Slovak J. Anim. Sci., 41, 2008 (3): 140 - 145

ROLE OF ANTIOXIDANT MICRONUTRIENTS ON INDUCTION OF RAT LIVER AND BRAIN CYTOCHROME P450 ENZYMES BY FENVALERATE

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ABSTRACT

The role of an association of micronutrients: omega 3 fatty acids (ω3) and selenium (Se), on the induction of rat liver and brain cytochrome P450 enzymes by fenvalerate (FEN) was investigated. The animals were housed in groups of 10 rats each and divided randomly into 4 groups. Treatments were carried out day by day for 20 days. The first group served as control (C) and injected orally with corn oil, group 2 was injected with a single dose of FEN (20 mg/kg, orally) for 24 h prior to decapitation, group 3 was administered orally with ω3 (20 mg/kg) and Se (4.10 mg/kg; ω+Se), group 4 was administered orally with FEN following the treatment with ω3 and Se (FEN+ω3 +Se). Treatment period extended for 3 weeks. The content of microsomal protein, drug metabolizing enzymes and thiobarbituric acid reactive substances (TBARS) were determined in liver and brain microsomes after treatment. Pretreatment of rats with repeated doses of ω3 and Se prior to the administration of FEN reduced the hepatic and brain content of cytochrome b_5, P450 and the level of TBARS. The activity of glutathione S-transferase (GST) was significantly induced in rat liver and brain in FEN treated group. Also the activity of NADPH cytochrome c-reductase was significantly decreased in liver microsome; while it increased in brain. Pretreatment of rats with ω3 and Se are more effective in decreasing the activity of amidopyrine N-demethylase in rat liver prior to FEN administration. Oral administration of FEN (20 mg/kg) did not cause mortality and had no significant effect on hepatic and brain microsomal protein. It may be concluded that association of micronutrients may reduce the toxic effects exerted by FEN upon liver and brain through inhibition of cytochrome P450 system.

Key words: Cytochrome P450, TBARS, omega 3 fatty acids, selenium

INTRODUCTION

The widespread use of pesticides in agriculture, public health and household environment poses human population to continuous exposure. Exposure to low-level of pesticides is known to produce a variety of biochemical changes, some of which may be responsible for the adverse biological effects reported in human and experimental studies (Lopes et al., 2007).

The biochemical changes induced after exposure to pesticides or their active metabolites, including target cells/receptor binding protein and DNA adduct formation, and induction or inhibition of pesticides effects, either by overproduction of free radicals or by alteration in antioxidant defense mechanisms (Abdollahi et al., 2004; Parakasam et al., 2001).

Synthetic pyrethroids constitute a unique group of insecticides having pyrethrum like structures with better performance characteristics and account for over 30% of insecticides used globally (Soderlund and Bloomquist, 1989). Synthetic pyrethroids are generally viewed as safe insecticides available due to their low acute toxicity to mammals (Parasanthi et al., 2005). Based on the symptoms produced in animals, pyrethroids are grouped into two distinct classes - Type I and Type II (Ecobichon, 1991).

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Received: February 18, 2008; Accepted: May 22, 2008

ISSN 1335-3686

Fenvalerate, [cyano(3-phenoxyphenyl)2-(4-chlorophenyl)-3-methylbutyrate] is a type II synthetic pyrethroid that has replaced other groups of insecticides due to its improved insecticidal potency (WHO, 1990). FEN displays moderate toxicity in mammal and is rapidly hydrolyzed in experimental animals to yield fenvaleric acid as a major metabolite (Kaneko et al., 1981). The potential of FEN to induce oxidative damage in various mammalian tissues in vivo have not been comprehensively investigated. Earlier demonstration of oxidative stress in various tissues of rats were detected following administration of single and multiple sub-lethal oral doses (Parasanthi, 2001).

Antioxidant micronutrients are being widely studied for their potentially beneficial properties in prevention of human diseases, including cancer, arthritis and cardiovascular diseases (Faure et al., 2007). Selenium is a structural component of several enzymes with physiological antioxidant properties, including glutathione peroxidase (GSH-Px) and thioredoxine (Perottoni et al., 2004). In recent years, there had been a great deal of studies carried out on selenium metabolism. In most of these studies the external selenium was given to experimental animals as sodium selenite (Shi et al., 2004). Because of the health problems induced by many environmental pollutants, efforts have been undertaken in evaluating the relative antioxidant potential of selenium (El-Demerdash, 2004).

