Pharmaceutical Research, Vol. 22, No. 4, April 2005 (© 2005)
DOI: 10.1007/s11095-005-2491-0

Research Paper

Nipecotic Acid: Systemic Availability and Brain Delivery After Nasal Administration of Nipecotic Acid and *n*-Butyl Nipecotate to Rats

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Received November 18, 2004; accepted December 28, 2004

Purpose. The purpose of this research was to characterize nipecotic acid pharmacokinetics in blood and brain after intravenous (i.v.) and nasal administration of nipecotic acid and its *n*-butyl ester.
Methods. Nipecotic acid and its *n*-butyl ester were administered to rats i.v. and intranasally (n = 5 rats/drug per route), and nipecotic acid pharmacokinetics in blood were characterized. Nipecotic acid concentration-time profiles were determined in blood by noncompartmental and compartmental methods. Nipecotic acid was also dosed i.v. and its *n*-butyl ester was dosed by nasal and i.v. routes, and brain levels of nipecotic acid over the subsequent 4 h (n = 5 rats/time point per route) were assessed.
Results. The absolute systemic availability of nipecotic acid after nasal dosing was 14%. After i.v. and nasal dosing of the *n*-butyl ester, nipecotic acid systemic availability was 97% and 92%, respectively. Both i.v. and nasal administration of the *n*-butyl ester resulted in a significantly longer terminal half-life and larger mean resident time and volume of distribution for nipecotic acid than was observed after an i.v. nipecotic acid dose. Total brain exposure to nipecotic acid was not significantly different after nasal and i.v. dosing of the *n*-butyl ester. However, the brain/blood nipecotic acid ratio declined significantly with time after i.v. and nasal dosing of the ester prodrug. Nipecotic acid was not detectable in brain after i.v. dosing of nipecotic acid.
Conclusions. The use of an ester formulation was crucial to delivering nipecotic acid to the brain. Preliminary evidence strongly suggests ester hydrolysis is rate limiting to nipecotic acid brain delivery. Once nipeoctic acid was formed, it displayed tissue trapping in brain. Parenteral dosing of nipecotic acid esters is unnecessary for systemic or brain delivery of nipecotic acid and possibly other CNS active zwitterion esters.

KEY WORDS: anticonvulsant; brain hydrolysis; GABA reuptake; tissue trapping.

INTRODUCTION

More than 30% of epileptic patients experience at least one uncontrolled seizure per year; usually in outpatient settings (1). Prodromal patients have no recourse but to simply let the seizure take its course. Those patients who develop status epilepticus must wait until they can be treated by i.v. administration of an anticonvulsant by a trained health care worker. A simple, rapidly active anticonvulsant treatment that could be administered in an outpatient setting either by the patient or those around them might offer these patients greater hope for a more normal lifestyle and some added protection from more serious brain damage (2–3).

Nasal drug administration might provide an alternative to i.v. dosing for rapid delivery of anticonvulsants to the brain. Although nasal therapy is limited, it remains an important treatment niche (4,5). The ideal candidate for nasal therapy is one that satisfies some of the following characteristics: 1) rapid systemic delivery of a drug; 2) equivalence or near equivalence to i.v. dosing when i.v. administration is the only other effective means for drug administration; and 3) a unique therapeutic benefit, unattainable by any other less invasive route for drug dosing. Nasal products also should exhibit some practical physicochemical properties: 1) excellent water and good lipid solubility, and 2) good stability/shelf-life. A previous effort to assess nasal anticonvulsant administration was hampered by selection of oral anticonvulsant agents that were not the most optimal candidates for nasal dosing based on these criteria (6).

Many anticonvulsants work by elevating gamma amino butyric acid (GABA; a major inhibitory neurotransmitter) in the brain. *Rac*-nipecotic acid (Fig. 1) is a specific inhibitor of the GABA reuptake pump in brain, but it is frankly a lousy drug. Nipecotic acid does not penetrate the blood-brain barrier easily due to its polarity even if given intravenously (7–13). To overcome this delivery problem, the pharmaceutical industry has used more lipid-soluble forms of nipecotic acid. Lipophilic analogues of nipecotic acid, like Tiagabine, (14–16) have been synthesized by adding very large bulky groups on the nitrogen atom of nipecotic acid, to improve liphophilicity and generate a more stable, orally effective anticonvulsant. The disadvantages of that approach, however, are the slower onset of drug action, the greater dependence on metabolism for elimination, the associated potential for drug-drug inter-

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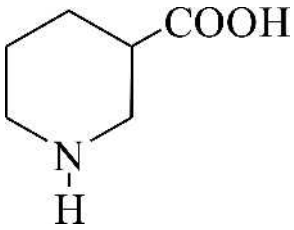

Fig. 1. Structure of nipecotic acid.

actions created by metabolism, and the possibility for broader binding to less desirable targets by either the drug and/or its liphophilic metabolite(s).

Nipecotic acid lipophilicity can also be enhanced by addition of alkyl groups to the carboxylic acid to form nipecotic acid ester prodrugs (7–13). The delivery of nipecotic acid to the brain is improved by parenteral administration of these prodrugs. Unfortunately, these esters are metabolized so rapidly in the body that parenteral dosing is the only means of ensuring their effective delivery to the brain. Injection of drugs has always limited their practical utility to extreme medical conditions due to the dependence on skilled health care workers, the pain associated with drug injections, and the risk for infection with i.v. dosing. In spite of years of studying ester prodrugs of nipecotic acid (7–13), none have ever evolved into serious marketable therapeutic agents.

