Role of Alpha Lipoic Acid in Oxidant /Antioxidant Status and Gene Expression of Glutathione Reductase in Hydrogen Peroxide Exposed Rats: (Part -2)

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Summary

This study was designated to evaluate the protective role of alpha lipoic acid against oxidative stress resulted by hydrogen peroxide on some oxidants/ antioxidants parameters and gene expression of glutathione peroxidase in adult Wistar rats. Forty adult male rats were randomly divided into four equal groups (10 rats /group) and were handled daily as follows for 56 days : Control group were intubated distal water and received ordinary tap water ; group T1 were intubated 60mg/kg B.W of alpha lipoic acid and received ordinary tap water ; group T2 were received hydrogen peroxide in tap water at concentration of 0.5% , while group T3 were intubated 60mg/kg B.W of alpha lipoic acid and received ordinary tap water containing 0.5% hydrogen peroxide. Fasting blood samples were collected at 0, 28 and 56 days of experimental periods for measurement of serum peroxynitrite and malondialdehyde concentrations, as well catalase activity. Furthermore, gene expression of glutathione reductase in liver was investigated. Administration of 0.5% hydrogen peroxide in drinking water (group T2) manifested a significant elevation in serum peroxynitrite and malondialdehyde with significant decrease in catalase and Glutathione, concentrations. Also, a significant decrease in gene expression of glutathione reductase was observed as compared to other treated groups. Nevertheless, rats in group T3 shows a significantly improvement in oxidant /antioxidant status with increase in folds changes of gene expression of glutathione reductase as compared to control and T2. In conclusion, supplementation of alpha lipoic acid to rats significantly reduced oxidative stress –induced by hydrogen peroxide and caused improvement of gene expression of glutathione reductase in liver via its antioxidant properties.

Keywords: Hydrogen peroxide, Alpha lipoic acid, Gene expression, Glutathione reductase.

Introduction

Reactive oxygen species (ROS) comprise both free radical and non-free radical oxygen containing molecules such as hydrogen peroxide (H2O2), superoxide (SO -), singlet oxygen (1/2O2), and the hydroxyl radical (•OH-),), there are also cellular reactive nitrogen (RNS) such as nitric oxide (•NO), peroxyl radicals (•ROO-), peroxynitrite (•ONOO-) , as well as iron, copper, and sulfur species which could attribute to increased ROS formation (1and 2). Furthermore, an evidence indicates that healthy cellular metabolism requires the generation of ROS by mitochondria, which plays a critical role in initiating cell death (3).

Oxidative stress (OS) is a state related to increased cellular damage caused by ROS, that is reflects an imbalance between the systemic ROS and the ability of biological system to detoxify or to repair the resulting damage (4). Activation of these ROS by different extracellular or intracellular stimuli, caused an elevation in ROS production (5and 6) .

Alpha lipoic acid is a naturally produced in small amounts by plants, animals, and humans (7and 8). It was found to be a co-actor for many mitochondrial enzyme complexes that are involved in energy production (9).Alpha Lipoic Alid (ALA),
, is a medium chain fatty acid with two sulfur atoms that is synthesized within human mitochondria by lipoic acid synthase (10). It was shown to have powerful antioxidant

abilities, equal to that of coenzyme Q 10, vitamin C, and vitamin E (11). Unlike other antioxidants, ALA has the unique ability to neutralize free radicals within aqueous and lipid regions of the cells, as well as in intracellular and extracellular environments (12). Both the oxidized (LA) and reduced (DHLA) forms of lipoic acid are capable of scavenging hydroxyl and nitric oxide radicals, peroxynitrite anions, and hydrogen peroxide and extinguishing single oxygen atoms. ALA caused modulation single transduction (13 and 14), up regulates antioxidant enzyme gene expression (15), metal chelator (16) and an anti-inflammatory (17).

Protection effect of antioxidant against the damaging effects of free radicals is carried out by enzymatic like catalase, glutathione reductase (GR), glutathione peroxidase (GPx) and superoxide dismutase (SOD) and non-enzymatic antioxidant system chains such as lipoid acid, L-arginine, coenzyme Q10, uric acid, bilirubin, albumin, and transferrin, vitamins A, E, and C, flavonoids, omega-3 and omega-6 fatty acids (18 and19). Meanwhile, glutathione system play an important place in this situate. In this system, glutathione peroxidase supply detoxification of inorganic and organic peroxides by using reduced glutathione (GSH). The regeneration of oxidized glutathione is carried out by GR which uses NADPH as reduced equivalents (20 and 21). Therefore, the current study was designated to explore the protective role of ALA against oxidative stress-induced by hydrogen peroxide (H_2O_2) on some oxidants/ antioxidants parameters and gene expression of glutathione peroxidase in adult Wistar male rats.

