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Mini-Review Article

Hylonome

Changes in bone collagen with age and disease

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The overall shape and optimal physical properties of the animal body depend on a framework of collagen. With increase in age these collagenous tissues change, as outwardly manifest in wrinkled skin, stiffened joints, shortening stature, but also internally in the stiffening of the vascular and pulmonary systems. The properties of collagen change with embryonic growth and development, for example, embryonic dermal collagen contains a high proportion of type III collagen but is predominantly type I in the adult, similarly endochondral ossification involves the replacement of type II collagen by type I. However, these are not age-related changes as defined by aging research, which is concerned with the aging of mature tissue. Collagenous tissues exist as a diverse array of structures from thick parallel fibers in tendon, through laminated sheets in bone and cornea to the thin transparent non-fibrous basement membranes in the lens capsule of the eye. This biological diversity has been accounted for by the identification of genetically distinct collagens[1], currently 20 types. All possess the characteristic triple helix based on three polyproline helices wound into a triple helix. These triple helices then aggregate extracellularly to form different supramolecular fibrous and non-fibrous structures. The major supporting collagens are type I, II and III in which the monomeric molecules polymerize to form striated fibers due to their parallel alignment in an end-overlap-quarter-staggered fashion. These fibers have no tensile strength and are immediately cross-linked between molecules[2] thereby preventing the rod-like molecules sliding past each other under stress and hence providing extreme resistance of the body framework to external mechanical stresses.

Maturation and aging of collagen

The change in properties has been shown to be due to two different cross-linking processes[3]. Firstly, an enzymic process involving lysyl oxidase in which intermolecular cross-links are formed at precise locations along the molecule emanating from the N and C-terminal regions to specific lysine/hydroxylysine residues in the helix due to the accurate alignment of the molecules in the fiber. In the case of bone collagen the initial cross-link is a Schiff base resulting from the reaction of an aldehyde, with the ε-amino group of lysine which is then stabilized by undergoing an Amadori rearrangement to a keto-imine. This cross-link is the major stabilizing bond in recently synthesised bone collagen and is a divalent bond linking two molecules.

Maturation

As the synthesis of collagen decreases following the slowing of growth at maturity an increasing proportion of the mature tri-valent cross-links, the pyridinolines or the pyrroles forms following the reaction of divalent cross-links with a further hydroxylysine or lysine aldehyde, respectively. Preliminary studies indicate that the pyrroles may form an interfibrillar bond since it correlates with the mechanical properties of cortical bone[4] glycation.

The second process is non-enzymic and occurs once the rate of collagen turnover decreases following maturation and involves the adventitious reaction of glucose with lysine and arginine side-chains. This second mechanism is known as glycation and is believed to play a central role in the pathogenesis of aging[5]. This is due to the formation of advanced glycation end-products, some of which are intermolecular cross-links and lead to the stiffening and consequent dysfunction of vulnerable tissues such as kidney, capillary basement membranes, and the cardiovascular and pulmonary systems. The initial reactions are well established but only some of the Advanced Glycation End-products (AGEs) have been identified and we have yet to determine the important functional glycation cross-link(s)[3,6]. The initial stage involves the reaction of glucose with the ε-amino side-chain of lysine and the guanidine side-chain of arginine. These glucose complexes may then be oxidised to more reactive compounds. Other glycation agents may be derived from oxidation of lipids, such as malondialdehyde[7], or the normal metabolite methylglyoxal[18], both

