SHORT COMMUNICATION

Glutathione Levels and Lipid Oxidative Damage in Selected Organs of Obese Koletsky and Lean Spontaneously Hypertensive Rats

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Summary

Koletsky rats, the genetically obese strain of spontaneously hypertensive rats (SHROB), are the well-accepted animal model of human metabolic syndrome. They are characterized by early onset obesity, spontaneous hypertension, hyperinsulinemia, hyperlipidemia, proteinuria and shortened life-span. One of the factors in the pathogenesis of metabolic syndrome is oxidative stress. The aim of the present study was to compare two parameters related to oxidative stress: the levels of the main intracellular antioxidant, reduced glutathione as well as the indirect indicator of lipid peroxidation damage, thiobarbituric acid-reactive substances (TBARS) in heart, renal cortex and medulla and liver in male lean spontaneously hypertensive rats (SHR) and obese Koletsky rats. We did not find any significant differences in these markers in heart and kidneys. However, we found significantly lower glutathione level in Koletsky rat liver compared with SHR (5.03±0.23 vs. 5.83±0.14 μmol/g tissue, respectively). On the contrary, we observed significantly higher TBARS levels in Koletsky rat liver compared with SHR (28.56±2.15 vs. 21.83±1.60 nmol/mg protein, respectively). We conclude that the liver is the most sensitive tissue to oxidative damage with the significantly decreased concentration of glutathione and the significantly increased concentration of TBARS in obese Koletsky rats in comparison with lean control SHR.

Key words

Thiobarbituric acid-reactive substances • Heart • Renal cortex • Renal medulla • Liver

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Koletsky rats, obese spontaneously hypertensive rats (SHROB), were established as a model of human metabolic syndrome [1,2]. They are characterized by hyperphagia and severe obesity (even when fed with standard diet), increased blood pressure, hyperinsulinemia, hyperlipidemia (characterized by a marked triacylglycerolemia and a moderate rise in plasma total and LDL cholesterol), altered carbohydrate and protein metabolism, nephropathy and premature vascular disease [3]. Koletsky rats have a non-functional leptin receptor due to a mutation causing a premature stop codon in the extracellular domain [4]. The mutation in the leptin receptor induces the incapability of leptin signaling leading to an extreme insulin resistance, which results in hyperinsulinemia and glucose intolerance in response to an oral glucose load [5]. Moreover, Molinar-Toribio *et al.* [6] demonstrated significant changes in markers of endothelial dysfunction (increase of intercellular adhesion molecule-1; ICAM-1), thrombotic activity (increase of plasminogen activator inhibitor-1; PAI-1), inflammation (increase of C-reactive protein; CRP) and changes of oxidative stress as well as antioxidant defense in plasma and distinct organs of the Koletsky rats in comparison with the Wistar-Kyoto (WKY) control rats [6]. The aim of our study was to monitor the levels of two oxidative stress markers – reduced glutathione (the main intracellular antioxidant) and thiobarbituric acid-reactive

substances; TBARS (degree of lipid peroxidation damage) in the heart, renal cortex and medulla and liver in lean spontaneously hypertensive rats (SHR) and obese Koletsky rats.

Homozygous male obese Koletsky (fa^k/fa^k) and control sex and age-matched lean SHR littermates were purchased from Charles River (Wilmington, USA). Rats were housed at 23 °C under a 12 h light/dark cycle period, fed a standard chow containing 58 % carbohydrates, 9 % fat, 33 % protein (Spezialdiäten GmbH, Soest, Germany) and given tap water *ad libitum*. All procedures and experimental protocols were performed in accordance with guidelines and practice established by the *Ethical Committee of the Institute of Physiology CAS*, conformed to the *European Convention on Animal Protection and Guidelines on Research Animal Use* (Directive 2010/63/EU). Rats aged 19 weeks were killed by decapitation and tissue samples were collected for analyses. Body weights of the rats were significantly higher in Koletsky rats (516±8 g) compared to SHR control (373±8 g) [7]. The absolute kidney and heart weights did not significantly differ between two genotypes but livers of Koletsky rats were more than twice heavier than livers of SHR [7].

The intracellular content of reduced glutathione in heart, kidneys and liver was determined immediately in fresh tissues colorimetrically at 412 nm according to the methods described earlier [8,9]. Briefly, the tissue samples were homogenized in 3 % sulfosalicylic acid and 10 % homogenates were centrifuged for 10 min at 3000× g. A portion of the supernatant was mixed with 0.02 M 5, 5'-dithiobis-(2-nitrobenzoic acid) in 0.1 M phosphate buffer (pH 8). The absorbance of a colored product was read at 412 nm, the concentration of glutathione was calculated from the standard curve prepared by a serial dilution of 1 mM stock solution. Lipid peroxidation in the samples was monitored by measuring TBARS formation [10]. The frozen-thawed 10 % homogenates were incubated with thiobarbituric and acetic acid at 95 °C for 45 min. The absorbance was measured at 535 nm using Tecan Infinite M200 multimode microplate spectrofluorometer. The results are expressed as the means ± SEM. The statistical differences were evaluated by a paired Student's *t*-test. Values of $P \leq 0.05$ were considered to be statistically significant.

