Pathogenesis of skeletal and connective tissue involvement in the mucopolysaccharidoses: glycosaminoglycan storage is merely the instigator

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Abstract

The mucopolysaccharidoses (MPSs) are a series of rare genetic disorders in which progressive bone and joint disease represents a key source of morbidity for patients. The recent introduction of enzyme replacement therapy for many of the MPSs has led to a need for increased physician awareness of these rare conditions in order to ensure that treatment is initiated at a time that leads to optimal benefit for patients. In addition, the current experiences of the clinical responsiveness of patient’s symptoms to enzyme replacement approaches have also fuelled an interest in the development of alternative and adjunctive therapeutic approaches directed particularly to the rheumatological aspects of disease. Understanding the underlying pathogenesis of the MPSs is a key element for advancements in both of these areas. This review highlights the current knowledge underlying the pathophysiology of disease symptoms in the MPSs and underscores the importance and role of pathogenic cascades.

Key words: Lysosomal storage disease, Mucopolysaccharidosis, Enzyme replacement therapy, Glycosaminoglycans, Dysostosis multiplex, Signal transduction, Inflammation, Apoptosis, Proteoglycans, Arthropathy.

Introduction

The mucopolysaccharidoses (MPSs) are a complex group of progressive storage disorders that ultimately affect most organ systems [1, 2]. A key component of disease morbidity relates to progressive involvement of bone and connective tissue, leading to significant health impact. Although the primary defect in glycosaminoglycan (GAG) metabolism for each of the MPSs is well characterized, the precise mechanisms underlying the disease symptoms, and in particular the symptoms that most significantly impact patients, are poorly understood [3]. Recent advances highlight an emerging concept that primary GAG storage leads to perturbation of cellular, tissue and organ homeostasis mediated through alterations of complex multifarious pathways, and that these secondary effects are important effectors of disease symptoms and progression (reviewed in [3]). In addition, once initiated, these secondary events may not be easily reversible by direct enzyme replacement strategies [4]. This concept underscores the critical importance of commencement of therapy early in the course of disease and highlights a need for additional and supplementary therapeutic strategies in the MPSs. Here we review the pathogenesis of the MPSs with an emphasis on rheumatological aspects of disease. An appreciation of the underlying pathogenesis of the connective tissue and skeletal involvement in these disorders provides insight into factors affecting the natural history of MPS disease, the optimal timing and impact of therapeutic intervention, as well as the identification of potential alternative therapeutic targets.

Novel concepts of disease mediators in the MPSs—the role and place of GAGs

Historically the pathogenesis of the MPSs has been considered as a simple storage phenomenon which assumed that GAGs are an inert storage material and this storage was the sole and direct mediator of disease symptoms. A key observation that questions this view comes from various MPS animal model studies which demonstrate that although disease symptoms in the MPSs are progressive, most organs and tissues do not show progressive GAG storage [5, 6]. This simple observation therefore challenges the primary storage or inert GAG-centric perspective of disease mediators and indicates that it is more...
accurate to consider the specific MPS disease symptoms or signs and then unravel the various complex homeostatic alterations that underlie the symptoms. This perspective presumes that a causal link to the defect in GAG degradation can be made, although recent evidence suggests that for some disease features the link may be complex and not necessarily linear or first degree in nature, but may be best conceptualized as a complex pathogenic cascade [7–13]. For many lysosomal storage diseases this concept of a pathogenic cascade has been clearly illustrated [14, 15]. In the case of the MPSs, one must consider the fact that GAGs are biologically active molecules both as free GAGs and when complexed with proteins forming proteoglycans. As such, GAGs are involved in many critical cellular and tissue pathways, including signal transduction (via their ability to modulate the function of cell surface receptors), sequestration of extracellular humoral factors and modulation of the cross talk between cells [3]. Thus there are numerous potential mechanisms underlying specific MPS disease symptoms as indicated in Table 1. The complexity of the mechanisms underlying the progressive features of MPS disease underpins the potential limitations and roles of therapeutic approaches.

