Phenobarbital Effects on Brain and Liver Tissues Enzyme Activity in Balb/C Mice

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Received October 8, 2001
Accepted June 19, 2002

Abstract

Yazar, E., O. Demir, M. Elmas, A. L. Bas, B. Tras: *Phenobarbital Effects on Brain and Liver Tissues Enzyme Activity in Balb/C Mice.* Acta Vet. Brno 71, 2002: 309-312.

The purpose of the present study was to investigate the effect of phenobarbital on some brain and liver tissue enzyme activities in Balb/C mice. Forty male Balb/C mice were used. Ten mice served as a control group, and thirty mice were administered with phenobarbital (80 $mg \cdot kg^{-1}$ body mass, single oral dose). Brain and liver samples were taken at 6, 12 and 24 h after drug administration. Brain and liver tissues alkaline phosphatase, aspartate aminotransferase, gamma glutamyl transferase and amylase activities were measured by auto-analyzer.

Phenobarbital did not affect gamma glutamyl transferase and amylase activities in the brain and liver, and alkaline phosphatase and aspartate aminotransferase activities in the liver. However, statistically significant ($p < 0.05$) increases of alkaline phosphatase and aspartate aminotransferase activities were observed in the brain. In general, after phenobarbital administration, serum alkaline phosphatase and aspartate aminotransferase activities increase and these increases are believed to be derived from the liver. These result suggest that brain may contribute to increased activities of these enzymes in serum in Balb/C mice.

Phenobarbital, liver, brain, enzyme, mouse

Phenobarbital (PB) is a major drug in the treatment of canine, feline and human epilepsy and can significantly reduce the severity of seizures. PB raises the threshold for seizure discharge and inhibits the initiation, diffusion, and spread of discharge from the neural focus. Drug is rapidly and completely absorbed after oral doses and clinically effective 12 to 24 h after oral administration. PB is metabolized by liver and extracted by the kidney (Barragry 1994; Foster et al. 2000; Gieger et al. 2000).

Common side effects of PB are ataxia, sedation, polyuria, polydipsia, polyphagia, and it may depress both the respiratory drive and the mechanisms responsible for the rhythmic character of respiration (Hobbs 1996; Booth 1998; Branum et al. 1998). In addition to these side effects, PB stimulates the division of liver cells (Alberts et al. 1998) and causes increases in serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) activities (Aiges et al. 1980; Luoma et al. 1980; Verma and Haidukewych 1994; Chauvet et al. 1995).

ALP, AST and GGT enzymes are accepted as indicators of hepatotoxicity (Boyd 1982; Isogai et al. 1994; Rosenthal 1997). Also, GGT and ALP are present in blood-brain barrier (Brust et al. 1994; Agrawal et al. 1996; El-Hafny et al. 1996; Lawrenson et al. 1999). Amylase (AM) is an especially accepted pancreatic enzyme. However, in the body, AM is present in a number tissues such as salivary glands, semen, testes, lungs, adipose tissue and liver. In addition to this, cerebral trauma has also been associated with hyperamylasemia (Moss and Henderson 1994).

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The aim of the current study was to investigate the effect of phenobarbital on ALP, AST, GGT and AM activities of brain and liver tissues in Balb/C mice.

Materials and Methods

Forty male Balb/C (aged approximately 5 months, body mass 38-44 g) mice were used in the experiment (Selcuk University, Experimental Medicine, Research and Application Center, Konya, Turkey). Mice were fed a standard pellet diet and tap water *ad libitum* during the entire experimental period. Ten mice were used as a control group. Thirty mice were administered PB (Luminaletten®, Bayer, Istanbul) via intragastric gavage needle at doses 80 $mg \cdot kg^{-1}$ body wt, orally (Yuschak and Gautieri 1993; Yazar et al. 2001). After the administration of PB, ten mice were randomly selected and killed at 6, 12 and 24 h.

Brain (cerebrum) and liver (300 mg, lobus hepatis sinister) samples were immediately removed and washed with cold (+4 ^{0}C) saline solution after killing. Samples were homogenized with 500 µl of cold (+4 ^{0}C) homogenate solution (0.25 M sucrose (Sigma) + 10 mM Tris (Sigma) + 1 mM EDTA (Pharmacia Biotech), pH 7.4) into ice (Vani et al. 1990; Yazar and Tras 2001). The homogenates were centrifuged (11.150 *g*, 15 min, +4 ^{o}C), and the supernatants were carefully removed and stored for analysis (-80 ^{o}C). Brain and liver tissues ALP (EC:3.1.3.1.), AST (EC:2.6.1.1.), GGT (EC:2.3.2.2.), AM (EC:3.2.1.1.) and protein levels were measured using an auto analyzer (Olympus 5200, Japan).

All values are expressed as mean ± SEM. The results were analyzed by a parametric Tukey HSD multiple range test (SPSS for Windows, release 6.0). In all cases, probability of error of less than 0.05 was selected as the criterion for statistical significance from the control values.

