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Review

Circulating elastin peptides, role in vascular pathology

Peptides d'élastine dans la circulation, rôle en pathologie vasculaire

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ABSTRACT

The atherosclerotic process starts with the degradation of elastic fibers. Their presence was demonstrated in the circulation as well as several of their biological properties elucidated. We described years ago a procedure to obtain large elastin peptides by organo-alkaline hydrolysis, κ-elastin. This method enabled also the preparation of specific antibodies used to determine elastin peptides, as well as anti-elastin antibodies in body fluids and tissue extracts. Elastin peptides were determined in a large number of human blood samples. Studies were carried out to explore their pharmacological properties. Similar recent studies by other laboratories confirmed our findings and arose new interest in circulating elastin peptides for their biological activities. This recent trend justified the publication of a review of the biological and pathological activities of elastin peptides demonstrated during our previous studies, subject of this article.

Mots clés :
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RÉSUMÉ

Le processus d'athérosclérose débute avec la dégradation des fibres élastiques. Leur présence a été démontrée dans le sang circulant, ainsi que leurs propriétés biologiques et pathologiques. Nous avons décrit, il y a des années, une méthode pour la préparation de gros peptides d'élastine, la κ-élastine. Cette méthode a rendu possible la préparation d'anticorps anti-élastine pour la détection et le dosage de peptides d'élastine dans le sang et les tissus. La concentration des peptides circulants a été déterminée dans un grand nombre de sérums normaux et pathologiques. Les propriétés pharmacologiques des peptides d'élastine ont été aussi étudiées. Des études similaires viennent d'être publiées récemment par plusieurs équipes, confirmant l'importance et l'actualité de ce sujet. L'objet de cette revue est la présentation de l'activité biologique des peptides d'élastine mis en évidence au cours de nos études.

1. Introduction

Among the most important modifications with age of the elastic arteries is the fragmentation of elastic fibers observed by early pathologists who carried out histological studies after autopsies, as currently practiced in Prof. Balo's Experimental Pathology Institute at the Semmelweis Medical University in Budapest, where one of us (LR) was trained (Fig. 1) [1]. Prof. Balo with his wife Ilona Banga discovered the first elastolytic protease, pancreatic elastase, and wrote extensive reviews on its role in atherogenesis [2,3].

Interest in elastin was slow to arise during the after-war decades of last century, mainly because of its extraordinary resistance and insolubility as shown also by its routine purification procedure by heating to 100 °C for 45 min. in 0.1 M NaOH solution [4,5]. Miles Partridge in Cambridge worked out a method to "solubilize" elastin by mild acid hydrolysis, refluxing fibers in 0.25 M oxalic acid, and obtained smaller and larger sized peptides, α and β elastin [6]. We described another procedure to "solubilize" elastin, using 1 M KOH in 80% aqueous ethanol [7,8]. Although elastin resists boiling in dilute aqueous alkaline solutions, in presence of organic solvents, it becomes easily degradable. This could be attributed to the strong hydrophobic interactions stabilizing elastin [8]. The large peptides obtained by degradation

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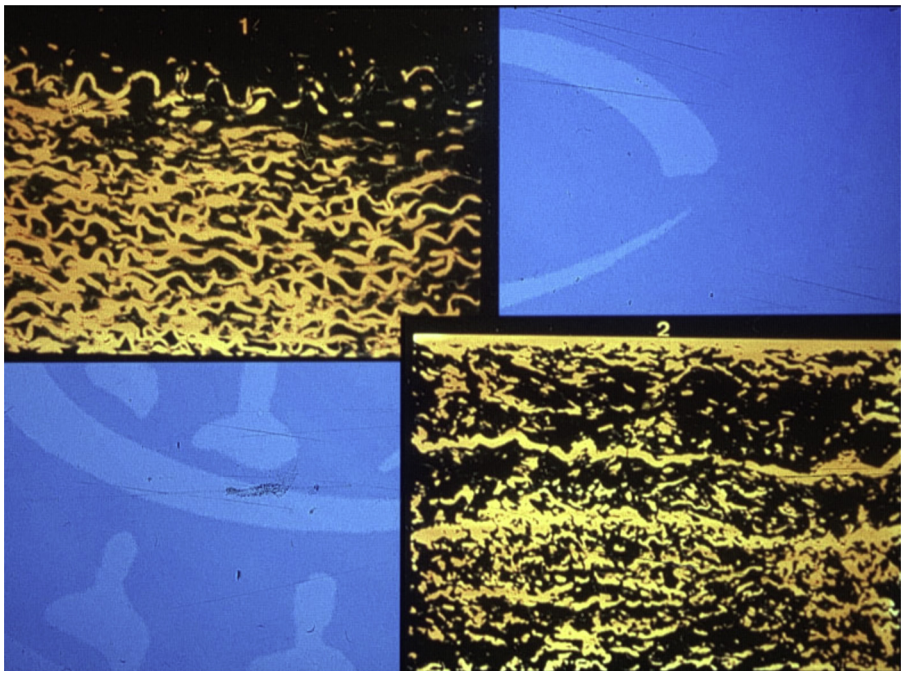

