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Abnormal alterations in the metabolic patterns of patients on valproate therapy

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Abstract

Four cases of abnormal metabolic patterns which were obtained from three infantile patients and one adult on valproate (valproic acid; 2-n-propyl-pentanoic acid) therapy are reported. Serum levels of valproate and 15 metabolites were measured by gas chromatography/mass spectrometry. A mentally retarded, 11-month-old boy developed an extremely altered metabolic profile after having been treated with valproate polytherapy for 3 months. The altered pattern included strongly elevated serum levels of the 4-ene as well as of the x-/x1-metabolites, with the b-metabolites (2-ene; 2,3′-diene) being diminished. Two samples obtained previously had shown a common pattern. The infant died 3 weeks after the last sample had been taken. Two boys of the same age showed similar but less intense deviations in their metabolic profiles at the onset of valproate therapy. Within a few weeks they approached, in a step-wise fashion, the average pattern common for children under 3 years of age. The striking alterations were paralleled by the metabolic profiles of an adult patient who suffered from intrahepatic metastasis and renal insufficiency. From the close resemblance of the abnormal metabolic patterns it was concluded that liver dysfunction results in alteration of the whole metabolic system. Regular inspection of the entire profile of an individual might help to recognize conspicuous alterations in time to avoid severe side effects.

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Introduction

Valproate (valproic acid, 2-n-propyl-pentanoic acid)-associated liver dysfunction occurs in two forms: a mild, reversible variety and an idiosyncratic form being difficult to reverse (Eadie et al 1988). The first variety appears within the first few days of treatment, the second turns up after a few weeks and usually within 6 months of therapy. One hundred and seventy-nine fatal cases of valproate-induced liver failure have been reported (Dreifuss et al 1987; Dreifuss 1989; König et al 1994, 1999; Bryant & Dreifuss 1996). Male infants under 3 years of age, suffering from severe seizures and mental retardation and being treated with high valproate doses (> 30 mg kg^{-1} daily) and polytherapy, were at particular high risk for the incidence of fatal liver disease. Much effort has been made to reveal valproate metabolites (Figure 1) which might be responsible for inducing the rare hepatotoxicity (Rettie et al 1987; Scheffner et al 1988; Kuhara et al 1990; Nau et al 1991; Siemes et al 1993; Dreifuss 1995; Sugimoto et al 1996). Biochemical studies showed that the 4-ene metabolite was cytotoxic to rat hepatocytes in culture (Kingsley et al 1983) and that the 4-ene and the 2,4-diene metabolites were potent inducers of microvesicular steatosis and inhibitors of fatty acid b-oxidation (Granneman et al 1984; Kesterson et al 1984).

Methods

The analytical background was described in detail elsewhere (Darius 1996; Darius et al 2000). Serum samples were collected at steady-state and stored frozen at -21°C until gas chromatography/mass spectrometry (GC/MS) analysis was performed. The HP 5989A MS-Engine was interfaced directly to an HP 5890 Gas Chromatograph which

