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The mechanisms and consequences of the maturation and ageing of collagen

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Abstract. The stabilisation of collagen fibres during development and through growth to maturation is now fairly well understood. It is a carefully controlled enzymic process which produces intermolecular cross-links at specific locations. In marked contrast, the changes in the physical properties that occur towards old age are stochastic and involve oxidative reactions that result in the formation of glucose mediated cross-links. This excessive and random cross-linking leads to a devastating loss of tissue functionality and deterioration of vital organs. In addition, specific residues involved in cell-matrix interactions may become modified. This can affect the expression of cells and lead to the formation of an inappropriate collagen matrix during its slower turnover in old age. This is exemplified in the ubiquitous disorders osteoporosis and osteoarthritis, age-related diseases in which we have noted gene regulated changes in the collagen deposited and also post-translational changes such as over-hydroxylation of lysine residues. Both of these effects can have a profound deleterious effect on the function of the matrix tissue.

Keywords. Collagen; ageing; cross-link; glycation; osteoporosis; osteoarthritis.

1. Introduction

The overall shape and function of the body depends on the basic framework of collagen polymers stabilised by intermolecular cross-links. As a consequence the fibres are virtually inextensible and provide exceptional tensile strength and resistance to mechanical forces. 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 [1,2]. Unlike most other proteins the turnover of collagen is extremely slow and can, therefore, reflect age-related changes. These are manifest by increased rigidity, increased resistance to enzymic degradation and modified cell-matrix interactions [3]. 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 [4].

2. Intermolecular cross-linking

The continual increase in the mechanical strength of collagen with age must involve the production of covalent intermolecular cross-links which prevent the molecules sliding

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past each other under stress. The most dramatic increases occur between birth and maturation followed by a progressive increase in old age when the size and turnover of the fibres is virtually static. It is now known that these changes in the mechanical properties can be related to two distinct mechanisms of intermolecular cross-linking.

The first mechanism is enzymatically controlled and stabilizes the collagen fibril through development to maturation. Initially, this results in the formation of divalent intermediate cross-links which then spontaneously combine to form multi-valent cross-links on maturation.

The second mechanism, known as glycation, involves the stochastic addition of glucose which as a consequence of the low turnover of mature collagen can undergo further oxidation to form intermolecular cross-links.

3. Enzyme-derived cross-links

The mechanism of enzyme derived cross-linking is now well-established and will be dealt with very briefly, only recent studies being emphasised. When fibrillar collagen molecules are aligned in the quarter-staggered end-overlap arrangement the enzyme lysyl oxidase can bind to a specific sequence in the triple helix (Hyl–Gly–His–Arg). When bound the enzyme oxidatively deaminates lysines in the N and C-terminal telopeptides of adjacent molecules producing reactive lysine-aldehydes. Highly specific cross-links then form spontaneously between the lysine-derived aldehyde and the hydroxylysine in the enzyme binding sequence[5]. There is no definitive evidence for cross-links in any other regions of the molecule. This location of the divalent cross-links has been identified by chemical means and confirmed the quarter-stagger alignment of the molecules in the fibril originally deduced from electron microscopy.

The nature of the cross-links depends primarily on the extent of the hydroxylation of the telopeptide lysine. In immature skin where the lysine is not significantly hydroxylated the major cross-link is dehydro-hydroxylysinonorleucine (deH-HLNL), figure 1. In bone and cartilage the telopeptide lysines are highly hydroxylated and results in the formation of dehydro-dihydroxylysinonorleucine (deH-DHLNL) which almost instantly undergoes an Amadori rearrangement to form hydroxylysino-keto-norleucine (HLKNL), figure 2. Both these divalent cross-links decrease in mature tissue by spontaneous reaction with additional groups. The ability of the divalent cross-links to react further is dependent upon the molecules of the adjacent fibril being in register rather than quarter-staggered i.e. bonding between microfibrils[5,6]. The formation of such inter-fibrillar cross-links would account for the increase in strength of the fibre during maturation.

