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The pharmacokinetics of commonly used antiepileptic drugs in immature CD1 mice

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Abstract

Rodents eliminate antiepileptic drugs (AEDs) faster than humans, creating challenges for designing clinically-relevant protocols. Half-lives of AEDs in immature mice are unknown. The pharmacokinetics of commonly-used AEDs were examined in CD1 mice using a single-dose protocol at post-natal day 19. Following intraperitoneal therapeutic dosing, blood serum concentrations spanning 1–48 hours post-administration and corresponding brain tissue concentrations at 4 hours were analyzed. Half-lives of valproate, phenobarbital, diazepam (and metabolites), phenytoin, and levetiracetam were 2.6, 15.8, 22.3, 16.3, and 3.2 hours respectively, compared to 0.8, 7.5, 7.7, 16.0, and 1.5 hours reported for adult mice. Brain-to-blood ratios were comparable to adult ratios. AEDs tested had longer half-lives and maintained therapeutic plasma concentrations longer than reported in mature mice, making clinically-relevant protocols feasible.

Keywords

Antiepileptic drugs; pharmacokinetics; plasma levels; brain-to-blood ratios; immature mice

INTRODUCTION

Neonatal stroke occurs approximately once per 4000 term births, and while most children survive their stroke, approximately 75% have sequelae including learning and memory problems. These strokes often present with seizures. In the pediatric population, strokes carry a significant risk of mortality and morbidity and also commonly present with seizures. They are commonly symptomatic, multifocal, and resistant to treatment with common antiepileptic drugs [1]. Anticonvulsants are commonly administered in the months following stroke, therefore animal models are a useful tool to determine consequences of this therapeutic stratagem [2] in the developing brain. Our group has previously investigated the

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ischemic stroke following unilateral carotid ligation in CD1 neonatal mice and characterized the associated post-stroke cognitive impairments [3]. With the intention of investigating the feasibility of designing effective drug administration protocols to evaluate the effects of anticonvulsants on the post-stroke developing brain, the current study was undertaken.

Pediatric patients have faster drug absorption times, higher peak drug concentrations, and more rapid clearing of anticonvulsants, leading to more adverse effects, less effective seizure control, or both [4]. Recent literature using rodent models has indicated the existence of possible negative consequences of anticonvulsant administration for brain development and cognition. Increased apoptosis and decreased proliferation have been shown throughout the immature brain after anticonvulsant administration [5,6]. Long-term cognitive deficits following neonatal drug administration have been demonstrated through behavioral tests targeting water maze learning and memory tasks [6]. Models of seizures in the immature brain are needed to understand the mechanisms of anticonvulsant effects upon seizures and brain development.

Clinically, antiepileptic drugs are given chronically and must maintain a therapeutic trough concentration. Rodents eliminate drugs at a quicker rate than humans, challenging the creation of therapeutic chronic-dosing protocols which would mimic clinical protocols. These protocols would help investigate hypotheses that provide insights into the effects of antiepileptic drugs on the developing brain. The shorter half-lives of commonly used antiepileptic drugs after single-dose administration have been reported for adult rats and mice (Table 1), but pharmacokinetic data for immature rodents is lacking.

Concentrations of anticonvulsants in the brain in humans are developmentally-dependent and different drug doses are efficacious at different ages [4]. Elements of the blood-brain barrier, and the tight junctions between the cerebral endothelial cells that make up the blood-brain barrier themselves, develop as the brain matures [7]. An associated decline in brain-to-blood ratios of small molecules has been demonstrated during development [7]. Anticonvulsants also have different mechanisms to cross the blood-brain barrier through the various drug transporters [8]. At therapeutic plasma concentrations, brain penetrations of different antiepileptic drugs in mature mice are variable (Table 2). Brain-to-blood ratio data for antiepileptic drugs in immature rodents is lacking.

This study evaluated serum concentrations at time-points spanning one to 48 hours, and brain concentrations at four hours post-injection in post-natal day 19 CD1 mice.

METHODS

All materials and methods were approved by the Johns Hopkins University Animal Care and Use Committee. Litters of CD1 mice were purchased from Charles River Laboratories Inc (Wilmington, MA), arrived at post-natal day 17 (P17), and acclimated for 2 days. Pups were housed in polycarbonate cages with the dam on a 12 hour light:dark cycle; food was provided ad libitum.

Antiepileptic drugs

Diazepam, levetiracetam, phenobarbital (phenobarbital sodium salt), phenytoin (5,5-Diphenylhydantoin), and valproate (valproic acid sodium salt) were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Diazepam was reconstituted to 1mg/ml in 100% ethanol, levetiracetam to 50mg/ml in sterile dH₂O, phenobarbital to 25mg/ml in sterile dH₂O, phenytoin to 25mg/ml in 3mls sterile dH₂O with 1μl 10N NaOH, and valproate to 100mg/ml in sterile dH₂O.

