INTERACTION BETWEEN ELASTIN AND ELASTASES AND ITS ROLE IN THE AGING OF THE ARTERIAL WALL, SKIN AND OTHER CONNECTIVE TISSUES. A REVIEW*

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SUMMARY

Elastic fibers are progressively lysed during maturation and aging and in an accelerated fashion in several aging diseases such as diabetes, arteriosclerosis, emphysema and several skin diseases. Several enzymes (elastase-type proteases) were isolated in recent years in our laboratory which appear to be involved in these processes. A cell membrane bound serine protease was isolated from arterial smooth muscle cells and was shown to increase with in vitro aging of the cells. A metallo-protease was isolated from skin fibroblasts and was shown to be capable of attacking the constituents of elastic fibers, mainly the microfibrillar glycoproteins and also the desmosine cross linked elastin in vivo. This partially purified fibroblast enzyme was shown to attack these elastic fibers when injected into the dermis. A new selective staining procedure was used to visualise and quantitate, by computerized image analysis, the skin elastic fibers in normal and pathological human or animal skin biopsies. This method, combined with the injection of elastase in rabbit skins, alone or together with inhibitors, enables the ex vivo/in vivo study of elastase action (and of its inhibition).

Key words: Elastin; Elastases; Aging; Artery; Skin; Extracellular matrix; Connective tissues

INTRODUCTION

Elastin is, phylogenetically speaking, the “youngest” fibrous protein of the extracellular matrix. It appears only from chordates and the earliest vertebrates (the fish),

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in contrast to collagen and structural glycoproteins which appear much earlier with the first metazoans and proteoglycans or glycosaminoglycans which appear in early invertebrates [1,2]. It may be a coincidence that this same fibrous protein, elastin, is also the first which is degraded, fragmented and lysed during the aging process in several tissues, such as the arterial wall, the dermis and the lung [3–5].

Several pathological processes are now known where this elastolytic process is accelerated. Such processes are arteriosclerosis, diabetes, several diseases of the skin and emphysema.

Recent studies carried out in our laboratory enabled us to elucidate some of the cellular and molecular mechanisms involved in these processes. They all depend on the regulation of the biosynthesis and inhibition of proteolytic enzymes capable of attacking elastic fibers, the elastases or elastase-type proteases [6,7].

The biological role of elastic fibers is mainly to confer a higher elasticity to tissues than proteoglycans or types I, II, III or IV collagen could do. As a matter of fact elastin is not the only polymer which shows rheological properties which can be qualified as elastic, hyaluronate gels or type IV collagen might also possess some elastic properties. Nevertheless, during phylogenesis elastin evolved and was concentrated in several tissues which need a much higher elastic recoil than others. Such tissues are the elastic cartilage, such as that of the ear, the trachea, the skin, lung and mainly the large arteries such as the aorta. At every systole the volume of the aorta increases considerably and the spontaneous elastic recoil exerts a considerable facilitating influence on the circulation and lowers the stress which is imposed on the myocard to ensure an efficient circulation of the blood. This elastic recoil of aorta is rendered possible through the coordinated extension and retraction of its concentric elastic laminae. During aging elastin is degraded and the collagen/elastin ratio increases. As a result, the elastic recoil of the vessel wall decreases and the aorta has to increase its volume to compensate for the decreasing elasticity of its wall. The result is an increase of the volume of the aorta with a rigid vessel wall, poorer in elastin and richer in collagen, impregnated with lipids and Ca²⁺ salts.

The influence of this progressive modification of the rheological properties of the aorta with age was quantitatively evaluated and recently reviewed by Bader [8] and Kohn [9].

The aging of the skin is also accompanied by a loss of the elastic fibers of the superficial dermis which were studied in great detail by Cotta-Pereira et al. [10], Bouissou et al. [4,11] and Robert [12]. It could be shown, using skin biopsies, that the destruction of the superficial elastic fibrillar network is accompanied by the deposition of lipids and lipoproteins of the LDL family in the dermis [11,13]. The parallelism between lipid deposition and elastolysis in the skin and the aorta justifies the use of skin biopsies for the evaluation of the degree of elastolysis and lipid deposition of the arterial wall [11,14].

