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TRIAL

Evidence of CFTR Function in Cystic Fibrosis after Systemic Administration of 4-Phenylbutyrate

Pamela L. Zeitlin,[1,*] Marie Diener-West,[3] Ronald C. Rubenstein,[5] Michael P. Boyle,[2] Carlton K. K. Lee,[4] and Lois Brass-Ernst[1]

Departments of [1]Pediatrics and [2]Medicine, Johns Hopkins University School of Medicine, [3]Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, and [4]Department of Pharmacy, Johns Hopkins Hospital, Baltimore, Maryland, 21287 USA
[5]Division of Pulmonary Medicine, Children's Hospital of Philadelphia, and Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA

**To whom correspondence and reprint requests should be addressed. Fax: 410-955-1030. E-mail: pzeitli@jhmi.edu.*

Most individuals with cystic fibrosis (CF) carry one or two mutations that result in a maturation defect of the full-length protein. One such mutation, ΔF508, results in a mutant membrane glycoprotein that fails to progress to the apical membrane, where the wild-type protein normally functions as a cyclic AMP-regulated chloride channel. 4-Phenylbutyrate (Buphenyl), an orally bioavailable short chain fatty acid, modulates heat shock protein expression and restores maturation of the ΔF508 protein *in vitro* and *in vivo*. We performed a randomized, double-blind, placebo-controlled, dose-escalation and safety study of Buphenyl in 19 adults with CF (homozygous ΔF508) to test the hypothesis that Buphenyl would be safe, well-tolerated, and associated with an increase in chloride transport in nasal epithelia. Three dose levels (20, 30, or 40 g divided t.i.d.) of drug or placebo were given for 1 week. Serial measurements of chloride transport by nasal potential difference (NPD) testing and metabolic safety testing were performed. A maximum tolerated dose of 20 g was defined based on minimal adverse reactions, the safety profile, and a statistically significant induction of chloride transport that was maximal by day 3. This short-term phase I/II study demonstrates proof of principle that modulation of ΔF508 CFTR biosynthesis and trafficking is a viable therapeutic approach for cystic fibrosis.

Key Words: butyrates, clinical trial, cystic fibrosis, mutation, chloride, sodium, sweating, CFTR, ΔF508, Buphenyl

INTRODUCTION

The ΔF508 CFTR mutation results in a mutant membrane glycoprotein that fails to progress to the apical membrane, where the wild-type protein normally functions as a cyclic AMP-regulated chloride channel [1,2]. Instead, the majority of nascent ΔF508 CFTR molecules becomes ubiquitinated and rapidly degraded from the endoplasmic reticulum [3]. More efficient folding and maturation of ΔF508 CFTR can be induced by high concentrations of glycerol [4] or by protein assembly at 27°C [5,6]. Both HSP70 and HSC70, distinct members of the 70 kDa heat shock protein family, interact with CFTR, and regulation of these heat shock protein–CFTR interactions can restore ΔF508 CFTR trafficking [7,8]. We recently demonstrated that 4-phenylbutyrate (Buphenyl), an orally bioavailable short chain fatty acid, modulates heat shock protein function and restores ΔF508 maturation *in vitro* and *in vivo* [7–9]. It is not known whether restoration of ΔF508 CFTR to the plasma membrane will be sufficient to reverse cystic fibrosis (CF) disease.

We performed a randomized, double-blind, placebo-controlled, dose-escalation, and safety study of Buphenyl in 19 adults with CF (homozygous ΔF508). We hypothesized that Buphenyl would be safe, well-tolerated, and associated with a gain in chloride transport in nasal epithelia as quantified by nasal potential difference (NPD) testing. Drug or placebo was administered in three oral dose levels (20, 30, or 40 g) divided thrice daily (t.i.d.) for 1 week. Serial measurements of NPD, sweat electrolyte concentration, metabolic and hepatic function, pulmonary function, and sputum microbiology were performed during the study period and during a 1 month washout period. Pharmacokinetics were evaluated during the first 72 hours of study drug administration and will be reported separately.

