Plasma and hepatic carnitine and coenzyme A pools in a patient with fatal, valproate induced hepatotoxicity

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Abstract

Reduced hepatic mitochondrial β -oxidation and changes in the plasma carnitine pool are important biochemical findings in valproate induced liver toxicity. The carnitine pools in plasma and liver and the liver coenzyme A (CoA) pool in a patient with fatal, valproate induced hepatotoxicity were measured. In plasma and liver the free and total carnitine contents were decreased, whereas the ratios short chain acylcarnitine/total acid soluble carnitine were increased. The long chain acylcarnitine content was unchanged in plasma, and increased in liver. The total CoA content in liver was decreased by 84%. This was due to reduced concentrations of CoASH, acetyl-CoA, and long chain acyl-CoA whereas the concentrations of succinyl-CoA and propionyl-CoA were both increased. The good agreement between the plasma and liver carnitine pools reflects the close relation between these two pools. The observed decrease in the hepatic CoASH and total CoA content has so far not been reported in humans with valproate induced hepatotoxicity and may be functionally significant.

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Valproate is a branched chain fatty acid composed of eight carbons and has no structural relation to other antiepileptic drugs. Its antiepileptic properties were discovered by chance by Meunier *et al.*,¹ and the drug was introduced into the anticonvulsant market in 1968 in Europe and 10 years later in the United States.² Shortly after introduction, cases of severe and occasionally fatal hepatotoxicity in patients treated with valproate have been reported.²⁻⁴ The frequency of fatal valproate induced liver damage decreases with age, averaging roughly 1:10 000 in children and 1:40 000 in adults, and is higher in patients receiving an antiepileptic polytherapy compared with monotherapy.⁴ The precise mechanism of valproate induced hepatotoxicity is currently not known. In vitro studies suggest that it is related to hepatic depletion of CoASH or microsomal production of metabolites such as 4-ene-valproate and 2,4-ene-valproate, or both⁵; both mechanisms ultimately lead to decreased

mitochondrial β -oxidation. In agreement with this concept, microvesicular steatosis, the principal histological finding in valproate induced hepatotoxicity,²⁻⁴ is also detected in other types of liver disease with decreased mitochondrial β -oxidation such as Reye's syndrome, Jamaican vomiting sickness, mitochondrial cytopathies, and acute fatty liver of pregnancy.⁵⁻⁷

It is well established that the plasma carnitine pool, which is believed to reflect changes in the hepatic carnitine and coenzyme A (CoA) pools,^{8,9} shows a shift towards short chain acylcarnitines when hepatic β -oxidation is decreased.¹⁰ This shift in the plasma carnitine pool has also been described in patients with severe valproate induced hepatotoxicity.¹¹ In this paper, we characterise for the first time the liver carnitine and CoA pools in a patient with fatal valproate induced hepatotoxicity.

Case report

This 39 year old woman was known for congenital, bilateral ptosis, and progressive paralysis of external eye muscles. She had suffered from a manic depressive disorder since the age of 20 but was receiving no current medical treatment for this disease. Five months before the actual illness, she developed a frontal status epilepticus with convulsions, and was treated successfully with valproate (2400 mg/day) and clonazepam (6 mg/day). A computer tomogram and magnetic resonance imaging of the brain showed multiple bifrontal and cerebellar lesions, suggesting a multi-infarct syndrome. Valproate and clonazepam serum concentrations were consistently in the therapeutic range, and the patient had initially a normal liver function (normal transaminase activity and no cholestasis). At the same time, treatment with aspirin (100 mg/day) was started. Four months after the start of the antiepileptic treatment, the patient developed progressive ataxia and became increasingly apathic. Treatment with carbamazepine (400 to 600 mg/day) was started, which failed to improve the clinical status and had to be stopped because of leucopenia. The patient developed progressive anaemia and thrombocytopenia, the prothrombin index decreased, and the serum bilirubin concentration and transaminase activities increased. Coagulation studies were compatible with disseminated intravascular coagulation and the patient was admitted to hospital. At entry, the patient was somnolent and hypothermic (34.8°C), had a blood pressure of 85/60 mm Hg, a bilirubin

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concentration of 145 μM , the alanine transaminase activity was 35 U/l, the aspartate transaminase activity 95 U/l, the prothrombin ratio 39%, the ammonia concentration 75 μM , and the thrombocyte count $47 \times 10^9/\text{l}$. Hypofibrinogenaemia with increased fibrin degradation products, increased partial thromboplastin time and thrombocytopenia were compatible with disseminated intravascular coagulation. Epileptic signs were not detectable, and a computer tomogram of the brain was unchanged in comparison with the earlier investigations. The serum concentrations of clonazepam and valproate were in the therapeutic range and the antiepileptic treatment was initially continued. Blood and spinal fluid cultures were negative for bacterial growth. Twelve hours after entry, the patient developed a cardiovascular collapse and was resuscitated and intubated. Her subsequent course of illness was noticeable for deteriorating liver function with hepatorenal syndrome and progressive disseminated intravascular coagulation. The patient died five days after entry with multiorgan failure. Necropsy was performed five hours after death.