Drug administration and sample collection

Mice were injected intraperitoneally with a single dose of 1mg/kg diazepam, 200mg/kg levetiracetam, 60mg/kg phenobarbital, 60mg/kg phenytoin, or 200mg/kg valproate. Doses necessary to achieve therapeutic efficacy in adult rodents were chosen [9]. Due to the limited total blood volume of neonatal mice, 19 day old mice were used in this study which allowed collection of blood sample volumes sufficient for individual quantification. The P19 mouse can be considered roughly equivalent to the juvenile human. Blood samples were obtained by open cardiac puncture to access the total blood volume, and sample collection occurred at times ranging from 1 to 48 hours (n=2–5 each) using a litter-based model [7]. Samples were centrifuged at $14,000g \times 15$ minutes, and serum was stored in sterilized EDTA tubes at 4°C. At 4h post-injection, corresponding brain samples [10] were harvested after trans-cardiac flushing with 0.9% NaCl. Brain samples were homogenized in 0.9% NaCl (1:5 w/v) and centrifuged at $10,000g \times 10$ minutes at 4°C. Supernatants were collected and stored at -80°C.

Sample analysis

Plasma samples and brain supernatants were thawed at room temperature. Benzodiazepines, phenobarbital, phenytoin and valproate were assayed in plasma and in brain supernatants using an automated polarized immunofluorescence assay (PIFA, Abbott Diagnostics, Dallas, TX). The benzodiazepine antibody detects diazepam and its active metabolites desmethyldiazepam, oxazepam, and temazepam. Accordingly, results were reported as benzodiazepines.

Levetiracetam was detected in plasma and in brain supernatant by high performance liquid chromatography (HPLC) using previously described methods [11] with modifications (column size 250 mm). Drug was detected using ultraviolet spectroscopy at 205 nm. Unknown concentrations in samples were quantified by comparison to standards prepared with known amounts of levetiracetam (Keppra®, UCB Pharma Inc, Smyrna, GA).

Data analysis

For each time point, drug concentration data was averaged (n=2–5). Composite drug concentration versus time data was subjected to standard non-compartmental pharmacokinetic analysis based on extravascular administration, using computer-assisted linear regression software (WinNonlin Professional, version 4.1, Pharsight Corp, Mountain View, CA). Disappearance half-life was determined by linear regression of the terminal component of the time versus concentration curve. For experiments in which metabolism occurred too quickly to allow non-compartmental analysis, i.e. with levetiracetam and valproate, disappearance half-life was determined from two time points using the equation: $t_{1/2} = 0.693 / k_{el}$ where k_{el} is the disappearance rate constant, equal to $[\ln(C_1/C_2)] / (t_2 - t_1)$, where C_1 and C_2 were the concentrations of the drug at times t_1 and t_2 respectively. Concentrations were reported as mean $\pm$ S.D.

RESULTS

Phenobarbital concentrations ($\mu\text{g/ml}$) post-injection (h) were 38.7 ± 3.2 (1h), 40.6 ± 7.2 (4h), 30.2 ± 3.2 (12h), and 5.5 ± 1.2 (24h). Phenytoin serum concentrations ($\mu\text{g/ml}$) increased from 20.4 ± 1.9 (1h) to 30.9 ± 1.2 (4h), persisting at 30.1 ± 3.3 (8h), and declining to 8.4 ± 3.2 (36h). Diazepam, levetiracetam, and valproate concentrations were highest one hour post-injection. Benzodiazepine concentrations (ng/ml) were 157.3 ± 13.8 (1h), 69.1 ± 7.2 (4h), 66.3 ± 18.3 (12h), 83.3 ± 41.1 (24h), and 26.9 ± 4.8 (36h). For levetiracetam, concentrations ($\mu\text{g/ml}$) post-injection were 127.4 ± 24.1 (1h), 23.3 ± 3.5 (4h), 0.8 ± 0.5 (12h), and 0.7 ± 0.9 (24h). Valproate serum concentrations ($\mu\text{g/ml}$) were 274.6 ± 37.9 (1h),

150.3 ± 14.4 (4h), 3.3 ± 1.0 (12h), and 0.2 ± 0.4 (24h) (Figure 1). Half-lives calculated from these serum concentrations were greater than their respective values in adult mice (Table 1).

Each drug had a different degree of penetration of the blood-brain barrier (Table 2). We found that four hours after intraperitoneal injection of these antiepileptic drugs, brain-to-blood ratios were comparable to data in adult rodents.

DISCUSSION

This study found that in comparison with adult mice, immature post-natal day 19 mice metabolized the five studied anticonvulsant drugs slower. In these mice, the anticonvulsants yielded longer half-lives and were at or above therapeutic plasma concentrations for extended periods. Human infants have been shown to be hypometabolic compared to adults [4]. Antiepileptic drugs are administered chronically to neonates presenting with seizures at doses designed to maintain therapeutic trough concentrations. We examined the feasibility of similar experimental dosing regimens that would accomplish this goal in immature CD1 mice. We found that while all drugs had longer half-lives, only diazepam, phenobarbital, and phenytoin could plausibly follow a clinically-relevant protocol with one or two injections daily. Diazepam with its metabolites had the greatest difference, with a half-life of 22.3 hours compared to 7.7 hours in adult mice [12].

