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Btk inhibition treats TLR7/IFN driven murine lupus

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ABSTRACT

Bruton's tyrosine kinase (Btk) is expressed in a variety of immune cells and previous work has demonstrated that blocking Btk is a promising strategy for treating autoimmune diseases. Herein, we utilized a tool Btk inhibitor, M7583, to determine the therapeutic efficacy of Btk inhibition in two mouse lupus models driven by TLR7 activation and type I interferon. In BXSB-*Yaa* lupus mice, Btk inhibition reduced autoantibodies, nephritis, and mortality. In the pristane-induced DBA/1 lupus model, Btk inhibition suppressed arthritis, but autoantibodies and the IFN gene signature were not significantly affected; suggesting efficacy was mediated through inhibition of Fc receptors. In vitro studies using primary human macrophages revealed that Btk inhibition can block activation by immune complexes and TLR7 which contributes to tissue damage in SLE. Overall, our results provide translational insight into how Btk inhibition may provide benefit to a variety of SLE patients by affecting both BCR and FcR signaling.

1. Introduction

Bruton's tyrosine kinase (Btk) is expressed in a large variety of immune cells including monocytes, macrophages, basophils, mast cells, and B cells. Btk regulates signaling downstream of the B cell receptor (BCR) and Fc receptors (FcR) and may also play a role in toll-like receptor (TLR) signaling [1,2]. Previous studies using Btk deficient mice and Btk inhibitors have demonstrated that loss of Btk activity can ameliorate disease in animal models of autoimmune disorders including rheumatoid arthritis [3] and systemic lupus erythematosus (SLE) [4–7]. Consequently, Btk is considered a promising drug target for treatment of these diseases.

SLE is an extremely heterogeneous disease that manifests with a wide variety of symptoms including fatigue, rash, arthritis, nephritis, and neurological dysfunction. The pathogenesis and etiology of the disease are also diverse with patients showing different environmental exposures, genetic predispositions, autoantibody profiles, and various cellular dysfunctions. Given the high degree of heterogeneity in SLE, it would be decidedly beneficial to use preclinical animal models that represent different patient populations to learn how inhibition of a certain pathway or target affects disease. One drug target of interest is Btk, as this enzyme regulates signaling in several pathways relevant to autoimmune disease. Btk inhibitors have previously been tested and found to be efficacious in the NZB/W [4,6], MRL/*lpr* [7], and B6.Sle1 [5] mouse models of SLE. However, these models do not recapitulate all the different subsets of lupus patients, and therefore there is a gap in knowledge as to how Btk inhibition may affect disease driven by TLR activation and type I interferon (IFN). Thus, we wished to test the potential for Btk inhibition to provide therapeutic benefit in SLE mouse models that are more TLR7-driven and have a higher IFN involvement.

Substantial evidence exists implicating TLR7 [8] and IFN [9] in the pathogenesis of human lupus and anti-IFN agents are currently being evaluated in clinical trials [10]. The IFN gene signature, which serves as a marker for high IFN activity, has been shown to be elevated in as much as 50% of all lupus patients. Polymorphisms in these pathways have been found to increase the risk for developing SLE [11] and females have demonstrated higher sensitivity to TLR7 activation [12], consistent with their greater propensity for SLE development. Additionally, there have been correlation studies of high IFN in SLE patients and those with high IFN are likely to develop more severe disease [10]. However, these patients have not been well represented in prior preclinical studies testing Btk inhibition and little is known about how Btk blockade might affect TLR7 and IFN-driven disease processes.

Abbreviations: Btk, Bruton's tyrosine kinase; TLR7, Toll-like receptor 7; BCR, B cell receptor; FcR, Fc receptor; SLE, systemic lupus erythematosus; IFN, interferon; FACS, fluorescence-activated cell sorting; MMF, mycophenolate mofetil.

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In the studies presented herein the BXSB-*Yaa* and pristane-DBA/1 models of lupus were utilized to test the potential efficacy of Btk inhibition for treating TLR7 and IFN-driven SLE. Previous results in related models have suggested that genetic Btk deficiency may affect disease in the context of *Yaa* duplication [13] or after pristane injection [14] and we wished to determine if a reduction in Btk activity by treatment with a pharmacological inhibitor could have similar efficacy. BXSB-*Yaa* mice have a duplication of the *Yaa* locus, which results in increased expression of TLR7 and spontaneous development of disease [13,15]. The lupus-like disease that develops is characterized by production of autoantibodies, lymphoid hyperplasia, and nephritis which leads to early mortality. Proteinuria is an early marker of nephritis in these mice and histological analysis of kidneys reveals evidence of severe glomerulonephritis. In the pristane-DBA/1 model, DBA/1 strain mice are injected in the peritoneum with the hydrocarbon pristane which induces disease development. The disease has mechanistically been characterized as highly TLR7 and IFN dependent [16] and involves generation of autoantibodies and development of arthritis [17]. The arthritis that develops is autoantibody-mediated and has similarities to that of SLE patients as it manifests as a mild erosive disease [18]. The pristane model is one of the very few mouse lupus models that has been demonstrated to display a robust IFN gene signature [17] and it therefore may be particularly relevant as a model for IFN high lupus patients. The autoantibody production in pristane-DBA/1 mice is biased toward reactivities against RNA-binding proteins such as RiboP and SmRNP, more so than against dsDNA or other DNA associated molecules, similar to what has been observed for SLE patients with high IFN signatures as they demonstrate a biased increase in RNA-binding reactivities [19,20]. Even though these two models both are highly TLR7 dependent, they may represent distinct subsets of lupus as they have different pathogenesis, autoantibody profiles, and end organ disease manifestations. Thus, utilizing these two different models allows for interrogating the effect of Btk inhibition on TLR7-driven disease with a variety of readouts.

