Valproic Acid and the Liver
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Valproic acid (VPA) is widely used as an anticonvulsant, but therapy with the drug has been associated with hepatotoxicity, either reversible hepatic dysfunction or irreversible hepatic failure. Both clinical and experimental studies have revealed several VPA-related biochemical abnormalities in the liver: inhibition of the β-oxidation and synthesis of fatty acids and inhibition of gluconeogenesis, urea synthesis, oxidative phosphorylation, and the glycine cleavage system. Other abnormalities noted include alteration in the protein conformation of the internal mitochondrial membrane, hyperammonemia, and increased bile flow. The mechanisms of such hepatotoxicity, whether mediated by VPA or by its metabolites, are still little understood. Susceptibility to VPA hepatotoxicity may be enhanced by such conditions as starvation, inborn errors of metabolism, additional neurological disease, and concomitant administration of enzyme-inducing drugs.

Additional Keyphrases: hepatotoxicity, fatty acids, glucose, urea, metabolism, mitochondrial membrane proteins, ammonia, bile, anticonvulsant drugs

Valproic acid (VPA)—also referred to as di-n-propylacetic acid, 2-propyvaleric acid, and 2-propylpentanoic acid—is a simple eight-carbon branched-chain fatty acid used in the treatment of various forms of epilepsy (Figure 1). It is not related structurally to any other anticonvulsant currently in therapeutic use. Since 1978, when VPA was licensed for use as an anticonvulsant in the United States, numerous reports have documented several undesirable side effects of the drug (1-15). One of the major adverse effects of VPA is its potential hepatotoxicity. As more information has become available, evidence has increasingly associated VPA with either a reversible hepatic dysfunction (16-20) or irreversible hepatic failure (19, 21-30).

Various aspects of the mechanisms of action, antiepileptic properties, pharmacology, and adverse effects of VPA have been surveyed elsewhere (19, 31-40). Here, we focus on metabolic and biochemical changes induced in the liver by VPA and its metabolites. We also discuss several other aspects of the drug’s actions, such as metabolic pathways of VPA and its interactions with other drugs.

Absorption, Distribution, and Metabolism

VPA is rapidly absorbed after oral and intraperitoneal administration (41-48). Absorption may be delayed if the drug is taken after a meal, but the extent of absorption is not affected (49, 50). Intraperitoneal administration of a mixture of [14C]VPA and [9H]VPA demonstrated that the concentration in the tissues peaked after 30 min; 64% of the total radioactivity in the liver was distributed mainly in the soluble and mitochondrial cell fractions (47).

VPA is bound to plasma proteins and has a small apparent volume of distribution. In normal humans, the free fraction of the drug is relatively small at therapeutic doses. At higher concentrations the proportion of free fraction of VPA increases, and it can displace other drugs from their binding site on serum albumin (51).

VPA is metabolized largely by the liver and is eliminated mainly in the urine, with minor amounts in the feces and expired air. In rodents, about 20% of an oral dose of the radioactive compound was found as glucuronide in the urine after 3 h, 68-73% within 24 h (42); 80% of [14C]VPA administered as a single oral dose was excreted as glucuronide in urine by 24 h (52). By 24 h, 97% or more of intraperitoneally administered labeled drug was gone from tissues (47). Between 3% (42) and 7-13% (52) of VPA is eliminated in the feces within 24 h after its oral administration. Of [14C]VPA orally administered, 2% was excreted as 14CO2 in the first 24 h (42). Other authors (53) have reported that as much as 13 to 18% of the radioactivity is excreted in the expired air, which suggests that VPA is oxidized via α or β-oxidation. VPA may be also excreted in the bile; 7% of

Fig. 1. Metabolic pathways of VPA

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the radioactivity administered orally as \(^{14}\text{C}\)carboxy-labeled VPA was found in the bile after 4 h (42).

Elimination of VPA is impaired in acute viral hepatitis and liver cirrhosis, and is enhanced in the presence of enzyme-inducing drugs (37). Several other factors—species, age, diet, and concomitant presence of other drugs—also have a major influence on VPA clearance (36, 54–57). VPA kinetics exhibit diurnal variation in both young and elderly adults, with clearances of total and unbound VPA being greater during the evening (58).