In skin deH-HLNL reacts with a histidine residue to form the mature cross-link histidino-hydroxylysinonorleucine (HHL), figure 1. In bone and cartilage the HLKNL reacts with another telopeptide aldehyde to form mature cross-links (figure 2). In cartilage the hydroxylation of the telopeptide lysine is virtually complete and the mature cross-link formed is hydroxylysyl-pyridinoline (HP) at a concentration of one HP per collagen molecule. However, in bone collagen there is an apparent deficiency in cross-linking, only one HP per 5 collagen molecules suggesting the presence of an additional cross-link. In recent studies we have shown that the deficiency could be made up by the putative pyrrole cross-link as determined by Ehrlich's reagent[7]. Although the structure of the pyrrole has not been confirmed, possible candidates have been proposed. Kuypers *et al*[8], postulated it could form from the reaction of a telopeptidyl lysine-aldehyde and the immature cross-link HLKNL. This mechanism would be in line with the mechanism of pyridinoline

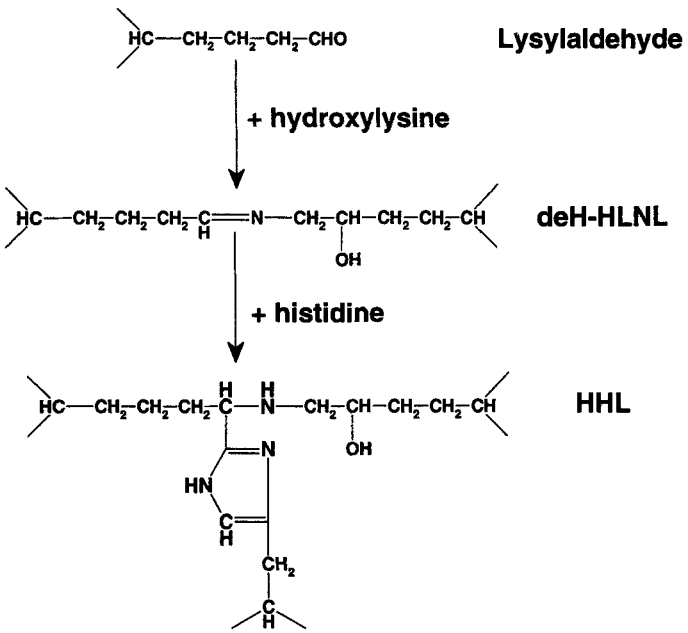

Figure 1. Aldimine pathway of enzymic cross-link formation in collagen, showing immature divalent aldimine and mature trivalent HHL.

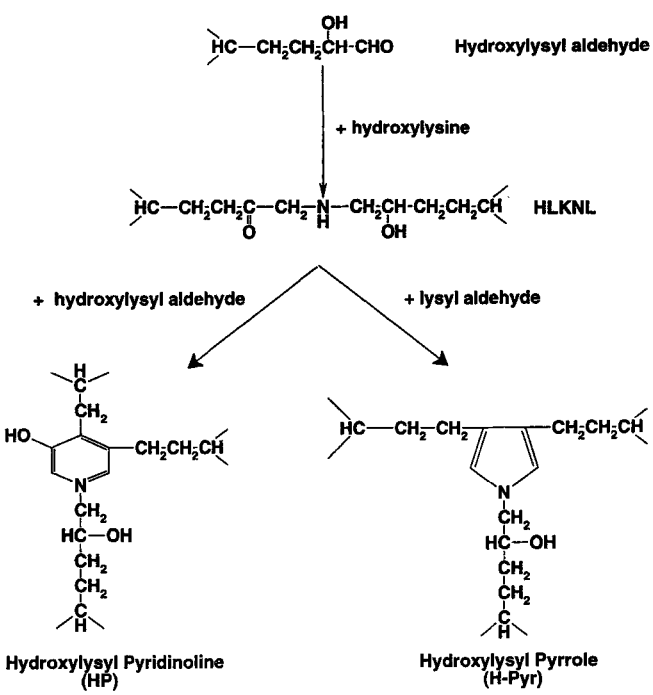

Figure 2. Ketoimine pathway of enzymic cross-link formation in collagen, showing immature keto-imine and mature pyridinolines and pyrroles.

formation postulated by Robins and Duncan[9]. If the telopeptides are highly hydroxylated pyridinoline would form, otherwise the pyrrole would form. Structural elucidation of the actual cross-link has proved difficult due to its inherent instability during the isolation procedures. Kleter *et al*[10] recently reported the isolation and characterisation of a modified pyrrole, a pyrrolinenone, in acid hydrolysates of bovine dental collagen, but this pyrrole does not react with Ehrlich's reagent.

