BSc/MSci Applied Medical Sciences

Title:

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Alternative to Doxycycline: Examination of Zinc Chelator Inhibition of Matrix Metalloproteinase, an ECM Degrading Protein, in Ehlers Danlos Syndromes

Abstract:

Concise summary of disease area, method and impact – 300 words maximum in box below

Ehlers Danlos Syndromes (EDS) encompass a group of 13 heterogenous connective tissue disorders in which genetic mutations contribute to defects in the structure, production, or processing of collagen. Clinical manifestations include hyperextensibility of the skin as well as soft, hypermobile joints associated with the absence of collagen. [1,2] The protein collagen functions to maintain extracellular matrix (ECM) stability and can be characterized by its high tensile strength, solubility, immunogenicity, and flexibility. While research regarding the function of collagen has been well established, there is still more to understand about ECM dysfunction. Recent EDS research has uncovered the therapeutic promise of inhibiting matrix metalloproteinase (MMP) production to protect the ECM. As it relates to EDS disorders, MMP1 is overexpressed and facilitates the breakdown of type I, II, III, VII, and X collagen. Trials of MMP inhibitor (MMPi), doxycycline, reverted EDS cells myofibroblast-like differentiation and halted ECM proteolysis. Based on these observations, it was predicted that in vivo studies using doxycycline would stop disease progression and potentially alleviate clinical symptoms. Although this progress offers hope to many EDS patients, it is important to note that these experiments were performed exclusively on Hypermobile Ehlers Danlos Syndrome (hEDS) cells in vitro. [1] Further, doxycycline is classified as a nonselective inhibitor with side effects damaging to bone health. In hopes of solidifying a treatment option for EDS, this proposed study looks to supplement prior research by testing zinc chelators, a known MMPi, on multiple EDS subtypes in vitro. Research of MMP inhibition would have direct impact on EDS patients and their families. Additionally, developments for EDS have the potential to improve treatment strategies for many other diseases including hereditary collagen disorders such as Osteogenesis Imperfecta, and Marfan Syndrome as well as cancers.

Introduction:

To include background and significance of the disease - 400 words maximum in box below.

The first classification of EDS was published in the 1960s with an international connective tissue nosology comprising of 9 subtypes. Now however, EDS has 13 subtypes classified with unique diagnostic criterion. Collectively, the prevalence of EDS disorders is about 1 in 3,500 people. [3,4] Hypermobile EDS (hEDS) has the highest prevalence, 1 in 5,000 people, compared to the second highest being classical EDS with an occurrence of 1 in 20-40,000 people. [3,5] The danger in a disease affecting the ECM is the risk posed to blood vessels, organs, skin, and bones. The symptoms associated with each EDS subtype correspond to specific mutations affecting specific types of collagens. Vascular EDS (vEDS), for example, is autosomal dominant and associated with a mutation in the COL3A1 gene and sometimes COL1A1 gene. The COL3A1 gene corresponds to type III collagen, a fibrillar collagen typically found in the walls of blood vessels, lungs,

cartilage and bone. The mutation of COL3A1 could involve duplication, splice site alterations, or substitutions of glycine at the triple helical region causing slow folding and more post translational modifications. These mutations alter mobility and affect the morphology of type III collagen leading to symptoms of vascular ruptures, gastrointestinal perforation, pulmonary pneumothoraces, and additional complications. [6,7] In the case of vEDS, a majority of individuals are diagnosed by the age of 18 owing to positive family history and early onset of symptoms. In other subtypes however, diagnosis can be delayed due to a later onset of symptoms or lesser severity. With EDS disorders being hereditary autosomal disorders, the relative risk of an individual being affected is equal regardless of age, sex, or race. Diagnosis of EDS disorders begins with a review of family history as well as a physical exam of skin hyperextensibility and joint hypermobility with reference to the Beighton scale. Following this, doctors will order for imaging such as magnetic resonance imaging (MRI), computerized tomography (CT), or echocardiography. Although these primary tests indicate a likelihood of EDS, confirmatory testing is performed through genetic analysis. [5] While most subtypes of EDS are detected through this pathway, some subtypes have a direct method for diagnosis. In particular, kyphoscolitic type (kEDS) can be diagnosed through a laboratory urine sample or skin biopsy sample. [8] Each subtype progresses differently; hEDS has milder symptoms, while 70% of vEDS individuals experience vascular rupture. With few therapies available, EDS disorders bring suffering to patients and their families.

