7) at an age of 12-14 weeks. The animals were housed under standard conditions: constant temperature 22 °C; 12/12 h light /dark cycle; free access to standard rat chow and tap water. Experiments were carried out in two periods: control period (before unilateral nephrectomy) and in the course of after unilateral nephrectomy (UN).

Surgical manipulation

The UN was performed under general anesthesia with pentobarbital sodium (Nembutal, Sigma) in a dose of 35 mg/kg b.w., applied intraperitoneally. Access to the right kidney was achieved by abdominal incision. Close to the hilum renale, a ligature was placed covering the ureter, renal vein and artery, and then the kidney was removed. The experiments were conducted in accordance with guidelines for the care and use of laboratory animals of the ethical standards at the Medical University - Sofia based on the Convention on Animal Protection and with the approval of Bulgarian Food Safety Agency (BFSA license №019/07.03.2017).

Investigated parameters

The urine samples from the experimental animals, needed for subsequent analysis, were collected in metabolic cages (Tecniplast, Italy) every day for 6 hours between 8.00 – 14.00 h AM. Urine flow rate was determined gravimetrically. The concentrations of urea and creatinine were measured spectrophotometrically using commercial kits (Giese diagnostics) according to the manufacturer's protocol. The urea and creatinine excretions were calculated.

Urine sample total antioxidant capacity estimation:

The assay was performed according to Re *et al*., [16]. The radical cation was pre-formed in buffer water by adding 14 mM ABTS stock solution to potassium persulfate (2.45 mM final concentration). The resulting mixture was allowed to stay overnight in the dark at 4 °C. When the reaction has stopped and a primary stock solution of the radical with stable absorbance was obtained, a working solution with absorbance 0.700 ± 0.005 at 734 nm of ABTS ●+ was prepared. The absorbance values were taken exactly 60 min after mixing 1 ml of the ABTS radical working solution with the tested urine samples. Calibration curve using the reference compound Trolox was prepared and on the base of the obtained

absorbance data the results were expressed as µmol Trolox equivalent (TE)/µl of urine.

All results were presented as mean ± SEM. Student's t-test was used for comparison between two means. Differences at a probability level of $p<0.05$ were considered significant.

RESULTS

We did not find differences in urine flow rate: 3.28±0.59 and 3.05±0.36 µl.min^{-1}.100 g b.w., excretions of creatinine: 141.83±11.44 and 144.97±13.95 ng.min^{-1}.100 g b.w.; and urea: 110.23±9.22 and 107.25±10.66 ng.min^{-1}.100 g b.w., between normotensive Wistar and spontaneously hypertensive rats under control conditions (Fig.1).

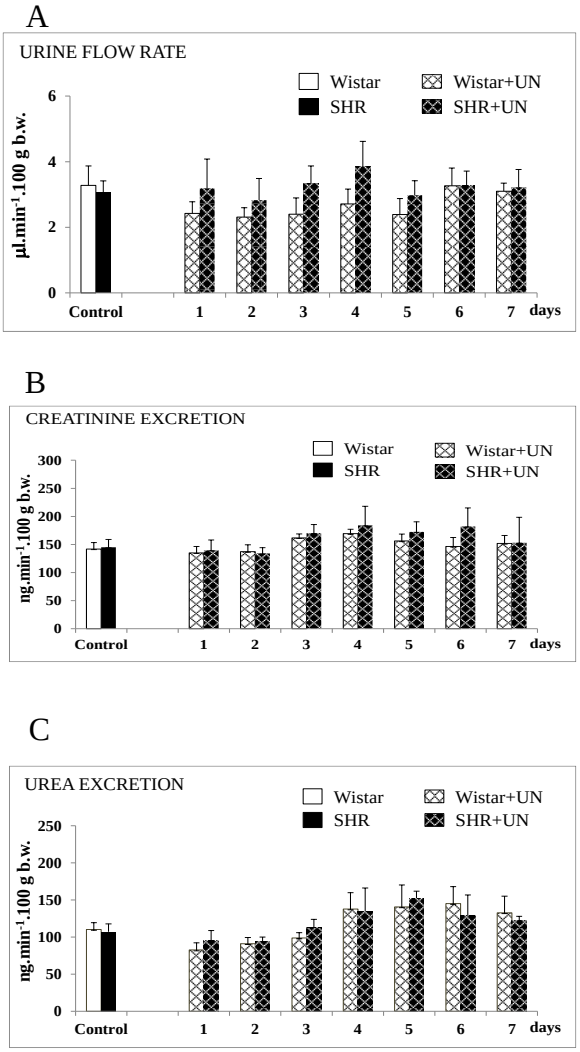

Figure 1. Urine flow rate (A), creatinine (B) and urea (C) excretion in normotensive Wistar rats (Wistar, n=7) and spontaneously hypertensive rats (SHR, n=7) in control period and for 7 days after unilateral nephrectomy (UN).