Materials and Methods

Forty adult male rats were randomly divided into four equal groups (10 rats /group) and were handled daily as follows for 56 days : Group C, rats in this group were intubated distal water plus received ordinary tap water and served as control ; Group T1 , rats in this group were intubated 60mg/kg B.W. of ALA as well as received ordinary tap water ; Group T2, rats were administered H_2O_2 in tap water at concentration of 0.5% and Group T3, rats in this group were intubated 60mg/kg B.W. of ALA and received ordinary tap water containing 0.5% H2O2.

Rats were deprived overnight; blood samples were collected and transferred to a gel tube without anticoagulant at zero, 28 and 56 days of experiment. Sera were isolated and frozen at -18 C until analysis to estimate the following parameters including: Peroxynitrite concentrations (22), malondialdehyde (MDA) (23), while, catalase as described by (24). Whereas, at the end of the experiment gene expression of GR in the liver tissues of all experimental groups was estimated using enzymatic kits including : (Total RNA Extraction Kit, AccuZolTM , Bioneer, Korea ; DNase I enzyme kit , promega, USA.; AccuPower® RocketScriptTM RT PreMix 96 plate, Bioneer, Korea and AccuPower ® Greenstar TM qPCR PreMix 96 plate , Bioneer, Korea . The real time (PCR) primers that used in this study were designed by using (National Centre for Biotechnology Information (NCBI) Gene Bank. The Primer pairs were design online and supported from (Bioneer, Korea Company) for GR gene:
TGCTTTGGCCTCATTCCAAG (Forward)
and
TATAGTCATCCGTCAGGTGTGC
(reverse).
For housekeeping gene:
ATCCCAGACCCCATAACAACG (forward) and
TTTTTGAGGGTGCAGCGAAC(Reverse).

Quantitative Reveres Transcription Real-Time PCR technique was performed for quantification of relative gene expression analysis for GR gene. These genes were normalized by using housekeeping gene (GAPDH). This technique was done according to method as described by (25). The data results of qRT-PCR for target and housekeeping genes were analyzed by the relative quantification gene expression levels (fold change) ΔCT Livak method according to (26). Statistical analysis of data was conducted on the basis of One-Way Analysis of Variance (ANOVA) utilizing a significant levels of ($P<0.05$). Specific group differences were determined using Least Significant Differences (LSD) as portrayed by Snedecor and Cochran (27).

Results and Discussion

A significant ($p<0.05$) increase in peroxynitrite radical concentration was observed after 28 day of the experiment in T2 and T3 groups which received H2O2 and H2O2 plus ALA as compared to control and T1 groups (Fig1.A). On the other hand, continuous received of hydrogen peroxide in tap water (group T2) for 56 day, caused a significant ($p < 0.05$) increase in this parameter as compared with other experimental groups. Besides, oral gavages of alpha lipoic acid to H2O2 treated rats (group T3) along the experimental period caused a significant ($p<0.05$) decrease in serum peroxynitrite concentration as compared to group T2 .

The treatment of rats with H2O2 in drinking water (group T2) showed a significant ($p < 0.05$) increase in serum MDA as compared to treated group (Fig 1.B). While treatment with alpha lipoic acid (T1) alone or in combination with H2O2 showed significant ($p < 0.05$) decrease in serum malondialdehyde concentration at two treatment period (28 and 56 days) as compared to group T1. The result also showed that the combination treatment (group T3) produced a significant ($p < 0.05$) decrease in MDA level at the end of the experiment compared to pretreatment period.

Exposure of rats to 0.5% of H_2O_2 in drinking water for 28 and 56 days caused a significant ($p < 0.05$) decrease in serum catalase activity in group T1 comparing to other experimental groups (Fig 1.C). Whereas treated of rats with ALA concurrently with H_2O_2(group T2) for 56 days showed a significant ($p < 0.05$) increased in this parameter as compare to (groupH_2O_2)

due to antioxidant effect of ALA was counteract the oxidative stress of H_2O_2 . At the same period a significant ($p < 0.05$) increase in this parameter was observed in group T1 as compared to other experimental groups. Within the time, a significant ($p < 0.05$) decrease in serum catalase activity in H_2O_2 treated group T2 and H_2O_2 plus ALA treated group H_2O_2 at 28 and day 56 days compared to zero time .