Key Challenges:

To cover the important areas to consider when confronting the disease and the main aim of your research - 300 words maximum in box below.

The limitations when it comes to EDS start with the variety in subtypes. While seven subtypes have autosomal recessive patterns of inheritance, five have autosomal dominant inheritance and one, myopathic EDS, has a blend of dominant and recessive components. Additionally, there are many genes involved, (COL5A1, COL5A2, COL1A1, COL3A1, TNXB, PLOD1, COL1A2, FKBP14, ADAMTS2, AEBP1 to name a few) all distributed across several chromosomes. This variety has posed an obstacle in diagnosis, for example the hEDS genetic component is still unidentified and some subtypes have a blend of mutations. In spite of these differences, therapy research through a general approach and seen success in in vitro trials. This breakthrough began with analysis of the RAC1 actin-dependent stimulation of matrix metalloproteinase (MMP) production pathway. MMPs function to degrade the ECM and, at low levels, aid in tissue remodeling. As it relates to EDS disorders, MMP1 is overexpressed and facilitates the breakdown of type I, II, III, VII, and X collagen worsening symptoms including joint hypermobility. [9] Inhibition of the pathway for MMP1 expression was discovered as a potential therapeutic target in the examination of hEDS fibroblast cell differentiation. The hEDS cells were treated with doxycycline, a nonselective MMP inhibitor, which partly reverted the myofibroblast-like differentiation. [1,10] This finding illustrates the underlying connection between MMP imbalance and ECM proteolysis, opening a new avenue for research. While doxycycline mediated the desired response in vitro, it poses a risk to MMP balance due to its nonspecific binding. Hindrance of the wrong MMP can prevent necessary healthy ECM degradation affecting tissue health. To continue the pursuit of EDS therapy options and make up for the shortcomings of doxycycline, this study aims to examine the efficacy of zinc chelators as MMPi across common EDS subtypes.

The Approach:

To provide a strategy of how you would go about undertaking a novel research study and increase understanding of the disease mechanism(s), describing methods to tackle the key challenges, and what you hope to achieve - 400 words maximum in box below.

MMP1 is a part of the collagenase family of MMP proteins and is credited for its involvement in tumorigenesis and angiogenesis in cancer as well as ECM proteolysis in several disorders. To date, the tetracycline group of drugs, including doxycycline, are the only MMPi therapies focused on in EDS specific research. However, general studies of MMPi have unveiled a host of molecules that have the potential to elicit more selective blockage. These molecules include bisphosphonates, synthetic MMPi, and zinc chelators. A review of MMP morphology indicates that targeting compounds to bind at the zinc active site will inhibit the enzyme activity. [11] As it applies to MMP1, the S1' specificity pocket is shallow and clinical use of a selective inhibitor must consider this. Zinc chelators are especially adept and will remain the focus of this study owing to the extensive development of zinc binding groups (ZBG) modifications for selectivity. Based on the literature available, thiocarbonyl squaric acid full-length ZGB has the highest MMP1 binding efficacy with an IC_{50} value of 15 μM. [12] With a substrate in mind, the study will proceed with in vitro models of unaffected fibroblasts (control), hEDS, vEDS, and classical EDS (cEDS) cells. To start, cells will be obtained from patients with the aforementioned EDS subtypes and run through purity-confirmation testing. The cells will then be evenly divided into groups for treatment with a placebo or squaric acid ZGB. Using Immunofluorescence imaging, the cells will be photographed and analyzed for conformational changes or reverted differentiation at various time points. In the event of successful MMPi, the respective cells will be analyzed through X-Ray crystallography and NMR spectroscopy to better understand the structural chemistry in the MMP1-ZBG complex. Using this method to find an alternative EDS MMPi is logical in its scientific approach using established knowledge on the chemistry of both the substrate and the enzyme. Further, it is both ethically and experimentally feasible due to the use of in vitro rather than in vivo cells. Although exact predictions for the outcome of this experiment cannot be made, confirmation of ZBG as a competitive inhibitor of MMP1 will introduce a treatment option for individuals with EDS. Noting the wide application of MMPi, research of ZBG may expand to impact other diseases. Conversely, should complications arise or ZGB is ineffective at inhibiting MMP, understanding its shortcomings will contribute to improve doxycycline selectivity for clinical practice.

References:

Add references in box below. A numbered referencing style is recommended (e.g. Vancouver).

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Word limit: 1,400 words (excluding title and references); see above for word limits to each section
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