International Journal of Cardiology 187 (2015) 291–298

Contents lists available at ScienceDirect

International Journal of Cardiology

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

Review

The relevance of estrogen/estrogen receptor system on the gender difference in cardiovascular risk

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ARTICLE INFO

Article history:
Received 10 January 2015
Received in revised form 7 March 2015
Accepted 15 March 2015
Available online 17 March 2015

Keywords:
Thrombogenesis
Estrogen receptors
Estrogen
Platelets
Megakaryocytes

ABSTRACT

It has been reported that the incidence of thrombotic events can display a gender disparity. In particular, a lower thrombotic risk has been described in female gender. The mechanisms underlying this disparity are still poorly understood. Of great interest is the hypothesis that hormones, estrogen in particular, could play a key role. In fact, the possibility that some hormonal factors could protect women from thrombotic events appears well documented in literature. For instance, several studies aimed at the analysis of the impact of estrogen and estrogen receptors in thrombogenesis claim for the implication of these hormones either in megakaryocyte differentiation or, more intriguingly, directly affecting platelet integrity and function. In consideration of the absence of the nucleus, platelet susceptibility appears quite striking and probably due to the non-nuclear estrogen receptor function. In this review we briefly summarize our knowledge as concerns the role of estrogen and estrogen receptors in determining megakaryocyte/platelet functions and thrombogenicity.

1. Introduction

Thrombosis is the commonest cause of death in developed countries. It is the basis of three major cardiovascular disorders: ischemic heart disease (acute coronary syndrome), stroke (arterial cerebral thrombosis) and venous thromboembolism (VTE). Depending on the various pathological *conditions* a different incidence of thrombotic events between men and women was found. For instance, stroke incidence appears as lower in females than in males until advanced ages, after which the rates rise dramatically in both sexes [1]. Similarly, the recurrent venous thrombosis appears as less frequent in females and, also for the first event, the male/female ratio is 2:1 [2]. These data seem to suggest that some hormonal factors, estrogen in particular, could protect women from thrombotic events. Several insights derive in fact from a number of studies carried out either in animals or in humans. For example, studies carried out in animal models have demonstrated that ovariectomy increases infarct size and that estradiol supplementation reduces injury [3]. In humans, a good example is that represented by the use of oral contraceptives and postmenopausal hormonal therapies, which contain various estrogens: it has been found that these treatments are associated with a weaker risk of both arterial and venous thrombosis [4]. On these bases, it was hypothesized that hormonal (i.e., estrogenic) factors could play a pivotal role in regulating thrombotic

Abbreviations: AA, arachidonic acid; ADP, adenosine-5′-diphosphate; AGO2, argonaute RISC catalytic component 2; AKT, protein kinase B; BAD, Bcl-associated death kinase; CHD, coronary heart disease; COL, collagen; CVD, cardiovascular disease; E2, 17β-estradiol; eNOS, endothelial nitric oxide synthase; EP, epinephrine; ERs, estrogen receptors; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; ERK, extracellular-signal-regulated kinases; ETS, E twenty-six protein; FOXA 1, forkhead box protein A1; GATA3, GATA binding protein 3; GPIIb–IIIa, glycoprotein IIb/IIIa complex; GPR30G, G protein-coupled receptor 30; Hsps, heat shock proteins; HRT, hormone replacement therapy; IP3, inositol trisphosphate; MAPK, mitogen-activated protein kinase; mERs, membrane estrogen receptors; miRNA, microRNA; MK, megakaryocyte; MPTP, mitochondrial permeability transition pore; MR, mineralocorticoid receptor; mtERs, mitochondrial estrogen receptors; nERs, nuclear estrogen receptors; NO, nitric oxide; PAC-1, pro-caspase activating compound; PI3K, phosphatidylinositol 3-OH kinase; RAS, membrane-associated guanine nucleotide-binding protein; RCT, randomized controlled trial; ROS, reactive oxidizing species; RUNX, runt-related transcription factor; TFPI, tissue factor pathway inhibitor; TRAP, thrombin receptor-activating peptide; TRBP2, transactivation responsive RNA binding protein 2; VTE, venous thromboembolism; VWF, von Willebrand factor

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http://dx.doi.org/10.1016/j.ijcard.2015.03.145
0167-5273/

events, also influencing the ensuing cardiovascular diseases. Herein, we discuss literature data dealing with this issue: the role of estrogen and estrogen receptors in determining megakaryocyte/platelet integrity and function and in the control of thrombogenicity.

