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Pharmacokinetics of sodium phenylacetate and sodium benzoate following intravenous administration as both a bolus and continuous infusion to healthy adult volunteers

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Abstract

Background: Ammunol® (sodium phenylacetate/sodium benzoate) is an intravenously administered, investigational drug used for the treatment of acute hyperammonemia in infants, children, and adults with urea cycle enzyme deficiencies. A pharmacokinetic study of sodium phenylacetate/sodium benzoate (NAPA/NABZ) was performed in two groups of normal healthy volunteers, following the dosing regimen used to treat hyperammonemia.

Methods: The first group of subjects ($n = 3$) received a bolus dose of 5.5 g/m^2 of NAPA/NABZ, over a period of 1.5 h. Following a seven-day washout, subjects then received the same bolus dose, followed by a continuous infusion of 5.5 g/m^2 over 24 h. A second group of different subjects ($n = 17$) received the same treatment regimen, but using doses of 3.75 g/m^2. Phenylacetate (PA) and benzoate (BZ), and their respective metabolites, phenylacetylglutamine (PAG), and hippurate (HIP) were measured over a 24-h period. An HPLC method was used for the measurement of all analyte concentrations. Non-compartmental analysis and modeling was performed using WinNonlin Professional®.

Results: Both BZ and PA displayed saturable, non-linear elimination, with a decrease in clearance with increased dose. During the bolus dose with continuous infusion regimen, plasma levels of both BZ and PA peaked at the end of the priming dose, and PA levels remained near peak for 5–9 h. In contrast, BZ plasma levels immediately fell following the priming dose, and became undetectable at 14.1 ± 4.2 and 26.8 ± 2.3 h in the low- and high-dose group, respectively. The formation of HIP occurred more rapidly than that of PAG. For both PA and BZ, metabolite formation increased in a linear fashion with the dose.

Conclusion: These data describe the pharmacokinetics of PA and BZ, and their respective metabolites, as observed in healthy adult volunteers, with the higher dose studied equivalent to that used to treat hyperammonemia. Dose optimization is required to maximize nitrogen removal, while minimizing the risk of toxicity, especially due to PA. Because of the slower elimination of PA, and the non-linear pharmacokinetic behavior displayed by both PA and BZ, only investigational protocol-specific doses should be used, and higher doses should be avoided unless blood level monitoring can be done promptly and frequently.

Keywords: Ammonul®; Pharmacokinetics; Sodium phenylacetate; Sodium benzoate; Intravenous administration; Hyperammonemia; Alternative pathway therapy; Urea cycle; Adult volunteers

Introduction

The investigational combination drug product (Ammonul®) containing 10% sodium phenylacetate (NAPA) and 10% sodium benzoate (NABZ) has been used for the intravenous treatment of acute hyperammonemia in patients with urea cycle enzyme deficiencies since the mid-1980s. The product is diluted in 10% dextrose and administered as a 90-min bolus dose infusion, followed by a 24-h maintenance dose. Both NAPA and NABZ remove nitrogen via alternate non-urea cycle enzymatic pathways, and are the only intravenous ammonia scavenger agents currently available for clinical use.

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In patients with limited or no urea synthesizing capacity, acute hyperammonemia is a medical emergency that can require multiple medical interventions depending on severity, and including, dialysis, administration of ammonia scavenger agents and urea cycle intermediates (arginine or citrulline), protein restriction and high caloric intake to counteract catabolism. The specific regimens used vary with patient presentation and specific urea cycle enzyme deficiency, and comprehensive reviews are available [1].

The individual pharmacokinetics of both NAPA and NABZ have been described in the literature [2–6], and the published pharmacokinetic parameters are summarized in Tables 1 and 2. To date, there has been no published work describing the pharmacokinetics of intravenous NAPA and NABZ, when co-administered in the same manner as is currently used to treat hyperammonemia. To better understand the pharmacokinetics of this combination drug product, a study was undertaken in normal healthy volunteers utilizing the dosage regimen recommended for treating hyperammonemia in urea cycle enzyme-deficient patients.

