

Blockade of mini-TrpRS for treatment of diabetic foot syndrome

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Abstract

Diabetic foot syndrome demonstrates wound chronicity due to impaired tissue perfusion in lower limbs. Previous studies showed interferon-gamma (IFN- γ), a central inflammatory mediator in diabetic foot syndrome, to induce the truncated form of tryptophanyl-tRNA synthetase (mini-TrpRS) that has strong angiostatic properties. Recently we reported that mini-TrpRS signalling could be blocked in the presence of IFN- γ with D-tryptophan *in vitro*. Here we discuss the IFN- γ /mini-TrpRS axis in the pathology of diabetic foot syndrome and emerging therapeutic options.

Background

Approximately 25% of patients with diabetes will develop diabetic foot syndrome [1]. This severe complication of progressing diabetes is responsible for approximately 85% of lower limb amputations associated with a dramatic decrease in quality of life and increased mortality [2]. The aetiology of diabetes can be traced to well-recognized risk factors such as genetic predisposition, ageing, male sex, and lifestyle factors, including unhealthy diet and low physical activity. Most of these traditional risk factors are either non-modifiable (genetics, ageing, sex) or hard to modify in diabetic foot syndrome patients such as exercise or even diet. The substantial evidence, however, suggests that inflammatory pathways are the principal and common pathogenic mediators in the course of diabetes and its complications under stimuli of traditional risk factors [3,4]. Among them, interferon-gamma (IFN- γ), an important pro-inflammatory cytokine primarily involved in the host defence against acute infections, is also chronically elevated in patients with diabetes and considered as a crucial risk factor for diabetic foot syndrome [5,6]. In particular, IFN- γ is a critical cytokine in angiostatic responses that impairs normal tissue perfusion and microvascular reaction to injury [7]. Despite the importance of the anti-angiogenic properties of IFN- γ , the molecular mechanisms by which IFN- γ elicits its actions on vasculature remained poorly understood. Our recent findings support novel thinking to how IFN- γ may only indirectly contribute to the pathology of diabetic foot syndrome by up-regulating angiostatic pathways and hold promising treatment implications for patients with this debilitating complication of diabetes.

The problem

Impaired tissue perfusion in lower limbs is a leading cause of wound chronicity in patients with progressing diabetes. Innovations in enhancing wound revascularization would lead to a significant improvement in patient care and their quality of life.

The rationale and solution

The *WARS1* gene-encoded human tryptophanyl-tRNA synthetase (TrpRS) exists as two forms; a full-length protein and a truncated

variant known as 'mini-TrpRS' in which most of the N-terminal domain is deleted during alternative splicing of the *WARS1* precursor mRNA [8]. IFN- γ strongly stimulates the production of mini-TrpRS, and it is the only human tRNA-ligase whose expression is induced by IFN- γ [9]. The significance of this phenomenon was recognized following the discovery that mini-TrpRS, unlike its full-length variant, is also a potent negative regulator of angiogenesis outside its primary role in aminoacylation [10-12]. Angiostatic activities of human mini-TrpRS were extensively studied using several human and animal models, including human umbilical vein endothelial cells (HUVEC) migration and proliferation, chick chorioallantoic membrane, mouse matrigel, and mouse retinal angiogenesis assays [10]. Importantly, our recent discovery shows that mini-TrpRS signalling in the presence of IFN- γ *in vitro* can be effectively blocked with D-tryptophan, a cognate amino acid acting as a decoy substrate for mini-TrpRS [13]. It has become evident that mini-TrpRS signalling inevitably occurs in the context of more complex regulation mediated by IFN- γ to execute specific outcomes depending on the pathological environment and the cell type involved [13,14]. An important question, whether a direct neutralization of mini-TrpRS would limit the progression of diabetic foot syndrome, is posed. In this regard, D-tryptophan would appear an ideal intervention to be investigated in patients with diabetic foot syndrome. Chemically, D-tryptophan is an enantiomer of L-tryptophan, an essential amino acid found in most human proteins, and a precursor to the neurotransmitter serotonin, the hormone melatonin, and vitamin B3 [15]. In experimental models, D-tryptophan is readily cleared from

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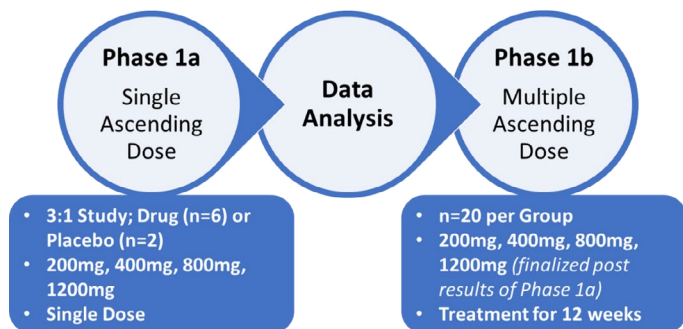


Figure 1. Schematic of Phase 1a/b clinical trial of D-tryptophan for treating patients with diabetic foot syndrome

plasma, and there is no appreciable conversion of D-tryptophan to metabolically active L-tryptophan [16,17]. As such, side effects such as unintended stimulation of serotonin production in the brain [16] or other metabolic functions of the L-tryptophan [17] would be expected to be negligible in treated patients with a diabetic foot ulcer. Indeed, D-tryptophan, also known as NLG8189 and Indoximod (methylated D-tryptophan), has not been trialled exactly in patients with diabetic foot syndrome but oncological patients; the drug was investigated at a dose up to 2,000 mg twice daily, was well tolerated, and no patients discontinued treatment due to toxicity [18]. A dose up to 800 mg showed good oral bioavailability with a linear dose-response of the area under the curve (AUC) and the maximum serum concentration (C_{max}) [18]. Within this frame of reference, studies investigating diabetic foot syndrome therapy based on D-tryptophan are planned for future work.

The therapy

We plan a double-blind, placebo-controlled clinical trial to evaluate the safety, tolerability, Pharmacokinetic (PK), Pharmacodynamics (PD), and explore the effect of D-tryptophan combined with standard wound care compared to placebo in patients with diabetic foot syndrome. In phase 1a, we will assess a single ascending dose (SAD) followed by a multiple ascending dose (MAD) study over 12 weeks in phase 1b (Figure 1). The development of a novel pharmacological treatment based on D-tryptophan is directed at combining with the current standard of wound care to aid with the clinical management of this debilitating complication of diabetes.

The primary objective is to assess the safety and pharmacokinetic tolerability of D-tryptophan in treated individuals (Phase 1a), with the secondary objective to assess the effect of D-tryptophan on biological markers of wound healing.

Authorship and contribution

Conceptualization, EB, VV, UHM, and CSM; Writing – Original Draft Preparation, EB and VV; Writing – Review & Editing, UHM, and CSM; Visualization, VV and CSM.

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Conflict of Interest

None.

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