1. Introduction

The shape, cellular arrangement and the tensile strength of organs are determined by their extracellular matrix (ECM) [1]. This consists of a variety of molecules, among which fibrillar collagens and proteoglycans quantitatively predominate. The cells of the connective tissue were formerly considered to be almost metabolically inactive, but are now recognised as being an active participant in the initiation and modulation of tissue growth and repair [1-4]. Under normal physiological conditions, the ECM undergoes constant maintenance, with a relatively low basic turnover. Damage to tissue, largely independent of aetiology and target organ, produces a uniform response resulting in repair, which dramatically increases the activity of connective tissue cells [5-7]. Fundamentally, the process of repair serves to minimise the extent of initial damage and to preserve organ function. Increased collagen synthesis is an important part of this process. Despite the intention to save organ function, the outcome may be a twofaced sword: in an effort to save the organ, re-establishment of normal function is impaired by increased amounts of collagen. In cardiovascular diseases, the reduced contractility in the zone adjacent to a myocardial infarct [8,9], the stiffness of the myocardium in hypertrophied cardiomyopathies, and the reduced compliance of the arteries in hypertension, are all examples of impaired function brought about by the increased amounts of deposited collagen. Therefore, a better understanding of the role played by the connective tissue cells in cardiovascular diseases is essential if further diagnostic and therapeutic improvements are to be achieved. The promising reports on the collagen modulating effect of ACE inhibition and specific angiotensin II antagonists, clearly prompts this. Furthermore, thrombolytic therapy of acute myocardial infarction represent a clinical situation with pronounced influence on collagen metabolism. The first step towards management of unwanted fibrosis, without interfering with necessary repair is the development and validation of non-invasive methods for sequential measurement of extracellular activity, i.e. deposition and degradation of collagen. Serological markers of the collagen metabolism seems to constitute a good tool for forthcoming clinical research in the field. The current knowledge of serological markers of the collagen metabolism in cardiovascular diseases, is reviewed in the present article.

2. Biology of fibrillar collagens

Collagens are a group of proteins, with repeated Gly-X-Y sequences: Gly being the amino acid glycine and X and Y other amino acids, often prolines and hydroxyprolines. Furthermore, these proteins form aggregates of supporting macromolecules. 19 types of collagen have been identified thus far [1,10]. Quantitatively predominant are type I and III collagen, which are of fibrillar type. They are composed of three α chains, forming a triple helix. Type I collagen is present in most tissues, and is the most abundant protein in the human body. Type III is the second most common of the collagens, found in association with type I collagen, except in bones, which almost solely contain type I collagen. Compared with other tissues, a relatively high ratio of type III to type I is found in the cardiovascular system. Several cells are capable of synthesising collagens (osteoblasts, endothelial cells, smooth muscle cells) but most derive from fibroblasts. Secreted type I and III procollagens have amino- and carboxyterminal extension peptides, which are cleaved off before fibrils are formed (Fig. 1). However, type III collagen retains the aminoterminal extension, the propeptide. After their spontaneous assembly into fibrils, collagen molecules are
Fig. 1. Synthesis and deposition of type III collagen. After secretion of procollagen from the cell, the PIICP (carboxyterminal extension peptide) is removed, and the type III pN-collagen (collagen fibril with aminoterminal extension peptide) is attached to the surface of the fibril. As the pN-collagen is attached, PIINP from the surface of the fibril is released. Under catabolic conditions, PIINP attached to the fibril is released as a consequence on degradation of existing fibrils.

Cross-linked by pyridinium and deoxy-pyridinium containing bonds. The function of propeptides in the ECM is unknown, but they may serve as modulators of fibril growth, or constitute feedback regulation [11,12]. Although only a small amount of procollagen is needed to maintain the existing collagen skeleton under steady state conditions, large amounts of procollagen are synthesised and excreted, but undergo immediate degradation [11,13]. Blockade of this degradation may thus be the initial cause of the increased deposition of collagen. Collagen synthesis may gradually increase, followed by increasing activation of degradation in the remodelling phase. The elevated amounts of type I and III mRNA and activation of latent collagenase by plasmin lend support to this concept [14,15].

Once formed, the collagen fibril is very robust, but can be degraded by collagenase. Enzymatic digestion of the type I collagen fibril gives rise to different fragments, among which the parts of the pyridinium containing cross-link are found. The propeptides, with a molecular weight of 42–100 kDa, are drained from the interstitial space mainly by lymphatics, with a minor part transported directly across the capillary wall [16,17]. In bones and contractile organs the main route for the propeptides is thought to be transcapillary. Once the circulation is reached, the liver and kidneys are the main organs for degradation [2]. Procollagens are present in almost all body fluids.

