J. Pharm. Pharmacol. 2000, 52: 1043–1047
Received January 28, 2000
Accepted April 20, 2000

© 2000 J. Pharm. Pharmacol.

Influence of Nanoparticles on the Brain-to-serum Distribution and the Metabolism of Valproic Acid in Mice

JOERG DARIUS, FRANK P. MEYER, BERNHARD A. SABEL* AND ULRIKE SCHROEDER*

*Institute of Clinical Pharmacology, Medical Faculty, Otto-von-Guericke-University Magdeburg and *Institute of Medical Psychology, Medical Faculty, Otto-von-Guericke-University, Magdeburg, Germany*

Abstract

The suitability of nanoparticles as a drug-carrier system for the antiepileptic valproic acid has been studied in mice. The aim of the study was to increase the brain-to-serum ratio of the drug to reduce dose-related side effects in the periphery. The influence of nanoparticles on the metabolism of valproic acid was also investigated.

The serum kinetics and the brain tissue levels of valproic acid were not altered by administration with nanoparticles. However, the nanoparticles did inhibit the metabolic degradation of valproic acid via mitochondrial β-oxidation but did not influence any other metabolic pathway.

It can be concluded that nanoparticles loaded with valproic acid may help to reduce the toxic side effects of valproate therapy, not by reducing the therapeutically necessary dosage but by inhibition of formation of toxic metabolites. Using their ability to selectively block a pathway nanoparticles may serve as a tool to investigate the metabolic origin of metabolites and their contribution to therapeutic efficacy and side effects.

Valproic acid (2-n-propylpentanoic acid) is a first line antiepileptic drug used for the treatment of generalized and focal seizures (Bourgeois 1995). However, among the most commonly used antiepileptics it is the one with the lowest ability to cross the blood–brain barrier (Löscher & Frey 1984). As a consequence, high doses of valproic acid are required to achieve effective concentrations in the CSF and brain tissue. In addition certain side effects occur in the periphery related to high valproic acid doses or high concentrations of several valproic acid metabolites (Baillie & Sheffels 1995; Dreifuss 1995). Among these side effects are tremor, excessive weight gain, loss of hair and nystagmus. On occasion these are sufficiently severe to require discontinuation of the treatment Dreifuss 1995).

Hepatotoxicity is among the most severe side effects associated with valproic acid-therapy. Although its mechanism is not well understood, it is likely that valproic acid metabolites are involved (Baillie 1992) because in-vitro studies have demonstrated a high cytotoxic potential of certain unsaturated metabolites (Baldwin et al 1996; Jurima-Romet et al 1996). The formation of possibly hepatotoxic metabolites in patients is dose-dependent (Sugimoto et al 1996). Therefore, it is desirable to improve the transport of valproic acid across the blood–brain-barrier to reduce the necessary valproic acid dose and, in turn, lower the risk of side effects.

The transport of drugs across the blood–brain-barrier (e.g. leu-enkephalin derivatives) can be achieved by the use of polybutylcyanoacrylate nanoparticles. These nanoparticles can be coated or stabilized with non-ionic detergents such as polysorbates to improve the ability of the drug-loaded nanoparticle to enter the CNS (Tröster et al 1990; Schroeder & Sabel 1996; Schroeder et al 1998). In addition, nanoparticles are able to protect the loaded drug against rapid opsonization and uptake by the reticuloendothelial system, and by macrophages in liver and spleen (Tröster et al 1990).

In this study we have investigated the suitability of butylcyanoacrylate nanoparticles to increase the brain tissue concentration of valproic acid in mice, and the influence of the nanoparticles on the metabolism of valproate. The metabolism of valproate was investigated to find out whether the formation of possible hepatotoxic metabolites

Correspondence: B. A. Sabel, Institut für Medizinische Psychologie, Universitaätsklinikum Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany.
E-Mail: bernhard.sabel@medizin.uni-magdeburg.de

could be altered by adsorption of valproic acid onto nanoparticles.