The unilateral nephrectomy did not cause changes in these parameters in either Wistar rats or SHR. On the first day after UN in Wistar rats, urine flow rate was 2.42±0.35 µl.min^{-1}.100 g b.w.; creatinine and urea excretion were 134.96±11.44 and 110.23±9.22 ng.min^{-1}.100 g b.w. respectively, and remained at this level until the end of the investigated period. The results obtained from the studied excretory parameters in SHR did not differ from those of Wistar rats and did not show statistically significant changes in the investigated period after the nephrectomy. On the first and seventh day after UN in SHR, the urine flow rate was: 3.19±0.89 and 3.22±0.54 µl.min^{-1}.100 g, creatinine and urea excretions were: 139.69±18.52 and 153.11±45.26; 95.99±12.81 and 121.79±6.32 ng.min^{-1}.100 g b.w.

The results concerning the estimation of the urinary total antioxidant capacity (TAC) before and after the unilateral nephrectomy in Wistar rats and SHR are presented on Fig. 2.

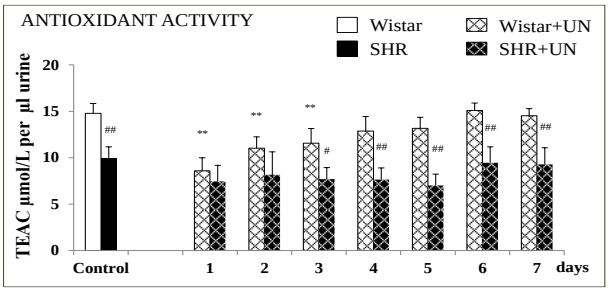

Figure 2. Total antioxidant capacity assessed using the ABTS model system in the urine of conscious, male normotensive Wistar rats, (n=7) and SHR (n=7) before (control period) and for 7 days after unilateral nephrectomy (UN). * ($p<0.05$); ** ($p<0.01$) - shows significant differences versus control; # ($p<0.05$); ## ($p<0.01$) - shows significant differences between Wistar rats and SHR.

We established differences between normotensive and spontaneously hypertensive rats in the control period of TAC, as well as alteration in dynamic changes of antioxidant potential after unilateral nephrectomy.

In the control period we observed lower TEAC in SHR: 9.90±1.27 µmol/L TE/µl, in comparison to Wistar rats: 14.77±1.07 µmol/L TE/µl, $p<0.01$.

In normotensive Wistar rats, the UN led to a statistically significant decrease of TEAC values in the first three days respectively: 8.58 ± 1.41; 11.02±1.22 and 11.56 ±1.59 µmol/L TE/µl urine, ($p<0.05$). For the rest of the tested period - day 4 do day 7 - the TEAC values were identical with the control. Differently from normotensive rats, in SHR the TEAC values were not affected by UN. The TEAC values vary from 7.45 ± 1.74 µmol/L TE/µl urine for day 1 to 9.26 ± 1.81 µmol/L TE/µl urine for day 7.

DISCUSSION

Our investigation concerns the follow-up of the effects of unilateral nephrectomy on urinary total antioxidant capacity (TAC) in normotensive and spontaneously hypertensive rats.

