The neuropharmacokinetics of valproate in pentylenetetrazol-kindled conscious epileptic rat hippocampus

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ABSTRACT
In the present communication, the neuropharmacokinetics of valproate in pentylenetetrazol-kindled epileptogenesis rat hippocampus have been examined by micordialysis. It was found that the maximum concentration and the area under the hippocampus concentration-time curve of valproate in pathological rats were significantly higher than those in the control group. Time to maximum concentration of valproate appeared at 45 min after drug administration, and then the concentration of valproate gradually declined. The results suggest that the pathological damages of rat brain induced by pentylenetetrazol may result in the increase of the valproate level in epileptic rat hippocampus, and the neuropharmacokinetic research of valproate in a chronic kindled conscious animal model with acute administration may help the clinic in the pharmacokinetic optimization of the valproate dosage schedule in epileptic patients.

Epilepsy represents the most common serious neurologic complications effecting approximately 0.7–1% in 150 people over their lifetime (10, 29). Neuropathological studies on either adult or child patients with repeated or sustained epileptic seizures have revealed that severe brain damage may occur in selectively vulnerable regions, chiefly the neocortex and limbic structures (9, 18). Especially the hippocampus has been demonstrated to be involved in initiation, propagation and termination of seizures (28, 33).

Valproate (VPA) is a major broad-spectrum antiepileptic drug (AED), effective against many different types of epileptic seizures (8, 16, 23). However, in clinical application, the value of pharmacokinetic optimisation with VPA is limited by its wide therapeuti c index, large fluctuations in the concentration-time profile and concentration-dependent protein binding.

The pentylenetetrazol (PTZ)-kindling models of epilepsy are currently the most widely used animal model for studying the epileptogenesis, which mimics the forms of epilepsy in humans (7, 13). The ability of VPA to inhibit PTZ-induced clonic and tonic convulsions has been demonstrated as having predictive efficacy against generalized absence and/or myoclonic seizures (23). Although the possible mechanisms of action and the blood-pharmacokinetics of this drug have been proposed (1, 2, 35), a limited number of studies on its neuropharmacokinetics has been carried out. Although serum concentrations are often measured, they are rarely subjected to pharmacokinetic interpretation (34). However, as a first-line broad-spectrum AED, the neuropharmacokinetic study of VPA is critical for optimization of therapy, ascertaining the modes and action mechanisms of drugs. Therefore, in present communication, the neuropharmacokinetics of VPA in freely-moving/epileptic rat hippocampus is detected and a possible mechanism involved is discussed.
MATERIALS AND METHODS

Animals and drug treatment. Male Sprague-Dawley rats (80–100 g) were employed for the experiments (Shanghai experimental animal center, Chinese Academy of Science). Five rats were kept in individual cages with water and food available ad libitum. The animal room was maintained at 21–23°C with a 12 h light-dark cycle. All experimental procedures were approved by the committee of Laboratory Animals, Chinese Academy of Science.

Rats were intraperitoneally (i.p) administered a subconvulsive dose of 1% pentylentetrazol (PTZ) (40 mg/kg; Sigma) dissolved in saline every other day. Control animals received the same number of saline injections. Rats were observed for 30 min after injection for the occurrence of generalized clonic seizures. The seizures were rated according to the following criteria (11, 12): Stage 0, no response; Stage I, ear and facial twitching; Stage II, myoclonic jerks without upright position; Stage III, myoclonic jerks, upright position with bilateral forelimb clonus; Stage IV, clonic-tonic seizure; Stage V, generalized clonic-tonic seizures, loss of postural control.

After 28 days, 87.1% of the rats were kindled successfully by PTZ. To maintain the epileptic state of the kindled rats, the same dosage of PTZ was administered once a week until microdialysis analysis.

