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Optimized administration of hetIL-15 expands lymphocytes and minimizes toxicity in rhesus macaques

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ABSTRACT

The common γ-chain cytokine interleukin-15 (IL-15) plays a significant role in regulating innate and adaptive lymphocyte homeostasis and can stimulate anti-tumor activity of leukocytes. We have previously shown that the circulating IL-15 in the plasma is the heterodimeric form (hetIL-15), produced upon co-expression of IL-15 and IL-15 Receptor alpha (IL-15Rα) polypeptides in the same cell, heterodimerization of the two chains and secretion. We investigated the pharmacokinetic and pharmacodynamic profile and toxicity of purified human hetIL-15 cytokine upon injection in rhesus macaques. We compared the effects of repeated hetIL-15 administration during a two-week dosing cycle, using different subcutaneous dosing schemata, i.e. fixed doses of 0.5, 5 and 50 μg/kg or a doubling step-dose scheme ranging from 2 to 64 μg/kg. Following a fixed-dose regimen, dose-dependent peak plasma IL-15 levels decreased significantly between the first and last injection. The trough plasma IL-15 levels measured at 48 h after injections were significantly higher after the first dose, compared to subsequent doses. In contrast, following the step-dose regimen, the systemic exposure increased by more than 1 log between the first injection given at 2 μg/kg and the last injection given at 64 μg/kg, and the trough levels were comparable after each injection. Blood lymphocyte cell count, proliferation, and plasma IL-18 levels peaked at day 8 when hetIL-15 was provided at fixed doses, and at the end of the cycle following a step-dose regimen, suggesting that sustained expansion of target cells requires increasing doses of cytokine. Macaques treated with a 50 μg/kg dose showed moderate and transient toxicity, including fever, signs of capillary leak syndrome and renal dysfunction. In contrast, these effects were mild or absent using the step-dose regimen. The results provide a new method of optimal administration of this homeostatic cytokine and may have applications for the delivery of other cytokines.

1. Introduction

Interleukin-15 is a gamma-chain cytokine important for the survival, proliferation, mobilization and function of many lymphocyte subsets including NK, CD8, IEL and CD4 [1–6]. IL-15 is produced by stromal cells in several tissues, some blood endothelial cells and antigen presenting cells [7–9]. IL-15 is co-produced in the same cells with a second polypeptide chain, named IL-15 Receptor alpha (IL-15Rα) [10–13], and the two proteins form stable heterodimers in the Endoplasmic Reticulum (ER) of the producing cell [14,15]. The heterodimeric complex is transported to the plasma membrane where the IL-15Rα chain is cleaved at the extracellular domain by membrane associated proteases [14,16,17]. The soluble IL-15:IL-15Rα complex is released in the extracellular space and circulates in the plasma in both mice and humans [18]. This soluble IL-15:IL-15Rα complex is bioactive and has a long plasma half-life [14,16,19–21], thus representing a long-acting gamma-chain cytokine form *in vivo*.

Recombinant human single-chain IL-15 has been produced in *E. coli* (*sch* rhIL-15) as a non-glycosylated monomer of ~12 kDa [22] and has been shown to be immunostimulatory in macaques and humans. *sch* rhIL-15 has been tested in preclinical studies in macaques upon intravenous (IV) [23], subcutaneous (SC) [24] and continuous intravenous infusion (CIV) [24]. The first-in-human clinical trial of *sch* rhIL-15 delivered IV has been concluded [25], while a phase I clinical study of CIV is currently on-going in patients with advanced cancer (NCT01572493). Administration of IL-15 resulted in increased frequency and proliferation of peripheral NK cells and effector memory $CD8^+$ T cells [23,26]. Increased proliferation and tissue migration of

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$CD4^+$ effector memory cells were also reported [6]. Although administration of IL-15 was overall well tolerated, some toxicity was observed. In both non-human primates and human cancer patients, IV IL-15 administration was associated with hypotension, fever, chills and rigors beginning 2 h after IL-15 administrations [25,27]. Transient neutropenia with hepatic granulocyte accumulation was also observed in animals receiving the higher dose. Toxicities associated with IL-15 administration resolved after discontinuation of the treatment [27]. Limitations for the clinical use of *sch* rhIL-15 are its rapid plasma clearance and potential immunogenicity [24,27]. These problems can be overcome by the use of the heterodimeric complex of IL-15:IL-15Rα (hetIL-15) [16]. This form represents the naturally produced IL-15 in humans and it is currently evaluated in clinical trials in patients with refractory metastatic or unresectable cancer (NCT02452268). In the present work, we evaluated the pharmacokinetic and pharmacodynamic profile and toxicity of hetIL-15 in rhesus macaques. We also compare different doses and administration schemes to provide a regimen for the sustained optimal expansion of lymphocytes in the body.

2. Material and methods

2.1. hetIL-15 source

hetIL-15 is a heterodimer cytokine consisting of IL-15 and soluble IL-15Rα and was produced and purified from a cloned cell line derived from the human embryonic kidney HEK293 cell line transfected with optimized plasmid DNA encoding IL-15 and IL-15Rα [14,16,28,29], and grown in serum-Free Medium (Lonza). Purified, lyophilized hetIL-15 was reconstituted to the desired concentration using water for injection.

