Pathologie Biologie 62 (2014) 337–341

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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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ARTICLE INFO

Article history:
Received 16 April 2014
Accepted 12 May 2014
Available online 4 September 2014

Keywords:
Elastin
κ-elastin
Elastin peptides
Elastases
Pharmacology of elastin peptides

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 :
Élastine
κ-élastine
Peptides d'élastine
Pharmacologie des peptides d'élastine

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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http://dx.doi.org/10.1016/j.patbio.2014.05.020
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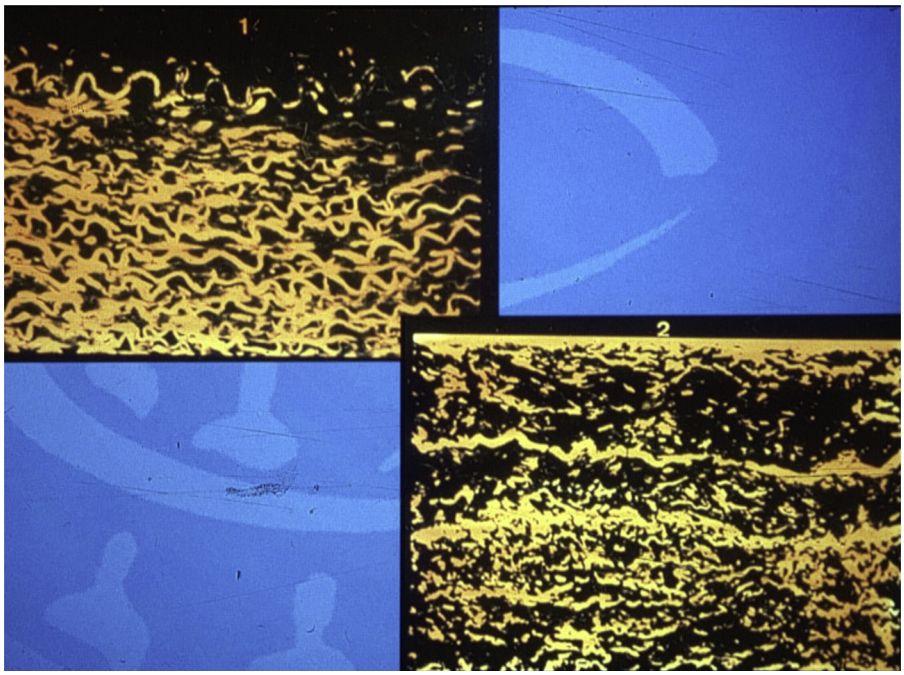

Fig. 1. Aging of the arterial wall, characterized by the fragmentation of elastic fibers. On top: thoracic aorta wall of a young (~20 years) man. Bottom: same from a 70 years individual. Notice the fragmentation of the elastic fibers. (Reproduced with permission from [7]).

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

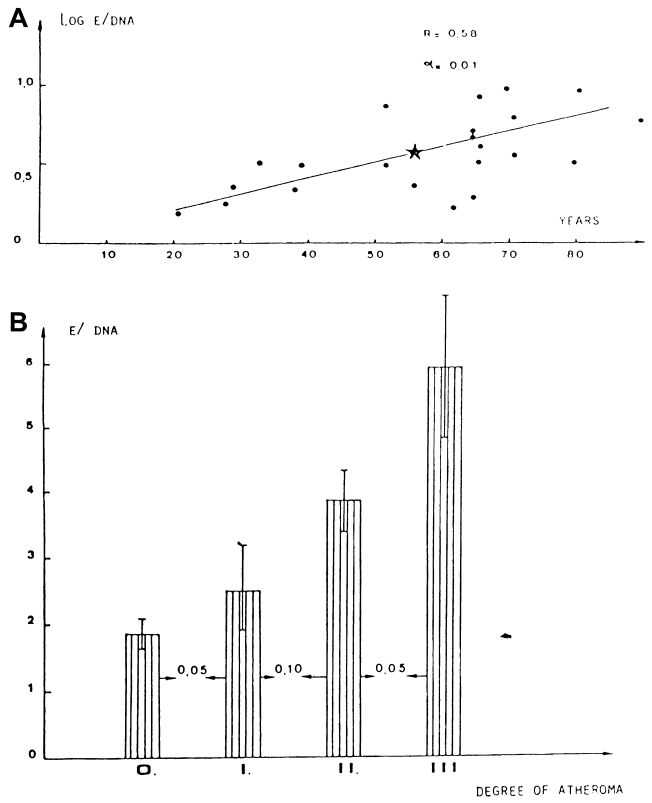

Fig. 2. Increase with age (top) and the degree of atherosclerosis (bottom) of the elastase-type protease activity, determined in post-mortem human aorta extracts. (reproduced with permission from [14]).

