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EARLY CHANGES IN HEPATIC REDOX HOMEOSTASIS FOLLOWING TREATMENT WITH A SINGLE DOSE OF VALPROIC ACID

DITA COTARIU,* SANDRA EVANS, JACOB L. ZAIDMAN and ORA MARCUS
Department of Biochemical Pathology, Assaf Harofeh Medical Center, Sackler School of Medicine, Zerifin, Israel

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Abstract—Changes in reduced glutathione (GSH) and pyridine nucleotide phosphate levels as well as in the activities of the glutathione peroxidase–reductase system and glucose-6-phosphate dehydrogenase have been studied in rats after a single i.p. administration of various doses of valproic acid (VPA). GSH level decreased in a dose-dependent relation. At the end of 180 min GSH levels either returned to control limits (lower doses) or showed a tendency to normalize (higher doses). GSH loss was paralleled by the reduction in glutathione reductase activity. A significant NADPH reduction was also seen after animal exposure to high VPA doses. At the end of 180 min a maximal NADPH decrease was reached. The activities of both glutathione peroxidase and glucose-6-phosphate dehydrogenase were suppressed irrespective of whether animals were given low or high VPA doses.

Valproic acid (VPA) is a widely used anticonvulsant effective in treating different forms of epilepsy. However, therapy with the drug has been associated occasionally with irreversible liver injury [1]. Despite the well-established association of VPA treatment with morphological and biochemical changes in the liver, the mechanisms underlying the hepatotoxicity are poorly understood. Severe hepatotoxicity is thought to emerge from multiple biochemical disturbances which ultimately assume different patterns of histopathological change [2]. An important factor may be injury mediated by oxidant stress. VPA and its unsaturated metabolite 4-en-VPA generated by the microsomal cytochrome P450-dependent mixed function oxidase system [3, 4] undergo further metabolic activation to electrophilic intermediates that bind covalently to liver macromolecules [5, 6]. This type of toxicity already reported for a number of xenobiotics may also involve reduced glutathione (GSH) depletion [7].

This study was undertaken to evaluate the effect of acute administration of varying doses of VPA on liver GSH and pyridine nucleotide phosphate levels as well as the effect on enzymes involved in the maintenance of these reducing equivalents.

MATERIALS AND METHODS

Female Charles River rats, weighing 150–200 g were maintained on a normal light–dark cycle and experiments were started between 8 and 9 a.m. Animals had free access to a standard diet. VPA (100, 300, 500 and 750 mg/kg, respectively) was administered i.p. Control rats received an equal volume of sterile saline and were killed at zero time (there is no difference between controls killed at various time intervals and controls measured at zero time). At various times thereafter, blood was drawn by cardiac puncture in heparinized syringes. Then, animals were killed by cervical dislocation. Livers were quickly removed, portioned into appropriate buffers or frozen in liquid nitrogen. Enzyme activities were measured at 37° in the supernatant fraction of 1:5 (w/v) tissue homogenate. The buffer contained 50 mM Tris–HCl, pH 7.4, 100 mM KCl and 1 mM EDTA. Activities are expressed as μmoles of NADPH oxidized or NADP reduced per min per mg protein.

Glutathione reductase activity was measured spectrophotometrically by following NADPH oxidation at 340 nm [8]. The reaction mixture consisted of 200 mM potassium phosphate buffer pH 7, 2 mM EDTA, 2 mM NADPH and 20 mM GSSG.

Glutathione peroxidase was estimated from the rate of NADPH oxidation at 340 nm, essentially as described by Beutler *et al.* [9]. The reaction mixture contained 48 mM potassium phosphate buffer, pH 7.6, 0.5 mM EDTA, 0.35 mM *t*-butylhydroperoxide, 2 mM GSH, 0.14 mM NADPH, and 70 mUnits glutathione reductase (Sigma Chemical Co., St Louis, MO).

Glucose-6-phosphate dehydrogenase activity was estimated from the rate of NADP reduction as monitored by the increase in absorbance at 340 nm [9]. The reaction mixture consisted of 100 mM Tris–HCl/0.5 mM EDTA buffer, pH 8.0, 100 mM $MgCl_2$, 2 mM NADP and 6 mM glucose-6-phosphate; 6-phosphogluconate dehydrogenase activity was measured in a similar fashion after substituting the reaction with 6 mM 6-phosphogluconate.

Protein content was determined by the method of Lowry *et al.* [10] with bovine albumin as standard.

GSH content was quantified after extraction with 10% metaphosphoric acid (w/v) containing 10 mM EDTA, by an enzymatic method, as described by Davies *et al.* [11].

* To whom correspondence should be addressed: Dr Dita Cotariu, Department of Biochemical Pathology, Assaf Harofeh Medical Center, Zerifin 70300, Israel.

