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Long-term administration of fatty acid amide hydrolase inhibitor (URB597) to rats with spontaneous hypertension disturbs liver redox balance and phospholipid metabolism

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ABSTRACT

Purpose: The effect of chronic administration of [3-(3-carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate (URB597), inhibitor of fatty acid amide hydrolase (FAAH) that hydrolyzes anandamide, on cross-talk between endocannabinoid system, oxidative status and pro-inflammatory factors in the liver of spontaneously hypertensive rats (SHRs) was investigated.

Materials/methods: Experiments were conducted using SHRs and normotensive control Wistar-Kyoto rats treated by intraperitoneal injection with URB597 for 14 days. The biochemical parameters were assayed in the rat's livers.

Results: In the liver of SHRs an increase in endocannabinoids level, the activity of enzymes degrading them and expression of the cannabinoid receptor type 2 (CB_2) receptor as well as a decrease in the expression of the CB_1 and vanilloid 1 receptor (TRPV1) were shown. These changes were related to inflammatory conditions as well as oxidative stress resulting from increased reactive oxygen species (ROS) generation due to enhanced activity of enzymes generating ROS accompanied by decrease in the effectiveness of transcription activity of nuclear factor erythroid 2 and the activity of antioxidant enzymes, as well as level of glutathione and vitamins. Chronic administration of URB597 to SHRs caused a decrease in FAAH activity and an increase in anandamide and *N*-arachidonoyl-dopamine level as well as a decrease in CB_2 and an increase in TRPV1 receptor expression. The levels/activities of pro- and antioxidant and inflammatory factors tended to normalize, but phospholipid peroxidation and DNA modifications were increased.

Conclusion: In conclusion, long-term chronic administration of URB597 to SHRs by altering interactions between endocannabinoid and redox systems enhances some liver metabolic disturbances observed in hypertension.

1. Introduction

The liver is a highly complex vascular organ and therefore may be characterized by impaired functioning in vascular diseases such as hypertension [1]. It plays a fundamental role in detoxification of endogenous and exogenous compounds including nitric oxide and reactive oxygen species (ROS) and thus can protect the body from oxidative disorders [2]. However it was observed that in hypertensive rats the hepatic antioxidants status is reduced [3]. Therefore in the development of hypertension, high level of ROS may affect multiple tissues metabolism either directly or through reaction of superoxide with nitric oxide: a critical endogenous vasodilator [4]. Although ROS are needed in normal cellular functions, overproduction of ROS may lead to increased inflammatory response through activation of pro-inflammatory molecules such as tumor necrosis factor alpha (TNFα), which in turn aggravates oxidative stress and initiates a chain of deleterious events eventually culminating in cellular dysfunction and death [5,6]. Oxidative stress occurs when there is an imbalance between ROS level and antioxidant defense [2] that results in oxidative modifications of cells components including proteins and lipids what have been found in human hypertension in the blood vessels, heart and kidney, as well as liver tissue of hypertensive rats [4,7,8].

The development of hypertension is also strongly associated with functioning of endocannabinoid system including endocannabinoids –

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phospholipid metabolites, their receptors and the enzymes degrading endocannabinoids [9,10]. It was shown that the level of the main endocannabinoid – anandamide was increased in the plasma of patients with hypertension, and in different tissues of rats with primary and secondary hypertension including liver of rats with secondary hypertension [9–12]. Endocannabinoids are agonists of cannabinoid receptors that are present in most tissues including the liver and whose activation modulates oxidative and inflammatory conditions [13–15]. It has been shown that activation of cannabinoid receptor type 1 (CB_1) increases, whereas cannabinoid receptor type 2 (CB_2) and vanilloid receptor 1 (TRPV1) activation diminishes the level of ROS and inflammatory mediators [16]. It was also indicated that CB_2 agonists suppressed pro-inflammatory cytokines level and oxidative stress as well as cell death in the liver, while inhibition of CB_1 prevented liver injury in a model of hepatic fibrosis [14,15,17]. However, it was also shown that anandamide promotes hepatocytes apoptosis by a mechanism unrelated to CB receptors [18]. The third elements of endocannabinoid system are enzymes degrading endocannabinoids and as a result are responsible for level of endocannabinoids.

Endocannabinoids, including anandamide, have been shown to be involved in the regulation of blood pressure [19] via cannabinoid receptors, mainly CB_1, non-cannabinoid receptors (including TRPV1 and peroxisome proliferator-activated receptor (PPARγ)) as well as their metabolites with the generation of vasodilatory compounds, including prostacyclins [20]. Because anandamide elevation promotes blood pressure lowering, it has been suggested that the use of anandamide metabolism inhibitors should reduce blood pressure. Moreover, it was found that in males the gene modification of FAAH 129T is accompanied by reduced blood pressure [21]. Therefore, inhibitors of fatty acid amide hydrolase (FAAH), the enzyme responsible for degradation of anandamide, have been suggested as potential antihypertensive compounds. It was also shown that single administration of two different FAAH inhibitors, [3-(3-carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate (URB597) [9] and 5-(4-hydroxyphenyl) pentanesulfonyl fluoride (AM3506) [10], to primary hypertensive rats (SHR) normalized blood pressure. In contrast, chronic URB597 administration to rats with secondary hypertension reduced blood pressure in a manner dependent on age [22].

In spite of the above, anandamide by increasing the expression of receptors it can modify the level of ROS and consequently redox balance as well as other ROS-dependent processes [16]. Considering this, as well as the fact that FAAH activity in rat liver belongs to the highest [23] one should analyze the effect of changes in FAAH activity on the cellular consequences of the redox balance. It was shown e.g. that enhanced endocannabinoid level results in the activation of the CB_1 hepatic receptor, causing an increase in liver lipogenesis [24]. Therefore, we suggest that URB597 through modulation the action of endocannabinoid system may change liver redox balance responsible for this organ functioning.

Considering that the chronic influence of the FAAH inhibitor on the endocannabinoid system and redox balance in the liver has not been studied, we decided to evaluate the interactions between these systems in the liver of rats with primary hypertension.

2. Materials and methods

2.1. Ethical approval

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. Animals

Experiments were conducted using male rats with primary hypertension (SHR/NHsd Inbred, Harlan Laboratories, USA) and normotensive control Wistar-Kyoto rats (WKY/NCrl, Charles River Laboratories, Germany), aged 8–10 weeks and weighing 270–350 g. Rats were kept under standard conditions (12-h light/12-h dark cycles) and fed a pelleted rat chow (Labofeed B – maintenance; feed producer 'Morawski', Poland). The diet formula was based on the recommendations of the National Research Council in the field of the Nutrient Requirements of Laboratory Animals (67%-carbohydrates, 25%-proteins, 8%-fats). More details can be found on the website https://www.sukces.info.pl/labofeed-b.

The rats were divided into 4 groups of six rats each:

group 1 [WKY]: WKY rats were treated by intraperitoneal injection with URB597 solvent (1 mL mixture of DMSO, Tween 80 and saline (0.9% NaCl) [1:2:7; v:v:v]) every 12 h for 14 days.

group 2 [WKY + URB597]: WKY rats were treated by intraperitoneal injection with URB597 (1 mg/kg body weight in 1 mL of URB597 solvent) every 12 h, for 14 days.

group 3 [SHR]: SHRs were treated by intraperitoneal injection with URB597 solvent (1 mL) every 12 h for 14 days.

group 4 [SHR + URB597]: SHRs were treated by intraperitoneal injection with URB597 (1 mg/kg body weight in 1 mL of URB597 solvent) every 12 h for 14 days.