Surgery & microdialysis. Rats (250–350 g) were anesthetized with chloral hydrate (300 mg/kg i.p.). Intracerebral guide cannulae were implanted into rat hippocampus (Coordinates: P = −5.2 mm; L/R = ± 4.4 mm relative to bregma; H = −5 mm relative to the dural surface) with a stereotaxic instrument. Before the microdialysis probe (outer diameter 0.22 mm, effective length 4 mm, molecular cut-off 50 Kd, materials purchased from Eicom company, Japan) was implanted, the relative recovery rate was measured to be from 10% to 15% while probes were perfused at a flow rate of 1 μL/min in vitro. After a 48-hour recovery period, rats were anesthetized by ether absolute and the probe was implanted into rat hippocampus through the guide cannula, then perfused by artificial cerebral spinal fluid (ACSF) containing (in mM): NaCl 140, KCl 2.4, MgCl: 1.0, CaCl: 1.2 and NaHCO3: 5.0 (pH 7.4) at a flow rate of 1 μL/min with a micro-syringe pump (BAS, West Lafayette, Indiana, USA). Acute administration of 200 mg/kg VPA is regarded as an effective dose (6, 21, 22, 24). Following an initial 2 h equilibrium period, rats were i.p co-administrated with 40 mg/kg PTZ and 200 mg/kg VPA. Dialysate samples were collected at the first 30 min, and then the samples were collected every 15 min from 45 min to 150 min. Finally, they were collected every 30 min from 180 to 210 min in the freely moving rats. The dialysate samples were stored and frozen (−70°C) until analysis.

When the experiments were terminated, a rat was given an overdose of chloralhydrate and the brain was fixed in 4% paraformaldehyde solution. Coronal sections (60 μm thick) were made to verify the placement of the probe.

VPA determination. Free (unbound) VPA hippocampus concentrations were determined by fluorescence polarization immunoassay (FPIA) technology using the Abbott AxSYM™ immmunoassay analyzer (Abbott Park, IL, U.S.A.).

Statistics analysis. An estimate of VPA absolute concentration in rat hippocampus was presented by the dialysate concentrations of VPA dividing the relative recovery rate of the probe. The ends of collection times were used as the relevant timepoints. Concentrations of VPA were expressed as mean ± s.e.m (μg/mL). The neuropharmacokinetic parameters of VPA analysed according to an optimal one-compartment kinetic model (27, 32) were used to compare the difference between the epileptic and control group. Student’s paired t-test was performed for group analysis. P < 0.05 was considered to be significant.

RESULTS

The time course of VPA concentration in epileptic and control rat hippocampus is shown in Fig. 1. The concentration of VPA increased rapidly after VPA administration, achieving a maximum at 45 min, followed by a progressive decline in both groups. The transporting rate constants of VPA (Ka) into epileptic and normal rat hippocampus were 0.0576 ± 0.0208 min⁻¹ (n = 8) and 0.0668 ± 0.0198 min⁻¹ (n = 5), respectively. There was no statistical difference in the elimination half-life between the epileptic group (32.12 ± 9.03 min) and saline-treated group (25.35 ± 2.15 min). The maximum concentration (Cmax) in the epileptic and control group was markedly different (235.3 ± 93.2 and 93.09 ± 54.82 μg/mL individually, P < 0.05). Corresponding to Cmax, the areas under the hippocampus concentration-time curve (AUC0→∞) values of VPA between the control and epileptic groups were also significantly
Concentration-time profiles in pentylenetetrazol-kindled epileptic (solid squares) and control (open squares) rats’ hippocampus after acutely intraperitoneal injection of 200 mg/kg valproate. Values are mean ± SEM of 8 and 5 rats respectively.

**Table 1** Neuropharmacokinetic parameters in saline-treated and pentylenetetrazol-kindled epileptic rat hippocampus

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N.S. Group (n = 5)</th>
<th>Epileptic Group (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (min⁻¹)</td>
<td>0.0275 ± 0.0023</td>
<td>0.0229 ± 0.0051</td>
</tr>
<tr>
<td>t_{1/2} (min)</td>
<td>25.35 ± 2.15</td>
<td>32.12 ± 9.03</td>
</tr>
<tr>
<td>T_{max} (min)</td>
<td>14.46 ± 9.83</td>
<td>23.74 ± 6.40</td>
</tr>
<tr>
<td>k_e (min⁻¹)</td>
<td>0.0668 ± 0.0198</td>
<td>0.0576 ± 0.0208</td>
</tr>
<tr>
<td>t_{max} (min)</td>
<td>23.36 ± 4.55</td>
<td>28.29 ± 6.73</td>
</tr>
<tr>
<td>C_{max} (µg/ml)</td>
<td>93.09 ± 54.82</td>
<td>235.3 ± 93.2*</td>
</tr>
<tr>
<td>AUC_{0→∞} (min·µg/ml)</td>
<td>6252 ± 3607</td>
<td>19286 ± 5219**</td>
</tr>
</tbody>
</table>

