FABAD J. Pharm. Sci., 18, 1-5, 1993

Effect of Acute Diphenylhydantoin (DPH) Administration on Ethanol Induced Lipid Peroxidation in the Liver, Brain and Serum of Mice

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Summary : In this study, the possible effect of acute administration of diphenylhydantoin (DPH) (in doses of 8,16 and 32 mg/kg) on lipid peroxidation in the liver, brain tissues and serum of mice was investigated. The mice were killed 24 hours after intraperitoneal injection (IP) and peroxidation was assayed by measurement of malondialdehyde (MDA) production. The 10 % ethanol solvent increased the level of lipid peroxide both in liver and the serum but not in the brain. On the other hand, DPH inhibited the ethanol induced lipid peroxidation in liver.

Received : 31.3.1992
Accepted : 14.4.1992

Keywords : Diphenylhydantoin, Lipid peroxidation

Akut Olarak Uygulanan Difenilhidantoin'in (DPH) Farelerin Karaciğer, Beyin ve Serumunda Etanolün İndüklediği Lipid Peroksidasyon Üzerine Etkisi

Özet : Bu çalışmada farelere 8,16 ve 32 mg/kg dozlarda uygulanan difenilhidantoin'in (DPH) serum, karaciğer ve beyin dokuları üzerinde lipid peroksidasyon oluşumuna etkisi araştırılmıştır. Intraperitoneal enjeksiyondan 24 saat sonra öldürülen farelerin, bu dokulardaki lipid peroksidasyon düzeyleri malondialdehit (MDA) ürünü ölçülerek test edilmiştir. Çözücü olarak kullanılan % 10'luk etanol, karaciğer ve serumda lipid peroksidasyon düzeyini arttırmış, fakat beyinde etki göstermemiştir. Karaciğerde etanolün indüklediği lipid peroksidasyon, DPH uygulamasıyla azalmıştır.

Anahtar sözcükler : Difenilhidantoin, Lipid peroksidasyon

Introduction

The antiepileptic drug diphenylhydantion (DPH, phenytoin) has been used widely for treatment of epilepsy. DPH exerts a stabilizing effect on excitable membranes of a variety of cells, including neurons and cardiac myocytes. It can decrease resting fluxes of Na^+ as well as Na^+ currents that flow during action potentials or chemically induced depolarizations[1].

DPH is associated with several side effects, including congenital abnormalities affecting a large number of infants, referred to as the fetal hydantoin syndrome and a high incidence of certain tumor types[2-5]. In vitro and in vivo experimental data suggest that DPH is both carcinogenic and teratogenic in humans[6-8].

The most relevant steps in the metabolic pathways of DPH are those leading to the formation and deactivation of epoxides[9]. These compounds can covalently bind to macromolecules of target tissues, producing cytotoxic, mutagenic or carcinogenic effects[10,11]. The DPH epoxidation is mediated by monooxygenases, involving cytochrome P-450 and P-448[7,11].

Lipid peroxidation in tissues and tissue fractions represents a degradative process, which is the consequence of the production and the propagation of free radical reactions primarily involving memb-

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rane polyunsaturated fatty acids, especially arachidonic acid[12]. Arachidonic acid is also metabolised by a cyclo-oxygenase to cyclic endoperoxides, which are then rapidly converted to stable prostaglandins, thromboxanes and malondialdehyde (MDA)[13,14]. The aldehydic products of lipid peroxidation, especially MDA, are the most extensively studied compounds.

The aim of this study was to provide further information on effect of DPH on liver, brain and serum lipid peroxidation in mice.

Materials and Methods

Swiss albino mice(60 day-old male), each weighing 25 to 30 g were used. They were randomly divided into two control and three experimental groups, each group consisting of ten mice.

First control group was injected with 0.9 % NaCl solution and the second control group with 10 % ethanol. Ethanol was used in this concentration for the dilution of DPH. Phenytoin was administered by intraperitoneal injection in doses of 8,16 and 32 mg/kg body weight.

The mice were killed 24 hours after i.p. injection by decapitation and blood samples were immediately taken, and the liver and brain tissues were removed. All blood samples were centrifuged at 2000 rpm for 5 min, and serum lipid peroxide (MDA) levels were determined by using the thiobarbituric acid assay (TBA) as described by Yagi[15]. The liver and brain tissues were washed in 0.9 % NaCl-solution and the tissues were homogenized, then centrifuged. The lipid peroxide levels of the homogenates were determined by the method of Uchiyama-Mihara[16].

The results were statistically analyzed by using Student's t-test.