Materials and Methods

Nanoparticle preparation and drug loading
An acidic polymerization medium containing different stabilizers (1% stabilizer in 0·01 M HCl) was used to prepare the nanoparticles. The stabilizers selected were dextran 70 000 and Tween85 (polysorbate 85). Butylcyanoacrylate (1%) was added under constant magnetic stirring at 600 rev min^{-1}. After a 4-h polymerization period the nanoparticle suspension was neutralized and ultracentrifuged. The determination of particle size was achieved by means of photon correlation spectroscopy with an AutoSizer Lo-c (Malvern Instruments Ltd, UK). The nanoparticle suspension was lyophilized in the presence of 4% mannitol as cryoprotector. Thereafter, 30 mg lyophilized nanoparticles were resuspended in 5 mL 10 mM phosphate-buffered saline (PBS). Valproic acid (Arzneimittelwerk Dresden, Germany) was added at a concentration of 30 mg mL^{-1} suspension and allowed to adsorb onto the nanoparticle surface for 3 h. For coating of the dextran 70 000 stabilized nanoparticles, 0·01% polysorbate 80 (Tween80) was added and incubated for 30 min. The drug-loaded nanoparticles were given intravenously (0·1 mL/10 g).

Animals
Male NMRI mice (n = 36, 30–34 g) were used and housed in plastic cages with food and water freely available. The mice were maintained in temperature- and humidity-controlled rooms with a 12-h light–dark cycle.

Drug administration and sampling
Valproic acid containing solutions/suspensions were given intravenously (0·1 mL/10 g body weight) in four application forms: group A, valproic acid alone; group B, valproic acid adsorbed to nanoparticles (dextran stabilized); group C, valproic acid adsorbed to nanoparticles (dextran stabilized) but coated with Tween80; and group D, valproic acid adsorbed to nanoparticles (Tween85 stabilized). Samples were taken 5, 20 and 60 min after the intravenous injection of the drug or drug-loaded nanoparticles. At each time point 12 mice were decapitated and the blood was collected into tubes and allowed to clot. Serum was taken after centrifugation. The brains were removed quickly, rinsed with ice-cold water to remove blood and immediately frozen. All samples were stored at −20°C until analysis.

Drug analysis
Together with the parent drug 14 valproic acid metabolites were quantified in serum and brain tissue: 2-n-propyl-(E)-2-pentenoic acid [(E)-2-en], 2-n-propyl-(Z)-2-pentenoic acid [(Z)-2-en], 2-n-propyl-(E)-3-pentenoic acid [(E)-3-en], 2-n-propyl-(Z)-3-pentenoic acid [(Z)-3-en], 2-n-propyl-4-pentenoic acid (4-en), 2-(n-(E)-2-propenyl)-(E)-2-pentenoic acid ((E,E)-2,3′-dien), 2-(n-(Z)-2-propenyl)-(Z)-2-pentenoic acid ((E,Z)-2,3′-dien), 2-(n-2-propyl)-(E)-2,4-pentdienoic acid ((E)-2,4-dien), 2-n-propyl-3-hydroxypentanoic acid (3-OH), 2-n-propyl-4-hydroxypentanoic acid (4-OH), 2-n-propyl-5-hydroxypentanoic acid (5-OH), 2-(n-propyl)-3-oxopentanoic acid (3-keto), 2-(n-propyl)-4-oxopentanoic acid (4-keto), and 2-(n-propyl)-glutaric acid (2-PGA).

The brain and serum levels of valproic acid and its metabolites were determined using a modified GC-MS assay (Darius 1996). Whereas the sample preparation was not changed, the chromatographic conditions were altered to allow the quantification of the double unsaturated metabolites. In the improved method a different GC capillary column (BPX35, 30 m × 0·25 mm i.d., 0·25-μm film thickness, SGE, Weiterstadt, Germany) and a split/splitless injector set to 250°C were used. The injection volume was 0·5 μL. The oven temperature started at 90°C, was raised 35 K min^{-1} to 120°C, then raised 3 K min^{-1} to 170°C, and finally raised 17 K min^{-1} to 290°C. All mass spectrometric conditions were identical to those described previously (Darius 1996). Preliminary experiments revealed that the addition of nanoparticles or Tween80 did not have any influence on the analytical results.