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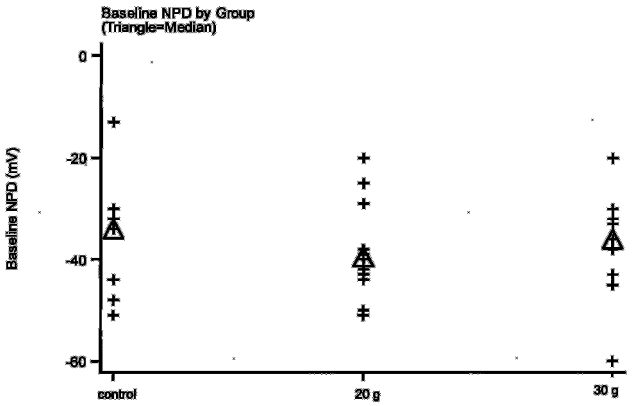

FIG. 1. Baseline NPD. Baseline NPD was recorded on day 0 during superfusion of Ringer's solution (triangle = median).

RESULTS

Demographics

There were 12 men and 7 women randomized in the study, and all 19 completed the final study visit. Mean age ± SD was 28.5 years ± 7.1, average weight was 62.6 kg ± 7.1, and average FEV_1 (% predicted) was 63.7 ± 17.0. There were no significant differences in gender, baseline age, weight, or FEV_1 among participants in the four groups (Kruskal–Wallis test, $P > 0.25$ for each comparison).

Adverse Events

As in our previous study, minor adverse events in the 20 g cohort included transient nausea, headache, and sleepiness after the initial dose, and body odor. The first three resolved with a dose of Tylenol, and hydration was encouraged. Body odor was an inconsistent complaint by family or friends of subjects. No dose adjustments were required. These complaints were also observed after the initial dose in the 30 g cohort. Several subjects reported visual disturbances that were transient after the first dose. One subject had severe headache that resolved with a reduction to 20 g daily. All three subjects in the 40 g cohort complained of nausea, headache, and visual disturbances, and one complained of cramps in the hands and fingers. One of these subjects tolerated 40 g of Buphenyl when it was divided into six daily doses. One tolerated a reduction to 30 g daily, and one subject found the symptoms to be so unpleasant that the drug had to be discontinued. The data and safety monitoring committee was convened to review the adverse event profile and recommended termination of the 40 g cohort. Although the maximum tolerated dose was 30 g daily, the number of tablets necessary and the side effect profile, as well as the physiologic outcome, suggest that the practical daily dose is 20

Nasal Epithelial Chloride Transport

NPD measurements were performed separately in the right and left nares. Several subjects presented with inflammation and tenderness on one side and NPD was not performed on that side if it was painful. Bilateral testing was resumed when the symptoms subsided. The baseline NPD did not differ between groups (Fig. 1). The means and standard deviations of the baseline sodium and chloride responses in each study group (Table 1) were comparable to data obtained in CF subjects in the Cystic Fibrosis Therapeutics Development Network (M.P.B. *et al.*, manuscript submitted), and there were no statistically significant differences in these baseline parameters between groups. In CF, the basal NPD is determined by the sodium potential which is unchecked (< –30 mV) in the absence of functional CFTR. Of the intended 38 measured basal NPDs, one naris in each of three subjects was too painful to allow measurement at the baseline. The baseline NPD was more positive than expected (–13 to –25) in 5 of 35 nares. This was due to technical difficulties related to patient reports of tenderness at the desired point of catheter placement. Although in those cases the catheter was repositioned for patient comfort and may not have represented the ideal site, these values were retained in the analysis. Average baseline NPD was similar across groups and indicative of CF.

