BIOCHEMICAL PHARMACOLOGY 75 (2008) 1007–1013

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Pharmacological regulation of factor XII activation may be a new target to control pathological coagulation

Inger Schousboe *

Department of Biomedical Sciences, Heart and Circulatory Research Section, The Panum Institute, University of Copenhagen, Blegdamsvej 3C, DK-2200 Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 20 August 2007
Accepted 3 October 2007

Keywords:
Factor XII
Pathological thrombosis
Contact activation
Deficiency

ABSTRACT

FXII was identified 50 years ago as a coagulation protein in the intrinsic pathway of blood coagulation as FXII deficient patients had marked prolongation of the *in vitro* surface-activated coagulation time. However, series of investigations have convincingly shown that FXII has no role in normal hemostasis. Recently, experimentally induced thrombosis in factor XII-knockout mice has provided evidence that factor XII (FXII) deficient mice are protected against ischemic brain injury after obstructive clot formation. Based on these experiments it has, therefore, been suggested, that blocking of FXII could be a unique target to prevent obstructive clot formation in arterial thrombosis without side effect of increased bleeding. FXII deficiency has, however, not convincingly been shown to protect against arterial thrombosis in humans. The target mentioned above may either be an inhibition of FXII activation or an inhibition of its proteolytic activity. FXII is a zymogen of the proteolytic enzyme, FXIIa, the substrates of which are factor XI and prekallikrein. Thus, FXIIa is not only involved in the activation of the coagulation system, but is also associated with the kallikrein/kinin system. The activation of the latter is deeply involved in inflammation and pain sensation. Furthermore, FXIIa binds to endothelial cells and to the extracellular matrix, indicating a role in vascular repair. Therefore, a complete evaluation of all these properties of FXII and FXIIa has to be considered when formulating a strategy for blocking FXII activation.

1. Introduction

Recently, experiments with knockout mice have changed the long-standing concept that the FXII-induced intrinsic coagulation pathway is not important for clotting *in vivo*. These experiments demonstrate that FXII-mediated fibrin formation is crucial for pathological arterial thrombosis but not for hemostasis, and therefore, suggest that FXII could be an ideal target for safe anticoagulation and a novel target for antithrombotic therapy [1–4]. With the reference to the experiments with the knockout mice, the present commentary will focus on the data obtained from cohort investigations of thromboembolic patients and point out that inhibition of FXIIa has a positive as well as a negative impact on other diseases, in addition to its putative role in protection against thromboembolism and possible subsequent ischemic injury.

2. Factor XII deficiency in knockout mice

The FXII-knockout mice showed no FXII plasma activity. Interbreeding resulted in normal litter size and did not increase fetal loss or affected pregnancy outcome, and the deficiency of FXII did not cause thrombophilia or impaired

* Tel.: +45 35327800; fax: +45 35367980.
E-mail address: schousboe@imbg.ku.dk.
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.10.003

fibrinolysis [1]. However, intravital fluorescence microscopy and blood flow measurements in the arteries of the knockout mice revealed a severe defect in formation and stabilization of platelet rich occlusive thrombi induced by different means of injuries. Infusion of FXII into the knockout mice reversed the effect. This established that FXII is of significance for proper thrombus formation [3,4]. Based on these observations, Johne et al. [2] initiated an investigation, which indicated that platelets promote the FXII-mediated proteolytic system in plasma. This investigation was supported by observations showing that inorganic polyphosphates secreted from activated platelets trigger clotting only in the presence of FXII [5]. The impact of these observations is that FXII may be a risk factor of thromboembolism and may very likely be the starting signal for a series of investigations elucidating the mystery of the biological function of FXII. The observations also very strongly indicate that inhibitors directed against the platelet-mediated activation of FXII may offer a selective and safe strategy for preventing stroke and other thromboembolic diseases.

In order to understand the role of FXII in pathological and physiological thrombus formation, a short description of the blood clotting system is provided. Proper blood clotting involves three phases: (i) the initiation, (ii) the propagation and (iii) the amplification (Fig. 1). *In vivo*, the system is initiated when circulating FVII and FVIIa (activated FVII) bind to tissue factor. Tissue factor is expressed in adventitia on the subendothelial layer of blood vessels and on extravascular cells and thus is exposed to the blood stream when normal vasculature is disrupted. Concomitant with this, platelets, which have become activated by binding to the collagen in the subendothelium expose negatively charged phospholipids on the platelet surface. As a consequence, the activation of FVII is enhanced, and the coagulation factors assemble on the surface of the platelets. Subsequently, FVIIa activates FX and FIX. By analogous interactions, FII (prothrombin) becomes activated by FXa. FIIa (thrombin) catalyzed proteolytic cleavage of the cofactors, FV and FVIII, enhances further the rate of FX activation by FIXa and the activation of prothrombin by FXa. Further enhancement of the thrombin generation is supported by reciprocal thrombin activation of FIX, FX and FXI. The final results of these reactions are the thrombin-catalyzed cleavage of fibrinogen and FXIII, generating a clot of fibrin.

