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Enzymatic Antioxidants Activity in Beta Thalassemia Major

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Abstract : Thalassemia is genetic disorder caused by globin mutation that reduces synthesis of globin chains. Chronic anemia is main character of beta thalassemia major that in some occasions required multiple transfusion to overcome the low hemoglobin level. This repeated treatment results in iron overload that is responsible to catalyze the production of reactive oxygen species, ROS. Antioxidants prevent further impact of ROS, firstly by iron scavenging.Superoxide dismutase plays role as the first line defense, while glutathione peroxidase plays important role in erythrocyte defense. Catalase, thioredoxin and peroxiredoxin are also included in enzymatic antioxidant system against ROS in beta thalassemia major.
Keywords : Thalassemia major, multiple transfusion, iron overload, enzymatic antioxidant, oxidative stress.

Introduction

Thalassemia is one of hemoglobinopathy with high prevalent inthe world. Around 56.000 people are diagnosed to have thalassemia major, while half of which are beta thalassemia major[1]. Beta thalassemia major is characterized by chronic anemia as a result of unbalancehemoglobin (Hb) structure. The structure has excess α-globin chain that remains unpaired and transformed into hemichrome when erythropoiesis occurs. The hemichromes bind to membrane protein and trigger coagulability factors activation. Hb dissociation also leases iron from heme and induces synthesis of reactive oxygen species (ROS)[2].

Iron is found mainly in erythrocytes complex and bound to heme in hemoglobin structure.When erythrocytes rupture, hemoglobin is recycled by macrophages in the reticuloendothelial system. Iron is released by heme oxygenase (HO-1) and bound to transferrin or ferritin pools. Iron has ability to play role in redox activity, either in ferrous (Fe^{2+}) or ferric (Fe^{3+}) form. Iron also catalizes generation of free radicals by interacting with superoxide radicals, a primary free radicals that is produced naturally in normal metabolism. Fenton reaction and Haber-Weiss reaction are known to be common mechanisms for iron to generate hydroxyl radicals. It is dangerous that iron in high level triggers more oxidative damages in cellular activity. Therefore,regarding ironquantity and distributionis necessary to control its toxicity in the body[3].

Unstable Hb structure and multiple transfussion allows high production of ROS resulting in systemic tissue damage. Heme and iron from degraded Hb can exacerbate oxidative stress in beta thalassemia major[4]. ROS which is produced from several metabolism reactions, including ineffective erythropoiesis and hemolysis, continuously exposes erythrocyte. Several enzymatic antioxidants that play role in erythrocyte are catalase, glutathione peroxidase, and peroxiredoxin-2. Under hypoxic condition, autooxidation of Hb occurs and triggers ROS generated more. Over production of ROS is early step to cause cellular damage by oxidative stressenhancing erythrocyte aging[2,5]. Oxidative stress in thalasemia is a result of iron overload. This is due to short survival of mature red blood cells (RBCs), hemolysis, multiple blood transfusion, and high absorption and accumulation of iron. Treatment of iron chelators and antioxidants, either separately or in combination, helps to improve oxidative stress[6].

Requirement of Transfusion and Iron Metabolism

In beta thalassemia major, erythrocytes are produced in massive capacity but do not mature, and mostly die in erythroblast stage[7].Multiple blood transfusion is acquired to overcome low Hb level. Iron overload is known to be negative side effect of multiple transfusion. The iron in transfused blood is absorpted by digestive organ and cause accumulation of iron that catalyzes ROS production. This condition supports creating pro-oxidantenvironment and facilitates oxidative stress. Oxidative stress can cause growth failure, liver, cardiovascular, endocrine, and other complications in beta thalassemia major patients[8].

Main mechanism in generating ROS is ineffective erythropoiesis which causes chronic anemia and iron overload in thalassemic patients. ROS which is generated from free globin chains and labile plasma iron (LPI) contributes to oxidative damage in cells and tissues. These damages can exacerbate complications in thalassemic patients.In patient with thalassemia major, reticuloendothelial system and parenchyma are targets of iron overload. The rate of iron loading in thalassemia major ranging between 0.3 and 0.6 mg/kg/day.Major cause of mortality in transfusion dependent thalassemia (TDT) is cardiac siderosis[9].Excessive iron is known to be accumulated in some organs, such as liver, heart, endocrine glands, resulting oxidative damage and severe complication in patients with thalassemia major[10].

During erythropoietic, erythroid regulator synthesizes erythroferrone (ERFE), a protein supressing hepcidin synthesis. In this condition, iron level increases as preparation to produce erythrocytes. Unfortunately, in thalassemia major, this is pathological mechanism of EFREin triggering iron overload[11]. Hepcidin binding to ferroportin plays important role in iron metabolism.When erythrocytes undergo hypoxia, hepcidin level decreases, stabilizes ferroportin that promotes iron absorption increases, and activity of reticuloendothelial system releasing iron increases.Non-transferrin bound iron (NTBI), whichis form of excess iron, has strong ability to catalize formation of formation of hydroxyl radicals[9,10]. Production of hepcidin increases as deposit iron level higher. This condition makes ferroportin internalized and degraded[12].

