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Fenvalerate-induced Oxidative Stress in Erythrocytes and the Protective Role of Quercetin

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Abstract: This study was designed to investigate the ameliorative effect of Quercetin on Fenvalerate-induced biochemical alterations in human erythrocytes invitro. Erythrocytes are useful model to study the interaction of pesticides with biological membranes. Pesticides are thought to exert damaging effect on biomembranes through free radical generation; therefore antioxidants can play a crucial role in offering protection against pesticide induced oxidative damage. Quercetin is a potential antioxidant, known to be able to protect cells against oxidative damage.

The biochemical parameters chosen to evaluate the effect of Quercetin were lipid peroxidation (LPO), selected antioxidant and membrane bound enzymes in erythrocytes. No statistical differences were found in the Quercetin treated groups compared with the control group. Following in vitro exposure, Fenvalerate caused a significant induction of oxidative damage in erythrocytes as evidenced by increased levels of thiobarbituric acid reactive substances (TBARS), and decreased levels of GSH. The activities of superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GPx) were found to be significantly reduced in Fenvalerate treated erythrocytes compared with the control erythrocytes. However, Quercetin pretreatment significantly prevented the decrease in the activities of antioxidant enzymes and membrane bound ATPases. The beneficial effects of Quercetin observed here presumably reflect the ability of this flavonoid to protect cells from the toxic effects of Fenvalerate.

Key words: Quercetin; Fenvalerate; Erythrocytes; Antioxidant activity.

Introduction

Since the discovery of DDT in 1939 numerous pesticides have been developed and used extensively worldwide. In industrialized countries, the Green Revolution of the 1960s significantly increased agricultural productivity by increasing the cultivated surfaces, mechanization, planting of hybrid crops with higher yields, and pest control (1). Pesticides are hazardous chemicals designed to repel or kill rodents, fungi, insects, and weeds that undermine farming. The main effects of pesticides represent a great benefit for human health. Indeed, they help control agricultural pests and plant disease vectors, human and livestock disease vectors and nuisance organisms. Moreover, they insure increased food production, a safe and secure food supply, and other secondary benefits (2) However, many first generation pesticides have been found to be harmful to the environment.

Synthetic pyrethroids, including Fenvalerate are now broadly recognized as a major class of

synthetic organic insecticides (3). Introduced commercially less than 20 years ago, synthetic pyrethroids now account for more than 30% of insecticide use worldwide (4, 5) in household, agricultural, and veterinary applications (6, 7). Pesticide exposure can alter the membrane permeability of erythrocytes (8). The involvement of reactive oxygen species (ROS) have been implicated in the toxicology of organochlorine (9, 10) and organophosphate (11, 12) pesticides. Fenvalerate has been shown to increase lipid peroxidation in liver and kidney of mice through free radical generation (13).

Flavonoids have long been considered inert and nonessential for human health; however, in the last few years it has been shown that these compounds affect a wide variety of biological systems in mammals, exhibiting antioxidant, anti-inflammatory, antiviral, antiproliferative, and anticarcinogenic effects (14, 15). Recently, much attention has been paid to their antioxidant properties since antioxidant defenses in the cell can temper the negative influence of free radicals and associated reactions and keep them in check (14, 15).Quercetin is commonly found in food products (16) and is one of the most abundant natural flavonoids. Administration of Quercetin has been shown to increase plasma antioxidant capacity in rats (17, 18). It posses the ability to prevent the oxidative damage induced in erythrocytes by a number of oxidizing agents (19). Thus Quercetin could significantly contribute to the antioxidant defense mechanisms in erythrocytes.

Materials and Methods

Chemicals

All fine chemicals including Quercetin, thiobarbituric acid (TBA), DTNB and reduced glutathione were purchased from sigma chemical Co., USA. All other chemicals were of good quality and of analytical grade.

Blood collection

Blood samples were collected into tubes containing EDTA-2Na from healthy adult individuals after informed consent. They were free of any medication, drugs or nutrient supplementation.

Experimental design

Erythrocyte suspensions were divided into four groups. One group served as normal. In the second group, previously prepared erythrocyte suspension was incubated for 15 min at a concentration of 100µg/ml of Fenvalerate at room temperature. The third group was incubated with Quercetin alone at a concentration of 25µM for 15 min at room temperature and the fourth group was pre incubated with Quercetin, followed by Fenvalerate incubation at indicated concentration.