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 $\geq$ 150 mmHg were considered hypertensive. URB597 treatment for 2 weeks did not modify SBP in SHR (187 $\pm$ 15 mmHg and 191 $\pm$ 49 mmHg) or WKY (117 $\pm$ 18 mmHg and 101 $\pm$ 10 mmHg) rats (before the first and after the final dose, respectively). The solvent for URB597 did not modify SBP both in SHR (184 $\pm$ 34 and 205 $\pm$ 43 mmHg) and in WKY (114 $\pm$ 18 and 110 $\pm$ 13 mmHg) before the first and the final injection.

2.3. Tissue preparation

At the end of the experimental period, rats were anesthetized with an intraperitoneal injection of pentobarbital (70 mg/kg body weight) and sacrificed. The livers were rapidly removed and prepared in three different ways: fresh tissue samples were used immediately for the determination of total ROS generation; part of the fresh tissue samples were frozen in liquid nitrogen and pulverized for determination of endocannabinoids, fatty acids and their metabolites as well as glutathione level and monoacylglycerol lipase and FAAH activity; the rest of liver was homogenized in 0.9% NaCl solution – 10% homogenates were centrifuged at 20,000 $\times$ *g* for 15 min at 4 °C and aliquots of the supernatants were taken for the measurement of other biochemical parameters.

2.4. Determination of ROS

Total ROS generation was measured by the production of a stable nitroxide CM-radical ($t_{1/2}$ = 4 h), using electron spin resonance (ESR) and the reaction between ROS and the spin probe CMH (1-hydroxy-3-methoxy-carbonyl-2,2,5,5-tetrame-thylpyrrolidine) [25].

2.5. Determination of prooxidant enzyme activity

NADPH oxidase (NOX – EC 1.6.3.1) activity was assayed using luminescence assay according to the method of Griendling [26]. Enzyme specific activity was expressed in RLU (Relative Luminescence Units) per milligram of protein.

Xanthine oxidase (XO – EC1.17.3.2) activity was measured spectrophotometrically at 290 nm by determination of uric acid generated from xanthine [27]. One unit of XO activity was defined as the amount of the enzyme which was required to release 1 μmol of uric acid per minute. Enzyme specific activity was expressed in U per milligram of protein.

2.6. Determination of antioxidant enzymes activity

Superoxide dismutase (Cu/Zn–SODase – EC.1.15.1.1) activity was assayed spectrophotometrically at 480 nm (as described by Sykes) [28] by the inhibition of the oxidation of adrenaline to adrenochrome. One unit of SODase was defined as the amount of enzyme which inhibits adrenaline oxidation to adrenochrome by 50%. Enzyme specific activity was expressed in U per milligram of protein.

Catalase (CAT–EC 1.11.1.9) activity was assayed spectrophotometrically at 240 nm by the rate of hydrogen peroxide decomposition [29]. One unit of CAT was defined as the amount of enzyme required to catalyze the decomposition of 1 μmol of hydrogen peroxide to water and oxygen in 1 min. Enzyme specific activity was expressed in U per milligram of protein.

Glutathione peroxidase (GSH-Px – EC.1.11.1.6) activity was assayed spectrophotometrically by following the oxidation of NADPH at 340 nm [30]. One unit of GSH-Px activity was defined as the amount of enzyme catalyzing the oxidation of 1 mmol NADPH per minute. Enzyme specific activity was expressed in U per milligram of protein.

Glutathione reductase (GSSG-R – EC.1.6.4.2) activity was determined spectrophotometrically by measuring NADPH oxidation at 340 nm [31]. One unit of GSSG-R was defined as the amount of enzyme that catalyzes the oxidation of 1 mmol of NADPH per minute. Enzyme specific activity was expressed in U per milligram of protein.

Thioredoxin protein (Trx) level was determined by enzyme-linked immunosorbent assay (ELISA) – absorption was read at 490 nm with the reference filter set to 620 nm [32]. The level of thioredoxin was assessed using a calibration curve and normalized for milligrams of protein.

Thioredoxin reductase (TrxR-EC, 1.8.1.9) activity was estimated spectrophotometrically at 412 nm by assessing the reduction of 5,5′-dithiobis (2-nitrobenzoic acid) to 5-thio-2-nitrobenzoic acid by NADPH using a commercial kit (Sigma-Aldrich, St. Louis, MO, USA) [33].

2.7. Western blot analysis

Western blot analysis of cellular proteins (Nrf2, Keap1, Bach1, KAP1, p21, p62, p-cJun (pSer63), p-ERK ½, HO-1, TNFα, NFκB-p52/RelB, CB_1, CB_2, TRPV1) was performed according to standard procedures [34]. Briefly, after SDS-PAGE and blotting, proteins on the membranes were detected by incubation with the following primary antibodies: HO-1 (H4535, host: mouse, 1:200), Bach1 (A08321, host: rabbit, 1:500), KAP1 (SAB4502351, host: rabbit, 1:1000), β-actin (A2228, host: mouse, 1:1000), TNF-α (T7539, host: mouse, 1:1000), p-cJun (pSer63) (J2128, host: rabbit, 1:1000), p-ERK ½ (SAB4301578, host: rabbit, 1:1000), CB_1 (SAB1305934, host: rabbit, 1:1000), CB_2 (SAB1306696, host: rabbit, 1:1000), TRPV1 (SAB1404527, host: mouse, 1:1000) and Na^+/K^+ ATPase (A3979, host: mouse, 1:1000) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies against Keap1 (SC-15246, host: goat, 1:1000) and NFκB-p52/RelB (SC-7386, host: mouse, 1:1000) were purchased from Santa Cruz Biotechnology, (Santa Cruz, CA, USA) and primary antibodies against Nrf2 (MAB3925, host: mouse, 1:500) were purchased from R&D system (Minneapolis, MN, USA). Primary antibodies against p21 (ab109199, host: rabbit, 1:1000) from Abcam (Cambridge, MA, USA), against p62 (orb89844, host: rabbit, 1:1000) from BiorByt (Cambridge, UK), Visualized protein bands were quantitated using the Versa Doc System and Quantity One software. The results are expressed as a percentage of the expression determined in control groups.

2.8. Detection of non-enzymatic antioxidant level

The level of reduced and oxidative glutathione (GSH, GSSG) was measured according to the procedure of Maeso using capillary electrophoresis (CE). The separations were performed on a fused-silica capillary with an ultraviolet detector set at 200 nm [35].

Determination of vitamins C [36], A, and E [37] were performed on RP-18 column using high-performance liquid chromatography (HPLC) with UV detection (250 nm and 294 nm respectively).

2.9. Determination of phospholipid metabolism and mediators

The levels of phospholipids and free fatty acids [arachidonic acid (AA), docosahexaenoic acid (DHA), linoleic acid (LA)] were measured using gas chromatography [38]. Fatty acids were extracted by the Folch method, and separated using thin layer chromatography. Fatty acid derivatives were analyzed by gas chromatography (GC) with a flame ionization detector (FID) (Perkin Elmer, Clarus 500). Nonadecanoic acid (19:0) and 1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine (19:0 PC) were used as internal standards for quantification.

