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Comparison of Aspirin's Antioxidant Effect with Selenium and Vitamin E by Measuring Mitochondrial Selenium Dependent Glutathione Peroxidase and Cytosolic Total Glutathione Peroxidase Activity in Mice Liver

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In this study, the antioxidant effect of aspirin was studied and compared with selenium and vitamin E on the activities of mitochondrial selenium dependent glutathione peroxidase (Se-GPX) and cytosolic total glutathione peroxidase (t-GPX). After the injection of selenium, vitamin E and aspirin intraperitonally to the mice, they were killed by servical dislocation at the 2, 4, 8, 12 and 24 h and their livers were removed. Enzyme fractions were obtained from livers. Mitochondrial fractions were used to find out the changes in the Se-GPX activities and cytosolic fractions were used to find out the changes in the total GPX activity. Aspirin increased cytosolic t-GPX activity in 12 and 24 h and mitochondrial Se-GPX activity in 8 h when compared with controls ($p < 0.05$). The t-GPX activity was less than vitamin E in 2 h and selenium in 12 h ($p < 0.05$). The study shows that aspirin has an antioxidant effect by increasing the cytosolic t-GPX and mitochondrial SeGPX activity. The antioxidant effect of aspirin is less than selenium and vitamin E.

Key Words: Aspirin, Selenium, Vitamin E, Liver, Selenium dependent glutathione peroxidase, Cytosolic total glutathione peroxidase.

INTRODUCTION

Aspirin (acetylsalicylic acid) is one of the most widely used drugs worldwide. It acetylates cyclooxygenases thereby irreversibly blocking the conversion of arachidonic acid to prostanoids[1]. After absorption from the stomach and small intestine, aspirin is rapidly hydrolyzed to salicylic acid in the liver and blood where it is tightly bound to plasma proteins and distributed to all tissues in the body[2]. Salicylic acid is a hormonal mediator of the systemic acquired resistance responses to pathogen attack, environmental and oxidative stress in plants[3]. It has been supposed that aspirin and its metabolite salicylic acid inhibit the oxidative stress[4].

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Selenium is an essential micronutrient for human health and part of seleno-proteins. It is found in selenosistein (SeCys) as amino acid. Some of them have important enzymatic functions[5]. All of these enzymes depend on Se and SeCys are located at the active center[6,7]. The best characterized selenoenzymes are the Se dependent glutathione peroxidase (SeGPX) and thioredoxin reductase (TrxR) families. The activities of which are responsible for the recognition of Se as an important dietary antioxidant[6].

Vitamin E is an essential fat-soluble vitamin, which includes different naturally occurring isomers. This nutrient is the most effective chain-breaking lipid soluble antioxidant in the biological membrane, where it prevents the propagation of free radical damage and contributes to membrane stability[8]. Vitamin E and Se interact synergistically as important antioxidant defense mechanisms of cells[9].

Glutathione peroxidase (GPX) is found in the cytosol and mitochondria of animal tissues. catalyzes the reduction of hydroperoxides, including hydrogen peroxide, by reduced glutathione and functions to protect the cell from oxidative damage. With the exception of phospholipid-hydroperoxide GPx, a monomer, all of the GPX enzymes are tetramers of 4 identical subunits. Each subunit contains a selenocysteine in the active site which participates directly in the two-electron reduction of the peroxide substrate. The enzyme uses glutathione as the ultimate electron donor to regenerate the reduced form of the selenocysteine[10]. GPX presents at least in 2 different types in several human tissues, one of which contains selenium in its active centre as known SeGPX and another which has been determined as cationic isoenzymes of glutathione S-transferases. Both of them is known total GPX (t-GPX). It shows high activity in liver[11].

In this article, the antioxidant effect of aspirin as compare Se and vitamin E has been examined by meausuring SeGPX activity in mitochondrial matrix and t-GPX activity in cytosole.

EXPERIMENTAL

Swiss albino type laboratory mice (*Mus musculus*), aging 6-7 weeks, weighing 20-35 g and fed *ad libitum*, obtained from the Department of Biology, Science Faculty, Ondokuz Mayis University, Samsun, Turkey were used. Mice were chosen from the same generation were fasted for 24 h before injections. All injections were made intraperitoneally. The first group was used as a control group. The second group was administered with Se (200 mg/kg)[12] the third group with vitamin E (500 mg/kg)[13], the fourth group with aspirin (200 mg/kg)[14]. After 2, 4, 8, 12 and 24 h, the injections, the mice were killed by cervical dislocation and their livers were removed and perfused with 0.9 % NaCl. The livers were stored in 0.25 M sucrose solution at -70 ºC until assayed. In order to measure mitochondrial Se-GPX and cytosolic t-GPX activity, supernatants were obtained from livers *via* homogenization (at 15,000 rpm for 30 s), ultrasonification (at 15 µ, 3 times, for 15 s) and ultracentrifugation (at 15,000 rpm for 20 min). The supernatants were removed and pellet resuspended