Methods

Study design

This was a prospective, open-label pharmacokinetic study of intravenous NAPA and NABZ, administered to normal healthy volunteers. It was conducted at a single

Table 1
Published pharmacokinetic parameters of sodium phenylacetate

Population	Route of administration and dose	Model	Parameter estimates	Ref.
18 Adults with cancer	Intravenous NAPA at 125 and 150 mg/kg, with some additional dose escalation	One-compartment model with Michaelis–Menten elimination noted that NAPA may induce its own elimination comparing day 1 vs day 12–14 AUC	V_{max} = 29 ± 6.3 mg/kg/h K_m = 106 ± 22 μg/ml V_d = 21 ± 4.81 L	[2]
17 Adults with cancer	Intravenous NAPA priming dose followed by prolonged, escalating continuous infusion	Non-linear kinetics	V_{max} = 24.1 ± 5.2 mg/kg/h K_m = 105.1 ± 44.5 μg/ml V_d = 19.2 ± 3.3 L	[3]

Table 2
Published pharmacokinetic parameters of sodium benzoate

Population	Route of administration and dose	Model	Parameter estimates	Ref.
2 Patients with hyperammonemia	Oral NABZ 130 and 150 mg/kg	*Post absorption data* One-compartment model with Michaelis–Menten elimination	*Period 1* V_{max} = 152 μg/ml/h K_m = 65.7 μg/ml *Period 2* V_{max} = 90 μg/ml/h K_m = 30 μg/ml	[4]
6 Healthy adult volunteers	Oral benzoic acid at 3 doses (40, 80, 160 mg/kg) 3 way cross-over	*Benzoate* One-compartment model with first-order rate absorption and Michaelis–Menten elimination *Hippurate* One-compartment model with first-order elimination	V_{max} = 101.9 μg/ml/h K_m = 10.5 μg/ml C_{max} 40 mg/kg = 99.7 μg/ml 80 mg/kg = 202.8 μg/ml 160 mg/kg = 336.5 μg/ml	[5]
4 Neonates with hyperammonemia	Intravenous NABZ 3.5 mmol/kg/day divided q6h	Assumed first-order, one compartment elimination model	Large inter-patient variability, low benzoate clearance associated with impaired renal function and hypotension V_d = 0.14 ± 0.07 L/kg (0.086–0.244) $t_{1/2}$ = 2.8 ± 3.1 h (0.75–7.4) Cl = 1.00 ± 0.61 ml/kg/min (0.33–1.56)	[6]

clinical research center, and was approved by the local institutional review board.

Subjects

Two volunteer cohorts were studied, each undergoing two separate treatment periods. The first cohort ($n = 3$), during period 1, received 5.5 g/m^2 of NAPA/NABZ as a 90-min infusion, with blood sampling at 0, 0.25, 0.5, 0.75, 1.5, 2, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5, 10.5, 11.5, and 12.5 h. After a one-week washout, during the second treatment period, a 90-min bolus dose of 5.5 g/m^2 was administered, followed by a 24-h continuous infusion of the same dose. Blood was sampled at 0, 0.25, 0.75, 1.5, 2.5, 4.5, 6.5, 8.5, 10.5, 12.5, 16.5, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29.5, 30.5, and 32.5 h. The second cohort of subjects ($n = 17$) underwent an identical sequence of drug administration and sampling; only, in this group, a dosage of 3.75 g/m^2 was used for each of the combination drugs. Infusions were administered via a peripheral venous catheter.

Sample handling and analytics

Ten milliliters of venous blood was drawn into sodium heparin vacutainers. They were centrifuged at 1600*g* at a temperature of 4 °C for 10–15 min. The plasma was separated into two storage tubes and frozen at −20 °C. Following precipitation with methanol and addition of internal standard the plasma samples were analyzed by reverse phase high-pressure liquid chromatography using ultraviolet absorption detection. For the analytes, phenylacetic acid, and benzoic acid, the calibration curve ranged from 5.000 to 500.0 μg/mL, and for the metabolites phenylacetylglutamine (PAG), and hippuric acid (HIP) the calibration curve ranged from 5.000 to 500.0 μg/mL.