The drug is metabolized before excretion. Only very small amounts are eliminated in urine as unchanged VPA. Four independent metabolic pathways have been demonstrated: glucuronidation (endoplasmic reticulum), \(\beta\)-oxidation (mitochondria), and \(\omega_1\)- and \(\omega_2\)-oxidation (endoplasmic reticulum, via cytochrome P-450) (Figure 1).

Glucuronidation seems to be the main route of clearance for VPA in the dose range normally used in humans (59). The proportion of glucuronide conjugation ranges from 7% to 80%, depending on the species investigated and the route of administration (36, 37, 42, 52, 60). The main product of \(\beta\)-oxidation of VPA is 3-oxo-VPA (37, 53, 61, 62) (see Figure 1). Other metabolites of this pathway identified so far are 2-en-VPA and 3-OH-VPA (36, 63, 64). There is also evidence of \(\omega\)-oxidation of VPA, leading to the excretion of 2-propyl-gluaric acid (36, 62, 65). A number of other \(\omega\)-oxidation intermediates—e.g., 4-OH-VPA, 5-OH-VPA, 4-en-VPA, and 3-en-VPA—have also been found (36, 64, 66–69).

Interactions with Other Drugs

VPA is often used in combination with other anticonvulsants. Their possible interactions may have important clinical implications (51). Concurrent administration of VPA and phenobarbital often increases the concentration of phenobarbital in plasma, necessitating a dosage reduction of the latter drug (70). This increase may be greater in children (71). An increase in phenobarbital’s biological half-life and a decrease in its metabolic clearance have been reported (72). VPA also inhibits phenobarbital hydroxylation in rat liver microsomes in vitro (73).

VPA has a dual mechanism of interaction with phenytoin: it can displace phenytoin from plasma-protein binding sites and can inhibit its oxidative metabolism. Both the clearance of the total phenytoin and the free phenytoin concentration in serum are thereby enhanced (74). The displacement interaction between the two drugs is subject to diurnal variation (75).

Several reports also indicate possible interactions between VPA and primidone, ethosuximide, carbamazepine, and clonazepam (51). VPA influences the plasma-protein binding of diazepam, its concentration in erythrocytes, and its kinetic profile (76). Conversely, the concurrent administration of other drugs may affect the metabolism of VPA, either by displacement from its plasma-protein binding sites (salicylates) or by enzyme induction of VPA metabolism (phenytoin, phenobarbital) (51). Salicylates also supposedly inhibit VPA \(\beta\)-oxidation by reducing the formation of valproyl–coenzyme A (77).

Metabolic and Biochemical Disturbances Induced by VPA

**Fatty acid metabolism.** That VPA interferes with fatty acid metabolism is supported by several experimental findings. The lipid content of the liver increases within 2 to 4 h after VPA administration (78, 79), as lipids preferentially accumulate in periporal regions (79). VPA inhibits ketogenesis at a site not yet determined. Concentrations of \(\beta\)-hydroxybutyrate decrease in plasma of infant mice (80), young rats (81), starved rats (82), and humans given VPA (83, 84). Total ketone-body concentrations in blood decrease in both fed and starved rats, the maximum decrease coinciding with the maximum VPA blood concentration (85). A similar decrease occurs in healthy humans after oral or intravenous administration of VPA (86). In perfused rat liver, VPA inhibits ketogenesis, predominantly in periporal regions of the liver lobule (79). VPA also decreases ketogenesis in isolated hepatocytes (87). Both human patients and rats given VPA excrete large amounts of \(C_4\)–\(C_{10}\) dicarboxylic acids in the urine, and starvation enhances this process. This suggests interference of the drug with oxidation of endogenous fatty acids (88, 89). The \(\beta\)-oxidation of long- and medium-chain fatty acids is inhibited by VPA and its unsaturated metabolite, 4-en-VPA (85, 87, 90, 91). These data relate to total hepatic fatty acid oxidation, but fatty acids undergo \(\beta\)-oxidation in liver peroxisomes as well as in mitochondria (92). Peroxisomal \(\beta\)-oxidation increases in the liver of both rats and mice fed for two weeks a diet containing 1% VPA (93). In rats in which peroxisomal \(\beta\)-oxidation has been increased by starvation, 3-hydroxybutyrate formation is inhibited 1 h after VPA administration. So, at low VPA concentrations peroxisomes might partly take over impaired mitochondrial function (94).

