The Effects of Aspirin and Vitamin E on Blood Antioxidant Enzymes of Rats during Experimental Liver Ischemia-Reperfusion

Birsen KILIÇOĞLU AYDIN Zafer EREN
Department of Biology, Faculty of Science and Art, Ondokuz Mayis University, Samsun TURKEY

birsenki@omu.edu.tr

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Abstract

Damage caused by free oxygen radicals is quite important in ischemia/reperfusion (I/R). Therefore, our study focuses on the effects of Aspirin and Vitamin E on the activity changes in erythrocyte radical scavenging enzymes in superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gpx) activities following liver ischemia/reperfusion in rats. Winstar albino male rats were divided into six groups as sham, isotonic saline solvent Aspirin, Vitamin E solvent corn oil injected control groups and Aspirin, Vitamin E and Aspirin+Vitamin E injected test groups. Rats subjected to ischemia were then grouped and exposed to reperfusion at the 0^{th}, 2^{nd}, 8^{th} and 24^{th} hours. After reperfusion, blood samples were taken via a dispossible injector from the heart at these hours and the enzyme activities were studied in erythrocyte fractions. Enzyme activity changes were seen during reperfusion periods, particularly the 2^{nd} hour of reperfusion more striking than in ischemia period. When Aspirin and Vitamin E were given separately caused enzyme activity changes but these changes more remarkable when Aspirin and Vitamin E given together. Considering these results, Aspirin and Vitamin E may be suggested to prevent post-ischemic damage owing to their potential therapeutic effects.

Key words: ischemia/reperfusion, superoxide dismutase, catalase, glutathione peroxidase

INTRODUCTION

Reactive oxygen species play the major role in ischemia/reperfusion (I/R) damage. The amount of reactive oxygen species is normally controlled by the cells, oxidative stress becomes apparent when the balance between their production and degragation is lost. Organisms have endogenous antioxidant enzyme systems such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in order to protect themselves from the harmful effects of reactive oxygen species.

I/R is an inevitable phenomenon in many surgical operations especially organ transplantations. Accumulation of toxic metabolic products (free oxygen radicals, lactic acid etc.) during ischemia may cause tissue necrosis or lysis[1]. Moreover, destruction of the membrane phospholipids increases arachidonic acid release during ischemia[2,3]. Consequently, arachidonic acid entering into cyclooxygenase metabolic pathway increases the formation of prostaglandins[4]. Arachidonic acid metabolism not only participates formation of free radicals but also disorders blood flow during reperfusion[5,6,7]. As a result, prostaglandin metabolism is closely related to free radical formation.

Reperfusion damage is mainly caused in two ways: One of them is the formation of free oxygen radicals and the second is disorders in the blood flow during the reperfusion. As blood flow is pressured for a long time during ischemia, a slowness and arrhythmia come into existence in circulation during reperfusion. Therefore, some drugs which is improved blood flow are suggested. Likewise, when dopamine having vasodilatator effect is injected to rats after hepatic ischemia has been observed an improvement in blood flow and liver mitochondrial functions[8]. Aspirin, another vasodilatator agent which we used in our study may show a similar effect. Aspirin has been used as analgesic and antipyretic for over a century and its basic effect mechanism is inhibition of cyclooxygenase enzyme providing synthesis of prostaglandins from membrane phospholipids from arachidonic acid [9-13].

Damages caused by free oxygen radicals are improved by using various radical scavengers. It is common knowledge that antioxidant therapy significantly reduces I/R damage. Vitamin E is an endogenous antioxidant playing an important role as chain-breaking in the lipid layers of cellular membranes [14,15]. An increase has been observed in mitochondrial respiration rate and tissue energy levels when vitamin E is injected before hepatic ischemia [16]. After vitamin E given, the cause of the improvement in tissue may due to the prevention of lipid peroxidation by vitamin E. Likewise, recent studies reveals that Aspirin has also antioxidant effect[17-20].

The effects of Aspirin and Vitamin E which have synergistic effects from the point of view their antithrombotic and antioxidant characteristics were studied on blood antioxidant enzymes in I/R treated rats.

