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Preclinical evaluation of the mono-PEGylated recombinant human interleukin-11 in cynomolgus monkeys

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ABSTRACT

The mono-PEGylated recombinant human interleukin-11 (rhIL-11) was evaluated for its pharmacology and toxicology profile in non-human primates. This PEGylated IL-11 (PEG-IL11) showed a much prolonged circulating half-life of 67 h in cynomolgus monkeys as compared to its un-PEGylated counterpart (~3 h) through subcutaneous administration, implicating that a single injection of the recommended dose will effectively enhance thrombopoiesis in humans for a much longer period of time compared to rhIL-11 in humans ($t_{1/2}$ = 6.9 h). The toxicokinetics study of single dose and multiple doses showed that systemic exposure was positively correlated with the dosing level, implying that efficacy and toxicity were mechanism-based. A single high dose at 6.25 mg/kg through subcutaneous route revealed tolerable and transient toxicity. Multiple-dose in monkeys receiving 0.3 mg/kg weekly of the drug developed only mild to moderate toxicity. Major adverse events and immunogenicity in monkeys were only observed in the overdose groups. Bones were positively impacted; while reversible toxicities in heart, liver, kidney and lung observed were likely to be consequences of fluid retention. In summary, the PEG moiety on rhIL-11 did not elicit additional toxicities, and the drug under investigation was found to be well tolerated in monkeys after receiving a single effective dose of 0.1–0.3 mg/kg through subcutaneous delivery, which may be allometrically scaled to a future clinical dose at 30–100 μg/kg, creating a potential long acting, safer, and more convenient treatment approach based on rhIL-11.

1. Introduction

After chemotherapy, pancytopenia represented major factors in the causation of adverse events and a rapid recovery of bone marrow function is essential and pivotal for eligibility of next cycle treatment regimen. As of today, safe and successful medical interventions for chemotherapy-induced thrombocytopenia (CIT) remains an objective for drug development. Oprelvekin (recombinant human interleukin-11, rhIL-11), the only approved growth factor stimulating the production of platelet, induces adverse effects including fluid retention which can lead to peripheral and pulmonary edema (NEUMEGA, 2011). Platelet transfusion remains the gold standard for treating CIT but it is often accompanied with allergic reaction, and in some cases, septic complications and severe anaphylactic reactions (Kiefel, 2008). Treatment of CIT is also costly as a recent pharmacoeconomic analysis in the United States indicated that direct incremental cost was over US$ 3000 and US $ 2366 for platelet transfusion and oprelvekin treatment respectively in an event of thrombocytopenia (Kuter, 2015).

Many pharmaceutical developments explored a new hematopoietic growth factor other than IL-11 for regulating megakaryocytopoiesis and platelet production. Thrombopoietin (TPO) and PEGylated megakaryocyte growth and development factor (PEG-MGDF) are two examples of the clinical trials that have been discontinued due to occurrence of life-threatening neutralizing antibodies (Kuter, 2009; Li et al., 2001). Other developments of modified rhIL-11 (Jung et al., 2010; Cox-III, 2012; Takagi et al., 2007) seem to be all halted in preclinical phase probably due to complexity in production and in some cases, repeated administrations may led to the loss of clinical benefit to patients. Therefore, modification of rhIL-11 to improve its pharmacokinetic profile with the potential of a longer half-life and safer profile may

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represent a logical approach to the treatment of CIT. We have successfully generated a series of PEG-IL11s (PEGylated IL-11s in various combinations of sizes, such as 10–40 kDa, and shapes, such as linear or branched of the PEG moiety) and based on the animal pharmacokinetic profile, we have selected the mono-PEGylated rhIL-11 with a single 40 kDa branched polyethylene glycol polymer at N-terminal amine as the lead drug candidate as preclinical research has demonstrated this molecule to be successful in preventing severe-thrombocytopenia while alleviating plasma expansion in rat models with just a single administration (Yu et al., 2016). This paper details the series of preclinical investigations that characterized this molecule.

2. Materials and methods

2.1. Preparation of the test article

The test article, PEG-IL11, was produced in a GMP facility in Shenzhen Kexing Biotech, China. The starting material, rhIL-11, was supplied by Hangzhou Jiuyuan Gene Engineering Company manufacturing according to China Pharmacopeia. The PEG reagent, supplied by Jenkem Technology, is the Y-shaped aldehyde-functionalized PEG with a molecular weight around 40 kDa (Catalog No. Y-PALD-40K).

The PEGylation reaction involves a stable amine bond formation mainly at the N-terminal amino acid residue with some minor reactivity for the internal lysine residues using the following conditions to optimize the PEGylation yield. A batch of 1.5 g of rhIL-11 at 5 mg/mL was mixed with 2-fold molar ratio of the PEG reagent in 50 mM NaH_2PO_4 pH 5.0 and 10 mM sodium cyanoborohydride (Aldrich #156-159). The reaction mixture was incubated at room temperature for 24 h, followed by quenching with 2 mM glycine. The resulting PEGylated conjugate was isolated by a cation-exchange column (5 × 10 cm, MacroCap SP resin from GE Healthcare Life Sciences #17-5440-01). Fractions containing mono-conjugated IL-11 were pooled and concentrated with a buffer exchange using ultrafiltration in 10 mM sodium phosphate pH 7 with 0.3 M glycine adhering to the Neumega's formulation. The protein concentration is directly determined by ultraviolet spectroscopy at wavelength 280 nm, using the absorbency value of 0.944 for a 0.1% (1 mg/mL) solution, and adjusted to 2 mg/mL. For the sterile fill-and-finish, each vial was filled with 0.75 mL of the PEGylated rhIL-11 solution under nitrogen gas and stored at 4 °C. Prior to animal administration, the test article was diluted with 0.9% saline solution to appropriate volume at 0.5 mL/kg.