Results

In our study we have used 8,16 and 32 mg/kg DPH dosages in order to be in accordance with therapeutic usage (300-600 mg/day in adults and 5-10 mg/kg in children). Since more than half of the consumed DPH dosages are inactivated through parahydroxylation by liver microsomal enzymes. Biological half-life of DPH is approximately 32 hours. Lipid peroxide levels are determined 24 hours after i.p. injection.

As shown in Figure 1, the liver lipid peroxidation level in the 10 % ethanol-treated controls was higher than those of saline-treated controls in the 8,

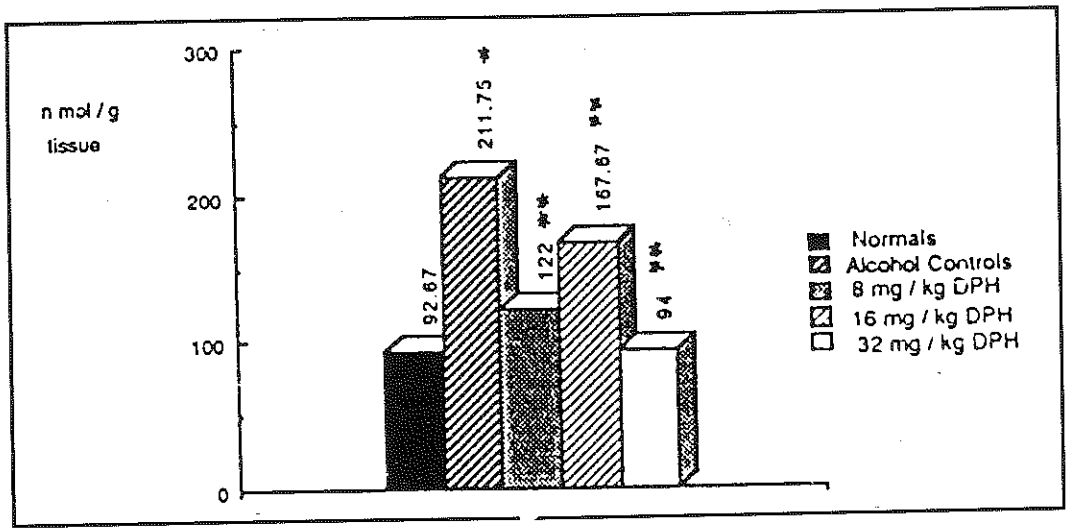

Figure 1. Influence of DPH treatment on ethanol induced lipid peroxidation in the liver of mice
(*) Significantly different from saline-treated control ($p<0.001$)
(**) Significantly different from ethanol-treated group ($p<0.001$)

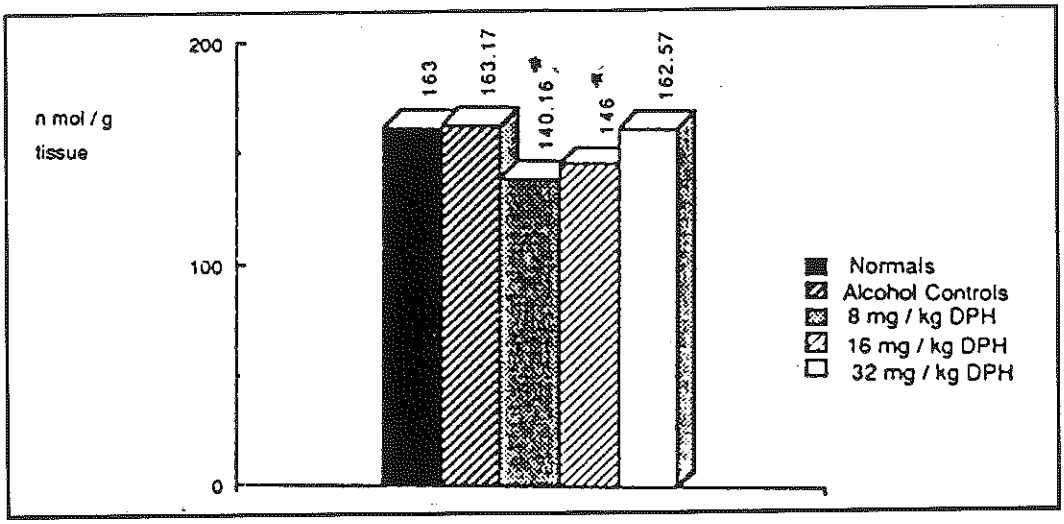

Figure 2. Brain lipid peroxide levels of mice after single i.p. injection of DPH
(*) Significantly different from controls ($p<0.05$)

16, and 32 mg/kg DPH treated groups. It was noticed that the highest dose (32 mg/kg) of DPH decreased the lipid peroxidation level to values seen in saline-treated controls.