MATERIAL AND METHOD

Animals and Experimental Design

Adult male Spraque-Dawley rats were were used in all experiments. Standart food and water were available ad libitium except on the drug-injection day when animals were deprived of food. Housing and experimental treatment of the animals were in accordance with The Experimental Research Ethic Commitie of Ondokuz Mayis University guidelines(TCAM 01/30).

Drug administration

Animals were randomized in to six groups each containing sixteen rats. Injection wasn't performed in sham group. The another five groups were given (i.p) saline solution (0,9% NaCI) of Aspirin, corn oil solution of vitamin E, Aspirin (200 mg/kg b.w) [21], VitaminE (500 mg/kg b.w) [22] and Aspirin + Vitamin E, respectively. Injection were performed five hours before ischemia.

Ischemia operation has been carried out as follows

Animals were anesthetized with xylazine (10 mg/kg body weight, intraperitoneal injection) and ketamine (90 mg/kg body weight, intramuscular injection)[23]. Then the abdomen was opened. However right portal braches of liver were not closed, left portal ven belonging to medium and

left lateral lobes, left hepatic artery and left bile duct were clamped. This way, partial ischemia operation was performed in 70% of the total weight of liver[24, 25]. Clamping operation was performed by passing a 0.3 mm thick surgical suture through a 1mm thick small plastic tube.

Animals which injected solvents and drugs were separated into four groups all over again according to the ischemia reperfusion period. Animals in sham group were exposed to only laparotomy, they weren't performed ischemia/reperfusion. Another five groups performed Ischemia or ischemia/reperfusion designated as follows: OR (45 min ischemia), 2R(45 min ischemia, 2 h reperfusion), 8R(45 min ischemia, 8 h reperfusion) and 24R(45 min ischemia, 24 h reperfusion). Animals subjected to the ischemia only(OR) were immediately sacrificed following the ischemic period. In contrast, to evaluate the effect(s) of reperfusion the vascular clamp was removed following the ischemic period to re-establish blood flow to the ischemic liver. The surgical incision was closed and the animals were revived. They were subsequently sacrificed after 2, 8 or 24 h of reperfusion. Blood was taken at these hours intracardiacally from rats.. Guamari *et al*.'s model [26] was modified and employed in erythrocyte haemosylate preparation.

Enzyme assays

Catalase (EC 1.11.1.6) activity was assayed in a reaction mixture (3 ml) composed of 60 mM K-phosphate buffer (pH 7,0) to which 30 % (m/v) H_2O_2 was added to reach , at 240 nm , an absorbance value in the range 0,520-0,550. Reaction was started by adding 100 µl crude haemosylate and activity determined by monitoring the degregation of H_2O_2 at 240 nm over 2 min.against haemosylate –free blank. Enzyme spesific activities was expressed as µmol H_2O_2 /mg protein/ml haemosylate[27]. SOD activity were assayed by the methods of Flohe and Otting[28]. The assay is based on reduction of cytocrome *c* by superoxide radicals which are generated by xanthine-xanthine oxidase system. Enzyme activity was expressed U per mg of protein/ml haemosylate (1 U nit of SOD is defined as the amount of enzyme required to inhibit the rate of cytochrome C reduction by 50%). Glutathione peroxidase (Gpx) activity was measured by the glutathione reductase coupled oxidation of NADPH using cumene hydroperoxide as a subsrate[29]. Activity was expressed as µmol NADPH min/mg protein/ml haemosylate. Protein was determined by the Lowry *et* al.[30] using bovine serum albumin as standard.

Statistical evaluation

Data were expressed as ± SD. Statistical differences between groups and hours were made using two-factors ANOVA analyses SPSS for Windows package software (version 10). Those with a P value less than 0,05 was considered statistically meaningful.

RESULTS

As a result of two-factors analysis of data, I/R treatment increased SOD activity and a significantly differences were found between groups and hours ($p<0,05$). Despite the lack of a significant difference between groups and hours, pretreatment with Aspirin improved SOD activity which increased with I/R, particularly during the 2nd hour reperfusion. Vitamin E decreased the SOD enzyme activity which increased with I/R ($p<0,05$), but this decrease disappeared within 24 hours. Pretreatment with Aspirin + Vitamin E together decreased SOD activity and this decrease were more than in the pretreatment with Aspirin and Vitamin E, individually($p<0,05$).

