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REVIEW ARTICLE

Oxidative stress and bovine liver diseases: Role of glutathione peroxidase and glucose 6-phosphate dehydrogenase

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Abstract

This article summarizes the different types of free radicals, antioxidants and the effect of oxidative stress on the activities of glutathione peroxidase and glucose 6-phosphate dehydrogenase in bovine liver diseases. A growing body of evidence suggests that the formation of reactive oxygen species is a common occurrence associated with most if not all disease processes. The overall importance of reactive oxygen species to the progression and severity of various disease states varies greatly depending on the conditions and whether the disease is acute or chronic. Free radical researches in animals are in progress and further investigations are needed to establish the involvement of reactive oxygen species in diseases affecting different animal species and the pathology they produce.

Key Words : Antioxidants, Bovine, Free radicals, G6PD, GSH-Px

Abbreviations : Glucose 6-phosphate dehydrogenase (G6PD), Glutathione peroxidase (GSH-Px), Glutathione reductase (GR), Hydrogen peroxide (H_2O_2), Hydroxyl radical ($^{\cdot}OH$), Hypochlorous acid (HOCL), Nicotinamide adenine diphosphate (NADPH), Nitric oxide ($NO^{\cdot}$), Reactive oxygen species (ROS), Superoxide anion ($O_2^{\cdot -}$), Superoxide dismutase (SOD).

Introduction

Oxidative stress is a process based upon the action of free radicals formed by oxygen or other molecules and fragments[42]. Free radicals are highly reactive substances produced continuously during metabolic processes and participate to a great extent in physiological

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events such as immune response, metabolism of unsaturated fatty acids and inflammatory reaction. However, their excess results in impairment of DNA, enzymes and membranes[74] and may induce changes in the activity of the immune system and in the structure of basic biopolymers, which may be related to mutagenesis and aging processes[44,51].

A number of endogenous highly reactive forms of oxygen exist in biological systems, which are summarized in Fig. 1. The most important include superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($^{\cdot}OH$) and hypochlorous acid (HOCL)[67]. HOCL is produced by the reaction of H_2O_2 with chloride ions and plays an important role in the leukocyte respiratory burst, which is involved in the host defense system[40]. Nitric oxide ($NO^{\cdot}$) acts as a free radical and as a biological mediator in biochemical reactions. Physiologically it is synthesized from L-arginine by NO synthase employing cofactor NADPH. In the host, $NO^{\cdot}$ arises in some pathological situations[12].

The oxygen radical is the most important of all biological free radicals. It is a diradical having two unpaired electrons each one located in a separate orbital. All aqueous systems that generate $O_2^{\cdot -}$ ultimately produce H_2O_2[40]. $O_2^{\cdot -}$ is readily converted to H_2O_2 and $^{\cdot}OH$ via Fenton-like reactions, which are catalyzed by redox cycling metal ions, including iron, copper, chromium and vanadium[68]. These metal ions have the ability to accept and donate single electrons, making them important catalysts of free radical reactions, the most widely distributed and most commonly studied transition metal ions are the cations iron and copper[67]. $O_2^{\cdot -}$ induces important reducing reactions in biological materials e.g. it reduces Fe^{3+} in metalloproteins such as ferritin. The reduction of protein bound iron is an important reaction in biological material, because if there is sufficient H_2O_2 available, a Fenton reaction between the resultant Fe^{2+} and H_2O_2 occurs and gives rise to the highly reactive $^{\cdot}OH$[40]. H_2O_2 traverses biological membranes and intracellularly targets phospholipids, carbohydrates, metalloproteins and DNA, and causes damage via Fenton reactions[55].