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of which, although only present in minute quantities, react very rapidly with lysine and arginine side-chains. Further reactions involving oxidation of these complexes leads to the formation of AGEs, some of which may be cross-links. One such cross-link, designated pentosidine, has been identified in collagenous tissues and has been shown to increase linearly with age[9]. However, the concentration of this cross-link at 1 per 100 collagen molecules is insufficient to account for the observed stiffening of the collagen fibre. The importance of AGEs was neatly demonstrated by the administration of a mixture of AGEs prepared *in vitro* to normal rats who subsequently underwent typical age-related changes, basement membrane thickening, stiffening of the aorta in the absence of hyperglycaemia[10]. The glycation reaction can be inhibited *in vivo* by competing reagents such as aminoguanidine, aspirin and more recently pyridoxine[11]. An attempt to cleave the AGE cross-links has been made by the use of phenyl-thiazolium compounds. Although the mechanism is not clear, these compounds have been shown to reverse the stiffening of the vascular system of diabetic rats[12]. The extent of the age-related changes, both the maturation of the enzymic cross-links and the formation of glycation cross-links obviously depends on the rate of turnover of the particular collagenous tissue. Thus the extent of glycation is high in articular cartilage where the biological half-life has been estimated at 100 years, less in the dermis which has a half-life of about 15 years whilst glycation is low in bone with a half-life of about 6 months.

Aging of bone

We have studied the effect of age on the collagen of trabecular bone by analyzing the changes in the iliac crest from about 100 individuals[13]. The iliac crest was chosen as it might be expected to reflect any change in bone quality due to its higher turnover. There was a clear decrease in bone density as assessed by pQCT, a decrease in maximum stress and Young's modulus with age; however, there was no corresponding change in the composition of the collagen. The reduction in mechanical properties of the bone was paralleled by the reduction in collagen content. There was no change in lysine hydroxylation, immature or mature cross-links or collagen type. The absence of age changes may be due to a high turnover of the iliac crest bone collagen, newly synthesized collagen being the predominant component. Glycation is therefore unlikely to be important in iliac bone. It is, however, possible that glycation occurs in some slow turnover cortical bones but is unlikely to be sufficiently extensive to stiffen the bone since even these bones have a significant turnover rate. We used these findings as a baseline for the study of changes in osteoporotic bone collagen since we reasoned that any observed changes would probably be due to the disease.

Osteoporosis

In contrast to normal aging of bone we found that the trabecular subchondral bone of human femoral heads revealed higher lysine hydroxylation, increased immature cross-linking, increased collagen synthesis and degradation and reduced mineralisation compared to age-matched non-osteoporotic controls. This study revealed increased turnover of collagen in osteoporosis despite an overall loss of collagen[14,15]. The over-hydroxylation leads to finer fibrils and modified cross-links, and reduced calcification, all these effects leading to a further increase in the fragility of the bone. The rate of bone collagen turnover in osteoporosis can be modified by oestrogen therapy. We found that 12 months treatment of established osteoporotic subjects the turnover of collagen was reduced as expected, and the collagen was found to be more mature and of higher bone density[16]. These changes clearly stabilized the bone. However, follow-up studies of the same cohort of women 6 years later revealed an increase in collagen synthesis, demonstrating for the first time an anabolic effect of oestrogen in long-term osteoporosis[17]. It is clear that not only is the quantity of bone collagen important for bone strength but also its quality, which in turn is determined by its rate of turnover. Attempts to correlate bone mineral density with the risk of bone fracture can only be very approximate, and the quality of the collagen would have to be taken into account for a more accurate prediction.

Paget's disease

Another common bone disease of the elderly is Paget's disease, which involves excessive and disorganized bone remodeling. The rates of turnover can be up to 20-fold. The newly synthesized collagen structures are disorganized producing both woven and lamellar bone, which is probably the cause of the most common complication, fracture after trivial injury. Changes in the collagen do not appear to have been studied, but clearly the increased metabolism could lead to high lysine hydroxylation thus affecting the fiber size and cross-linking, both leading to increased fragility. Whether the genetic type of collagen changes has not been investigated, but it is probable that type I homotrimer levels are increased.

The relatively high turnover of bone even in the aged maintains, to a large extent, the mechanical properties of bone, the major deleterious changes occurring due to a metabolic disorder of the collagen.

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