In our studies we utilized a small molecule Btk inhibitor, M7583, as a tool to assess the potential for Btk inhibition to treat lupus in the BXSB-*Yaa* and pristane-DBA/1 mouse lupus models. We first present a characterization of the compound to demonstrate its suitability as an effective Btk specific inhibitor suitable for in vivo use, and then demonstrate the efficacy of Btk inhibition for treatment of disease in the two models. We also performed studies using FACS, gene expression analysis, and human immune cells to further characterize the mechanism of action of Btk inhibition efficacy and how this might translate in humans. This work demonstrates the potential for Btk inhibitors to provide benefit to lupus patients of different disease subsets and provides mechanistic insight as to how Btk inhibitors may affect multiple immunological processes mediating disease pathogenesis.

2. Methods

2.1. Mouse lupus models

All procedures using animals were performed in accordance with the EMD Serono Institutional Animal Care and Use Committee (IACUC) and all local and national laws and regulations regarding animal care. Female DBA/1 mice used for pristane model studies were purchased from Jackson Labs. To induce disease development DBA/1 mice were injected with 0.5 ml of pristane (Sigma) i.p. at 11–12 weeks of age. Beginning at 2 months after pristane injection mice were fed chow formulated with M7583 at a concentration of 25 mg of compound/kg of chow. The consumption of chow was measured weekly and body weights were recorded and these two values were used to calculate the dose of compound received per mouse which averaged 3.35 mg/kg over the course of the study. Mice were also fed chow formulated with mycophenolate mofetil (MMF) (Selleck Chemicals) at a concentration of 500 mg/kg of chow which resulted in a dosage of 63.6 mg/kg. Vehicle group mice were fed chow of the same diet (Harlan Teklad 2018) without any added compounds. Chow formulations were made by Research Diets, Inc. At 6 months after pristane injection mice were euthanized via CO_2 asphyxiation and blood was collected via the vena cava. Spleens were collected and split in half for FACS analysis or gene expression analysis, and paws were preserved in formalin for histology analysis.

Male BXSB-*Yaa* mice were purchased from Jackson Labs and at 9 weeks of age treatment was initiated using the same formulated chows described above fed to the DBA/1 mice. Based on chow consumption and body weights, BXSB-*Yaa* mice received on average 3.7 mg/kg of M7583 and 74.4 mg/kg of MMF. At 20 weeks of age mice were euthanized via CO_2 asphyxiation and blood was collected via the vena cava. Spleens were collected and split in half for FACS analysis or gene expression analysis, and kidneys were preserved for histology analysis.

2.2. Arthritis and nephritis assessments

Arthritis development in pristane-injected mice was monitored over time and clinical scores were assigned based on inflammation and swelling similar to a scoring system previously detailed [21]. Mice were scored on a scale of 0–4 per paw with 4 being the most severe arthritis, and scores from all 4 paws were summed for a total score. At the conclusion of the study paws were removed, fixed in formalin and shipped to HistoTox Labs where they were processed for toluidine blue staining and scored for histological evidence of damage by a trained pathologist. For monitoring of proteinuria in BXSB-*Yaa* mice, urine was collected by bladder massage in the morning on 2 consecutive days and the samples were pooled. The levels of albumin and creatinine in the urine were determined using the Advia 1800 clinical chemistry analyzer (Siemens). The urinary albumin-to-creatinine ratio (UACR) was calculated as the ratio of milligrams of albumin per gram of creatinine per deciliter of urine. At the conclusion of BXSB-*Yaa* studies kidneys were collected, fixed in formalin and shipped to HistoTox Labs where they were processed for hematoxylin and eosin staining and scored for histological evidence of damage by a trained pathologist. The scoring system used was modified from a previously published system [22] and evaluates kidney sections based on glomerular crescents, protein casts, interstitial inflammation, and vasculitis and a total histology score is obtained based on a composite score of these parameters.

2.3. In vitro assays

The potency for M7583 against Btk was determined using purified rBtk (Carna Biosciences). The Btk protein was diluted in buffer to a final of 0.05 ng/μl with 75 μM ATP and 1 μM of the KinKDR peptide FITC-AHA-EEPLYWSFPAKKK-NH2 (Tufts Core Facility, Boston MA). Various concentrations of M7583 were also included. Reactions were performed at 25 C for 90 min and halted by addition of stop solution containing 0.5 M EDTA. Plates were then read on the Caliper LabChip 3000 (Caliper Life Sciences) and the data was loaded into GeneData Screener for generation of IC_{50} curves.

Kinase selectivity for M7583 was determined in the Kinase Profiler™ screening panel (EMD Millipore) that tested the inhibitory activity of the compound at 1 μM against 270 kinases. The biological selectivity of M7583 was assessed in vitro using primary human cells with BioMap® profiling by BioSeek Inc. The activity of the compound was assessed using a concentration range of 1 nM to 1 μM in 12 different primary cell co-culture assay systems according to previously published methods [23].

The ability of M7583 to block FcR signaling was determined using basophils in whole blood. Human blood was collected with citrate as an anticoagulant and transferred to 96 well plates. Blood was pretreated for 30 min at 37 °C with dilutions of M7583 before activation with anti-IgE (Beckman Coulter) added to a final of 2 μg/ml and incubated at 37 °C for 5 min. After activation, cells were stained for 15 min with anti-CD63-FITC (BD Biosciences) and then PBS-EDTA (20 mM) was