Oxidative stress results when reactive forms of oxygen are produced faster than they are safely neutralized by antioxidant mecha-

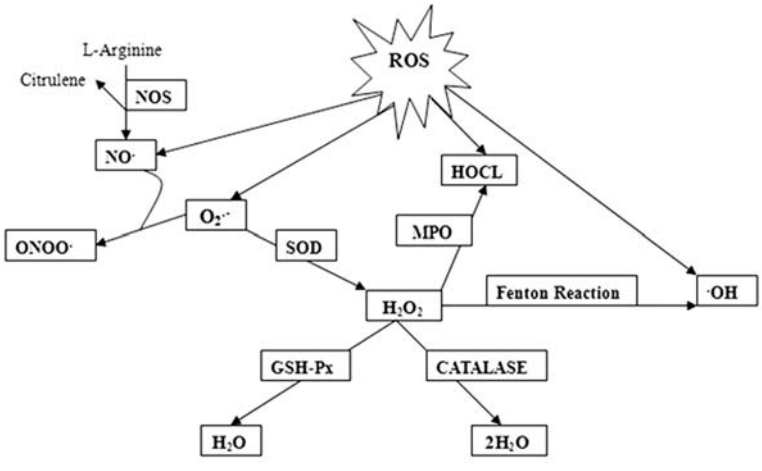

Fig. 1. Shows different types of reactive oxygen species (ROS). Abbreviations : GSH-Px. glutathione peroxidase ; HOCl. hypochlorous acid ; H_2O_2. hydrogen peroxide ; MPO. myeloperoxidase ; NO. nitric oxide ; NOS. NO synthase ; $O_2^{\cdot -}$. superoxide anion ; $^{\cdot}OH$. hydroxyl radical ; ONOO. peroxynitrite anion ; SOD. superoxide dismutase.

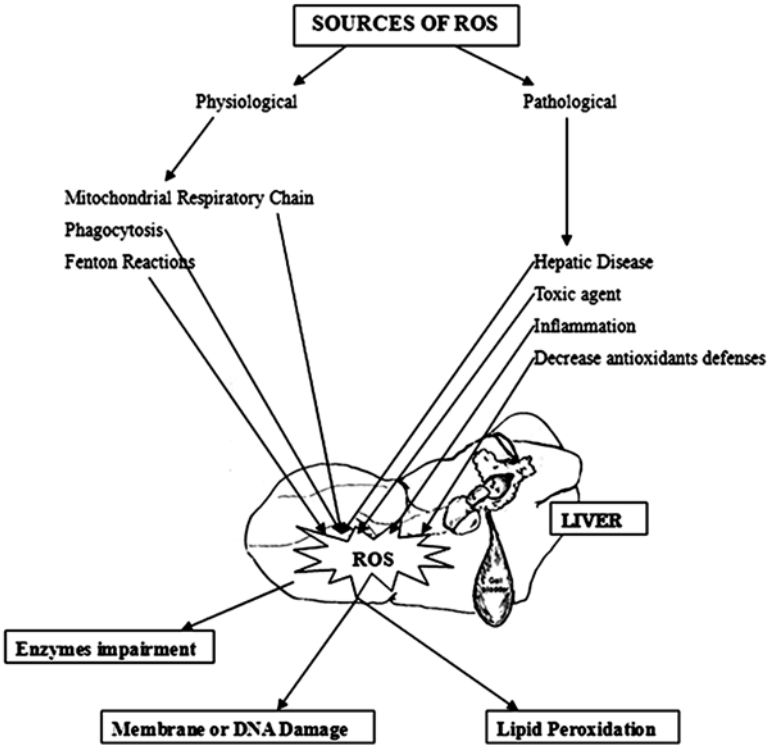

Fig. 2. Sources of reacive oxygen species (ROS) in the liver.

nisms[63] and/or from a decrease in antioxidant defense that may lead to damage of biological macromolecules and disruption of the normal metabolism and physiology[72]. Although a growing body of evidence indicates that free radicals and/or reactive oxygen species may be involved in toxicity and disease, it is not clear whether reactive metabolites and/or reactive oxygen species are early events that lead to the compromise of defense mechanisms resulting in a cascading response and in cell damage or whether they are late occurring events in biological processes associated with toxicity and disease[67].

I. Antioxidants and free radicals

Antioxidants are agents that scavenge free radicals, prevent their formation or repair the damage they cause[29]. Free radical scavengers and inhibitors of lipid peroxidation (antioxidants) play a central role in protection against reactive oxygen species[50]. Pathological free radical reactions do not necessarily cause cell and tissue damage, as antioxidants of cells and tissues are able to prevent free radical injury[26]. The antioxidant system consists of antioxidant enzymes, including superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px), glutathione, ancillary enzymes such as glutathione reductase (GR), glutathione S-transferase and glucose 6-phosphate dehydrogenase (G6PD), metal-binding proteins such as transferrin, ceruloplasmin and albumin, vitamins such as alpha-tocopherol, ascorbate and beta-carotene, flavonoids and urate[30]. Antioxidants may act by directly scavenging the radicals, sustaining the activity of antioxidant enzymes or inhibiting the activity of oxidizing enzymes[61]. On the intracellular level, ROS formation and metabolism can be summarized as shown in Fig. 1.