Nasal administration of CNS active esters of zwitterions like nipecotic acid, however, has never been reported. An simple *n*-butyl ester of nipecotic acid has a shelf-life in a solution tolerated by the nasal cavity of 2 ± 1 years (17). This ester also has good water and lipid solubility with a log (octanol/water) partition coefficient (P) = 0.93 ± 0.10, vs. a P = 0.0060 ± 0.0002 for nipecotic acid (17). Simple esters of nipecotic acid have also been reported to be active anticonvulsants but only after subcutaneous and, to a lesser extent intraperitoneal administration to mice and rats (7–10). The purpose of this study was to assess nipecotic acid systemic and brain availability in rats after the intravenous and nasal administration of nipecotic acid and one of its stable esters, *n*-butyl nipecotate.

MATERIALS AND METHODS

Materials

Rac-Nipecotic acid was obtained from Sigma (St. Louis, MO, USA). Sodium pentobarbital (50 mg/ml) was obtained from Abbott Labs (North Chicago, IL, USA). Saline (0.9%, injectable) was purchased from Baxter Healthcare Corp. (Deerfield, IL, USA). Butyl alcohol, bis(trimethylsily)trifluoroacetamide (BSTFA; derivatization grade), and trifluroacetic acid anhydride (99+%) were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). Waters Oasis MCX LP extraction columns (3 cc, 60 mg) were purchased from Waters Corporation (Milford, MA, USA). Heparinized caraway capillary tubes were purchased from Baxter Healthcare Corporation (McGraw Park, IL, USA). Polyethylene tubing (I.D. 0.58 mm) was obtained from the Becton-Dickinson Company (Sparks, MD, USA), and silastic tubing (I.D. 0.50 mm) was obtained from Dow Corning Corporation (Midland, MI, USA). A Hamilton syringe 10–100 μl was purchased from Waters Corporation. A 200-μl positive displacement pipette and pipette tips were purchased from Wheaton (Midville, NJ, USA).

Synthesis of Nipecotic Acid *n*-Butyl Ester

The *n*-butyl ester of nipecotic acid was prepared according to a previously reported method (7). The crystals collected were filtered and recrystallized from an acetone-petroleum ether mix (1/5, v/v). The structure and purity of the final hydrochloride salt of the *n*-butyl nipecotic acid ester was confirmed by NMR spectra (in $CDCl_3$–δ-9.66, 1H, s; δ-4.08, 2H, t; δ-3.51, 2H, dd; δ-2.90, 3H, m; δ-2.07, 3H, m; δ-1.98, 1H, s; δ-1.57, 3H, m; δ-1.34, 2H, m; δ-0.90, 3H, t), GC-MS (single peak and molecular ion; m/z 185), melting point (70–73°C), and elemental analysis (C 54.17%; H 9.09%; N 6.32%; O 14.43%).

Animals

Institutional animal care and use committee (IACUC) approval was obtained prior to the initiation of this research and during its execution. Male Sprague-Dawley rats weighting 250–300 g (Harlan Sprague Dawley, Inc., Indianapolis, IN, USA) were used throughout this research effort.

Animal Model and Operation

A previously described *in vivo* experimental technique for nasal drug administration was used for this study (18). Identical surgical procedures were performed on all rats, and animals remained under anesthesia by intraperitoneal injection of pentobarbital (100 mg/kg) for the duration of these studies. Two cannulae were implanted in the rat and exteriorized for attachment to a syringe (19–20). One cannula was inserted into the jugular vein for i.v. drug dosing, and a second cannula was inserted into the femoral vein and threaded into the inferior vena cava for purposes of blood sampling. A tracheotomy was performed on each rat and the nasal cavity sealed.

Drug Administration and Sample Collection

Nipecotic acid and the *n*-butyl ester prodrug solutions were prepared fresh at the appropriate concentration with 0.05 M phosphate buffer (pH 6.0). For nasal administration, 50 μl/300 g rat of drug solution was placed in the nostril (25 μl/each nostril) with a microsyringe. For intravenous administration, a sterile drug solution was prepared by filtration (0.2-μm filter) and injected into the jugular vein cannula followed by a 0.2–0.3 ml flush with sterile saline.

Nipecotic acid (10 mg/kg dose) was administered intravenously, and a 40 mg/kg dose was given intranasally (5 rats/route) in random order for assessing nipecotic acid pharmacokinetics. In a separate experiment, nipecotic acid *n*-butyl ester (5.8 mg/kg nipecotic acid equivalent dose) was administrated i.v. or intranasally (5 rats/route) in random fashion to define nipecotic acid pharmacokinetics. For brain disposition studies, 10 mg/kg of nipecotic acid was administered i.v., and the ester dose was reduced to a 4.6 mg/kg nipecotic acid dose equivalent. Rats were sacrificed at preselected times according to a separate random study design. Preliminary work was carried out in a small group of rats to initially define the duration of blood sampling after each drug and the dose that

produced a readily measured nipecotic acid blood level. The pharmacologic activity of the ester prodrug (respiratory depression) prevented the use of a larger dose equivalent of nipecotic acid in anesthetized rats and was also responsible for lowering the ester dose used for brain disposition work.