in sucrose solution and were centrifuged again (at 15,000 rpm for 20 min). The supernatants were first processed by Lowry method[15] for total protein determination then by Lawrence-Burk method for Se-GPX activity and t-GPX activity[16]. Se-GPX and t-GPX values were calculated by their enzymal activity in Unit/milligram protein/millilitre (IU/mg protein/mL) measured by ultraspectrophotometry at 340 nm.

Substances were provided from the following firms: Aspirin from Bayer; EDTA, H_2O_2, KH_2PO_4 and azide from Merck W. Germany; NADPH from Calbiochem in Germany; Glutathione (GSH), glutathione reductase (GR), (Bovine serum albumin) BSA, Cumene hydroperoxid from Sigma in England; K_2HPO_4 from Monplet-Esteban; Se as sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) from Merck and Vitamin E (d-α-tokoferol) from Roche.

Measurement of Se-GPX activity: Lawrence and Burk's method were used for the measurement of Se-GPX activity[16]. The reaction mixture was 50 mM phosphate buffer (pH = 7), 5 mM EDTA, 1 mM sodium azide (NaN_3), 0.2 mM NADPH, 1 EU/mL GSSG-reductase and 2 mM GSH. The reaction mixture and sample solution were mixed and incubated for 5 min at 37 ºC. Then the reaction was initiated with H_2O_2 and reported NADPH absorbance at 340 nm for 5 min. A unit of enzyme activity was evaluated as the amount of enzyme which consumes 1 mmol NADPH in a minute.

Measurement of t-GPX activity: Lawrence and Burk's method were used for the measurement of t-GPX activity[16]. The reaction mixture was 50 mM phosphate buffer (pH = 7), 5 mM EDTA,1 mM sodium azide (NaN_3), 0.2 mM NADPH, 1 EU/mL GSSG-reductase and 2 mM GSH. The reaction mixture and sample solution were mixed and incubated for 5 min at 37 ºC. Then the reaction was initiated with cumene and reported NADPH absorbance at 340 nm for 5 min. A unit of enzyme activity was evaluated as the amount of enzyme which consumes 1 mmol NADPH minute.

Statistical analysis: 5X4 factorial design two-way ANOVA was conducted to assess time X group interaction. Post-hoc multiple comparisons were done with Duncan Multiple Comparison Test with a significance level of 0.05. Statistical analysis were done using SPSS 13 software (Statistical Package for the Social Science Program) program. $p < 0.05$ admitted as meaningful. Values shown are means ± SD.

RESULTS AND DISCUSSION

Aspirin increased cyt-t-GPX activity in all times and mit-Se-GPX activity at 2, 8, 12 and 24 h compared with control group. The level of increase was 22-231 % for mit-Se-GPX and 81-554 % for t-GPX. However, the amount of increase was statistically significant only at 8 h for Se-GPX ($p < 0.05$); at 2 and 24 h for t-GPX ($p < 0.05$). Vitamin E in comparison to control group induced to increase Se-GPX activities at 2, 4, 12 and 24 h but none of them was statistically significant ($p > 0.05$). It also induced t-GPX activity in all times, which were statistically significant at 2, 4 and 8 h ($p < 0.05$). Although Se caused higher activities of Se-GPX than control group at 2, 4, 8 and 12 h, it was statistically significant at 2 and 12 h ($p < 0.05$),

whereas increased t-GPX activities in all of times; 2, 4, 12 and 24 h were important as statistically ($p < 0.05$). Details of enzyme levels are shown in Table-1.