Therefore, the objective of this study was to evaluate the role of selected antioxidant micronutrients - selenium and omega3 fatty acids on rat liver and brain cytochrome P450 enzymes induced by fenvalerate.

MATERIALS AND METHODS

Chemicals

Fenvalerate (purity 98%) was purchased from Chem. Service Inc. (West Chester, PA). Reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), reduced nicotinamide adenine dinucleotide phosphate (NADPH), amidopyrine, cytochrome C, thiobarbituric acid and all other chemicals were purchased from Sigma Chemical Company (Saint Louis, USA). Use of fenvalerate was approved by the Animal Care Committee and met all guidelines for its use.

Animals

Male rats (n = 40) with average body weight of 200±50 g were obtained from National Research Institute, Cairo, Egypt and acclimatized for 2 weeks prior to the experiment. They were assigned to four groups and housed in Universal galvanized wire cages at room temperature (22-25 °C) and in a photoperiod of 14 h light/10 h dark per day. Animals were fed with standard balanced commercial diet containing 18% crude protein, 14% crude fiber, 2% fat and 2600 kcal DE/kg feed was used and water was provided ad libitum.

Experimental design

The animals were housed in groups of 10 rats each and divided randomly into 4 groups. Treatments were carried out day by day for 20 days. The first group served as control (C) and injected orally with corn oil, group 2 was injected with a single dose of FEN (20 mg/kg, orally) for 24 h prior to decapitation, group 3 was administered orally with ω 3 (20 mg/kg) and Se (4.10 mg/kg; ω+Se), group 4 was administered orally with FEN following the treatment with ω3 and Se (FEN+ω3+Se). Treatment period extended for 3 weeks.

Tissue preparations and assays

Preparation of liver microsomes

Rats were fasted for 24 h prior to each designated time period and were then sacrificed by cervical dislocation. The abdominal cavity and the head were opened immediately; liver and brain were removed, washed with cold 0.1 M phosphate buffer (pH 7.4), weighed and chilled on ice. All the following procedures were carried out in cold conditions. A 33% (W/V) crude homogenate was prepared in 0.1 M phosphate buffer (pH 7.4) by homogenization with a Teflon pestle, using 5 strokes. The crud homogenate was then centrifuged at 11,000 xg for 20 min at 4 °C to remove the intact cells, nuclei and mitochondria. The supernatant was subsequently centrifuged at 105,000 xg for 60 min at 4 °C to sediment the microsomal pellet. The pellet was then resuspended in 0.1 M phosphate buffer (pH 7.4), kept in ice bath and used as the enzyme source.

Determination of proteins

The protein concentration of the hepatic and brain microsomal fractions was determined by the method of Lowery et al. (1951).

Enzyme assays

Liver and brain microsomal cytochrome P450 and b_5 were determined according to Omura and Sato (1964), using molar extinction coefficient 91 $cm^{-1}mM^{-1}$ for P450 and 185 $cm^{-1}mM^{-1}$ for cytochrome b_5, respectively. The activity of microsomal NADPH – cytochrome C reductase was assayed according to the method of Williams and Kamin (1962). The total volume of incubation mixture was 2.2 ml and contained 0.5 M potassium phosphate buffer (pH 7.5), 100 ηM NADPH, 50 ηM cytochrome C, and 33% microsomal enzyme. The rate of reduction of cytochrome C was measured at 0 and 30 seconds

after addition of NADPH at wavelength of 550 ηm. The activity of this enzyme was calculated by using extinction coefficient of 21 $cm^{-1}mM^{-1}$.