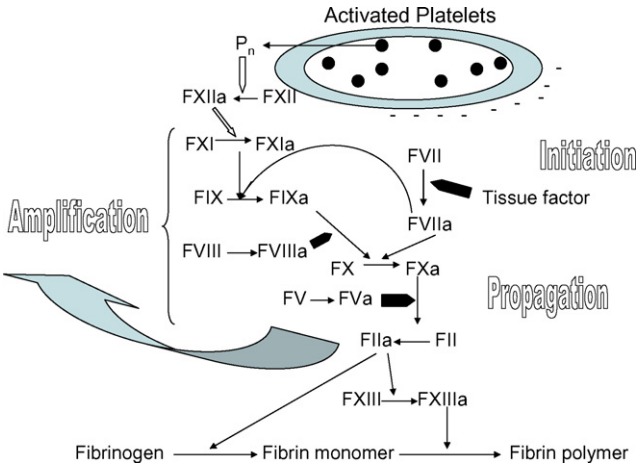

Fig. 1 – Schematic drawing of the coagulation system *in vivo* and the possible involvement of FXII for stabilization of a thrombus. The *in vivo* coagulation consists of three phases. The initiation phase, in which factor VII (FVII) becomes activated in contact with tissue factor in the subendothelium; the propagation phase, at which factor X (FX) and factor IX (FIX) are activated by FVIIa, and prothrombin (FII) is activated by FXa in the absence of factor Va (FVa), and the amplification phase, at which thrombin (FIIa) activates FV, factor VIII (FVIII), and FXI. Thrombin (FIIa) activates fibrinogen and factor XIII (FXIII) generating fibrin. At the top of the figure, a recently described mechanism for activation of FXII by inorganic polyphosphates (P_n) secreted from the dense granules (●) in the activated platelets is shown. Following that the generated FXIIa enhances *via* activation of FXI the rate of fibrin generation, and thus increases the density of the net of fibrin, by which the clump of aggregated platelets becomes further stabilized.

3. Activation of FXII and pharmacological aspects of its inhibition

The (patho) physiological significance of the activation of FXII has been questioned for more than 50 years. The reason for this is that hereditary deficiency of FXII has never been associated with abnormal bleeding or other pathological states in clinical observations [6]. However, although FXII activation does not initiate blood clotting, recent findings strongly indicate that activation of FXII plays a role in stabilizing the clot formation by FXIIa-mediated activation of FXI [3]. This activation may *in vivo* be promoted by aggregating platelets and nucleic acids derived from damaged cells [2,7].

FXIIa is generated by activation of FXII, either by auto-activation on a surface of negatively charged compounds or by activation by kallikrein. FXIIa enhances blood clotting *via* activation of FXI in the intrinsic pathway of coagulation, and participates in inflammatory reactions *via* activation of prekallikrein in the kallikrein/kinin system. Thus, a reciprocal activation loop enhances the rate of both FXIIa and kallikrein generation (Fig. 2). Activation of FXII, whether by autoactivation or by kallikrein activation results in cleavage of the Arg353–Val354 bond, generating a heavy and a light chain containing 353 and 243 amino acid residues, respectively, and held together by a disulfide bond. FXII consists of a sequence of domains (Fig. 3). The heavy chain is responsible for binding to negatively charged surfaces involving the positively charged amino acid sequence within residues 39–47 in the fibronectin type II domain; the light chain contains the catalytic domain [8–10]. The 39–47 sequence could be a possible drug target for inhibiting the activation of FXII (see below).

In vitro several anionic surfaces have been shown to bind to and activate FXII. These include kaolin, dextran sulfate, acidic phospholipids and sulfatides (glycocerebroside sulfates), but although a series of studies have indicated that acidic phospholipids and sulfatides expressed in platelets [10,11], may activate FXII, *in vivo*, this has never convincingly been shown.