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Pharmacokinetics, disease-modifying activity, and safety of an experimental therapeutic targeting an immunological isoform of macrophage migration inhibitory factor, in rat glomerulonephritis

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ABSTRACT

New therapeutic agents are needed to overcome the toxicity and suboptimal efficacy observed in current treatment of glomerulonephritis (GN). BaxB01 is a fully human monoclonal antibody targeting a disease-related immunologically distinct isoform of Macrophage migration Inhibitory Factor (MIF), designated oxidized MIF (oxMIF) and locally expressed in inflammatory conditions. We report the pharmacokinetic profile of BaxB01, and its dose and exposure-related disease-modifying activity in experimentally induced rat GN.

BaxB01 bound to rat oxMIF with high affinity and reduced rat macrophage migration in vitro. After intravenous administration in rats, BaxB01 demonstrated favorable pharmacokinetics, with a half-life of up to nine days. Disease modification was dose-related (≥ 10 mg/kg) as demonstrated by significantly reduced proteinuria and diminished histopathological glomerular crescent formation. Importantly, a single dose was sufficient to establish an exposure-related, anti-inflammatory milieu via amelioration of glomerular cellular inflammation. Pharmacodynamic modeling corroborated these findings, consistently predicting plasma exposures that were effective in attenuating both anti-inflammatory activity and reducing loss of kidney function. This pharmacologic benefit on glomerular function and structure was sustained during established disease, while correlation analyses confirmed a link between the antibody's anti-inflammatory activity and reduced crescent formation in individual rats. Finally, safety assessment in rats showed that the experimental therapeutic was well tolerated without signs of systemic toxicity or negative impact on kidney function.

These data define therapeutically relevant exposures correlated with mechanism-based activity in GN, while toxicological evaluation suggests a large therapeutic index and provides evidence for achieving safe and effective exposure to a MIF isoform-directed therapeutic in nephritis-associated disease.

1. Introduction

Crescentic glomerulonephritis (GN) presents with anti-glomerular basement membrane (GBM) antibodies or immune complexes deposited in glomeruli, but also pauci-immune without deposits of immunoglobulins or complement in glomeruli, and is a major cause of rapidly progressive renal failure and severe glomerulosclerosis (Tam et al., 1999), and is associated with activation of complement and Fc receptors and recruitment and infiltration of leukocytes in particular T-cells, neutrophils and monocytes (Kurts et al., 2013). These cells are a major constituent of the crescentic lesions and persist locally within the inflammatory site producing cytokines that propagate inflammatory tissue damage and rapidly progressive nephritis (Peterson and Winchester, 2005).

Conventional immunosuppressive therapy of inflammatory kidney disease is associated with considerable toxic effects and suboptimal efficacy. Therefore, new therapeutic agents that overcome these issues are needed, and biologic agents offer exciting opportunities (Smith

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et al., 2010). While current treatment induces initial remission in many patients, the actual renal relapse rates are still high and favor prolonged immunosuppression (Houssiau, 2012). Sequelae of inflammatory kidney diseases, such as ANCA-Vasculitis, Goodpasture disease or Lupus nephritis (LN), also encompass a potential for rapid progressive GN and therefore disease management might require attenuation of inflammation to curtail further renal damage and suppression of autoimmunity to prevent exacerbation of disease (Parikh and Rovin, 2016).

Macrophage Migration Inhibitory Factor (MIF) is a regulatory cytokine of the innate and adaptive immune response and is produced, stored, and secreted by macrophages and other cells of the immune system (Lolis and Bucala, 2003). MIF is therefore considered a promising and rational therapeutic target across inflammatory conditions (Hoi et al., 2007; Leng et al., 2011; Lang et al., 2015; Bruchfeld et al., 2016). Anti-MIF treatment has previously been shown to prevent loss of renal function and to inhibit renal leukocyte infiltration and activation in an experimental model of rat GN (Yang et al., 1998) induced by administration of nephrotoxic serum (NTS). The murine NTS model is dependent on kidney-infiltrating T helper (Th) 17 cells and then followed by infiltrating Th1 cells, which recruit neutrophils and macrophages, respectively, and cause sustained kidney inflammation. In a later phase of disease, regulatory T cells (Tregs) might infiltrate the kidney in an attempt to limit disease activity (Kurts et al., 2013; Artinger et al., 2017). Nonetheless, the pleiotropic functions and ubiquitous cellular synthesis of MIF by non-immune and immune cells pose conceptual difficulties in developing safe and targeted pharmacological interventions.

BaxB01 is a previously described fully human monoclonal IgG1 antibody (mAb) and has been shown to neutralize the biologic function of MIF (Kerschbaumer et al., 2012). Furthermore, BaxB01 has been shown to ameliorate inflammatory conditions including GN and to bind with high specificity to oxMIF, a redox dependent and immunologically distinct conformational isoform of MIF, present in the circulation of SLE patients and in the urine of patients with acute LN (Thiele et al., 2015).