I . 1 . Glutathione peroxidase (*GSH-Px*)

Glutathione is an important non-protein thiol in cell systems that influence various cell functions. It occurs mainly intracellularly, primarily in the reduced form. Reduced glutathione (GSH) fulfills several essential functions such as detoxification of oxygen-derived free radicals and storage and transfer of cysteine[37]. Glutathione protects cells against reactive oxygen species by a variety of mechanisms, one mechanism is the GSH-Px reaction[20]. There are two types of glutathione peroxidase; selenium dependent and selenium independent, which differ in their requirement for selenium, which is an essential component of selenium dependent GSH-Px[36]. The selenium dependent enzyme (GSH-Px) is active with both hydrogen peroxides and organic hydroperoxide. The selenium independent enzyme also possesses glutathione peroxidase activity and is active with organic hydroperoxide, but not with hydrogen peroxide. This selenium independent activity is principally expressed by the alpha class of glutathione S transferase (GST)[31]. It appears that glutathione peroxidase activity of both types of enzyme is important in cellular defense against a wide variety of hydroperoxides[41].

Tissues having both types include liver, lungs, adrenal glands, testis, kidney medulla and kidney cortex, however; spleen, cardiac muscles, erythrocytes, brain, thymus, adipose tissue and striated muscles of calves contain only the selenium dependent GSH-Px[61]. Liver possesses the greatest GST activity and spleen the greatest GSH-Px activity. The adrenals contain large amounts of these glutathione-dependent enzymes, but significant differences are observed between the cortex and medulla[31]. Furthermore, approximately 98% of GSH-Px activity in peripheral blood is associated with erythrocytes[59]. GSH-Px activities are considerably high in ruminants[69].

GSH-Px detoxifies peroxides in the cell (Fig. 1). As peroxides decompose to form highly reactive radicals, GSH-Px plays a critical role in protecting the cell from free radical damage, particularly lipid peroxidation[41]. The cellular GSH-Px system is a highly efficient and indispensable constituent of cellular mechanisms protecting against oxidative stress[73].

I . 2 . Glucose 6-phosphate dehydrogenase (*G6PD*)

Glucose 6-phosphate dehydrogenase is the first enzyme in the pentose phosphate pathway. It is widely distributed in nature, being found in almost all animal tissues and microorganisms. It is present in large amounts in blood cells, smaller amounts in liver, kidney and heart and in trace amounts in serum[21]. As shown in Fig. 3, G6PD catalyzes an oxidation/reduction reaction, which transfers electrons from one molecule to another, where G6PD catalyzes the oxidation of G6P to 6-phosphoglucolactone, while concomitantly reducing $NADP^+$ to NADPH[75]. G6PD may be

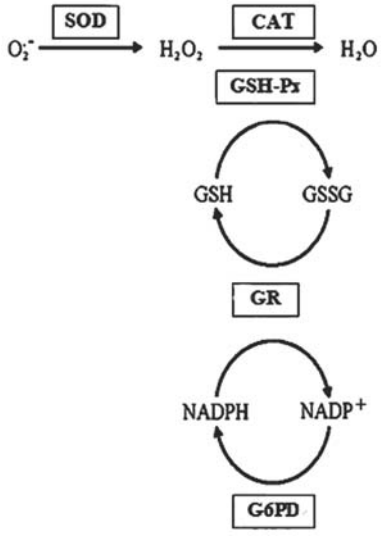

Fig. 3. The relation between G6PD, glutathione peroxidase (GSH-Px), catalase (CAT), glutathione reductase (GR) and NADPH.

responsible for the antioxidant function, as generation of NADPH is required for the activity of GR and GSH-Px[58]. NADPH is used to keep glutathione (a tri-peptide) in its reduced form, GSH acts as a scavenger for dangerous oxidative metabolites in the cell and it converts harmful hydrogen peroxide to water with the help of the enzyme GSH-Px[32,75]. Recent investigations have demonstrated that G6PD plays a protective role against reactive oxygen species in cells that possess alternative routes for the production of NADPH[54]. In addition, G6PD may play a dominant role in controlling the output of GSH and thus in maintaining the intracellular redox potential[54].