Fig. 1. Aging of the arterial wall, characterized by the fragmentation of elastic fibers. On top: thoracic aorta wall of a young (~20 years) man. Bottom: same from a 70 years individual. Notice the fragmentation of the elastic fibers. (Reproduced with permission from [7]).

in 1 M KOH in 80% ethanol were designated κ-elastin (kappa-elastin) and widely used since for research and industrial purposes [7]. The discovery of the ethanolic KOH-procedure for the preparation of κ-elastin was a typically serendipitous event. When one of us (LR) joined Prof. Dische at the Biochemistry Department of Columbia University's College of Physicians and Surgeons for a post-doctoral stay on leave from the French National Research Center (CNRS) as a carrier investigator in the early 1960's, Dr Dische proposed to use either heating tissues in dilute TCA or hydrolyzing in an ethanolic KOH solution in order to separate proteins from attached carbohydrates (glycoproteins), a procedure currently used in his laboratory for the study of the composition of glycans of glycoconjugates. Before doing it, it was important to test this procedure with purified macromolecules of the extracellular matrix (ECM). The well-known resistance of elastin to alkaline hydrolysis disappeared in presence of organic solvents, systematically tested to explore the hydrophobic nature of elastin [5,8]. It appeared during these tests that insoluble, fibrous elastin was degraded in alkaline ethanol at room temperature to large peptides designated κ-elastin [4,8]. This was the beginning of a long series of experiments, undertaken after return to Paris, resulting in a number of publications on the properties of κ-elastin, first of all, the presence of elastin peptides in human sera [9,10].

Elastase-type enzymes degrading elastin fibers were demonstrated in other tissues as the pancreas where they were discovered [2,3], among them, the vascular wall, the skin, produced by smooth muscle cells, fibroblasts and mononuclear cells, as those present in atherosclerotic lesions of the vascular wall [9,11–14]. It was also demonstrated that their activity is increasing with age, also with severity of atherosclerosis (Fig. 2) as well as with passage number in cell cultures (Fig. 3) [10–14].

2. Immunochemical studies

κ-elastin was shown to be antigenic, antibodies were raised in rabbits and used for the determination of elastin peptides in human blood serum [9,10]. A large number of human sera were analyzed as part of an epidemiological study in collaboration with Dr Annick Alperovitch – the EVA study (Étude du Vieillissement Artériel – study of vascular aging) [10]. Using κ-elastin fore calibration, it was found that all human sera contained elastin peptides. Their concentration showed a relatively wide dispersion both for males and females at all ages (Fig. 4). Anti-elastin antibodies were also detected in human sera [15]. During these studies, we could show that rabbits immunized with elastin in complete Freund's adjuvant developed sever atherosclerotic lesions [16,17]. An immunological theory of atherosclerosis was proposed and further confirmed by the demonstration in human sera of specific anti-elastin antibodies [15] and their role in atherogenesis. Age-dependent modifications of the vessel wall could also be attributed to similar mechanisms, such as the upregulation of vascular elastase activity[13,14]. Similar mechanisms could be proposed for the age-dependent alterations of the vascular wall [18–20].

All these experiments were largely facilitated by the use of κ-elastin both for immunization as well as for testing and titrating antibodies.

3. Pharmacological properties of κ-elastin

As a result of the rapidly expending use of elastin peptides in biochemistry, it became important to establish its relevant pharmacological properties. This was accomplished [21], the results will be summarized. One of the important observations was the strong affinity of κ-elastin for collagen fibers covered by strongly adhering elastin peptides as shown in vitro and confirmed in vivo on shaved rat skin where the area of the dermis composed essentially by collagen fibers, treated with κ-elastin took up the specific staining of elastin [21]. As the rheological properties of the treated skin improved, it could be assumed that the κ-elastin treatment was efficient in this respect also, improving the rheological properties of the treated skin. ^{3}H-labelled κ-elastin was administered i.v. and also percutaneously to rats [21,22]. After i.v. administration, radiolabelled elastin peptides were first rapidly excreted in the urine ($t_{1/2}$ = 7.9 min) followed by a slower phase ($t_{1/2}$ = 162 min). The strong affinity of elastin peptides for the skin