Here, we describe for the first time the pharmacokinetics (PK) of BaxB01 in the rat, and dose-related disease-modifying activity in experimental immune GN. We also deduced minimally effective dose levels and applied pharmacodynamic (PD) modeling to define exposure levels for optimal attenuation of disease progression. The data link posology with anti-inflammatory mechanism and ameliorated glomerular histopathology. Finally, we provide evidence for the safety and tolerability of this novel therapeutic approach in the treatment of inflammatory kidney disease.

2. Materials and methods

2.1. Materials

Recombinant human or rat MIF was produced in *Escherichia coli* BL21 (Stratagene, La Jolla, CA) and monoclonal anti-oxMIF antibody BaxB01 was produced in Chinese hamster ovary cells and purified as previously described (Kerschbaumer et al., 2012). Nephrotoxic anti-rat GBM serum (NTS) was prepared by subcutaneous immunization of New Zealand White rabbits with 1 mg of GBM in 1 ml of complete Freund's adjuvant (Sigma-Aldrich, St. Louis, MO); and anti-rat RBC antibodies were absorbed as previously described (Tam et al., 1999). LAL test results on NTS indicated endotoxin levels < 0.3 EU/ml.

All animal experiments were approved by a local IACUC and conducted in compliance with applicable national laws and regulations in AAALAC accredited facilities.

2.2. BAXB01 affinity

BaxB01 or a non-binding control IgG1-antibody was immobilized to Biacore CM5 optical sensor chips (GE Healthcare, Piscataway, NJ) using standard amine coupling conditions. Rat or human recombinant MIF were diluted in HBS-EP buffer (GE Healthcare) to concentrations of 50, 75, 100, or 150 nM in the presence of 0.2% Proclin300 (active component 5-chloro-2-methyl-4-isothiazolin-3-one; Sigma) to transform MIF into an oxMIF surrogate (Thiele M et al., 2015). Proclin treated MIF was applied to immobilized BaxB01 and affinity measured with a Biacore™ 3000 Instrument (GE Healthcare). The kinetics of the concentration series were analyzed by local simultaneous association/dissociation fitting of each binding curve to the iterative Langmuir 1:1 interaction model with mass transfer compensation provided by the BiaEvaluation software (GE Healthcare).

2.3. Rat monocyte cell migration assay

BaxB01 inhibition of random migration of rat monocytes was tested in a Transwell™ Chemotaxis assay (Corning, Tewksbury, MA 01876). Briefly, rat alveolar macrophages ([AgC11 × 3A, NR8383.1]; ATCC® CRL-2192) were seeded at a density of 10^6 cells/ml in serum-free culture media (F12K; Life Technologies, Carlsbad, CA) in the top chambers of porous Transwell™ inserts (8 μm; Corning, Tewksbury, MA). Cell migration towards BaxB01 (0.04–30 nM) in the lower chamber was determined in five separate experiments using microscopy after 16 h at 37 C. IC50 values were calculated by 4-parameter logistic nonlinear regression of the number of migrated cells normalized to buffer negative control (chemotactic index) using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

2.4. PK analysis in rat plasma

Briefly, sheep anti-human IgG1 (The Binding Site, Birmingham, UK; TBS AU006), diluted (1:500) in 0.1 M carbonate buffer (pH 9.5) was coated to 96-well Maxisorp F96 plates (Nalge Nunc, Rochester, NY) overnight. After a washing step with PBST (phosphate-buffered saline with 0.05% Tween 20), the wells were blocked with PBST with 0.1% gelatin, 2 mM benzamidine, at 37 °C for 1 h. Then samples or standards (CRM470 IgG1 calibration curve ranging from 15.7 to 252 ng/ml in five serial dilutions) were loaded in duplicate. After 15 min-incubation at room temperature (RT), affinity-purified sheep anti-human IgG1 antibody peroxidase conjugate (The Binding Site, Birmingham, UK; AP-006) was added and incubated at RT for 1 h. After washing, tetramethylbenzidine substrate SureBlue (KPL, Gaithersburg, MD) was added and the peroxidase reaction stopped with 1.5 M sulfuric acid. Absorbance was determined in an ELISA reader at 450/620 nm. Quantitative evaluation was based on a double-logarithmic calibration curve approach correlating blank-corrected mean optical densities (ODs) of the standard's concentrations with their IgG1 concentrations. This calibration curve was used to calculate IgG1 concentrations of the samples.

2.5. Pharmacokinetics (PK) study in healthy and diseased Wistar Kyoto rats

Male Wistar Kyoto (WKY) rats (Charles River, Sulzfeld, Germany) received a single IV bolus injection of BaxB01 (50 mg/kg) and blood samples (0.4 ml) were drawn from the ventral tail artery (batch design, n = 3/time point) three days before and 15 min and 1, 3, 7, 10, and 14 days after administration. Similarly, blood from diseased animals was sampled at designated time points starting four days after disease induction with rabbit NTS (described below). Whole blood was collected and processed as 3.8% trisodium citrated plasma and stored in 100 μL aliquots at ≤ −20 °C. To compare PK parameters from healthy and diseased rats, the same nominal dose of BaxB01, rat strain, body weight, and time points were used.

2.6. PK/PD efficacy study in diseased Wistar Kyoto (WYK) rats

104 male WKY rats (Charles River, Stone Ridge, NY) were assigned