Statistics
Data were analysed for significance by the Mann–Whitney U-test.

Results

The valproic acid serum kinetics are shown in Figure 1. The serum kinetics of the parent drug were not altered by the use of nanoparticles.

As depicted in Figure 2, the brain tissue kinetics of valproic acid paralleled those in serum. Therefore, there was no elevation of the valproic acid brain tissue levels by nanoparticles.

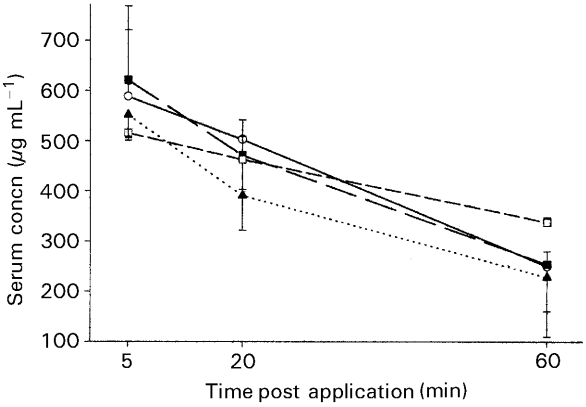

Figure 1. Serum kinetics of valproic acid in mice after intravenous injection of 300 mg kg^{-1} valproic acid. A, valproic acid alone (control, ○); B, valproic acid adsorbed to nanoparticle (Dextran stabilized, ■); C: as in B but nanoparticle coated with Tween80 (□); D, valproic acid adsorbed to nanoparticle (Tween85 stabilized, ▲). Symbols represent means, error bars represent standard deviations (n = 3).

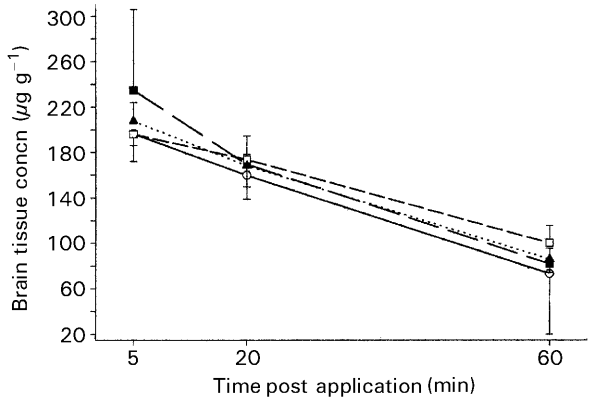

Figure 2. Brain tissue kinetics of valproic acid in mice after intravenous injection of 300 mg kg^{-1} valproic acid. A, valproic acid alone (control, ○); B, valproic acid adsorbed to nanoparticle (Dextran stabilized, ■); C: as in B but nanoparticle coated with Tween80 (□); D, valproic acid adsorbed to nanoparticle (Tween85 stabilized, ▲). Symbols represent means, error bars represent standard deviations (n = 3).

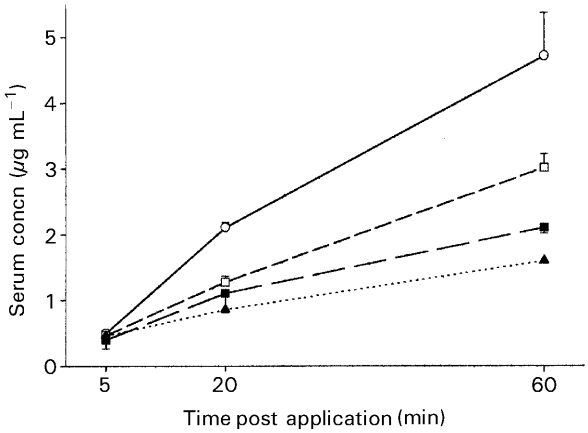

Figure 3. Serum kinetics of (E)-2-en in mice after intravenous injection of 300 mg kg^{-1} valproic acid. A, valproic acid alone (control, ○); B, valproic acid adsorbed to nanoparticle (Dextran stabilized, ■); C: as in B but nanoparticle coated with Tween80 (□); D, valproic acid adsorbed to nanoparticle (Tween85 stabilized, ▲). Symbols represent means, error bars represent standard deviations (n = 3).

