Dalazatide (ShK-186), a Kv1.3 Inhibitor that Targets Effector Memory T Cells: Ex Vivo Studies in Pediatric Systemic Lupus Erythematosus

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Background: T cell activation depends upon a calcium signaling cascade that is regulated by voltage-gated potassium channels. Effector memory T cells (TEMs), which are implicated in the immunopathogenesis of a range of autoimmune diseases, express the potassium channel Kv1.3. Dalazatide is a potent peptide inhibitor of the Kv1.3 channel that has recently shown efficacy in a Phase 1/2 placebo-controlled trial. Evidence suggests that inflammatory cytokine producing TEM cells might be involved in the immunopathogenesis of lupus nephritis.

Objectives: Provide proof-of-principle ex vivo data for therapeutically targeting chronic T cell activation in systemic lupus erythematosus (SLE) by using Dalazatide to block the Kv1.3 channel and to evaluate the effect of dalazatide on the in vitro immunoregulatory production of TEM cells from SLE patients.

Methods: Peripheral blood mononuclear cells from pediatric and adult SLE patients and as well as healthy controls were studied. T lymphocyte subsets were assayed ex vivo for Kv1.3 expression by flow cytometry. The effect of dalazatide on PMA/ionomycin-induced inflammatory cytokine expression by TEM cells was evaluated by intracellular cytokine staining.

Results: Kv1.3 expression by CD4+ TEM cells was significantly higher in patients with active lupus nephritis when compared to patients with inactive SLE or healthy controls. Dalazatide inhibited TNF-α, IL-17 and IFN-γ production by both CD4+ and CD8+ TEM cells from SLE patients in a dose-dependent manner. Higher levels of dalazatide-mediated inhibition were observed in IFN-γ- and TNF-α- expressing CD4+ TEM cells from patients with active SLE when compared to samples from SLE patients with inactive disease.

Conclusions: Our ex vivo studies suggest that dalazatide inhibition of Kv1.3 may be a therapeutic target for SLE. In addition, Kv1.3 expression may be a useful biomarker of disease activity in SLE.

1. Dalazatide Targets Kv1.3 Channels on Autoeffecter Effector Memory T Cells

Kv1.3 and KClα1 are K+ channels needed to maintain membrane potential (by allowing K+ efflux) during T cell activation and the requisite increase in intracellular Ca²⁺.

While KClα1 is important in activation of naive and central memory T cells, Kv1.3 is required for activation of effector memory T cells which are pathogenic in several autoimmune disorders including Psoriasis, Psoriatic Arthritis, Multiple Sclerosis, Type 1 Diabetes and Rheumatoid Arthritis. [Chu et al., 2012]

DALAZ is a highly potent and specific (37-amino acid) peptide inhibitor of the Kv1.3 channel. It is the first Kv1.3 blocker to have entered clinical trials.

2. Kv1.3 in SLE

During times of disease, lupus nephritis patients show an increase of urinary (8-diol) TAME. [Oyewale et al., 2012]

KClα1 is the main K+ channel responsible for Ca²⁺-homestasis in hyperactive SLE T cells. [Nicolaou et al., 2007, 2010]

Blocking Kv1.3 with SHK-186 could return hyperactive T cells to a resting state.

3. Immunofluorescence studies of Kv1.3 in lupus nephritis patient samples

A. Activated CD4+ TEM cells express Kv1.3.

B. Kv1.3 on TEM activated in vitro

C. Kv1.3 on SLE urinary lymphocytes

CD4+ T memory cells activated in vitro were analyzed by flow cytometry (A), and immunofluorescence (B). Kv1.3 (green), DAPI nuclear stain (blue). C. Four fields of urinary cells from a patient with lupus nephritis.

4. Kv1.3 expression elevated in SLE T effector memory Cells

5. Dalazatide (ShK-186) Effect on Cytokine Expression

Dalazatide decreases proinflammatory cytokine production in CD4+ TEMs.

6. Current Status and Future Plans

Current Status

- Validation of dalazatide blockade in-vitro in SLE patient cells
- Development of assays to identify novel dalazatide-sensitive biomarkers
- Immunofluorescence to quantify Kv1.3 expression in urinary T cells and renal biopsy samples in SLE

Future Plans

- Proof of concept studies to work towards an adult and pediatric SLE clinical trial
- KPI and Kineta are currently seeking a partner(s) to maximize the potential of the dalazatide / ShK franchise.

References

[Adapted from Chu et al., 2007; Nicolaou et al., 2007; Nicolaou et al., 2010]