Blood samples (200 μl) were collected at time points: 0, 1, 3, 5, 10, 20, 30, 40, 60, 120, 240, and 360 min after the administration of nipecotic acid, and at the continuing time points of 480 and 600 min after administration of the *n*-butyl ester for the initial pharmacokinetic studies. Blood samples were immediately extracted and stored at –20°C, until analyzed (21). Rats were sacrificed at 5, 10, 20, 30, 60, 120, 180, and 240 min after nasal and i.v. administration of the *n*-butyl ester. Fewer time points than initially planned were evaluated after i.v. administration of nipecotic acid because of an inability to detect any nipecotic acid in those rat brains. Blood and brain (minus olfactory bulb) were immediately collected from each rat (n = 5 rats per time point) at the time of sacrifice. Blood was processed as previously described (21). Brain was cleaned of surrounding blood vessels, washed, weighed, and homogenized in 4 parts phosphate buffer to 1 part brain tissue. Duplicate 200 μl aliquots of brain homogenate were assayed for nipecotic acid using the same assay used for the blood (21).

In Vitro Rat Brain Homogenate Hydrolysis Rate of the *n*-Butyl Ester

Healthy rats were killed, their brains excised, blood vessels and tissue surrounding the brain removed, the brain washed, weighed, and homogenized in 2 volumes of phosphate buffer (0.05M, pH 7.4) to 1 g of brain. Final dilutions of this homogenate (½; ⅓; ¼; 1/5; 1/7.5; 1/10) were used to assess the linearity in nipecotic acid formation from the ester (50 μg/100 μl) as a function of brain homogenate (900 μl) concentration (total volume 1 ml). Samples were incubated for 30 min at 37°C and processed as before to assess nipecotic acid formation.

Fresh rat brain was obtained and diluted to a final brain homogenate concentration of 1 part brain/4 parts buffer and used to assess the linearity in nipecotic acid formation with time. Ester (50 μg/100 μl) was added to 900 μl of brain homogenate and incubated for 5, 10, 20, 30, and 60 min at 37°C before processing to assess nipecotic acid formation with time of incubation.

The maximal rate for ester brain hydrolysis was assessed by taking 5 fresh rat brains and incubating the brain homogenate for 5 min at 37°C with 200 μg/100 μl of the *n*-butyl ester. Samples were immediately processed and assayed for nipecotic acid formation.

Data Analysis

Concentration-time profiles of nipecotic acid after i.v. administration of nipecotic acid were evaluated by a noncompartmental method and also fit to a two-compartment model with i.v.-bolus input. The blood concentration-time profiles of nipecotic acid after nasal administration of nipecotic acid to each rat were evaluated by a noncompartmental method with extravascular input and also fit to a two-compartment model with first-order and zero-order inputs by the same program. The blood concentration-time profiles of nipecotic acid after i.v. and nasal administration of the *n*-butyl ester were only evaluated by a noncompartmental method with intravascular and extravascular input, respectively, due to the complex nature of the nipecotic acid blood concentration-time profile (Fig. 2B).

The terminal half-life of nipecotic acid ($t_{½}$) was calculated by dividing 0.693 by the terminal rate constant (β) obtained from the fitted concentration-time data. Nipecotic acid's area under the blood concentration time (AUC) profile was determined by the linear-trapazoidal method prior to peak drug levels and the log-trapazoidal method after peak drug levels with extrapolation to infinite time (C_{last}/β). The area under the first moment curve (AUMC) for nipecotic acid was determined by the linear-trapezoidal method with extrapolation to infinite time (C_{last}/β^2). The systemic clearance (Cl) after the intravenous nipecotic acid dose was determined by Eq. (1):

