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

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ABSTRACT

PRM-151, recombinant human Pentraxin-2 (PTX-2) also referred to as serum amyloid P (SAP), is under development for treatment of fibrosis. A First-in-Human (FIH) 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 ELISA 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 $t_{1/2}$ 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.

1. 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 dyspnea, 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 PDGF, TGF-β, IL-1, MCP-1 and 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 remodeling [8–10]. Elevated levels of fibrocytes are associated with increased fibrosis

Abbreviations: AE, adverse events; ELISA, enzyme linked immunoassay; ERS/ATS, European Respiratory Society/American Thoracic Society; FACS, fluorescence activated cell sorter; FIH, firt-in-human; IIP, idiopathic interstitial pneumonia; IL-1, interleukin-1; IPF, idiopathic pulmonary fibrosis; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; PBMC, peripheral blood mononuclear cell; PD, pharmacodynamics; PDGF, platelet derived growth factor; PF, pulmonary fibrosis; PK, pharmacokinetics; PTX-2, pentraxin-2; SAP, serum amyloid-P; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

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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 (IV) 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.

2. Methods

2.1. Subjects

Single ascending doses of PRM-151 were administered as an intravenous infusion to twenty-six 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 ERS/ATS 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 (BMI) of 18–33 kg/m^2 and a body weight $\geq$50 kg. The use of any over-the-counter drugs, including herbal supplements (except for the occasional use of paracetamol and vitamins $\leq$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 three weeks before test article administration. Exclusion criteria for healthy volunteers included history of amyloidosis, any active inflammatory condition and screening 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 FVC $<$ 45% predicted and history of amyloidosis, connective tissue disorder, 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.

2.2. 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 (2 healthy subjects, 3 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 FDA guidelines and based on the NOAEL in rats and cynomolgus monkeys. The selected PRM-151 dose range was based on the pharmacokinetic behavior 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.

2.3. 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 analyze the baseline-corrected plasma concentrations of PRM-151.

2.4. 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