Iron Overload and ROS

Free radicalis molecule with unpaired electron that is considered to be reactive in certain environment. ROS is a group of reactive molecule in pro-oxidant environment that is responsible to cause oxidative damage. Free radicals in biologicalsystems include superoxide ion radical ($O_2\bullet^-$), hydroxyl radical (OH•), peroxyl (ROO•), alkoxyl radicals (RO•) and a single oxygen ($^1O_2$) (Fibach, 2010).The superoxide ion radical is primary product of oxidative reaction.This ROS should be neutralized by superoxide dismutase (SOD), unless it produces H_2O_2, a nonradical ROS. In pro-oxidant environment, iron catalyzes H_2O_2 to produce hydroxyl radical by either Fenton reaction or Haber–Weissreaction. Hydroxylradicals cause oxidative damage more than superoxide radical, as no radical scavenger known to cut its reaction.Production of ROS can be limited by ironchelators[13].

Oxidative damage can be induced as high level iron migrates to some organs.Iron from blood transfusion is absorpted by intestine to liver, and accumulated in the reticuloendothelial system and then transferred to parenchymatous organs, such as the heart and endocrine organs. Therefore, myocardiopathy, liver cirrhosis, and endocrine complications are among the long term consequences of iron overload. The excess iron is deposited in transferrin. When transferrin reaches its saturated capacity, iron is exported as non-transferrin bound iron (NTBI) which is highly toxic[14] and its redox active form labile plasma iron (LPI) in the plasma and as labile iron pool (LIP) in the cells[15]. Ferritin has capacity as iron storage up to 4500 iron atoms. Some proteins contribute in iron transport, such as transferrin (Tf), Transferrin receptor (Tfr), Divalent transporter 1 (DMT1), heme carrier protein (HCP1), and ferroportin (FPN)[16]. Iron exporter is function of FPN, while cell membrane iron transportation is function of Tf, Tfr, and HCP1[17].

Erythrocyte is responsible to transport oxygen and carbon dioxide around the body. O_2 binding affinity is deacreased when heme iron (Fe^{2+}) is oxidized to iron (Fe^{3+}) to form methemoglobin[18]. In reduced level of Hb, iron within prostethic group group of Hb need to be noticed because of its ability to catalyze ROS production[19]. Hemoglobin is considered to be source of oxidants in erythrocytes. In 24 hours normal cycle, 3% of Hb undergoes autooxidation and generates superoxide radicals. Ferric form from methemoglobin is also oxidant source that has possibility to produce hydroxyl radicals through Haber-Weiss and Fenton reaction. Haber-Weiss reaction is limited by activity of ferritin. Reaction between Hb and H_2O_2 contributes to heme degradation and release of free iron[18].

Enzymatic Antioxidants

Antioxidant is defensive system in the body against ROS. There are two type of antioxidants, enzymatic and nonenzymatic antioxidants that are either obtained from the diet or from selfsynthesis[20]. Enzymatic antioxidants remove ROS by metabolic conversion. Superoxide dismutase, glutathione, catalase, and thioredoxin systems are the main cellular enzymatic antioxidants systems[21] (Figure 1).Enzymatic antioxidant system is considered to be biomarker for oxidative stress, including in thalassemia. Erythrocytes is known to have selfprotective enzymatic antioxidants systems, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), and nonenzymatic antioxidants systems, including vitamins EandC. This enzymatic mechanisms allow erythtocytes to minimize oxidative damage by ROS when ineffective erythropoiesis and hemolysis occur. Furthermore, it is helpful to prevent other tissues and organs damage[22].

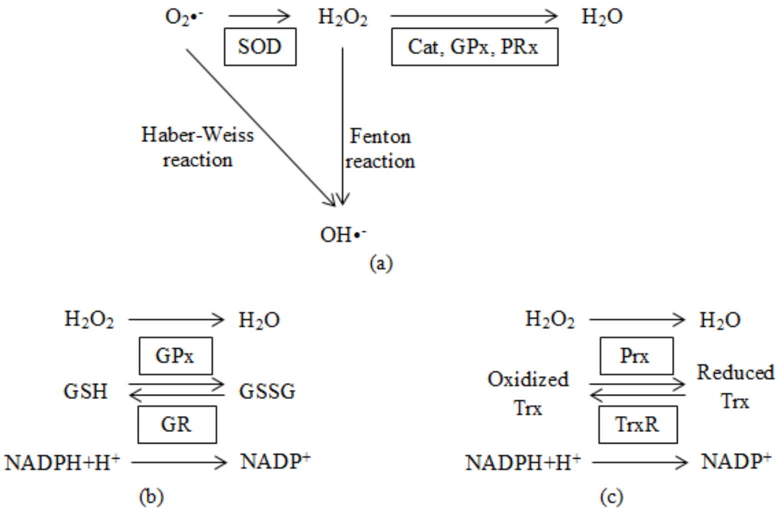

Figure 1. (a) Scheme of enzymatic antioxidants against ROS production; (b) Mechanism of glutathione system; (c) Mechanism of thioredoxin system.