Assessment of lipid peroxidation was determined by measuring the levels of 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) by GC MS/MS [39] and F_2-isoprostanes (8-isoPGF$_{2\alpha}$) by liquid chromatography–mass spectrometry (LC MS/MS) [40]. Derivatives of MDA and 4-HNE were detected using the selected ion-monitoring (SIM) mode. Transitions of the precursor to the product ion were as follows: *m/z* 204.0 and 178.0 for MDA-PFB, *m/z* 333.0 and 181.0 for 4-HNE-PFB-TMS and *m/z* 307.0 for IS (benzaldehyde-D_6) derivatives. 8-isoPGF$_{2\alpha}$ was extracted using solid phase extraction (SPE). 8-iso PGF$_{2\alpha}$–d_4 was used as an internal standard for quantification. Multiple reaction monitoring (MRM) in negative-ion mode was used to analyze 8-iso-PGF$_{2\alpha}$. Transitions of the precursor to the product ion was as follows: *m/z* 353.2→193.1 for 8-isoPGF$_{2\alpha}$ and 357.2→197.1 for 8-isoPGF$_{2\ \alpha}$-d_4.

Endocannabinoids levels: anandamide (AEA), 2-arachidonoylglycerol (2-AG) and *N*-arachidonoyl-dopamine (NADA) were estimated using ultra-performing liquid chromatography tandem mass spectrometry (Shimadzu, LCMS 8060) [41]. AEA-d_8, 2-AG-d_8, and NADA-d_8 were used as internal standards for quantification. Endocannabinoids were extracted using SPE and analyzed in positive-ion mode (MRM mode). The precursor to the product ion transition was as follows: *m/z* 348.3→62.1 for AEA, *m/z* 379.3→287.2 for 2-AG, *m/z* 440.0→137.0 for NADA, 356.3→63.1 for AEA-d_8, *m/z* 387.0→295.0 *m/z* for 2-AG-d_8 and *m/z* 448.0→137.0 for NADA-d_8.

FAAH (EC-3.5.1.99) activity was measured spectrophotometrically at 410 nm in the liver homogenates by determination of *m*-nitroaniline (m-NA) formation [42]. Enzyme specific activity was expressed in nmoles of m-NA/min/mg protein.

Monoacylglycerol lipase (MAGL) (EC 3.1.1.23) activity was measured spectrophotometrically at 412 nm by determination of 5′-thio-2-nitrobenzoic acid (TNB) formation [43]. Enzyme specific activity was expressed in nmoles of TNB/min/mg protein.

Cyclooxygenase 1 and 2 (COX1, COX2) activity was measured spectrophotometrically at 412 nm by following the oxidation of TMPD using a commercial assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) [44].

2.10. Determination of protein and DNA modifications

Carbonyl group (CO) levels were measured spectrophotometrically at 412 nm using 2,4-dinitrophenylhydrazine [45]. Carbonyl groups were quantified using a calibration curve and normalized for milligrams of protein.

DNA oxidative modifications, after isolation of genomic DNA, were estimated by the level of 8-hydroxy-2′-deoxyguanosine (8-OHdG) using LC MS/MS [46].

2.11. Statistical analysis

Data are expressed as mean ± S.D. Statistical comparisons were performed by two-way analysis of variance (ANOVA) followed by a post hoc Tukey test. The results were considered statistically significant if the p-values were 0.05 or less.

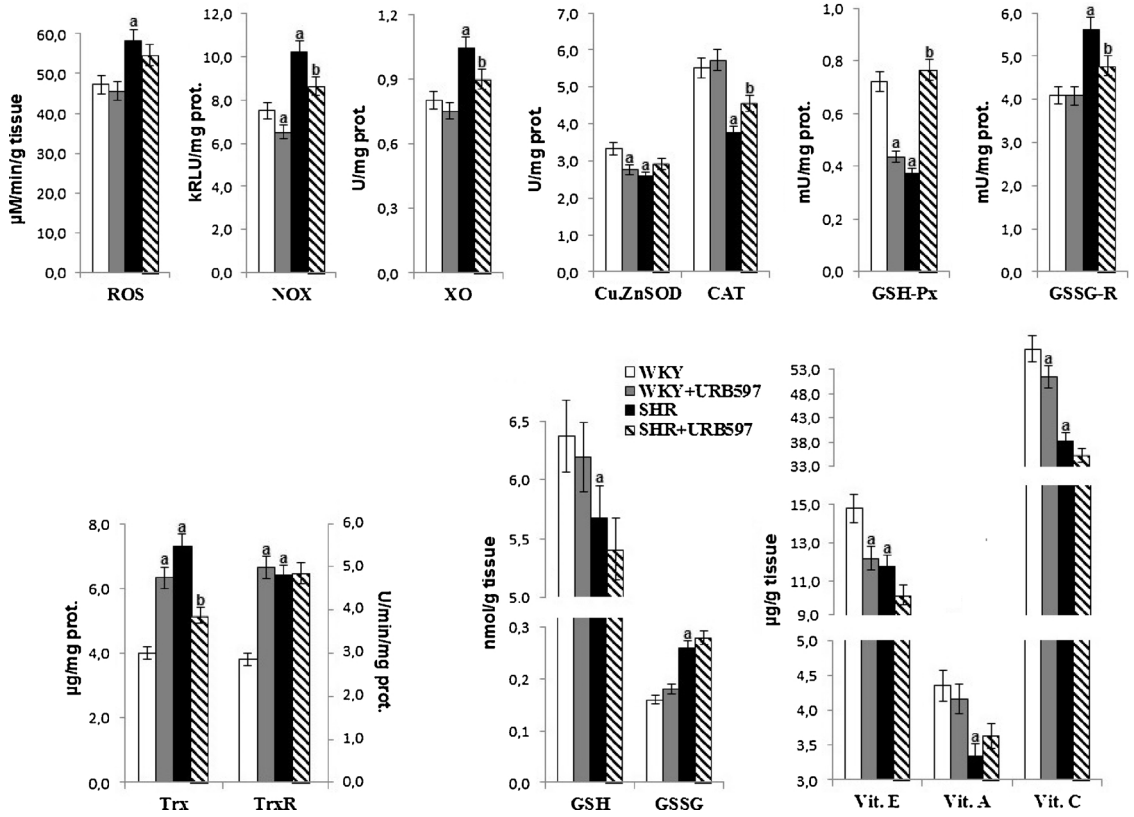

Fig. 1. ROS level, the activity of ROS-generating enzymes [NOX and XO] and the activities/levels of antioxidant parameters in the liver of normotensive and hypertensive rats (SHR) and rats after URB597 administration.
Data points represent the mean ± SD, n = 6; (a – significantly different from WKY group, $p < 0.05$; b – significantly different from SHR group, $p < 0.05$).

3. Results

This study has shown that redox imbalance in the liver of rats with primary hypertension occurs compared to control rats (Fig. 1). Significant increases in the activity of XO and NADPH oxidase and consequently higher total ROS level were observed. Decreases in the activities of the antioxidant enzymes such as Cu,Zn-SOD, CAT and GSH-Px and the levels of all non-enzymatic antioxidants (GSH, vitamins E, A, and C) were also indicated. However, the activity of GSSG-R and TrxR, and the level of Trx were increased.

Chronic treatment of normotensive rats with URB597 led to a decrease in the activity of prooxidant enzyme – xanthine oxidase as well as antioxidant enzymes such as Cu,Zn-SOD and GSH-Px and non-enzymatic antioxidants including vitamin C and E. However the components of thioredoxin system (Trx and TrxR) level/activity were enhanced in comparison to control rats. Chronic treatment of SHRs with URB597 caused a decrease in the activity of xanthine oxidase and NADPH oxidase and antioxidant enzymes such as GSSG-R as well as level of Trx in comparison to hypertensive rats. Moreover in the liver of hypertensive rats receiving URB597 an increase in CAT and GSH-Px activity was observed.