The results showed that H_2O_2 in drinking water (group T2) induced significant elevation in serum peroxynitrite and MDA concentrations with significant decrease in catalase activity compared to control group that give impression of induced oxidative stress. This results is in agreement with other studies (28-30). Peroxynitrite (ONOO_) which is formed by the diffusion-controlled reaction of O2●− and NO has been shown to be strong reactive oxidant that oxidize proteins, sulfhydryl, lipids and DNA leading to cellular injury (31 and 32). Peroxynitrite, when generated in excess, may damage cells by oxidizing or nitrating cellular components and oxidation of cofactors of antioxidant enzyme either by direct or free-radical-dependent mechanisms lead to antioxidant depletion of superoxide dismutase, glutathione reductase, and glutathione (32 and 33). MDA, which is one of the final products of membrane lipid peroxidation(LPO), which can be measured via the thiobarbituric acid assay, and it is one of the oldest and most widely used direct assays for assessing sperm membrane oxidation(34 and 35) .

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. Likewise, one catalase molecule can catalyzes the decomposition of millions hydrogen peroxide to water and oxygen each second. It is a very important enzyme in protecting the cell from OS by ROS (36). The mechanisms underlying the decreased CAT activity was proposed by different investigators who suggested that NADPH is important in maintaining CAT activity, and that the loss of NADPH due to exposure to H_2O_2 would adversely affect against erythrocyte CAT enzyme activity (37).

Administration of ALA concurrently with H_2O_2 caused a significant decrease in serum MDA concentration and increase in catalase activity in T3 treated groups compared to control group, indicating the antioxidant activity of ALA. These results came in accordance with many researches (38 - 40). The protective effect of ALA was indicted by the prevention of both, the increased lipid peroxide and the decreased enzymatic antioxidant activity and non-enzymatic antioxidant levels. Several similar mechanisms have been proposed to explain the protective efficacy of ALA against the liver oxidative

stress induced by certain agents, drugs (41-44) and from excessive production of LPO (45).