The first observation in our study was that in the control period, total antioxidant capacity was significantly (by more than 30%) lower in SHR, compared to Wistar rats. This fact is not surprising because several research studies demonstrated that essential hypertensive patients, as well as different animal models of hypertension produce excessive amount of ROS [17-20] and have abnormal levels of antioxidant status [17]. It has been demonstrated that increased ROS production in hypertension is accompanied by reduced nitric oxide level and antioxidants bioavailability [21]. The critical role of NO/NO-synthase (NOS) pathway in the regulation of blood pressure has been undoubtedly established [22]. Endothelial NOS catalyzes NO production from L-arginine and oxygen - a reaction that requires essential cofactor tetrahydrobiopterin (BH_4). The absence of BH_4 is associated with uncoupling of the L-arginine/NO pathway resulting in decreased formation of NO, and increased eNOS-mediated generation of superoxide. Superoxide leads to BH_4 oxidation, which provokes NO-uncoupling and increases ROS production. Its interaction with NO results in the formation of peroxynitrite [23]. In turn, peroxynitrite oxidizes and destabilizes eNOS which produces more superoxide [24, 25]. Decreased BH_4 and uncoupled NOS have been implicated in hypertension [26]. Decreased production of the fast and powerful vasodilator NO, as a result of absence of BH_4 and impaired L-arginine/NO pathway, led to endothelial dysfunction and to increase of blood pressure. It has been established that oxidative stress and hypertension in SHR were linked with the presence of functionally abnormal antioxidant enzymes [27]. On the other hand, the hypertension is associated with kidney redox imbalance resulting in enhanced reactive oxygen species (ROS) and enzymes-dependent phospholipid metabolism [28]. It is a possible that the reduced urine antioxidant capacity in SHR may also be due to established differences in their metabolic characteristics that can affect antioxidant defense system potency [29].

The decreased total antioxidant capacity in SHR, established in our study, by using ABTS assay in urine samples, confirms the abnormal level of antioxidant status found in other studies in various forms of hypertension in humans and in animal models [9, 10, 20].

In the next step of our experimental protocol we established an effect of unilateral nephrectomy on urinary total antioxidant capacity. Unilateral nephrectomy is a surgical procedure inducing renal mass reduction without direct pathological changes of the remaining kidney. We established that after UN the remained kidney in both experimental groups compensates the investigated major renal excretory functions – urine flow rate, creatinine and urea excretion. Despite this, several days are needed to restore the urinary antioxidant status in normotensive Wistar rats decreased after UN. Differently from Wistar rats, in SHR unilateral nephrectomy did not affect antioxidant capacity. The compensatory responses in the remaining kidney after unilateral nephrectomy are associated with a variety of factors such as reactive oxygen species, growth factors, and cytokines [30, 31]. The available experimental data demonstrated that after UN in mice superoxide formation in the remaining kidney significantly increased, peaking at 3 days and then gradually decreased over time [32]. Our results validate the need for 3 days for recovery of urine antioxidant capacity of normotensive Wistar rats after UN. In addition to our studies of "sham" operated normotensive rats, in which kidney was not removed, we tested the hypothesis for the possible effect of surgery on urinary antioxidant capacity. In this experiment, we detected a decrease in antioxidant capacity only on the first postoperative day. We hypothesize that the established extended period (3 days) of decrease of antioxidant capacity after nephrectomy is a result of dynamic compensation processes caused by renal removal which includes various factors responsible for maintaining homeostasis in the body. It has been found that nephrectomy changes the response of the kidney to Ang II but the mechanism of this effect has not been fully clarified [33]. The involvement of the renin angiotensin system in the compensatory responses of the kidney remaining after nephrectomy cannot be excluded, since the renin–angiotensin system (RAS) is responsible for preserving the fluid balance and vascular tone of the body. Available experimental data evidence the stimulating effect of Angiotensin II, which is an important component of the RAS, on NADPH oxidase (NOX), leading to an increase of ROS [34]. It is a possible, that the decreased in the first 3 days urinary total antioxidant capacity in Wistar rats, established in our study, may be a result of compensatory activated renin–angiotensin system, leading to an increase of ROS and decrease of TAC. It has been established that kidneys are rich sources of ROS, derived primarily from NADPH oxidase (NOX) [34].

Differently from normotensive Wistar rats, in SHR unilateral nephrectomy did not cause changes in total antioxidant capacity. There is substantial evidence suggesting that angiotensin II plays an important role in elevating blood pressure of spontaneously hypertensive rats, despite normal plasma renin activity [35]. We suggested that the lack of changes of TAC in SHR after nephrectomy, possibly mediated by RAS, is due to an inappropriately high Ang II–generating activity found in SHR [36], established up-regulated NOX, as well as increased ROS in hypertension [34, 37, 38]. These facts give us reason to believe that the reduced antioxidant capacity of SHR under control conditions is exhausted and does not have the capacity to a compensatory response to nephrectomy.

We suggest that the lack of response of urinary antioxidant activity to unilateral nephrectomy in SHR could be attributed to the baseline limited total antioxidant capacity as a result of uncoupling of the L-arginine/NO pathway, imbalance between Ang II levels and Ang II–generating activity, as well as to the altered metabolic and antioxidant systems characteristic for hypertensive states.

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