was confirmed by their slow elimination after percutaneous administration, 30 to 40% of the labelled peptides were still present in the dermis 48 h after administration. Besides the skin, only the liver and lung contained significant radioactivity (4% and 2% resp. of the administered dose) at 48 h. Urinary elimination concerned about 5% of the administered dose. Histochemical studies on the skin showed the diffuse presence of elastin peptides in the dermis several weeks after repeated percutaneous administration as described, strongly associated with the collagen fibers [21,22]. Part of this increase was the result of the stimulation of elastogenesis by the fibroblasts. Table 1 shows the organ distribution after i.v. administration of elastin peptides. Less than 70% of total radioactivity

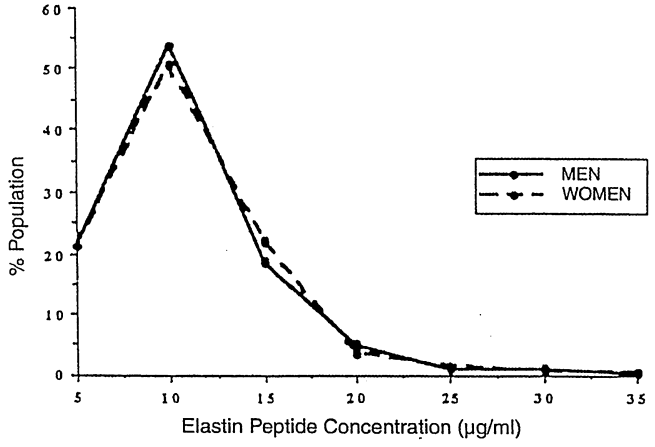

Fig. 3. Distribution of elastin peptide concentrations in the sera of normal (non pathological) individuals. Abscissa: elastin peptide concentration in µg/µL. Ordinates: frequency, %. (reproduced with permission from [14]).

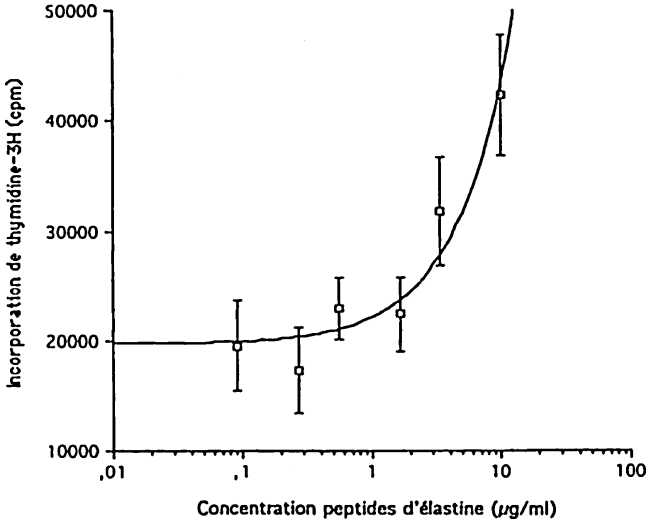

Fig. 4. Stimulation of cell proliferation by elastin peptides determined by ^{3}H-thymidine incorporation in Chinese Hamster fibroblasts (CCL39). Abscissa: elastin peptide concentration in µg/µL, log κ-elastin. Ordinates: radioactivity, dpm (counts per minute). (reproduced with permission from [7]).

administered could be recovered from the organs of the animals. The largest part of the non-excreted fraction is localized in the skin. These experiments confirmed and extended the biological importance of the strong affinity of elastin peptides for collagen fibers.

4. Role in atherogenesis

One of the important properties exhibited by elastin peptides was their strong stimulation of cell proliferation as shown in Fig. 4 with Chinese Hamster fibroblasts. Similar results were obtained with vascular smooth muscle cells. It is therefore tempting to extrapolate these findings to the process of "intimal proliferation" observed at the early phase of atherogenesis [18–20] (Fig. 5). We demonstrated the presence of elastolytic proteases of the vessel wall and their strong upregulation with age and developing atherosclerosis, both effects acting independently with a strong increase of the degradation of vascular elastin [11–13].

