A Study on the Consequences of Maturation and Ageing of Collagen

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***Abstract*: Collagen is a major type of fibrous protein in higher vertebrates making up one-third or more of the total body protein. The larger and heavier the animal, the greater the fraction of its total proteins contributed by collagen.**

Collagen fibrils are arranged in different ways, depending on the biological function of the particular type of connective tissues. The overall shape and function of the body depends on the basic framework of collagen polymers stabilized by intermolecular cross-links. As a consequence the fibers are virtually inextensible and provide exceptional tensile strength and resistance to mechanical forces. Unlike most other proteins the turnover of collagen in extremely slow and can therefore reflect age - related changes. These are manifested by increased resistance to enzymic degradation and modified cell-matrix interactions. These changes result in wrinkling of the skin, stiffness of the joints and cardiovascular system as well as a reduction in the elasticity and permeability of the basement membranes in capillaries and kidneys. These changes can be accounted for primarily by the formation of glycation intermolecular cross - links in the collagen.

Introduction

The loss of functionality of collagenous tissues during ageing, in terms of increased stiffness, enzyme resistance, and loss of permeability can be accounted for, by excessive intermolecular cross-linking following the reduction in the rate of turnover. Two mechanisms have been shown to be involved, enzymic and non-enzymic, the latter predominating in old age, although the precise nature of the glucose-derived non-enzymic cross-links have not been elucidated. Collagenous tissues exist as a diverse array of structures, from parallel fibres in tendons, through laminated structures such as cornea and skin, to non-fibrous collagen in thin basement membranes.[1]

A potentially important age effect is the modification of the expression of the cells due to a change in the environment, which is enhanced by glycation of the cell-matrix interaction sites.

Changes in collagen properties may play a role in the common age-related diseases such as Osteoporosis and Osteoarthritis, in which changes in the quality of the newly synthesized collagen have been demonstrated that are deleterious to the functioning of the bone collagen. The changes in the bone collagen of both diseases represents a new approach to these diseases.

Age-related diseases:

There are deleterious changes in the properties of collagen that could lead to age-related disorders, that is, loss of functionality due to increased stiffness and a change in the expression of the cells to produce dysfunctional matrix during the normal slow turnover in old age. Inappropriate matrix formation with age is of particular importance in Osteoporosis (OP) and Osteoarthritis (OA), two very common age related diseases, both of which cause major health problems and disability in the elderly.

Bone is a two-phase system of a collagenous framework which acts as a support for the second mineral phase. The collagen is predominantly type I with only very small amounts of types III, V and VI and allows intermolecular cross-linking to take place, providing the structural strength. Bone turns over throughout life and it is essential that the newly synthesized bone even in old age, is identical to the pre-existing collagen since any change would result in weaker bone and poor mineralization. However, in diseases such as OP and OA, it has been shown that the characteristics of the collagenous matrix are altered.

Osteoporosis:

The loss of bone, particularly in post-menopausal women, can be sufficiently extensive to lead to fractures, although there is only a weak correlation between bone density and fractures. The residual bone is generally believed to be normal bone but we have shown that there is a change in the quality of the collagen following loss of oestrogen and cytokines in post-menopausal women. The collagen was found to be over-hydroxylated with respect to lysine, and to possess a reduced level of immature cross-links.[2] Over-hydroxylation has also been shown to result in narrow fibres, reducing the strength of the bone even further.

In a similar study of collagen on the intervertebral bone from osteporotic subjects, Muller and his colleagues also found an increase in lysine hydroxylation particularly in the a_2 chains, and they related the extent of the change to the density of the bone.

The change in the post-translational modifications of collagen in osteoporosis is therefore highly significant. The bone is not the same as the pre-existing bone as claimed in many text-books but the quality is reduced as a consequence of the change in environment of the collagen-producing osteoblasts.

The loss of bone can be readily arrested by hormone replacement therapy (HRT) or by biophosphonates, but the question arises as to whether the lost bone can be replaced. In collaboration with Studd and his colleagues we reported that HRT inhibited the loss of collagen and that after 12 months treatment the collagen matured and the bone density increased, thus reducing the risk of fracture. However, after 6 years on HRT we noted the formation of newly synthesized collagen by the presence of immature cross-links and this was supported by histomorphometry, clearly demonstrating the anabolic effect of estrogen.