Pharmacokinetic analysis

Ucyclyd Pharma (Scottsdale, AZ) supplied analyte concentration data as Microsoft Excel® 4.0 spreadsheets. Data were analyzed using WinNonlin Professional® (version 4.1). The concentration:time data for phenylacetate and benzoate for each dose group (periods 1 and 2 for each cohort) were analyzed using non-compartmental methods, and the results are presented as treatment group means. For each active drug and its metabolite, the following model-independent parameters were calculated: AUC, C_{max}, and T_{max}. Data from each cohort and treatment period were then modeled individually, using WinNonlin Michaelis–Menten model 302 (one-compartment with constant IV input and Michaelis–Menten output), with fitting via the Gauss–Newton (Levenberg and Hartley) algorithm.

Results

The first cohort of three volunteers received 5.5 g/m^2, initially as a single bolus dose (period 1), and then as a bolus dose, followed by a continuous infusion (period 2). During the second treatment period, significant nausea, vomiting, and somnolence occurred, and as a result no additional volunteers were treated at this dose level. The dose was then lowered to 3.75 g/m^2; 17 volunteers received this bolus dose (period 1), and 14 of them received the combined bolus followed by the continuous infusion (period 2).

Time–concentration curve graphs of the bolus dose followed by continuous infusion are provided for phenylacetate and PAG (Figs. 1 and 2), as are graphs for benzoate and HIP (Figs. 3 and 4). Non-compartmental analysis results for each compound and its corresponding metabolite are provided (Tables 3 and 4). Because the bolus dose followed by continuous infusion mimics the manner in which the drug is infused according to the investigational drug protocol, only these modeled data are presented here. Graphs of modeled data are provided (Figs. 5 and 6) for phenylacetate and benzoate respectively.

Sodium phenylacetate and phenylacetylglutamine

Following the bolus dose alone (period 1), plasma levels of phenylacetate peaked at the end of the 1.5-h infusion, with a maximum plasma concentration of 2225 (± 309) and 3022 (± 220) μmol/L, in the low- and high-dose groups, respectively (Table 3).

When the bolus dose was followed by a 24-h continuous infusion, a plateau phase was observed following

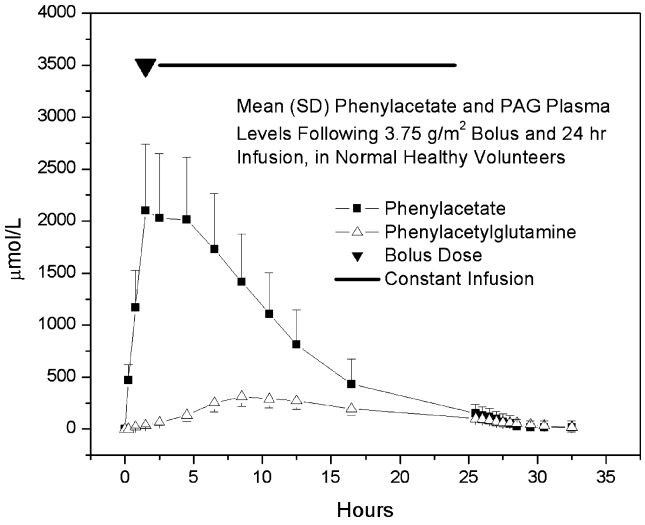

Fig. 1. Mean phenylacetate and PAG plasma levels at the 3.75 g/m^2 dose level.