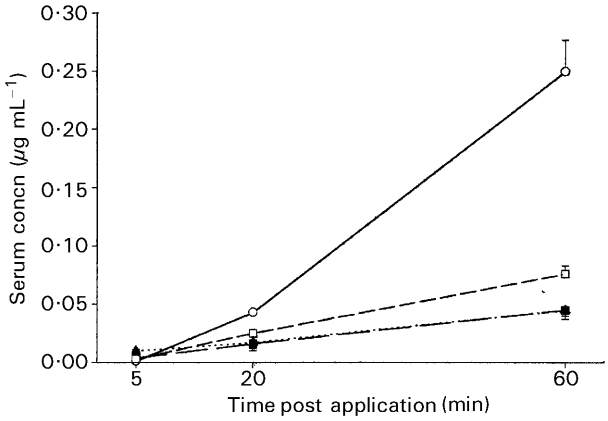

Figure 4. Serum kinetics of (E)-2,4-dien in mice after intravenous injection of 300 mg kg^{-1} valproic acid. A, valproic acid alone (control, ○); B, valproic acid adsorbed to nanoparticle (Dextran stabilized, ■); C: as in B but nanoparticle coated with Tween80 (□); D, valproic acid adsorbed to nanoparticle (Tween85 stabilized, ▲). Symbols represent means, error bars represent standard deviations (n = 3).

The nanoparticles did have a tremendous influence on the metabolism of valproic acid. As can be seen from Figures 3 and 4 and from Table 1 the formation of β-oxidation metabolites was significantly reduced after injection of drug-loaded nanoparticles (groups B–D) compared with valproic acid alone (group A). The ω-oxidation metabolites remained largely unaffected. In Table 1 the mean concentrations at the last time point (60 min post application) are listed. Due to accumulation after the valproic acid injection, all metabolites exhibited the highest concentration within the study period at this point.

The brain tissue concentration profiles of the valproic acid metabolites mainly reflect the changes observed in serum. In group A higher tissue concentrations were found for the majority of those β-oxidation metabolites already showing higher levels in the corresponding serum samples.

Interestingly, the (Z)-isomers of the monounsaturated valproic acid metabolites were not influenced by the nanoparticles, indicating that, in contrast to their (E)-counterparts, (Z)-2-en and (Z)-3-en were not formed via the microsomal β-oxidation pathway or a successive reaction (Figure 5).

Discussion

The passage of valproic acid across the blood–brain-barrier is mediated by diffusion as well as

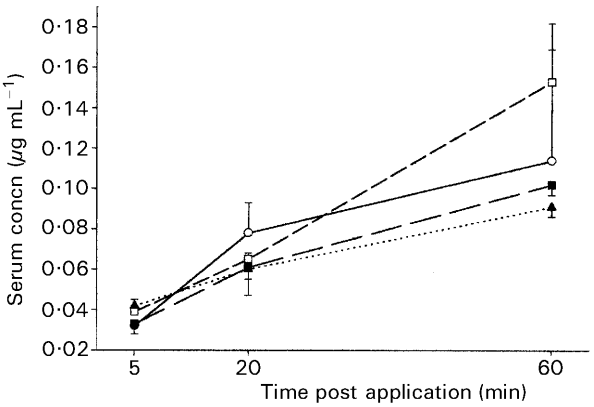

Figure 5. Serum kinetics of (Z)-2-en in mice after intravenous injection of 300 mg kg^{-1} valproic acid. A, valproic acid alone (control, ○); B, valproic acid adsorbed to nanoparticle (Dextran stabilized, ■); C: as in B but nanoparticle coated with Tween80 (□); D, valproic acid adsorbed to nanoparticle (Tween85 stabilized, ▲). Symbols represent means, error bars represent standard deviations (n = 3).

by a transporter system (Adkison et al 1995). The adsorption of valproic acid to nanoparticles does not improve the brain-to-serum ratio and, in turn, does not help to reduce the therapeutically-necessary valproic acid dosage. Whether this is due to lack of transport of valproic acid into the CNS or due to an effective transport process out of the CNS, cannot be answered at this time.