We have shown that increasing age in non-osteoporotic subjects does not necessarily lead to increased hydroxylation and reduced cross-linking, at least in iliac crest bone.[3]

After 6 years on HRT, the formation of newly synthesized collagen by the presence of immature cross-links was noted and this was supported by histomorphometry, clearly demonstrating the anabolic effect of estrogen.[4]

The role of collagen in osteoporosis is relatively unexplored, but the recent evidence for the anabolic effect of estrogen suggest that regeneration of new bone is an achievable goal and deserves greater research input.

Osteoarthritis:

The characteristic feature of osteoarthritis is the fibrillation of the articular cartilage and consequently biochemical research has concentrated on the degradative mechanisms involved in the destruction of the cartilage. These studies have in the main concentrated on the proteoglycans, although, it is only when the collagen is degraded that the disease becomes irreversible.

Studies on bone collagen have demonstrated a thickening of the subchondral bone which Radin postulated would increase the shear stress on the cartilage and accelerate fragmentation. Similarly Dieppe et al have shown that there is increased bone activity, detected by technetium scintigraphy, in osteoarthritic patients who subsequently develop severe OA, as judged by radiographic narrowing of the joint space.[5] We recently demonstrated that the elevated activity was due to a several-fold increase in collagen metabolism as determined by collagen & degradation increased synthesis was demonstrated by higher levels of C-terminal procollagen peptide levels, increased ratio of immature to mature cross-links and increased bone specific alkaline phosphatase. Degradation was demonstrated by increased MMP serine Proteinase and Cathepsin. The overall balance was in favour of increased collagen deposition as evidenced by the thickening of the subchondral bone. The levels of TGFb, which is known to promote collagen synthesis while inhibiting degradation, were found to be increased four fold, and other factors such as connective tissue binding factor, which stimulates TGFb production, are also likely to be increased.

The homotrimer has previously been reported in embryonic and tumor derived collagens.[6]

The change in environment of the osteoblasts in this fibrotic situation is also likely to lead to changes in the function of the cell. Indeed, Westacott et al reported that osteoblasts from the subchondral bone are capable of degrading proteoglycans, unlike osteoblasts from normal subjects.

The increased collagen content and the change in the expression of the osteoblasts suggested to us that the collagen synthesised by these osteoblasts may well be different from normal. We found that the increased metabolism resulted in over-hydroxylation and hypo-mineralization of the collagen.

The evidence for the thickening of the subchondral bone in the femoral head is sound but it is observed at a late stage of OA and its role in OA is difficult to assess. Whether the thickening occurs prior to, or during, degradation of the articular cartilage cannot be determined. However, considerable evidence is accumulating from studies on animal models that thickening of the subchondral bone precedes cartilage fibrillation. The most convincing evidence to date comes from the studies of Carlosn et al on the cynomolgus macaques where thickening does occur prior to cartilage fibrillation and the extent of the thickening can be related to the amount of fibrillation.

Recently,Loughlin et al found that the data did not support association of any alleles or genotypes of the Vitamin D receptor gene or the oestrogen receptor gene.[7]

The majority of the cells respond to mechanical stimulation by modulating biochemical pathways but the nature of the mechano-receptor is unknown.[8]

We found increased collagen metabolism in both the bones and the ligaments prior to any sign of OA as assessed by radiographic narrowing of the joint space.[9]

Conclusion

The loss of functionality of collagenous tissues during ageing, in terms of increased stiffness, enzyme resistance and loss of permeability can be accounted for by excessive intermolecular cross-linking following the reduction in the rate of turnover. Two mechanisms have been shown to be involved, enzymic and non-enzymic, the latter predominating in old age, although the precise nature of the glucose - derived non enzymic cross-links have not been elucidated.

A potentially important age effect is the modification of the expression of the cells due to a change in the environment, which is enhanced by glycation of the cell-matrix interaction sites.

Change in collagen properties such as osteoporosis and osteoarthritis, in which we have demonstrated changes in the quality of the newly synthesized collagen that are deleterious to the functioning of the bone collagen. The change in the bone collagen of both diseases represents a new approach to these diseases.

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