Spontaneous hypertension modified liver antioxidant defence not only at protein level but also at level of transcription factor Nrf2 pathway (Fig. 2). In the liver of SHRs expression of Nrf2 activators, such as KAP1, p62 and inhibitor Keap1 as well as Nrf2 target protein – HO-1 levels were decreased compared to control rats. However the levels of p-cJun and p-ERK1/2 were increased.

Administration of FAAH inhibitor to WKY rats resulted in increased levels of Nrf2 and HO-1, while in SHRs liver only HO-1 level was increased. In both groups of rats (control and hypertensive) given URB597 an increase in the expression of examined activators (KAP1, p21, p62) was shown. However URB597 decreased liver level of Nrf2 inhibitors (Keap1 and Bach1) in WKY rats but increased in SHRs liver. Treatment of WKY rats as well as SHRs with URB597 led to a decrease in kinase p-cJun and an increase in kinase p-ERK1/2 levels comparing to control and hypertensive group, respectively.

Primary hypertension modified liver phospholipid metabolism was shown by the levels of phospholipid and free fatty acids in comparison to control rats (Fig. 3). Phospholipid AA and free fatty acids – AA and LA levels were decreased in SHRs livers, which resulted from significantly enhanced oxidative modifications; revealed by enhanced levels of lipid peroxidation products (4-HNE and 8-isoprostanes). In addition, oxidative damages also involved protein and DNA; demonstrated as enhanced level of protein carbonyl groups and DNA modification measured as 8-OHdG.

Administration of URB597 to WKY rats decreased level of all free fatty acids in the liver, while URB597 given to SHRs caused a decrease in the level of free and phospholipid AA and an increase in the levels of free DHA and LA. These changes were accompanied by increased level of MDA and 8-OHdG in both groups of rats comparing to WKY rats and SHRs. A decrease in the level of 4-HNE was observed after URB597 administration to normotensive rats. Moreover in both groups of rats receiving URB597 the level of protein carbonyl groups was reduced.

Regardless of ROS-dependent phospholipid metabolism, SHRs had higher endocannabinoids (AEA, 2-AG and NADA) levels (Fig. 4). Moreover, a significant increase in the activities of endocannabinoids-degrading enzymes, namely FAAH and MAGL was observed. At the same time the level of expression of CB_2 receptor was higher, while CB_1 and TRPV1 receptor levels were lower in the liver of SHRs comparing to WKY rats.

Treatment with URB597 led to an increase in the level of AEA and a decrease in FAAH activity in the liver of both SHRs and WKY rats. In addition, URB597 raised 2-AG level in WKY rats as well as NADA level in SHRs. Expression of all examined endocannabinoid receptors were

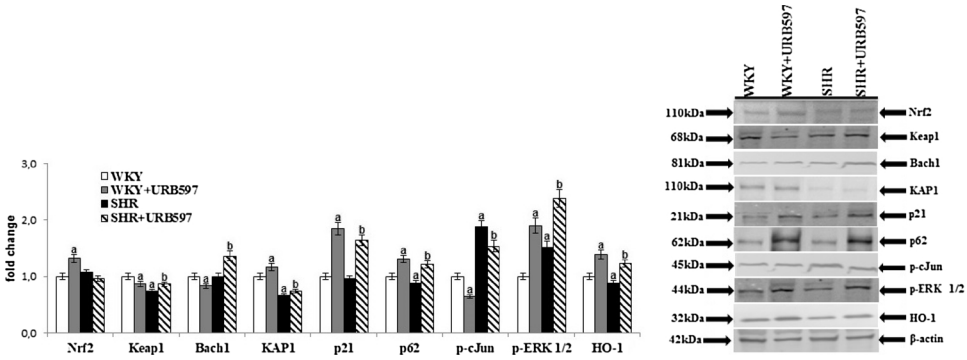

Fig. 2. The levels of Nrf2 and its activators [KAP1, p21, p62, p-cJun, p-ERK 1/2] and inhibitors [Keap1, Bach1] as well as HO-1 in the liver of normotensive and hypertensive rats (SHR) and rats after URB597 administration. The expression of the examined proteins is shown compared to the control groups.
Data points represent the mean ± SD, n = 6; (a, significantly different from WKY group, $p < 0.05$; b, significantly different from SHR group, $p < 0.05$).

decreased in the livers of WKY rats. However expression of CB_2 receptor was diminished, while TRPV1 was enhanced in the liver of SHRs.

Hypertension was also associated with an increase in expression of liver pro-inflammatory proteins such as NFκB, and a product of its transcription activity – TNFα (Fig. 5), while the activity of COX1 was decreased. However URB597 given to WKY and SHRs led to decreased levels of pro-inflammatory proteins NFκB and TNFα, and activation of inducible cyclooxygenase (COX2).

4. Discussion

Spontaneous hypertension in rats corresponds to the human arterial hypertension and it is known that oxidative stress and inflammation are involved in the development of this disease [4]. Considering that the redox balance is also regulated by the components of the endocannabinoid system, in the present study we have analyzed the effect of the URB597, a selective FAAH inhibitor, on cross talk between redox

