FACTORS WHICH DETERMINE SKELETAL RESPONSE TO THERAPEUTIC GROWTH HORMONE IN OSTEOGENESIS IMPERFECTA

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Osteogenesis Imperfecta (OI) encompasses a group of connective tissue dysplasias characterized by osseous fragility leading to fractures. OI is heterogeneous and at least 4 phenotypes, OI types IA, IB, IVA and IVB contribute to presentations of non-lethal OI of mild-moderate severity.

Fifteen children have participated in a prospective study of the effect of Genotropin on fracture frequency, bone density as assessed by Dual Energy X-ray Absorbtiometry (DEXA) as well as growth response in various skeletal elements. To better characterize the molecular mechanisms underlying response to synthetic growth hormone, collagen synthesis and secretion by cultured skin fibroblasts from each of these subjects or an affected parent was studied. There were 6 patients with OI type IA, 5 patients with OI type IVA and 4 patients with OI type IVB.

All patients showed reduced type I collagen synthesis. Electrophoretic mobility of pepsin digested type I collagen chains was normal in all but three patients who are being investigated further, one with OI type IA and 2 with OI type IVB. In screening patients in general with OI for collagen protein electrophoretic mutation, the majority of patients with OI type I have shown no abnormality in electrophoretic mobility of pepsin digested collagens. However we have recently observed 2 patients with OI type IB with abnormalities of electrophoretic mobility similar to those observed in the 3 patients in the growth hormone study. These two patients with OI type IB have been demonstrated to have multi-exon deletions in one COL1A2 allele. Differential inclusion of mutant collagens into tissues/cell lines has been demonstrated both *in vitro* and *in vivo* The technique involves the measurement of the proportion of mutant versus normal collagen incorporated (included) into ascorbate induced matrix in long term culture.

A surprising finding from the DEXA studies of the skeleton has been that bone density has been normal for age in some subjects with OI in the Genotropin trial. Thus osteopenia (lit. less bone) may not be a primary consequence of some type I collagen mutations which result in bone fragility but osteopenia may result from a combination of factors including immobilisation after fractures. Further studies of the extent of *in vitro* incorporation *i.e.* inclusion of mutant collagens into bone are needed to explain the findings in OI of mid-moderate severity and the effects of growth factors such as Genotropin on collagenous matrices.

THE COLLAGEN PROTEIN FAMILY: MECHANISMS OF INTRACELLULAR FOLDING, CHAIN ASSOCIATION AND THE GENERATION OF DIVERSE SUPRAMOLECULAR STRUCTURES

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The name "collagen" is used as a generic term to cover a wide range of protein molecules which share the basic structural motif of three polypeptide chains wound in a characteristic triple helical configuration and form highly organised supramolecular assemblies in the extracellular matrix. Some 19 different collagen types comprised of at least 33 individual genetically distinct polypeptide chains have been identified although not all have been well characterised. The most commonly occurring collagens are types I, II, and III which form the long-recognised structural fibrils in which adjacent molecules are staggered with respect to each other and stabilised by covalent intermolecular crosslinks. The other collagen types, including the fibril associated collagens (types IX, XII, XIV and XVI), short chain collagens (types VIII and X), basement membrane collagen (type IV), anchoring fibril collagen (type VII), and microfibrillar collagen (type VI), share the triple helical motif but this may be the only thing these collagens have in common with the fibril-forming collagens. Many have large non-collagenous domains which often remain after deposition of the collagens into the extracellular matrix and direct the formation of diverse supramolecular aggregates. These non-collagenous domains may have important roles in interacting with cells and other matrix components.

Intracellular assembly of fibrillar procollagens (types I, II, III, V and XI) is thought to occur via the folding of individual subunit C-propeptide domains and the formation of intrachain disulphide bonds, followed by association and alignment of the chains, stabilisation of the trimeric structure by interchain disulphide links, and finally the zipper-like folding of the triple helix from the C- to N-terminus. The recognition events important for assembly have not been characterised, but are presumed to occur via specific protein-protein interactions between the C-propeptides involving sequence motifs within the propeptide and/or conformational determinants of the folded individual propeptides. Procollagen chains assemble in a highly regulated type-specific manner, despite the sequence homology between the C-propeptide domains and their similar predicted tertiary structure. The goal of our studies is to begin to dissect procollagen folding pathways and the mechanisms directing selective chain association in cells expressing more than one fibrillar collagen type.