TABLE-1
EFFECT OF SELENIUM, VITAMIN-E AND ASPIRIN ON MITOCHONDRIAL SeGPX AND CYTOPLASMIC tGPX

		Mitochondrial SeGPX**				Cytoplasmic tGPX***			
		Mean	SD*	Min	Max	Mean	SD*	Min	Max
2 h	Control	83.0^{b}	1.00	82	84	74.0^{c}	0.00	74	74
	Selenium	339.0^{a}	247.00	92	586	338.0^{b}	236.00	102	574
	Vitamin E	117.7^{b}	9.50	108	127	866.0^{a}	294.00	572	1160
	Aspirin	161.0^{b}	101.00	60	262	373.7^{b}	36.50	337	410
4 h	Control	97.0^{a}	2.00	95	99	53.0^{b}	0.00	53	53
	Selenium	117.7^{a}	16.50	101	134	414.7^{a}	365.50	49	780
	Vitamin E	125.0^{a}	35.51	89	160	347.0^{a}	164.00	183	511
	Aspirin	80.0^{a}	0.00	80	80	96.0^{b}	0.00	96	96
8 h	Control	116.0^{b}	5.29	112	122	44.0^{b}	0.00	44	44
	Selenium	235.0^{ab}	183.00	52	418	140.0^{ab}	1.00	139	141
	Vitamin E	94.7^{b}	2.52	92	97	311.3^{a}	7.77	305	320
	Aspirin	384.0^{a}	0.00	384	384	171.0^{ab}	56.00	115	227
12 h	Control	81.0^{b}	9.54	71	90	57.0^{b}	1.00	56	58
	Selenium	705.3^{a}	248.02	459	955	578.0^{a}	72.00	506	650
	Vitamin E	141.0^{b}	0.00	141	141	233.0^{b}	0.00	233	233
	Aspirin	186.7^{b}	133.50	53	320	264.3^{b}	36.23	226	298
24 h	Control	97.0^{a}	6.24	90	102	46.0^{b}	0.00	46	46
	Selenium	49.7^{a}	7.51	42	57	294.7^{a}	39.50	255	334
	Vitamin E	159.0^{a}	63.00	96	222	125.0^{ab}	23.00	102	148
	Aspirin	119.0^{a}	36.00	83	155	301.7^{a}	186.00	115	487

Meanings which have different letters are significantly different in any hour intervals ($p < 0.05$) *Standard deviation; **Mitochondrial Se-GPX activity 10^{-5} U/mg. protein; ***Cytoplasmic t-GPX activity 10^{-5} U/mg protein.

As regard to rise of Se-GPX activity, there were statistically significant rises at 2 and 12 h. In case of t-GPX activity increased at 4 and 12 h in favour of Se when compared with aspirin injected groups ($p < 0.05$). On comparison with aspirin and vitamin E, aspirin induced to increase Se-GPX activity at only 8 h, whereas vitamin E increased t-GPX activities at 2 and 4 h ($p < 0.05$).

In present study, it is demonstrated that aspirin has antioxidant property comparing with two important antioxidants *i.e.*, Se and vitamin E. There are some studies that non-steroidal antiinflammatory drugs such as aspirin can inhibit chemically induced tumours of the colon, liver and esophagus in labaratory animals[17-19]. However, some epidemiologic studies proposed that daily aspirin usage decrease prostate cancer incidance in USA[20]. This mechanism has not been exactly clarified yet. But some researches explanining the mechanism that aspirin scavenges some free radicals in

the cell[2]. In an electron spin resonans study, it was reported that aspirin was an efficient ^{-}OH radical scavenger[21] unlike for $^{-}O_2$ and H_2O_2. In present study, it is also showed antioxidant capacity of aspirin in mice by measuring cytoplasmic t-GPX and mitochondrial Se-GPX. Supplementation of mice with aspirin led to an increase in activity of these enzymes in mice liver (Table-1). It is known that Se and vitamin E are good antioxidants and work synergistically[22]. Se supplementation more increased cytosolic t-GPX activity and mitochondrial Se-GPX activity rather than vitamin E and aspirin in mice liver (Table-1). This is correlated that Se has more powerful antioxidant capacity. However, vitamin E has also led to rise in cytosolic t-GPX activity in mice liver. In fact, α-tocopherol, a known biological antioxidant, protects membranes from oxidative stress[23]. In present study, a significant elevation in the cytosolic t-GPX and mitochondrial Se-GPX is observed in aspirin injected mice, we can say that aspirin has antioxidant effect. But this effect is lower than Se and vitamin E. For example, in a study, when vitamin E deficient rats were supplemented with aspirin, reduction was observed in the levels of increased colonic oxidative stress and prostaglandin production. Aspirin is capable of activating the NO-cGMP signaling pathway in endothelial cells. In another study, increased levels in NO and cGMP by aspirin were causally related to antioxidant protection and improved integrity of the endothelium[24]. Previous results show similarities and different with other studies[25-27]. This was associated increase in GPX activity on supplementation with salicylic acid[2].

This study suggested that although injection of aspirin rises cytosolic t-GPX and mitochondrial Se-GPX activities in mice liver, these augmentations is less than Se and vitamin E injected groups. Overall, it is concluded that aspirin has antioxidant effect.

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