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Stimulation of gene expression and activity of antioxidant related enzyme in Sprague Dawley rat kidney induced by long-term iron toxicity

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ABSTRACT

The trace elements such as iron are vital for various enzyme activities and for other cellular proteins, but iron toxicity causes the production of reactive oxygen species (ROS) that causes alterations in morphology and function of the nephron. The present study was designed to determine the effect of long-term iron overload on the renal antioxidant system and to determine any possible correlation between enzymatic and molecular levels. Our data showed that reduced glutathione (GSH) levels, which is a marker for oxidative stress, strikingly decreased with a long-term iron overload in rat kidney. While renal mRNA levels of glucose 6-phosphate dehydrogenase (*G6pd*), 6-phosphogluconate dehydrogenase (*6pgd*) and glutathione peroxidase (*Gpx*) were significantly affected in the presence of ferric iron, no changes were seen for glutathione reductase (*Gsr*) and glutathione S-transferases (*Gst*). While the iron affected the enzymatic activity of G6PD, GSR, GST, and GPX, it had no significant effect on 6PGD activity in the rat kidney. In conclusion, we reported here that the gene expression of *G6pd*, *6pgd*, *Gsr*, *Gpx*, and *Gst* did not correlate to enzyme activity, and the actual effect of long-term iron overload on renal antioxidant system is observed at protein level. Furthermore, the influence of iron on the renal antioxidant system is different from its effect on the hepatic antioxidant system.

1. Introduction

The kidney is a structurally and functionally complex organ that plays an essential role in mammals, such as the regulation of electrolytes and acid–base balance of blood, resulting in blood pressure homeostasis, the production of hormones, and the reabsorption of water, glucose, and amino acids (Ring et al., 2005; Tokonami et al., 2013). The basic structural and functional unit of the kidney is the nephron. The functional defects of the nephron are related to the toxic damages (toxic metals, pesticides, herbicides, various drugs used in medicine, etc.) or the inherited malfunctions of specific genes and proteins (Sabolic, 2006; Reggiani et al., 2007; Bonventre et al., 2010).

Iron, an indispensable trace element for life due to its crucial functions, was involved in oxygen transport, biosynthesis, respiration, detoxification, and other processes; however, it can also be toxic because of its ability to donate and accept electrons within the cell. Thus, iron homeostasis is tightly regulated to organize a complex biochemical network in the body (Lee et al., 2006; Munoz et al., 2011). It is well published that many biological diseases have been associated with iron deficiency and overload such as anemia (Ganz and Nemeth, 2012), cancers (Durigova et al., 2012; Lalefar and Ozeran, 2012), neurodegenerative disorder (Graham et al., 2006), cardiovascular diseases (Zhao et al., 2010; Arora and Ghali, 2014), and diabetes (Maritim et al., 2003).

The human diet normally contains either organic (Ferrous, Fe^{2+}) or inorganic (Ferric, Fe^{3+}) iron. Although intracellular iron is usually in the ferrous form, non-bound ferric iron is potentially highly toxic to the cells. Thus, Fe^{3+} must be oxidized to Fe^{2+} via the Fenton reaction (Fig. 1). As a result of this reaction, the production of reactive oxygen species (ROS) is induced in the presence of high levels of ferric iron, which can lead to damage of cellular organelles, DNA, enzymes and lipids (Morel and Barouki, 1999; Valko et al., 2005). Thus, preventing oxidative damage via antioxidant system is indispensable for cell survival (Yu et al., 2007).

Several organs including the kidney is a target for iron accumulation. The kidney has a highly active oxidative metabolism. Although renal toxicity induced by iron overload has been recognized, the intracellular mechanisms of this nephrotoxicity are still unclear (Sabolic, 2006). Recent in vivo and in vitro studies have shown that necrosis, oxidative stress, and apoptosis are affected by heavy metals in kidney (Sponsel et al., 1996; Dimitriou et al., 2000; Barbier et al., 2005; Ekong et al.,

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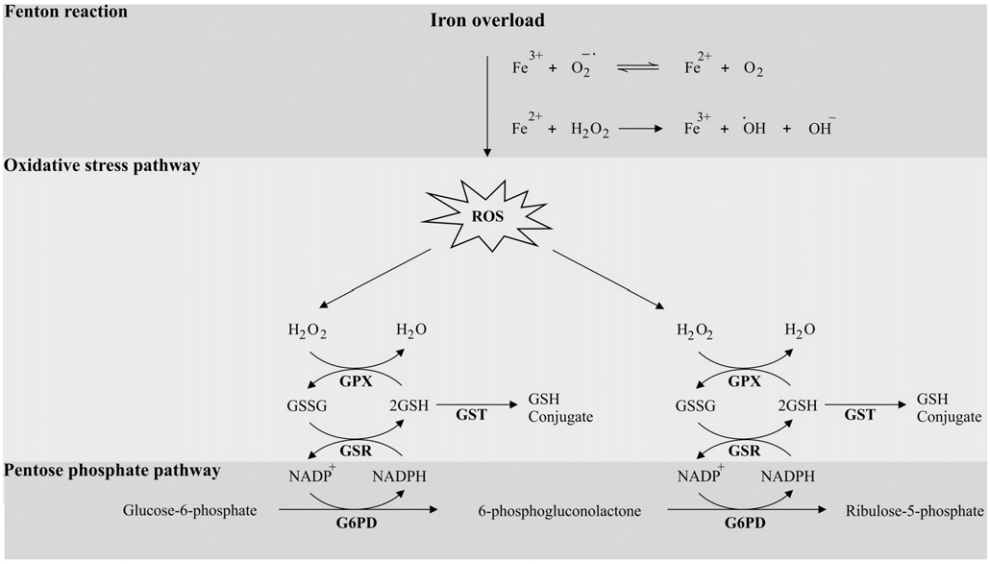

