

Effects of Phenobarbital on Serum and Liver Paraoxonase and Arylesterase Activities in Rats

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Abstract: This study was carried out to determine the effects of phenobarbital, administered orally and intraperitoneally over different periods, on paraoxonase, arylesterase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and total protein levels in the serum and liver. Thirty-six rats, 16-18 weeks old and weighing 240-260 g, were used. Phenobarbital was applied both orally and intraperitoneally at a dose of 100 mg/kg. Paraoxonase and arylesterase activities in serum and liver samples were measured spectrophotometrically using paraoxon and phenylacetate substrates, respectively. Although no significant changes were determined in serum paraoxonase and arylesterase activities, liver paraoxonase and arylesterase activities and total protein levels increased significantly in both the orally and intraperitoneally treated groups. An increase was observed in serum ALT and AST activities, which may indicate the presence of liver damage. In conclusion phenobarbital, known as a classical enzyme inducer, may increase the activities of the aforementioned enzymes by increasing the synthesis of new proteins in liver microsomes.

Key Words: Rat, phenobarbital, paraoxonase, arylesterase

Ratlarda Serum ve Karaciğer Paraoksonaz ile Arilesteraz Aktiviteleri Üzerine Fenobarbitalin Etkileri

Özet: Bu çalışma farklı periyotlarda oral ve periton içi yolla uygulanan fenobarbital'in serum ve karaciğer paraoksonaz, arilesteraz, aspartat aminotransferaz (AST) ve alanin aminotransferaz (ALT) aktiviteleri ile total protein düzeyleri üzerine olan etkilerinin belirlenmesi amacıyla yapıldı. Çalışmada 36 adet, 16-18 haftalık, 240-260 g ağırlıklarında rat kullanılmıştır. Fenobarbital hem ağızdan hem de periton içi yolla 100 mg/kg dozunda uygulanmıştır. Serum ve karaciğerde paraoksonaz ile arilesteraz aktiviteleri sırasıyla paraokson ve fenilasetat'ın substrat olarak kullanılmasıyla spektrofotometrik olarak belirlenmiştir. Fenobarbital verilen gruplarda serum paraoksonaz ve arilesteraz aktivitelerinde önemli değişmelerin olmadığı, buna karşın karaciğer paraoksonaz ve arilesteraz aktiviteleri ile total protein düzeylerinde önemli artışların olduğu belirlenmiştir. Karaciğer toksisitesinin bir göstergesi olarak serum AST ve ALT aktivitelerinin arttığı görülmüştür. Sonuç olarak klasik enzim indükleyicisi olarak bilinen fenobarbitalin muhtemelen karaciğer mikrozomlarında yeni proteinlerin sentezini artırarak karaciğerde söz konusu enzimlerin aktivitelerinde bir artışa yol açabileceği düşünülmektedir.

Anahtar Sözcükler: Rat, fenobarbital, paraoksonaz, arilesteraz

Introduction

Esterases play major roles in the hydrolysis of a number of prodrugs in humans and experimental animals. They are classified into 3 groups (A- B- and C-esterases) on the basis of their reactivity with organophosphorus compounds such as paraoxon and diisopropyl fluorophosphate. A-esterases, including arylesterases, hydrolyse organophosphates rapidly, whereas B-esterases, including acetylcholinesterase and nonspecific carboxylesterase, are inhibited by organophosphates. C-esterases such as acetylcholinesterase do not interact with

organophosphates. Inter-individual variation in the activity of esterases is an important factor which influences both the pharmacological and toxicological effects of prodrugs in humans and animals (1-3).

Oxidised low density lipoproteins (LDLs) are believed to play an important role in the events associated with the initiation of atherosclerosis. High density lipoproteins (HDLs) have been shown to prevent oxidative modification of LDLs in vitro and in vivo (4,5). Paraoxonase and the platelet-activating factor acetylhydrolase, associated with HDLs, are responsible for

its anti-oxidative and anti-inflammatory properties. Studies indicate that paraoxonase can significantly lower lipid peroxidative generation during LDL oxidation and that therefore, it may provide HDL-associated protection against the atherosclerotic process (4,6-8).

Enzyme inducers can be used as tools when one wishes to evaluate the relative importance of different enzymatic systems in xenobiotic metabolism (9,10). Phenobarbital is a classical inducer of the cytochrome P450 system, also including some other subgroups. Phenobarbital is known to moderately induce cholinesterases, carboxylesterases and hepatic paraoxonase activities. It is reported to decrease the toxicity of some organophosphates as a result of an increase in paraoxonase activity. In addition, a number of xenobiotics increase serum AST and ALT activities and are used as markers of liver toxicity. It is reported that phenobarbital treatment increased these activities and protein levels in the liver (10-13).

The aim of this study was to investigate the effects of orally and intraperitoneally administered phenobarbital on paraoxonase and arylesterase activities which induce a number of biochemical events in humans and animals.