VPA also inhibits the synthesis of fatty acids from endogenous substrates in isolated hepatocytes. This inhibition may be reversed by octanoate and butyrate (82).

VPA is thought to inhibit \(\beta\)-oxidation by sequestering coenzyme A (CoA), and by the accumulation of non-acetyl CoA esters, possibly valproyl-CoA (81, 84). The decrease in the concentrations of free CoA would limit the activities of some CoA-dependent enzymes. One result would be a fall in acetyl-CoA, the final product of \(\beta\)-oxidation. The concentration of acetyl-CoA indeed decreases after treatment with VPA (84, 95). Acetyl-CoA is one of the factors controlling the rate of ketogenesis, and its depletion may explain the decrease in \(\beta\)-hydroxybutyrate that is found both in vitro and in vivo.

**Carnitine.** Carnitine is an essential factor in the transport of fatty acids across the inner mitochondrial membrane, the site of \(\beta\)-oxidation. Treatment with VPA has been associated with decreased carnitine in the serum (96–99) and in the liver (83). A high ratio of acylcarnitine to free carnitine is seen in VPA-treated patients. A renal loss of carnitine is supposed to occur via carnitine esters (99–100), as already described for other organic acids. Carnitine deficiency is found in patients with Reye’s syndrome (101). The Reye’s-like syndrome associated with VPA administration could also be due to carnitine depletion, which would lead to impairment of fatty acid metabolism (102). Acetyl carnitine and valproylcarnitine have been detected in the urine of patients receiving VPA (103). In other cases, VPA-induced hepatic failure was not associated with carnitine deficiency (89).

**Carbohydrate metabolism.** VPA administration decreases the concentration of glucose in the blood of young rats (81), infant mice (84), adult female mice (104), and starved rats (85). Hepatic glucose is also decreased (83). The hepatic glycogen concentration declines (80), even after a single therapeutic dose of VPA (84). However, glycogenolysis seems not to be affected by VPA, as judged by rates of glucose accumulation (82). VPA promotes a 50 to 80%
increase in liver pyruvate, lactate, and \( \alpha \)-ketoglutarate and a 53 to 60% decrease in citrate and malate (84). Lactate and pyruvate also accumulate in isolated rat hepatocytes (82, 87). This effect may be due to either an increased rate of glycolysis or a reduced rate of pyruvate utilization by mitochondria. VPA inhibits competitively pyruvate uptake by mitochondria (105) and pyruvate oxidation in isolated hepatocytes (85). Several short- and medium-chain acyl-CoA esters inhibit pyruvate oxidation at the stage of pyruvate dehydrogenase (106), and valproyl-CoA inhibits purified pyruvate dehydrogenase from pig-heart (107). There is no significant change in pyruvate or lactate concentrations in the plasma of rats after intraperitoneal administration of VPA (85). In normal humans, however, after either oral or intravenous VPA administration, serum lactate and pyruvate concentrations initially increase, then decline (86).

Glucogenes...
intoxication is the development of a fatty liver. Microvesicular steatosis appears in 80% of the patients, first in periporal regions of the liver lobule (23, 24). Mature rats, but not young or weanling rats, also develop steatosis after subcutaneous administration of very large (750 mg/kg) doses of VPA alone or lower (350 mg/kg) doses of VPA plus phenobarbital (145). In adult female rats, prolonged oral administration of VPA also induces perportal steatosis. However, concomitant administration of phenobarbital reduces the steatogenic capacity of the drug (140). The authors suggest that, owing to enzyme induction by phenobarbital, VPA is more rapidly metabolized to final harmless products.