The aggregate data for the measured low chloride/isoproterenol response over time of the placebo (Fig. 2A), 20 g (Fig. 2B), and 30 g (Fig. 2C) cohorts were assessed separately by group. No change was observed for the placebo group during the first 7 days. In contrast the low chloride/isoproterenol responses increased at days 2, 3, 4, and 7, respectively, for the 20 g (Fig. 2B) and 30 g (Fig. 2C) cohorts. The primary outcome variable, the change in the low chloride/isoproterenol response from baseline levels on each study day, was then compared across groups for days 2 (Fig. 3A), 3 (Fig. 3B), 4 (Fig. 3C), and 7 (Fig. 3D). A statistically significant induction of chloride transport potentials was observed in a dose-dependent relationship on day 3 (Fig. 3B), but this difference diminished by day 4 (Fig. 3C). The mean difference in low chloride/isoproterenol change between the 20 g cohort and the control group was –2.2 mV (95% confidence interval (CI): –10.1,

TABLE 1: Basal NPD parameters

Group	*n*	Amiloride inhibition (mV)	Low chloride response (mV)	Isoproterenol response (mV)	Low chloride/isoproterenol response (mV)
Control	7	22.3 (9.7)	3.9 (4.3)	0.1 (1.6)	4 (4.2)
20 g	11	23.9 (9.1)	4.4 (7.2)	3 (2.0)	7.5 (8.0)
30 g	11	25.4 (14.6)	4.5 (6.8)	1.4 (2.9)	5.9 (5.7)
40 g	6	27.2 (7.8)	–2 (4.1)	3.7 (4.6)	1.7 (5.5)

Data are expressed as mean (SD)