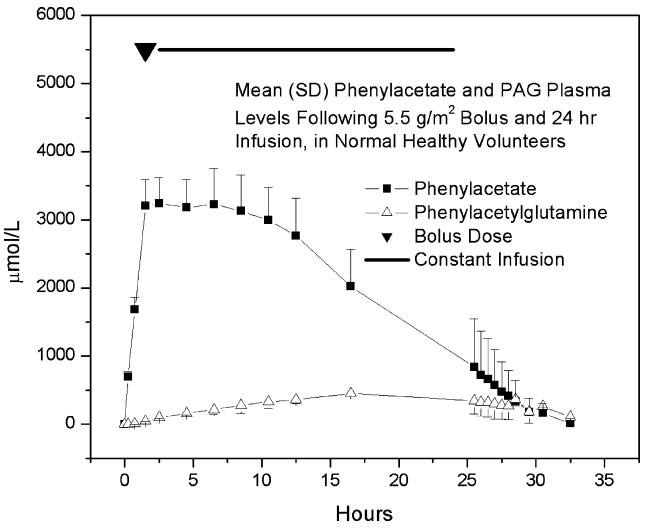

Fig. 2. Mean phenylacetate and PAG plasma levels at the 5.5 g/m^2 dose level.

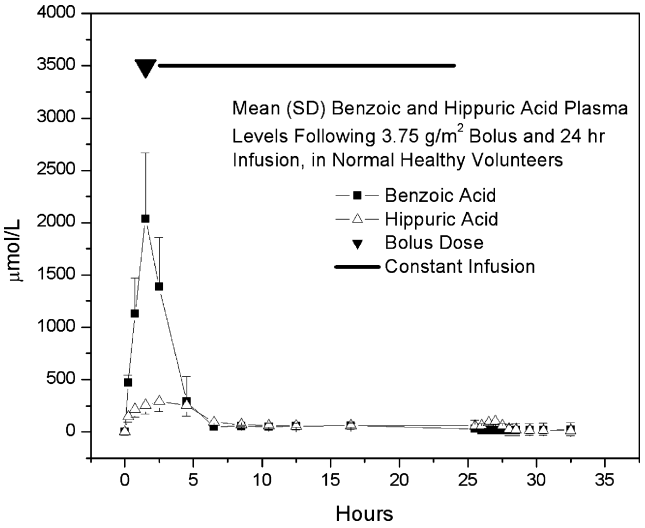

Fig. 3. Mean benzoic and hippuric acid plasma levels at the 3.75 g/m^2 dose level.

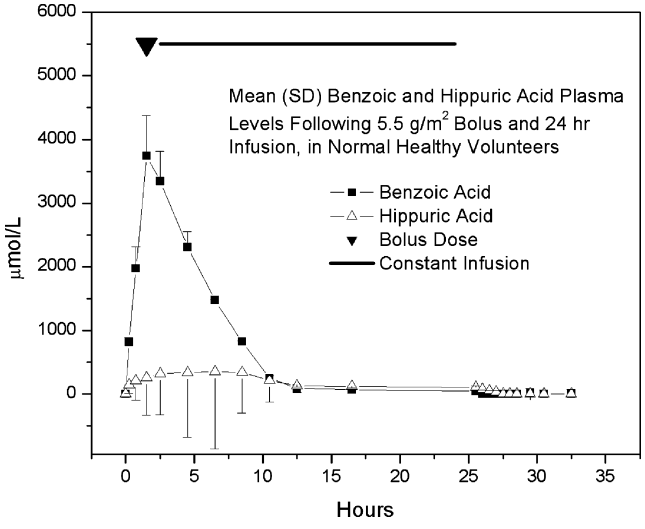

Fig. 4. Mean benzoic and hippuric acid plasma levels at the 5.5 g/m^2 dose level.

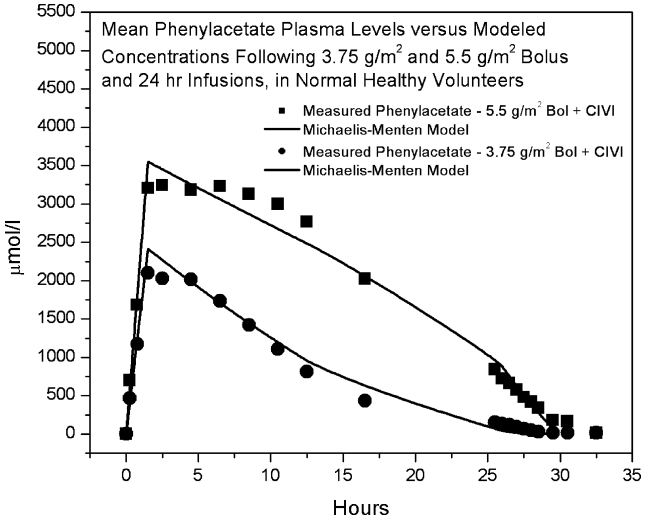

Fig. 5. Modeled phenylacetate concentrations at the 3.75 g/m^2 and 5.5 g/m^2 dose levels.

