Pathol. Oncol. Res. (2017) 23:455–458
DOI 10.1007/s12253-017-0199-1

LETTER TO THE EDITOR

Platelets, Micro-Particles and Elastase. A Review with Extrapolation to the Mechanism of Generation and Bio-Pathology of Platelet Fragments

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Received: 12 November 2016 / Accepted: 16 January 2017 / Published online: 23 January 2017

Introduction

Over the recent years it was realized that cells, among them transformed cells, can be degraded to yield "micro-particles" (MP-s) which on their turn can penetrate other cells, inducing modifications, among them malignant transformation [1, 2]. Similar results were described for blood platelets (BP-s). They also can be degraded to yield micro-particles exhibiting original biological properties which will be succinctly reviewed. Some years ago, working on BP-s, our team identified a neutral, elastase-type endopeptidase which was then intensively studied in collaboration with the team of Prof. J. Caen at the Hayem Institute in the Saint Louis Hospital Medical School in Paris. We shall review some of the characteristics of this enzyme and propose for it an active role in the production of platelet-derived micro-particles. Finally, we shall discuss the biological and pathological processes, especially in the vessel wall during atherogenesis, where these platelet-derived micro-particles may well play an important role.

Detection and Characterization of an Elastase-Type Endopeptidase in Human Blood Platelets

We observed in 1969 a platelet-derived elastase-type endopeptidase, active at neutral pH degrading a variety of proteins, among them elastin [3]. This enzyme, present in its pro-form in platelets, was then studied in our laboratory, in collaboration with the team of Prof. Caen (ref-s from [4–17]). Suspected first by British colleagues to be a contamination by polynuclear neutrophyle (PMN)-elastase, isolated at that time, [[18] for a review], but convincingly attributed to platelets by a series of investigations [4–17], confirmed by James et al. [19] in the USA. The pro-form of this enzyme was purified and its activation to fully active elastase carried out in vitro, followed by a series of detailed investigations of its properties [4–17] as well as its role in the liberation of platelets from megakaryocytes [20]. The liberation of this enzyme during the atherosclerotic process after the adherence of platelets to the de-endothelialysed vascular wall, especially to collagen fibers followed by the release of platelet constituents may well play an important role in the progression of the atherosclerotic process with aging [21, 22]. These findings triggered an intense activity in our laboratory to further investigate tissue derived elastolytic enzymes involved in the remodeling of the vascular wall during the progression of the lesions as described by pathologists and histologists [21, 22]. During these investigations we discovered and studied in detail an elastase-type protease in human skin fibroblasts as well as in vascular smooth muscle cells, some of them became known as metallo-endopeptidases (MMP-s) [23]. The effect of in vitro aging of cells was also studied according to Len Hayflick's protocole [24] testing elastase activity in successive serial cell cultures of fibroblasts and smooth muscle cells. These studies revealed an important fact: elastase type enzyme activity was systematically upregulated in successive subcultures using the Hayflick-protocol, as shown on Fig.1 [25]. The elastase-type endopeptidase of fibroblasts and smooth muscle cells was then studied in order to elucidate their role in the atherogenic process. Addition of hyaluronan to fibroblast cultures was shown to increase elastase-production [26]. These processes might well play an important role in the age-dependent

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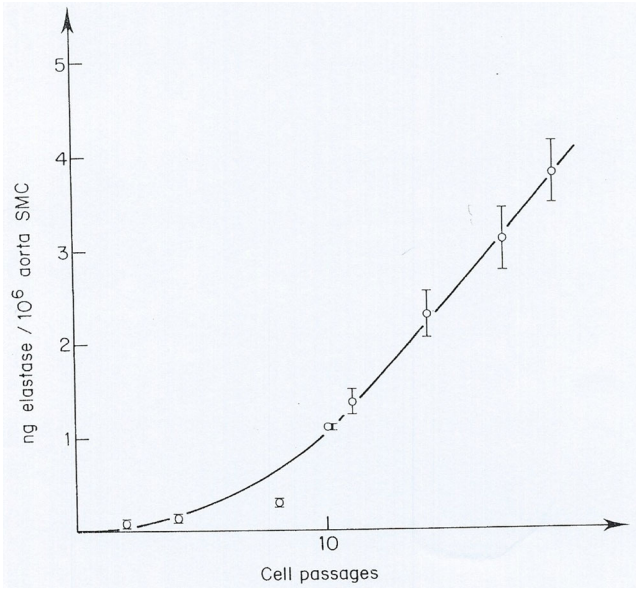

Fig. 1 Increase of elastase-type protease production by human skin fibroblasts as a function of successive cell passages. Abscissa: cell passages; Ordinates: µg elastase produced by 10^6 rabbit aorta smooth muscle cells. (Reproduced with permission from ref. [25])

modifications of the composition of extracellular matrix (ECM) and its role in atherogenesis [21, 22].