Glutathione-S-transferase activity was assayed according to the method of Habig et al. (1974). The incubation mixture contained 30 µg protein of the supernatant fraction, 0.5 ml of reduced glutathione (0.5 mM), 0.1 M sodium phosphate buffer (pH 7.3). After preincubation at 37 °C for 5 min the reaction was initiated by adding 50 µl of 1-chloro-2,4-dinitrobenzene (CDNB; 0.5 mM) and incubated at 37 °C for another 5 min; the reaction was terminated by the addition of 0.2 ml of trichloroacetic acid solution (33% W/V). After centrifugation, the CDNB conjugate was measured spectrophotometrically at 340 ηm. Calculations were done using a molar extinction coefficient of 9.60 $cm^{-1}mM^{-1}$. A unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 µM of CDNB conjugate per milligram of protein per minute under the assay conditions.

The activity of amidopyrine N-demethylase was measured according to Nash (1953). The method is based on measurement of the concentration of formaldehyde produced by oxidative N-demethylation of amidopyrine in microsome. The incubation mixture (1.71 ml) contained 0.4 ml of 0.1 M Tris-HCl buffer (pH 7.4), 0.4 ml of 2.5 µM magnesium chloride, 0.2 ml of 1 mM NADPH, 0.1 ml of microsomal suspension and 0.11 ml of 80 mM amidopyrine. After incubation at 37 °C for 20 min, the reaction was stopped by adding 0.25 ml of 25% zinc sulphate and 0.25 ml of aqueous solution of barium hydroxide. After centrifugation (3,000 rpm for 10 min) formaldehyde was determined spectrophotometrically from changes in the colour intensity of the supernatant at 412 ηm. The enzyme activity was then expressed as µM of formaldehyde/min x kg liver/brain sample.

The activity of aniline 4-hydroxylase was measured according to Kato and Gillette (1965). The incubation mixture (1.71 ml) contained 0.4 ml of 0.08 mol Tris-HCl buffer, pH 7.4, 0.4 ml of 0.16 mol magnesium chloride, 0.2 ml of 0.03 mol NADPH, 0.1 ml of microsomal suspension and 0.11 ml of 0.03 mol aniline. After incubation at 37 °C for 20 min, the reaction was stopped by adding 0.5 ml of 15% trichloroacetic acid. After centrifugation (at 1,000 xg for 10 min), 1 ml of supernatant was added to 0.5 ml of 10% sodium carbonate and 1.5 ml of 2% phenol. After incubation at 37 °C for 30 min, the color developed was measured spectrophotometrically at 630 ηm.

Thiobarbituric acid reactive substances (TBARS) were measured according to the method described by Tapel and Zalkin (1959). The colour intensity of the TBARS reactants was measured at 532 ηm and a molar extinction coefficient of 156,000 $cm^{-1}mM^{-1}$ was used for calculation of the concentration.

Statistical analyses

Statistical analyses were done to obtain the standard deviation and standard errors of mean. The data for the treated animals were compared with data for the control and FEN treated animals by using the Student's t-test.

RESULTS AND DISCUSSION

Treatment with FEN didn't cause mortality in the group treated with a single dose of FEN (20 mg/kg). No significant change was observed in liver body weight ratio in the group treated with FEN. A slight increase in the brain body weight ratio was observed in treated group. Also, oral administration of FEN had no significant difference on hepatic and brain microsomal protein (Table 1 and 2). In agreement with our results Morisseau et al. (1999) indicated no toxic effect attributable to fenvaleric acid II and no significant difference in the protein content of hepatic cytosolic, peroxisomal and microsomal fractions for any of the doses administered.

Oral administration of FEN was found to produce a significant increase in the hepatic content of b_5 and P450 by 48.90% and 26.90%, respectively. However, as compared with liver, the magnitude of induction in b_5 and P450 contents in rat brain was found to be much higher (55.90% and 47.90%, respectively). On the other hand, pretreatments of rats with ω3 and Se as repeated doses prior to administration of FEN was found to decrease the hepatic and brain contents of b_5 and P450 (Table 1 and 2). Inhibition of cytochrome P450 after repeated dose treatments of rats with ω3 and Se may protect the liver against the toxicity of FEN (Sheweita et al., 2001). Cytochrome b_5 plays a great role in the reduction of cytochrome P450 substrate complex by providing electrons through the NADH pathway. Therefore, the increase in the hepatic and brain contents of cytochrome b_5 in the group treated with a single dose of FEN may increase the rate of biotransformation of drugs and carcinogens (El-Banna, 2006).