Table 1. Serum and liver valproic acid content after i.p. administration

VPA mg/kg	Serum (μg/mL) 30 min		Serum (μg/mL) 60 min		Liver (μg/g) 30 min		Liver (μg/g) 60 min	
100	91 ± 16	(5)	—		38 ± 1.5	(4)	—	
300	339 ± 22	(4)	157 ± 6	(5)	123 ± 36	(6)	61 ± 9	(5)
500	470 ± 27	(7)	402 ± 15	(7)	220 ± 37	(5)	126 ± 24	(7)
750	531 ± 41	(7)	507 ± 41	(8)	683 ± 58	(5)	294 ± 26	(7)

Values are mean ± SE with the number of animals in parentheses.

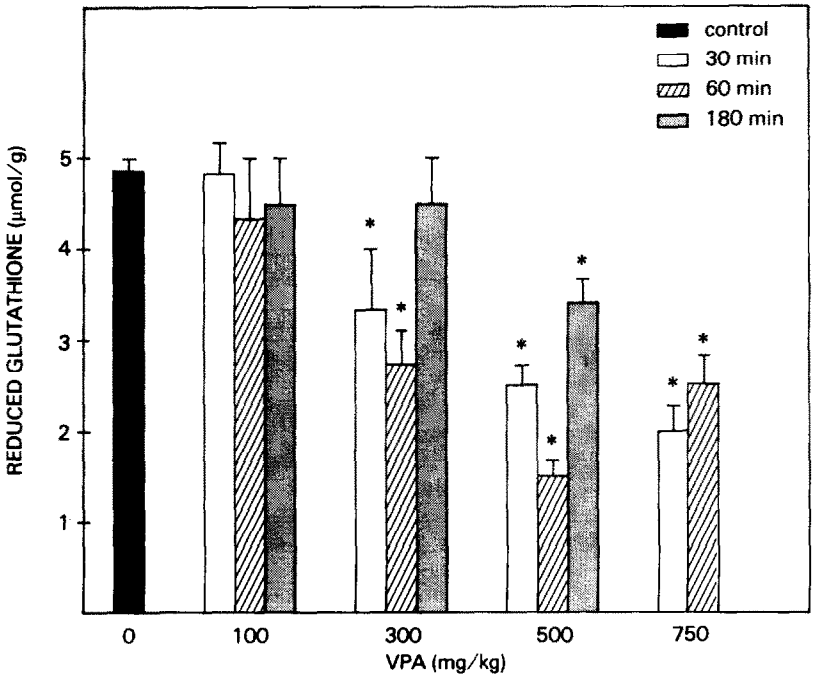

Fig. 1. Effect of valproic acid on liver GSH level. Each bar represents the mean ± SE of determinations from 5–12 animals. *P < 0.05 with respect to saline-injected control animals.

Oxidized and reduced pyridine nucleotides were selectively prepared from quick-frozen livers, in acid and alkaline extracts, respectively. The extracted pyridine nucleotides were then measured spectrophotometrically, as shown in Ref. 12.

Serum and liver VPA was determined by a commercial kit (Emit, Syva, Palo Alto) and results are summarized in Table 1. VPA concentration peaks in rat liver at 30 min after i.p. administration [13]; the half-life of a single dose ranges from 0.3 to 1 hr [14].

Statistics. Results are given as the mean ± SE. Data were analysed by one-way analysis of variance followed by Bonferroni's test. P values of less than 0.05 were considered as significant.

RESULTS

The dose–response relation of GSH content of rat liver versus VPA dose is given in Fig. 1. The concentration of GSH at the end of 30 and 60 min, respectively, following injection of large doses (300, 500, 750 mg/kg) was significantly diminished compared to control animals. At the end of 180 min the GSH level either returned to control limits (300 mg/kg VPA) or tended to revert to normal, but was still significantly reduced (500 mg/kg VPA). No effect whatsoever on GSH level was seen in animals given a non-toxic dose (100 mg/kg). A fairly good correlation is seen between GSH decrease and the activity of glutathione reductase (Fig. 2) which is not affected by the low-VPA dose (100 mg/kg) but is significantly reduced by larger doses. This VPA-mediated reduction in glutathione reductase activity is seen at the end of both 30 and 60 min after administration. Glutathione peroxidase activity in rat liver as a function of dose and time after VPA administration is shown in Fig. 3. The enzyme activity is reduced significantly even at low VPA doses (100 mg/kg). This decrease ranges with the various doses from 30 to 55% of control values and is maintained over the 60-min treatment period. Hepatic glucose-6-phosphate dehydrogenase activity as a function of VPA dose and time of exposure is given in Fig. 4. The enzyme activity decreases significantly at all doses studied. The levels of NADPH decrease