$$Cl = Dose/AUC^{0-\infty} \quad (1)$$

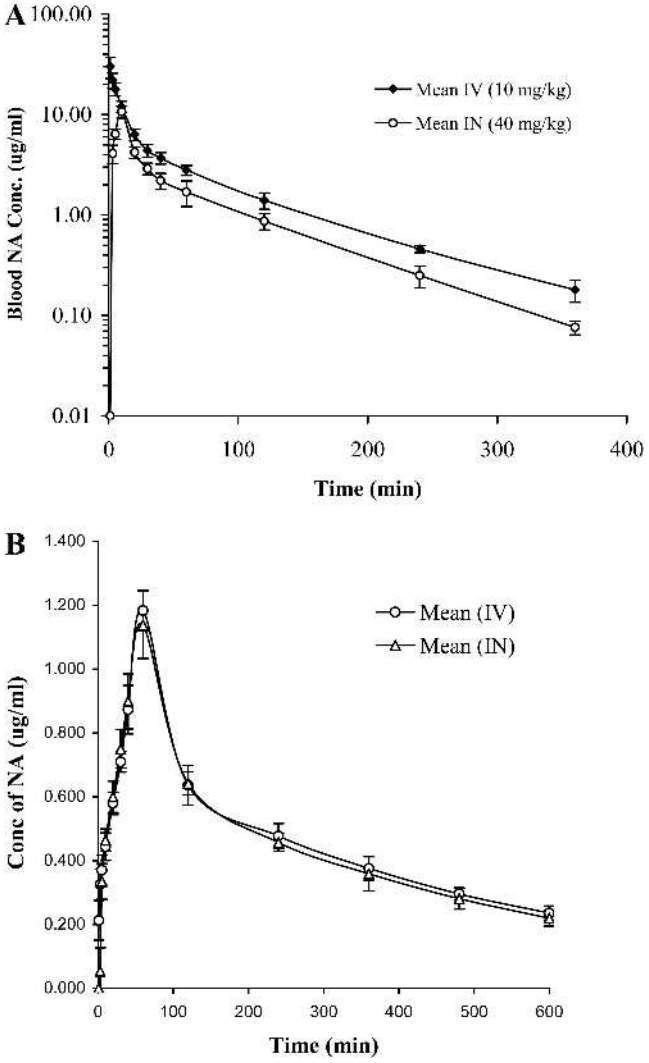

Fig. 2. (A) Blood nipecotic acid levels after intravenous (10 mg/kg) and nasal administration (40 mg/kg) of nipecotic acid (n = 5). (B) The nasal (IN) and intravenous (IV) profiles of nipecotic acid after a 5.8 mg/kg nipecotic acid equivalent dose of the butyl ester (n = 5 rats/route). All values show the mean ± SD of the mean value.

The volume of distribution at steady state (V_{SS}) for the intravenous nipecotic acid was determined from the Eq. (2):

$$V_{SS} = \text{Dose} \times \text{AUMC}/(\text{AUC})^2 \quad (2)$$

The systemic availability (F) was determined from Eq. (3):

$$F_1 = (\text{Dose}_{iv,NA} \times \text{AUC}_1)/(\text{Dose}_1 \times \text{AUC}_{iv,NA}) \quad (3)$$

where AUC_1 and $Dose_1$ represent the $AUC^{0-\infty}$ and total dose of nipecotic acid expressed in dose equivalents after the nasal nipecotic acid and intravenous and nasal *n*-butyl nipecotic acid doses. Statistical analysis was performed by a two-tailed Student's *t* test with the significance level set at $\alpha = 0.05$.

The concentration-time profiles of nipecotic acid in brain homogenate after iv and nasal ester administration were converted to brain amount-time profiles. The amount-time profiles were then analyzed by a non-compartmental model with extravascular input and fit to a two-compartment model with first-order input. Brain and blood concentration-time points and the brain/blood ratio at each time point were compared by a two-way ANOVA with $\alpha = 0.05$. All pharmacokinetic parameters were fit using the WinNonlin program (Pharsight Corp., Cary, NC, USA).

RESULTS

Pharmacokinetics of Nipecotic Acid After Intravenous and Nasal Administration

The mean ± SD blood levels (n = 5 rats/route) of nipecotic acid after its intravenous (10 mg/kg) and nasal (40 mg/kg) administration are shown in Fig. 2A. The concentration-time profiles were analyzed by a noncompartmental method with appropriate input, and the pharmacokinetic parameters determined from that noncompartmental approach. The AUCs were 720 ± 80 μg min ml^{-1} for the intravenous dose and 390 ± 60 μg min ml^{-1} for the nasal dose. The dose corrected bioavailability after nasal administration was an estimated 14%. The terminal half-life of nipecotic acid was similar after i.v. (75 ± 11 min) and nasal (71 ± 7 min) dosing. Table I summarizes the relevant pharmacokinetic parameters after both i.v. and nasal administration of nipecotic acid after correction for differences in the dose administered.

Table I. Mean Pharmacokinetic Parameters of Nipecotic Acid After i.v. and Nasal Administration (n = 5 Rats/Route of Administration)

		i.v.		Nasal		
Parameters	Unit	Mean	±SD	Mean	±SD	p value[a]
V_c	ml	93	26	86	21	0.47
$t_{1/2}$	min	75	11	71	7	0.38
AUC_{inf}	min μg/ml	720	80	98[b]	15	**0.0005**
V_z	ml	550	110	450	96	0.13
Cl	ml/min	4.2	0.5	4.4	0.7	0.48
MRT_{inf}	min	82	9	78	3	0.21
V_{ss}	ml	349	75			
F	%	100		14		

[a] Two-tailed *t* test: The only significant difference found was for the dose-corrected area under the concentration-time profiles after intravenous and nasal doses of nipecotic acid.
[b] Dose corrected AUC = 391/4.

Pharmacokinetics of Nipecotic Acid After *n*-Butyl Ester Intravenous and Nasal Dosing

The mean ± SD blood levels for nipecotic acid following intravenous and nasal administration of the *n*-butyl ester (5.8 mg/kg nipecotic acid dose equivalents) are shown in Fig. 2B (n = 5 rats/route). The peak level of nipecotic acid appears at 60 min. Analyzing the concentration-time profile in each rat by a noncompartmental method results in mean nipecotic acid AUCs of 410 ± 45 μg min ml^{-1} after i.v. and 390 ± 46 μg min ml^{-1} after the nasal ester dose. The absolute systemic availability of nipecotic acid after i.v. and nasal administration of the *n*-butyl ester was 97% and 92%, respectively. The relative systemic availability of nipecotic acid for nasal to i.v. *n*-butyl ester doses was 95%. Table II summarizes the pharmacokinetic parameters for nipecotic acid after both i.v. and nasal administration of the *n*-butyl ester. No significant difference was noted in any pharmacokinetic parameters of nipecotic acid after ester administration by nasal and i.v. routes.

Comparison of Pharmacokinetics of Preformed vs. Nipecotic Acid Formed *in Vivo*

A two-tailed *t* test compared differences between nipecotic acid half-lives, AUCs (corrected for dose), volume of distribution, clearance, and mean resident time for nipecotic acid administered i.v. vs. nipecotic acid formed from the i.v. *n*-butyl ester to assess their significance. The results are summarized in Table III. Nipecotic acid half-life and mean residence time were significantly longer and its volume of distribution significantly larger after administration of the *n*-butyl ester dose.