In recent studies we have shown that the pyrrole content correlated with bone strength, whilst in contrast, we could not find a correlation with the pyridinoline cross-link[7]. We have therefore suggested that the pyrrole forms interfibrillar cross-links which increase the strength of the fibre above that of the intrafibrillar divalent keto-imine[11]. The pyridinoline presumably forms an intra-fibrillar cross-link and would not enhance the strength of the fibril above that provided by the divalent keto-imine. Indeed there is some evidence that the pyridinoline although trivalent only cross-links two molecules[12,13], although this contrasts with Eyres' evidence for three molecules[14]. Evidence for our proposal that the pyrrole links two molecules in the fibril with one in another fibril remains to be demonstrated.

The telopeptide lysines of tendons and ligament are only partially hydroxylated and consequently form both aldimine and keto-imine intermediates in the immature tissue and HHL and HP in the mature tissue. The ratio of the cross-links present depends upon the particular tissue and may relate to tissue function such as resisting the tensile and compressive forces exerted on the tissue.

Following maturation there is little change in the concentration of the mature cross-links, but there remains a steady increase in the stiffness of the tissue, clearly indicating a second cross-linking mechanism. This process causes the overwhelming deterioration of tissues with age. It is distinct from the precise cross-link formation observed during development and results in the deleterious formation of excessive cross-links along the triple helix.

4. Glycation-derived cross-links

In a follow up to our studies on controlled collagen cross-linking, we have demonstrated that the second mechanism is non-enzymic and involves the adventitious accretion of glucose. This process, known as glycation, plays a central role in the pathogenesis of ageing[15,16]. Due to the slow turnover of mature collagen these glucose adducts can react further to form cross-links via complex oxidative reactions.

The formation of glycation-derived cross-links results in the stiffening of tissues and concomitant loss of functional properties and causes the most serious late complications of ageing. Tissues such as the basement membrane, the cardiovascular system and the capillary system, particularly of the retina, are the most severely affected. In addition, modification of single amino acid residues can lead to a change in the charge profile of the collagen which can seriously perturb its interaction with matrix components. These age-related changes are accelerated in diabetic subjects due to hyperglycaemia and are a major cause of morbidity and mortality in these subjects[17]. It is essential that the mechanisms involved in glycation are clarified if the ageing process is to be fully understood. This will greatly increase the likelihood of alleviating the deleterious effects associated with ageing.

Glucose exists predominantly in the ring form and therefore reacts slowly with the ε-amino group of lysine to form glucosyl-lysine as the equilibrium slowly changes to the

reactive open-chain form. Ribose exists predominantly in the open chain form and reacts 200 times faster than glucose. Whether there is specificity in any particular lysines reacting is not clear although there is recent evidence that there may be some preference. We have carried out a detailed analysis and found that there is certainly a preference for the α1CB 7 peptide [18]. Identification of the glycated residues within this collagen peptide is nearing completion in our laboratory.

The hexosyl-lysines spontaneously form keto-imine structures following the Amadori rearrangement and then react further by undergoing oxidative reactions to form advanced glycation endproducts (AGEs). The term AGE is used to describe a protein-bound moiety detected after formation of the unstable Schiff-base that appears to be a final product. Thus an AGE can involve a one-step process from the Schiff base to form carboxymethyl-lysine, or undergo a series of complex reactions to form an intermolecular cross-link such as pentosidine. These complex reactions can arise from oxidative break-down of the hexosyl-lysine to produce more reactive sugars such as 3-deoxyglucosone or glyoxal which can then complex with other amino acid side chains [19]. These oxidative reactions involve free-radical and metal ion-induced mechanisms as confirmed by the inhibition of AGE formation following addition of radical scavengers and anti-oxidants [20].

The presence of AGEs in collagenous tissues has been confirmed by so-called AGE specific antibodies. However, it should be noted that in the majority of cases the AGE labelled is unknown since the antigen comprised a mixture of AGEs produced by *in vitro* glycation of bovine serum albumin rather than an isolated AGE of known structure.