To examine the importance of disulphide bond formation in procollagen folding, assembly and secretion we have assessed the ability of proα1(I) chains containing site-directed mutations of cysteine residues to fold and form stable triple-helical molecules. In additional experiments, fibroblasts were incubated with 10mM DTT to completely prevent the formation of disulphide bonds. Together, these data demonstrate that inter- and intrachain disulphide bonds are not required for folding of the triple helix and secretion of proα1(I) homotrimers, however, correct C-propeptide folding and the formation of intrachain disulphide bonds are critical for the heterotrimeric association of proα1(I) and proα2(I) chains.

METABOLISM OF LIVER COLLAGEN IN DIMETHYLNITROSAMINE INDUCED HEPATIC FIBROSIS

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Hepatic fibrosis is characterized by an accumulation of connective tissue components, especially collagen in the liver. In order to obtain more information about the alteration of connective tissue metabolism during the progression of hepatic fibrosis, the biosynthesis and metabolic degradation of liver collagen were investigated in dimethylnitrosamine (DMN) induced liver fibrosis in rats. The metabolism of liver collagen was studied after a single intraperitoneal injection of ^{3}H-proline in a dose of 1110 KBq/100 g body weight. The incorporation of ^{3}H-proline into collagen as ^{3}H-hydroxyproline was measured as an index of collagen biosynthesis. The total and ^{3}H-hydroxyproline were determined after extraction and fractionation of liver collagen into neutral salt soluble, acid soluble and pepsin solubilized fractions. The urinary levels of total and ^{3}H-hydroxyproline were also studied in order to assess the metabolic degradation of liver collagen. A significant increase was noticed in the rate of biosynthesis of liver collagen in all DMN treated animals with a maximum on the 21st day. About 4 fold raise was recorded in the amount of total liver collagen. The urinary excretion of total and labeled hydroxyproline were also increased significantly with a maximum excretion on the 7th day. No correlation was noticed between the increase in liver hydroxyproline content and the increase in urinary excretion of hydroxyproline. The results of the present study indicated a significant increase in the biosynthesis and metabolic degradation of liver collagen during DMN induced hepatic fibrosis. It also revealed that the balance between synthesis and degradation was almost maintained in the early stages of fibrosis as a self defence mechanism, but it was totally impaired in the acute phase of the disease with a net result of accumulation of collagen in the liver.

PROTEOGLYCAN STRUCTURE AND FUNCTION IN NORMAL AND DISEASED CARTILAGE

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Cartilage matrix metabolism undergoes pronounced shifts during the development of OA. These alterations, in interplay with mechanical loading and other exogenous and endogenous factors, over time lead to deterioration of joint function in OA. Experimental evidence points to a marked increase by the chondrocytes of both synthesis and degradation of matrix molecules in the early phases of OA, with little or no net loss of the major matrix molecules. Later, there may still be evidence for an increased synthesis of matrix components, but due to defects in the structure of new molecules or their extracellular assembly, or shifts in degradative activity, net loss of matrix occurs. In the end, the compensatory efforts mounted by the chondrocytes collapse while degradation continues and the matrix and joint fail. The 'point of no return' on this pathway has not been defined and may be different in different joints, individuals and experimental models of OA. Aging may cause a shift of the 'point of no return' so that a lesser joint insult may initiate the OA process in the aged cartilage than in the young cartilage.

During cartilage matrix degradation molecular fragments and other products of tissue metabolism are released into the synovial fluid and subsequently other body fluid compartments. These 'markers' may be used to monitor the dynamics of matrix metabolism in vivo in the human and in animal models. Analysis of the structure of the released molecular fragments may help to elucidate the disease mechanisms in OA.

We have shown the increased release of fragments of aggrecan and COMP and of MMP-1 and MMP-3 to human joint fluid after knee injury and in OA. MMP-3 levels are 15-70 fold that of MMP-1. MMP concentration in synovial fluid is a sensitive indicator of joint pathology, especially when combined with other markers. Serum concentrations of MMP-3 increase after joint injury. Concurrent with these indications of increased matrix degradation, we find increases in other synovial fluid markers which suggest an increased synthesis of both aggrecan and collagen II.