The overall recovery of each batch was about 25%. The product purity was 95% as determined by the reverse-phase HPLC. Using the 7TD1 (DSMZ #ACC23) cell-based assay (Karow et al., 1996) the bioactivity of the PEG-IL11 was found to be 10–14% of its un-PEGylated counterpart. This was expected due to the steric hindrance of the PEG-moiety. However, animal pharmacokinetics studies have shown that the PEGylated IL-11 is more stable and thus has a longer half-life in the serum than its un-PEGylated counterpart (Takagi et al., 2007). A total of three separate batches of PEG-IL11 were produced according to the regulatory guidance of China FDA. The pharmacokinetics, pharmacodynamics, safety pharmacology and toxicology were carried out in both rats and monkeys. This report is to describe the results from studies done in monkeys. The un-PEGylated rhIL-11 was used as a reference standard and was acquired from Hangzhou Jiuyuan Gene Engineering Company (Lot# 20160302).

2.2. Animals and animal husbandry

Clinically healthy, socially housed adult cynomolgus monkeys (3–4 years old) were purchased from Yueyuan Animal Breeding Farm, Guangdong, China. Animals were sub-caged in a conventional clean, GLP-certificated facility of New South Center of Safety Evaluation for Drugs in Guangzhou University of Chinese Medicine, China and were maintained at a 12-hour light and dark cycle with constant temperature (23–25 °C) and humidity control (51–68%). Animals were kept in the facility for 48 days prior to commencement of the first dosing. The in life portion of the experiments was carried out with procedures adhered to relevant local regulation on the care and use of laboratory animals. Research protocols were approved by relevant research committees for animal care and use based on 3R principal (Reduction, Replacement, and Refinement). At the end of study, animals were euthanized by intraperitoneally injecting sodium pentobarbital solution at 40 mg/kg, followed by rapid exsanguination via abdominal aorta.

2.3. Pharmacokinetics (PK) studies

Two groups of 6 monkeys each with equal gender ratio were given a single subcutaneous injection, one group with the drug product at 0.1 mg/kg while the other group with un-PEGylated IL-11 at 0.1 mg/kg. For the group given the drug product, blood samples were collected before the injection and after injection at the following time-points: 1, 3, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144, 192, 240, 288, 336 h. For the group given the un-PEGylated IL-11, blood samples were collected before the injection and after the injection at the following time-point: 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48 h. For comparison, a group of 6 monkeys (3 males and 3 females) was also given the drug product at 0.1 mg/kg but with single intravenous administration. Their blood samples were collected pre-injection and post-injection at 0.0333, 0.0833, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 h, and were measured for the level of IL-11. For all blood collection, 0.5 mL of blood samples was collected in an EDTA-anticoagulation tube. After 10 min of centrifugation at 3000 rpm, plasma was collected and stored at 4 °C. These plasma samples containing PEGylated rhIL-11 were stable up to 30 days under a storage condition of 2–8 °C (data not shown).

The concentration of immunoreactive IL-11 in plasma samples was determined with the DuoSet ELISA kit for human IL-11 (R&D Systems, Cat. No. DY218), using 10% blank plasma in the Reagent Diluent provided as diluent. Data acquisition and processing of ELISA were performed using the software packages, ELISA Calc (Customized Applications, Inc.) with 4-parameter logistic regression. All pharmacokinetic calculations of the study were carried out with the drug and statistics (DAS) pharmacokinetic program 3.2.8 (Chinese Pharmacology Society), using noncompartmental methods. The bioavailability (F%) of subcutaneous administration was yielded by the following formula.

$F\% = (AUC_{0-\infty}\text{ of SC} \times \text{Dose of IV}) / (AUC_{0-\infty}\text{ of IV} \times \text{Dose of SC}) \times 100\%$.

2.4. Single dose toxicity studies

These studies were performed in a facility being certified for good laboratory practice by China FDA. Three groups of monkeys comprising one male and one female per group, were given a single subcutaneous injection, one group with a saline solution, while the other two with the drug product at a low dose (0.1 mg/kg) and a high dose (6.25 mg/kg) respectively. The group administered with saline solution was the vehicle control (saline solution). Notes for symptoms of toxicity as judged by the physical shape and behavior or bodily secretions were taken for next 6 h post-injection, followed by a twice daily observation period of 14 days. The amount of food consumption was noted once every week. Body weight was measured on D0 (day zero when given the drug), D1, D7 and D14, while hematological indices such as platelet count and biochemical indices such as alkaline phosphatase level were investigated on D-2 (day minus 2, i.e. 2 days before drug injection), D7 and D14. For hematology study, 2 mL of blood was collected in an EDTA-anticoagulation tube, while for biochemical analyses 3 mL of blood was collected. For hematology study, an automated blood analyzer (Siemens ADVIA2120i) was used. For biochemical analyses, an automated biochemical analyzer (Hitachi 7080) and commercial assay kits were used. For example, kits for alkaline phosphatase (ZCSEPO-012) and serum albumin (ZCJUNO-009) were purchased from Shanghai