These results are in favor of an important role of elastin peptides in the generation and progression of the atherosclerotic process.

Recent studies by several laboratories confirmed and extended the pharmaco-biological properties of elastin peptides. According to Gayral et al. [23], elastin peptides potentiate atherosclerosis development, this effect was shown to be mediated by the immune Neu1-PI3 K_y pathway. These results can be considered as a confirmation of our previous studies demonstrating the production in rabbits of strong atherosclerosis lesions by immunization with elastin peptides [16,17]. The team of M.P. Jacob demonstrated that potassium channel openers stimulate elastin production by SMCs in the rat aorta and reversed the genetic elastin deficit in BN rats [24]. Elastin peptides were shown also to produce insulin resistance in mice and contribute to the development of type II diabetes [25]. The team of Hornebeck demonstrated the role of elastolysis in inflammation associated with aging [26,27]. The team of Debelle further extended these results to several pathologies and confirmed the importance of elastin peptides in atherogenesis [28–30]. All these recent studies from several laboratories confirm and extend our demonstration of the presence and importance of circulatory breakdown products of vascular elastic fibers and their role in age-related pathologies.

Table 1
Distribution of the radioactivity in the organs of rats, 24 h after I.V. injection of radioactive κ-elastin (28 mg proteins, 644 cpm). The results of 2 independent experiments are given. For details see "methods".

Organ	Weight of organs		Total radioactivity per organ		% of total radioactivity administered	
	Experiment no. 1 (370 g)	Experiment no. 2 (475 g)	Experiment no. 1	Experiment no. 2	Experiment no. 1	Experiment no. 2
Lung	1.5	1.9	22.155	4.242	0.61	0.75
Liver	11.5	14.7	3.282	22.450	4.14	4.01
Heart	0.913	0.983	2.491	2.935	0.46	0.52
Spleen	0.519	0.494	1.970	2.009	0.36	0.35
Aorta	0.095	0.079	1.823	1.318	0.34	0.23
Testicles	3.165	2.97	5.922	6.300	1.10	1.12
Skin	3.71	2.49	7.778	5.253	1.45	0.93
Muscles	2.94	3.7	5.907	6.721	1.10	1.20
Kidney	2.06	2.62	25.731	50.461	4.81	9.02
Intestine	0.524	1.25	1.933	3.001	0.36	0.53
Stomach	1.28	1.67	3.445	3.864	0.84	0.69

5. Discussion

Over the recent decades, a new principle was demonstrated, consisting in the appearance of original biological activities by breakdown products of matrix macromolecules. Among the first, the biological activities of elastin peptides were explored as summarized above. Proteolytic fragments of fibronectin were also shown to exhibit original biological activities, absent from the intact molecule [31,32]. It is easily degraded by proteolytic enzymes and degradation products were demonstrated by immunoblot and other procedures in human blood. Among these studies, those of Barlati in Italy can be cited, who showed for some fibronectin fragments tumor potentiation among other properties [31]. Vera Keil at the Paris Pasteur Institute demonstrated proteolytic activity for some proteolytic FN-fragments. Homandberg demonstrated articular inflammation p-
roduced by FN-fragments. Finally some other FN-fragments were shown to upregulate FN biosynthesis, creating all the elements of a vicious circle [31]. FN-fragments were detected by immunoblot in the blood of elderly patients in a geriatric hospital, but were shown to be absent from the blood of centenarians without overt pathologies [32]. More recently, the Reims-school around FX Maquart demonstrated biological and pathological activities of other matrix-degradation products and proposed the term Matrikins for their collective designation [33–41]. Table 2 gives an overview of the most important biological activities of elastin peptides, mostly mediated by the activation of the elastin receptor (8,14,19,22]. The elastin receptor was demonstrated by immunorhodamin staining on several cell types, all of those involved in vascular pathology (see the above-cited references).