Fig. 1. A simplified scheme of oxidative stress induced by ferric iron. Reactive oxygen species (ROS) are produced in the cells by iron overload activating signaling pathways, such as the pentose phosphate pathway (PPP) and the activation of antioxidant response. This response neutralizes the ROS to protect cells against any damage.

2006; Gobe and Crane, 2010). Histological assessments showed that three month intraperitoneal (ip) administration of ferric iron causes oxidative renal injury in rats and induces nephrotoxicity (Ebina et al., 1986). Ferric iron treatment (ip) causes the rapid and temporary accumulation of membrane lipid peroxidation products in rat kidney, and then the accumulation of iron induces acute renal proximal tubular necrosis resulting in oxidative tissue damage (Fukuda et al., 1996). Four hours after ferric nitrilotriacetate treatment (ip), the blood urea nitrogen and serum creatinine levels markedly increased. However, GSH level and superoxide dismutase (SOD) activity decreased in rat kidneys. It also produced significant renal morphological alterations (Gupta et al., 2009). A recent study has shown that 12 h after the treatment (ip) of ferric nitrilotriacetate (Fe-NTA) it induces tissue necrosis as a result of lipid peroxidation (LPO) and oxidative damage (Ansar et al., 2014). So far, we have not encountered any studies on the impact of long term Fe^{3+} administration on gene expression and enzyme activities of antioxidant system in rat kidney.

A limited amount of ROS is produced in the cell due to normal metabolic processes, but increased ROS production by iron overload causes the depletion of reduced glutathione (GSH) and the inhibition of the activity of several antioxidant enzymes. As seen in Fig. 1, cellular GSH level is important for the reduction of hydrogen peroxide and oxygen radicals via glutathione system to protect cell against ROS. Since any change of GSH level in the cell might be an indication for the accumulation of ROS that induces oxidative stress (Ercal et al., 2001; Xu et al., 2003), GSH/glutathione disulfide (GSSG) ratio is firmly regulated by the antioxidant enzymes including glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione S-transferases (GST) (Akkemik et al., 2012; Karaman et al., 2012). GPX metabolizes hydrogen peroxide to water by using GSH as a substrate. GR catalyzes the conversion of GSSG to GSH, using the cofactor nicotinamide adenine dinucleotide phosphate (NADPH) produced by the pentose phosphate pathway (PPP) with the enzymatic function of glucose 6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) (Johansson et al., 2004; Rhee et al., 2005; Pannala et al., 2013). Thus, the G6PD and 6PGD have also been accepted as antioxidant enzymes like GSR, GPX, and GST (Reiter et al., 1997; Akkemik et al., 2010b; Morgan et al., 2014).

The extent of tissue damage caused by heavy metals depends on the nature, the dose, route, and duration of exposition (Reyes et al., 2013). Most of the heavy metals are not biodegradable and therefore they persist in the environment in the long term (Wang et al., 2003). Thus, the heavy metal toxicity on living organisms is the focus of current intense investigation (Akkemik et al., 2012; Budak et al., 2014a,b). Thus, the objective of this study was to provide a better understanding of how the iron overload affects both gene expression and enzyme activities of some renal antioxidant enzymes and show any possible correlation between enzymatic and molecular levels.

2. Materials and methods

2.1. Iron preparation

The maximum permissible limit (MPL) of iron in drinking water is approximately 0.87 ppm (Jadhav et al., 2007). The World Health Organization (WHO) released the second edition of *Guidelines for drinking-water quality, Health criteria and other supporting information*, Geneva, 1996. In this edition, the median iron concentration in rivers and groundwater has been reported to be 0.5–10 ppm, the concentration of iron can sometimes be found up to 50 ppm. The effects of toxic doses of iron are about 800–2000 mg/kg of body mass in the rat. Iron (III) chloride hexahydrate (Sigma) was dissolved in deionized water (mpMinipure dest up, MES) 1 h before the experiment. The final concentration of solutions was obtained for 0.87, 3, 30, and 300 ppm iron, respectively.

2.2. Animals and experimental design

Male Sprague Dawley rats (*Rattus norvegicus*; n = 15) were purchased from Atatürk University Medical Experimental Application and Research Center. The rats were housed in standard conditions as follows: temperature (22 ± 2 °C), humidity (40–60%), and a 12/12 h light–dark cycle. Before starting the experimental procedure, the rats were acclimated to our facilities for one week. After the acclimation period, the rats were randomly divided into five groups. First group, considered as control, was given only deionized water. Other groups were exposed daily to the different concentrations of iron (0.87, 3, 30 and 300 pmm, respectively) with drinking water for 4 months. The rats were given ad libitum access to water and food. No animal died and showed any visible sign of toxicity. The rats were anesthetized by intraperitoneal injection of ketamine/xylazine cocktail after 4 months