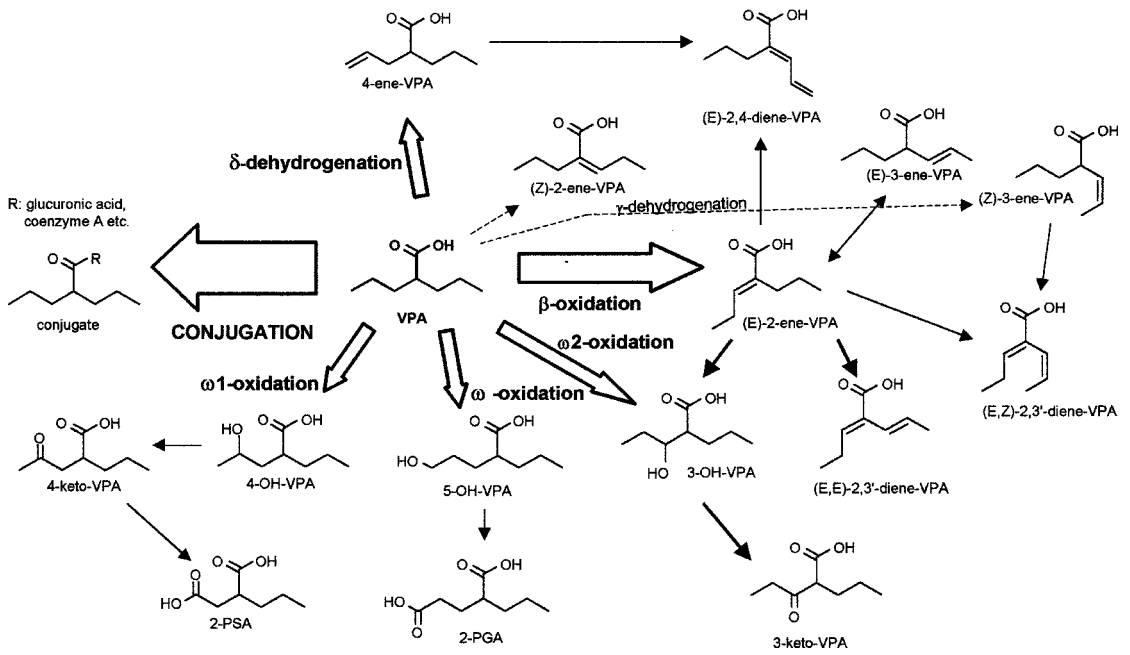

Figure 1 Pathways of valproate biotransformation.

was equipped with a capillary splitless injector and an HP 7673A automated sampler. The 15 metabolites measured in the assay were 2-n-propyl-(E)-2-pentenoic acid ((E)-2-ene), 2-n-propyl-(Z)-2-pentenoic acid ((Z)-2-ene), 2-n-propyl-(E)-3-pentenoic acid ((E)-3-ene), 2-n-propyl-(Z)-3-pentenoic acid ((Z)-3-ene), 2-n-propyl-4-pentenoic acid (4-ene), 2-n-propyl-(E)-2,4-pentadienoic acid ((E)-2,4-diene), 2-[(E)-1′-propenyl]-(E)-2-pentenoic acid ((E,E)-2,3′-diene), 2-[(E)-1′-propenyl]-(Z)-2-pentenoic acid ((E,Z)-2,3′-diene), 3-hydroxy-2-n-propyl-pentanoic acid (3-OH), 4-hydroxy-2-n-propyl-pentanoic acid (4-OH), 5-hydroxy-2-n-propyl-pentanoic acid (5-OH), 3-keto-2-n-propyl-pentanoic acid (3-keto), 4-keto-2-n-propyl-pentanoic acid (4-keto), 2-n-propylsuccinic acid (PSA) and 2-n-propylglutaric acid (PGA).

Results

Large differences existed in individual serum levels among our epileptic population (200 individuals; age range 0.7–65.1 years). Nevertheless, the uniform overall course of metabolic profiles as shown in Figure 2 evolved (the arrangement of metabolites is voluntary). The 3-keto, E-2-ene and (E,E)-2,3′-diene metabolites represented the major products of phase I metabolism (Figure 1), regardless of mono- or polytherapy, age, dose, etc. In all profiles, the 4-ene level formed a trough. The metabolic pattern of a specific individual remained unchanged unless therapy underwent strong modification. Individual deviations from the average pattern in the subgroup of children under 3 years of age (19 individuals; 9 undergoing monotherapy and 10 undergoing polytherapy; mean age 1.4 ± 0.7 years) were common. Even though intra-individual differences were observed, the overall course of the profiles did not alter. We tried to establish the specific metabolic pattern for every patient when therapy had been started or substantially modified. Thus, the base reference pattern represented the normal condition of the individual (Kreher et al 2001). A boy (patient HSM), who had been a triplet premature birth, was admitted to the Department of Pediatrics at the age of 8 months, suffering from BNS-like seizures (Blick-Nick Salaam: saltatory spasm, infantile massive spasm, jacknife seizures) and cerebral motor disturbance (Table 1). The (E)-2-ene and (E,E)-2,3′-diene levels were unusually low ($t_{(E)\text{-}2\text{-}ene} = 6.17 > t_{0.95,18} = 2.1$, where $t_{(E)\text{-}2\text{-}ene}$ represents the results of the Student's *t*-test for the metabolite and $t_{0.95,18}$ is where $P = 0.95$, $F = 18$) in the HSM 1 and HSM 2 (day 44) samples (Figure 2). No further conspicuous deviation was found. The HSM 2 profile (day 44) showed slight elevation in the x-/x1-metabolites (4-OH, 5-OH, 4-keto, PGA, PSA). This might have been caused by phenobarbital co-medication which had been applied temporarily. The HSM 3 pattern (day 85) showed a completely different course. The most significant increase, covering almost an order of magnitude, was observed in the serum levels of the primary products of x- and x1-hydroxylation (4-OH, 5-OH) and the 4-ene. The 4-ene level amounted to 0.491 l g mL^{-1} (1.24% of valproate serum level) which was an extremely high, but not unparalleled, value. However, compared with the 4-ene levels of HSM 1 (0.120 l g mL^{-1}; 0.25% of valproate serum level) and HSM 2 (0.142 l g mL^{-1}; 0.29% of valproate serum level), a four-fold increase had taken place. The trough usually expected for the 4-ene within the profile disappeared. Additional increase in the levels of 4-keto and PGA occurred. The levels of 4-ene, 4-OH, 5-OH and 4-keto were significantly different from those established for the sub-