Nipecotic Acid Brain Levels After Nasal and Intravenous Administration of the Ester

Mean ± SD nipecotic acid brain concentrations (μg/g brain, excluding olfactory bulb) were not noticeably different after intravenous or nasal administration of the ester prodrug (Fig. 3). Peak brain levels of nipecotic acid were achieved in 10 min and fell below detectable levels after 3 h. Nipecotic acid brain levels could be fit to a two-compartment model with first-order input. The average pharmacokinetic param-

Table II. Comparison of Pharmacokinetic Parameters of Nipecotic Acid After i.v. and Nasal Administration of *n*-Butyl Ester (5.8 mg/kg Nipecotic Acid Equivalent Dose, n = 5 Rats/Route)

		i.v.		Nasal		
Parameters	Unit	Mean	SD	Mean	SD	p value[a]
T_{max}	min	60	0	60	0	—
C_{max}	ng/ml	1180	40	1140	40	0.10
$t_{1/2}$, terminal	h	6.2	1.4	5.9	1.6	0.35
AUC_{inf}	min μg/ml	410	45	390	46	0.22
V_z	ml	2150	270	2060	300	0.32
Cl	ml/min	4.1	0.5	4.1	0.4	1.00
MRT_{inf}	min	510	90	490	120	0.40
$F_{absolute}$	%	97		92		

[a] Two-tailed *t* test: There is no significant difference in any pharmacokinetic parameter after i.v. and nasal administration of the *n*-butyl ester.

Table III. Comparison of Pharmacokinetic Parameters of Nipecotic Acid After an i.v. Dose of Nipecotic Acid (NA; 10 mg/kg) and the *n*-Butyl Ester (B-NA; 5.8 mg/kg Nipecotic Acid Equivalent Dose, n = 5 Rats/Drug)

		Dosing NA		Dosing B-NA		
Parameters	Unit	Mean	SD	Mean	SD	p value[a]
$t_{1/2}$, terminal	min	75	11	370	84	**0.003**
AUC_{inf}/D	min μg/ml	420	44	410	45	0.42
V_z	ml	550	110	2150	270	**0.0005**
Cl	ml/min	4.2	0.5	4.1	0.5	0.38
MRT_{inf}	min	82	9	512	90	**0.0008**

[a] Two-tailed *t* test: Significant differences between these dosage forms were noted in the half-life, volume of distribution, and mean resident time of nipecotic acid.

eters obtained by fitting nipecotic acid brain concentrations over time are provided in Table IV. A two-way ANOVA found no significant difference in nipecotic acid brain concentrations with route of ester administration ($F_{0.05,1,56} = 0.50$, significant F = 4.02).

The brain/blood ratio of nipecotic acid after administration of the ester by the intravenous and nasal routes was similar at each time point (Table V). However, nipecotic acid brain/blood ratio declined with time. Intravenous administration of nipecotic acid did not result in a detectable brain concentration of the drug at any time point (Fig. 3).

Brain Hydrolysis of *n*-Butyl Nipecotic Acid Ester

The formation of nipecotic acid from the ester was linear in fresh rat brain homogenate at homogenate concentrations of up to 20% for a period of no more than 5 min. Under these conditions, it was not possible to quantify nipecotic acid formation over a range of ester concentrations for the purpose of estimating both V_{max} and K_m for brain ester hydrolysis. Thus,

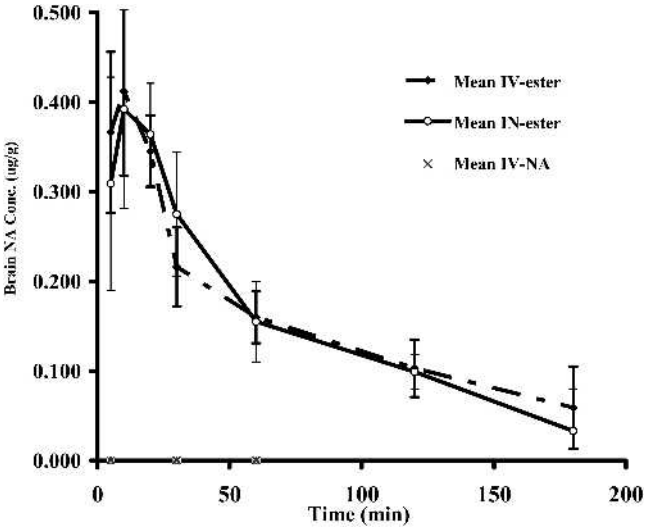

Fig. 3. Brain concentrations of nipecotic acid after an intravenous and nasal dose of the butyl ester (4.6 mg/kg nipecotic acid equivalent dose of the ester). Nipecotic acid was also administered by the intravenous and nasal routes but could not be detected in the brain at any time point (values are plotted on the *x*-axis). All values show mean ± SD of the mean value.