The destruction of the fine elastic fibrils of the papillary dermis is not the only process observed during the aging of the skin. The collagen bundles also become looser and fibroblasts show signs of degenerative modifications [11]. Nevertheless one of the
most conspicuous modifications which can be quantified is this rarefaction of elastic fibers.

A recent modification of the Verhoeff staining enabled our laboratory to propose a new method [15] for staining of elastic fibers keeping a complete white background which can then be quantitated by automatic or semi-automatic image analysis.

Figure 1 shows an example of this procedure which can be used for the evaluation of the aging process, but also for the study of the elastolytic enzymes involved in this process, using either in vivo injections of such enzymes in the skin followed by a biopsy and histochemical evaluation of the lysis or the action of these enzymes on skin sections obtained on the cryostat. This method also enables the study of natural and synthetic inhibitors on the elastolytic process [16].

Fig. 1. Human skin, paraffin embedded, stained acc. to Godeau [15] to visualize the papillary elastic network. x500. OXY = oxytalan fibers, Elau = elaunin fibers, ELAS = elastic fibers.
Fig. 2. Skin biopsy of a Werner patient (B) and of a control age matched skin biopsy (A) stained acc. to a modified technique of Unna [16]. Notice the strong decrease of oxytalan and elaunin fibers in (B) as compared to (A). × 375.
Elastic Fibers of the Skin in Werner's Syndrome

Recently we had the opportunity to study a skin biopsy of a Werner patient (40 years) of Professor DeWulder of the Lille Medical School. Figure 2 shows a section of the skin of this patient stained according to a modified method of Unna [16].

The arborescent fine elastic network of the papillary dermis is absent. The oxtalan fibers were no more detectable and only scarce and fragmented elaunin fibers could be detected under the dermo-epidermal basement membrane. There is, however, a dense elastic network in the deeper dermis.

The collagen fibers were also abnormal as shown by the picro-Syrius-red staining according to Junquiera et al. [17]. A strong increase of fine collagen fibers stained in green (type III) could be seen in the papillary dermis together with a strong increase of fibronectin (in preparation).

These results confirm the strong analogy between the modifications of the dermis in Werner's syndrome and those found in much older (65–70 years of age) normal persons or in younger diabetic patients.

The pulmonary elastic fibrils also play an important role in the elastic recoil of the lung during the respiratory process. Emphysema is accomplished by the destruction of the alveolar walls and especially of the elastic laminae which contribute effectively to the elastic recoil of the organ during the respiratory movements.

The experimental method which is widely used to study emphysema in animals is the intratracheal instillation of elastolytic proteases such as pancreatic elastase or papain [18]. This is followed by the rapid destruction of the alveolar elastic structures and although in a few weeks elastin is resynthesised, a chronic disease similar to human panalveolar emphysema is obtained (Fig. 3).

Recent studies showed that one of the mechanisms by which smoking accelerates emphysema in man is due to the fact that the α1-protease inhibitor (α1-antitrypsin) is inactivated by the oxidation of a methionine residue in its active centre [19].

Recent studies in our laboratory showed that this is not the only mechanism because there is an age-dependent decrease of the efficiency of elastase inhibitors in the sera of smokers which is not seen in the sera of non-smokers (Fig. 4). These results cannot be explained only by the oxidation of a methionine residue in the antiprotease molecule because the turnover of this protein is sufficiently rapid to replace the inactive molecules by active ones.

The results shown in Fig. 4 can, however, be explained by several other hypothesis. One of them would be a negative feedback of the oxidised inhibitor on its own synthesis. Another possibility is the acceleration of the "molecular aging" of the inhibitor. It was shown that a score of proteins and enzymes show an age-dependent decrease of their efficiency [20,21]. A more or less important fraction of the enzymes or proteins synthesised in the aging organism exhibit a lower specific activity than those isolated from "young" organisms. In agreement with these findings, we could show that by immunological criteria there is no decrease in the sera of smokers in the amount of circulating
elastase inhibitors (α₁-protease inhibitor and α₂-macroglobulin). Only the activity of these inhibitors as determined by a plaque assay [22] showed a strong decrease [23]. This decrease of activity per molecule of circulating inhibitors is similar to the reported decrease of activity of a score of enzymes during the aging process [20,21].