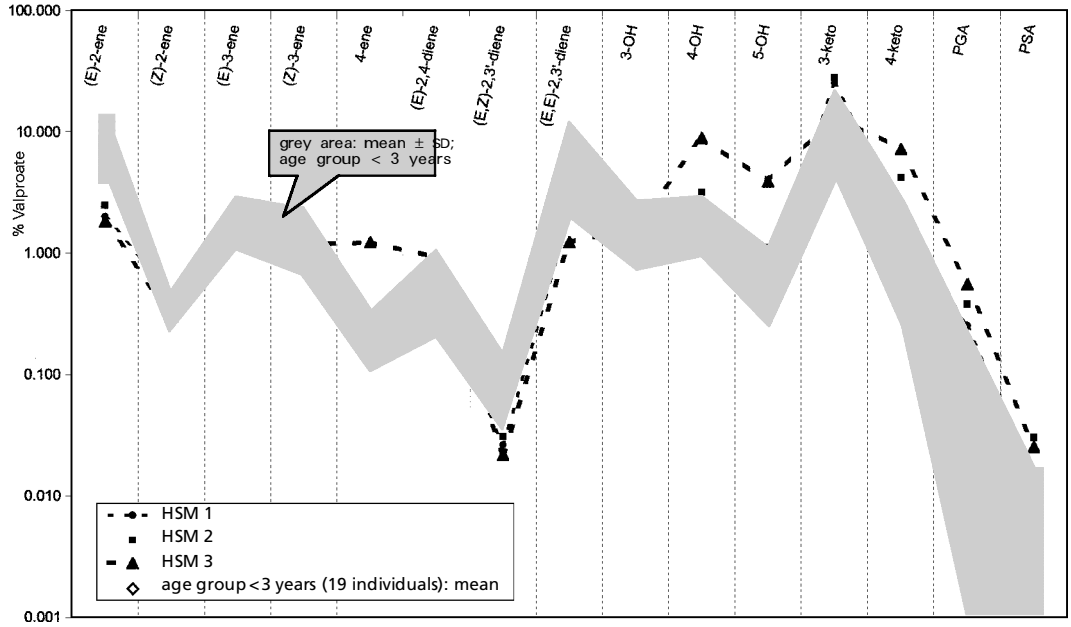

Figure 2 Three metabolic profiles (using serum samples taken at intervals of approximately one month) of patient HSM, who had been a triplet premature birth, admitted to hospital at the age of 8 months, suffering from BNS-like seizures and cerebral motor disturbance. The arrangement of metabolites is voluntary. The grey area represents the range of means$\pm$s.d. for the age group of children $<$ 3 years. All HSM profiles exhibited low (E)-2-ene and (E,E)-2,3′-diene levels. Only the HSM 3 pattern had strongly elevated levels of 4-ene, 4-OH and 5-OH. Note the logarithmic scale of the%- valproate axis.