Isolation of erythrocytes and erythrocyte membranes

Erythrocytes and their membranes were isolated from the control and experimental groups according to the method of Dodge et al. [20] with slight modifications. Packed cells were washed with isotonic saline to remove buffy coat. Different aliquots of packed cells were thoroughly washed with tris-buffer, 0.31 M and pH 7.4. These were used for the assay of various biochemical parameters. Then, another aliquot of packed cells were subjected to hemolysis by adding hypotonic Tris-buffer, 0.015 M, pH 7.2. After 4-6 h, the erythrocyte ghosts were sedimented by centrifugation at 12,000 rpm, for 40-45 at 4° C. the supernatant (hemolysate) was used for the analysis of antioxidants. The erythrocyte membrane pellets were suspended in 0.02 M Tris-buffer, pH 7.2 and used for various other biochemical estimations.

Oxidative stress measurement

Hemolysis was determined by the method of senturk et al. [21]. TBARS levels were determined by a modified version of the method described by Ohkawa et al. [22]. The data are expressed as µ moles of TBARS produced/mg protein. Reduced glutathione (GSH) was estimated by the method of Ellman [23] and the amount of glutathione is expressed as µ moles/mg protein.

Determination of antioxidant enzymes and membrane bound enzymes

Superoxide dismutase (SOD) was measured by the method of Misra and Fridovich (24) and activity of SOD is expressed as units/mg protein. Catalase (CAT) was determined by the method of Abei (25) and activity of CAT is expressed as µmoles of H_2O_2 decomposed/min/mg protein. Glutathione peroxidase (Gpx) was assayed by the method of Rotruck et al. (26) and the activity of GPx was measured expressed as nmoles of glutathione oxidized/min/mg protein. The protein content was estimated by the method of Lowry et al. (27).Activities of Na^+/K^+ and Ca^{2+}-ATPase from erythrocyte membranes were determined by the method of the Bonting [28] and Hjerten and Pan [29], respectively. The activities were indirectly measured by estimating the phosphorous liberated

after the incubation of erythrocytes membrane in a reaction mixture containing the substrate ATP. The phosphorus content was then estimated by the method of Fiske and Subbarow [30] and the activity of ATPases were expressed as µg of phosphorus liberated/min/mg protein.

Statistical Analysis

All data were analyzed with SPSS/10 student software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by LSD. The values are expressed as the mean ± SD for 5 different sets of experiments and results were considered significantly different if $p < 0.05$.

Results

No statistical differences were found in the Quercetin treated groups compared with the control group as shown in the Table 1 & 2. The level of TBARS was significantly increased in Fenvalerate treated erythrocytes compared to control. However, TBARS were found to be significantly reduced in erythrocytes pre incubated with Quercetin. A significant decrease in the activities of all three antioxidant enzymes and membrane bound ATPases along with Glutathione were noted in Fenvalerate treated erythrocytes while erythrocytes pre incubated with Quercetin showed significant increase in the activities of antioxidant and membrane bound enzymes compared to Fenvalerate only group.

Table 1: levels (mean ± SD) of Hemolysis, TBARS and GSH of control and experimental erythrocytes

Parameters	Control Erythrocytes	Erythrocytes +QE	Erythrocytes + FEN	Erythrocytes + QE + FEN
% Hemolysis	8.08±0.73	[a]11.12±0.92	[b]66.02±1.35	[c]35.32±1.60
Lipid peroxides (µ moles of TBARS formed/l)	2.42 ± 0.08	[a]2.36±0.18	[b]13.44±1.30	[c]6.08±0.30
GSH(µ moles/mg protein)	2.96±0.12	[a]3.8±0.1	[b]1.85±0.07	[c]2.95±0.09

Abbreviations; FEN-Fenvalerate, QE-Quercetin

[a] Non significant vs. control erythrocytes, [b] $p<0.05$ vs. control erythrocytes, [c] $p<0.05$ vs. erythrocytes+ FEN.