The principal finding of this study is that nanoparticles were able to protect the drug from being metabolized by microsomal β-oxidation. The reduced formation of certain metabolites may have therapeutic implications. The most striking effects were seen for (E)-2-en, a metabolite known to be neurotoxic (Elmazar et al 1993), and the di-unsaturated valproic acid metabolites shown to be cytotoxic and, therefore, possibly hepatotoxic agents (Baldwin et al 1996; Jurima-Romet et al 1996). From this pilot study it cannot be elucidated whether this effect is due to an unselective or selective inhibition of fatty acid β-oxidation. If it was a selective inhibition of the formation of toxic metabolites, this would lower the risk of valproic acid-associated side effects and would increase the therapeutic index of valproic acid.

The potential contribution of unsaturated metabolites to the therapeutic efficacy of valproic acid has been the subject of a long-standing debate. We suggest that nanoparticles may be used as a tool to evaluate the contribution of metabolites formed exclusively by microsomal β-oxidation after injection of valproic acid-loaded nanoparticles.

Whether the (Z)-isomers of 2-en and 3-en are formed via β-oxidation has resulted in controversial discussion in the literature. Granneman et al (1984) expected (Z)-2-en to be a product of the mitochondrial β-oxidation; Adkison et al (1995) mentioned that both 3-en isomers are formed by reversible isomerization of (E)-2-en. Our data do not support either hypothesis. As for 3-OH, which is not exclusively formed in the mitochondria and whose serum concentrations were consequently not influenced by the nanoparticles, for (Z)-2-en and (Z)-3-en an additional metabolic pathway should exist.

It can be concluded that valproic acid-loaded nanoparticles may help to reduce the toxic side

Table 1. Mean (s.d.) of serum concentrations of valproic acid and its metabolites in mice 60 min after the intravenous injection of 300 mg kg^{-1} valproic acid.

Compound	Treatment groups			
	A. Valproic acid alone (control)	B. Valproic acid adsorbed to nanoparticle (Dextran stabilized)	C. As in B but nanoparticle coated with Tween80	D. Valproic acid adsorbed to nanoparticle (Tween85 stabilized)
Valproic acid	249·8 (140·0)	254·0 (25·4)	337·9 (2·1)	228·4 (68·0)
(E)-2-en	4·72 (0·66)	2·11 (0·09)*	3·02 (0·21)*	1·60 (0·02)*
(Z)-2-en	0·114 (0·055)	0·102 (0·005)	0·153 (0·029)	0·091 (0·005)
(E)-3-en	1·48 (0·15)	0·54 (0·03)*	0·66 (0·09)*	0·34 (0·04)*
(Z)-3-en	0·152 (0·039)	0·143 (0·011)	0·199 (0·044)	0·105 (0·012)
4-en	0·729 (0·390)	0·702 (0·042)	0·903 (0·091)	0·648 (0·045)
(E)-2,4-dien	0·250 (0·027)	0·045 (0·008)*	0·076 (0·007)*	0·045 (0·005)*
(E,Z)-2,3′-dien	0·052 (0·023)	0·022 (0·004)	0·035 (0·002)	0·025 (0·002)
(E,E)-2,3′-dien	0·334 (0·032)	0·072 (0·012)*	0·101 (0·011)*	0·052 (0·002)*
3-OH	9·73 (3·39)	10·88 (2·88)	16·10 (6·03)	8·81 (0·33)
4-OH	9·31 (5·52)	12·35 (1·39)	14·48 (4·69)	12·99 (1·91)
5-OH	19·74 (8·26)	29·05 (3·99)	27·99 (8·99)	24·39 (1·55)
3-keto	1·86 (0·22)	0·65 (0·04)*	0·73 (0·11)*	0·42 (0·02)*
4-keto	3·64 (0·78)	4·22 (0·75)	5·52 (1·65)	4·73 (0·41)*
PGA	4·50 (1·33)	5·64 (0·65)	7·63 (1·60)*	6·02 (1·09)

*Significantly different from control (group A) ($P < 0·05$, Mann–Whitney U-test).

effects of valproic acid therapy, not by reducing the therapeutically-necessary valproic acid dosage but by inhibition of the formation of toxic metabolites.

