Phenytoin-Induced Hepatic 8-Hydroxydeoxyguanosine in DNA of Balb/C Mice and Its Reduction by Curcumin

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Abstract: Phenotion, PT (5,5-diphenylhydratoin), is widely used anticonvulsant agent. The aim of this study was to investigate the toxicity of this drug by measuring the ability of PT to induce oxidative DNA damage through the formation of 8-hydroxydeoxyguanosine (8-OH-dG) using HPLC with electrochemical detection. PT initiates reactive oxygen species (ROS) formation followed by damage to DNA and other cellular compartments. Curcumin is a free radical scavenger, markedly inhibited the formation of 8-OH-dG. Results showed that curcumin reduce the toxicity of PT by 6 folds. These findings are consistent with conclusion that curcumin has highly protective power against PT toxicity and the protective action relates, at least in part to its direct free radical scavenging ability.

Key words: Phenytoin · Curcumin · Antioxidant · 8-hydroxydeoxyguanosin · DNA damage

INTRODUCTION

Phenytoin (PT) is an aromatic compound commonly used as an antiepileptic drug [1,2], since it acts to suppress the abnormal brain activity seen in seizure by reducing electrical conductance among brain cells by stabilizing the inactive state of voltage-gate sodium channels [3,4]. However, recent studies have proved its effectiveness in treating various types of neuropathic pain syndrome [5], anxiety syndromes [6] and behavioral problems [7]. This drug has significant adverse effects since it was proved that it is a hepatotoxic compound [8,9]. Its use was associated with idiosyncratic hepatotoxicity (type B reaction), which in some cases may be fatal [10].

The major route of phenytoin metabolism is a hydroxylation to a para hydroxyphenyl phenyl hydantion (4HPPH), which is considered as the main metabolite [11]. This process is catalyzed primarily by CYP2C9 and to much lesser by CYP2C18 and CYP2C19 [4]. It was found that most of PT is bio-transformed by the liver [12,14] and less than 5% is eliminated unchanged in the urine [7]. The mechanism(s) of phenytoin-initiated toxicity is unknown. PT can be enzymatically bioactivated to a reactive intermediate leading to increased formation of reactive oxygen species (ROS) [13,14], which can oxidize lipid,

protein and DNA [11,15]. Oxidative stress is strictly involved in the pathogenesis of many types of liver injuries, including drug-induced hepatotoxicity, but molecular mechanisms are not really defined [16]. There is a growing body of evidence suggesting that idiosyncratic drug-induced hepatotoxicity may be mediated, at least in part, by oxidative stress, characterized by enhanced levels of ROS [2,17].

Biochemical features of PT hepatotoxicity are variable but generally include abnormal serum alanine aminotransferase (ALT), glutamyl-oxaloacetic transaminase (SGOT) and glutamyl-pyruvic transaminase (SGPT) activities [18,19]. These parameters reflect damage to hepatocytes and are considered to be highly sensitive and fairly specific preclinical and clinical biomarkers of hepatotoxicity. PT is taken up into hepatocytes by multi-specific transporters [14] whereas it may induce the production of potentially harmful free radicals and their reactive oxygen species [20,21]. These reactants interact with DNA strand breaks, DNA-protein cross links and oxidative DNA base modification such as the formation of 8-hydroxydeoxyguanosine (8-OH-dG) [22-24]. 8-OH-dG is a key biomarker [25-27] relevant to carcinogenesis because the formation of 8-OH-dG in DNA causes misincorporation during replication and subsequently leads to G-T transversion [28]. The toxic

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potential of PT is related to its ability to generate the hydroxyl radical ($^{\bullet}$OH?) [29]. This highly toxic free radical targets DNA resulting in oxidative DNA base adducts such as 8-OH-dG) [30].

Recently, a number of natural antioxidants have been evaluated for their chemopreventive effects in various pathological states [31]. Curcumin (differuloylmethane), a phenolic compound and major component of *Curcuma longa* Linn is a major yellow pigment in turmeric ground rhizomes which is used widely as a spice and coloring agent in several foods, as well as cosmetics and drugs [32-34]. It was defended as a non-vitamin antioxidant with its high oxygen-radical scavenging and quenching capacities is very useful in living tissues to reduce the risk of adverse reaction that produced by hydroxyl radicals.