**Primary arthropathy in the MPSs**

Each of the MPSs has progressive joint involvement, but there are significant differences in the specific joint symptomatology and rate of progression of the joint disease in the individual disorders. The joint symptomatology in each of the MPSs results from both primary effects of the disease on articular cartilage and the surrounding connective tissue structures as well as primary bone disease. For illustrative purposes, the underlying pathogenesis of the articular disease and bone disease will be considered separately, although overlapping mechanisms are clearly at play. The joint disease in the MPSs is characterized as progressive joint disease in the absence of significant clinical signs of inflammation [16–17]. MPS I, II, VI and VII patients show progressive joint stiffness involving all joints, with formation of joint contractures and eventual destructive joint disease [18, 19]. The resulting progressive loss of hand dexterity, spinal alignment, joint pain and joint mobility represents a significant source of morbidity for patients. MPS III patients have less severe but similar joint involvement. MPS IV patients also have progressive joint involvement, but in contrast they have joint hyperextensibility, joint hypermobility and severe skeletal dysplasia [1, 16, 20].

Studies of articular chondrocytes and synovial tissues from various MPS animal models, where dermatan sulphate is stored, reveals an age-dependent increase in articular chondrocyte apoptosis with associated increased nitric oxide and inflammatory cytokine release [21]. This enhanced apoptosis in MPS appears to be specific for articular chondrocytes. Dermatan sulphate, by virtue of its structural similarity to lipopolysaccharide (LPS), has been proposed and demonstrated to be a direct mediator of this process by activation of the LPS signal pathway with evidence of elevated expression of LPS binding protein (LBP), Toll-like receptor 4 (TLR4), CD14 and the chemokine receptor CXCR4, all of which are essential elements of the LPS signal transduction pathway [9, 13, 22]. This LPS activation leads to activation of TNF-α, IL-1β and macrophage inflammatory protein (MIP)-1α with evidence of increased TNF-α and IL-1β in SF samples. In addition the collagenase MMP-13 has been noted to be overexpressed in MPS synoviocytes, as is RANK ligand (receptor activator of nuclear factor-κB), ceramide and sphingosine-1-phosphate [9]. Thus a complex cascade leading to chondrocyte apoptosis, synovial hyperplasia and inflammatory joint destruction mediated directly by MPS synoviocytes as well as via cytokine and chemokine recruitment of macrophages appears to underlie the progressive arthropathy in MPS I, II, III, VI and VII. Therefore, despite the lack of clinical evidence of joint inflammation, activation of inflammatory pathways represents a significant component of joint disease in these MPSs.

Limited research has been directed towards the elucidation of the mechanisms underlying the joint disease in MPS IV, where the primary storage material is keratan sulphate. Unfortunately, clinically relevant animal models for MPS IV are not available for study. Biochemical and histological characterization of articular cartilage in two individuals with MPS IVA revealed evidence of wider collagen fibre diameter with decreased lysyl hydroxylation and total amount of pyridinolines, in addition to an altered arrangement of proteoglycans in the extracellular matrix surrounding articular chondrocytes [23]. A report of two siblings with MPS IVA, where femoral condyle biopsies were studied, revealed that in addition to detection of
keratan sulphate there were significant increases in type I collagen at both the protein and mRNA level as well as decreases of type II collagen and the proteoglycan aggrecan at both the protein and mRNA level [24]. These studies serve to illustrate that in MPS IVA there also appear to be perturbations in protein components of articular cartilage, but the underlying mechanisms and how the observations relate to the primary defect in keratan sulphate degradation remain unknown.

Primary bone disease in the MPSs

Each of the MPSs is associated with primary skeletal dysplasia that is referred to by the descriptive term dysostosis multiplex [1]. Unfortunately, this term is relatively poorly defined and does not lend itself to objective severity assessment. The main clinical manifestations of skeletal dysplasia in the MPSs are short stature, which can be particularly severe in MPS IV and VI, and progressive skeletal deformation, leading to spinal misalignment, long bone deformation, macrocephaly, hip dysplasia, chest deformity and facial dysmorphism. Osteopenia is described in MPS VI and VII.