the end of the bolus dose, during which phenylacetate concentrations remained essentially unchanged for 5 and 9 h, respectively. In the high-dose group, the mean T_{max} occurred at 7.2 (± 5.0) h, well after the initial bolus dose and C_{max} was 2261 (± 272) and 3422 (± 365) µmol/L, in the low- and high-dose groups, respectively (Table 3). Thereafter, plasma level concentrations declined over the remaining 24-h period, during the ongoing continuous infusion. For phenylacetate, AUC increased disproportionately with dose, while this parameter for PAG was approximately dose-proportional (Fig. 7). PAG was not detected until 0.75 h after the start of the infusion, and plasma levels increased slowly, with T_{max} occurring at 8.8 (± 1.7) and 19.5 (± 5.2) h, in the low- and high-dose continuous infusion groups, respectively.

Sodium benzoate and hippurate

In all dose groups, peak plasma levels of benzoate occurred at the end of the 1.5-h infusion (Table 4). During period 1, C_{max} was 2136 (± 319) and 3444 (± 286) and during period 2, it was 2182 (± 298) and 3746 (± 593) µmol/L, in the low- and high-dose groups, respectively. Also, in all dose groups NABZ concentrations declined rapidly following T_{max}, and approached the limit of detection in these treatment groups at 6.5 and 12.5 h, respectively (Figs. 3 and 4). In contrast to PAG, plasma levels of HIP were detectable at the first sampling point, 15 min following the start of the bolus dose, in all groups. As with PAG, the AUCs of HIP increased in proportion to the dose (Fig. 7), while the AUC for the parent drug, benzoate, increased disproportionately with dose. This

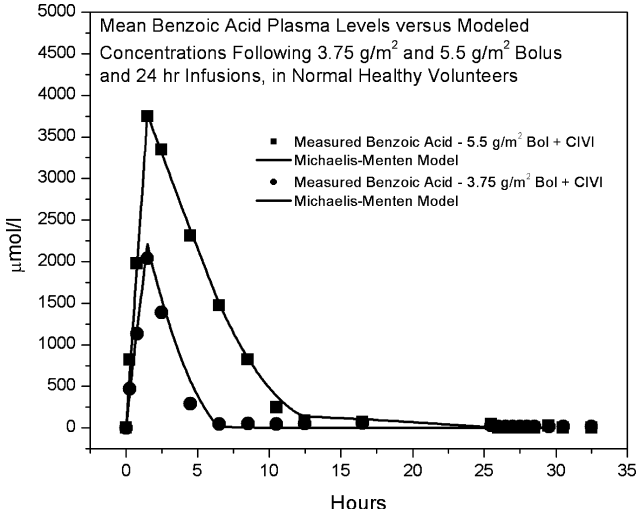

Fig. 6. Modeled benzoic acid concentrations at the 3.75 g/m^2 and 5.5 g/m^2 dose levels.

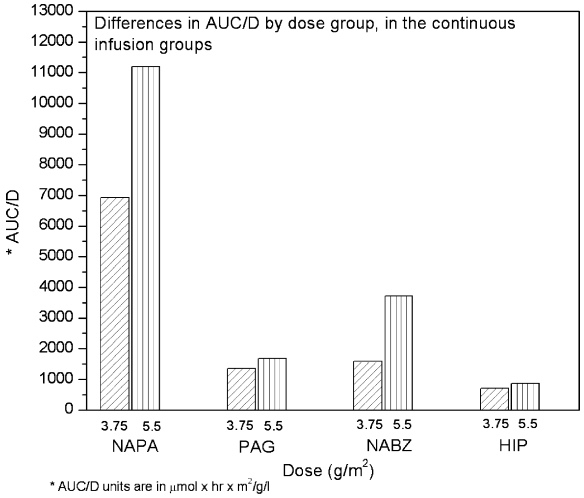

Fig. 7. Comparison of parent drug and metabolite AUCs at each dose level.