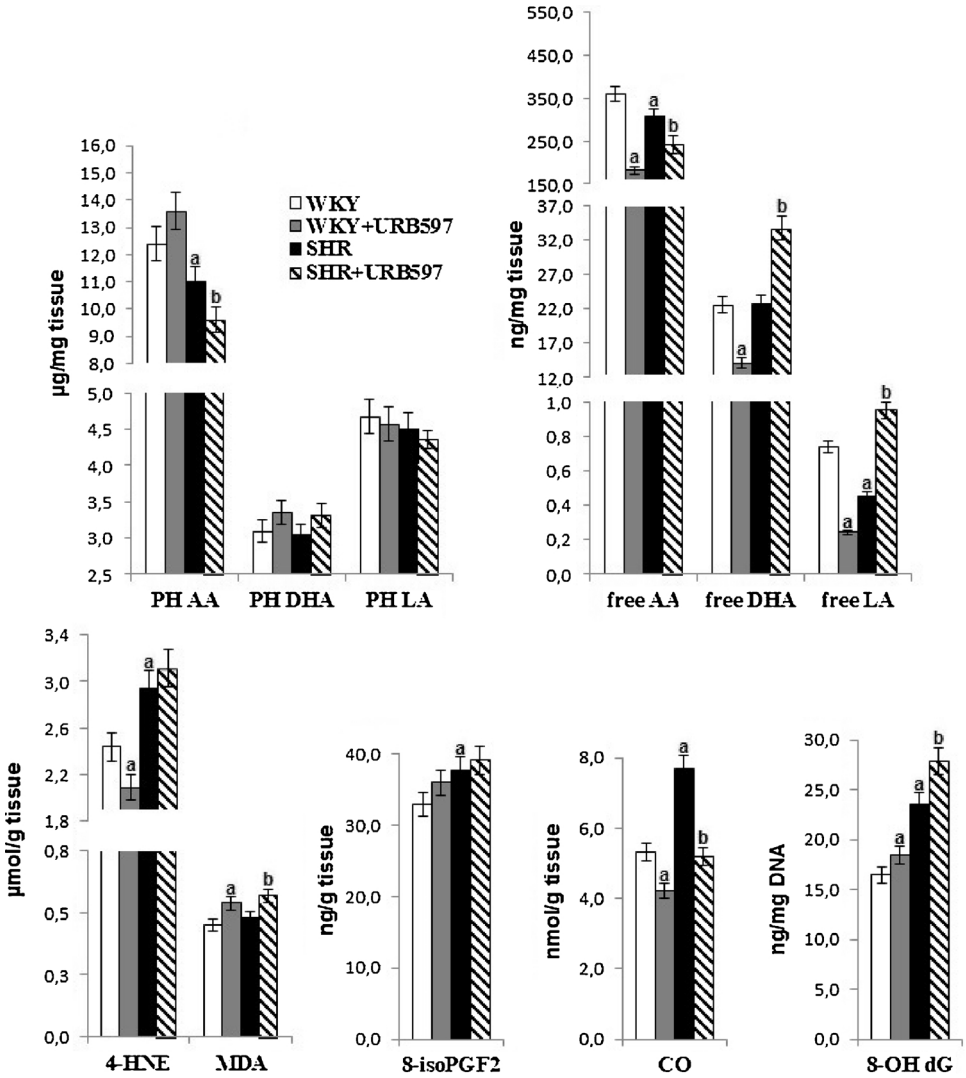

Fig. 3. The levels of phospholipid and free fatty acids and the levels of lipid peroxidation products [4-HNE, MDA, 8-isoPGF$_2$] as well as the levels of oxidative modification products of protein [CO] and DNA [8-OH dG] in the liver of normotensive and hypertensive rats (SHR) and rats after URB597 administration.
Data points represent the mean ± SD, n = 6; (a, significantly different from WKY group, $p < 0.05$; b, significantly different from SHR group, $p < 0.05$).

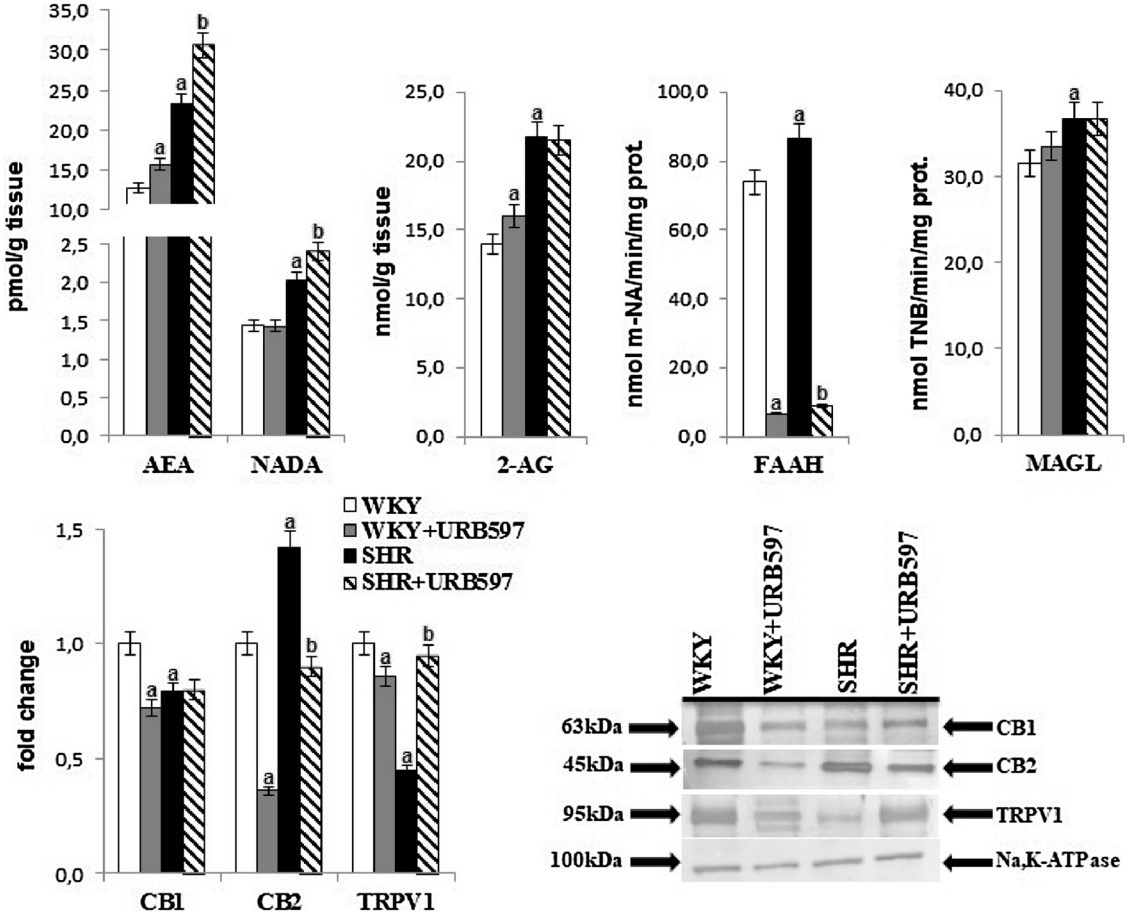

Fig. 4. The levels of endocannabinoids [AEA, 2-AG and NADA], their receptors [CB_1, CB_2, TRPV1], activities of enzymes degrading endocannabinoids [FAAH, MAGL] in the liver of normotensive and hypertensive rats (SHR) and rats after URB597 administration. The expression of cannabinoid receptors is shown compared to the control group.
Data points represent the mean ± SD, n = 6; (a, significantly different from WKY group, $p < 0.05$; b, significantly different from SHR group, $p < 0.05$).

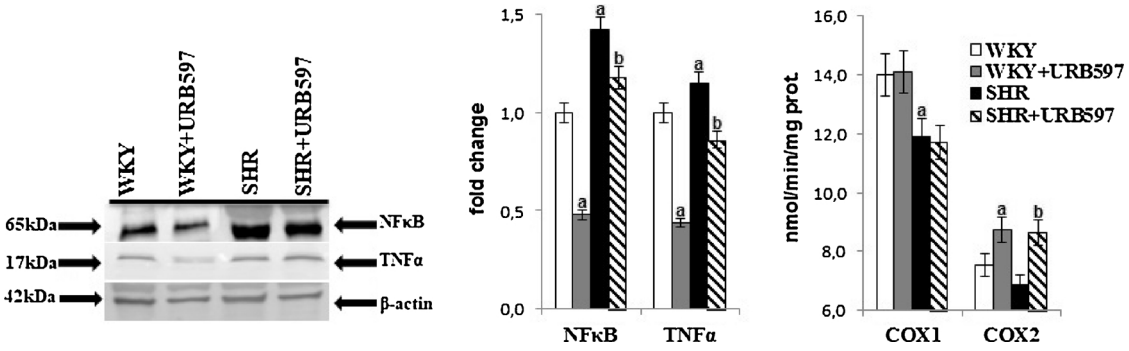

Fig. 5. The levels of NFκB and TNFα and the activities of COX1 and COX2 in the liver of normotensive and hypertensive rats (SHR) and rats after URB597 administration. The expression of NFκB and TNFα is shown compared to the control group.
Data points represent the mean ± SD, n = 6; (a, significantly different from WKY group, $p < 0.05$; b, significantly different from SHR group, $p < 0.05$).

and endocannabinoid systems in the liver of rats with genetic hypertension (SHRs).