THE NATURE OF ELASTOLYTIC PROTEASES INVOLVED IN THE AGE-DEPENDENT PATHOLOGIES OF ELASTIC TISSUES

The first protease shown attacking rapidly elastic fibers was the pancreatic elastase
Fig. 4. Age dependent decrease of serum elastase inhibitors in smokers and absence of significant variation in non-smokers [23].
isolated and described in detail by Banga and Balo [24,25] in the early 50's. Several other proteases endowed with elastolytic activity were since then described (for a review, see ref. [6] and [7]), such as leukocyte elastase which is a lysosomal enzyme, platelet derived protease, and the macrophage elastase [26]. More recently we could isolate and characterise an elastase-type protease from smooth muscle cells of the aorta [27–30] and from fibroblasts of the skin [16]. Table I shows some of the characteristics of these enzymes.

The first general consideration suggested by Table I is that it is highly improbable that the only biological role of these enzymes would be the degradation of elastic fibers. As a matter of fact, such catabolic processes are useful for proteins which have a measurable

| TABLE I |
| PROPERTIES OF THE ELASTASE-TYPE PROTEASES ISOLATED FROM ARTERIAL SMOOTH MUSCLE CELLS AND FROM FIBROBLASTS |

<table>
<thead>
<tr>
<th>Aorta smooth muscle cells</th>
<th>Fibroblasts (skin, vulva, chick embryo)</th>
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</thead>
<tbody>
<tr>
<td>Class of the protease</td>
<td>Serine</td>
</tr>
<tr>
<td>Substrates</td>
<td>Suc(Ala)₃NA</td>
</tr>
<tr>
<td></td>
<td>Z Ala, ONap</td>
</tr>
<tr>
<td></td>
<td>Ac(Ala)₃ProAlaNa</td>
</tr>
<tr>
<td></td>
<td>Elastin(s)</td>
</tr>
<tr>
<td></td>
<td>Azocasein</td>
</tr>
<tr>
<td>Optimum pH of activity</td>
<td>~8.0</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>30 kD (rat aorta)</td>
</tr>
<tr>
<td>Inhibitors</td>
<td>α₁, Pi</td>
</tr>
<tr>
<td></td>
<td>α₂ M</td>
</tr>
<tr>
<td></td>
<td>MeOSuc(Ala)₂ProAlaCH₂Cl</td>
</tr>
<tr>
<td>Effectors (synthesis)</td>
<td>Cycloheximide</td>
</tr>
<tr>
<td>inhibitors</td>
<td>LDL</td>
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<td>activators</td>
<td>VLDL</td>
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<td>Properties and</td>
<td>Increases with cell passages.</td>
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<td>implication in diseases</td>
<td>Arterial diseases.</td>
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*Abbreviations: Suc(Ala)₃NA: succinyl-trialanine paranitroanilide; Z Ala, ONap: benzyloxycarbonyl L-alanine-2 naphthol ester; Ac(Ala)₃ProAlaNA: acetyl bis-alanine proline-alanine-nitroanilid; α₁, Pi: alpha 1 proteinase inhibitor; α₂ M: alpha 2 macroglobulin; MeOSuc(Ala)₂ProAlaCH₂Cl: methoxy-succinyl bis-alanine-proline-alanine-chloro methyl ketone; and Sgp mf: structural glycoproteins microfibrils.*
turnover, which is not the case of elastic fibers. Although the synthesis of elastin remains possible during the whole life, the elastic structures deposited during morphogenesis do not appear to have a measurable turnover. It seems much more probable to assume that these enzymes, quite different in their nature and relative activity, have completely different biological roles, and, by accident, may happen also to degrade elastic fibers if the conditions enable them to do so.