Unsaturated VPA metabolites—e.g., 4-en-VPA and 2,4-dien-VPA—are potent inducers of microvesicular steatosis in young rats (81). VPA is thought to induce hepatic steatosis by depressing endogenous fatty acid β-oxidation, as already shown.

Choleretic effects. A dose-related enhancement of bile flow occurs after a single injection of VPA in rats and monkeys (78, 146–148). This choleretic effect of VPA has been explained by the osmotic activity of VPA–glucuronide conjugates in bile (148). Inhibitors of glucuronidation both decrease the biliary excretion of VPA and prevent the increase in the bile flow (149). Cholesterosis might be enhanced by the VPA-induced increase in inorganic ion excretion (148).

Miscellaneous. VPA treatment is also associated with thrombocytopenia (15, 150); lymphocytopenia (151); platelet dysfunction and bleeding (152); changes in the metabolism of zinc (153, 154), selenium, and copper (153); induction of urinary d-glucaric acid excretion (155); and higher concentrations of high-density lipoprotein cholesterol (156), total proteins, and α2- and β-globulin fractions (157) in serum.

Hepatotoxicity of VPA

VPA is widely used, both alone and as a component of multidrug regimens. There is now a large body of evidence showing that therapy with VPA may be associated with a reversible hepatic dysfunction; with irreversible hepatic coma in which the histological pattern resembles that of Reye's syndrome, often accompanied by hepatocellular necrosis; or with hyperammonemia, usually without clinical evidence of hepatic injury (16–30). Up to 44% of VPA-treated patients have abnormal results for liver-function tests (40), which normalize after the dose is decreased or the drug is discontinued. Such results are more common in patients who are receiving valproate as part of a multidrug regimen.

The overall incidence of fatal hepatotoxicity is low. Frequencies of 1/20 000 (38) or around 1/5000 (29) have been suggested. The highest frequency (1/6000) was found in children up to two years old who were receiving the drug as part of a multidrug regimen (40). The incidence declines with age and is as low as 1/37 000 in patients who are receiving VPA alone (40). The mechanism by which VPA causes liver damage is still not well understood. VPA hepatotoxicity is believed to be mediated by some of its metabolites, and particularly by its unsaturated biotransformation product, 4-en-VPA, because this metabolite is structurally similar to methyleneacyclopropylacetic acid and 4-pentenoic acid (Figure 2). The former is the metabolite of hypoglycin, a constituent of unripe akee fruits that produces the Jamaican vomiting sickness; this disorder shares some features with Reye's syndrome. 4-Pentenoic acid produces a Reye's-like syndrome in rats. It potently inhibits mitochondrial fatty acid metabolism by destroying 3-ketocetyl-CoA thiolase (EC 2.3.1.16), the last enzyme in the β-oxidation complex (158, 159).

VPA undergoes desaturation to 4-en-VPA in liver microsomes, presumably via cytochrome P-450 (160); 4-en-VPA may undergo further enzymatic activation to chemically reactive species with hepatotoxic properties (161). This metabolic transformation might be enhanced by previous or concomitant treatment with enzyme inducers such as phenobarbital.

VPA and its two α-oxidation metabolites, 4-en-VPA and 4-OH-VPA, show dose-related toxicity in isolated rat hepatocytes (162, 163). 4-en-VPA, a potent inhibitor of fatty acid β-oxidation (91), induces microvesicular steatosis (81), presumably by depressing fatty acid oxidation, and destroys cytochrome P-450 (136–138). It was found in large amounts in a fatal case of VPA-induced liver failure (28). However, other VPA biotransformation products such as 4-OH- and 5-OH-VPA (163, 164), 2,4-dien-VPA (81), oxo-VPA metabolites, and 2-propylglutarate (165) have also been associated with hepatotoxicity. As already mentioned, VPA may sequestrate CoA or carnitine as valproyl–CoA (81, 82, 84, 85) or valproyl–carnitine (103) and thereby inhibit metabolic pathways that depend on CoA or carnitine. Thus, it appears that the various forms and degrees of VPA-induced liver injury might be mediated by different mechanisms. Several conditions such as starvation, congenital abnormalities, and neurological disease (in addition to the seizure disorder) may enhance individual susceptibility to the drug.

References


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