4.1. Redox balance and endocannabinoid system interactions in the liver of SHRs

The results of this study show that in the liver of a spontaneously hypertensive rats, significant activation of pro-oxidant enzymes, such as NADPH oxidase and xanthine oxidase, results in an increase of ROS generation. This is in agreement with previous report indicating an increase in superoxide and hydrogen peroxide levels in the endothelial cells of the aorta in rats with spontaneous hypertension [47]. Enhanced activity of both enzymes generates superoxide, indicating an increased generation of this radical, but the increase in the level of hydrogen peroxide is also suggested, because despite the reduced activity of superoxide dismutase catalyzing the conversion of the superoxide into hydrogen peroxide, the activities of catalase and glutathione peroxidase participating in the degradation of hydrogen peroxide are also significantly decreased. In addition, the level of non-enzymatic antioxidants, such as glutathione and vitamin C and E, is reduced, which clearly indicates a deterioration of protection of cell membranes. Vitamin E as a lipophilic antioxidant is responsible for the protection of

membrane phospholipids, and after oxidation it is regenerated by the related action of GSH and vitamin C [48]. Under oxidative conditions, observed in hypertension, this protective action is reduced. Therefore liver's defense against ROS–dependent phospholipid peroxidation – the glutathione-related system consisting of reduced glutathione and GSH-related enzymes – is also suppressed. Consequently, enhanced lipid peroxidation products, with increased level of reactive non-saturated aldehyde (4-HNE) and prostaglandin derivatives such as 8-isoprostanes generation is observed. It was earlier shown that 4-HNE is a selective inhibitor of glutathione peroxidase selenol center but also creates adducts with cysteine, histidine, lysine of proteins leading to decrease their biological activity [49].

As a result of ROS-dependent protein structure modifications, there are also changes in the functions of Kelch-like ECH-associated protein 1 (Keap1) – cytosolic inhibitor of nuclear factor erythroid 2 (Nrf2), transcription factor, which regulates a large number of genes responsible for cell protection, particularly against pro-oxidative conditions [50]. In physiological conditions, Nrf2 remains under the control of the inhibitors: Keap1 and basic leucine zipper transcription factor 1 (Bach1) as well as activators KRAB-associated protein-1 (KAP1) and nucleoporin p62 (p62) [51]. In the liver of the hypertensive rat, the Nrf2 pathway is suppressed, which is confirmed by the heme oxygenase 1 (HO-1) level, as well as the decreased activity of classical antioxidant enzymes. We, and other authors, also observed that hepatic antioxidant status is decreased in the liver of another strain group of hypertensive rats – DOCA-induced hypertensive rats – seeming to confirm that hypertension is closely-related with oxidative stress [3,8]. Independent of the above changes, spontaneous hypertension enhanced the function of the thioredoxin system responsible for liver detoxification activity [52], by increasing the level of Trx and its reductase activity.

Oxidative stress in the liver of SHRs is revealed not only by an increase in ROS-dependent phospholipid peroxidation, but also as an intensification of enzymatic metabolism of phospholipids, which results in elevated levels of endocannabinoids such as AEA, 2-AG and NADA. This is despite the enhanced activity of the main enzymes degrading endocannabinoids (FAAH and MAGL) what suggests a significant increase in endocannabinoid biosynthesis. Earlier reports showed the involvement of endocannabinoids, mainly 2-AG, in inhibiting oxidative stress and associated inflammation in hepatic tissue [53]. In turn, it was found that AEA can induce ROS formation, which can contribute to direct, independent from cannabinoid receptors, modification of Nrf2 pathway activation, and induction of HO-1 transcripts [54]. However, in SHR, despite elevated AEA levels, there is no elevated HO-1 expression but elevated levels of Trx and activity of TrxR are found. It is believed that AEA is a partial or full agonist of the CB_1 receptor, depending on tissue and conditions, and is suggested to have low efficacy on CB_2 receptors, whereas 2-AG is a full agonist for both CB_1 and CB_2 receptors [55]. However, an elevated level of endocannabinoids is accompanied by CB_1 receptor down-regulation and significant CB_2 receptor up-regulation. This confirms that changes in the redox balance dysregulating phospholipid metabolism in the liver of SHRs, estimated as the level of endocannabinoids as well as phospholipid peroxidation products and consequently leading to disturbed liver cell metabolism observed as inflammation.

4.2. Effect of chronic administration of URB597 on redox and endocannabinoid systems interactions

Long-term administration of URB597 almost completely reduces FAAH activity in the liver of control (WKY) and spontaneously hypertensive rats. This is particularly important in the context that rat liver FAAH activity is one of the highest in comparison to other organs [42]. Observed increase in AEA proves the metabolic efficiency of URB597 in relation to the liver tissue of both groups of rats. At the same time, no effect of URB597 on MAGL activity indicates that the isoform of MAGL in the liver of rats with genetically induced hypertension is not sensitive to URB597, in contrast to Wistar rats with DOCA-salt induced hypertension [8]. However, the level of another endocannabinoid, NADA, increased after URB597 treatment. This may indirectly result from the inhibition of FAAH activity leading to the increase level of dopamine which is conjugated with arachidonic acid, supplying the NADA biosynthesis pathway [56]. Such a suggestion may be justified in the observed, in this study results, significantly reduced level of free arachidonic acid. Because metabolic dependence of the redox and endocannabinoid systems as well as pro-inflammatory factors is demonstrated in the liver of SHRs, observed changes in endocannabinoids level caused by URB597 may be involved in the changes of the redox balance and the level of pro-inflammatory mediators. It was earlier shown that hepatocytes from FAAH−/− knock-out mice displayed increase in AEA-induced ROS formation [42], while in human cardiomyocytes pretreated by FAAH inhibitor anandamide induced cell death [57]. These results suggest that FAAH play a key role in controlling the tissue injury, which is, at least in part, mediated by the activation of cannabinoid receptors by endocannabinoids [57]. URB597 does not influence CB_1 receptor expression that is decreased as a consequence of hypertension. Such situation is not favorable for the liver because it was suggested the involvement of CB_1 in the control of liver regeneration [58], however it is supported by down-regulation of the CB_2 receptor responsible for the reduction of ROS and TNFα level [15]. Attention should also be paid to the increased expression of TRPV1 which, like the CB_2 receptor, is responsible for the reduction of pro-oxidative and pro-inflammatory conditions [59]. Previously, a similar effect has been demonstrated as a consequence of the reduced production of the cytokine interleukin 17 (IL-17), what may contribute to liver regeneration by accelerating hepatocyte proliferation [60]. However, there are also reports suggesting that activation of the TRPV1 receptor potentiates apoptosis by increasing intracellular calcium and mitochondrial superoxide level [61]. It was indicated that AEA promoting oxidative and pro-inflammatory conditions may also lead to the activation of caspase 3 mediated apoptosis [62]. On the other hand, the observed increase in inducible COX2 activity may indicate on increase in the level of AEA degradation products, such as pro-inflammatory prostaglandins E2 and D2 [63]. Therefore, it is difficult to clearly determine the metabolic consequences of changes in the endocannabinoid system in the liver of SHRs.

Analysis of the basic parameters responsible for the redox balance suggests that under the influence of URB597, there is a slight shift in redox equilibrium into the reduction, which is indicated by an increase in the expression of HO-1, whose level is dependent on Nrf2 transcriptional activity [64]. The increased activity of Nrf2 (although protein levels were unchanged) may be due to changes in activator (cyclin-dependent kinase inhibitor 1 – p21 and KAP1) expression, leading to upregulation of antioxidant gene expression [54]. It was shown that FAAH inhibition by URB597 induced Nrf2 nuclear translocation and HO-1 expression in breast cancer cells [54], while HO-1 and its products possess anti-inflammatory and anti-apoptotic functions [65].

The higher efficiency of some of the antioxidant proteins may be due to decreased protein oxidation observed as the level of protein carbonyl groups. However, the reduced expression of Trx, which is a component of the thioredoxin system responsible for protection against toxic compounds, indicates a reduction by URB597 of the protective capacity of the liver. The level of non-enzymatic antioxidants, including GSH, was not improved under the influence of URB597. As a consequence, the efficiency of the liver GSH-dependent system responsible for the protection of membrane phospholipids decreased, as evidenced by the increased level of lipid peroxidation products, particularly MDA. This corresponds to a reduction in the level of free arachidonic acid, which is the main substrate for the production of lipid peroxidation products [66]. Phospholipid membrane disturbances may be additionally enhanced by anandamide action [67].