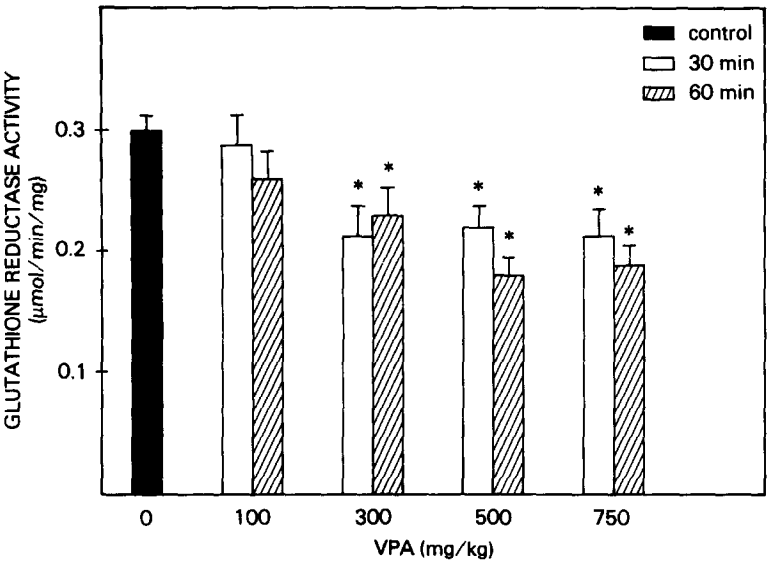

Fig. 2. Effect of valproic acid on liver glutathione reductase activity. Each bar represents the mean ± SE of determinations from 5–15 animals. *P < 0.05 with respect to saline-injected control animal.

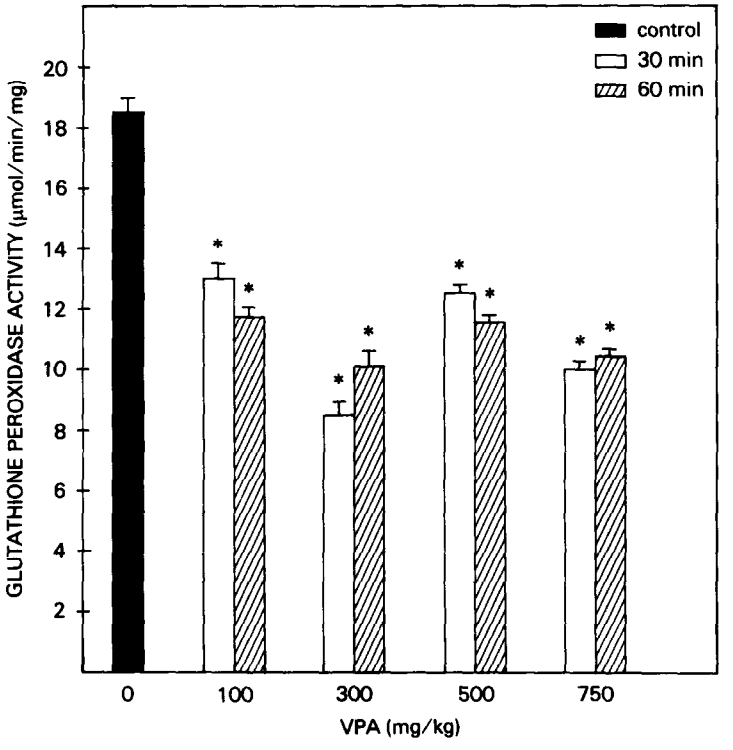

Fig. 3. Effect of valproic acid on liver glutathione peroxidase activity. Each bar represents the mean ± SE of determinations from 5–12 animals. *P < 0.05 with respect to saline-injected control animals.

in a dose-dependent manner after VPA administration (Fig. 5), without an apparent increase in the level of NADP. NADPH decrease is even more marked at the end of 180 min after toxic doses (500 mg/kg). The NADPH/NADP ratio at this time point is 2.3 (N = 5) with respect to 5.23 (N = 7) in controls.

