Clinical pharmacology of immuno-modulatory biotherapeutics : innovations in early drug development

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CHAPTER 3

Recombinant human serum amyloid p in healthy volunteers and patients with pulmonary fibrosis

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ABSTRACT

PRM-151, recombinant human pentraxin-2 also referred to as serum amyloid P (SAP), is under development for treatment of fibrosis. A first-in-human trial was performed to assess the safety, tolerability, and pharmacokinetics of single ascending intravenous doses of PRM-151 administered to healthy subjects, using a randomized, blinded, placebo controlled study design. Each cohort included three healthy subjects (PRM-151:placebo; 2:1). SAP levels were assessed using a validated enzyme linked immunoassay method, non-discriminating between endogenous and exogenous SAP. At a dose level of 10 mg/kg, at which a physiologic plasma level of SAP was reached, two additional healthy volunteers and three pulmonary fibrosis (PF) patients were enrolled enabling comparison of the pharmacokinetic SAP profile between healthy volunteers and PF patients. In addition, the percentage of fibrocytes (CD45+/Procollagen-1+ cells) in whole blood samples was assessed to demonstrate biological activity of PRM-151 in the target population.

PRM-151 administration was generally well tolerated. In two pulmonary fibrosis patients non-specific, transient skin reactions (urticaria and erythema) were observed. PRM-151 administration resulted in a 6- to 13-fold increase in mean baseline plasma SAP levels at dose levels of 5, 10, and 20 mg/kg. The estimated terminal half-life ($t_{1/2}$) of PRM-151 in healthy volunteers was 30 h. Pharmacokinetic profiles were comparable between healthy volunteers and PF patients. PRM-151 administration resulted in a 30-50% decrease in fibrocyte numbers 24 h post-dose. This suggests that administration of PRM-151 may be associated with a reduction of fibrocytes in PF patients, a population for which current pharmacotherapeutic options are limited. The pharmacological action of PRM-151 should be confirmed in future research.

Introduction

Idiopathic pulmonary fibrosis (IPF) is the most common idiopathic interstitial pneumonia (IIP) (1). It is a chronic, progressive, irreversible and lethal disease that generally occurs in middle-aged and elderly adults. IPF is a disease of unknown cause although recurrent epithelial injury and aberrant wound healing are thought to lead to fibrosis. Symptoms of IPF include chronic and progressive exertional dyspnoea, cough, a poor quality of life and eventually death. Therapeutic options are limited for all forms of pulmonary fibrosis (2), and the only treatment proven effective in prolonging survival is lung transplantation with a post-transplantation 5-year survival for IPF patients of approximately 44% (3). Efficacious therapy for pulmonary fibrosis remains elusive (4), and particularly pharmacotherapeutic options are limited.

In IPF, monocyte-derived cells play a central role in the fibrotic scarring process, as they take part in the production of (excess) collagen and cytokines such as platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), interleukin-1 (IL-1), monocyte chemoattractant protein-1 (MCP-1) and tumour necrosis factor-α (TNF-α) (5-7). The fibrocyte is a unique mesenchymal progenitor cell that differentiates from monocytes, and may be an important source of (myo) fibroblasts during tissue repair and tissue remodelling (8-10). Elevated levels of fibrocytes are associated with increased fibrosis and adverse clinical outcomes. The mean survival of IPF patients with fibrocyte counts exceeding 5% of total blood leukocytes was 7.5 months compared with 27 months for IPF patients with lower fibrocyte counts (11). Therefore, the fibrocyte may be a target for therapy in IPF, with fibrocyte counts as possible biomarker (10, 12).

The differentiation of circulating monocytes into fibrocytes (13-15) and pro-fibrotic (M2) macrophages (16) is controlled by serum amyloid P (SAP, also called pentraxin-2, PTX-2), a naturally occurring protein that circulates in the bloodstream with a crucial role in regulating wound healing (17). It has been shown that maintaining an elevated level of SAP in blood or locally at a site of injury can prevent excess scarring and the progression of fibrosis. Indeed, exogenous administration of SAP has been shown to reduce fibrosis in various animal fibrosis models such as in rodent models of ischemia reperfusion injury (18), bleomycin-induced lung fibrosis and lung fibrosis mediated by TGF-β overexpression (19), by decreasing the numbers of fibrocytes and pro-fibrotic M2-macrophages (15, 19, 20). The decreased accumulation of fibrocytes by SAP might be due to reduced leukocyte recruitment via lowering the levels of inflammatory cytokines (15).