Table 1 Clinical data for patient HSM.

Day	Anticonvulsant therapy (daily doses in mg)	Daily valproate dose (mg kg^{-1} daily)	Other medication	Sample no.	Time elapsed since last dose given (h)
3	Carbamazepine 75–0–150 Vigabatrin 500–0–250 Valproate 0–0–37.5				
9	Vigabatrin 500–0–500 Valproate 150–0–150		Paraffin		
20	Vigabatrin 500–0–0 Valproate 150–0–225	34.1	Paraffin, ambroxol	HSM 1	12.8
29	Valproate 225–0–225		Paraffin, ambroxol, erythromycin		
33	Valproate 225–0–300 Phenobarbital 30–30–30		Ambroxol, prednisolone, cefuroxime, baclofen		
44	Valproate 225–0–300 Phenobarbital 15–0–15	46.9	Baclofen	HSM 2	12.0
80	Valproate 240–0–350		Erythromycin		
85	Valproate 240–0–350	48.0	Ambroxol, erythromycin, amoxicillin, lactulose	HSM 3	12.5
105	Valproate 300–0–350		Ambroxol, cisapride, ranitidine		

Patient HSM had been a triplet premature birth and was admitted to hospital at the age of 8 months, suffering from BNS-like seizures and cerebral motor disturbance.

group ($t_{4\text{-ene}} = 11.4$, $t_{4\text{-OH}} = 10.0$, $t_{5\text{-OH}} = 7.1$, $t_{4\text{-keto}} = 5.6 > t_{0.95,18} = 2.1$). In contrast to the HSM 1 and HSM 2 samples, 3-keto and 3-OH were diminished, and further decrease in the (E,E)-2,3′-diene level had taken place. A block in b-oxidation must be assumed, with the system taking alternate metabolic routes instead. In the HSM 3

Table 2 Serum levels of valproate and its metabolites in the HSM samples, and means±s.d. calculated for the subgroup of children under 3 years of age.

Sample	HSM 1		HSM 2		HSM 3		Age group < 3 years	
	l g mL^{-1}	% Valproate	l g mL^{-1}	% Valproate	l g mL^{-1}	% Valproate	l g mL^{-1}	% Valproate
Valproate	47.600	–	48.984	–	39.733	–	83.222±33.646	–
(E)-2-ene	0.966	2.03	1.220	2.49	0.729	1.84	6.326±2.846	8.40±4.22
(Z)-2-ene	0.214	0.45	0.191	0.39	0.120	0.30	0.271±0.115	0.35±0.13
(E)-3-ene	0.594	1.25	0.676	1.38	0.814	2.05	1.494±0.584	1.99±0.91
4-ene	0.120	0.25	0.142	0.29	0.491	1.24	0.184±0.119	0.22±0.12
(E)-2, 4-diene	0.243	0.51	0.303	0.62	0.372	0.94	0.499±0.326	0.66±0.45
(E,E)-2,3′-diene	0.960	2.02	1.241	2.53	0.493	1.24	4.920±3.201	6.83±4.93
3-OH	1.191	2.50	1.251	2.55	0.644	1.62	1.186±0.482	1.66±1.01
4-OH	1.192	2.50	1.552	3.17	3.555	8.95	1.448±0.791	1.88±0.95
5-OH	0.289	0.61	0.543	1.11	1.546	3.89	0.611±0.637	0.74±0.60
3-keto	12.013	25.24	13.651	27.87	5.319	13.39	8.499±4.273	11.74±7.93
4-keto	0.922	1.94	2.026	4.14	2.869	7.22	1.160±0.821	1.60±1.36
PGA	0.119	0.25	0.184	0.38	0.219	0.55	0.142±0.259	0.17±0.24
PSA	0.006	0.01	0.015	0.03	0.010	0.03	0.031±0.169	0.03±0.16

% Valproate = weight%. HSM 1, dose: 34 mg kg^{-1} daily; HSM 2, dose: 47 mg kg^{-1} daily; HSM 3, dose: 48 mg kg^{-1} daily; Age group < 3 years, mean dose: 33±12 mg kg^{-1} daily, mean±s.d. (19 individuals).