A small molecular weight glutathione produced in the cells effectively scavenges free radicals and other reactive oxygen species directly and indirectly through enzymatic reactions [11,12]. We observed the lowest concentrations of reduced glutathione in the heart and the highest in the liver (Table 1) similarly as we found in our previous studies in different strains of rats, such as Wistar [13], Hannover Sprague Dawley and Ren-2 transgenic rats of both sexes and various ages [9,14] or in salt-resistant and salt-sensitive Dahl rats [15]. In the current study we found significantly lower glutathione levels in the liver of Koletsky rats compared to lean SHR littermates. In contrast to our results Molinar-Toribio *et al.* [6] demonstrated a significant increase in the glutathione level in Koletsky rat liver in comparison with WKY control, similar results were reported for the abdominal fat and brain. However, Molinar-Toribio *et al.* [6] compared Koletsky rats with WKY rats, but we compared obese Koletsky rats with lean SHR – the original rat strain from which Koletsky rats were derived. Moreover, the sex and age of experimental rats were different: female vs. male in our experiments and younger rats (11-14-week-old) vs. older animals (19-week-old) in our experiments.

Concerning TBARS, we found no significant differences in the heart and kidneys between lean SHR and obese Koletsky rats but we found significantly higher content of TBARS in the liver of Koletsky rats compared with SHR (Table 2). Molinar-Toribio *et al.* [6] did not find any significant differences in these three tissues between Koletsky rats and WKY.

Metabolic syndrome is frequently associated with non-alcoholic fatty liver disease (NAFLD), which is mainly characterized by excessive fat accumulation and which affects males more than females. Koletsky rats show hepatomegaly with fatty liver [16]. Similarly, our previous study indicated liver weight more than twice greater and the content of liver triacylglycerols nearly six times higher in Koletsky rats compared to SHR [7]. Méndez *et al.* [17] revealed differences in the proteomic profiles of liver carbonylated proteins belonging not only to lipid metabolism but also to redox regulation and chaperone activity between Koletsky and Wistar female rats. These results show that the final oxidative stress is very complex process and not just a simple imbalance between oxidants and antioxidants [17]. The experiments performed in male and female Koletsky rat liver demonstrated the increased *de novo* lipogenesis. Sex-specific differences revealed a more efficient fatty acid transport and esterification and greater insulin sensitivity in females that facilitate a less severe liver steatosis. Moreover, many hepatic genes involved in lipid biosynthesis and metabolism are regulated differentially

Table 1. Concentration of glutathione (-SH groups) in heart, renal cortex and medulla and liver of SHR and Koletsky rats.

	SHR	Koletsky rats
Number of rats	10	10
-SH in the HEART (μmol/g tissue)	2.06 ± 0.06	2.01 ± 0.03
-SH in the RENAL cortex (μmol/g tissue)	3.73 ± 0.11	3.59 ± 0.07
-SH in the RENAL medulla (μmol/g tissue)	2.85 ± 0.14	2.96 ± 0.14
-SH in the LIVER (μmol/g tissue)	5.83 ± 0.14	5.03 ± 0.23*

All values are means ± SEM. Significantly different: * $P \leq 0.05$ vs. SHR; -SH, thiol groups.

Table 2. Concentration of thiobarbituric acid reactive substances (TBARS) in heart, renal cortex and medulla and liver of SHR and Koletsky rats.

	SHR	Koletsky rats
HEART	42.08 ± 3.42	59.64 ± 11.92
(nmol/mg protein)	(n=5)	(n=5)
RENAL cortex	23.80 ± 0.72	25.89 ± 1.82
(nmol/mg protein)	(n=8)	(n=8)
RENAL medulla	53.68 ± 7.01	59.36 ± 8.07
(nmol/mg protein)	(n=6)	(n=6)
LIVER	21.83 ± 1.60	28.56 ± 2.15*
(nmol/mg protein)	(n=8)	(n=8)

All values are means ± SEM. Significantly different: * $P \leq 0.05$ vs. SHR.

in males and females [16]. We used male rats for our experiments and therefore the differences between SHR and Koletsky rats were significantly pronounced.

We conclude that our results in SHR and Koletsky male rats show the liver as the most sensitive tissue to the oxidative damage with the significantly decreased concentrations of glutathione and the significantly increased concentrations of TBARS.

Conflict of Interest

There is no conflict of interest.

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