In patients with IPF, the SAP level has been implicated to correlate with lung function (19). Furthermore, SAP directly inhibited M2-macrophage differentiation of monocytes into a pro-fibrotic phenotype (19). Taken together, these data suggest that the targeting of pro-fibrotic macrophages and fibrocytes by SAP-directed therapies might be a reasonable approach to the treatment of IPF. PRM-151, the recombinant form of human SAP (rhSAP), is such a compound that could potentially be used to prevent, treat, and reduce fibrosis. Preclinical data using human serum-derived SAP and PRM-151 demonstrated a potent anti-fibrotic activity of SAP in models of lung injury, skin injury, kidney injury and radiation-induced injury (15-21). We performed a first-in-human (FIH) trial to provide an initial assessment of the safety, tolerability, and pharmacokinetics (PK) of PRM-151 after administration of single intravenous doses. Importantly, a rational study design was chosen, consisting of 1) an efficient single ascending dose part in small cohorts of healthy subjects to assess the safety, tolerability, and pharmacokinetics of PRM-151, aiming to cover a range of PRM-151 doses resulting in plasma SAP levels with expected anti-fibrotic activity, and 2) an expanded cohort at a PRM-151 dose level that resulted in a desired SAP plasma level in the first study part. In this second study part two additional healthy volunteers and three pulmonary fibrosis (PF) patients were included, which not only allowed an initial comparison of the compound's pharmacokinetic and safety profile between healthy subjects and fibrosis patients, but also allowed the selection of a suitable biomarker for initial demonstration of biological activity of PRM-151 in PF patients. Especially the latter is of crucial importance for modern drug development, as the availability of such a pharmacodynamic measure will enable a more rational and efficient future development of the compound in the target population.

Methods

SUBJECTS Single ascending doses of PRM-151 were administered as an intravenous infusion to 26 healthy volunteers. In addition, three PF patients (one female, two males) were enrolled to compare pharmacokinetics of PRM-151 between healthy volunteers and the target population. One patient had a diagnosis of IPF according to the current European Respiratory Society/American Thoracic Society consensus statement (22) and two other (related) patients were diagnosed with Familial Interstitial Pneumonia. In the PF patients, fibrocytes were assessed as a pharmacodynamic parameter to demonstrate biological activity of PRM-151 early in the clinical development.

The healthy volunteers were aged 18-53 years (inclusive) and the PF patients were aged 29-72 years (inclusive), all subjects with a body mass index of 18-33 kg/m^2 and a body weight ≥50 kg. The use of any over-the-counter drugs, including herbal supplements (except for the occasional use of paracetamol and vitamins ≤100% of the recommended daily allowance) within 72 h before study day 1 was prohibited.

After signing an informed consent, subjects were medically screened within 3 weeks before test article administration. Exclusion criteria for healthy volunteers included history of amyloidosis, any active inflammatory condition and screening electrocardiogram (ECG) conduction intervals that were not within the gender specific normal range (QTc male <430 ms and females <450 ms). Exclusion criteria for PF patients included forced vital capacity (FVC) <45% predicted and history of amyloidosis, connective tissue disorder, chronic obstructive pulmonary disease (COPD), cystic fibrosis, tuberculosis or sarcoidosis. The study was conducted in accordance with the Declaration of Helsinki and Guideline for Good Clinical Practice, and was approved by the Ethics Review Board of the Leiden University Medical Centre, The Netherlands.

STUDY DESIGN This was a randomized, blinded, placebo controlled, inpatient/outpatient, sequential-group study of ascending single doses of 0.1, 0.25, 0.5, 1, 2, 5, 10, and 20 mg/kg PRM-151 (or placebo), administered to healthy subjects as a continuous intravenous infusion over 30 min under fasting conditions. Each cohort included three healthy subjects. Within each cohort, two subjects received PRM-151 and one subject received placebo. The subjects included in the expansion cohort (two healthy subjects, three patients) received a single continuous intravenous infusion of 10 mg/kg PRM-151 over 30 min under fasting conditions in an open label, inpatient/outpatient portion of the study. The starting dose for this FIH study was selected according to the US Food and Drug Administration (FDA) guidelines and based on the no-observed-adverse-effect level (NOAEL) in rats and cynomolgus monkeys. The selected PRM-151 dose range was based on the pharmacokinetic behaviour of PRM-151 as observed in preclinical models and an anticipated therapeutic PRM-151 dose that would result in a systemic SAP level of at least twice the normal circulating level, expected to result in anti-fibrotic activity as based on animal studies.