The important role of AGEs in the loss of tissue function has been neatly demonstrated by administration of a mixture of AGEs prepared *in vitro* to normal rats which in the absence of hyperglycaemia subsequently displayed characteristics associated with age/diabetes i.e. basement membrane thickening, glomerular hypertrophy and an increase in mesangial volume [21].

The identification of the intermolecular cross-links formed by glycation is a subject of intense research as it is this type of AGE that is likely to cause the most profound tissue damage. Cross-linking definitely occurs as shown by the increased stiffness, decreased solubility and insensitivity to degradative enzymes, and consequently reduces the optimal functioning of the tissue [22,23]. This is particularly damaging to the basement membrane where it leads to both decreased flexibility and loss of permeability [24].

The glycation cross-link pentosidine (figure 3) is derived from lysine, arginine and ribose and has been identified in several collagenous tissues and shown to increase with age in the dura mater [25]. Unfortunately, the concentration of pentosidine in tissue only amounts to about 1 pentosidine for 2–300 molecules of collagen and at this level would not be expected to account for the obvious changes in the physical properties of the collagen fibre [26]. It is now generally regarded as a biomarker of glyco-oxidation.

The absence of a significant fluorescent cross-link in glycated collagen led us to search for non-fluorescent components present at substantial concentrations. This led to the identification of a component, nonfluorescent component-1 (NFC-1), present at the level of 1 NFC-1 to 1 collagen molecule and its increase with age suggested that it may be an important intermolecular cross-link [26]. More recently we have shown that NFC-1 is a complex mixture of imidazolones derived from the reaction of glyoxal and methylglyoxal with arginine. The complex also contains a high molecular weight component which has not been completely characterised but is believed to be a cross-link moiety [27].

Employing compounds, such as N-hippuryl-lysine, glyoxal and methyl glyoxal the formation of model compounds capable of acting as cross-links has been demonstrated.

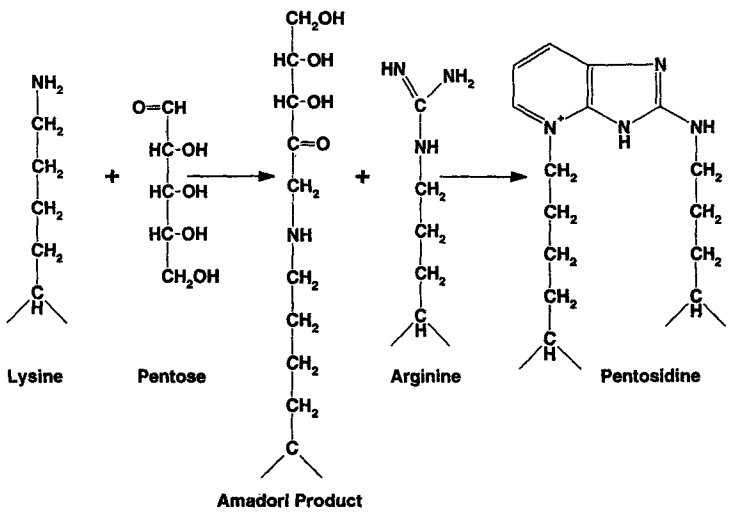

Figure 3. Formation of pentosidine from pentose, lysine and arginine.

These compounds have been designated GOLD and MOLD (figure 4) respectively [28,29] but have not been isolated from aged or diabetic tissue. Similarly, a series of compounds designated Vesperlysines A, B and C have been detected *in vitro* glycated bovine serum albumin [30]. Again the six carbon skeleton of glucose is not incorporated indicating its glyco-oxidation to fragments. Another putative cross-link Crossline (A and B) derived from N-acetyl lysine and glucose has also been described but it is acid labile and consequently has not yet been isolated from *in vivo* glycated tissue [31].

The formation of cross-links in collagen fibres *in vivo* is controlled by the accessibility of the participating groups and the environmental conditions, as such it is more than likely that many of these putative cross-links identified *in vitro* are not present *in vivo*.