Loscher [9] has previously discussed a three times daily injection regimen for controlling seizures in rodents, highlighting the caveat of a requisite half-life of at least 5 hours for the anticonvulsants. With higher dose administration and repeated dosing, a half-life of approximately 5 hours could possibly be achieved with levetiracetam and valproate in immature mice. Their half-lives are approximately 3 hours after a single dose, and the half-life of valproate is known to increase with repeated dosing in adult rodents. More work is planned with higher repeated doses to determine this.

Generally brain-to-blood ratios were comparable to those reported in mature mice. The immature blood-brain barrier develops throughout the post-natal period with maturation of the characteristic cerebral endothelial tight junctions [7,13–15]. Drug transporters develop concurrently. P-glycoprotein, a drug efflux transporter which acts on phenytoin, phenobarbital, and levetiracetam, is first detected in the rat brain at P7 and gradually increases to plateau at adult levels around P28, analogous to the developmental pattern within late gestational and neonatal humans [16,17]. The main factors for penetration of the blood-brain barrier in the mature brain are lipid solubility and the dissociation constant of the compound in question [18]. Therefore, ratios were not expected to vary widely between adults and the P19 immature mice. The discrepancy between the phenytoin ratios reported here and ratios previously published [19] can most likely be explained by the difference in dosing amounts, as phenytoin is known to accumulate in the brain. As diazepam and phenobarbital are very lipophilic, the increased amounts of lipids due to myelination in the adult brain could explain the greater concentrations of drug in the brain, and therefore higher brain-to-blood ratios, compared with the immature rodents.

CONCLUSION

This study demonstrates that a long-term clinical dosing strategy for anticonvulsants is feasible in immature CD1 mice. Antiepileptic drugs tested had longer half-lives and maintained therapeutic levels longer in the immature mice than in their mature counterparts. Blood-brain barrier penetration for all antiepileptic drugs in the study was similar to that reported in the mature rodent. Results from this study indicate that with a clinically-relevant

dosing protocol, therapeutic drug levels, in contrast to adult rodents, can be maintained in immature mice.

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Figure 1.

Pharmacokinetics of commonly used antiepileptic drugs in immature CD1 mice. Drug concentration data was averaged ($n=2-5$) and is displayed as mean $\pm$ S.D. as a function of time. Average concentrations of (a) phenobarbital after a single 60mg/kg i.p. injection; (b) phenytoin after a single 60mg/kg i.p. injection; (c) diazepam and its metabolites in the blood after a single 1mg/kg i.p. injection; (d) levetiracetam after a single 200mg/kg i.p. injection; and (e) valproate after a single 200mg/kg i.p. injection. Trends for all of the anticonvulsants are displayed together in (f); concentrations are in $\mu\text{g/ml}$, except for diazepam, which is in ng/ml .

Table 1

Half-lives and therapeutic concentrations of 5 commonly used antiepileptic drugs

Drug	T _{max} , hours	C _{max}	t _{1/2} , hours			Therapeutic Serum Concentrations	
			P19 CD1 mice	Mature mice, Strain	Humans	Rodents	Humans
Diazepam + metabolites	1	157.3 ± 13.8	22.3	7.7, CF-1	40 – 100	80 – 120	200 – 1000
Levetiracetam	1	127.4 ± 24.1	3.2	1.5, MRI	6 – 8	6 – 20	10 – 37
Phenobarbital	4	40.6 ± 7.2	15.8	7.5, NMRI	50 – 150	13 – 29	15 – 40
Phenytoin	4	28.7 ± 2.0	16.3	16, CFW	12 – 36	7 – 12	10 – 20
Valproate	1	274.6 ± 37.9	2.6	0.8, NMRI	8 – 15	240 – 270	50 – 100

Concentrations are in µg/ml except for diazepam, which is expressed in ng/ml. Half-lives for immature mice are compared to values in mature mice [11,12,20–22] and in humans [23]. Plasma concentrations reach therapeutic levels in rodents [9,24]; and are compared to human values [23].

Table 2

Brain-to-blood ratios after 4 hours in immature mice, compared to mature mice.

Drug	Average concentration of drug in brain, 4hr	Average concentration of drug in serum, 4hr	Ratio, Brain-to-Blood in P19 CD1 mice	Ratio, Brain-to-Blood in Mature Mice; Strain
Diazepam + metabolites	151.0 ± 33.5	85.1 ± 24.7	1.77	1.99; CF-1
Levetiracetam	40.9 ± 4.0	49.6 ± 5.2	0.82	0.8; NMRI
Phenobarbital	22.9 ± 1.9	39.6 ± 4.4	0.58	0.81; NMRI
Phenytoin	13.2 ± 3.8	24.2 ± 6.2	0.54	0.3; FVB
Valproate	17.0 ± 10.8	104.4 ± 36.7	0.16	0.2; NMRI

Concentrations are in µg/ml except for diazepam, which is expressed in ng/ml. Brain-to-blood ratios for immature mice are compared to mature mice [11,12,19,21,22].