PHARMACOKINETIC ANALYSIS The concentrations of PRM-151 in plasma were determined using a validated enzyme linked immunoassay (ELISA) method (Charles River Laboratories). This validated analytical method did not differentiate

between PRM-151 and endogenous human SAP. As a result, plasma concentrations measured in day 1 pre-dose (0 h) samples were a measure of baseline endogenous SAP levels and all the plasma concentrations obtained post-dose (at 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 30, 36, 48, 72, 96 h) measured endogenous SAP plus PRM-151 levels. For pharmacokinetic analysis, the baseline SAP concentration was subtracted from all post-dose concentrations to generate baseline-corrected PRM-151 plasma concentrations. Baseline-corrected PRM-151 values that were negative were assumed to be zero. A non-compartmental analysis PK method was used to analyse the baseline-corrected plasma concentrations of PRM-151.

PHARMACODYNAMIC ANALYSIS The pharmacodynamic effect of PRM-151 was investigated in three PF patients by assessing the percentage of fibrocytes (CD45+/Procollagen-1+ cells) in whole blood samples collected before, 24 h and 3 weeks after the administration of PRM-151. Briefly, Ficoll isolated peripheral blood mononuclear cells (PBMCS) were stored at -150°C until use. After thawing, cells were washed twice, resuspended in RPMI 1640 culture medium, supplemented with 10% fetal calf serum (FCS), 50 µg/mL gentamycin (GIBCO) and cultured with lipopolysaccharide (LPS, 1 µg/mL Sigma O26:b6) for 48 h at 37°C and 5% CO_2. Cells were then stained with biotinylated anti-CD45 and streptavidin APC-Cy7/efluor-780 (eBioscience). After paraformaldehyde fixation and saponin permeabilization, intracellular staining was performed with anti-Rat Procollagen-1 (Millipore) and Goat anti Rat Qdot® 605 (Molecular Probes Life Technologies). Fixable Aqua Dead Cell Stain kit (Invitrogen, Molecular Probes) was used as a live/dead marker. Flow cytometry was performed using the LSRII Becton Dickinson fluorescence activated cell sorter (FACS) machine with DIVA™ software, analysis was performed with Flowjo Software.

Results

SAFETY Only mild and transient adverse events (AEs) were observed and these were equally distributed between PRM-151 and placebo treatment. In one of the PF patients, three circumscribed non-specific urticarial lesions were observed during drug administration. The urticaria resolved spontaneously within 2 h upon stopping the infusion. There were no signs of anaphylaxis or dyspnoea or changes in blood pressure, heart rate, saturation or taste. A second PF patient experienced a non-specific skin erythema 5 days after dosing, which resolved spontaneously.

PHARMACOKINETIC RESULTS, HEALTHY VOLUNTEERS AND PF PATIENTS The mean baseline plasma concentration of SAP in the healthy volunteers was 20.4 ± 6.7 µg/mL. Based on the observations in healthy volunteers (periodic sampling of plasma SAP concentrations over a 24 h period prior to dosing, and subjects receiving placebo treatment), there was no indication for a significant diurnal rhythm in endogenous plasma SAP concentrations. Administration of PRM-151 doses of 2, 5, 10, and 20 mg/kg resulted in a distinct increase over baseline SAP levels, lasting up to 24 h (2 mg/kg) and 72 h (5, 10 and 20 mg/kg) after administration of study medication. At a dose level of 20 mg/kg, maximal plasma SAP concentration was approximately 13 times higher than the measured mean baseline concentration. The mean baseline plasma SAP concentration in PF patients was 15.0 ± 7.5 µg/mL. Pharmacokinetic profiles of healthy volunteers and PF patients receiving 10 mg/kg SAP were comparable (Figure 1). However, it should be noted that patient sample number was small and individual SAP concentration-time profiles were variable between patients (data not shown). As a result, no reliable $t_{1/2}$ of PRM-151 could be estimated in PF patients. Calculated pharmacokinetic parameters corrected for individual baseline SAP levels for healthy volunteers (three highest dose levels) and PF patients (10 mg/kg) are provided in Table 1. At a comparable dose level, the mean maximum concentration (C_{max}, µg/mL) of PRM-151 was similar between the two study populations (healthy volunteers, 145 ± 24.8 *versus* PF patients, 115 ± 31.1). The estimated $t_{1/2}$ of PRM-151 in healthy volunteers was 30 h.

PHARMACODYNAMIC RESULTS Fibrocytes (CD45+/procollagen-1+ cells) were identified in PBMCs collected from the PF patients (Figure 2). Before administration of PRM-151, 1.5-3.3% of all PBMCS consisted of CD45+/procollagen-1+ cells. At 24 h after PRM-151 administration, the percentage of fibrocytes was diminished in all three PF patients by 30-50%. At day 22, the number of fibrocytes was still reduced in two subjects, whereas for the third subject the number returned to baseline levels.