3. Assays for determination of collagen markers

When Goldberg and Taubman in 1974 reported the first assay for the determination of a procollagen propeptide [18], the variety of collagens and their extensions was undiscovered. Since then, increasing knowledge has led to the development of a variety of assays for the determination of collagen metabolism, some of which are commercially available. Owing to differences in the antigenic profile of these assays, it is important to be clear what exactly each particular assay determines. Gel chromatography is necessary to provide such information and is mandatory when implementing a new assay in the laboratory. Furthermore, the character and the limitations of an assay per se should be borne in mind. As an example of an important pitfall in radioimmunoassays, iodination may change the steric configuration and thereby the immunoreactivity of the tracer molecule [19].

To interpret data generated by markers of type I and III collagen in the circulation, several considerations are necessary. Serum levels of the carboxyterminal propeptide of type I procollagen (PICP) mirrors synthesis, and the carboxyterminal telopeptide of type I collagen (ICTP) reflects degradation of type I collagen. Circulating levels of the type III collagen marker, the aminoterminal propeptide of type III procollagen (PIINP), may originate from ongoing synthesis or degradation of type III collagen fibrils with PIINP on their surface. The actual state of the body, anabolic or catabolic, determines what is reflected. In general, circulating PIINP should be interpreted as expressing the turnover of type III collagen. The relation between the deposition rate of type III collagen and the serum concentration of PIINP disappears under catabolic conditions [20]. Given an adequate stimulus, the levels of circulating propeptides reflect the basic metabolic rate plus the process of healing. Theoretically, the mathematical integral of the serum concentrations over time corrected for the basal level, i.e. the area under the curve, but above the basal level, may give an estimate of the amount of collagen deposited. For this to hold true, it is assumed that 100% of the propeptides liberated reach the circulation and that there is a stoichiometrical relation between propeptides and deposited collagen. Both have found experimental support [17,20–23]. The sensitivity of the measurements represents a problem in view of the slow deposition of collagen over a prolonged period of time. In such cases,
huge amounts of collagen can be deposited, without detectable changes in the levels of circulating collagen markers.

The relation between quality of healing, and increase in collagen deposition rate appears to be a bell-shaped curve (Fig. 2). Unexpectedly high levels of PIIINP indicate a constant high drive on collagen synthesis, owing to insufficient modulation of the healing processes. This kind of disorganised healing results in inadequate granulation tissue, with possible low strength, for example leading to the formation of ventricular aneurysms after acute myocardial infarction [21]. Inadequate triggering of the collagen synthesis represents the opposite alternative, also resulting in insufficient scarring. The ideal healing sequence begins with a fast increase in collagen synthesis within 1 week, followed by a reduced activity within the 2nd week, and then reaching a plateau at a lower level, still above the initial, for months.

4. Monitoring collagen turnover in cardiovascular disease

Myocytes account for 80% of the left ventricular volume, but are far outnumbered by fibroblasts, which may constitute up to two-thirds of all cells of the heart [4]. The coordinated function of the healthy heart is dependent on a collagen skeleton, which however, could be formed and maintained by a much smaller number of fibroblasts. Survival after myocardial infarction with necrosis of even several grams of tissue is common, a fact that stresses the importance of the tensile strength provided by the fibrillar network and the ability to replace the infarcted area with fibrotic tissue to ensure the integrity of the heart. These characteristics mainly depend on the qualities of fibrillar collagens, and thus emphasise the relevance of non-invasive evaluation of collagen metabolism for the understanding and assessment of the repair and remodelling occurring during the course of myocardial infarction. In this sense it is important to realise that coronary enzymes provide information about the extent of cellular damage, whereas collagen markers gives information about the healing processes triggered by the injury. The two processes will often, but not always, be associated. A small infarct followed by inadequate repair constitutes such an example of disassociated myocardial damage and healing, and may have a less good prognosis than a larger infarct with normal healing. The concept of regarding the damage and healing as two essentially different events may fruitful, and collagen markers provides a non-invasive tool for monitoring the repair processes. Not as an alternative, but in combination with coronary enzymes in serum, this may provide improvement on diagnostic ability and information on prognosis. The serum levels of procollagen propeptides (PIIINP) display a characteristic sequential pattern following non-thrombolysed AMI. Following uncomplicated AMI, the increase in serum PIIINP correlates to the extent of myocardial damage expressed by enzymatic release [21]. This correlation seems to disappear in case of development of aneurysms. showing that serum PIIINP provides additional information to the determination of the myocardial enzymes. Prognosis after AMI relates to the extent of damage and the quality of repair, with collagen involved in both the replacement fibrosis and subsequent remodelling of the left ventricle; and serum levels of PIIINP have recently been reported to provide information on prognosis after AMI [24].

