THE EFFECTS OF VIGABATRIN ON RAT LIVER ANTIOXIDANT STATUS

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SUMMARY

The anti-epileptic drug vigabatrin was developed as an inhibitor of gamma-aminobutyric acid transaminase, and its ability to increase inhibition in the central nervous system led to its testing in an animal model. In animal models chronic use of vigabatrin is associated with irreversible myelin vacuolation. Antioxidant drugs change the antioxidant capacity of the body. Oxidative stress of the body increased when valproic acid and carbamazepine were used chronically. To assess whether vigabatrin may affect protein oxidation and lipid peroxidation, glutathione, glutathione peroxidase (GPx), and glutathione-*S*-transferase (GST) levels were studied in the livers of 57 rat fetuses after administration of vigabatrin to the mothers (19 in the first week of pregnancy, 20 in the second week, and 18 in the third week) and in 19 control rat fetuses without vigabatrin. We compared the results of administration of vigabatrin in each group with the controls. Rat fetus protein oxidation in group I (0.686 nmol/mg protein) and group II (0.723 nmol/mg protein) was higher than in the control group (0.388 nmol/mg protein). Lipid peroxidation (0.209, 0.224, 0.253 nmol/mg protein, respectively) and GPx levels (345.4, 329.0, 283.2 nmol/mg protein, respectively) of groups I, II, and III were higher than in the control group (0.104, 167.2 nmol/mg protein, respectively). GST in group II (79.2 nmol/mg protein) and group III (77.8 nmol/mg

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protein) were not different from that in the control group (78 nmol/mg protein). It was found that vigabatrin affected all the parameters that were studied, especially in group I, which was given the drug in the first week of pregnancy.

KEY WORDS

vigabatrin, rat, lipid peroxidation, protein oxidation, glutathione-*S*-transferase

INTRODUCTION

Vigabatrin is an anti-epileptic drug used for treatment of partial and secondarily generalized tonic clonic seizures. Vigabatrin acts as an irreversible substrate for gamma-aminobutyric acid (GABA) transaminase that causes elevated brain GABA levels /1/. This drug has been notable for association with adverse effects. It can cause the development of intramyelination in chronic toxicity studies in rodents and dogs /2/. Systemic adverse effects have been very infrequent /3/. Some gastrointestinal complaints have been observed in older patients /4/. This compound is absorbed from the gastrointestinal tract and eliminated mostly by renal excretion. It is not significantly metabolised by the liver, but it causes a depression in blood platelet level /5/. It was reported that this drug might increase the intrarenal GABA level and cause optic nerve atrophy /6/. Vigabatrin has teratogenic effects and decreases the methionine level in the embryo /7/.

Anti-epileptic drugs (AEDs) have been found to affect the antioxidant system /8/. Many AEDs are metabolised to generate reactive metabolites with the capability of covalent binding to proteins and other macromolecules /9/. Until now there has been no study to investigate the relationship between vigabatrin and the antioxidant system. Therefore this study was undertaken to evaluate the effect of vigabatrin on protein oxidation, lipid peroxidation, glutathione (GSH), glutathione peroxidase (GPx), and glutathione-*S*-transferase (GST) levels in fetal rats from mothers which received vigabatrin during pregnancy.

MATERIALS AND METHODS

Female Wistar albino rats (n = 21) (215-240 g) were used in this study. The rats were housed in individual cages at a temperature of 22 ± 1°C with 50% humidity. The animals were fed commercial pellets and water *ad libitum*. Rats have a 3-week gestation period. A total of 57 rat fetuses were derived from rats which had taken vigabatrin, and 19 rat fetuses without vigabatrin administration were used as a control group.

Pregnant rats were randomly divided into four groups. Group I received vigabatrin during first week of gestation, group II received the drug during the second week of gestation, and group III received the drug during the third week of gestation. One group of rats served as untreated controls. The vigabatrin dose was 100 mg/kg/day dissolved in 0.9% saline. The daily dose was administered by dividing the dose into two. All doses were administered orally. The rats were sacrificed and fetuses taken on the 22nd day of gestation.

Rat livers were taken and stored at -70°C until assay. Livers were thawed and homogenised (10% w/v) with 0.15 KCl at 4°C, then centrifuged at 10,000 *g* for 1.5 h. The supernatant was used as the source of experimental product. Glutathione was determined by the method of Beutler *et al.* /10/ with 5,5-dithio-bis-nitrobenzoic acid as substrate. GPx was estimated using the method of Beutler *et al.* /11/. GST activity was measured according to the method of Habig *et al.* /12/. Rat liver homogenate lipid peroxidation was measured by Thayer's method /13/. Protein oxidation was determined according to the technique which was reported by Levine *et al.* /14/. All the techniques used were spectrophotometric techniques.

SPSS for Windows Version 11 was used for evaluation of the raw experimental data and comparison of groups. Means of the groups were compared using independent t-test. If the F value of this test was higher than 0.05, the Mann-Whitney U-test was applied. $p < 0.05$ was taken as significant.