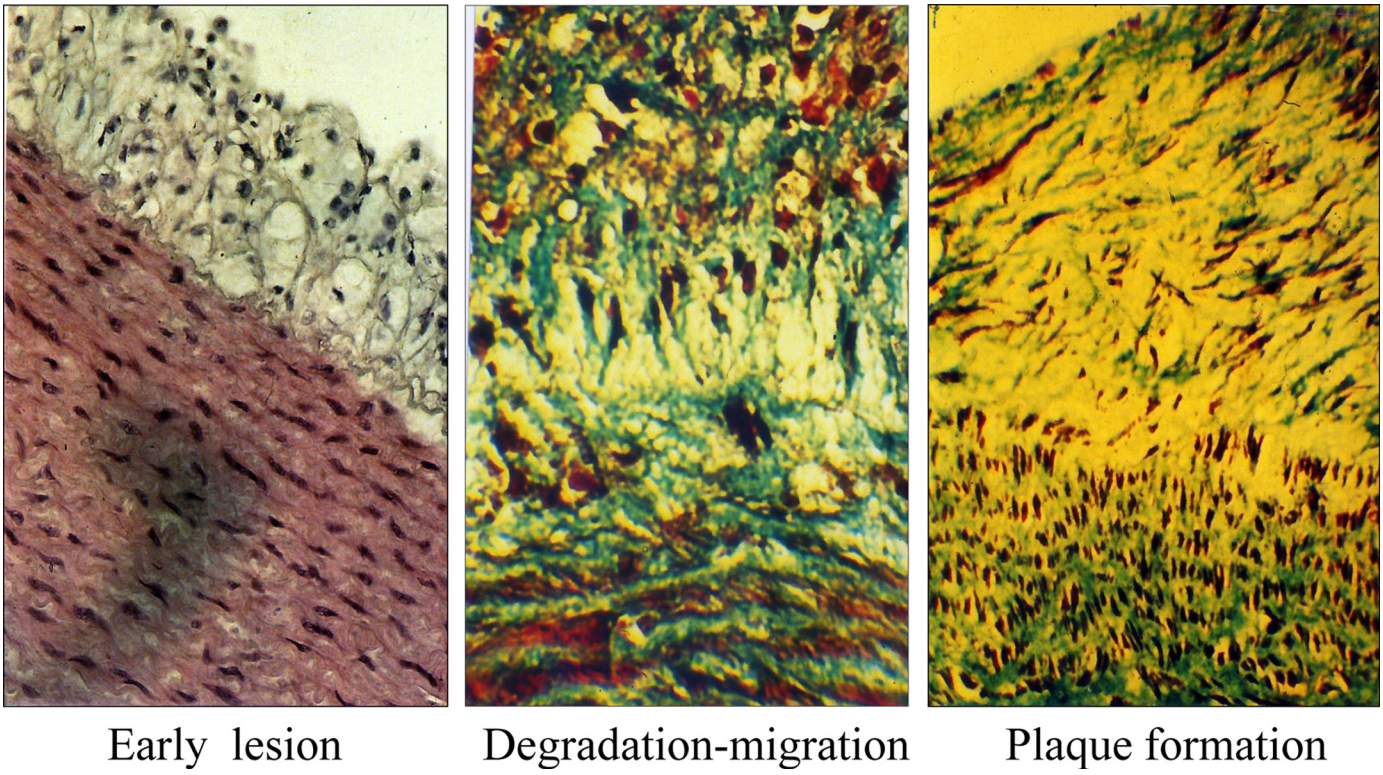

Fig. 5. Atherosclerotic lesions of the thoracic aorta of cholesterol-fed rabbits, stained by Masson's trichrome procedure. Left: early lesion, intimal proliferation with foam cells. Middle: degradation of ECM (collagen in green), migration of SMCs to the intima. Right: cell proliferation, matrix synthesis and lipid deposition produce the atherosclerotic plaque. The author's microphotos [16,17], G = × 160.

Table 2
Biological activities, mediated by elastin peptides through the activation of the elastin receptor (modified from ref [42]).

Activity	Cell type
Chemotactic migration	Monocytes Fibroblasts SMCs
Modifications of ion fluxes Increase of Ca++, Na+ influx Decrease of K+ influx	Fibroblasts SMCs Mononuclear cells
Release of lysosomal enzymes Increased synthesis of membrane-bound matrix metalloproteases and elastases	Mononuclear cells Fibroblasts SMCs
Increased oxygen consumption Free radical release	Mononuclear cells
Induction of adhesion of cells to Cells to elastin fibers	SMCs Fibroblasts Malignant cells
Release of NO Vasorelaxation	Endothelial cells
Growth factor-like activity	Fibroblasts

All these observations enlarge and complete the results yielded by the early demonstration of circulating elastin peptides and of their biological activities, summarized in this review. The physiopathological importance of the above discussed matrikin actions justifies specific research projects for the control of proteolytic activity responsible for their in vivo production and modulation.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgements

The authors wish to thank Dr. MP Jacob for the communication of references and helpful discussions.

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