There are multiple coronary risk factors associated with atherosclerosis but the increased incidence in ageing and diabetes strongly suggests that glycation plays a significant role, for example by direct glucose derived cross-link formation causing stiffening of the vascular collagen [32–34]. We have also suggested an indirect role for glycation in atherosclerosis since it has been shown that glycated collagen accelerates the oxidation of low density lipoprotein (LDL) to produce malondialdehyde, which is capable of reacting with the supporting collagenous framework of the aorta. It is interesting to note that MDA has in the past been reported to react with proteins to form a cross-link containing two Schiff bases via reaction of the two aldehydes. In our hands the reaction of MDA results in the formation of a dihydro-pyridine. This compound possesses two aldehyde side-chains which are capable of forming cross-links [35].

5. Inhibition of glycation cross-links

There is no doubt that glycation cross-links are formed *in vivo* and cause many of the catastrophic changes in body tissues with age and disease. Considerable efforts have therefore been made to find means of inhibiting their formation and cleaving existing cross-links. The isolation and characterisation of target molecules has proved to be very difficult and, as a consequence, development of precise pharmaceutical intervention has

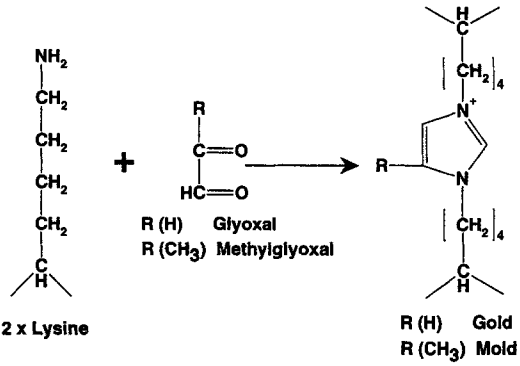

Figure 4. Pathway of imidazolium cross-link formation derived from lysines and glyoxal.

been hampered. To date the most promising route of intervention is with compounds such as aminoguanidine, which has been shown to inhibit AGE formation and reduce the characteristic damage to the tissue[36]. Aminoguanidine is currently undergoing patient trials in the USA. The mechanism is unclear and may involve reaction with the initial keto-imine, or with the oxidation products of glucose, thus inhibiting further reaction to form AGEs. Other reagents including aspirin, carnosine, thiazolidine, organic germanium compounds, flavonoids and possibly anti-oxidants such as Vitamin E may also have some therapeutic potential.

Cleavage of the intermolecular cross-link once formed is another approach and Vasan *et al*[37] recently demonstrated that N-phenylthiazolium bromide (PTB) cleaves covalent cross-links with the basic α-diketone structure and reported that cross-linked collagen could be cleaved by this reagent. A stable derivative of PTB, ALT 711, has recently been used successfully to reverse diabetes induced stiffening of large arteries, although, the exact mechanism by which this occurs is unknown[38]. At the present time there is no definitive evidence for α-diketone cross-links, and in view of their reactivity they would be expected to be precursors of ultimately more stable cross-links.

Macrophages have been reported to possess specific receptors to AGEs hence they can be targetted and removed[39]. The body itself may, therefore, have a means of removing the AGEs and it should be possible to further stimulate this system.

6. Cell-matrix interactions

In addition to the formation of cross-links, modification of single amino acid side-chains can lead to AGE formation. For, example, lysine side-chains can be modified to carboxymethyl-lysine (CML), carboxyethyl-lysine (CEL) and pyrraline. The arginine residues are similarly modified to form imidazolones, figure 5. These reactions with the side-chains clearly modify the charge profile of the molecule which could be important during interactions with other matrix components and cells.

The modification of arginine in particular raises the question of whether changes in crucial cell-matrix interaction sites, which generally involve arginine (e.g. RGD), are sufficiently extensive to seriously affect cell attachment and subsequent remodelling of the collagen. This effect is likely to be the most significant in basement membranes which not only interact with but act as a support for a variety of cells in different tissues, for example, the capillaries and the glomeruli. Arginine residues have been shown to be