The activity of G6PD is much higher in camel and buffaloes compared to that in other ruminants such as sheep, goats and cattle[3]. The liver and heart of cattle have the largest specificity of G6PD activity[71]. Clinically, the red blood cell G6PD is of great importance, since erythrocytes lack the citric acid cycle, depending on the pentose phosphate pathway as the only supply for NADPH[21].

GSH-Px and G6PD are two enzymes that protect cells from oxidative damage and are used in many studies either simultaneously or in association with other antioxidants enzymes as indicators for oxidative stress in a variety of diseases such as hepatic dysfunction in cattle[1,2,11,56] and rats[65,66], kidney diseases in humans[23] and rats[25,62], parasitic infestations in cattle[7,17,27,43], cattle mastitis[24,48], carcinogenesis[5,53] and in cows exposed to stress[8].

II. The role of free radicals in hepatic dysfunction

The liver is well protected against free radicals. It is one of the best antioxidant supplied organs. An important function of the liver is the detoxification of drugs, chemicals and toxic materials, with the subsequent release of free radicals[26,46]. Reactive oxygen intermediates are formed in many parts of liver cells. A balance between free radical reactions and antioxidant activities is very important for normal liver functioning. This balance is altered in pathological processes[10]. The production of free radicals has been implicated in a variety of liver diseases where they can damage cellular macromolecules and therefore, may participate in hepatocellular injury. In addition, free radical-initiated lipid peroxidation may play a role in hepatic fibrogenesis, perhaps through an effect of aldehydic peroxidation products on Kupffer cells and lipocytes[13].

Oxygen free radicals might play a role in the pathogenesis of tissue damage in many pathological conditions including liver diseases where antioxidant tissue systems are reduced. The leading mechanism of free radical toxicity is the peroxidation of membrane phospholipids[6,9,51]. Lipid peroxidation is initiated by the formation of lipid peroxide or hydroperoxides. Peroxy radicals are formed in the presence of oxygen to start a chain reaction (propagation). Various pathogenic effects occur as a result of the degradation of membrane lipids. The interaction of degradation products with various cellular macromolecules and the production of new reactive oxygen species during the course of the chain reaction process[67] may lead to membrane damage, protein damage, enzyme dysfunction and DNA or RNA damage[74]. Sources of reactive oxygen species (ROS) in liver tissue are summarized in Fig. 2.

The importance of oxidative stress, including lipid peroxidation, in the physiopathology of liver diseases has been established. Studying oxidative stress in the hepatic dysfunction of cattle revealed a significant increase in hepatic G6PD activity and a significant decrease in hepatic GSH-Px activity in

cows suffering from severe fatty degeneration[2], which indicates increase in the intensity of the hepatic lipoperoxidation process and a low antioxidative status[45]. The significant decrease in GSH-Px activity in the case of fatty liver may be attributed to increased free radical stress in the liver tissue, which inhibits the enzyme activity[2]. It has been reported that increased oxidative stress results in impairment of enzymes containing thiol groups and cell membranes[44,51]. Free radicals can oxidize proteins, the amino acids being oxidized to their hydroxy derivates; for example, phenylalanine can be oxidized to hydroxyphenylalanine, such oxidation can cause enzymatic inactivation[19]. It has been reported that G6PD activity in blood and in liver tissues may serve as a useful biochemical test specific for fatty liver in cows[11]. Elevated expression of G6PD is also important in the support of major antioxidant pathways, as the generated NADPH is the reducing coenzyme for peroxidases in the case of fatty liver[65].

Liver abscesses in fattening steers occur mainly due to intensive feeding of highly concentrated rations[39]; consumption of a carbohydrate-rich diet stimulates G6PD expression in endothelial and parenchymal cells[35,65]. Since G6PD supports reactive oxygen metabolism, the response may represent an antioxidant pathway in the hepatic cell populations that target sinusoid born reactive oxygen species during infections[1,65].