Previous studies have shown that reduced glutathione (GSH) level and glutathione S-transeferase (GST) activity can reduce the covalent binding of the activated forms of well known carcinogens, e.g. benzo α-pyrene, N-nitrosodimethylamine, aflatoxin B1; to DNA and other macromolecules (Gopalan et al., 1992 and Maloveille et al., 1981). The activity of GST was significantly induced in rat liver and brain in FEN treated group by 62.70 % and 36.80 %, respectively (Table 1 and 2). The higher activity of glutathione S-transeferase in the treated group suggested that GST might play an important role in the detoxication of FEN.

The activity of NADPH - cytochrome C reductase significantly decreased by 53.60% in liver microsome in the present study, meanwhile, this activity

Table 1: Effects of omega 3 fatty acids (ω_3) and selenium (Se) on fenvalerate (FEN) induced oxidative stress, on hepatic mixed function monooxygenases and TBARS of rat liver (Data expressed as Mean ± SEM)

Parameter \ Group	Control [a]	FEN[a]	ω_3+Se[a]	FEN+ω_3+Se[b]
Liver body weight ratio	0.040± 0.001	0.046± 0.005	0.049± 0.005	0.055± 0.006
		ρ >0.05	ρ < 0.01	ρ < 0.001
Liver microsomal protein	2.20± 0.237	2.36± 0.280	2.77± 0.361	2.80± 0.352
		ρ >0.05	ρ < 0.05	ρ < 0.05
Cytochrome b_5	2.62± 0.278	3.90± 0.112	2.14± 0.300	1.93± 0.507
		ρ < 0.001	ρ < 0.05	ρ < 0.001
Cytochrome P450	5.84± 0.102	7.41± 0.509	4.17± 0.204	4.84± 0.611
		ρ < 0.01	ρ < 0.001	ρ < 0.01
Glutathione S transferase	5.55± 0.495	9.03± 0.871	10.47± 1.571	8.80± 0.881
		ρ < 0.001	ρ < 0.01	ρ >0.05
NADPH - cytochrome C reductase	316.5± 10.6	146.8± 9.3	285.0± 29.0	115.8± 1.6
		ρ < 0.001	ρ < 0.05	ρ < 0.001
Amidopyrine N-demethylase	0.233± 0.006	0.210± 0.010	0.214± 0.022	0.172± 0.010
		ρ < 0.05	ρ > 0.05	ρ < 0.01
Aniline-4-hydroxylase	0.030± 0.007	0.026± 0.001	0.044± 0.005	0.028± 0.003
		ρ > 0.05	ρ < 0.05	ρ < 0.05
TBARS	1.52± 0.028	1.76± 0.109	1.49± 0.074	1.63± 0.089
		ρ < 0.01	ρ >0.05	ρ < 0.01

Hepatic microsomal protein content was expressed as mg protein/g liver, cytochrome b_5 and cytochrome P450 contents were expressed as ηM cytochrome/mg microsomal protein, Glutathione S-transferase activity was expressed as units/mg protein, NADPH - cytochrome C reductase activity was expressed as ηM cytochrome C reductase/mg protein/min, amidopyrine N-demethylase in liver microsomes was expressed as μM/min x kg liver sample, aniline-4-hydroxylase was expressed as μM/min/mg protein, and thiobarbituric acid reactive substances (TBARS) were expressed as μM TBARS/g tissue.a, vs. control; b, vs. FEN

significantly increased by 32.40% in brain microsome in FEN administered group (Table 1 and 2). NADPH - cytochrome C reductase activity is a component of the microsomal mixed-function oxidase system which catalyses hydroxylation reaction, and this process is of prime importance in the metabolism of lipids, drugs and other foreign compounds (Vermilion et al., 1981). The induction of NADPH - cytochrome C-reductase in rat brain in the present study could be one of the defense mechanisms of the animals to increase the rate of reduction of cytochrome P450 substrate complex (Sheweita et al., 2001).

Pretreatments of rats with repeated doses of ω3 and Se are more effective in decreasing the activity of amidopyrine N-demethylase prior to FEN administration in rat liver (Table 1). Inhibition of cytochrome P450 content and amidopyrene N-demethylase activity may play a significant role on the reduction of oxidative damage induced by FEN.