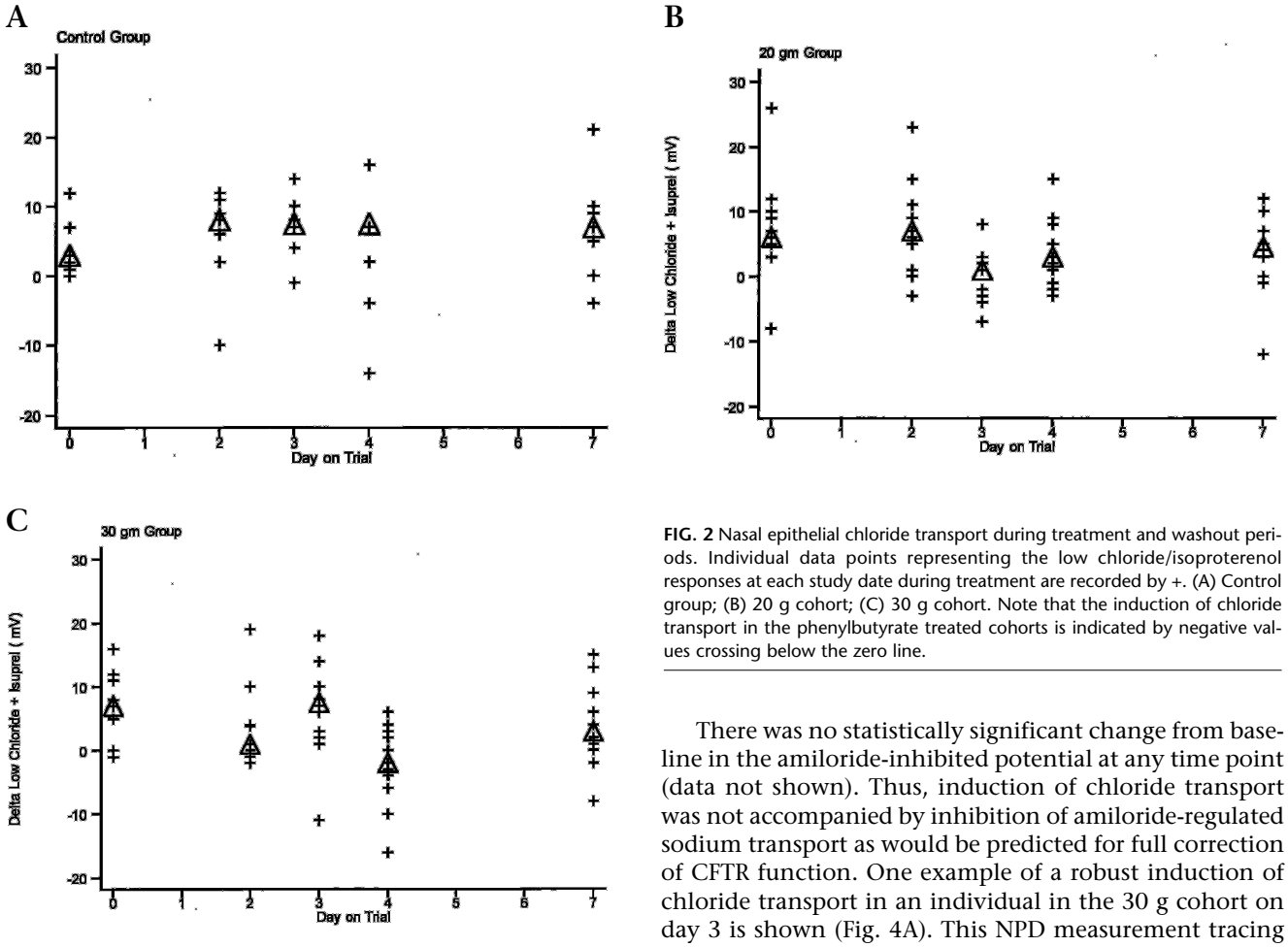

FIG. 2 Nasal epithelial chloride transport during treatment and washout periods. Individual data points representing the low chloride/isoproterenol responses at each study date during treatment are recorded by +. (A) Control group; (B) 20 g cohort; (C) 30 g cohort. Note that the induction of chloride transport in the phenylbutyrate treated cohorts is indicated by negative values crossing below the zero line.

5.8) and –11.9 mV (95% CI: –18.9, –5.0) on days 2 and 3, respectively. This difference decreased to –1.8 mV (95% CI: –10.0, 6.3) by day 4. A similar trend was observed in the comparison of the low chloride/isoproterenol change between the 30 g cohort and the control group from baseline to day 2 (–5.0 mV (95% CI: –14.3, –4.2)) and to day 3 (–6.3 mV (95% CI: –16.7, 4.1)).

Analysis of the isoproterenol response alone, without the previous low chloride maneuver, revealed the same patterns. A statistically significant, dose-dependent improvement of isoproterenol-stimulated chloride transport was observed on days 2 and 3, but this difference diminished by day 4. The mean difference in isoproterenol change between the 20 g cohort and the control group was –5.6 mV (95% CI: –10.2, –0.9) and –6.2 mV (95% CI: –12.1, –0.3) on days 2 and 3, respectively. This difference decreased to –2.8 mV (95% CI: –10.3, 4.7) by day 4. A similar trend was observed in the comparison of the difference in isoproterenol change on day 2 between the 30 g cohort and the control group (–5.6 mV (95% CI: –9.4, –1.7)) although the difference was not statistically significant by

There was no statistically significant change from baseline in the amiloride-inhibited potential at any time point (data not shown). Thus, induction of chloride transport was not accompanied by inhibition of amiloride-regulated sodium transport as would be predicted for full correction of CFTR function. One example of a robust induction of chloride transport in an individual in the 30 g cohort on day 3 is shown (Fig. 4A). This NPD measurement tracing demonstrates the persistently high basal level of NPD in spite of normal levels of chloride transport, which again suggests that induction of chloride transport has no significant effect on amiloride-regulated sodium transport.

Urine and plasma collected on day 7 demonstrated the presence of study drug metabolite in all treated subjects and not in controls. Phenylbutyrate was efficiently converted to phenylacetate and excreted in the urine as phenacetylglutamine in the 20 g cohort. Increasing the dose by 33% from 20 g to 30 g daily was associated with a doubling in the AUC_{24} (11.9 ± 5.9 and 22.6 ± 5.0 mm*h). Examples of the plasma phenylbutyrate (Fig. 4B) and phenylacetate (Fig. 4C) profiles for the same subject displayed in Fig. 4A are given. These graphs demonstrate the three expected daily peaks in phenylbutyrate concentration and more sustained levels of the first metabolite during the waking hours.

