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Research Paper

Redox system and phospholipid metabolism in the kidney of hypertensive rats after FAAH inhibitor URB597 administration

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ABSTRACT

Primary and secondary hypertension is associated with kidney redox imbalance resulting in enhanced reactive oxygen species (ROS) and enzymes dependent phospholipid metabolism. The fatty acid amide hydrolase inhibitor, URB597, modulates the levels of endocannabinoids, particularly of anandamide, which is responsible for controlling blood pressure and regulating redox balance. Therefore, this study aimed to compare the effects of chronic URB597 administration to spontaneously hypertensive rats (SHR) and rats with secondary hypertension (DOCA-salt rats) on the kidney metabolism associated with the redox and endocannabinoid systems. It was shown fatty acid amide hydrolase (FAAH) inhibitor decreased the activity of ROS-generated enzymes what resulted in a reduction of ROS level. Moreover varied changes in antioxidant parameters were observed with tendency to improve antioxidant defense in SHR kidney. Moreover, URB597 administration to hypertensive rats decreased pro-inflammatory response, particularly in the kidneys of DOCA-salt hypertensive rats. URB597 had tendency to enhance ROS-dependent phospholipid oxidation, estimated by changes in neuroprostanes in the kidney of SHR and reactive aldehydes (4-hydroxynonenal and malondialdehyde) in DOCA-salt rats, in particular. The administration of FAAH inhibitor resulted in increased level of endocannabinoids in kidney of both groups of hypertensive rats led to enhanced expression of the cannabinoid receptors type 1 and 2 in SHR as well as vanilloid receptor 1 receptors in DOCA-salt rats. URB597 given to normotensive rats also affected kidney oxidative metabolism, resulting in enhanced level of neuroprostanes in Wistar Kyoto rats and reactive aldehydes in Wistar rats. Moreover, the level of endocannabinoids and cannabinoid receptors were significantly higher in both control groups of rats after URB597 administration.

In conclusion, because URB597 disturbed the kidney redox system and phospholipid ROS-dependent and enzymatic-dependent metabolism, the administration of this inhibitor may enhance kidney disorders depending on model of hypertension, but may also cause kidney disturbances in control rats. Therefore, further studies are warranted.

1. Introduction

The kidneys play a crucial role in blood pressure regulation and are therefore involved in the progression of hypertension. However, the consequences of hypertension include renal oxidative stress leading to kidney damage, while renal oxidative stress resulting from an imbalance between reactive oxygen species (ROS) generation and antioxidant defense mechanisms may also be involved in the development of hypertension [1,2]. Moreover, oxidative conditions promote inflammatory processes by activating pro-inflammatory molecules such

Abbreviations: 2-AG, 2-arachidonoylglycerol; 4-HNE, 4-hydroxynonenal; 8-isoPGF$_{2\alpha}$, F$_2$ -isoprostanes; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; AA, arachidonic acid; AEA, N-arachidonoylethanolamine, anandamide; AM3506, 5-(4-hydroxyphenyl) pentanesulfonyl fluoride; Bach1, basic leucine zipper transcription factor 1; CAT, catalase; CB$_1$, cannabinoid receptor type 1; CB$_2$, cannabinoid receptor type 2; CO, carbonyl groups; COX1, cyclooxygenase 1; COX-2, cyclooxygenase 2; cPLA2, cytosolic phospholipase A2; Cu/Zn–SOD, superoxide dismutase; DHA, docosahexaenoic acid; DOCA, deoxycorticosterone acetate; FAAH, fatty acid amide hydrolase; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; GSSG-R, glutathione reductase; HO-1, heme oxygenase 1; KAP1, KRAB-associated protein-1; Keap1, Kelch-like ECH-associated protein 1; LOX, lipoxygenase; MAGL, monoacylglycerol lipase; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; NADA, N-arachidonoyl dopamine; NOX, NADPH oxidase; NPs-A$_4$/J$_4$, A$_4$/J$_4$-neuroprostanes; Nrf2, nuclear factor erythroid 2; p21, cyclin-dependent kinase inhibitor 1; p62, nucleoporin p62; p-cJun, phosphorylated Jun proto-oncogene; ROS, reactive oxygen species; SBP, systolic blood pressure; SHR, spontaneously hypertensive rats; TNF-α, tumor necrosis factor alpha; TRPV1, vanilloid receptor 1; URB597, [3-(3-carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate; WKY, Wistar Kyoto rats; XO, xanthine oxidase