Discussion

PRM-151, recombinant human SAP, is being developed as a novel anti-fibrotic agent. PRM-151 could attenuate monocyte differentiation into M2-macrophages and fibrocytes, and thus be a potential treatment for a variety of fibrotic diseases such as pulmonary fibrosis, scleroderma, cirrhosis, and cardiac fibrosis.

We performed an FIH dose escalation study to provide an initial evaluation of PRM-151 tolerability, safety and pharmacokinetics in healthy volunteers. Importantly, we also included a small cohort of pulmonary fibrosis patients to allow comparison of PRM-151 pharmacokinetics between healthy volunteers and patients, and early assessment of biological activity of PRM-151 in the target population.

Single intravenous doses of PRM-151 (10 mg/kg PRM-151 administered in PF patients, and up to 20 mg/kg in healthy volunteers) were well tolerated. Study drug administration did not raise any serious safety concerns and did not result in any clinically significant changes in blood or urinary laboratory parameters, nor in vital signs or ECG recordings. All reported adverse events were mild and transient and did not require medical intervention. The most common adverse events were fatigue and headache. Adverse events were equally distributed between PRM-151 and placebo treated patients, suggesting that these adverse events were not related to test article. Neither the nature nor the frequency of reported AEs increased with increasing doses of PRM-151. In one PF patient, a mild allergic reaction (urticaria) developed which resolved spontaneously.

The pharmacokinetics of PRM-151 was well-comparable between healthy volunteers and PF patients. The mean baseline plasma concentrations of SAP measured in the healthy volunteers and PF patients included in this study were 20.4 ± 6.7 µg/mL and 15.0 ± 7.5 µg/mL, respectively. These values are consistent with SAP levels reported in literature (19, 23). Interestingly, Murray et al. demonstrated a reduction of plasma SAP levels in IPF patients correlated with disease severity (19), underlining the potency of circulating SAP levels as a therapeutic target. In our study, administration of 5, 10 and 20 mg/kg PRM-151 to healthy subjects resulted in peak plasma concentrations that were almost 6-13 times higher than the mean baseline plasma SAP levels. Administration of 10 mg/kg PRM-151 to PF patients resulted in a 9-fold increase in mean baseline SAP level, indicating that circulating SAP levels can successfully be raised by PRM-151 treatment.

Next, we explored whether an increase in circulating SAP could influence the prevalence of monocyte-derived cell types that play a key role in fibrosis, or their soluble markers. SAP inhibits the differentiation of monocytes into M2-macrophages and fibrocytes (13-16). In three PF patients, we investigated the effect of PRM-151 treatment on the proportion of fibrocytes, as assessed by FACS analysis (CD45+/procollagen-1+ cells). Before PRM-151 treatment, we observed PBMC fibrocyte proportions of 1.5-3.3%. This is in line with results reported in literature for patients with stable IPF: in 51 patients, an average fibrocyte count of 2.72 ± 0.34% was observed (11). The same research group reported an average fibrocyte count of 1.0 ± 0.12% in a control group of seven healthy volunteers. Administration of

PRM-151 resulted in a 30-50% reduction in the percentage of fibrocytes (24 h post-dose). We cannot exclude the possibility that the presence of LPS in the culture medium may have influenced our results, however, an earlier report did not show significant effects of *Escherichia coli*-derived LPS on fibrocyte differentiation in a PBMC culture (24). Although the number of subjects was small, these results indicate a potential beneficial effect of PRM-151 in patients with elevated numbers of fibrocytes.

The minimum target therapeutic dose of PRM-151 to induce anti-fibrotic activity was estimated to be approximately double the normal circulating concentration, based on preclinical animal studies. This indicates that the selected dose level of 10 mg/kg in our study, resulting in a 9-fold increase of mean baseline SAP level and the associated decrease in fibrocyte number, might be unnecessarily high to induce the intended pharmacological effect. This should be confirmed or invalidated in future clinical studies.

In conclusion, we demonstrated that single intravenous doses of PRM-151 were generally well tolerated in healthy volunteers and a small group of PF patients. The administration of PRM-151 resulted in a 6- to 13-fold increase in circulating SAP levels at the highest dose levels tested. Circulating SAP levels remained elevated for a considerable period of time. Pharmacokinetic behaviour of PRM-151 did not differ between PF patients and healthy volunteers. Importantly, our data suggest that administration of PRM-151 may be associated with a reduction of fibrocytes in PF patients. Given the fact that the patient number was small, it is important that the pharmacological action of PRM-151 is confirmed in a multiple ascending dose study in IPF patients in the near future.