Table 2: levels (mean ± SD) of Antioxidant and Membrane bound enzymes of control and experimental erythrocytes:

Parameters	Control Erythrocytes	Erythrocytes +QE	Erythrocytes + FEN	Erythrocytes + QE+ FEN
SOD(units/mg protein)	1.81±0.12	[a] 1.8±0.01	[b] 1.45±0.02	[c] 1.6±0.03
CAT(µmol/min/mg protein)	25.2 ± 1.78	[a] 22.2 ± 3.11	[b]20.2±1.9	[c] 23.22 ±5.80
Gpx (nmole/min/mg protein)	150.2 ±1.64	[a] 144.8±1.48	[b] 96.8±1.92	[c] 121.2±1.92
Na^+/K^+- TPases(units/mg protein)	7.25±0.14	[a] 6.84±0.09	[b] 3.3±0.07	[c] 4.9±0.07
Ca^{2+}-ATPases (µmol/min/mg protein)	13.6±1.08	[a]13.9±0.74	[b]4.36±0.25	[c]9.36±0.29

Abbreviations; FEN-Fenvalerate, QE-Quercetin

[a] Non significant vs. control erythrocytes, [b] $p<0.05$ vs. control erythrocytes, [c] $p<0.05$ vs. erythrocytes+ FEN

Discussion

Following in vitro exposure, Fenvalerate caused a significant induction of oxidative damage in erythrocytes as evidenced by increased percentage of Hemolysis and increased levels of thiobarbituric acid reactive substances (TBARS). The activities of superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx) were found to be significantly reduced in Fenvalerate treated erythrocytes compared with the control erythrocytes. However, Quercetin pretreatment significantly restored the activities of antioxidant enzymes and the levels of TBARS.

The activity of Na^+ -K^+ & Ca $^{2+}$ATPase was reduced in the erythrocytes treated with Fenvalerate alone while pre incubation with Quercetin before the incubation of Fenvalerate prevented this fall in the activity of Na^+ -K^+ & Ca $^{2+}$ATPase. In the previous studies, treatment of erythrocytes with organophosphate pesticides was found to be associated with decrease in the activity of Na^+ -K^+ & Ca $^{2+}$ATPase (8).

Pesticides have been shown to enhance the production of reactive oxygen species (ROS), which in turn generate oxidative stress in different tissues (31). In fact, one of the molecular mechanisms underlying the toxicity of some pesticides seems to be lipid peroxidation (LPO). As a consequence, these compounds can disturb the biochemical and physiological functions of the red blood cells (RBC) (32). Oxidative stress results when the balance between antioxidant systems and ROS is lost (33). Oxidative damage of membrane results in increased membrane fluidity, compromised integrity and inactivation of membrane bound receptors and enzymes (34). The release of iron initiates a chain of reactions leading to lipid peroxidation and consequent hemolysis.

The antioxidant enzymes in erythrocytes such as SOD which dismutates $O_2^{\cdot -}$ and CAT which decompose H_2O_2 may counteract pyrethroid induced oxidative stress, resulting in the consumption of these enzymatic antioxidants. Increase in the level of enzymatic antioxidants followed by the incubation of erythrocytes could be due to the prevention of consumption of antioxidants by Quercetin through scavenging the free radicals. The antiradical and chelating effects are thought to be behind the protective role of Quercetin (35). Quercetin can chelate released iron and can prevent lipid peroxidation and hemolysis (19).Moreover, the decrease of serum TBARS was significantly correlated with the increase of serum free flavanoids by Quercetin administration in a previous study by nakumaura et al., (36).

GSH required to reduce H2O2 via glutathione peroxidase may also have an important function in mitigating the toxic effects of ROS.GSH is a major antioxidant and its level can be altered by various chemical agents (37).In the present work GSH content was depleted in samples treated with Fenvalerate. Sing et al reported depletion in GSH content of Erythrocytes after the administration of pesticides (8). In samples treated with Quercetin a reduction in the oxidative damage was observed, with elevation in the level of GSH and enzymatic antioxidants (37) which may be due to the fact that Quercetin can reduce the consumption of GSH under oxidative stress condition. Its antioxidant effect was documented in many in vitro and in vivo experimental studies (38).

The beneficial effects of Quercetin observed here presumably reflect the ability of this flavonoid to protect erythrocytes from Fenvalerate-induced oxidative stress. In conclusion, Quercetin appears capable of protecting cells from the toxic effects of Fenvalerate.

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