References

Adkison, K. D. K., Shen, D. D. (1996) Uptake of valproic acid into rat brain is mediated by a medium-chain fatty acid transporter. J. Pharmacol. Exp. Ther. 276: 1189–1200

Adkison, K. D. K., Ojemann, G. A., Rapport, R. L., Dills, R. L., Shen, D. D. (1995) Distribution of unsaturated metabolites of valproate in human and rat brain–pharmacologic relevance? Epilepsia 36: 772–782

Baillie, T. A. (1992) Metabolism of valproate to hepatoxic intermediates. Pharm. Weekbl. [Sci.] 14: 122–125

Baillie, T. A., Sheffels, P. R. (1995) Valproic acid–chemistry and biotransformation. In: Levy, R. H., Mattson, R. H., Meldrum, B. S. (eds) Antiepileptic Drugs. 4th edn, Raven Press Ltd, New York, pp 589–604

Baldwin, G. S, Abbott, F. S., Nau, H. (1996) Binding of a valproate metabolite to the trifunctional protein of fatty acid oxidation. FEBS Lett. 384: 58–60

Bourgeois, B. F. D. (1995) Valproic acid–clinical use. In: Levy, R. H., Mattson, R. H., Meldrum, B. S. (eds) Antiepileptic Drugs. 4th edn, Raven Press Ltd, New York, pp 633–639

Darius, J. (1996) On-column gas chromatographic-mass spectrometric assay for metabolic profiling of valproate in brain tissue and serum. J. Chromatogr. B 682: 67–72

Dreifuss, F. E. (1995) Valproic acid–toxicity. In: Levy, R. H., Mattson, R. H., Meldrum, B. S. (eds) Antiepileptic Drugs. 4th edn, Raven Press Ltd, New York, pp 641–648

Elmazar, M. M. A., Hauck, R.-S., Nau, H. (1993) Anticonvulsant and neurotoxic activities of twelve analogues of valproic acid. J. Pharm. Sci. 82: 1255–1258

Granneman, G. R., Wang, S. I., Machinist, J. M., Kesterton, J. W. (1984) Aspects of the metabolism of valproic acid. Xenobiotica 14: 375–387

Jurima-Romet, M., Abbott, F. S., Tang, W., Huang, H. S., Whitehouse, L. W. (1996) Cytotoxicity of unsaturated metabolites of valproic acid and protection by vitamin C and E in glutathione-depleted rat hepatocytes. Toxicology 112: 69–85

Löscher, W., Frey, H.-H. (1984) Kinetics of penetration of common antiepileptic drugs into cerebrospinal fluid. Epilepsia 25: 346–352

Schroeder, U., Sabel, B. A. (1996) Nanoparticles, a drug carrier system to pass the blood-brain barrier, permit central analgesic effects of i.v. dalargin injections. Brain Res. 710: 121–124

Schroeder, U., Sommerfeld, P., Ulrich, S., Sabel, B. A. (1998) Nanoparticle technology for delivery of drugs across the blood–brain barrier. J. Pharm. Sci. 87: 1305–1307

Sugimoto, T., Muro, H., Woo, M., Nishida, N., Murakami, K. (1996) Metabolite profiles in patients on high-dose valproate monotherapy. Epilepsy Res. 25: 107–112

Tröster, S. D., Müller, U., Kreuter, J. (1990) Modification of the body distribution of poly(methyl metacrylate) nanoparticles in rats by coating with surfactants. Int. J. Pharmacol. 61: 85–100