Thrombolytic therapy, however, alters the association between serological markers of myocardial repair and damage, owing to the increased levels of circulating plasmin, which activate latent collagenases and consequently induce degradation of collagen during the early stage of inflammation following AMI [25,26]. Thrombolytic therapy results in a non-organ specific release of collagen metabolites, which is independent on the confirmation of AMI, and which peaks 1–4 h after thrombolysis [27,28]. This is followed (after 3–5 days) by sequential changes, predominantly relating to the process of healing [24]. The nonspecific degradation of collagen fibrils induced by thrombolysis may, in part, explain the increased incidence of cardiac rupture after thrombolytic therapy [29]. The patency of the infarct-related coronary artery following thrombolysis appears, at least to some extent, to depend on the fibrin specificity of the thrombolytic agent. Thus, tissue-plasminogen activator (t-PA) appears to be superior in terms of the induction of initial recanalisation of the thrombosed coronary artery, but without adjunctive heparin therapy will be followed by reocclusion more often than would occur with streptokinase [30]. The degradation of collagen consequent on thrombolytic therapy with the accompanying release of collagen metabolites to the circulation appears to be more pronounced with streptokinase than with t-PA [25]. Much of the collagen affected by thrombolytic therapy originates from the vessel wall where exposed collagen acts as a potent thrombogenic factor. This may point to a role for collagen metabolites (PIIINP) as markers of the risk of reoclusion of the infarct-related coronary artery. Furthermore, thrombolytic therapy of acute myocardial infarction may per se interfere with the repair processes, increasing the risk of ventricular rupture, which could possibly be monitored by circulating collagen markers. The beneficial effect of thrombolysis on survival after acute myocardial infarction is indisputable, and some of this effect may be attributable to nonspecific ‘shaving’ of thrombogenic collagen in atherosclerotic vessel walls thereby reducing all-round thrombogenicity. Hence, prophylactic thrombolysis of high risk patients with known extensive atherosclerosis may be an idea for the future. The given dose of the thrombolytic agent might very well be markedly reduced in order to minimize the side effects, with out interfering with the effect on the collagenous components.
Aspects of myocardial pathology other than myocardial infarction have also brought collagen into focus. Thus, collagen is increasingly becoming recognised as a key determinant in the pathophysiology of general atherosclerosis, cardiomyopathy and hypertensive heart disease. As a natural consequence, interest has risen in serological monitoring of collagen metabolism during various aspects of cardiovascular disease. Serum levels of PIIINP in atherosclerotic patients apparently differ from those of controls [31]. Furthermore, elevated levels of serum PICP and PIIINP in hypertensive patients appear to be normal after 6 months of ACE inhibitor therapy [32]. In cardiomyopathy (dilated or ischaemic) correlations have been found between circulating levels of type I collagen markers of synthesis and degradation, type III collagen turnover, the basement membrane component laminin, and the severity of heart failure and prognosis [33]. Immediately after heart transplantation the serum concentration of PIIINP increases dramatically, probably because of collagen deposition as part of the surgical wound healing process, with levels remaining elevated for several months [34]. Severe rejection also appears to be reflected by the serum concentrations of PIIINP [34].

The renin-angiotensin-aldosterone and bradykinin systems have a profound influence on collagen. It is very possible that the ACE inhibitors and angiotensin II receptor blockers specifically inhibit collagen deposition and, through this mode of action, decreases circulating levels of collagen metabolites. Diez et al. [32] found a correlation between the mass of the left ventricle and the type I procollagen propeptide, which, however, does not entirely fit in with the concept of serum levels as estimates of the rate of deposition, rather than the amount of collagen deposited. The correlation is especially interesting, because only a small fraction of the total body content of type I collagen is found in the heart; the major part is located in bones, skin, and vessels. Interpretation of serological measurements of ECM components must take into account, the hemodynamic conditions and medication. Heart failure per se can affect organs involved in the metabolism of the ECM components, most importantly the liver, leading to impaired degradation of circulating markers and/or increased fibrosis in other organs. The significance of ACE inhibition has been mentioned above, and will be further elucidated in coming articles of the ‘Mystery Series’. The influence of the immunomodulating medicine given before and after transplantation has not been investigated in detail, but it is known that therapy with corticosteroid is followed by a decrease in the level of circulating types I and III procollagen propeptides [35].

5. Perspectives

Serological markers of collagen turnover have proved to be a valuable scientific tool, whereas the clinical implica-

References