Table 3
Pharmacokinetic parameters for PA and PAG determined using non-compartmental methods

NAPA dose (g/m^2)		
PK parameter[a]	3.75 ($n = 20$)	5.5 ($n = 3$)
Bolus Period		
PA		
C_{max} (µmol/L)	2225 (309)	3022 (220)
T_{max} (h)	1.7 (0.3)	1.7 (0.3)
AUC (µmol/L × h)	15004 (2445)	28156 (3791)
PAG		
C_{max} (µmol/L)	281 (42)	NR
T_{max} (h)	7.9 (0.9)	NR
AUC (µmol/L × h)	2047 (300)	NR
Continuous IV infusion period		
PA		
C_{max} (µmol/L)	2261 (272)	3422 (365)
T_{max} (h)	2.0 (1.1)	7.2 (5.0)
AUC (µmol/L × h)	25970 (5041)	61560 (7834)
PAG		
C_{max} (µmol/L)	333 (50)	462 (58)
T_{max} (h)	8.8 (1.7)	19.5 (5.2)
AUC (µmol/L × h)	5099 (858)	9213 (2120)

NR, not reported due to limited 12 h sampling period.
[a] Values are mean (SD).

Table 4
Pharmacokinetic parameters for BZ and HIP determined using non-compartmental methods

NABZ dose (g/m^2)		
PK parameter[a]	3.75 ($n = 20$)	5.5 ($n = 3$)
Bolus period		
BZ		
C_{max} (µmol/L)	2136 (319)	3444 (286)
T_{max} (h)	1.5 (0.0)	1.7 (0.3)
AUC (µmol/L × h)	4666 (859)	13216 (3827)
HIP		
C_{max} (µmol/L)	351 (65)	435 (113)
T_{max} (h)	3.0 (0.5)	5.2 (0.6)
AUC (µmol/L × h)	1330 (278)	2912 (257)
Continuous IV infusion period		
BZ		
C_{max}(µmol/L)	2182 (298)	3746 (593)
T_{max} (hr)	1.5 (0.0)	1.5 (0.0)
AUC (µmol/L × h)	5949 (1150)	20430 (7255)
HIP		
C_{max}(µmol/L)	323 (69)	419 (120)
T_{max} (h)	3.4 (1.0)	5.8 (1.2)
AUC (µmol/L × h)	2666 (640)	4725 (1284)

[a] Values are mean (SD).

relationship between sodium benzoate dose, plasma benzoate and hippurate, and urinary hippurate has been previously described [5]. HIP concentrations reached C_{max} more quickly than those of PAG, and appeared to plateau at 10.5 h during period 2, remaining at approximately 0.2 µmol/L until the end of the continuous infusion.

Modeled parameters

Michaelis–Menten parameters for phenylacetate and benzoate derived from the continuous infusion regimens are provided in Tables 5 and 6. In the 5.5 g/m^2 dose group, the following parameters (mean (SEM)) were derived for phenylacetate, V_d 10.27 (0.3439) L/m^2, V_{max} 0.2780 (0.03046) µmol/m^2/h, and k_m 67.55 (135.6) µmol/L, and for benzoate, V_d 9.722 (0.4179) L/m^2, V_{max} 0.7417 (0.1064) µmol/m^2/h, and k_m 304.1 (250.0) µmol/L. Similar values were computed for the respective lower-dose groups. The data fit the model well. The largest variability in individual parameters was seen in K_m.

Discussion

When used for the treatment of hyperammonemia, the objective of NAPA/NABZ therapy is to bind and

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