Results

Brain and liver tissues ALP, AST, GGT and AM activities are given in Table 1. Brain tissue ALP and AST activities increased ($p < 0.05$) at 12 and 24 h when compared with control group.

Table 1
Effect of phenobarbital (PB) on brain and liver tissues alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) and amylase (AM) activities in Balb/C mice (mean ± SEM, n = 40)

	Control (n= 10)	6th hour (na = 10)	12th hour (n = 10)	24th hour (na = 10)
Brain				
ALP $U \cdot mg^{-1}$ Tissue protein	60.90 ± 4.63	70.77 ± 9.21	87.06 ± 4.19 *	89.29 ± 6.65*
AST $U \cdot mg^{-1}$ Tissue protein	121.67 ± 8.11	124.62 ± 5.52	147.07 ± 5.13*	151.74 ± 9.63*
GGT $U \cdot mg^{-1}$ Tissue protein	4.85 ± 0.51	4.61 ± 1.25	5.01 ± 1.43	4.78 ± 1.51
AM $U \cdot mg^{-1}$ Tissue protein	4.36 ± 0.59	5.22 ± 0.55	5.47 ± 0.62	5.57 ± 0.48
Liver				
ALP $U \cdot mg^{-1}$ Tissue protein	11.01 ± 0.99	9.30 ± 1.10	8.45 ± 0.55	8.25 ± 0.66
AST $U \cdot mg^{-1}$ Tissue protein	709.00 ± 25.40	661.50 ± 41.97	667.44 ± 32.72	616.43 ± 43.05
GGT $U \cdot mg^{-1}$ Tissue protein	4.33 ± 0.65	3.94 ± 0.30	4.37 ± 0.61	4.92 ± 0.43
AM $U \cdot mg^{-1}$ Tissue protein	8.07 ± 1.15	8.82 ± 0.49	6.29 ± 0.52	6.69 ± 0.52

*$p < 0.05$ significantly different from control group

Discussion

In the present study, it was observed that while brain tissue ALP and AST activities increased significantly ($p < 0.05$) at 12 and 24 h, no significant alterations were observed in hepatic ALP and AST activities after PB administration. ALP and GGT were strongly present in cortical capillaries of blood-brain barrier (Brust et al. 1994; Agraval et al. 1996; Lawrenson et al. 1999). PB has been evaluated in rodent models of epilepsy in many studies (Loscher et al. 1988; King and LaMotte 1989; Bac et al. 1998) and caused increases in serum ALP, AST and GGT activities (Luoma et al. 1980; Aiges et al. 1980; Verma and Haidukewych 1994; Chauvet et al. 1995). In addition, AST activity alterations were mainly due to PB (Verma and Haidukewych 1994). Many studies reported that PB caused increases in serum AST (Fortman and Witte 1985; Haidukewych and John 1986; Kitchin and Brown 1987) and ALP activities (Chauvet et al. 1995; Filardi et al. 2000; Foster et al. 2000). In the present study, brain tissue ALP and AST activities increased significantly, but no changes were observed in the liver. ALP and AST are present in many organs such as heart, skeletal muscle, intestine, bone and kidney excluding liver (Rosenthal 1997). Damage occurring in these tissues caused by drugs can cause increases in serum ALP and AST activities (Tardivel et al. 1992; Rosenthal 1997). GGT activities in brain and liver tissue were not affected by PB in the current study. It was earlier stated that only phenytoin seemed to be responsible for the elevation of GGT activity (Sano et al. 1981), and no effect in the serum GGT activity was observed following PB administration in healthy dogs (Gieger et al. 2000).

It is concluded that PB caused increases in brain tissue ALP and AST activities. Increased serum ALP and AST levels may not be derived from the liver only after PB treatment, other tissues possessing these enzymes may contribute to their increases serum activities.

Vliv fenobarbitalu na aktivitu některých enzymů jaterní a mozkové tkáně myší Balb/C

Předmětem studie bylo posouzení účinku fenobarbitalu na aktivitu některých enzymů jaterní a mozkové tkáně myší Balb/C. Bylo použito čtyřicet myší Balb/C samčího pohlaví. Deset zvířat sloužilo jako kontrolní skupina a čtyřiceti myším byl podán fenobarbital (80 mg/kg tělesné hmotnosti, perorálně, individuálním podáním). Vzorky mozkové a jaterní tkáně byly odebrány 6, 12 a 24 hodin po podání preparátu.

Ve tkáních mozku a jater byla stanovena na autoanalyzátoru aktivita alkalické fosfatasy , aspartátaminotransferasy, gama-glutamyltransferasy a amylasy. U žádné z těchto tkání fenobarbital neovlivnil tkání aktivitu gamma-glutamyltransferasy a amylasy, ani aktivitu jaterní alkalické fosfatasy a aspartátaminotransferasy. Způsobil však zvýšení aktivity alkalické fosfatasy a aspartátaminoransferasy v mozkové tkáni. Zvýšení aktivity těchto enzymů v mozku se může podílet i na zvýšení jejich aktivity v krevním séru po podání fenobarbitalu.

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