Table IV. Comparison of Brain Pharmacokinetic Parameters Based on Mean Brain Concentrations of Nipecotic Acid After *n*-Butyl Ester Dosing by the Nasal and Intravenous Routes

Parameter	Unit	i.v.	Nasal
V_{brain}	g	0.94	0.68
A_{max}	ng/g brain	413	392
$t_{1/2}$	min	83	75
$AUC^{0-\infty}$	min μg/g	36	31
Clearance	g brain/min	0.0174	0.0198
MRT	min	111	92

only the maximum rate of ester hydrolysis was determined which is assumed to represent $V_{max} = 47 \pm 31$ ng min^{-1} g^{-1} brain (n = 5 rats), as the amount of metabolite formed (90–300 ng) was far below the amount of ester added (20,000 ng) to brain incubates. This rate can be interpreted as the V_{max} if brain binding of the ester is not rate limiting to nipecotic acid formation, or as the maximal rate of hydrolysis under these incubation conditions if binding is rate limiting for ester hydrolysis. We cannot distinguish between these two possibilities.

DISCUSSION

The absolute systemic availability of nipecotic acid was enhanced substantially by its administration as a nasal *n*-butyl ester (92%) vs. nasal nipecotic acid (14%). This indicates absorption from the nasal cavity was significantly improved by administrating nipecotic acid as an ester prodrug than as the more polar nipecotic acid. The systemic concentration-time profiles for nipecotic acid after nasal and intravenous administration of the ester prodrug were almost superimposable (Fig. 2B). This indicates a nasal nipecotic acid ester formulation is effective in delivering nipecotic acid to the blood. The less invasive nasal administration of nipecotic acid esters may represent a more optimal approach for nipecotic acid systemic delivery. The *n*-butyl ester disappeared from blood and brain rapidly and fell below 100 ng/ml or 100 ng/g brain after 10 min. The analytical method was never optimized to follow the ester in biological fluids or tissues because it was always assumed the prodrug ester would disappear rapidly *in vivo*.

Nasal and intravenous *n*-butyl nipecotic acid resulted in the death of rats when the nipecotic acid dose equivalents approached the dose used for intravenous nipecotic acid administration (10 mg/kg). In all cases, rats died of respiratory arrest 10–15 min after *n*-butyl nipecotic acid administration.

Table V. Brain-to-Blood Ratio (ng/g Tissue to ng/m Blood) for Nipecotic Acid After Intravenous (i.v.) and Nasal Administration of the *n*-Butyl Ester (4.6 mg/kg Nipecotic Acid Equivalents; n = 3 Animals per Time Point)

Time (min)		5	10	20	30	60	120	180
i.v.	Brain/blood	1.23	1.17	0.78	0.45	0.17	0.20	0.11
	± SD	0.21	0.16	0.13	0.05	0.03	0.05	0.10
Nasal	Brain/blood	1.11	1.06	0.88	0.51	0.18	0.18	0.08
	± SD	0.31	0.22	0.16	0.04	0.04	0.05	0.07

Differences between the nasal and i.v. dose were not significant based on a two-way ANOVA, with $\alpha = 0.05$, $p = 0.27$.

This adverse outcome and the timing of its onset was unaffected by the route of ester administration. The effect from the ester on respiration in anesthetized rats was so problematic it was necessary to allow the rat limited recovery from surgical anesthesia prior to administering the ester. Acute dosing of the ester returned the rat to a state of surgical anesthesia within 10 min after both nasal and intravenous administration.

The timing of this pharmacological effect correlates well with peak levels of nipecotic acid in brain, but not blood. Peak blood levels of nipecotic acid were not reached until 1 h after administration of the *n*-butyl ester (Fig. 2B). Nipecotic acid is a potent inhibitor of the GABA reuptake pump, and GABA is a major inhibitory neurotransmitter in the brain. Esters of nipecotic acid do not bind to the GABA reuptake pump and should not result in respiratory inhibition. Moreover, the effect appears more closely correlated with peak nipecotic acid appearance in brain, not peak *n*-butyl ester brain levels. No rats ever died from nipecotic acid injected intravenously, even when the nipecotic acid dose was twice the amount used in this study (20 mg/kg). This observation no doubt reflects the poor penetration of iv administered nipecotic acid across the blood brain barrier (Fig. 3).

The extremely slow time to reach a peak nipecotic acid blood concentration was unexpected based on the rapid disappearance of the *n*-butyl ester from blood and brain (below 100 ng/ml after 10 min). There are two possible reasons for the delay in peak nipecotic acid blood concentrations. One possibility is the ester is rapidly distributed and tightly bound to tissues that slowly release the ester into blood where it is rapidly hydrolyzed to nipecotic acid. Alternatively, the *n*-butyl nipecotic acid ester may be rapidly distributed and hydrolyzed throughout the body, but nipecotic acid formed in tissues is trapped and only slowly transported/released back into blood. Tissue trapping has been reported for similar types of polar metabolites (22–24) and could explain the complex concentration-time profile for nipecotic acid seen after administration of the *n*-butyl ester. The longer terminal half-life (5.9 h vs. 1.2 h), larger volume (2200 ml vs. 550 ml) and longer mean residence time (8.5 h vs. 1.4 h) for nipecotic acid formed *in vivo* vs. preformed nipecotic acid (Table III) are consistent with a drug affected by tissue trapping (22–24). This hypothesis is further supported by the declining brain/blood ratio of nipecotic acid seen after administration of the ester (Table IV). For lipophilic drugs, a constant tissue/blood ratio is expected after drug distribution. A declining tissue/blood ratio indicates drug movement between tissue and blood is not a passive, but rather a restricted process.