Superoxide Dismutase

Superoxide radical is generated naturally by respiration cycle and stay for long time in body. Superoxide dismutase is responsible to catalize superoxide radicals to hydrogen peroxide. Iron in ferric form (Fe^{3+}) reacts to superoxide radicals, resulting Fe^{2+} and oxygen by Haber–Weiss reaction. The reduced iron then reacts to hydrogen peroxide to produce Fe^{3+}, oxygen hydroxide, and hydroxyl radicals by Fenton reaction[6]. Hydroxyl radicals is very speedy reacting with the surrounding molecules, including protein, lipid or DNA[16].The higher iron produced, the higher DNA damage occurs[23]. SOD activity can be found in mitochondrial, cytoplasmic, and extracellular.Superoxide is produced by one electron reduction of oxygen. Accumulaton of superoxide is prevented as its harm to cause oxidative stress[24].

SOD1 (CuZn-SOD), SOD2 (Mn-SOD), and SOD3 (EC-SOD) are three forms of SOD. SOD1 is primary antioxidant to maintain erythrocyte lifespan. Under lack of SOD1 condition, erythrocyte is more fragile to suffer oxidative damage, resulting in ineffective erythropoiesis[25]. SOD1, SOD2, and SOD3 exists exclusively in intracellular cytoplasmic spaces, mithocondrial spaces, and extracellular spaces, respectively[26]. Pavlova et al.investigated SOD level in beta thalassemia major patients decreases more than 30% compared to controls[27]. SOD level in thalassemic patients can reach 1,5times lower than control[28]. High production of ROS might deplete SOD activity and cause oxidative stress.

Glutathione

Enzymatic system of glutathione is known to be major protective antioxidant mechanism. Glutathione in reduced form, GSH, donates electron to scavenge ROS by activity of some enzymes, including glutathione peroxidase (GPx) and glutathione-S-transferase (GST). Consequently, GSH is oxidized to GSSG. Glutathione reductase (GR) enables GSSG to convert to GSH. Ratio of GSH/GSSG is commonly used as indicator of oxidative stress. The more GSH converts to GSSG, the higher oxidative stress indicated. The intracellular GSH level depends on levels of GSH synthesis, utilization, and recycling. GPx and GST, two enzymes in glutathione system, have different mechanism against ROS. GPx plays role as antioxidant that reduce H_2O_2 to H_2O, while GST plays role in detoxifying xenobiotics, including metabolites from oxidative reactions[20]. The study of GPx activity was conducted and showed beta thalassemia major had lower GPx level than controls. Excessive hydroxyl radical production inhibites GPx activity[29,30].

Catalase

Catalase is intracellular enzyme containing four porphyrin heme groups[31]. This enzyme catalyzes H_2O_2, product of SOD acticity, to H_2O in cells. The lower activity of catalase, the higher H_2O_2concentration[32]. This condition is in accordance with oxidative stress condition and causes damage of oxidation sensitive tissues that may contribute to the manifestation of various diseases such as diabetes mellitus and anemia[33]. Catalase activity in beta thalassemia patients was lower than control. Activity of lipid peroxidation is considered to be associated with catalase activity[34].

Thioredoxin

Another protective enzymes in RBCs is thioredoxin system[13]. This system includes thioredoxin (Trx), thioredoxin reductase (TrxR, TXNRD, TXN, TR) and NADPH, that is responsible to reduce oxidized proteins and role as electron donor to some enzymes, such a peroxiredoxin[35]. Oxidized form of thioredoxin is reduced by thioredoxin reductase, a selenium-containing flavoprotein[36].Peroxiredoxin (Prx) responses to catalytic activity of hydrogen peroxide[37]. A pair of cysteine residues controls Trx activity in its active site, which exists in the oxidized (disulfide) or reduced (dithiol) state. This redox mechanisms allows Trx to maintain oxidative status in cell[38]. Thioredoxin system is essential by its antioxidative, protein-reducing, and signal-transducing activities to maintain redox status, immune function, and other diseases including cardiovascular disease[39].Previous study shows Trx level was lower in beta thalassemia patient. Decreased activity of Trx indicates higher oxidative stress occurs[36].

Conclusion

Heme undergoes degradation and releases iron catalyzing ROS production. Multiple transfusion should be received to increase Hb level in thalassemic patients. Negative effect of iron content in blood transfusion should not be neglected. Frequent transfusion allows iron absorption increased and contributes in generating higher level of ROS. SOD and GPx are main free radicals scavengers in erythrocyte. Oxidative damage begins when antioxidants activity is lower than free radical attacks. Thus, patients with beta thalassemia major might suffer liver, heart, endocrine glands dysfunction and other clinical complications. Advanced research of iron chelation is still in progress and is important to remark.

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