Studies of growth plate and bone tissues from various MPS animal models reveal primary morphogenic abnormalities in the developing growth plate as well as the structure of cortical bone. In young MPS I and VII mice, as well as MPS VI rat and cat models, there is evidence of disordered growth plate chondrocyte organization and trabecular architecture [8, 9, 22, 25, 26]. Studies by Wilson et al. [27] using the MPS I murine model have demonstrated a defect in endochondral ossification, which is secondary to osteoclast dysfunction. The primary mediator of the ossification defect appears to be the inhibition of the collagenase activity of cathepsin K by the local accumulation of heparan and dermatan sulphate. Paradoxically, cathepsin K protein levels appear to be up-regulated in the subgrowth plate area of MPS I mice, but its ability to cleave type II collagen is inhibited by the accumulation of GAGs. A primary defect in osteoclast function has also been demonstrated in murine MPS VII [9, 28]. Studies of the MPS VII murine growth plate show significant accumulation of chondroitin-4-sulphate and reduction in the number of cells in the proliferative zone, likely due to reduced chondrocyte proliferation rather than increased apoptosis [8]. mRNA expression studies of the growth plate show a marked decrease in expression of IL-6 family members, including leukaemia inhibitory factor (LIF), oncostatin M (OSM), cardiotrophin 1 and cardiotrophin-like cytokine factor 1, with more modest reductions in the cytokines IFN-γ and IL-1β, proteins involved in the TNF-α pathway and various negative regulators of signal transducer and activator of transcription (STAT). Increases were noted in the proteases cathepsin S and MMP-3 and reduced levels of tyrosine-phosphorylated STAT3. Taken as a whole, these data suggest that the bone shortening in MPS VII results from the primary accumulation of chondroitin-4-sulphate and is mediated through alteration of key components of the Janus kinase/STAT (JAK/STAT) signalling and transduction pathways. As chondroitin-4-sulphate does not accumulate in the other MPSs, the mechanisms responsible for growth plate dysfunction and bone shortening in the other MPSs are largely unknown, but the comprehensive studies in MPS VII indicate the complexity of the homeostatic alterations that potentially underlie the growth plate dysfunction in MPS.

Pathogenetic insights from studies of other organ systems

Cardiac

The cardiac manifestations of the MPSs consist of progressive valvular thickening leading to valvular incompetence as well as primary myocardial involvement; the former is universal, whereas the latter is uncommon [29–32]. Studies in MPS I mice, dogs and humans reveal an abnormality in cardiac and aortic vessel elastogenesis, which is likely secondary to both decreased elastic fibre assembly or synthesis as well as augmented elastic fibre fragmentation [7, 12, 29]. Decreased elastin synthesis and elastin fibre assembly has been demonstrated in vitro in MPS I and is hypothesized to be caused by accumulation of dermatan sulphate, which ultimately leads to deficiency of elastin binding protein, a key chaperone for elastin sorting and elastin fibre assembly [33]. A defect in elastin assembly in vivo has not been reported. Studies in the MPS I murine model reveal that increased elastin fragmentation is likely caused by increased expression of two elastindegrading proteins, MMP-12 and cathepsin S [7]. In addition, key transcriptional regulators of MMP, STAT1 and STAT3 showed evidence of activation in tissues of affected animals; these latter results show similarity to the activation of the STAT pathway in chondrocytes. The direct mediators responsible for activation of the STAT pathway in the aorta are not known, but may relate to the abnormal biomechanical properties of the vessel wall.