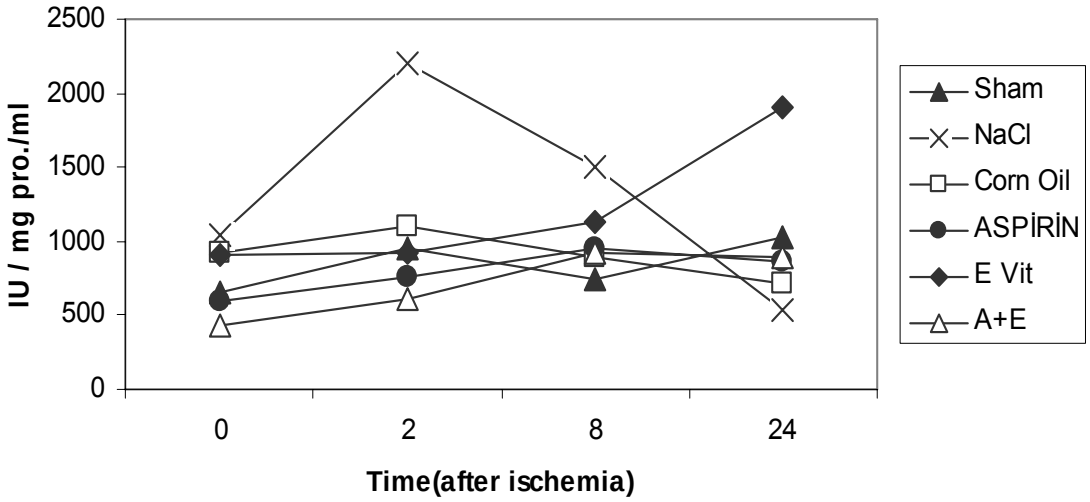

Figure 1. Erythrocyte superoxide dismutase(SOD) activity changes by the time in control and experimental groups

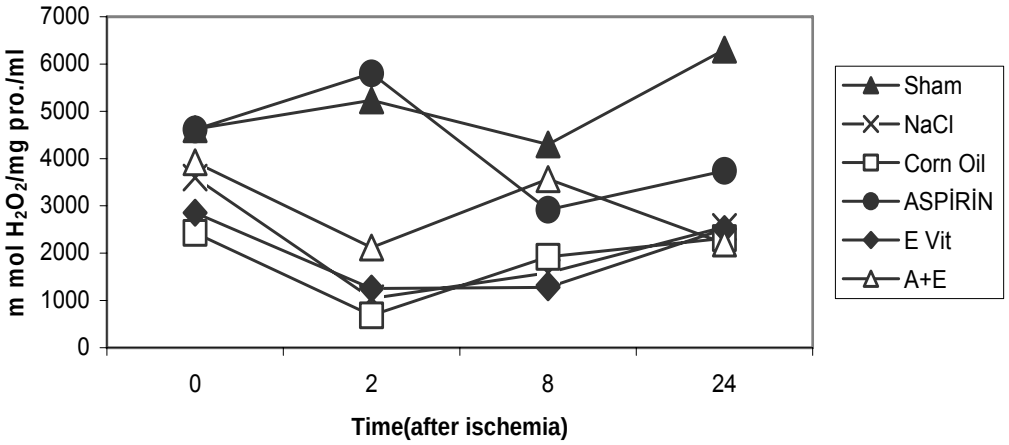

Figure 2. Erythrocyte catalase(CAT) activity changes by the time in control and experimental groups.

CAT activity decreased with I/R treatment and significantly differences were found between groups and hours ($p<0,05$). (Figure 2)

Pretreatment with Aspirin improved CAT activity which decreased with I/R, particularly during the 2nd hour reperfusion($p<0,05$). Not only pretreatment with vitamin E individually but also pretreatment with Aspirin + vitamin E together did not significant hanges in CAT activity which decreased with I/R($p>0,05$).

No significant difference was observed on GPx activity which treatment with I/R ($p>0,05$). (Figure 3).Although a marked decrease was observed at 24th hour pretreatment with Aspirin, no significant difference was observed between groups and hours($p<0,05$). Although decrease was observed in GPx activity at ischemic period pretreatment with vitamin E, increases were seen in different reperfusion periods. Pretreatmant with Aspirin + Vitamin E caused remarkable increase in Gpx activity, particularly during the 2nd hour reperfusion($p<0,05$).