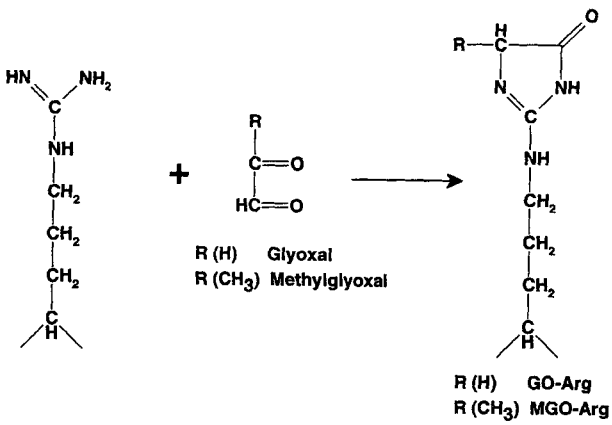

Figure 5. Pathway of imidazolone formation following reaction of glyoxals with arginine.

involved in recognition sites for the two matrix integrins $\alpha 1\beta 1$ and $\alpha 2\beta 1$ which form the physical link between the cells and the matrix[40]. In preliminary experiments we have demonstrated that the interaction of cells with collagen is significantly affected by prior incubation with methylglyoxal which we employed since it preferentially targets the arginine, compared to glucose and ribose which tend to produce a complex mixture of AGEs. We have already demonstrated that the interaction of MG63 and HT1080 cell lines with type I collagen is dramatically affected by prior incubation of the collagen with methylglyoxal. Both adhesion and cell spreading are reduced in a manner that is dependent upon the extent of collagen modification (figure 6). More recent studies have also shown that adhesion of primary endothelial cells to basement membrane is similarly perturbed by prior modification with glucose or methylglyoxal. This effect could prove to be as important in the malfunctioning of tissues than the more obvious changes in physical properties due to cross-linking. Further studies in this area are certainly warranted.

7. Age-related diseases

As we have shown above 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. The quarter-staggered end-overlap alignment of the type I fibre is crucial to provide a nucleation site in the gap region for the growth of the calcium apatite crystals, and simultaneously allows intermolecular cross-linking to take place, providing the structural strength. Bone turns over throughout life and it is essential that the newly synthesised bone, even in old age, is identical to the pre-existing collagen since any change would result in weaker bone and poor mineralisation. However, in

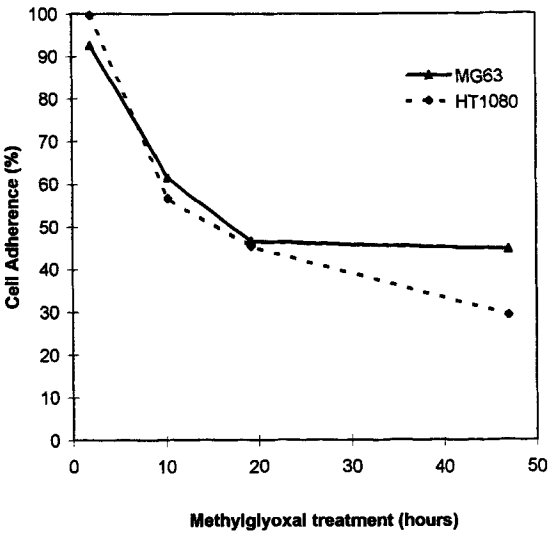

Figure 6. The effect of methylglyoxal modification of type I collagen upon cell adherence of two cell types.

diseases such as OP and OA, we have shown that the characteristics of the collagenous matrix are altered.

8. 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[41]. As discussed above the increase in telopeptide hydroxylysine would increase the pyridinoline content but reduce the pyrrole content, thus effectively weakening the mechanical strength of the bone. 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 osteoporotic subjects, Muller and his colleagues[42] also found an increase in lysine hydroxylation particularly in the $\alpha 2$ chains, and they related the extent of the change to the density of the bone.

Over-hydroxylation of lysine could arise from several mechanisms, increased lysyl hydroxylase activity as the tissue is turning over more rapidly (as in developing and fibrotic tissues); by a retardation of triple helix formation (as in some forms of osteogenesis imperfecta); or by the loss of specific proteins (such as molecular chaperones that aid the formation and stabilisation of the triple helix). However, the increase in metabolism of the bone collagen suggests that over-hydroxylation may be due to increased lysyl hydroxylase activity.

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[43].