5. Conclusion

The results of this study indicate that long-term administration of the FAAH inhibitor URB597 intensifies the dysregulation of cross-talk between the endocannabinoid and redox systems despite a decrease in the pro-inflammatory response in the liver of spontaneously hypertensive rats. However a decrease in CB_2 receptor expression affecting the antioxidant response may lead to the progression or formation of metabolic disorders, in particular related to phospholipid metabolism, which may ultimately lead to liver failure.

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Conflicts of interest

The authors declare no conflict of interest.

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References

[1] Mathwig C, Willaert K. Vascular architecture of the liver in SHR and WKY rats. JUR 2007;7(1):10 Available at: http://cornerstone.lib.mnsu.edu/jur/vol7/iss1/10.
[2] Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong C, et al. The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci 2015;16:26087–124.
[3] Hemalatha G, Pugalendi KV, Saravanan R. Modulatory effect of sesamol on DOCA-salt-induced oxidative stress in uninephrectomized hypertensive rats. Mol Cell Biochem 2013;379:255–65.
[4] Loperena R, Harrison DG. Oxidative stress and hypertensive diseases. Med Clin N Am 2017;101(1):169–93.
[5] Yang YY, Liu H, Nam SW, Kunos G, Lee SS. Mechanisms of TNFα-induced cardiac dysfunction in cholestatic bile duct-ligated mice: interaction between TNFα and endocannabinoids. J Hepatol 2010;53(2):298–306.
[6] Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal 2014;20(7):1126–67.
[7] Biernacki M, Ambrożewicz E, Gęgotek A, Toczek M, Bielawska K, Skrzydlewska E. Redox system and phospholipid metabolism in the kidney of hypertensive rats after FAAH inhibitor URB597 administration. Redox Biol 2018;15:41–50.
[8] Biernacki M, Łuczaj W, Gęgotek A, Toczek M, Bielawska K, Skrzydlewska E. Crosstalk between liver antioxidant and the endocannabinoid systems after chronic administration of the FAAH inhibitor, URB597, to hypertensive rats. Toxicol Appl Pharmacol 2016;301:31–41.
[9] Bátkai S, Pacher P, Osei-Hyiaman D, Radaeva S, Liu J, Harvey-White J, et al. Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. Circulation 2004;110:1996–2002.
[10] Godlewski G, Alapafuja SO, Bátkai S, Nikas SP, Cinar R, Offertáler L, et al. Inhibitor of fatty acid amide hydrolase normalizes cardiovascular function in hypertension without adverse metabolic effects. Chem Biol 2010;17:1256–66.
[11] Li D, Chen BM, Peng J, Zhang YS, Li XH, Yuan Q, et al. Role of anandamide transporter in regulating calcitonin gene-related peptide production and blood pressure in hypertension. J Hypertens 2009;27(6):1224–32.
[12] Wang Y, Kaminski NE, Wang DH. Endocannabinoid regulates blood pressure via activation of the transient receptor potential vanilloid type 1 in Wistar rats fed a high-salt diet. J Pharmacol Exp Ther 2007;321(2):763–9.
[13] Fonseca BM, Costa MA, Almada M, Correia-da-Silva G, Teixeira NA. Endogenous cannabinoids revisited: a biochemistry perspective. Prostaglandins Other Lipid Mediat 2013;102:13–30.
[14] Bátkai S, Osei-Hyiaman D, Pan H, El-Assal O, Rajesh M, Mukhopadhyay P, et al. Cannabinoid-2 receptor mediates protection against hepatic ischemia/reperfusion injury. FASEB J 2007;21(8):1788–800.
[15] Horváth B, Magid L, Mukhopadhyay P, Bátkai S, Rajesh M, Park O, et al. A new cannabinoid CB2 receptor agonist HU-910 attenuates oxidative stress, inflammation and cell death associated with hepatic ischaemia/reperfusion injury. Br J Pharmacol 2012;165(8):2462–78.
[16] Lipina C, Hundal HS. Modulation of cellular redox homeostasis by the endocannabinoid system. Open Biol 2016;6:150276.
[17] Giannone FA, Baldassarre M, Domenicali M, Zaccherini G, Trevisani F, Bernardi M, et al. Reversal of liver fibrosis by the antagonism of endocannabinoid CB1 receptor in a rat model of CCl 4-induced advanced cirrhosis. Lab Invest 2012;92(3):384–95.
[18] Biswas KK, Sarker KP, Abeyama K, Kawahara KI, Iino S, Otsubo Y, et al. Membrane cholesterol but not putative receptors mediates anandamide-induced hepatocyte apoptosis. Hepatology 2003;38(5):1167–77.
[19] Malinowska B, Baranowska-Kuczko M, Schlicker E. Triphasic blood pressure responses to cannabinoids: do we understand the mechanism? Br J Pharmacol 2012;165(7):2073–88.
[20] Stanley C, O'sullivan SE. Vascular targets for cannabinoids: animal and human studies. Br J Pharmacol 2014;171(6):1361–78.
[21] Sarzani R, Bordicchia M, Salvi F, Cola G, Franchi E, Battistoni I, et al. A human fatty acid amide hydrolase (FAAH) functional gene variant is associated with lower blood pressure in young males. Am J Hypertens 2008;21(8):960–3.
[22] Toczek M, Baranowska-Kuczko M, Grzęda E, Pędzińska-Betiuk A, Weresa J, Malinowska B. Age-specific influences of chronic administration of the fatty acid amide hydrolase inhibitor URB597 on cardiovascular parameters and organ hypertrophy in DOCA-salt hypertensive rats. Pharmacol Rep 2016;68:363–9.
[23] Deutsch DG, Ueda N, Yamamoto S. The fatty acid amide hydrolase (FAAH). Prostaglandins Leukot Essent Fatty Acids 2002;66(2-3):201–10.
[24] Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Bátkai S, et al. Endocannabinoid activation at hepatic CB 1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J Clin Invest 2005;115(5):1298–305.
[25] Kuzkaya N, Weissmann N, Harrison DG, Dikalov S. Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitricoxide synthase. J Biol Chem 2003;278:22546–54.
[26] Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 1994;74:1141–8.
[27] Prajda N, Weber G. Malignant transformation-linked imbalance: decreased xanthine oxidase activity in hepatomas. FEBS Lett 1975;59:245–59.
[28] Sykes JA, McCormac FX, O'Breien TJ. Preliminary study of the superoxide dismutase content of some human tumors. Cancer Res 1978;38:2759–62.
[29] Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–6.
[30] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158–69.
[31] Mize CE, Langdon RG. Hepatic glutathione reductase. Purification and general kinetic properties. J Biol Chem 1962;237:1589–95.
[32] Lovell MA, Xie C, Gabbita SP, Markesbery WR. Decreased thioredoxin and increased thioredoxin reductase levels in Alzheimer's disease brain. Free Radic Biol Med 2000;28(3):418–27.
[33] Holmgren A, Bjornstedt M. Thioredoxin and thioredoxin reductase. Methods Enzymol 1995;252:199–208.
[34] Eissa S, Seada LS. Quantitation of bcl-2 protein in bladder cancer tissue by enzyme immunoassay: comparison with Western blot and immunohistochemistry. Clin Chem 1998;44:1423–9.
[35] Maeso N, Garcia-Martinez D, Ruperez FJ, Cifuentes A, Barbas C. Capillary electrophoresis of glutathione to monitor oxidative stress and response to antioxidant treatments in an animal model. J Chromatogr B 2005;822:61–9.
[36] Ivanović D, Popović A, Radulović D, Medenica M. Reversed-phase ion-pair HPLC determination of some watersoluble vitamins in pharmaceuticals. J Pharm Biomed Anal 1999;18:999–1004.
[37] Vatassery GT, Brin MF, Fahn S, Kayden HJ, Traber MG. Effect of high doses of dietary vitamin E on the concentrations of vitamin E in several brain regions, plasma, liver, and adipose tissue of rats. J Neurochem 1988;512:621–3.
[38] Preparation of ester derivatives of fatty acids for chromatographic analysis. In: Christie WW, editor. Advances in lipid methodology – two. Dundee: Oily Press; 1993. p. 69–111 Available at: http://lipidlibrary.aocs.org/Analysis/content.cfm?ItemNumber=40374.
[39] Luo XP, Yazdanpanah M, Bhooi N, Lehotay DC. Determination of aldehydes and other lipid peroxidation products in biological samples by gas chromatography-mass spectrometry. Anal Biochem 1995;228:294–8.
[40] Coolen SA, Van Buuren B, Duchateau G, Upritchard J, Verhagen H. Kinetics of biomarkers: biological and technical validity of isoprostanes in plasma. Amino Acids 2005;29:429–36.
[41] Lam PM, Marczylo TH, El-Talatini M, Finney M, Nallendran V, Taylor AH, et al. Ultra performance liquid chromatography tandem mass spectrometry method for the measurement of anandamide in human plasma. Anal Biochem 2008;380:195–201.
[42] Siegmund SV, Seki E, Osawa Y, Uchinami H, Cravatt BF, Schwabe RF. Fatty acid amide hydrolase determines anandamideinduced cell death in the liver. J Biol Chem 2006;281:10431–8.
[43] Ulloa NM, Deutsch DG. Assessment of a spectrophotometric assay for monoacylglycerol lipase activity. AAPS J 2010;12:197–201.