The aim of this study was to investigate the toxicity of PT and the role of curcumin pre-treatment in the protection against PT-induced liver oxidative damage in Balb/c mice.

MATERIALS AND METHODS

Chemicals: All chemicals used in this study were of analytical grade and purchased from Sigma Co, USA.

Animals and Treatment: 40 Balb/c male mice (6-7 weeks old and around 30g weight each) were used in this study. Mice were obtained from the Animal House of Yarmouk University and kept on standard laboratory diet and tab water *ad libitum* throught the experiments. Five animals were housed stainless metal cages under 12:12h light-dark cycle and room temperature of 23-26°C. Mice were randomly assigned into 4 groups (10 mice each); control group (CON), mice were received neither PT nor curcumin but only normal standard diet and water. Group 2 was curcumin control group (CUR), mice were received curcumin supplementation (200mg/kg body weight) orally [32] for three weeks, prior to scarification. Group 3 was PT control group (PTC), mice were supplemented with 300 mg/kg/day PT (dissolved in water) orally for three weeks then sacrificed and the fourth group is the experimental group (PTCUR), mice were supplemented with 200mg/kg body weight curcumin orally and also PT (300mg/kg body weight/orally) for three weeks then sacrificed.

Toxicological Studies: After scarification of all mice, blood was collected, serum isolated and frozen in-70°C for further studies. The livers were immediately removed and perfused with Hanks-buffered saline to remove excess

blood. To assay 8-OH-dG, the method described by Al-Jassabi and Khalil [15] was used. Alanine transaminase (ALT) level was determined in the serum according to the method recommended by Al-Jassabi and Khalil [35]. Activities of glutamyl-oxaloacetic transaminase (SGOT) and glutamyl-pyruvic transaminase (SGPT) were determined according to method described in [36]. Activity of antioxidant enzyms, Catalase (CAT) was determined using the method reported by Tomoko *et al.* [37], Superoxide dismutase (SOD) using the method of Stief [38] and Glutathione peroxidase (GSHpx) by the method recommended by Leopold and Wolfgang [39].

For the measurement of lipid peroxidation, the thiobarbituric acid (TBA), method described by Reddy *et al.* [40] was used by measuring the amount of TBA reactive substances present in the liver homogenate. Commercial detection kits for malondialdehyde (MDA) levels examination were carried out using Shimadzu ultraviolet spectrophotometer (Model S441U). Protein phosphatase (PP1) activity was determined by measuring the rate of colour production from the dephosphorylation of *para*-nitrophenolphosphate (*PNPP*) substrate as a function of time using the microtitre plate reader [41,42].

Statistical Analysis: Results are expressed as mean± standard deviation. For comparison between groups, data were analyzed by one-way ANOVA; *P*= 0.05 was considered statistically significant.

RESULTS

The present results revealed non significant alterations in the body weighs of mice of the various treated groups. PT treated mice liver showed a little increase in the liver body mass index ratio due to massive intra-hepatic hemorrhage and pooling of blood in the liver, making the liver appear darker in colour when compared with the other groups, which were all within the normal values.

Serum values of ALT, SGOT and SGPT were utilized to evaluate liver injury. PT administration increased serum values of ALT (about 3-fold), SGOT (about 2-fold),and SGPT (about 3-fold) compared to those in CON mice, while pretreatment with curcumin significantly (P < 0.05) inhibit the rise of these enzymes induced by PT as shown in Table 1.

Antioxidant enzymes (CAT, SOD and GSHpxp) activities were decreased significantly (P<0.05) in mice treated with PT only, while these activities were near the normal after pretreatment with curcumin.

