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Nonlinear pharmacokinetics of high-dose recombinant fusion protein CD4-IgG₂ (PRO 542) observed in HIV-1–infected children

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To the Editor

The use of therapeutic proteins, such as Ig-based fusion proteins, is assuming an important place in medicine.^{1,2} Because of the IgG Fc region of these proteins, there is a possibility that interactions with Fc receptors may interfere with dose-dependent therapeutics.³ PRO 542 (CD4-IgG₂) is a tetravalent fusion protein designed to prevent the attachment of the virus through its glycoprotein 120 to the CD4 molecule on monocytes/macrophages and T cells.^{4,5} Our first study of PRO 542 in HIV-1–infected children documented that it was a safe therapeutic; exhibited linear dose-concentration pharmacokinetic behavior in 0.2, 1.0, 5.0, and 10.0 mg/kg studies; and possessed antiviral properties as assessed by plasma HIV-1–RNA

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levels and the number of infectious units per million cells.⁶ We designed a second PRO 542 study in HIV-1–infected children given twice (20 mg/kg) the previous highest dose to determine whether its antiviral properties would be increased proportionally. The bioanalytic technology used in the first and second PRO 542 did not change and was validated throughout. Also, different lots of PRO 542 were used in the 2 studies, but they continued to meet the same product specifications.

Study subjects (n = 13) were perinatally HIV-1–infected children, 2 to 12 years of age, receiving stable antiretroviral medications for at least 3 months, and exhibiting a RNA viral load of $\geq 10,000$ copies/mL. Patient plasma and sera were preserved in liquid N₂ (–140°C). Assessments of patient safety, peripheral blood CD4⁺ T-cell counts (cells/ μ L) and percents, plasma viral load (copies/mL), and pharmacokinetics were performed as previously described; pharmacokinetic parameters were determined from 6 samples obtained over a period of 14 days after the last dose.^{6,7}

Because of the small sample size, descriptive statistics, overall and classified by cohort, were computed for the following baseline characteristics: sex, race/ethnicity, age (y), weight (kg), CD4⁺ T-cell count/percent, and HIV-1–RNA viral load; and pharmacokinetic characteristics: area under the curve (AUC), total body clearance (CL), elimination half-life (T_{1/2}), highest concentration (C_{max}), and concentration 7 days after last dose (C-7 days). Kruskal-Wallis tests were used to determine whether the pharmacokinetic characteristics at baseline and virologic and immune responses at several time points differed between the 2 doses. Virologic and immune responses at several time points were calculated using median log₁₀ RNA changes and CD4 percent changes from baseline, respectively. Nonparametric analyses to test for significance were performed to avoid normality assumptions. The level of significance used was $\alpha = 0.05$.

Informed consent was obtained from parents or caretakers, and assent was obtained from children ≥ 7 years old where required. Human experimentation guidelines of the US Department of Health and Human Services and of the authors' institutions were followed in the conduct of this research.

Patients' baseline characteristics did not differ significantly across patients (n = 6) involved in the first study (PRO 542; 10 mg/kg) and those (n = 13) in the second study (PRO 542; 20 mg/kg).⁷ The median CD4⁺ T-cell percent was 23 (range, 1–46). The median RNA was 24,969 copies/mL (range, 2590–167,025 copies/mL).

The mean (median) predose serum concentrations of PRO 542 (20 mg/kg) at 0, 1, 2, 3, and 4 weeks (no infusion) were <0.04 (<0.04), 7.64 (6.48), 8.27 (7.60), 6.92 (6.71), and 10.26 (8.77) μ g/mL, respectively. The pharmacokinetic characteristics (median values) for PRO 542 obtained at 20 mg/kg in 13 patients were compared with those obtained at 10 mg/kg in 6 patients (Table I). The AUC, C_{max}, and C-7 days of PRO 542 at the 20-mg/kg dose were not significantly different from those of the 10-mg/kg dose; CL, however, was approximately 2-fold faster ($P = .0009$).