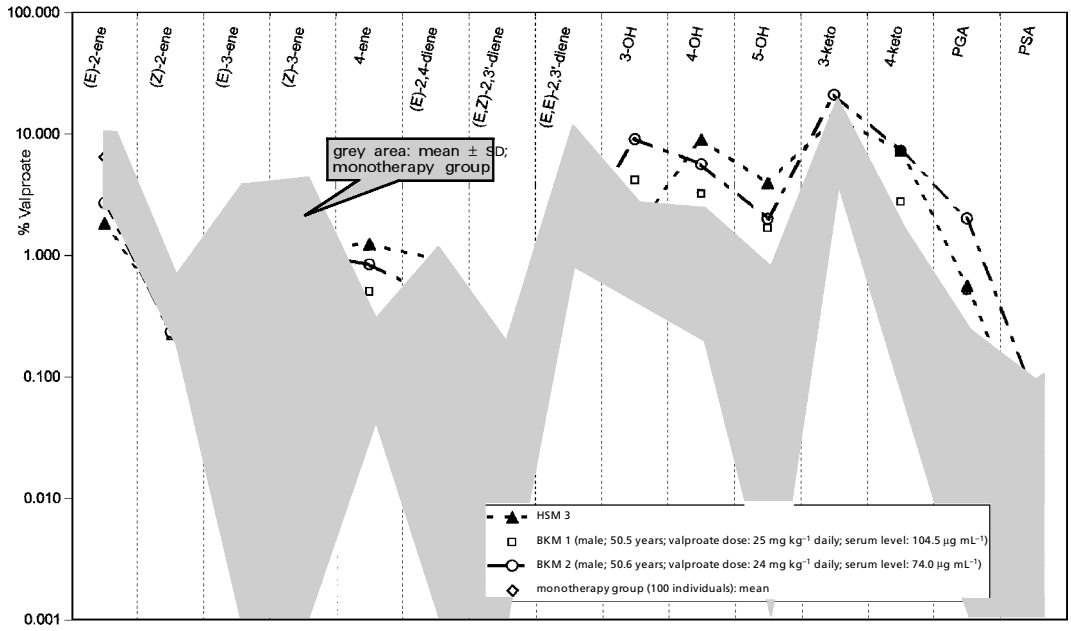

Figure 3 Comparison of the abnormal HSM 3 profile with two metabolic profiles of the adult patient, BKM, who suffered from hepatic metastasis and renal insufficiency. The grey area represents the range of means±s.d. calculated for the entire monotherapy group. Note the logarithmic scale of the%- valproate axis

pattern, the 3-keto level almost matched the mean calculated for epileptic patients under 3 years of age (Table 2). Hence, assessing metabolite levels purely on the basis of their absolute values appears to be insufficient since striking alterations with respect to the whole metabolic pattern run the risk of being overlooked.

The metabolic profiles which were obtained from a 50-year-old patient (BKM) receiving valproate monotherapy, and suffering from intrahepatic metastasis and renal insufficiency, showed features similar to the HSM 3 pattern (Figure 3). Compared with the metabolism patterns of the whole monotherapy group, enhanced 4-ene levels ($t_{4\text{-ene}}$ =