Materials and Methods

Animal Material: Thirty-six rats, 16-18 weeks old and weighing 240-260 g, were used in this study. The animals were adapted to the laboratory conditions before the experiment. Water and food were given ad libitum. The rats were divided into 6 groups, each containing 6 animals.

Group 1: (Control group): Isotonic sodium chloride administrated orally and intraperitoneally for 3 days.

Group 2: Phenobarbital administered orally for 1 day.

Group 3: Phenobarbital administered orally for 3 days.

Group 4: Phenobarbital administered orally for 7 days.

Group 5: Phenobarbital administered intraperitoneally once.

Group 6: Phenobarbital administered intraperitoneally 3 times at 24 h intervals.

Drug Administration and Sample Collection: Phenobarbital (Merck) was dissolved in water (1 g/l) and

the animals were allowed free access to drinking water for 1, 3 and 7 days (groups 2, 3 and 4). Phenobarbital was given at a dose of 100 mg/kg. In groups 5 and 6 a phenobarbital solution (20 mg/ml) was used. These groups were treated intraperitoneally with the same dose of the drug suspended in 1 ml of distilled water. Rats were decapitated following the drug administrations in groups 2, 3, 4 and 24 h after the last treatment in groups 5 and 6. Liver samples were homogenised in sucrose solution (0.25 mol/l) in a 1:4 ratio. The homogenates were centrifuged at 12,500 rpm and the supernatant was obtained for analysis (14,15).

Determination of Enzyme Activities: The determination of paraoxonase activity is based on the spectrophotometric measurement of p-nitrophenol levels released as a result of the enzymatic hydrolysis of paraoxon (14,15). The measurement of paraoxonase activity was carried out in the presence and absence of sodium chloride. The levels of paraoxonase without sodium chloride were calculated by adding 350 µl of a mixture consisting of paraoxon (2 mmol/l), calcium chloride (2 mmol/l) and Tris HCl buffer (pH 8.0) in 10 µl of serum. In the measurement of paraoxonase stimulating with sodium chloride, 1 mmol/l of sodium chloride was added to the above mixture. Phenylacetate was used as substrate to determine arylesterase activity. Paraoxonase and arylesterase activities were measured by spectrophotometry (Shimatsu UV 1200) at 405 and 270 nm, respectively.

The levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined as preceding liver damage by autoanalyser (Olympus-600) using the relevant test kits (Randox) with standard methods. Total protein levels were ascertained using the method described by Lowry et al. (16).

Statistical Analysis: The results were evaluated using a 't' test in the SPSS for Windows program.

Results

Paraoxonase, arylesterase, AST, ALT and total protein levels in animals treated with phenobarbital, orally and intraperitoneally, are shown in Tables 1-4. In the oral administrations there were no statistically significant changes in serum paraoxonase and arylesterase activities among the control and experimental groups (groups 2, 3 and 4). On the other hand, the levels of AST, ALT and

total proteins increased significantly in the experimental groups (Table 1). Significant increases were also obtained in the levels of liver paraoxonase, arylesterase and total protein in the experimental groups compared with the control group (Table 2).

In the intraperitoneal administrations, although decreases were observed in serum paraoxonase and

arylesterase activities in the experimental groups (groups 5 and 6), AST, ALT and total protein levels increased compared with the control group (Table 3). The levels of liver paraoxonase and arylesterase and total protein increased significantly in the experimental groups (groups 5 and 6) (Table 4).

Table 1. The effects of phenobarbital on serum paraoxonase, arylesterase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and total protein levels in the orally treated groups.

	Paraoxonase (mU/ml)	NaCl-stimulated paraoxonase (mU/ml)	Arylesterase (kU/l)	AST (u/l)	ALT (u/l)	Total protein (g/dl)
Control	140 ± 10.23	263 ± 15.29	107 ± 3.02	134 ± 5.66	86 ± 2.36	7.42 ± 0.23
Group 2	141 ± 12.54	260 ± 34.31	105 ± 4.98	140 ± 4.34	91 ± 5.45	7.32 ± 0.35
Group 3	143 ± 9.58	266 ± 26.64	104 ± 0.96	167 ± 8.77*	98 ± 5.69	7.83 ± 0.11
Group 4	124 ± 18.36	245 ± 15.37	105 ± 1.25	234 ± 9.25***	112 ± 5.48*	8.15 ± 0.44*

*P < 0.05 **P < 0.01 *** P < 0.001

Table 2. The effects of phenobarbital on liver paraoxonase, arylesterase activities and total protein levels in the orally treated groups.