The brain lipid peroxide levels in ethanol-treated and saline-treated controls in the 32 mg/kg DPH-treated group were found to be similar. However, the levels in animals treated with low and middle doses DPH were lower than that of other group (Figure 2).

Serum lipid peroxide levels in ethanol-treated control and drug-treated group were higher than that of the saline-treated control group (Figure 3).

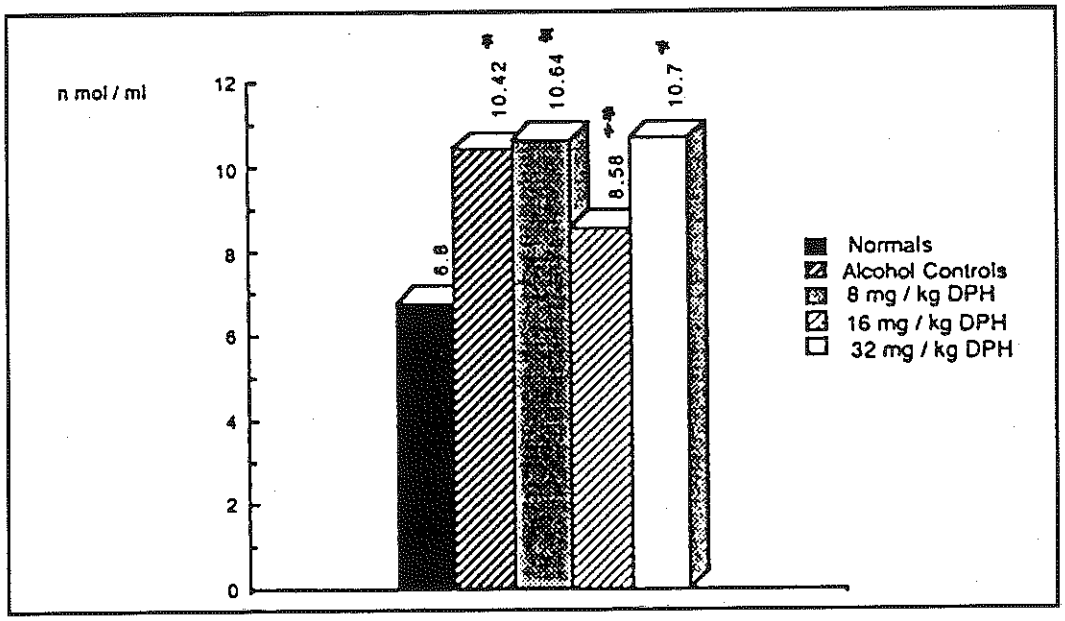

Figure 3. Influence of DPH treatment on ethanol induced lipid peroxidation in serum of mice
(*) Significantly different from saline-treated control ($p<0.001$)
(**) Significantly different from ethanol-treated group ($p<0.05$)

Discussion

Different DPH doses were investigated for their effects on liver, brain tissues and serum. Since ethyl alcohol was used as a DPH solvent, two control groups were established the former group receiving 0.9 % NaCl solution and the latter, 10 % ethanol. In the alcohol control group, ethanol increased the level of MDA in the liver and serum but not in brain. The role of lipid peroxidation in the hepatotoxic effect of ethanol has been shown[17-19]. DPH decreased the level of MDA which ethanol induced in liver.

John et al. reported that DPH, which was administered to 10-20 days and 60-120 days aged rats at the doses of 100-150 mg/kg, increased lipid peroxidation in younger rats and decreased it in older ones[20]. Hassell et al. (1984) described the formation of a reactive epoxide during the metabolism of phenytoin; the detoxification mechanisms including conjugation reactions are fully developed in adult animals only[21].

DPH applied in doses of 8 and 16 mg/kg, decreased the brain MDA levels. At the highest dose (32 mg/kg), result was found to be equal to both controls. These results support the findings of Willmore et al. that DPH at the dose of 100 mg/kg inhibited the seizures, but left brain lipid peroxidation level unchanged in experimental traumatic epilepsy[22].

In serum, at 16 mg/kg, DPH prevented the increased MDA levels induced by ethyl alcohol, but showed no effect at the highest and lowest doses. Ethyl alcohol could interfere with the therapeutic actions of a wide variety of drugs, altering their metabolism. For example, acute ingestion of ethyl alcohol reduced the clearance of DPH because both drugs compete for the same hepatic microsomal oxidase system[23].

In conclusion, our results show that ethyl alcohol increase the liver and serum MDA levels. These increases may well be dependent on the peroxidation of arachidonic acid and/or products of TXA_2 which is induced by ethyl alcohol, and MDA is also known to be a product of TXA_2. Our results show that DPH might decrease alcohol induced lipid peroxidation in liver and serum and normal brain lipid peroxidation.

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