Total brain levels of nipecotic acid were not significantly different whether the ester prodrug was administered by the nasal or intravenous routes. The olfactory bulb was purposely eliminated from these brain measurements to avoid biasing the outcome due to an expected higher olfactory bulb nipecotic acid concentrations after nasal dosing (25–29). Drug delivery to the olfactory bulb would not be anticipated to provide any substantial anticonvulsant protection. The similarity in nipecotic acid total brain concentrations was somewhat surprising since the earliest measured *n*-butyl ester concentrations in brain tissue were actually higher after the i.v. ester (3.2 μg/g brain) compared with the nasal ester dose (1.9 μg/g brain). However, the maximal rate of ester hydrolysis in total rat brain homogenate studies suggests nipecotic acid formation is extremely slow (assumed V_{max} = 47 ng min^{-1} g^{-1} brain). If the *n*-butyl ester can be hydrolyzed at only a maximal rate of 47 ng min^{-1} g^{-1} brain tissue, and ester concentrations fall from over 1 μg/g brain to below 100 ng/g total brain after 10 min, nipecotic acid formation (assuming a zero-order process over the first 10 min) would quickly decline after 10 min as ester levels become undetectable. Peak brain nipecotic acid levels are estimated to reach about 470 ng/g brain by 10 min assuming a zero-order rate process based on *in vitro* brain homogenate data. Actual peak nipecotic acid brain levels averaged 413 and 392 ng/g brain at 10 min after i.v. and nasal administration of the ester, respectively. The total brain levels of ester and nipecotic acid suggest the ester prodrug distributes into and then out of the brain with only a portion of the ester actually converted to nipecotic acid in brain. The similarity in total brain nipecotic acid levels with route of ester administration is not really due to equivalence in brain delivery of the ester prodrug but rather appears to result from an ester hydrolysis in rat brain that is rate limiting for nipecotic acid brain delivery.

The terminal rate constant for nipecotic acid disappearance from brain tissue was 0.008–0.009 min^{-1}. It is notable the rate constant for GABA elimination from the brain is 0.0714 min^{-1} (30).

This implies GABA may be transported with an efficiency about 10 times greater than that of nipecotic acid, assuming a similar volume of distribution for both zwitterions.

CONCLUSIONS

Nasal dosing of the *n*-butyl ester of nipecotic acid provides a viable approach for delivering nipecotic acid zwitterions to the brain. A pharmacological response (rats stopped breathing and died) was observed only after dosing the ester, but not after i.v. nipecotic acid administration. There was no significant difference in nipecotic acid total brain levels after i.v. or nasal ester dosing, suggesting the nasal route is as effective as the i.v. route for delivery of nipecotic acid to the total brain via this ester. There was strong evidence to suggest nipecotic acid formed *in vivo* from the ester may undergo tissue trapping. Tissue trapping in the brain may provide a unique advantage for esters of CNS active zwitterions by providing a mechanism for prolonged residence of the pharmacologically active zwitterions in brain tissue.

REFERENCES

1. R. J. Porter. Therapy of epilepsy. *Curr. Opin. Neurol. Neurosurg.* **1**:206–211 (1988).
2. G. A. Baker, A. Jacoby, D. Buck, C. Stalgis, and D. Monnet. Quality of life of people with epilepsy: a European study. *Epilepsia* **38**:353–362 (1997).
3. A. C. Hui, G. M. Joynt, H. Li, and K. S. Wong. Status epilepticus in Hong Kong Chinese: aetiology, outcome and predictors of death and morbidity. *Seizure* **12**:478–482 (2003).
4. L. Illum. Nasal drug delivery – possibilities, problems and solutions. *J. Control. Rel.* **87**:187–198 (2003).
5. A. Hussain. Intranasal drug delivery. *Adv. Drug Deliv. Rev.* **29**: 39–49 (1998).
6. L. Li, S. Gorukanti, Y. M. Choi, and K. H. Kim. Rapid-onset intranasal delivery of anticonvulsants: pharmacokinetic and pharmacodynamic evaluation in rabbits. *Int. J. Pharm.* **199**:65–76 (2000).