This bone is used to monitor bone changes and it turns over faster than most other bones and would have been expected to reflect changes in bone quality, but we could find no change in the quality of the collagen, despite extensive overall loss of bone with age. 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 bisphosphonates, but the question arises as to whether the lost bone can be replaced. In collaboration with Studd and his colleagues[44] 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 synthesised collagen by the presence of immature cross-links and this was supported by histomorphometry, clearly demonstrating the anabolic effect of oestrogen[45].

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

9. 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[46] postulated would increase the shear stress on the cartilage and accelerate fragmentation. Similarly Dieppe *et al*[47] 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. We recently demonstrated that the elevated activity was due to a several-fold increase in collagen metabolism as determined by collagen synthesis and 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 levels[48]. The overall balance was in favour of increased collagen deposition as evidenced by the thickening of the subchondral bone. The levels of TGF-β, which is known to promote collagen synthesis whilst inhibiting degradation, were found to be increased four fold, and other factors such as connective tissue binding factor, which stimulates TGF-β production, are also likely to be increased.

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*[49] 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-mineralisation of the collagen. We also noted an increase in the ratio of the $\alpha 1/\alpha 2$ type I chains from 2:1 to 4:1, suggesting the presence of additional $\alpha 1$, chains possibly as type I homotrimer[50]. The homotrimer has previously been reported in embryonic and tumour derived collagens[51]. We have isolated the homotrimer and are currently attempting to demonstrate whether it is a genetically distinct type I collagen, as in the tumour, or a typical bone type I $\alpha 1$ chain.

The effects of over-hydroxylation of the lysine, hypomineralisation and the presence of the homotrimer are not fully understood. We know that over-hydroxylation could result in the formation of narrow fibrils and hypomineralisation would result in a reduction in the stiffness of the bone. The presence of the homotrimer is also likely to be deleterious. A mouse model of osteogenesis imperfecta has been shown to possess the type I homotrimer and studies on this collagen have demonstrated that it has a reduced efficiency to self-assemble, reduced ability to mineralise and reduced mechanical strength of the bone[52]. Similarly, the mechanical strength of the tail tendons were found to be reduced by 50% compared to controls[53].

The effect of over-hydroxylation and the presence of type I homotrimer would therefore most certainly have a deleterious effect on the mechanical properties of the subchondral bone in osteoarthritis. The proposal by previous workers that the sclerosis stiffens the bone may need to be re-examined. Indeed, a recent study indicated that the bone below the subchondral plate is materially weaker, in contrast to other reports[46] on the whole bone.

The finding of the homotrimer and the post-translational modifications demonstrate that OA is not a simple destructive disorder as previously believed but that there is a definite genetic predisposition involving the collagen genes. Recently Loughlin *et al*[54] found that the data did not support association of any alleles or genotypes of the Vitamin D receptor gene or the oestrogen receptor gene. However, they did find moderate evidence of an association of the Col1A1 marker with OA in females. The latter finding is consistent with the increased type I homotrimer production.

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 Carlson *et al*[55] 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.

The initial impetus for the increased turnover of the subchondral bone is unknown but bone is susceptible to changes in the tension exerted on it through the tendons and ligaments, and possibly by the compressive forces through the cartilage. Indeed, the majority of cells respond to mechanical stimulation by modulating biochemical pathways, but the nature of the mechano-receptor is unknown[56].

On the basis that the tendons and ligaments may have undergone subclinical damage and consequently reduce the tension on the bone we have recently investigated the changes in the spontaneous OA in the STR/ORT mouse. We found increased collagen metabolism in both the bone and the ligaments prior to any sign of OA as assessed by radiographic narrowing of the joint space[57]. Although preliminary this is clearly an interesting avenue of research into the aetiology OA.

The recent evidence for the changes in the metabolism and quality of the bone collagen in OA demonstrate that OA can no longer be considered as a 'wear and tear' disorder of the articular cartilage with increasing age. The work also suggests that some research effort should be re-directed from cartilage to bone and ligaments.

10. Concluding remarks

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.

Changes in collagen properties may play a role in the common age-related diseases such as osteoporosis and osteoarthritis, in which we have demonstrated changes in the quality of the newly synthesised collagen 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.

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