Similar to what was observed previously in pediatric patients treated with PRO 542 (10 mg/kg),⁶ more than half of the patients treated with PRO 542 at 20 mg/kg tended to have a decrease in HIV-1–RNA shortly after infusions; however, these modest reductions were not sustained over the 1-week dosing interval and therefore were not appreciably compounded on repeat dosing. For example, at 7 days posttreatment with PRO 542 at 20 mg/kg versus 10 mg/kg, the median log₁₀ HIV-1 RNA changes were +0.02 versus –0.01, +0.02 versus +0.07, and –0.04 versus +0.08 for doses 1, 2, and 3, respectively. Thus it appears that the likely explanation, on the basis of the available data, for the lack of efficacy of a doubled dose of PRO 542 in the current versus previous study is the property of nonlinear pharmacokinetics whereby the

increase in PRO 542 dose from 10 to 20 mg/kg did not achieve an increase in drug concentration.³ The volume of distribution of IgG molecules (PRO 542 in this case) critically depends on the affinity of the IgG for tissue sites containing the Fc receptors (FcγR) I, II, and III. A large apparent volume of distribution can be expected, therefore, where there is a high affinity. In addition, the tissue transport (Brambell) receptor for IgG (FcRB) might be saturated at high concentrations of IgG, thus providing a mechanistic explanation for the increased clearance rate of IgG with increasing concentration of IgG.^{8–10} Very large doses of therapeutic IgG are necessary to change the effective concentration of IgG significantly, and that in turn may increase the rate of elimination of endogenous and exogenous IgG. Finally, host antibody responses to fusion proteins such as PRO 542 may lower their effective serum concentration, although this appears unlikely in the current case given that nonlinear effects were observed after the first injection of PRO 542 and C_{max} values were similar between the first and final injections. Our observations of nonlinear pharmacokinetics of PRO 542 in HIV-1–infected children are not a reflection of young age. Single-dose studies of PRO 542 in adult HIV-1–infected patients of 10 mg/kg⁴ and 25 mg/kg⁵ yielded 2-hour C_{max} mean values of 564 (454–674) μg/mL and 590 (299–814) μg/mL, respectively, indicating nonlinear behavior.

This current pediatric study of the pharmacokinetics of a fusion protein containing the Fc component of IgG demonstrates that simply doubling of dosage at high concentrations does not enhance antiviral properties of the drug, most likely because of unique properties of the IgG component (Fc fragment). PRO 542 was designed with the Fc component of IgG₂, the IgG subclass that exhibits the least FcγR binding, but at high concentrations, binding may become appreciable. This hypothesis is supported by the current data in that because the T_{1/2} was similar between the 10-mg/kg and 20-mg/kg doses, an increase in the volume of distribution, perhaps because of increased binding, was responsible for the increase in CL. Our data suggest that the pharmacokinetics of PRO 542 are not dose-proportioned at higher dose levels similar to the nonlinear effects reported in other investigations of the pharmacokinetic and pharmacodynamic properties of IgG.^{3,8–10} Our findings have relevance to the numerous other applications of fusion proteins in medicine in which enhancing efficacy by increasing the dose is desired. The application of these designer proteins to the treatment of allergic and immunologic disorders will give clinicians exciting new therapeutic reagents whose advances and limitations must be understood in terms of their fundamental biological properties.¹¹

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TABLE I
Pharmacokinetic characteristics (median and range) of PRO 542 after multiple doses every 7 days

	PRO 542 20 mg/kg (n = 13)	PRO 542 10 mg/kg (n = 6) [†]	P value
AUC	11,714 (5964–17,870) µg*h/mL	11,362 (8531–13,124) µg*h/mL	.7257
CL	1.71 (1.12–3.35) mL/h/kg	0.88 (0.76–1.17) mL/h/kg	.0009
T _{1/2}	1.82 (1.22–2.43) days	2.13 (1.54–2.58) days	.1144
C _{max}	337 (84.8–517.8) µg/mL	274 (229–322) µg/mL	.2926
C-7 days	8.77 (1.90–22.3) µg/mL	6.95 (2.87–14.7) µg/mL	.5393

[†]Values from Shearer et al.⁶