The term "elastase" is justified by the extraordinary resistance of purified elastin to proteases isolated from biological fluids as trypsin, chymotrypsin and others, acting at a neutral, physiological pH [27]. The first elastase was demonstrated in pancreatic extracts by Balo and Banga at the Pathology Institute of the Budapest Medical University [28]. This enzyme, degrading rapidly elastin at neutral pH, is however a digestive enzyme, present only in trace amounts in the circulating blood, insufficient to produce the massive degradation of elastic fibers as seen in atherosclerotic blood vessels. Elastase-type proenzymes were then isolated from aorta and other tissues as discussed below.

Elastin is routinely purified by heating tissues (aorta, nucheal ligament of cattle) in 0,1 N NaOH for 45 min to boiling temperature, which eliminates all impurities [29]. It is however rapidly "solubilized", degraded to large peptides in alcoholic 1 M KOH (NaOH is insoluble in ethanol or other organic solvents) [30]. Using this method, we obtained large elastin peptides of about 70 kDa designated as κ-elastin. Synthetic peptide substrates were also used for the determination of elastase activity [31]. Elastase-type enzymes were isolated from human aorta extracts [8] as well as from other cells and tissues, among them fibroblasts (for an exhaustive literature see ref. [31]). Similar elastase-type endopeptidases degrading elastin at neutral pH, demonstrated in fibroblasts [25, 26] were isolated from a variety of tissues and characterized [31]. We demonstrated the age- and pathology-related increase of aorta and cellular (SMC-s, fibroblasts) elastases [8, 31, 32] and their involvement in number of pathologies such as atherosclerosis [33].

We could show also that purified elastin was immunogenic in rabbits. Immunization of rabbits with purified elastin peptides (κ-elastin) induced an atherosclerotic process in large arteries [33]. As elastin peptides were demonstrated in human blood serum [34, 35] this type of immune-process was proposed to be involved in human atherosclerosis also [36]. As platelets play an important role in thrombotic processes of the vessel wall during atherogenesis and coronary thrombosis, platelet elastase may well be also an active player in this type of vessel wall injury. Platelet aggregation in contact with extracellular matrix components during atherogenesis is involved in the production and progression of this all-too frequent vascular pathology [36–38].

Potential Role of Platelet Elastase in the Production and Biological Activity of Platelets and their Micro-Particles

The production of platelets requires a proteolytic process for this highly regulated degradation of megakaryocytes protoplasma. This might well be the origin and role of this endopeptidase of blood platelets. During activation of platelets by aggregation and thrombus formation, this enzyme is released and produces degradation of components of the vessel wall and contribute to the production of the atherosclerotic plaque. Platelet-derived micro-particles might well play an active role in this process also. Their smaller size compared to platelets may well facilitate their penetration in the vascular wall and also in cells with consequences still to be explored.

Discussion

Cell-derived micro-particles and their biological properties redirected the attention of a number of cell-biologists to this new subject of cell- and molecular biology and pathology. Among these newly described mechanisms the problem of the transmission of malignancy by micro-particles deserves certainly attention. Other pathologies as atherosclerosis and thrombotic vascular diseases are of great epidemiological importance. The role of platelets and their interaction with macromolecules of the vascular wall attracted a great deal of interest as described above. Cardiovascular pathology where thrombotic processes play an important role, are on top of the list of the most frequent players in morbidity and mortality in western societies. For these reasons the more recently

discovered cell- and platelet-derived micro-particles certainly deserve further attention.

Conclusions

We described years ago an original elastase-type endopeptidase in blood platelets [4–17]. In this short review we propose an original role for this enzyme in the production of platelet-derived micro-particles in blood-vessel pathology where thrombotic processes play an important role. With the recent increase of life expectancy these types of pathology concerning tissues rich in extracellular matrix as the vessel wall [39] deserve a special interest. One consequence of these mechanisms is to command new investigations to find therapeutic responses to this recently described role of platelet micro-particles and their implementation in routine therapeutics.

Compliance with Ethical Standards

Conflict of Interest The authors declare there is no conflict of interest.

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