	Paraoxonase (U/g)	NaCl-stimulated paraoxonase (U/g)	Arylesterase (U/g)	Total protein (g/dl)
Control	1928 ± 54.31	2334 ± 69.41	384 ± 10.28	10.24 ± 1.25
Group 2	2166 ± 82.12*	2556 ± 58.25*	481 ± 21.54*	13.04 ± 0.98*
Group 3	2288 ± 39.47*	2575 ± 79.32*	515 ± 14.59*	12.12 ± 2.02*
Group 4	2474 ± 20.56**	2871 ± 57.39**	573 ± 36.25**	13.46 ± 0.65*

*P < 0.05 **P < 0.01 *** P < 0.001

Table 3. The effects of phenobarbital on serum paraoxonase, arylesterase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and total protein levels in the intraperitoneally treated groups.

	Paraoxonase (mU/ml)	NaCl-stimulated paraoxonase (mU/ml)	Arylesterase (kU/l)	AST (u/l)	ALT (u/l)	Total protein (g/dl)
Control	140 ± 3.55	263 ± 9.57	107 ± 2.98	134 ± 0.54	86 ± 1.65	7.42 ± 0.65
Group 5	135 ± 4.68	262 ± 7.84	98 ± 5.64	312 ± 1.02***	105 ± 5.25*	9.33 ± 0.24
Group 6	128 ± 15.69*	248 ± 19.25	87 ± 3.42*	308 ± 1.36***	121 ± 10.2*	8.96 ± 1.23*

*P < 0.05 **P < 0.01 *** P < 0.001

Table 4. The effects of phenobarbital on liver paraoxonase, arylesterase activities and total protein levels in the intraperitoneally treated groups.

	Paraoxonase (U/g)	NaCl-stimulated paraoxonase (U/g)	Arylesterase (U/g)	Total protein (g/dl)
Control	1928 ± 45.29	2334 ± 54.62	384 ± 3.56	10.24 ± 0.45
Group 5	2518 ± 98.36*	2700 ± 45.21*	421 ± 9.87*	11.76 ± 0.97*
Group 6	2569 ± 80.03**	2858 ± 78.65***	492 ± 11.85**	11.94 ± 1.23*

*P < 0.05 **P < 0.01 *** P < 0.001

Discussion

Paraoxonase, synthesised in the liver, is a serum esterase related to calcium. Although this enzyme plays an important role in the metabolism of various xenobiotics, its role in physiological events has not yet been clarified. Phenobarbital significantly stimulates metabolic pathways in liver microsomes, and is therefore used as a microsomal enzyme inducer (9-11).

Hernandez et al. (12) reported that while phenobarbital increased liver paraoxonase and arylesterase activities by 58% and 61%, respectively, by inducing microsomal enzymes, the activities of these enzymes in plasma were not affected. Microsomal proteins showed an increase of 7% following the administration of phenobarbital, and the increase in microsomal enzyme activities is explained by the synthesis of new proteins (10,12). In this study, serum paraoxonase activities were not changed in groups 2 or 3, whereas a decrease was observed in group 4, treated orally with phenobarbital. On the other hand, increases were seen in liver paraoxonase and arylesterase activities in all groups orally treated with phenobarbital. In the 2 intraperitoneally treated groups, while serum paraoxonase levels decreased, they increased in the liver. Liver total protein levels also increased significantly when compared with the control group, which supported the observations of the above researchers.

In other studies (9,13,15), it has been reported that although phenobarbital, unlike other esterases, reduced serum paraoxonase activity, it increased liver paraoxonase activity. This indicates that there is no association between liver and serum activities. The findings of this study showed that although phenobarbital significantly increased liver paraoxonase activities, it did not cause significant changes in serum paraoxonase activities. The

intraperitoneal administrations were more effective than the oral applications owing to their easy access to the blood stream and prominent effect on the liver. In contrast, in the oral application some quantities of the drug might have been discharged before absorption through the intestines. Parallel to the results of other researchers, no significant changes were observed in serum paraoxonase activity in any orally or intraperitoneally treated group.

Phenobarbital has been reported to affect arylesterase activities in 2 different ways. First, it induces enzymes which metabolise cytochromes or other phase I drugs. Second, it induces some microsomal A-esterase isoenzymes which have access to the blood (11, 12). The findings of this study, that despite significant increases being obtained in liver arylesterase activities, there were no significant changes in serum, support the opinions of the above researchers.

Serum AST and ALT activities are used as markers of liver toxicity (17). However, Kaliste-Korhonen et al. (9) reported that phenobarbital increased serum AST and ALT activities. In our study, increases were also observed in these activities in both oral and intraperitoneal administrations.

A-esterases play important roles in the detoxification of organophosphates and in the prevention of atherosclerosis due to their association with HDLs (4). The metabolism of many xenobiotics, particularly organophosphate compounds, may be altered by the induction of these enzymes (9,10). In conclusion, although an increase was detected in paraoxonase and arylesterase activities in the liver, no changes were observed in serum. This is thought to be the result of an increase in new protein synthesis in the liver by phenobarbital.

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