RESULTS

Rat fetus liver protein oxidation values were found to be 0.388, 0.686, 0.723 and 0.429 nmol/mg protein for control, and groups I, II, and III, respectively. The mean values of lipid peroxidation were

0.104, 0.290, 0.224 and 0.253 nmol/mg protein. The mean levels of GST were 78.8, 140.7, 79.2 and 77.8 nmol/mg protein; the mean values of GPx were 167.2, 375.4, 329.0 and 283.3 nmol/mg protein; and mean values of GSH were 0.068, 0.035, 0.034 and 0.053 nmol/mg protein, respectively. These values with their standard deviations are shown in Table 1. Protein oxidation values of groups I and II were significantly higher than in the control group ($p < 0.001$). Lipid peroxidation and GPx activity levels were significantly higher in all vigabatrin treated groups when compared with controls ($p < 0.001$). GSH levels in group I (first week of gestation) and group II (second week of gestation) were significantly lower than that in the control group ($p < 0.001$). There were no significant differences found in GST levels in the second and third groups. Furthermore, group III's levels of GSH and protein oxidation were not significantly different from those of the control group.

TABLE 1

Glutathione-*S*-transferase (GST), glutathione peroxidase (GPx), glutathione (GSH), protein oxidation and lipid peroxidation in groups I, II, III and controls

	Controls (n = 19)	**Group I** (n = 19)	**Group II** (n = 20)	**Group III** (n = 18)
GST	78.0 ± 8.3	140.7 ± 28.7*	79.2 ± 9.8	77.8 ± 7.1
GPx	167.2 ± 4.3	345.4 ± 33.4*	329.0 ± 48.0*	283.3 ± 40.7*
GSH	0.068 ± 0.01	0.035 ± 0.007*	0.034 ± 0.003*	0.053 ± 0.003
Protein oxidation	0.388 ± 0.08	0.686 ± 0.077*	0.723 ± 0.008*	0.429 ± 0.037
Lipid peroxidation	0.104 ± 0.02	0.290 ± 0.005*	0.224 ± 0.003*	0.253 ± 0.046*

Values are means ± SD (nmol/mg protein).
* $p < 0.001$ compared to the control group.

DISCUSSION

Some epileptic drugs may change antioxidant parameters in humans and experimental animals /8,13/. Recent studies suggest that membrane lipid peroxidation and protein oxidation may be causally involved in some forms of epilepsy /15,16/. However, studies of the effects of vigabatrin, which is increasingly used nowadays, on the antioxidant system and on its use in pregnancy are very limited. A study carried out by Abdulrazzaq *et al.* /7/ reports that vigabatrin passes through the placenta and decreases the methionine level in the fetus, and that this decrease probably has a teratological effect. Other studies report that vigabatrin causes spina bifida, myelin vacuolisation and blindness in adults /2,4-6/. In our literature search, we found no study on the effect of vigabatrin on the antioxidant system. The present study suggests that the increased protein oxidation, lipid peroxidation, GPx and GST in the fetuses of the group of pregnant rats treated by vigabatrin during the first week of pregnancy indicate that the drug passes through the placenta and affects the oxidant status of the rat fetus. It is reported that a decrease of GSH level, especially in the first trimester of pregnancy in humans, may cause increased drug toxicity. Through enzymatic or direct action, GSH blocks the destructive alteration of proteins, lipids and nucleic acids by free radicals, heavy metals, and other toxic drugs. It has also been reported that decreasing GSH and alteration of antioxidant enzyme activities by valproic acid may enhance drug toxicity /17,18/. Our present results suggest, because of the increased GPx and lipid peroxidation, that this effect is continued to some extent also in the second and third weeks of pregnancy in rats. These results show that the antioxidant enzymes in the liver are particularly affected during the early part of pregnancy. It is well known that a drug that can pass through the placenta has a greater potential for teratogenic effects during the early part of pregnancy (the first trimester in humans; the first week in rats) in comparison with later periods /19/.

Antioxidant enzyme levels were found different in studies carried out on valproic acid. In one study, by Seckin *et al.*, they showed that GST activities were highly increased in the liver, but GPx and superoxide dismutase (SOD) activities were not affected /20/. Tabatabei and Abbott /21/ reported that valproic acid caused oxidative stress and cytotoxicity *in vitro*. Cotario *et al.* /22/ and Pippenger *et al.* /23/

reported that GPx activity was decreased in their studies in humans, whereas Cengiz *et al.* reported that GPx activity was increased /24/. Valproic acid and its metabolites may increase reactive oxygen species, which bind proteins and DNA molecules that are primarily targets of alkylating agents /9/. When increase of free radicals cannot be compensated for by the antioxidant system, the free radicals can cause various toxic effects. Increased protein oxidation and lipid peroxidation is evidence that the liver cells' antioxidant defence mechanism is not sufficient.

This study showed that during the intrauterine period, the administration of vigabatrin in rats changes certain antioxidant parameters and enzyme levels; however, we were unable to gather enough information about vigabatrin use during pregnancy in humans. The authors suggest that vigabatrin, which is being increasingly used, should not be used during the first trimester of pregnancy until further studies have shown that it is safe during pregnancy in humans.

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