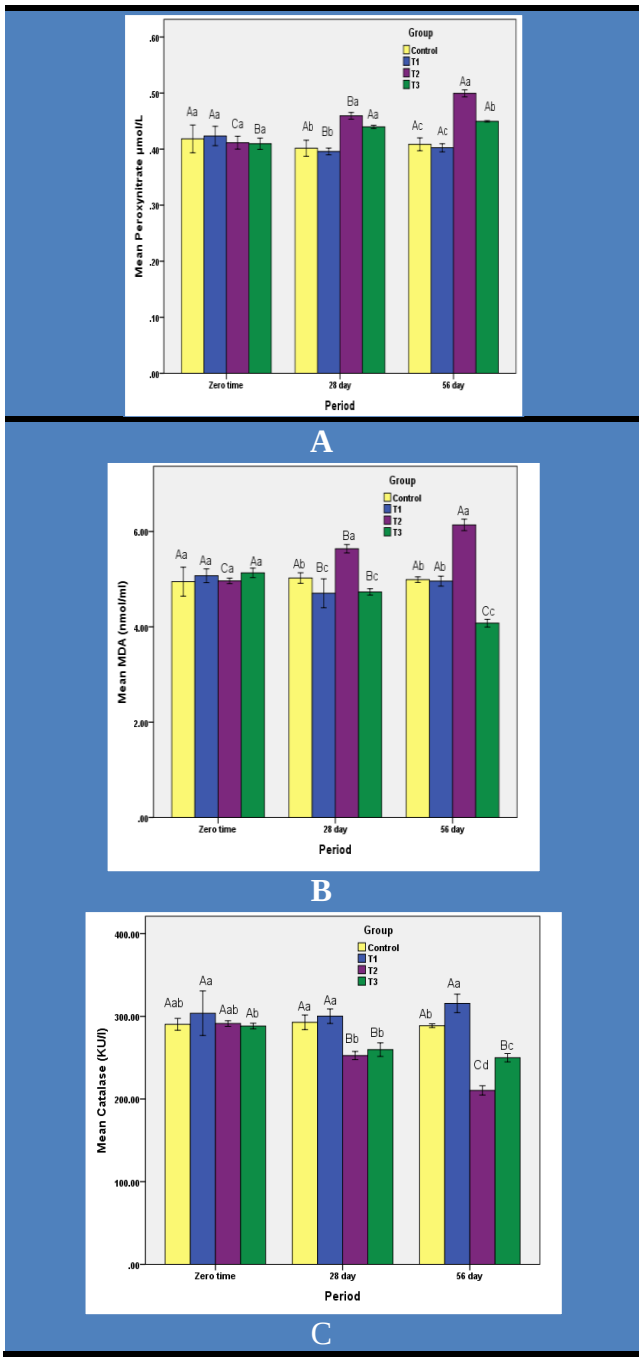


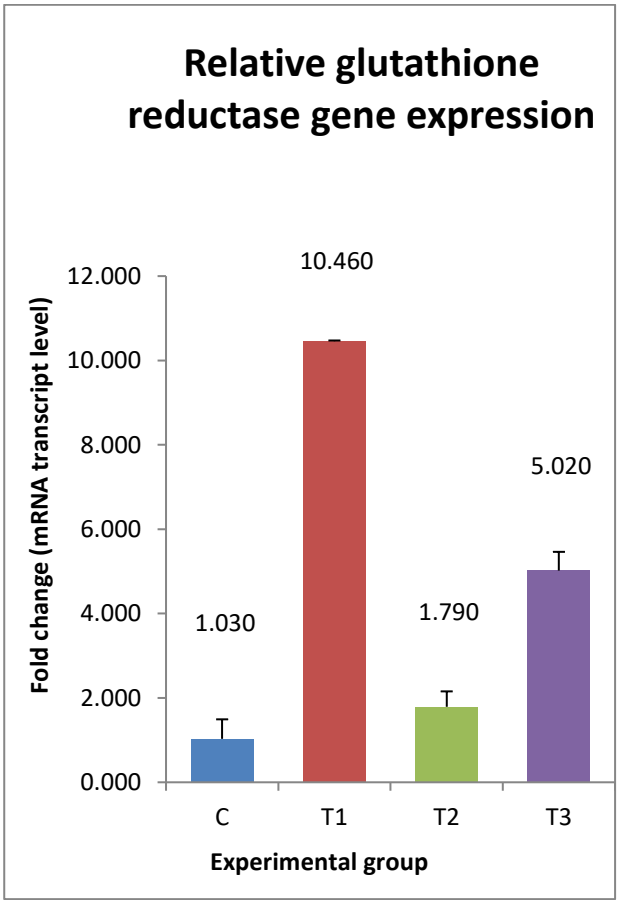
Figure1. Effect of alpha lipoic acid (ALA) for 28 and 56 days on serum peroxynitrite (A), (MDA) (B), catalase(C) Concentrations of hydrogen peroxide treated male rats. Values are expressed as means ±SE. n = 7/ group C: control received drinking tap water. T1: gavages alpha lipoic acid (ALA) (60 mg/ kg B.W). T2: received 0.5% H_2O_2 in drinking tap water. T3: received 0.5% H_2O_2 in drinking tap water plus 60 mg / kg B.W of ALA. Means with different small letters denote significant differences ($p < 0.05$) between Groups. Means with different capital letters denote significant differences ($p < 0.05$) within Groups.

Figures (2) illustrated the result of gene expression of glutathione reductase, Rats in group T2 that received H_2O_2 in drinking water registered significant ($P<0.05$) decrease (1.79±0.25) in folds of gene expression of GR reductase as compared to T1 (10.46±0.40) and T3 (5.02±0.17) treated groups. Meanwhile, group of animals that administrated 60mg/kg B.W. of ALA plus H_2O_2 (group T3) showed non-significant ($P>0.05$) differences in folds of GR gene expression as compared to control group. The results also manifested a slight significant increase in this parameter in group T2 as compared to control group

The results showed decrease in GR genes expression in liver tissues of H_2O_2 treated rats as compared to both T1(ALA) and T3 (ALA+H_2O_2) , indicating occurrence of oxidative stress. A temporary increase in the activity of GP and GR after intoxication of rats by xenobiotic was reported previously (46). Down-regulation of GR results in cellular GSSG content increase, and reduction of GSH/GSSG ratio is involved in many responses against oxidative stress (47). As mentioned previously, the data showed that H_2O_2 caused an increased in serum lipid peroxidation as expressed by increased levels of MDA, this will cause an increased accumulation of H_2O_2 which could further stimulate lipid peroxidation (48). H_2O_2 not only increase the free radical formation but also decrease its ability to detoxify ROS, attenuating of antioxidant enzymes such as GSH- reductase and GSH-Px , and that caused depression in gene expression of these enzymes leading to hepatocellular damage (49). Many researches have demonstrated that oxidants, radiation, heat shock, heavy metals, and chemotherapeutic agents can increase GSH concentrations by induction of g-GCS-HS expression in various cell types (50 and 51). In this study, the results showed for the first time, induction gene expression of GR by H_2O_2 in the liver tissue.