Underfeeding in cattle induces changes in the antioxidant systems in liver manifested by lowering hepatic G6PD and SOD activities, which result in depletion of antioxidant defense mechanisms and render the hepatocytes more susceptible to the lethal effects of endogenous or exogenous peroxides, and indicates that the generation of lipid peroxides in cattle in poor nutritional condition exceeds the antioxidant capacity of the liver cells, generating a situation of oxidative stress and peroxidation[56].

Increased oxidative stress in hepatic dysfunction has also been reported in patients with cirrhosis and hepatocellular carcinoma, shown by the decrease of GSH-Px activity in cirrhotic and liver cancer tissues compared with adjacent normal liver tissue[15]. In addition, the hepatic activities of SOD and GSH-Px are decreased in patients with chronic hepatitis C (CHC), which reflects both a decrease in the synthesize capacity of liver and the antioxidant defense of patients with CHC. Increased lipid peroxidation may be caused by inflammation related to viral infection and decreased antioxidant levels may be an early marker of oxidative stress. The lipid peroxides formed may be chemotactic for the neutrophils causing increased inflammation, which further drives oxidant-mediated injury in the liver[22]. In rats, G6PD activity significantly increases in liver when macronodular cirrhosis is induced by long-term thioacetamide administration[57].

The effect of hepatic dysfunction on erythrocytic oxidative status was studied in cows through measuring the erythrocytic activities of GSH-Px and G6PD. The results reveal that there are no effects for hepatic glycogen degeneration, fatty degeneration[2] or liver abscesses[1] on erythrocytic oxidative status. Many studies have been performed on humans to determine the effect of hepatic dysfunction on erythrocytic oxidative stress; some of these studies have reported non-significant differences in red cell GSH-Px activity between liver cirrhosis and alcoholic liver disease compared with healthy controls[4,34,70]. However, other studies have reported that red cell GSH-Px concentrations are significantly lower in the patients with chronic liver disease compared with the controls[16,28]. Furthermore, GSH-Px activity was

diminished in the blood serum and the erythrocytes in patients with abnormal liver function[14]. Lower activities of erythrocyte GSH-Px and SOD activities have been reported in patients with acute hepatitis B[47]. On the other hand, impaired pentose phosphate pathway metabolism has been reported to be a common, acquired RBCs abnormality in patients with severe liver disease[64]. The cause of such contradictory results may be related to the degree of hepatic dysfunction or the presence or absence of selenium deficiency. Significant decreases in plasma selenium level and erythrocyte GSH-Px have been reported in patients with chronic liver disease[16]. In rats, mean erythrocyte GSH-Px and SOD activities have been reported to be significantly lower in the CCl4 induced group compared with the control group[33].

It has been demonstrated that G6PD activity is strongly elevated in chemically induced hepatocellular carcinoma in rat liver and that the characteristics of the activity of the enzyme are dramatically changed in these lesions[18]. The SH groups in G6PD protein, which are essential for its activity, may have been altered in the lesions. The role of G6PD in proliferation has been shown in studies using the non-competitive G6PD inhibitor dehydroepiandrosterone. Proliferation of cancer cells is slowed down by G6PD inhibitor[49]. Moreover, it has been demonstrated that the majority of ribose recovered from the nucleic acids of tumor cells arrives directly or indirectly through the non-oxidative part of the pentose phosphate pathway controlled by transketolase activity[52]. Therefore, it seems unlikely that G6PD is important for the supply of riboses for the proliferation of (pre) neoplastic cells. Furthermore, the formation of NADPH may induce tumor growth by the influence of the redox state on transcription factors as proposed by Kuo et al.[38].

Conclusion

This paper discusses the effects of bovine liver diseases on the activities of glutathione peroxidase and glucose 6-phosphate dehydrogenase. Two enzymes (GSH-Px and G6PD) are examined in relation to the oxidative stress in the blood and hepatic tissues of cattle suffering from liver disease, which indicates increased oxidative stress in the liver tissues in some hepatic dysfunction but not in the blood. However, further research is required to elucidate the status of the two enzymes in different degrees of hepatic dysfunction and in various hepatic diseases.

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