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Research paper

An oral administration of a recombinant anti-TNF fusion protein is biologically active in the gut promoting regulatory T cells: Results of a phase I clinical trial using a novel oral anti-TNF alpha-based therapy☆

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ABSTRACT

Background: An orally administered BY-2 plant cell-expressed recombinant anti-TNF fusion protein (PRX-106) consists of the soluble form of the human TNF receptor (TNFR) fused to the Fc component of a human IgG1 domain.

Aim

This study aim at determining the safety and the immune modulatory effect of an oral administration of PRX-106 in humans.

Methods: Three different doses (2, 8 or 16 mg/day) of PRX-106 were orally administered for five consecutive days in 14 healthy volunteered participants. Subjects were followed for safety parameters and for an effect on T lymphocytes subsets and cytokine levels.

Results: An oral administration of PRX-106 was safe and well tolerated. The PK study showed that PRX106 is not absorbed. No effect on white blood cells and lymphocytes counts were noted. A dose dependent effect was noted on systemic lymphocytes. The oral administration of all three dosages was associated with an increase in CD4 + CD25+ and CD8 + CD25+ subset of suppressor lymphocytes. A marked increase in CD4 + CD25 + FoxP3 regulatory T cells was noted in the 8 mg treated group. In addition, NKT regulatory cells, CD3 + CD69+ and CD4 + CD62 lymphocyte subsets increased with treatment. No changes in serum TNF alpha were observed.

Conclusion: An oral administration of the non-absorbable recombinant anti-TNF fusion protein, PRX-106, is safe, not associated with immune suppression, while inducing a favorable anti-inflammatory immune modulation. The PRX-106 may provide a safe orally administered effective anti-TNF alpha-based immune therapy for inflammatory bowel diseases and non-alcoholic steatohepatitis, as well as other autoimmune, TNF-mediated diseases.

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1. Introduction

Etanercept is a recombinant dimeric soluble tumor necrosis factor receptor (TNFR) fusion protein that blocks the soluble TNF, but not the membrane-bound TNF. Parenteral administration of Etanercept is used for the treatment of various immune-mediated disorders (Wiedmann et al., 2009; Hoy and Scott, 2007). Nevertheless, parenteral TNF-based therapies are associated with immune suppression, thus carrying a risk for side effects such as: infusion reactions, opportunistic and non-opportunistic infections, hematological and neurological complications, hepatotoxicity, and malignancies (Stallmach et al., 2010; Rongioletti et al., 2010). Additionally, a parenteral administration of TNF antagonists is connected to a primary response failure in some of the patients (Bendtzen et al., 2009). Others may have a secondary response failure; a situation where they respond initially, but have later relapses (Bendtzen et al., 2009).

An oral immune therapy is a method for delivering signals which are generated at the level of the gut immune system affecting the systemic immune system (Ilan, 2009a). This therapeutic method makes use of the inherent ability of the gut immune system to inhibit or to promote its reactions towards the orally administered compounds (Lefrancois and Puddington, 2006; van der Heijden et al., 1987). One of the goals of this method of therapy for immune-associated diseases is induction

Abbreviations: TNFα, tumor necrosis factor alpha; TNFR, TNF receptor; Tregs, regulatory T cells; TNFR-Fc, TNF receptor-Fc fusion protein; PK, pharmacokinetics; ConA, Concanavalin A; NKT, natural killer T cells; TCR, T-cell receptor; NASH, nonalcoholic steatohepatitis; LAP+, latency associated peptide.

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of regulatory T cells (Tregs) that mediate tolerance, suppress inflammation, while omitting the generalized immune suppression. As a result, the development of the compounds which are able to make use of the gut immune system for generating beneficial systemic immune signals, is an attractive approach for the treatment of immune-mediated disorders.

The orally administered plant cells (BY-2) expressing recombinant anti-TNF fusion protein (PRX-106), have an amino acid sequence identical to Etanercept (Enbrel™), and consist of the soluble form of the human TNF receptor (TNFR). It is fused to the Fc component of a human antibody IgG1 domain. It was previously presented that PRX-106 binds to TNF alpha and is stable in the gastric environment and crosses epithelial barriers. The PRX-106 survives the gastric environment and showed beneficial effects in animal models of immune-mediated hepatitis and colitis (BYaA et al., 2013; BYaA et al., 2014).

The aim of the present study was to determine the safety and immune modulatory effect of the oral administration of PRX-106 in humans. The data shows that short term oral administration of plant cells expressing recombinant anti-TNF fusion protein is well tolerated and biologically active in the gut. While not absorbed, it exerts a systemic immune modulatory effect by promoting Tregs without induction of immune suppression.

2. Methods

2.1.1. Study design and product

The clinical trial was a phase 1, open-label randomized study, which examine the safety and pharmacokinetics of oral PRX-106 in healthy volunteers. The subjects were enrolled in a single-center trial at the Hadassah-Hebrew University Medical Center in Jerusalem, Israel. The study drug was PRX-106 which consists of lyophilized genetically modified plant cells expressing the human tumor necrosis factor receptor-Fc fusion protein (TNFR-Fc), a dimeric soluble fusion protein of the TNF-2 receptor and Fc portion of IgG1. The product was manufactured by the sponsor, Protalix Ltd. (Carmiel, Israel). The trial was registered at ClinicalTrials.gov, NCT02107833.

