

NON-LINEAR PHARMACOKINETICS OF HIGH DOSE RECOMBINANT FUSION PROTEIN CD4-IgG2 (PRO542) OBSERVED IN HIV-1-INFECTED CHILDREN

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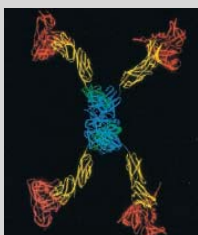
ABSTRACT

Rationale: Doubling the dose of rC4-IgG2 (10mg/kg to 20 mg/kg) in HIV-1 infected children failed to enhance its anti-viral effects.
Methods: Study subjects (n=13) were perinatally HIV-1-infected children, 2-12 years of age, receiving stable antiretroviral medications for at least 3 months, with RNA of $\geq 10,000$ copies/mL. Descriptive statistics were used to summarize the results. Kruskal-Wallis tests and non-parametric analyses were used to test for significance ($\alpha=0.05$).
Results: PRO 542 (20 mg/kg) was given intravenously at 4 weekly intervals to 13 HIV-1-infected children. This doubled dose of PRO 542 did not boost the anti-viral effect of PRO 542 at 10 mg/kg given previously to 6 children. PK analysis of serum samples yielded the following median and range values AUC (area under the curve), 11,714 (5,964 - 17,870) $\mu\text{g}\cdot\text{h}/\text{mL}$; CL (clearance), 1.71 (1.12 - 3.35) mL/kg; $T_{1/2}$ (half-life), 1.82 (1.22 - 2.43) days; Cmax (maximum concentration), 337 (84.8 - 517.8) $\mu\text{g}/\text{mL}$; and C-7 days (concentration 7 days after last dose), 8.77 (1.90 - 22.32) $\mu\text{g}/\text{mL}$. Except for a CL of 0.88 (0.76 - 1.17) mL/h/kg (P=0.0009), these values were not appreciably different from those obtained at a dose of PRO 542 at 10 mg/kg.
Conclusions: Increased CL of PRO 542 at 20 mg/kg is likely due to increased high affinity Fc:FcR binding, increased apparent volume of distribution, and saturation of the transport (Brambell) receptor (FcRB) at high concentrations of PRO 542. The non-linearity in PRO 542 PK (an inherent property of Fe-based fusion proteins) is likely responsible for the lack of higher dose effect in HIV-1-infected children.

INTRODUCTION

The use of therapeutic proteins, such as immunoglobulin-based fusion proteins is assuming an important place in medicine. Because of the immunoglobulin G (IgG) Fc region of these proteins, there is a possibility that interactions with Fc receptors may interfere with dose-dependent therapeutics.

PRO 542 (CD4-IgG2) is a tetraaval fusion protein designed to prevent the attachment of the virus through its glycoprotein 120 (gp120) to the CD4 molecule on monocytes/macrophages and T-cells.



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 mg/kg studies, and possessed antiviral properties as assessed by plasma HIV-1-RNA levels and the number of infectious units per million cells.

We designed a second PRO 542 study in HIV-1-infected children given twice (20 mg/kg) the previous highest dose to determine if its antiviral properties would be increased proportionally. The bioanalytical 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 two studies but they continued to meet the same product specifications.

METHODS

Study subjects (n=13) were perinatally HIV-1-infected children, 2-12 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/ μL) and percents, plasma viral load (copies/mL) and pharmacokinetics were performed as previously described; pharmacokinetic parameters were determined from six samples obtained over 14 days following the last dose (Shearer, et al. *J Infect Dis* 2000;182:1774-1779).

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

RESULTS

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). The median CD4+ T cell percent was 23 (range 1 to 46). The median RNA was 24,969 copies/mL (range 2,590 to 167,025 copies/mL).

The mean (median) pre-dose serum concentration of PRO 542 (20 mg/kg) at 0, 1, 2, 3, and 4 weeks (no infusion) were <0.04 (<0.4), 7.64 (6.48), 8.27 (7.60), 6.92 (6.71), and 10.26 (8.77) $\mu\text{g}/\text{mL}$ 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, Cmax, 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=0.0009).