Methods

Sample preparation

Blood samples (5 ml) were withdrawn in heparinised tubes, centrifuged at $600 \times g$ for five minutes, and the plasma was removed and frozen at -20°C until analysis. The blood sample from the patient was obtained three days before death. The blood samples from control subjects were obtained after an overnight fast. Liver samples from the patient and from control subjects (different from control subjects used for the determination of plasma carnitine) were removed at necropsy (about five hours after death), and frozen at -70°C until analysis. Control subjects died after an accident and had macroscopically and microscopically a normal liver.

Assay methods

Free, total acid soluble and long chain acylcarnitine concentrations in plasma and liver were determined by the radioenzymatic assay originally described by Cederblad¹² with the modifications described by Brass and Hoppel.^{8,13} The method has coefficients of variation below 10%.¹³ Plasma and liver

TABLE II Characterisation of the liver carnitine pool. Liver tissue was obtained at necropsy five hours after death. The carnitine content was determined by radioenzymatic analysis as described in the methods section. Carnitine concentrations are given as nmol/mg non-collagen protein. Data are presented as mean (95% confidence intervals).

	Patient	Controls (n=3)
Free carnitine	2.98	5.51 (0.70)
Short chain acylcarnitine (SCA Cn)	1.19	0.82 (0.65)
Total acid soluble carnitine (TAS Cn)	4.17	6.33 (0.70)
SCA Cn/TAS Cn	0.29	0.13 (0.08)
Long chain acylcarnitine	0.80	0.48 (0.19)
Total carnitine	4.97	6.81 (0.48)

samples were prepared in perchloric acid and centrifuged for two minutes at $10\,000 \times g$. In the supernatant, the free and the total acid soluble carnitine concentrations were determined. The short chain acylcarnitine concentration was calculated as the difference between the total acid soluble and the free carnitine concentrations. In the pellet, the long chain acylcarnitine content was determined. The total carnitine concentration was calculated as the sum of the total acid soluble and the long chain acylcarnitine concentrations.

The hepatic concentrations of CoASH, short chain acyl-CoAs, and total acid soluble CoA were determined in neutralised perchloric acid extracts by high performance liquid chromatography (HPLC) as described previously. The total acid soluble CoA concentrations were determined after alkaline hydrolysis of the perchloric acid supernatants and the short chain acyl-CoA concentrations were calculated as the difference between the total acid soluble CoA and the CoASH concentrations. The long chain acyl-CoA concentrations were determined in the hydrolysed pellet of the perchloric acid extracts by HPLC.¹⁴ The total CoA concentration was calculated by addition of the total acid soluble and the long chain acyl-CoA concentrations. The results obtained by HPLC (CoASH, total acid soluble CoA, and long chain acyl-CoA) were validated by the radioenzymatic method described by Cederblad.¹⁵ The results between the two methods differed by less than 10%.

Liver non-collagen protein concentrations were determined according to Lowry¹⁶ with bovine serum albumin as a standard after alkaline hydrolysis of collagen.¹⁷ All analyses were performed in duplicate with the average of the two values reported in the result section. Results are given as mean (95% confidence intervals).

Results

Histological examination of liver samples obtained at necropsy showed microvesicular steatosis, a finding that is compatible with valproate induced hepatopathy. Together with the clinical findings such as progressive ataxia and apathia, and disseminated intravascular coagulation, valproate toxicity was considered to be the most probable explanation for the rapid deterioration in this patient.

In plasma obtained three days before death, the free and total acid soluble carnitine, long

TABLE I Plasma carnitine concentrations. Blood was withdrawn three days before death. The plasma carnitine concentration was determined by radioenzymatic analysis as described in the methods section. Carnitine concentrations are given as $\mu\text{mol/l}$. Data are presented as mean (95% confidence intervals).

	Patient	Controls (n=10)
Free carnitine	18.8	35.8 (4.0)
Short chain acylcarnitine (SCA Cn)	5.8	5.2 (2.8)
Total acid soluble carnitine (TAS Cn)	24.6	41.0 (3.2)
SCA Cn/TAS Cn	0.24	0.13 (0.05)
Long chain acylcarnitine	2.8	3.7 (1.1)
Total carnitine	27.4	44.7 (7.1)

TABLE III Characterisation of the liver coenzyme A pool. Liver tissue was obtained at necropsy five hours after death. The coenzyme A content was determined by high performance liquid chromatography and radioenzymatic analysis as described in the methods section. Coenzyme A concentrations are given as pmol/mg non-collagen protein. Data are presented as mean (95% confidence intervals).

	Patient	Controls (n=3)
CoASH	101	1530 (180)
Succinyl-CoA	89	36 (19)
Acetyl-CoA	20	89 (80)
Propionyl-CoA	32	4 (4)
Total acid soluble CoA	278	1850 (80)
Long chain acyl-CoA	42	211 (90)
Total CoA	320	2060 (130)

chain acylcarnitine, and total carnitine concentrations were decreased, and the short chain acylcarnitine concentration was unchanged (Table I). The ratio short chain acylcarnitine/total acid soluble carnitine was increased.