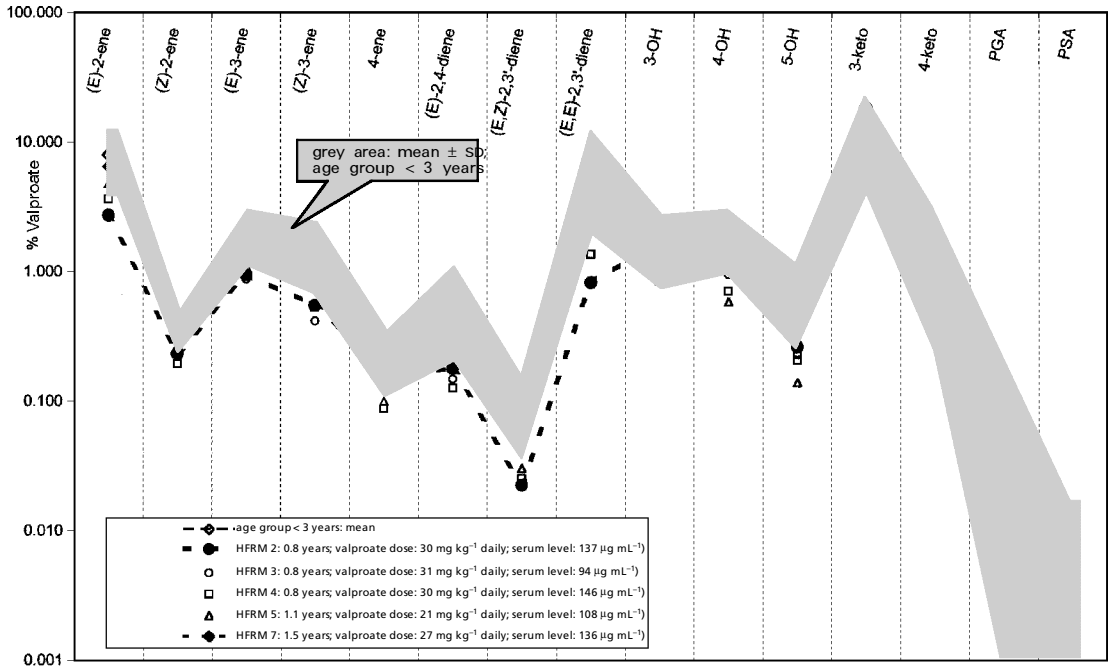

Figure 4 Alterations occurred in the metabolic profile of patient HFRM during therapy (HFRM was a 9-month-old male infant who suffered from near sudden infant death syndrome and mental retardation). The grey area represents the range of means±s.d. for the age group of children< 3 years. After initial disorder (HFRM 2), the metabolic profile approached, in a step-wise fashion, the overall course being typical for the age group. Note the logarithmic scale of the% valproate-axis.

$3.0 > t_{0.95,99} = 1.99$) and a strong increase in the x-/x1-metabolites (4-OH, 5-OH, 4-keto, PGA, PSA) were present in the BKM patterns ($t_{4\text{-OH}} = 2.6$, $t_{5\text{-OH}} = 8.6 > t_{0.95,99} = 1.99$), and the levels of (E)-2-ene and (E,E)-2,3′-diene were diminished.

Two boys under 3 years of age developed abnormal features immediately after the start of valproate therapy. A 9-month-old male infant (HFRM), who suffered from near sudden infant death syndrome and mental retardation, received a constant valproate dose of 30 mg kg^{-1} daily (150–0–150 mg; monotherapy). Samples were collected approximately 12 h after last valproate intake. The HFRM 2 sample exhibited low (E)-2-ene as well as low (E,E)-2,3′-diene levels (Figure 4; no peak existed for (E,E)-2,3′-diene; $t_{(E)\text{-2-ene}} = 3.8$, $t_{(E,E)\text{-2,3′-diene}} = 2.6 > t_{0.95,18} = 2.1$). The 4-ene level was elevated. The overall course of subsequent patterns (HFRM 3, 1 week; HFRM 4, 2 weeks; HFRM 5, 3.5 months; HFRM 7, 9 months later) approached the average pattern of the subgroup step by step.

Discussion

A general tendency towards metabolization rates of infants exceeding those of adults was reported previously (Nau et al 1991; Fisher et al 1992; Darius & Meyer 1996). Comparison of our entire monotherapy group (100 individuals; mean age: 16.1±14.6 years) with the subgroup of infantile patients showed that, in this subgroup, metabolites of microsomal x- and x1-oxidation pathways (4-OH, $t_{4\text{-OH}} = 2.8 > t_{0.95,99} = 1.99$; 5-OH, $t_{5\text{-OH}} = 2.6 > t_{0.95,99}$; 4-keto; PGA), as well as the 4-ene (formed by d-dehydrogenation) and the 2,4-diene, were enhanced, whereas mitochondrial b-oxidation (2,3′-diene; 3-OH; 3-keto) and the 3-enes were less affected (Kreher et al 2001).