FIGURE 1 Mean plasma concentration-time profiles in healthy volunteers (A; *n*=5) and pulmonary fibrosis patients (B; *n*=3), with standard deviation as error bars. SAP: serum amyloid P.

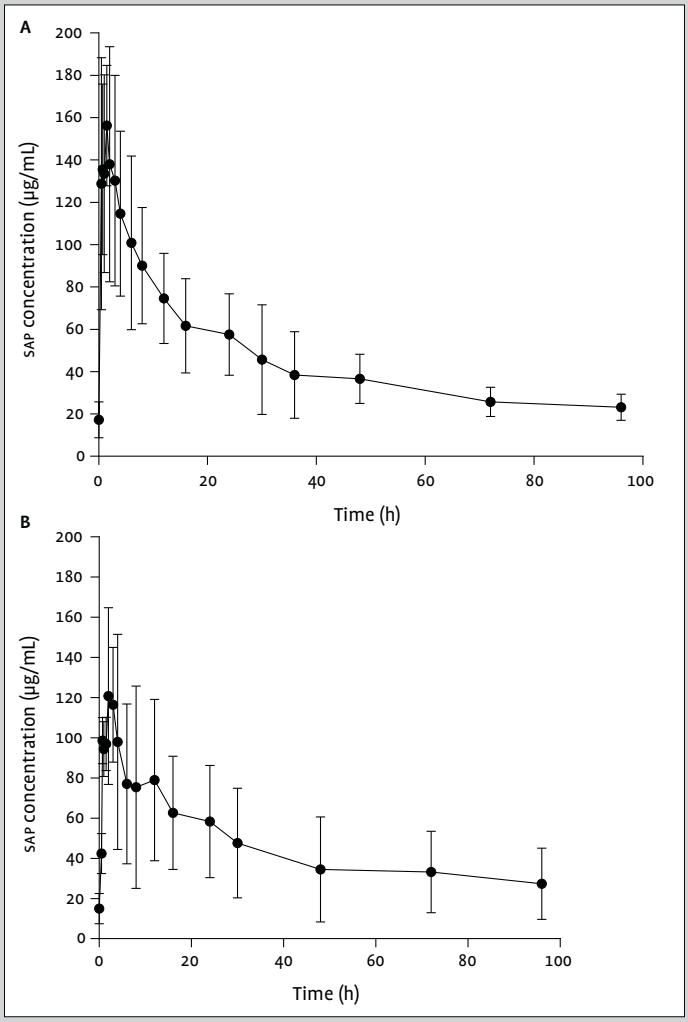

FIGURE 2 Effect of PRM-151 on fibrocytes in pulmonary fibrosis patients.

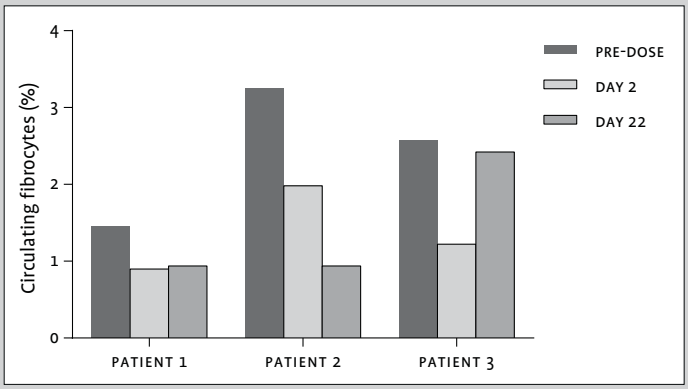

TABLE 1 Pharmacokinetic parameters in healthy volunteers and pulmonary fibrosis patients.

	Healthy volunteers			
Pharmacokinetic parameter	**5 mg/kg *n*=2**	**10 mg/kg *n*=4**	**20 mg/kg *n*=2**	**PF patients *n*=3**
t_{max} (h)	0.75 ± 0.354	1.25 ± 0.645	1.25 ± 0.354	1.50 ± 0.500
c_{max} (µg/mL)	89.6 ± 22	145 ± 24.8	243 ± 60.2	115 ± 31.1
$t_{1/2}$ (h)	ND	29.7 ± 7.66	20.3 ± 18.42	ND
AUC_t (µg*h/mL)	1721 ± 378	2672 ± 1128	5547 ± 1301	2943 ± 2025

Data are presented as mean ± standard deviation. AUC: area under the curve; c_{max}: maximum concentration; ND: not determined; PF: pulmonary fibrosis; $t_{1/2}$: terminal half-life; t_{max}: time at observed maximum concentration.

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