2.2. Treatment of rhesus macaques with hetIL-15

Rhesus macaques (*Macaca mulatta*) of Indian or Chinese origin were housed and handled in accordance with the Institutional Animal Care and Use Committee. Animals were housed at BIOQUAL, Inc. (Rockville, MD, US; animal welfare assurance no. A3086-01; protocol numbers 14-A478-11 and 17-024 and USDA Certificate number 51-R0036) and at Wuxi AppTec Animal Facility protocol #206-0001-TX (Suzhou, China). Animals were treated with hetIL-15 at the indicated doses either by IV or SC routes. The dose of hetIL-15 was calculated and expressed as the equivalent amount of single-chain IL-15 found within the heterodimer. Both single and repeated injection treatments were performed. A cohort of 48 monkeys was randomly assigned to 4 groups of 6/sex/group to determine the pharmacokinetics, pharmacodynamics and toxicity of hetIL-15 when administered in two dosing cycles over 6 weeks by the subcutaneous injection route. hetIL-15 was provided at 3 different doses, 0.5, 5, or 50 μg/kg, and the same dose was used for all the injections (fixed-dose regimen). The control group was administered vehicle (saline). Animals were randomly assigned to groups using a computer-generated randomization method (Provantis) based on body weight. Dosing cycle 1 was conducted on days 1, 3, 5, 8, 10 and 12 and dosing cycle 2 was conducted on days 29, 31, 33, 36, 38, and 40. The first day of dosing was designated as day 1. Animals were sacrificed at day 41 and 68. In additional studies with animals housed at BIOQUAL, Inc., monkeys were enrolled in a 2-week cycle following a doubling step-dose regimen. Injections were performed on days 1, 3, 5, 8, 10, and 12 at doses of 2, 4, 8, 16, 32 and 64 μg/kg, respectively (step-dose regimen). Animals were sacrificed at day 15. A list of animals enrolled in the protocols is shown as Supplementary Tables 1, 2 and 3.

2.3. Cytokine measurements in rhesus macaques

Rhesus macaques were bled at different time points prior, during and after either single or repeated IL-15 administrations and plasma was collected for cytokine measurements. IL-15 plasma levels were evaluated using a colorimetric immunoassay (Quantikine, D1500; R&D Systems), according to the manufacturer's instructions. IL-18 plasma levels were evaluated using a colorimetric immunoassay (MBL International), according to the manufacturer's instructions. These assays detect both human and rhesus macaque cytokines and allow the determination of endogenous plasma IL-15 and IL-18 levels prior to treatment or in control animals.

2.4. Analysis of lymphocyte subsets in blood and tissue

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation and cryopreserved in liquid nitrogen until analysis. Immunophenotypic analysis was performed using the following directly conjugated anti-human antibodies: APC-Cy7 CD3 (Clone SP34-2) and V500 CD4 (clone L200) obtained from BD Biosciences; AF405-CD8 (Caltag; clone 3B5). Cell proliferation was monitored by staining with AF700- or FITC-Ki-67 Ab in cells permeabilized with the Foxp3 Staining Buffer Set (eBioscience). All the samples were acquired in a LSR II Flow Cytometer (BD) and analyzed using FlowJo software (Tree Star, Inc., Ashland, OR).

2.5. Physical examinations and clinical pathology

2.5.1. Body temperature

Body temperature was recorded pre-dose and at approximately 4–6 and 24 h post-dose.

2.5.2. Body weight

Each animal was weighed once during pretest, on Day -1, and then once weekly throughout the dosing phases.

2.5.3. Hematology and clinical chemistry

All study animals were evaluated for hematology and clinical chemistry at the indicated time points. Hematology blood samples were also collected on days 1, 3, 8 and 15 for lymphocyte counts. Blood samples for hematology, coagulation, and clinical chemistry evaluations were obtained from a cephalic vein. Animals were compared to control animals assayed at the same time in the same facility.

2.6. Data and statistical analysis

Pharmacokinetic analysis of hetIL-15 plasma concentration–time data was performed using WinNonlin v6.3 (Pharsight Corp, Cary, NC). AUC was calculated using the linear up/log down trapezoidal rule by noncompartmental methods from drug-treated animals only. AUC and C_{max} ratios were used to evaluate dose–proportionality and drug accumulation. Differences were evaluated by 1-way ANOVA or unpaired student's *t* test. The *p*-values were corrected for multiple comparisons using Holm-Sidak test. Prism 6.0c software package (GraphPad Software, Inc., La Jolla, CA) was used for analysis.

3. Results

3.1. Administration of hetIL-15 via SC route results in increased half-life and reduced C_{max} in comparison to IV route

We have previously shown that administration of purified human hetIL-15 in mice resulted in sustained IL-15 plasma levels and in robust expansion of NK and T cells [16], demonstrating *in vivo* stability and bioactivity superior to *E. coli* derived single-chain *sch* rhIL-15. In this study, we evaluated the pharmacokinetic/pharmacodynamic profiles and toxicity of hetIL-15 upon administration in rhesus macaques. To determine the hetIL-15 pharmacokinetics, a total of 16 macaques received a single injection of the cytokine via either IV or SC routes. At the dose of 5 μg/kg delivered IV, the peak IL-15 plasma level was determined as ~55 ng/ml at 10 min after injection. The exponential