Compared with the common state, the metabolic system was altered in the initial serum sample obtained from the infantile patient HFRM (HFRM 2). b-oxidation producing the (E)-2-ene and its successor (E,E)-2,3′-diene was impaired, and d-dehydrogenation (4-ene) and the x-/x1-metabolic routes were intensified. Both the 3-OH and 3-keto levels, which had been average or slightly elevated in the initial HFRM 2 profile, decreased in the samples obtained later on. This supports the contention that initially x2-hydroxylation played an important role in the production of 3-OH (and its successor 3-keto), while b-oxidation via the 2-ene was suppressed (Heinemeyer et al 1985; Prickett & Baillie 1986). From HFRM 2 towards HFRM 7, microsomal x-, x1- and x2- hydroxylations and d-dehydrogenation were reduced in favour of mitochondrial b-oxidation. Whereas the metabolic system approached the normal state in patient HFRM, as well as in another 8-month-old boy, within a few weeks, infantile patient HSM showed drastic changes in the HSM 3 sample compared with patterns determined previously. A large increase in the 4-ene and the x-/x1-metabolites occurred while the main metabolic route of b-oxidation was impaired, as was

found by Kuhara et al (1990). Recently, McLaughlin et al (2000) reported impaired b-oxidation in a surviving adult patient during the peak of hepatotoxicity, even though there were no remarkable alterations in the 2-ene and 4-ene levels found relative to simultaneous plasma valproate concentrations. The 3-keto levels were unusually high early in the toxicity (b-oxidation blocked beyond 3-keto stage) and abnormally low at its peak (b-oxidation blocked between (E)-2-ene and 3-keto stages). This bears resemblance to previous reports (Kochen et al 1983; Siemes et al 1993) and to the HSM case. However, precursors within the b-pathway (2-ene, 3-OH) and the (E,E)-2,3′-diene were diminished in the HSM 3 pattern while the 3-keto level was low.

The metabolic profile obtained from adult epileptic BKM, who suffered from hepatic metastasis, matched the HSM 3 profile. The conclusion from this resemblance might be that the metabolic system of patient HSM did indicate liver dysfunction. In this context, the steady flatulence of the abdomen, nausea and diarrhoea, which persisted despite intensive care, appear to have been manifestations of the dysfunction. Autopsy revealed low-grade centrilobular lesion of the hepatic parenchyme with the beginnings of cell necrosis. This finding was explained by venous congestion caused by pneumonia and endocarditis. On the other hand, hepatic cell necrosis in the centrilobular zone has appeared in several cases of valproate-induced fulminant liver failure (Zimmermann & Ishak 1982; Eadie et al 1988). Pneumonia, sepsis and myocarditis were reported to have been ultimate causes of death, possibly as a consequence of the poor condition of the patients (Scheffner et al 1988). Our patient, HSM, belonged to the high risk population (age, sex, developmental retardation, valproate dose, polytherapy), and disorder of the metabolic profile emerged 3 months after the start of valproate therapy. However, there remains an uncertainty about interactions between valproate and erythromycin causing toxicity, since rare interactions of dubious clinical importance have been reported (Redington et al 1992; Rosensteil & Adam 1995). There is no evidence of whether the abnormal HSM 3 profile represents either a reversible, passing condition or whether it represents a final life-threatening state because of a lack of clinical data.

The 4-ene is suspected to induce fatal liver disease (Sugimoto et al 1983; Rettenmeier et al 1985; Baillie 1988). Therefore, any marked rise in its concentration is an alarming signal. Nau et al (1991) argued that elevated 4-ene/valproate ratios in some patients might have been caused by retarded elimination of the 4-ene after valproate administration had been discontinued. No such reason can be put forward in the case of HSM since this patient received valproate continuously until death. The time points of sampling relative to dosing were uniform (Table 1).

Since alterations of single metabolite levels may turn up just within the specific pattern of the individual, we suggest regular monitoring of the entire metabolic profile of patients at high risk. Detecting abnormal alterations (more than 2-fold decrease or increase in serum levels) with respect to the previously established profile of the individual (i.e., not only with respect to means and ranges derived from large populations) might help to protect patients from severe side effects or life-threatening condition.

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