The hepatic non-collagen protein concentration was 50.3 mg/g in the patient and 113 (16) mg/g in control subjects.

Table II shows the hepatic carnitine concentrations. In comparison with control livers, free, total acid soluble and total carnitine concentrations were decreased, the short chain acylcarnitine concentration was unchanged, and the long chain acylcarnitine concentration was increased. The short chain acylcarnitine/total acid soluble carnitine ratio was increased. Table III shows the hepatic CoA concentrations. With the exception of propionyl-CoA and succinyl-CoA, all other CoA species were decreased in the liver of the patient. In comparison with control livers, the hepatic CoASH content was decreased by 93% and the total CoA content by 84%.

Discussion

It is well established that treatment with valproic acid can be associated with decreased free and total plasma carnitine concentrations in patients with^{11 18–20} or without hepatopathy.^{21–24} Valproate undergoes hepatic metabolism with the formation of acyl-CoAs, which can be converted to the corresponding acylcarnitines.^{5 25} In contrast with acyl-CoAs, acylcarnitines can be exported from the liver and can be excreted by the kidneys, causing significant carnitine losses, eventually leading to secondary carnitine deficiency.^{10 26 27} In addition, it is known that the loss of free carnitine is also increased in patients excreting large amounts of acylcarnitines, as acylcarnitines such as valproylcarnitine can inhibit the renal reabsorption of filtered carnitine.^{20 27 28} In comparison with the findings in our patient, the plasma carnitine concentrations in patients with alcohol induced liver disease^{29 30} or end stage primary biliary cirrhosis³¹ has been shown to be increased, suggesting that the plasma carnitine concentration in patients with liver disease depends on the cause of liver disease.

With the exception of the long chain acylcarnitines, which were increased in liver and not affected in plasma of our patient, the hepatic

carnitine pool showed qualitatively similar changes as the plasma carnitine pool, supporting the concept that the liver and plasma carnitine pools interact closely.^{8 9} As discussed, the decrease in the free and total carnitine content in the liver of our patient is therefore most probably a consequence of increased renal carnitine excretion. In agreement with our findings, short term administration of valproate to mice has been shown to decrease the free and total carnitine content and to increase the long chain acylcarnitine content in the liver,^{32–34} whereas longterm administration of valproate did not affect the hepatic carnitine pool in rats.^{35–37} In comparison, in patients or rats with liver cirrhosis, the total hepatic carnitine content seems to be affected by the cause of liver disease, showing a decrease in patients with end stage alcoholic liver cirrhosis,³⁸ an increase in rats with secondary biliary cirrhosis,³⁹ and no change in rats with carbon tetrachloride induced liver cirrhosis.⁴⁰

The analysis of the hepatic CoA pool showed a considerable decrease in the CoASH and total CoA contents and increases in succinyl and propionyl-CoA. In comparison with our findings, short term administration of valproate to mice or rats,^{32 34 41} isolated hepatocytes⁴¹ or isolated mitochondria⁴² decreased the CoASH but not the total CoA content, and induced a shift from CoASH to acyl-CoAs. While the increase in the hepatic succinyl- and propionyl-CoA contents may be explained by the metabolism of valproate or by inhibition of hepatic mitochondrial metabolism, or both,^{5 25} the decrease in the total CoA content of our patient remains unexplained. Interestingly, the total CoA content in livers from rats with secondary biliary cirrhosis, an animal model of chronic liver disease with impaired hepatic fatty acid metabolism,⁴³ was also found to be decreased.³⁹ On the other hand, the hepatic coenzyme A pool in rats with carbon tetrachloride induced liver cirrhosis was not different from control rats (S Krähenbühl and E Brass, unpublished data), showing that the cause of liver disease is important for the induction of changes in the CoA pool. As CoASH is a critical substrate for β -oxidation, a decrease in the hepatic CoASH content could lead to impaired hepatic fatty acid metabolism, which is considered to represent the major mechanism in valproate induced hepatotoxicity.^{5 25}

In contrast with the reversible inhibition of hepatic energy metabolism (including fatty acid oxidation), which is normally associated with the administration of valproate to humans or experimental animals,^{5 32 35–37 41 42 44} fatal hepatic toxicity is rare.^{2–4} Two factors known to increase the risk of severe hepatic toxicity are concomitant administration of substances inducing hepatic cytochrome P450 isoenzymes and young age.⁴ Our findings support the concept that reduced hepatic mitochondrial metabolism represents another risk factor for valproate induced hepatotoxicity.²⁸ Our patient was treated with low dose acetylsalicylic acid, a drug known to inhibit hepatic β -oxidation.⁴⁵ In addition, our patient showed

signs compatible with a disorder of mitochondrial energy metabolism such as ptosis and progressive external ophthalmoplegia. We therefore propose that valproic acid should not be given to epileptic patients with inborn deficiencies of fatty acid metabolism or to patients treated with drugs known to inhibit hepatic fatty acid oxidation, or both.

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