Accumulation of phenylacetate in the plasma was observed in one individual in the 30 g cohort. This suggests that in this subject, phenylbutyrate may have saturated the metabolic pathway to conversion to phenacetylglutamine, thus suggesting a maximum tolerated dose of

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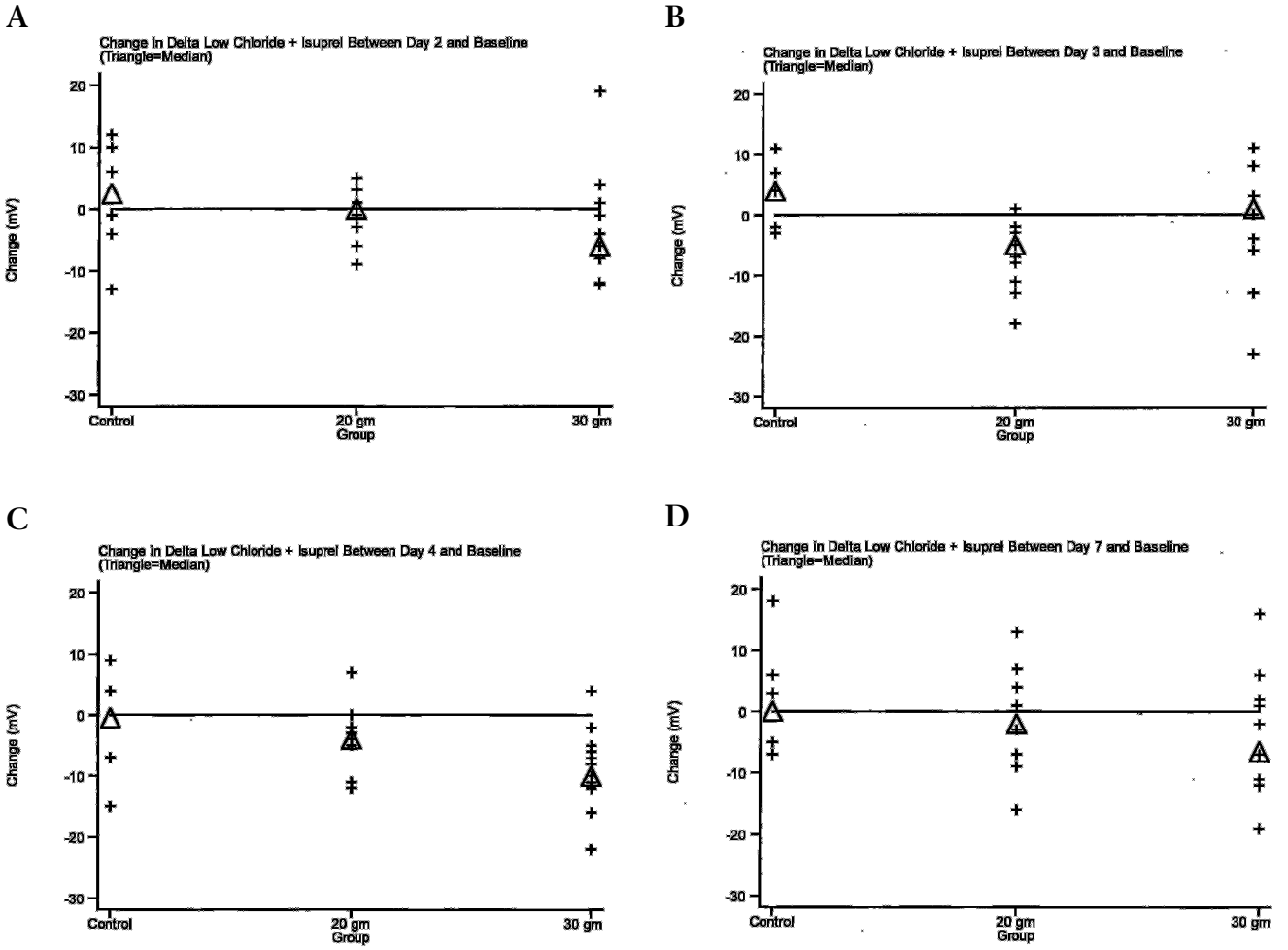

FIG. 3. Change from baseline in nasal epithelial chloride transport (triangle = median). Nasal epithelial chloride transport was measured on day 2 (A), day 3 (B), day 4 (C), and day 7 (D). Days 2 and 3 resulted in maximal inductions of chloride transport for 20 and 30 g cohorts.