THE SMOOTH MUSCLE CELL ELASTASE-TYPE PROTEASE

Cultures of smooth muscle cells derived from the aorta of rats and pigs were studied in this respect [27–30]. A serine protease could be extracted which was shown to be localized on the cell membrane [31]. Table I shows some of its properties. It can be seen that it is inhibited by the usual serine protease inhibitors. Although calcium ions have some effect, it does not seem to be a metallo-protease. It may be considered as a calcium modulated serine protease. Its membrane localisation and the absence of any detectable secretion of this enzyme in tissue culture condition suggests that

![Diagram](image)

Fig. 5. A: Atherosclerotic plaque of rabbit aorta induced by cholesterol and primrose oil feeding (from Robert et al. [33]) showing the disorganised subendothelial layers, lysed elastic laminae and the migrating smooth muscle cells which form the fibrous plaque by oversynthesising the extracellular matrix macromolecules. This migration-oversynthesis may be the result of a “phenotypic modulation” [32] which is schematically represented on the drawing (B). From sessile and contractile the smooth muscle cells become migrating and over synthesising matrix elements.
this enzyme attacks elastic fibers only when the smooth muscle cells are "modulated" from sessile and contractile to become migrating and biosynthetic [32]. This phenotypic modulation was proposed by the Campbell's as one of the basic mechanisms involved in atherogenesis (Fig. 5).

As a matter of fact, the intimal elastic plaques (Fig. 5) are composed of such "modulated" smooth muscle cells which have a variable but often important biosynthetic activity and oversynthesize the extracellular matrix macromolecules such as collagen, proteoglycans, elastin and glycoproteins [3,33].

A strong activation of the biosynthesis of this elastase-type enzyme was shown to occur with subsequent passages of the smooth muscle cells (in vitro aging) and also by adding LDL to the culture medium. HDL had no such effect [34].

These in vitro modifications parallel surprisingly well those which were found in vivo and ex vivo using human aortas of individuals of different ages and pathological conditions [35,36]. As a matter of fact, in human aortas we could also show an age-dependent increase of the elastase-type activity in the aorta extracts [35]. It is therefore tempting to speculate that the age-dependent increase of this enzyme activity in the aorta is the result of its increased synthesis by the smooth muscle cells of the vessel wall. This increase is further potentiated by the presence of LDL in the proximity of the cells and by the "phenotypic modulation" of the cells during their migration from the media towards the intima. During the migration they degrade the elastic laminae of the aorta as a result of the continuous contact between the cell membrane and the elastic fibers. The released elastic fibers have a chemotactic effect and may well attract macrophages and promote the migration of the smooth muscle cells.

THE ELASTASE-TYPE PROTEASE OF HUMAN SKIN FIBROBLASTS

Fibroblasts were cultured from human skin and vulva and an elastase-type protease could be extracted from these fibroblasts which behave as a metallo-protease [16]. Table I shows some of the characteristics of this enzyme.

While the smooth muscle cell enzyme is not secreted, this enzyme is secreted into the medium, where about 25–30% of the total activity could be recovered. It is inactivated by metal complexing agents such as α,α'-dipyridyl and EDTA, and reactivated by calcium ions. It seems likely that it is involved in the abovementioned degradation of elastic fibers of the skin. The fine subpapillary elastic fibers are "microfibrils" which are composed of structural glycoproteins [37] and according to Cotta-Pereira only the deep dermis contains the mature elastic fibers [10].

The fibroblast enzyme, when injected in the dermis, was shown to be able to act not only on the mature elastic fibers but to degrade also the microfibrils. This degradation of the microfibrils was shown to be much faster with the fibroblast enzyme than the degradation of mature elastic fibers [16]. Therefore this fibroblast enzyme seems to be a good candidate for the age-dependent degradation of the elastic fibers of the skin and may also be involved in such pathological processes as Lichen sclerosus et atrophicus of the vulva, where a destruction of elastic fibers and fibronectin could be demonstrated [6]. More details on these and similar pathological skin conditions can be found in a recent review [39].
It was demonstrated previously by Bouissou et al. [11] and Robert and Robert [3] that there is a parallelism between the degradation of elastic fibers in the dermis and in major blood vessels, such as the aorta [3]. It appears now that two different enzymes may well be involved in this process in the two different tissues. We now have to look for a mechanism which might coordinate the increase and/or liberation of these different enzymes. Local increase in tissue deposited LDL may be one factor. Hormonal mechanisms may well be possible candidates also.

The abovementioned studies on the elastase-type proteases and their role in the degradation of elastic fibers will certainly enable us in the near future to propose new types of inhibitors to control this elastolytic processes and slow down the age dependent degradation of elastic fibers. Such inhibitors were recently synthesised in our laboratory and preliminary in vitro and in vivo experiments gave promising results [36].

REFERENCES