Similar to what was observed previously in pediatric patients treated with PRO 542 (10 mg/kg), 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 one-week dosing interval and therefore were not appreciably compounded upon repeat dosing. For example, at 7 days post-treatment with PRO 542 at 20 mg/kg vs. 10 mg/kg, the median log₁₀ HIV-1 RNA changes were +0.02 vs. -0.01, +0.02 vs. +0.07, and -0.04 vs. +0.08 for doses 1, 2, and 3, respectively.

RESULTS (Cont'd.)

| TABLE I Pharmacokinetic Characteristics (median and range) of PRO 542 Following Multiple Doses Every 7 Days | | | |
|--|--|--|---------|
| | PRO 542 20 mg/kg (n=13) | PRO 542 10 mg/kg (n=6)‡ | p-value |
| AUC | 11,714 (5,964 - 17,870) $\mu\text{g}\cdot\text{h}/\text{mL}$ | 11,362 (8,531 - 13,124) $\mu\text{g}\cdot\text{h}/\text{mL}$ | 0.7257 |
| CL | 1.71 (1.12 - 3.35) mL/h/kg | 0.88 (0.76 - 1.17) mL/h/kg | 0.0009 |
| $T_{1/2}$ | 1.82 (1.22 - 2.43) days | 2.13 (1.54 - 2.58) days | 0.1144 |
| Cmax | 337 (84.8 - 517.8) $\mu\text{g}/\text{mL}$ | 274 (229 - 322) $\mu\text{g}/\text{mL}$ | 0.2926 |
| C-7 days | 8.77 (1.90 - 22.3) $\mu\text{g}/\text{mL}$ | 6.95 (2.87 - 14.7) $\mu\text{g}/\text{mL}$ | 0.5393 |

AUC: area under the curve; CL, clearance; $T_{1/2}$, half-life; Cmax, maximum concentration; C-7days, concentration 7 days after the last dose.
 ‡Values from Shearer, et al. *J Infect Dis* 2000;182:1774-1779.

DISCUSSION

It appears that the likely explanation, based on the available data, for the lack of efficacy of a doubled dose of PRO 542 in the present vs. previous study is the property of non-linear pharmacokinetics whereby the increase in PRO 542 dose from 10 to 20 mg/kg did not achieve an increase in drug concentration.

The volume of distribution of IgG molecules (PRO 542 in this case) critically depend upon the affinity of the IgG for tissue sites containing the Fc receptors (FcR), 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.

Very large doses of therapeutic IgG are necessary to significantly change the effective concentration of IgG 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 present case given that non-linear effects were observed after the first injection of PRO 542 and Cmax values were similar between the first and final injections.

Our observations of non-linear 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/kg and 25 mg/kg yielded 2h Cmax mean values of 564 (454-674) $\mu\text{g}/\text{mL}$ and 590 (299-814) $\mu\text{g}/\text{mL}$, respectively, indicating non-linear behavior.

DISCUSSION (Cont'd.)

This present 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 due to 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 FcR binding, but at high concentrations, binding may become appreciable.

This hypothesis is supported by the present data in that because the $T_{1/2}$ was similar between the 10 and 20 mg/kg doses, an increase in the volume of distribution, perhaps because of increased binding, was responsible for the increase in CL.

CONCLUSIONS

- Our data suggest that the pharmacokinetics of PRO 542 are not dose-proportionated at higher dose levels similar to the non-linear effects reported in other investigations of the pharmacokinetic and pharmacodynamic properties of IgG.
- Our findings have relevance to the numerous other applications of fusion proteins in medicine where enhancing efficacy by increasing the dose is desired.
- The application of these designer proteins to the treatment of HIV and many other chronic disorders will give clinicians exciting new therapeutic agents whose advances and limitations must be understood in terms of their fundamental biological properties.

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INFORMED CONSENT

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.

CONFLICT OF INTEREST

The authors do not have a commercial or other association that might pose a conflict of interest.

PUBLICATION

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