Sweat Electrolytes

Although several individuals exhibited a decrease in sweat chloride while taking the study drug with restoration of pre-drug values during the washout, there was significant inter-subject variability and no statistically significant difference for the groups as a whole (Table 2).

Hepatic Enzymes

One subject had an isolated elevation in alkaline phosphatase at baseline which persisted. There was a trend toward reduction in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the 20 g and 30 g cohorts and a statistically significant drop in total bilirubin by day 7 for the 30 g cohort (Table 2).

Uric Acid

Uric acids levels became mildly elevated while on study during the washout period (Table 2). Phenacetylglutamine and urate may compete for the same transporter in the kidney.

Pulmonary Function Test

There was no statistically significant difference from baseline in FEV1 levels beetween groups, according to measurements made on day 0, 3, 4, or 7 within each group.

Microbiology

Pseudomonas aeruginosa and *Staphylococcus aureus* were scored semi-quantitatively at baseline and day 7 using a Likert scale from 0 (none) to 6 (heavy). Median score for *P. aeruginosa* at baseline was 4.5 in the controls, 2 in the 20 g cohort, and 5 in the 30 g cohort. There were no statistically significant differences in these scores at the baseline measurement between groups or between baseline

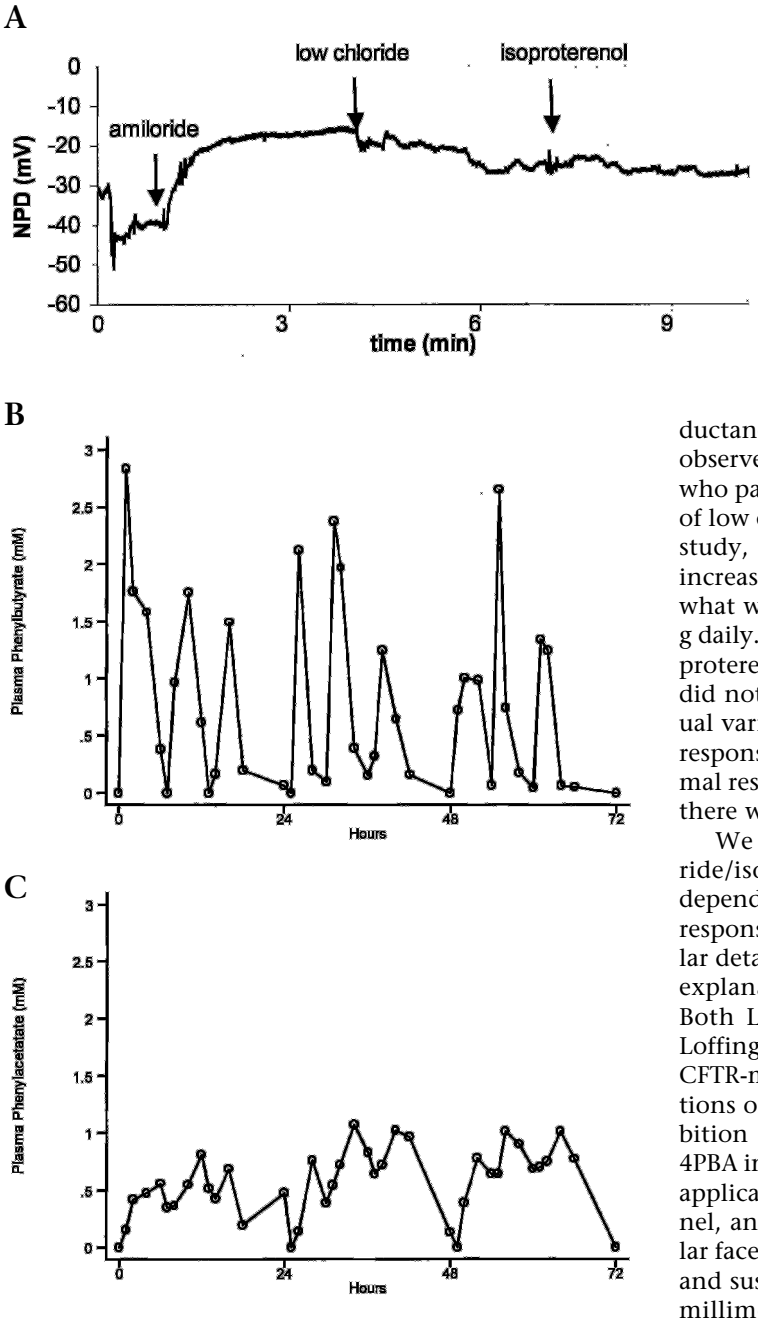

FIG. 4. Example of a NPD in a subject taking 30 g phenylbutyrate daily for 3 days. (A) NPD. (B) Plasma phenylbutyrate levels for study days 1–3. (C) Plasma phenylacetate levels for study days 1–3.

DISCUSSION

Magnitude of Chloride Response in CF Nasal Epithelia

The combined low chloride/isoproterenol response in the NPD test has been recognized to be the most discriminatory measure of CFTR dysfunction and cystic fibrosis [10]. Homozygous ΔF508 subjects (uniformly pancreatic insufficient) would be expected to have negligible, or at most,

chloride/isoproterenol responses). CF can be associated with slightly higher or intermediate levels of low chloride/isoproterenol response, particularly in the setting of one or more pancreatic sufficient mutations [11–14]. In our study, restoration of the ΔF508 CFTR to the plasma membrane may have been associated with a sub-maximal response because this