CNS

MPS III, MPS VII and a portion of MPS I and II patients have progressive CNS disease manifesting as neurodegeneration [1]. Analysis of murine models of MPS IIB and MPS I reveal a central role of inflammation in which activation of microglia and astrocytes are noted, with increased CNS expression of CD38, lysozyme M, cathepsins S and Z, cytochrome b558, DAP12 and complement factors C1q and C4 [34]. Accumulation of gangliosides (GM2 and GM3) as well as cholesterol, ubiquitin, mitochondrial ATPase subunit c and aggregates of paired helical filament P-tau has also been demonstrated in various MPS models [35–37]. These studies highlight the complex secondary metabolic alterations that underlie CNS disease in the MPSs (reviewed in [38]).

Conclusion

Rheumatological symptoms and disease complications represent a significant contribution to disease burden for MPS patients. The elucidation of the complex
Pathophysiological processes that underlie these symptoms in the MPSs underscore discussions related to therapeutic approaches in these conditions (Fig. 1). The complex pathological cascade underlying the progressive joint and bone involvement in the MPSs would imply that initiation of treatment early in the course of disease would afford the best outcome and have the greatest chance to significantly impact quality of life. Conversely, delayed diagnosis and late initiation of treatment would likely lead to the patient entering a stage of disease where the bone and joint pathology is unlikely to be reversed and may in fact continue to progress despite treatment. Therefore, methods need to be developed to diagnose MPS patients early in the course of disease by increasing awareness among physicians and consideration of either newborn or early targeted population screening.

Therapy for the MPSs is reviewed elsewhere in this supplement [39]. Experience with haematopoietic stem cell transplantation (HSCT) in severely affected MPS patients indicates that bone and joint disease is minimally impacted, even when HSCT is initiated early in the course of disease [40–42]. Long-term experience with enzyme replacement therapy (ERT) and specifically long-term ERT started very early in the course of disease for patients with attenuated disease is limited. Two recent reports of sibling pairs with MPS I and MPS VI illustrate the potential for significant alteration of bone and joint disease in attenuated patients when treatment is initiated early in the course of disease. In the case of MPS I, experience with 5 years of laronidase (rhIDUA) treatment in a sibling pair with one sibling beginning treatment at 5 months of age and the other at 5 years of age reveals minimal joint and bone disease in the younger sibling as compared with the older sibling at the same age [43]. In the case of MPS VI, the comparison of siblings receiving N-acetylgalactosamine-4-sulphatase (rhASB) beginning at 8 weeks and 3.6 years of age, respectively, and followed for 3.6 years revealed evidence of stabilization of severe scoliosis in the older sibling and prevention of scoliosis, hand contractures and facial dysmorphism in the younger sibling [44]. On the other hand, corneal clouding continued to progress, as did radiological evidence of dysostosis.

A key factor highlighted by these cases is the uncertainty of the appropriate dose of recombinant enzyme that is required to prevent disease, as clinical trials have utilized patients with pre-existing and advanced disease. It is possible that higher doses or more frequent dosing of ERT is required to impact skeletal and joint disease in the MPSs, particularly during periods of rapid growth.

The identification of the complex pathogenic cascade underlying the pathogenesis of the MPSs and, in particular, the identification of the TLR4 and STAT signal pathways, as well as inflammation and altered collagenase activity of cathepsin K, as key elements in the pathogenesis of the rheumatological aspects of the MPSs should lead to potential additional therapeutic approaches for these disorders. It is likely that therapeutics targeted to these key pathways will augment enzyme replacement strategies.

**Fig. 1** Mechanisms of bone and joint disease in the MPSs.

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<td>• Inhibition of collagenase activity of cathepsin K</td>
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<td>• Alteration of STAT pathway</td>
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<td>• Decreased IL-6 and IL-6 family chemokines</td>
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References


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Rheumatology key messages

- Defective GAG degradation in the MPSs leads to a complex cascade of pathophysiological events.
- Secondary consequences of GAG storage can be irreversible; early intervention may improve long-term outcome.
- The complex pathophysiology of the MPSs need not necessitate targeting and combining treatments for optimal outcome.

Pathogenesis of mucopolysaccharidoses