*Significant difference, P < 0.05; **P < 0.01; *Acutely intraperitoneal injection of 200 mg/kg valproate. K: elimination rate constant of drug from rat hippocampus; t_{1/2}: terminal half-life; T_{max}: time between valproate dosing and the occurrence of the first quantifiable concentration of valproate in rat hippocampus; K_e: rate constant of transporting into rat hippocampus; t_{max}: time to maximum concentration; C_{max}: maximum concentration; AUC_{0→∞}: area under the rat hippocampus concentration-time curve.

**DISCUSSION**

It has been demonstrated that the influx of VPA into the brain is predominantly mediated by active transport using a medium- and long-chain fatty acid selective anion transporter. Diffusion should only play a minor role (15, 31). Interestingly, free fatty acids in the rodent brain markedly increased during or within seconds of convulsions induced by PTZ or electroshock (4, 26). The high levels of fatty acids could inhibit transport of VPA into the brain (19). Thus, it is possible that the increase of the fatty acid levels induced by PTZ could result in the decrease of VPA levels in epileptic rat hippocampus. In contrast to the deduction, in the present study, it is found that C_{max} and AUC_{0→∞} of VPA in epileptic rat hippocampus are significantly higher than those in the control group. Obviously, besides the inhibitory modulation mentioned above, it could be affirmed that up-regulation mechanisms are involved in the influx of VPA in epileptic rat hippocampus. The following possible mechanisms are considered: 1) the pathological damage induced by PTZ, such as loss of neuron cells and modification of neuronal plasticity (3, 30), could weaken the function of blood-brain and blood-CSF barriers (3) and increase the capillary transparency which enable the influx of VPA to
be increased; 2) the pathological influence of PTZ on rat hippocampus may result in the functional potentiation and synthesizing increase of anion transporter (14); 3) PTZ may decrease epileptic cerebral blood flow, thereby increasing both the cerebral capillary transit time and the opportunity for VPA diffusion across capillary endothelium; 4) The PTZ decreases the systemic of valproate and increases systemic exposure, leading to an increase in hippocampus extracellular fluid (ECF) concentration; Consequentially, the VPA level in epileptic rat hippocampus was markedly enhanced. After VPA acute injection, the drug was totally eliminated at 180 min in rat. The result is well in accordance with amino acid transmitter release influenced by VPA in rat hippocampus. The level of major amino acid neurotransmitters turned to be the lowest or highest point at exact time point in PTZ-kindled epileptic rat hippocampus co-administrated with VPA and PTZ (20).

A disadvantage of VPA therapy is the relatively poor correlation of serum concentration with clinical effectiveness and adverse reaction compared with other AEDs. Therapeutic monitoring does not predict clinical efficacy as accurately as with other AEDs. The pharmacological activity of VPA can persist for days to weeks after the drug is cleared from serum (25). A central active drug such as VPA could interact with molecular entities, including neuronal receptors, transmitter uptake carriers and ion channels, localized in the outer cell membrane (24). If so, the extracellular levels of central active drugs in the brain are of far more importance than those in the tissue. Thus, drugs may be evaluated not by their accumulation in the brain, but rather whether ECF levels are sufficient to obtain pharmacological responses (17). Measuring VPA concentration of brain ECF by in vivo microdialysis could reflect the real concentration of VPA at its active sites.

In summary, the pathological damages of rat brain induced by PTZ may result in the increase of the VPA level. The VPA neuropharmacokinetic research in chronic kindled epileptic animals with acute administration has the potential to be useful for optimizing VPA therapy and preventing toxic effects.

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REFERENCES