mutation carries a shorter open time and lower conductance than wild-type CFTR [15]. We had previously observed, in subjects of this genotype taking 19 g daily who participated in a pilot study, a modest improvement of low chloride/isoproterenol response [8]. In the present study, a dose escalation was undertaken to attempt to increase the low chloride/isoproterenol response above what we observed in our first pilot clinical trial using 19 g daily. Whereas some subjects achieved low chloride/isoproterenol responses approaching normal values, others did not. Our results suggest additional genes or individual variation in pharmacokinetics may play a role in the response, but that on average, we have achieved a maximal response using 20 g daily. Our data also indicate that there was no additional advantage to taking 30 g daily.

We observed peak improvement of the low chloride/isoproterenol response between 3 and 4 days, depending on the dose, and a slight diminution in this response by day 7 in all dose groups. While the molecular detail of this observation is not known, two potential explanations are supported by published observations. Both Linsdell [16], in single channel recordings, and Loffing *et al.* [17], in Calu-3 cells, observed inhibition of CFTR-mediated Cl^- transport with millimolar concentrations of 4-phenylbutyrate (4PBA), suggesting direct inhibition of the channel by 4PBA. Inhibition of CFTR by 4PBA in single channel recordings was only observed with application of 4PBA to the cytoplasmic face of the channel, and not with application of 4PBA to the extracellular face. For this mechanism to apply *in vivo,* a significant and sustained intracellular accumulation of Buphenyl at millimolar concentrations would need to occur. Only transient plasma concentrations of Buphenyl greater than 1 mM (Fig. 4B) are observed. Rapid metabolism of Buphenyl is also observed in patients with urea cycle disorders, and our pharmacokinetic data (reported elsewhere), reporting quantitative conversion of Buphenyl to phenylacetate and phenacetylglutamine before excretion in the urine, argues against such a mechanism. Furthermore, patients with urea cycle disorders who have taken Buphenyl daily for many years have not developed lung disease, bronchiectasis, or phenotypic features of CF

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