Table 1: Summery of the results of the effects of curcumin on the toxicity of PT

	CON	PT	CUR	PTCUR
ALT (U/L)	61062±3.103	348.33±0.961	57.66±1.593	98.691.008
LPO (MDA) μM	0.066±0.001	3.266±0.001	0.044±0.001	0.805±0.002
PP1 (U/mg)	0.574±0.002	0.165±0.001	0.553±0.001	0.313±0.001
8-OH-dG/10^5 2dG	4±0.014	38±0.299	3±0.013	17±0.57
SGOT (U/L)	11.28±1.003	68.04±3.122	10.66±1.001	23.41±1.664
SGPT (U/L)	10.80±1.001	66.12±2.608	10.77±1.001	19.67±1.322
SOD (U/mg protein)	66±3.330	21±1.202	68±3.007	54±2.804
CAT (U/min/mg protein)	67±3.103	38±1.886	71±3.557	66±3.004
GSHpx (umol/min/mg protein)	0.87±0.004	0.22±0.002	0.89±0.008	0.67±0.003

MDA is a product of oxidative damage to lipids and in this study, the concentration of MDA in liver homogenate is considered as a biomarker of PT toxicity. Results in Table 1 shows that liver homogenate of mice exposed to PT contained higher levels of MDA (about 12-fold increase) when compared with CON values. These levels decreased significantly (P < 0.05) in liver homogenates of mice supplemented with curcumin.

Results of spectrophotometric measurements of protein phosphatase activity of liver homogenates for all groups are presented in table 1. The findings show that the activity of PP1 was significantly (P< 0.05) inhibited in group that received PT (group 3) only.

There was a significant increase (P < 0.05) in the level of 8-OH-dG in PT-treated mice. Table 1 shows that curcumin inhibited PT-induced formation of 8-OH-dG.

DISCUSSION

Phenytoin, 5,5-diphenylhydantoin, is widely used as an anticonvulsant agent [2]. Recently, it has received much attention concerning its variety of toxicities [13]. Phenytoin toxicity is thought to be due to the formation of four oxidative metabolites, 4-hydroxylated (4-HPPH), 3-hydroxylated (3-HPPH), a catechol (3,4diHPPH) and the 3,4-dihydrodiol [5], which raised the production of ROS [10]. However, there is a tendency now to limit the clinical use of this drug brcauseof its several adverse effects, mainly idiosyncratic hepatotoxicity [19]. The level of serum ALT, SGOT, SGPT activities reflect damage to hepatocytes and indicates the increased cellular permeability [43] and are considered to be highly sensitive and fairly specific preclinical and clinical biomarkers of hepatotoxicity [23]. The results show that curcumin provides a hepatoprotective effect by reversing the changes produced by PT.

Our results show that PT increase lipid peroxidation with high levels of MDA as a main product of lipid break down, but curcumin has been proved by others to be a powerful antioxidant, reduces the formation of MDA likely via its ability to scavenge free radicals [44,45].

The generation of 8-OH-dG resulted from oxidative modification of DNA [27]. A strong correlation between higher amounts of 8-OH-dG and greater degree of oxidative stress, DNA strand break, or DNA damage has been reported [30]. As expected, the antioxidant curcumin reduced the level of 8-OH-dG. H_2O_2 is a normal metabolite in the cell; its steady state concentrations range from 10^{-9}-10^{-8} M [46]. Although H_2O_2 may not cause DNA damage under physiological conditions, it participates in the metal ion-catalyzed Haber-Weiss reaction and generates the highly reactive hydroxyl radical, which can target DNA resulting in oxidative DNA damage [27].

In this study, the reduction or prevention of oxidative hepatic injury caused by phenytoin metabolites has been proposed to be achieved through increasing host antioxidant defense system by supplementation with curcumin. The body has defense mechanisms of antioxidants to limit damage caused by different species of ROS. These protective mechanisms consist of enzymatic species, SOD, CAT, and GSHpx and our results showed that the activities were effected by phenytoin, but supplementation of curcumin attenuated their activities to the about normal. SOD is considered as the first line of defense to scavenge superoxide anions generated in cytosolic and mitochondrial compartments of the cell [46]. CAT and GSHpx causes direct breakdown of hydrogen peroxide to oxygen and water [37]. Thus, our results indicate that PT treatment disrupted the antioxidant defense mechanism of the liver cells causing oxidative damage.

It was concluded that curcumin, widely used spice especially in Asia, acts as a very potent antioxidant against the toxicity of phenytoin an antiepileptic drug widely used all over the world for treating several types of seizure.

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