[44] Kulmacz RJ, Lands WE. Requirements for hydroperoxide by the cyclooxygenase and peroxidase activities of prostaglandin H synthase. Prostaglandins 1983;25(4):531–40.
[45] Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990;186:464–78.
[46] Dizdaroglu M, Jaruga P, Rodriguez H. Measurement of 8-hydroxy-2'-deoxyguanosine in DNA by high performance liquid chromatography-mass spectrometry: comparison with measurement by gas chromatography-mass spectrometry. Nucleic Acids Res 2001;29:e12.
[47] Friques AG, Arpini CM, Kalil IC, Gava AL, Leal MA, Porto ML, et al. Chronic administration of the probiotic kefir improves the endothelial function in spontaneously hypertensive rats. J Transl Med 2015;13(1):390.
[48] Wefers H, Sies H. The protection by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. FEBS J 1988;174(2):353–7.
[49] Łuczaj W, Gęgotek A, Skrzydlewska E. Antioxidants and HNE in redox homeostasis. Free Radic Biol Med 2017;111:87–101.
[50] Lopes RA, Neves KB, Tostes RC, Montezano AC, Touyz RM. Downregulation of nuclear factor erythroid 2–related factor and associated antioxidant genes contributes to redox-sensitive vascular dysfunction in hypertension novelty and significance. Hypertension 2015;66(6):1240–50.
[51] Sun H, Zhu J, Lin H, Gu K, Feng F. Recent progress in the development of small molecule Nrf2 modulators: a patent review (2012-2016). Expert Opin Ther Pat 2017;27(7):763–85.
[52] Prigge JR, Coppo L, Martin SS, Ogata F, Miller CG, Bruschwein MD, et al. Hepatocyte hyperproliferation upon liver-specific co-disruption of thioredoxin-1, thioredoxin reductase-1, and glutathione reductase. Cell Rep 2017;19(13):2771–81. https://doi.org/10.1016/j.celrep.2017.06.019.
[53] Cao Z, Mulvihill MM, Mukhopadhyay P, Xu H, Erdélyi K, Hao E, et al. Monoacylglycerol lipase controls endocannabinoid and eicosanoid signaling and hepatic injury in mice. Gastroenterology 2013;144:808–17.
[54] Li H, Wood JT, Whitten KM, Vadivel SK, Seng S, Makriyannis A, et al. Inhibition of fatty acid amide hydrolase activates Nrf2 signalling and induces heme oxygenase 1 transcription in breast cancer cells. Br J Pharmacol 2013;170:489–505.
[55] Mallat A, Teixeira-Clerc F, Lotersztajn S. Cannabinoid signaling and liver therapeutics. J Hepatol 2013;59:891–6.
[56] Murillo-Rodríguez E, Vázquez E, Millán-Aldaco D, Palomero-Rivero M, Drucker-Colin R. Effects of the fatty acid amide hydrolase inhibitor URB597 on the sleep-wake cycle, c-Fos expression and dopamine levels of the rat. Eur J Pharmacol 2007;562(1–2):82–91.
[57] Mukhopadhyay P, Horváth B, Rajesh M, Matsumoto S, Saito K, Bátkai S, et al. Fatty acid amide hydrolase is a key regulator of endocannabinoid-induced myocardial tissue injury. Free Radic Biol Med 2011;50(1):179–95.
[58] Mukhopadhyay B, Cinar R, Yin S, Liu J, Tam J, Godlewski G, et al. Hyperactivation of anandamide synthesis and regulation of cell-cycle progression via cannabinoid type 1 (CB1) receptors in the regenerating liver. PNAS 2011;108(15):6323–8.
[59] Hao X, Chen J, Luo Z, He H, Yu H, Ma L, et al. TRPV1 activation prevents high-salt diet-induced nocturnal hypertension in mice. Pflügers Arch Eur J Physiol 2011;461:345–53.
[60] Rao R, Graffeo CS, Gulati R, Jamal M, Narayan S, Zambirinis CP, et al. Interleukin 17–producing γδT cells promote hepatic regeneration in mice. Gastroenterology 2014;147(2):473–84.
[61] Sun Z, Han J, Zhao W, Zhang Y, Wang S, Ye L, et al. TRPV1 activation exacerbates hypoxia/reoxygenation-induced apoptosis in H9C2 cells via calcium overload and mitochondrial dysfunction. Int J Mol Sci 2014;15(10):18362–80.
[62] Mukhopadhyay P, Rajesh M, Bátkai S, Patel V, Kashiwaya Y, Liaudet L, et al. CB1 cannabinoid receptors promote oxidative stress and cell death in murine models of doxorubicin-induced cardiomyopathy and in human cardiomyocytes. Cardiovasc Res 2010;85:773–84.
[63] Weber A, Ni J, Ling KHJ, Acheampong A, Tang-Liu DD, Burk R, et al. Formation of prostamides from anandamide in FAAH knockout mice analyzed by HPLC with tandem mass spectrometry. J Lipid Res 2004;45(4):757–63.
[64] Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell Mol Life Sci 2016;73(17):3221–47.
[65] Ryter SW, Choi AM. Heme oxygenase-1/carbon monoxide: from metabolism to molecular therapy. Am J Respir Cell Mol Biol 2009;41(3):251–60.
[66] Else PL, Kraffe E. Docosahexaenoic and arachidonic acid peroxidation: It's a within molecule cascade. Biochim Biophys Acta 2015;1848(2):417–21.
[67] Siegmund SV, Uchinami H, Osawa Y, Brenner DA, Schwabe RF. Anandamide induces necrosis in primary hepatic stellate cells. Hepatology 2005;41:1085–95.