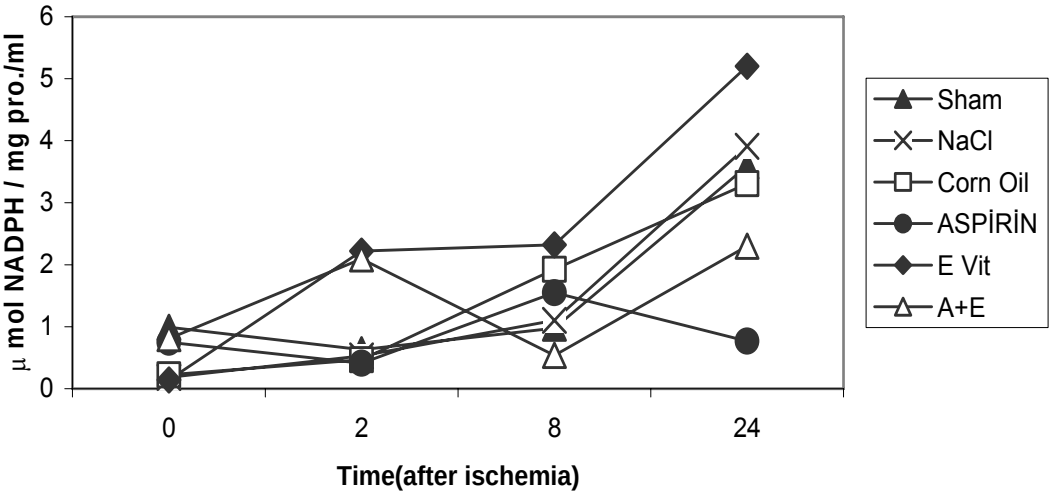

Figure 3. Erythrocyte glutathione peroxidase(Gpx) activity changes by the time in control and experimental groups.

DISCUSSION

Toxic metabolites which occur during ischemia period pass into blood circulation in reperfusion periods. This affects the circulation system negatively, particulary neutrophils and erythrocytes. Liver has quite high blood turnover which is 1/3 of total bodyblood passes from the liver in a minute[31]. Therefore, antioxidant enzyme activity changes in erythrocytes that are highly sensitive to free radicals give us important clues about the peripheral damage of I/R. Iron ions influx increase in to the tissue in ischemia and reperfusion following ischemia süperoxide (O^-_2) radical and hydrogen peroxide(H_2O_2) are produced. H_2O_2 is a weak oxidant and is relatively stable. However, unlike superoxide, H_2O_2 can rapidly diffuse across cell membranes, and in the precence of transistion metal ions it can be converted to toxic hydroxyl radicals via Fenton chemistry[37] as follows:

$$Fe^{2+} + H_2O_2 \xrightarrow{} Fe^{3+} + OH^{\cdot} + OH^-$$

As a result, O^-_2 and H_2O_2 products yields the highly toxic hydroxyl radical that may cleave covalent bonds in proteins and carbohydrates, cause lipid peroxidation, and destroy cell membranes[4,38].

In our study, no important change occurred in erythrocyte antioxidant enzymes just after ischemia (0th hour reperfusion) but significant difference was observed especially at the 2nd hour reperfusion. This may be explained by erythrocytes facing a radical attack during the first hours

of reperfusion, and by trying to eliminate these radicals with antioxidant enzymes at advanced stages of reperfusion.

Although ischemia causes important damages in tissues and cells, it is known that damage during reperfusion is much more marked than ischemia period[32]. Experimental evidence shows that there are two distinc phase of liver reperfusion. The early phase covers the first hours after reperfusion. During this phase the main event is the activation of Kuppfer cells. Complement activation and recruitment of CD_4^+ T lymphocytes are factors that enable the activation of the Kuppfer cells[33]. It has been observed that superoxide radical (O^-_2) increased 9 times during the 40-minute reperfusion period following 60-minute liver ischemia in rats[34]. It has been observed that the most marked SOD and GPx activitity changes occurred at least two hours later after transplantation in blood samples received from patients subjected to liver transplantation[35]. Additionally, in another study, it has been observed that there were no important differences in antioxidant enzymes activity in blood received from patients just after the kidney transplantation [36].

