Age-related changes in antioxidant defence mechanisms and peroxidation in isolated hepatocytes from spontaneously hypertensive and normotensive rats

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Abstract

The effects of age and hypertension on the antioxidant defence systems and the lipid peroxidation in rat isolated hepatocytes were studied. Four different age groups (1, 3, 6 and 12 months) were considered in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. Age-associated changes were observed on vitamin E status, glutathione (GSH) level, MDA formation and glutathione peroxidase (GSH-Px) activity in both strains. Maximal levels or activities of these parameters were found at 3 and 6 months, except for MDA which was low at 3 months. Then, a fall was observed at 12-month-old compared to 6-month values. In addition, GSH-Px activity was significantly lower in SHR than in WKY rats, except at the age of one month. The decrease of this enzyme activity could induce an increased cellular generation of radical species and lipid peroxidation, which might be link to hypertension. (Mol Cell Biochem 132: 25–29, 1994)

Key words: hepatocytes, Hypertension, age, GSH-Px

Introduction

The free radical theory of aging [1, 2] is a widely accepted explanation for the progressive accumulation of age-related cell constituent damages. Some studies have shown an age-dependent increase of lipid peroxidation in liver and other tissues of rat and mouse [3, 4], and the hepatic dysfunction caused by products of lipid peroxidation [5]. In addition, lipid peroxidation is a causal contributor to the pathophysiology of many diseases [6], including vascular diseases [7], among which hypertension represents a major cardiovascular risk factor [8, 9].

Under physiological conditions, there is a continuous production of reactive oxygen species, controlled by a number of defensive enzymatic and non-enzymatic systems. Among the antioxidant enzymes, glutathione per-
oxidase (GSH-Px) protects the cell from the damaging effects of oxidizing species like organic hydroperoxides and hydrogen peroxide. Non enzymatic defences are provided by antioxidant molecules, the most important being vitamin E and reduced glutathione (GSH) which act as a radical scavenger and a reductant, respectively.

The purpose of this report is twofold, first to investigate the effects of age on the antioxidant defence systems and the lipid peroxidation in rat isolated hepatocytes and second to investigate the effects of hypertension on these parameters.

Materials and methods

Animals

Male spontaneously hypertensive rats (SHR) and male normotensive Wistar-Kyoto (WKY) rats were purchased from IFFA-CREDO (L’Arbresle, France). Animals were fed a commercial standard pellet (Sourriffarat, UAR, Epinay sur Orge, France) and received tap water ad libitum. The composition of the diet was 21% proteins, 53.5% glucides, 4% fats, 4.5% cellulose, 11.5% water, 5.5% ashes, mineral mix (27.8 g/kg), vitamin mix (16,000 IU/kg vitamin A, 2,000 IU/kg vitamin D3 and 170 mg/kg vitamin E). The systolic blood pressure was measured by the tail cuff method [10]. Experiments were performed at different ages (1, 3, 6 and 12 months).

Cells

Isolated hepatocytes were prepared according to the method of Seglen [11] as modified by Skrede et al. [12]. The liver was first perfused in situ through veina porta with perfusion buffer pH 7.4. While still being perfused, the liver is cut from the carcass, and the perfusion is then switched over to collagenase buffer, according to the method of Seglen [11]. The liver is then transferred to a Petri dish and gently dispersed in Krebs-Henseleit solution with a stainless steel comb. The suspension is then purified by a succession of filtration and centrifugation [11] in order to remove the non-parenchymal cells, damaged cells, subcellular debris and small clumps of non perfused tissue. The cells are then resuspended in Krebs-Henseleit buffer as described [12]. Counting and viability of the cells (measured by exclusion of trypan blue) were performed in a hemocytometer and revealed that 90-95% of cells were viable. The final suspension was frozen before analysis in presence of ascorbic acid (0.5%).

Determination of GSH-Px

The enzyme activity was measured on hepatocytes solubilized with lubrol by the method of Paglia and Valentine [13] as modified by Chaudière and Gérard [14]. The measurement was performed at 37° C in Tris-HCl buffer (50 mM), containing reduced glutathione (2 mM), glutathione-reductase (1 U/ml), NADPH (0.14 mM) and tert-butyl hydroperoxide (0.2 mM) as the hydroperoxide substrate. GSH-Px activity was spectrophotometrically determined in following the oxidation of NADPH at 340 nm (spectrophotometer Beckman UV-DU8, Fullerton, USA).

Glutathione measurement

Total glutathione was determined according to the method of Neuschwander-Tetri and Roll [15]. Disulfides were reduced with dithiothreitol and derivatized with ortho-phthaldialdehyde (OPT), and the GSH-OPT complex was quantified by reverse phase high-performance liquid chromatography (rp-HPLC). HPLC was performed using a Kontron LC pump T-414 equipped with a Nucleosil C18 15 cm x 4.6 mm (5 µm particle size) column (SFCC/Shandon, Eragny, France), a Kontron Uvikon 735 LC detector (Kontron, Zurich, Switzerland), connected with a Shimadzu GR3A integrator (Kyoto, Japan). Chromatographic conditions are those reported in [16].

Vitamin E status

Vitamin E determinations were done according to a method previously reported [17]. Briefly, isolated hepatocyte suspensions were mixed with two volumes of ethanol/water (1:1, v/v) containing tocol as an internal standard. After extraction twice with three volumes of hexane, the vitamin E extract was analyzed by rp-HPLC.

Thiobarbituric acid (TBA)-malondialdehyde (MDA) complex determination

Isolated hepatocytes suspensions were treated by