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as transcription factors (NFκB) and cytokines (TNF-α and IL-6) [3]. Oxidative stress and inflammation are strongly implicated in inducing kidney hypertrophy, end-stage renal disease, arteriosclerosis, and peripheral vascular disease, which lead to kidney failure in animal models and humans [4]. ROS, generated during hypertension, change ROS- and enzymatic-dependent phospholipid metabolism. Hypertension is frequently accompanied by hyperlipidemia, which enables ROS-mediated lipid peroxidation and the generation by oxidative fragmentation electrophilic aldehydes such as malondialdehyde (MDA) and by oxidative cyclisation prostaglandin derivatives such as 8-isoprostanes, which constrict blood vessels [5–7]. However enzymatic phospholipid metabolism leads among others to the generation of endocannabinoids that in turn are involved in the regulation of ROS and inflammatory factor levels [1,8]. The main endocannabinoids and their receptors are present within human and animal kidneys [9,10]. Anandamide and 2-arachidonoylglycerol (2-AG), ligands of G protein–coupled receptors (mainly CB1/2 and TRPV1), are synthesized on demand from phospholipid arachidonic acid [8]. Endocannabinoids system, mainly endocannabinoids and enzymes metabolizing them, likely is involved in the regulation of renal blood flow and hemodynamics and of tubular sodium and fluid reabsorption but also participates in modulation inflammation and redox balance [11]. It is known that cannabinoid receptors take part in the regulation of redox balance as follows: CB1 activation enhances oxidative stress and may promote tissue injury by enhanced inflammation, MAPK activation, and cell death, while CB2 and TRPV1 activation prevents ROS generation and may play a protective role in preventing renal injuries, possibly by inhibiting the inflammatory response and endothelial cell activation e.g. in hypertension [12]. Moreover the existence of the crosstalk between ROS and endocannabinoids has been proven in different organs [13]. The above data indicate that cooperation of the redox and endocannabinoid systems may be due to metabolic changes during the progression of hypertension.

The levels of endocannabinoids, particularly anandamide, are regulated by the fatty acid amide hydrolase (FAAH) enzyme, which is mainly responsible for anandamide degradation [14]. Therefore, FAAH inhibitors are postulated to be antihypertensive agents. The acute administration of FAAH inhibitors [3-(3-carbamoylphenyl)phenyl] N-cyclohexylcarbamate (URB597) and 5-(4-hydroxyphenyl) pentanesulfonyl fluoride (AM3506) to spontaneously hypertensive rats (SHRs) normalized the blood pressure and decreased the cardiac contractility [15]. However, the chronic administration of URB597 to rats with secondary hypertension (DOCA-salt hypertensive rats) decreased but did not normalize blood pressure and cardiac and renal hypertrophy in an age-dependent manner [16]. Moreover, the chronic administration of URB597 disturbs redox metabolism in the liver of DOCA-salt hypertensive rats [17]. However, no studies have compared the metabolic effects of chronic FAAH inhibition caused by URB597 in the kidneys of rats with two different types of hypertension.

Thus, the aim of this study was to show the effects of chronic administration of the FAAH inhibitor URB597 to rats with primary (SHRs) and secondary (DOCA-salt hypertensive rats) hypertension on changes in the redox system resulting in phospholipid metabolism in the kidney.

2. Materials and methods

2.1. Animals

The experiment was performed using rats with primary hypertension (SHRs) and rats with secondary hypertension induced by the administration of DOCA (11-deoxycorticosterone acetate) and salt. All procedures and experimental protocols were approved by the local Animal Ethics Committee in Białystok, Poland [resolution No. 4/2012 of 25.01.2012].

2.2. Spontaneously hypertensive rats

Experiments were performed on 8–10-week-old male (270–350 g) SHRs and normotensive control Wistar Kyoto (WKY) rats. The animals were housed with free access to standard pelleted rat chow and water (unless otherwise stated) and maintained under a 12-h light-dark cycle.