Shi, X. *et al*.[18] report in their studies that Aspirin has much more effective antioxidant on (O^-_2) radicals than the (OH^-) and H_2O_2 radicals [18]. In our study, pretreatment with Aspirin important SOD enzyme activity changes were observed especially at the 2nd hour reperfusion, however, there weren't seen significant changes in Gpx and CAT activity. This may be explain that Aspirin is much more effective antioxidant on O^-_2 radicals than the H_2O_2 radicals.

In this study, Aspirin decreased SOD enzyme activity ischemia and during reperfusion periods, particularly at the 2nd hour of reperfusion. This may be related to Aspirin's antioxidants properties. Recent studies reveal some evidence that Aspirin has also antioxidant properties in addition to its analgesic, antipyretic and antiinflammatory properties [39,17,40,18]. Erythrocytes are very sensitive cells to oxygen radicals, particularly to lipid peroxidation damage. A marked increase was observed in Gpx activity towards the 24th hour with the effect of Aspirin. There is no citric acid cyclus in erythrocytes and they their energies by means of hexose monophosphate pathway. $NADPH_2$ which is coenzyme for Gpx is produced in this metabolic pathway and erythrocyte membrane damage as a result of lipid peroxidation may cause a decrease on Gpx activity. That's why the cause of the increase on Gpx activity during 24th hour reperfusion might be considered prevent lipid peroxidation and maintain membrane integrity with Aspirin pretreatment. Shimizi *et al*., [41] report that malonaldehyde which is lipid peroxide end-product decreased significantly(60%) after six hours being injected Aspirin(50 mg/kg body weight) to the rats. Accordingly this study, we injected Aspirin six hours before treatment ischemia and so lipid peroxidation during reperfusion was prevented. In the light of these data, the cause of the decrease in SOD and Gpx activities during ischemia/reperfusion might be the prevention of erythrocyte lipid peroxidation pretreatment with Aspirin.

While SOD activity was increased in the pretreatment with vitamin E after the 8th hour, there were no important changes in CAT and Gpx activity. Our studies have correlated with previous studies [4,42]; Fışkın *et al*.[42] injected Vitamin E (50mg/kg body weight) to rats by intraperitoneally 15 minutes before 45-minute global liver ischemia and they observed no changes in blood SOD, CAT and Gpx activity after 15-minute reperfusion.

Inhibition of platelet aggregation is very important in the prevention of I/R-caused thrombosis and for blood flow arrangement in liver. It is known that, depending on arachidonic acid concentration, more (O^-_2) and H_2O_2 is released from platelets [43]. It is reported that Aspirin affected the arachidonic acid metabolism] by inhibited the collagen glycosyltransferase enzyme in platelet membrane[44,41,10], inhibited platelet aggregation by blocking ADP release[46] and increased the amount of NO released from neurophils [19]. Injection of Aspirin together with Vitamin E is not only important for the inhibition of platelet aggregation [47], but also in that it shows a synergistic effect increasing the amount of prostacyclin in arachidonic acid metabolism [20]. When Aspirin and Vitamin E were given separately caused enzyme activity changes but these changes more remarkable when Aspirin and Vitamin E given together. This may be considered that radical formation and radical damages might be more effectivelly improved when Aspirin and Vitamin E were given together. Our findings show parallelism with the findings of many researchers [20,47,17].

In conclusion, blood antioxidant enzymes responded differently to 0th, 2nd, 8th and 24th hour reperfusions following 45-minute liver ischemia. Cells have different enzymes in different subcellular organelles in order to protect themselves from the harmfull effect of free radicals. Variation of responses of antioxidant enzymes to radicals may be regarded as the adaptation of cells created against oxidative stress in different subcellular compartments. Considering that radical formation increased depending on the ischemia process [34,23,48] and that radical formation might be high during the initial hours of reperfusion [49,50], we believe that Aspirin and Vitamin E might be used for prophylactic purposes in operations in which I/R damage is possible.

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