The level of TBARS significantly decreased in rat liver and brain by 7.40% and 9.90%, respectively in the group pretreated with repeated doses of ω3 and Se prior to FEN administration. Meanwhile, FEN induced significant increases in lipid peroxidation in rat liver and brain was by 15.80% and 14.40%, respectively (Table 1 and 2). Lipid peroxidation may be due to oxidation of molecular oxygen to produce superoxide radicals. This reaction is also the source of H_2O_2, which causes the production of malondialdehyde (MDA) by initiating the peroxidation of unsaturated fatty acids in the membrane. Both H_2O_2 and O_2^- produce highly reactive hydroxyl radical that can initiate lipid peroxidation leading to loss of membrane structure and function (Kale et al., 1999). The increase in the concentration of TBARS and the activity of GST in FEN treated group (Table 1 and 2) suggested that FEN and its metabolites may be detoxified in these tissues (Atessahin et al., 2003). Selenium, in the form of glutathione peroxidase (GSH-Px), plays an

Table 2: Effects of omega 3 fatty acids (ω_3) and selenium (Se) on fenvalerate (FEN) induced oxidative stress on brain mixed function monooxygenases and TBARS of rat brain. (Data expressed as Mean ± SEM)

Parameter \ Group	Control [a]	FEN[a]	ω_3+Se[a]	FEN+ω_3+Se[b]
Brain body weight ratio	0.009± 0.0006	0.012± 0.0028	0.012± 0.0011	0.011± 0.0015
		ρ<0.05	ρ < 0.01	ρ > 0.05
Brain microsomal protein	0.868± 0.084	0.744± 0.119	0.498± 0.107	0.740± 0.081
		ρ >0.05	ρ < 0.001	ρ > 0.05
Cytochrome b5	2.47± 0.208	3.85± 0.470	1.94± 0.280	2.06± 0.284
		ρ < 0.01	ρ < 0.05	ρ < 0.001
Cytochrome P450	5.51± 0.289	8.15± 0.597	4.49± 0.294	5.18± 0.185
		ρ < 0.01	ρ < 0.001	ρ < 0.001
Glutathione S transferase	15.96± 0.990	21.83± 0.893	35.83± 1.604	20.55± 2.024
		ρ < 0.001	ρ < 0.001	ρ >0.05
NADPH - cytochrome C reductase	852.1± 64.5	1128.51± 27.6	1377.4± 148.6	691.7± 18.2
		ρ < 0.001	ρ < 0.001	ρ < 0.001
Amidopyrine N-demethylase	0.186± 0.015	0.185± 0.011	0.165± 0.005	0.175± 0.009
		ρ > 0.05	ρ < 0.05	ρ > 0.05
Aniline-4-hydroxylase	0.165± 0.008	0.197± 0.015	0.313± 0.032	0.124± 0.011
		ρ < 0.01	ρ < 0.001	ρ < 0.01
TBARS	2.29± 0.130	2.62± 0.110	2.59± 0.121	2.36± 0.055
		ρ < 0.05	ρ < 0.05	ρ < 0.05

Brain microsomal protein content was expressed as mg protein/g brain, cytochrome b_5 and cytochrome P450 contents were expressed as ηM cytochrome/mg microsomal protein, Glutathione S-transferase activity was expressed as units/mg protein, NADPH - cytochrome C reductase activity was expressed as ηM cytochrome C reductase/mg protein/min, amidopyrine N-demethylase in brain microsomes was expressed as μM/min x kg brain sample, aniline-4-hydroxylase was expressed as μM/min/mg protein, and thiobarbituric acid reactive substances (TBARS) were expressed as μM TBARS/g tissue. a, vs. control; b, vs. FEN.

important role in the protection of tissues from oxidative damage and can antagonize the toxic effects of some chemical substances (Raneva et al., 2002).

In conclusion, the present study provides direct evidence that micronutrients association has a beneficial effect on protecting the liver, brain and probably other organs against the toxicity and oxidative damage of fenvalerate through inhibition of cytochrome P450 and other members of drug metabolizing enzymes.

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