2.1.2. Study population

Eligible subjects included healthy 18–45 years of age males, body mass index (BMI) of 20–25 kg/m^2, negative laboratory tests for human immune deficiency virus (HIV), hepatitis C and hepatitis B viruses. The subjects were naïve to any previous recombinant protein therapy (or anti-TNF therapy), did not suffer from any type of acute of chronic disorders and did not receive any immunomodulatory medication, and provided written informed consent.

Exclusion criteria included clinical evidence of any active significant disease that could potentially compromise the ability of the investigator to interpret the effects of the study treatment on safety assessment, therefore, increasing the risk to the subject to unacceptable levels; Presence of any acute or chronic diseases; Suspected tuberculosis (previous, active or latent); History of any allergies or protein-drug hypersensitivity; Exposure to long-term steroid treatment within the last 12 months before the study; the Subject had a major operation in the last 6 months; Subjects who had received immunosuppressive treatment prior to the study or had chronic use of any medication including vitamins; Participation in another clinical trial during the previous three months; Reported history of alcohol or drug abuse; Subjects with short bowel.

2.1.3. Efficacy and safety assessments

The primary endpoint was the safety of each of the three dosages of PRX-106 as assessed by adverse events, physical examination, vital signs, concomitant medications and laboratory tests results. The secondary endpoint was the pharmacokinetics (PK) profile of TNFR-Fc in each of the three doses of PRX-106, and the effect of PRX-106 on immune parameters assessed by serum cytokines and analysis of surface markers of peripheral lymphocytes. Adverse events were evaluated at each study visits. These were defined as, any unfavorable and unintended signs, symptoms or disease that appeared during the study period whether or not considered related to the study drug, accidental injuries, change in medications, reasons for admission to hospital or reasons for surgical procedures and any laboratory abnormality. The adverse events were gathered from the start of the treatment until 30 days following the final visit dose.

2.1.4. Study design

Fourteen healthy volunteers of 18 years and above were registered and were allocated to one of the three dose cohorts, receiving PRX-106 doses equivalent to 2 mg, 8 mg or 16 mg of TNFR-Fc. Subjects received once daily orally administered PRX-106 for five consecutive days. On day 1, the subjects were followed for 12 h succeeding PRX-106 administration for PK sampling. The subjects were followed daily for drug administration and study procedures. Moreover, all the subjects were divided into three groups each to receive a different oral dose of PRX106 for 5 consecutive days. Group A ($n = 4$) received 2 mg, Group B ($n = 5$) received 8 mg, while Group C ($n = 5$) received 16 mg. The study drug was administered to all the participants orally for 5 consecutive days in the morning following 8 h of fasting. Vital signs (systolic and diastolic blood pressure, pulse rate, body temperature and respiratory rate) were measured at screening, before each administration of the study drug and at day 10. Physical examination and blood samples (total protein, albumin, ALT, AST, ALP, GGTP, LDH, cholesterol, uric acid, creatinine, BUN, sodium, potassium, fasting glucose, total bilirubin, complete blood count with differential) performed at screening and at days 1, 2, 5, and 10. Adverse events and concomitant medications were evaluated at each study visit.

2.1.5. Pharmacokinetics study

Blood samples of TNFR-Fc levels were measured twice at screening visit (2 h apart) and before drug administration on day 1 of the study at the following time points: pre-administration, at 2, 4, 6, 8, 10, 12, 24, 48, 72 and 98 h post-administration of PRX-106, and on day 10.

2.1.6. Flow cytometry analysis of peripheral blood lymphocytes

The patients were followed for the immune modulatory effects of oral PRX106 of lymphocytes subsets by flow cytometry analysis and serum cytokine levels at day 1, 2, 4, and day 10. The Flow cytometry analysis was performed as described with the following modifications (Shevach et al., 2001): Cells were suspended in 100 μl FACS Buffer (1% Bovine serum albumin, BSA, in PBS) and incubated with the following surface anti-human antibodies: CD4-FITC, CD25-PE, CD8-eFlour450, CD56-FITC, CD3-APC, CD69. and CD62-PE (e-Biosciences, USA) for 30 min. Tubes with intracellular staining for Foxp3 antibody (APC, e-Biosciences, USA) were fixed and permeabilized according to manufacturer's instructions (e-Biosciences, USA) for 40 min and then incubated for 30 min with Foxp3 antibody. Cells were washed twice with FACS buffer and incubated at 4 °C until they were analyzed. Cell phenotyping was performed by a LSR-II (Becton Dickinson) and analyzed by FACS-DIVA software . Only the live cells were counted, and background fluorescence from non-antibody-treated lymphocytes and isotype control was subtracted.

2.1.7. Serum cytokines

To investigate the effects of the orally administrated PRX-106 on cytokine measurements, serum levels of TNF-α, IFN-γ, IL-4, IL-10 and IL-12 (p70) were determined by high sensitive enzyme-linked immunosorbent assay according to the manufacturer's instructions. (Quantikine R&D Systems) (Rizos et al., 2008). TNF-α ELISA kit (Human TNF-alpha Quantikine HS ELISA R&D Systems HSTA00D) was used for the assessment of TNF serum levels.

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