2.2.1. Experimental protocol

The rats were divided into following four groups of six rats each:

→ group 1A [WKY]: during the last 14 days, WKY rats were treated intraperitoneally (*i.p.*) with solvent for URB597 [1 mL] every 12 h;

→ group 2A [WKY + URB597]: during the last 14 days, WKY rats were treated *i.p.* with URB597 [1 mg/kg b.w. in 1 mL of URB597 solvent] every 12 h;

→ group 3A [SHR]: during the last 14 days, SHRs were treated *i.p.* with solvent for URB597 [1mL] every 12 h; and

→ group 4A [SHR + URB597]: during the last 14 days, SHRs were treated *i.p.* with URB597 [1 mg/kg b.w. in 1 mL of URB597 solvent] every 12 h.

Systolic blood pressure (SBP) was measured in conscious rats using the tail-cuff method before and after URB597 (or solvent) treatment. Rats with SBP values ≥ 150 mmHg were considered hypertensive. Two-week URB597 administration did not modify SBP in SHR (187 ± 15 mmHg and 191 ± 49 mmHg) and WKY (117 ± 18 mmHg and 101 ± 10 mmHg) rats before its first and the final dose, respectively. The solvent for URB597 did not modify SBP both in SHR (184 ± 34 and 205 ± 43 mmHg) and in WKY (114 ± 18 and 110 ± 13 mmHg) before the first and the final injection.

The kidney hypertrophy index values appointed after URB597 (or solvent) treatment were as follows: WKY, 3.9 ± 0.2 mg/g; WKY + URB597, 3.9 ± 0.2 mg/g; SHR, 3.9 ± 0.2 mg/g; and SHR + URB597, 3.7 ± 0.2 mg/g. There were no significant intergroup differences in the index hypertrophy values.

2.2.1.1. DOCA-salt hypertensive rats. Four- to 5-week-old (100–140 g) male Wistar rats were used in the experiment. The animals were housed with free access to standard pelleted rat chow and water (unless otherwise stated) and maintained under a 12-h light-dark cycle. The rats were anesthetized *i.p.* with pentobarbital (70 mg/kg b.w.) and unilaterally nephrectomized. After a 1-week recovery period, hypertension was induced for 6 weeks through subcutaneous (*s.c.*) injections of DOCA (25 mg/kg b.w. in 0.4 mL of N, N-dimethylformamide [DMF]/kg b.w.) twice weekly and the replacement of drinking water with a 1% NaCl solution. After 4 weeks, the DOCA-salt rats were injected *i.p.* with URB597 (1 mg/kg b.w. in 1 mL of URB597 solvent) every 12 h for 14 days [18,19].

2.2.2. Experimental protocol

The rats were divided into following four groups of six rats each:

→ group 1B [Wistar]: twice weekly for 6 weeks, uninephrectomized rats were treated *s.c.* with 0.4 mL of DMF/kg b.w.; during the last 14 days, the rats were treated *i.p.* with solvent [1 mL/kg b.w.] every 12 h;

→ group 2B [Wistar + URB597]: twice weekly for 6 weeks, uninephrectomized rats were treated *s.c.* with 0.4 mL of DMF/kg b.w.; during the last 14 days, they were treated with URB597 [1 mg/kg b.w. in 1 mL of URB597 solvent] every 12 h;

→ group 3B [DOCA-salt]: twice weekly for 6 weeks, uninephrectomized rats were treated *s.c.* with 25 mg of DOCA /kg b.w. in 0.4 mL of DMF/kg b.w. and received drinking water with a 1% NaCl solution; during the last 14 days, they were treated *i.p.* with solvent for URB597 [1 mL/kg b.w.] every 12 h; and

→ group 4B [DOCA-salt + URB597]: twice weekly for 6 weeks, uninephrectomized rats were treated *s.c.* with 25 mg of DOCA /kg b.w. in 0.4 mL of DMF/kg b.w. and received drinking water with a 1% NaCl solution; during the last 14 days, the rats were treated *i.p.* with URB597 [1 mg/kg b.w. in 1 mL of URB597 solvent] every 12 h.