DISCUSSION

GSH and NADPH are two important substrates

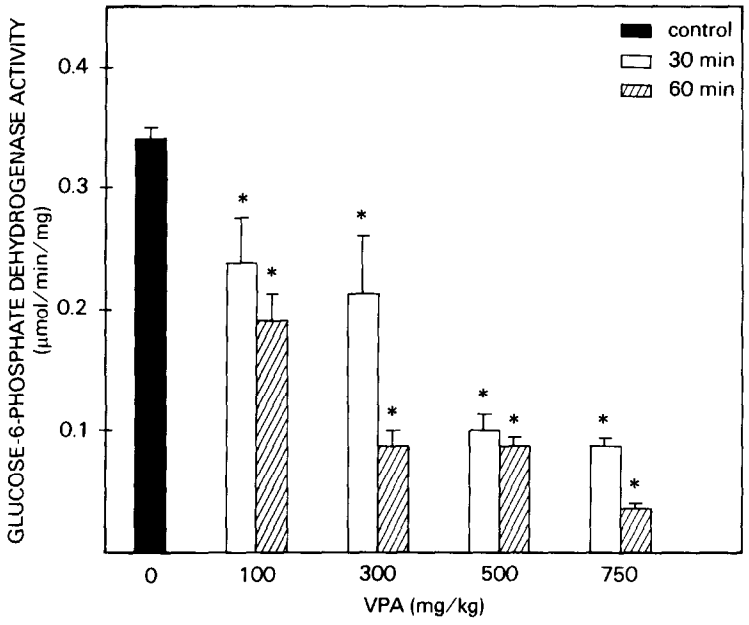

Fig. 4. Effect of valproic acid on liver glucose-6-phosphate dehydrogenase activity. Each bar represents the mean ± SE of determinations from 5–12 animals. *P < 0.05 with respect to saline-injected control animals.

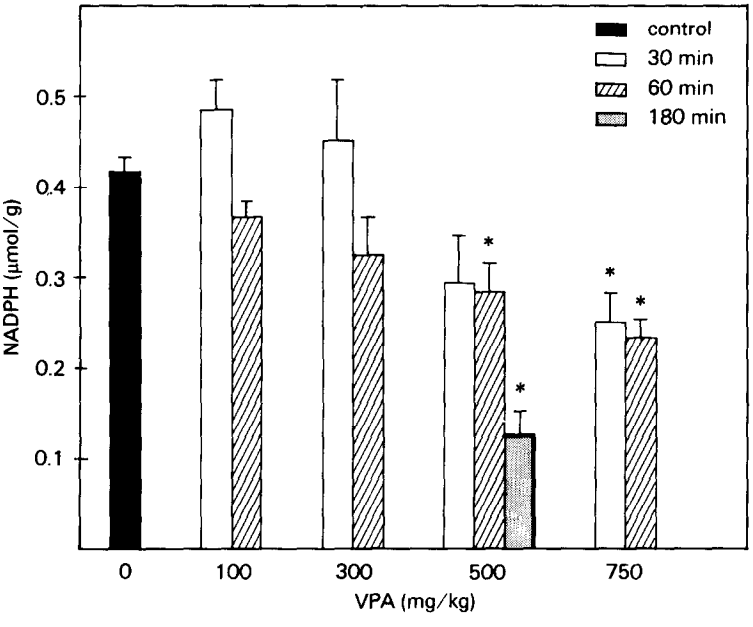

Fig. 5. Effect of valproic acid on liver NADPH level. Each bar represents the mean ± SE of determinations from 5–10 animals. *P < 0.05 with respect to saline-injected control animals.

for the reductive detoxification of reactive intermediates generated during biotransformation of xenobiotics. The glutathione peroxidase–reductase system plays a salient role in the oxidation and reduction of glutathione and detoxification of peroxides. This may be of critical importance in protecting cells against oxidants. The operation of the system is dependent on the supply of NADPH which is needed for glutathione reductase activity. Glucose-6-phosphate dehydrogenase replenishes NADPH through activation of the pentose phosphate pathway. Changes in its activity are expected to affect both NADPH and GSH levels. The present evidence underlies the ability of VPA to change both the redox potential of liver tissue and the activities of enzymes involved in the maintenance of reducing

equivalents when administered to rats *in vivo* as a single dose. The changes concern a transient loss of liver GSH in a defined dose- and time-response relation. No loss occurs at non-toxic doses but the loss becomes apparent at toxic doses. GSH depletion may be attributable to the VPA-mediated suppression of the glutathione peroxidase–reductase system. This early suppression also indicates a loss in the capacity of liver tissue to detoxify peroxides. The suppression of glucose-6-phosphate dehydrogenase activity by about 70–90% at large VPA doses results in a fall of NADPH.

The loss of vital stores of cellular reducing equivalents may result in various adverse effects on biologically important components like proteins and membranes. In a recent article Porubek *et al.* [6] demonstrate that both VPA and 4-en-VPA bind irreversibly to proteins, the liver being the primary target organ for alkylation *in vivo*. However, it should be mentioned that the formation of chemically reactive metabolites represents a relatively minor pathway of VPA metabolism since glucuronidation seems to be the main route of clearance for the drug.

Nevertheless, the shift in the redox potential of liver tissue to less reducing conditions seen after a single large dose of VPA may be of clinical relevance in chronically treated patients. The study of liver redox status following chronic exposure of rats to VPA which is now in progress will undoubtedly further our understanding on this matter.

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