Plants with antioxidant activity have been found to potentiate the activities and gene expression of many antioxidant enzymes including GR (52 and 53). Transcription of

antioxidant enzymes is regulated by antioxidant response elements (AREs). It was shown that Nrf2 (NF-E2-related factor 2) and Nrf1 are transcription factors that bind to AREs and activate these genes (54 and 55). Some studies have reported that the exogenous use of ALA and other antioxidants increases SOD activity and GSH levels with decrease MDA levels (56-58), which may be through elevation of gene expression of GSH reductase . However, an animal study examining the physiological activity of a-lipoic acid affecting GR expression in hydrogen peroxide treated rats has been lacking. Accordingly, further studies are necessary to explore the mechanism of action of ALA.



Figure, 2: Effect of alpha lipoic acid or 56 days on gene expression of glutathione reductase enzyme in liver tissue of hydrogen peroxide treated male rats.

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تأثير حمض الفا لايبوك على حالة الاكسدة/مانعات اللأكسدة ومستوى التعبيرالجيني للكلوتاثايون ريدكتيز في الجرذان المعرضة لبيروكسيد الهيدروجين (الجزء الثاني)

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الخلاصة

صممت هذه الدراسة لتقويم الدور الوقائي لحمض الفا ليبويك ضد الكرب التأكسدي المستحدث بإعطاء بيروكسيد الهيدروجين (H_2O_2) في ماء الشرب للجرذان البالغة . أستُعمِل أربعون من الجرذان الذكور البالغه وقسمت عشوائيا إلى أربع مجاميع متساوية وعوملت يوميا ولمدة 56 يوما على النحو الآتي: مجموعة السيطرة اعطيت الماء المقطر فضلاً عن مياه الصنبور؛ مجموعة المعالجة الأولى فأعطيت 60 ملغم /كغم من وزن الجسم من حمض الفا ليبويك مع مياه الصنبور العادية؛ في حين أعطيت المجموعة الثانية ماء الصنبور الحاوي على بيروكسيد الهيدروجين بتركيز 0.5٪ ، أما المجموعة الثالثة أعطيت 60 ملغم /كغم من وزن الجسم من حمض الفا ليبويك مع ماء الصنبور الحاوي على بيروكسيد الهيدروجين بتركيز 0.5٪ . بعد تصويم الحيوانات جمعت عينات الدم للمدد 0 و 28 و 56 يوما من التجربه لتحديد تركيز بيروكسينتريت و مالونديالدهيد و نشاط الكاتالازفي مصل الدم. فضلا عن ذلك تم حساب التغيير في التعبير الجيني للغلوتاثيون ريدكتديز في الكبد . أظهرت النتائج وجود أرتفاع معنوي في تركيز البيروكسينتريت و مالونديالدهيد مع انخفاض معنوي في الكلوتاثايون و نشاط الكاتالاز في مصل الدم وفي التعبير الجيني للغلوتاثيون ريدكتديز في الكبد في المجموعة T2 مقارنة مع المجاميع الأخرى. ومع ذلك بينت النتائج وجود تحسن معنوي في معايير حالة الكسدة/ مانعات الكسدة في مصل الدم قيد الدراسة في مصل الدم مع زيادة في م مستوى التعبير الجيني للغلوتاثيون ريدكتديز في الكبد في المجموعة المعالجة الثالثة مقارنة مع المجموعتين السيطرة ومجموعة المعالجة الثانية . يستنتج من ذلك أن أضافة حمض الفا ليبويك أدى الى حدوث تحسن معنوي في الأنزيمات المانعه للأكسدة مع زيادة التعبير الجيني للغلوتاثيون ريدكتديز في الجرذان المعرضة للكرب التأكسدي المستحدث بأعطاء بيروكسيد الهيدروجين بسبب فعاليته المضادة للاكسدة.

الكلمات المفتاحية: بيروكسيد الهيدروجين، حمض الفاليبويك،التعبير الجيني، كلوتاثايون ريدكتيز