7. A. M. Crider, T. T. Tita, J. D. Wood, and C. N. Hinko. Esters of nipecotic acid and isonipecotic acids as potential anticonvulsants. *J. Pharm. Sci.* **71**:1212–1219 (1982).
8. A. M. Crider, J. D. Wood, K. D. Tschappat, C. N. Hinko, and K. Seibert. Gamma-aminobutyric acid uptake inhibition and anticonvulsant activity of nipecotic acid esters. *J. Pharm. Sci.* **73**: 1612–1616 (1984).
9. C. N. Hinko, A. M. Crider, and J. D. Wood. A comparison of prodrug esters of nipecotic acid. *Neuropharmacol* **27**:475–483 (1988).
10. C. N. Hinko, K. Seibert, and A. M. Crider. Anticonvulsant activity of the nipecotic acid ester, (+/−)-n-nitrophenyl-3-piperidinecarbooxylate. *Neuropharmacol.* **23**:1009–1014 (1984).
11. S. Manfredini, B. Pavan, S. Vertuani, M. Scaglianti, D. Compagnone, C. Biondi, A. Scatturin, S. Tanganelli, L. Ferraro, P. Prasad, and A. Dalpiaz. Design, synthesis and activity of ascorbic acid prodrugs of nipecotic, kynurenic and diclophenamic acid, liable to increase neurotropic activity. *J. Med. Chem.* **45**:559–562 (2002).
12. H. H. Frey, C. Popp, and W. Loscher. Influence of inhibitors of the high affinity GABA uptake on seizure thresholds in mice. *Neuropharmacol.* **18**:581–590 (1979).
13. F. P. Bonina, L. Arenare, F. Palagiano, A. Saija, F. Nava, D. Trombetta, and P. de Caprariis. Synthesis, stability, and pharmacological evaluation of nipecotic acid prodrugs. *J. Pharm. Sci.* **88**:561–567 (1999).
14. L. J. S. Knutsen, K. E. Andersen, J. Lau, B. F. Lundt, R. F. Henry, H. E. Morton, L. Naerum, H. Petersen, H. Stephensen, P. D. Suzdak, M. D. B. Swedberg, C. Thomsen, and P. O. Sorensen. Synthesis of novel GABA uptake inhibitors. 3. Diaryloxime and diarylvinyl ether derivatives of nipecotic acid and guvacine as anticonvulsant agents. *J. Med. Chem.* **42**:3447–3462 (1999).
15. K. E. Andersen, J. L. Sorensen, J. Lau, B. F. Lundt, H. Petersen, P. O. Huusfeldt, P. D. Suzdak, and M. D. B. Swedberg. Synthesis of novel γ-aminobutryic acid (GABA) uptake inhibitors. 5. Preparation and structure-activity studies of tricyclic analogues of known GABA uptake inhibitors. *J. Med. Chem.* **44**:2152–2163 (2001).
16. K. E. Andersen, J. Lau, B. F. Lundt, H. Petersen, P. O. Huusfeldt, P. D. Suzdak, and M. D. B. Swedberg. Synthesis of novel GABA uptake inhibitors, Part 6: preparation and evaluation of N-Ω asymmetrically substituted nipecotic acid derivatives. *Biorganic Med. Chem.* **9**:2773–2785 (2001).
17. H. Wang. 2003. The brain delivery of nipecotic acid by the administration of its ester prodrug via the nasal route: Physicochemical Properties. Ph.D. Thesis, University of Kentucky, Lexington, KY, pp. 71–85.
18. S. Hirai, T. Yashika, T. Matsuzawa, and H. Mima. Absorption of drugs from the nasal mucosa of rat. *Int. J. Pharm.* **7**:317–325 (1981).
19. P. J. Wedlund, S. L. Chang, and R. H. Levy. Steady-state approach to examination of pulmonary metabolism: application to carbamazepine. *J. Pharm. Sci.* **2**:860–862 (1983).
20. S. L. Chang, K. Emmick, and P. J. Wedlund. Characterization of antipyrine autoinduction in rat utilizing a new microsampling technique for serial blood sample collection. *J. Pharm. Sci.* **74**: 456–458 (1986).
21. H. Wang, A. A. Hussain, J. S. Pyrek, J. Goodman, and P. J. Wedlund. Assay for nipecotic acid in small blood samples by gas chromatography-mass spectroscopy. *J. Pharm. Biomed. Anal.* **34/35**:1063–1070 (2004).
22. K. S. Pang, W. F. Cherry, J. A. Terrell, and E. H. Ulm. Disposition of enalapril and its diacid metabolite, enalaprilat, in a perfused rat liver preparation. Presence of a diffusional barrier for enalaprilat into hepatocytes. *Drug Metab. Dispos.* **12**:309–313 (1984).
23. T. Haradahira, M. Maeda, A. Kato, Y. Kanazawa, M. Yamada, Y. Torii, Y. Ichiya, and K. Masuda. Metabolic pathway of 2-deoxy-2-[18F]fluoro-D-talose in mice: trapping in tissue after phosphorylation by galactokinase. *Nucl. Med. Biol.* **21**:269–276 (1994).
24. A. M. Evans. Membrane transport as a determinant of the hepatic elimination of drugs and metabolites. *Clin. Exp. Pharmacol. Physiol.* **23**:970–974 (1996).
25. H. D. Kao, A. Traboulsi, S. Itoh, L. Dittert, and A. Hussain. Enhancement of the systemic and CNS specific delivery of L-DOPA by the nasal administration of its water soluble prodrugs. *Pharm. Res.* **17**:978–984 (2000).
26. T. C. A. Kumar, G. F. X. David, A. Sankaranarayanan, V. Puri, and K. R. Sundram. Pharmacokinetics of progesterone after its administration to ovariectomized rhesus monkeys by injection, infusion, or nasal spray. *Proc. Natl. Acad. Sci. USA* **79**:4185–4189 (1982).
27. A. Czerniawska. Experimental investigations on the penetration of ^{198}Au from nasal mucous membrane into cerebrospinal fluid. *Acta Otolaryng.* **70**:58–60 (1970).
28. T. Sakane, M. Akizuki, M. Yoshida, S. Yamashita, T. Nadai, M. Hashida, and H. Sezaki. Transport of cephalexin to the cerebrospinal fluid directly from the nasal cavity. *J. Pharm. Pharmacol.* **43**:449–451 (1991).
29. H. H. S. Chow, Z. Chen, and G. T. Matsuura. Direct transport of cocaine from the nasal cavity to the brain following intranasal cocaine administration in rats. *J. Pharm. Sci.* **88**:754–758 (1999).
30. B. A. Kakee, A. H. Takanaga, H. T. Terasaki, T. M. Naito, M. T. Tsuruo, and Y. Sugiyama. Efflux of a suppressive neurotransmitter, GABA, across the blood-brain barrier. *J. Neurochem.* **79**: 110–118 (2001).