2. Sex differences and thrombosis

2.1. Hormones and thrombosis

The crucial role of hormones in thrombosis and hemostasis in men and women has been analyzed in several works [2,5]. These works underscore that, although obviously occurring in both sexes, i) the intrinsic risk of VTE is two-fold higher in men than in women; ii) hormonal contraceptives increase the risk of VTE in women and that iii) this risk varies per type, dose, and hormone administration route. On this basis, avoidance of some hormonal contraceptives (as well as thrombosis prophylaxis during pregnancy which also increases the thrombotic risks) has been suggested [2].

2.2. Cardiovascular disease, gender and thrombosis

Cardiovascular disease (CVD) develops in women even 10 years later than men [6,7] and peripheral arterial diseases are less relevant in women in comparison with men (18% versus 27%) [8]. In women, estrogen deficiency is the principal factor that doubles the risk of CVD and a younger age at menopause is related to increased risk of morbidity and mortality [9–11]. After menopause, endogenous ovarian estrogen levels decrease and the risk for VTE increases. However, the mechanisms underlying this increase are still obscure [11]. As concerns stroke, men have a higher incidence (33% higher) and higher prevalence of stroke (41% higher) compared with women until advanced age: the mean age at first-ever stroke is 68.6 years among men, and 72.9 years among women. Nevertheless, the stroke tends to be more severe in women, with a 1-month case fatality of 24.7% compared with 19.7% for men [1,3]. About thromboembolic stroke associated with atrial fibrillation, women have recently been hypothesized to be at a higher overall risk than men and suffer more disability in association with stroke [12,13]. Different etiological bases of the stroke, i.e. the occurrence of atheroembolism (i.e., cholesterol crystals) or thromboembolism (i.e., formed in prevalence by fibrin and platelets), should however be taken into account in considering these data [14]. For many years, the shift in ischemic risk with age in women was attributed to the loss of hormones, especially estrogen, at the time of menopause. Recently, however, an important con-cause has been identified in accelerated atherosclerosis and metabolic syndrome, which have been suggested to represent a risk factor for stroke [15].

As concerns pediatric age, few contrasting data are available with regard to stroke. Mallick and coworkers [16] report no significant risk disparity with sex, whereas Golomb and coworkers [17] report a male predominance. Instead, no sex differences have been shown in male and female children as concerns VTE [18].

3. Sex differences in platelet function

Platelets can be activated by circulating factors in the blood or by the sub-endothelial collagen during endothelial injury. The interaction between exposed atherosclerotic plaque components, platelet receptors and coagulation factors eventually leads to platelet activation, aggregation and the subsequent formation of a superimposed thrombus (i.e. atherothrombosis), which may compromise the arterial lumen leading to the presentation of acute ischaemic syndromes [19]. Hormones can influence these processes thus determining a gender disparity.

3.1. Platelet aggregation mechanisms in a gender perspective

Several lines of evidence point at sex differences in platelet function in animals and humans. In the case of animals, conflicting results have been obtained. Morikawa and coworkers observed greater in vitro aggregation of platelets from male than female rats [20]. Accordingly, Ajayi et al. highlighted that the thromboxane A_2-mimetic U46619 elicits greater thrombus burden and more death in male rats [21]. Conversely, other authors found that platelets isolated from female mice bind more fibrinogen and have a greater maximal extent of aggregation in response to weak and low-dose agonists than platelets isolated from male mice [22]. In humans, studies on platelet function have observed heightened responsiveness of platelets isolated from women in comparison with men. In particular, platelets of women showed higher responses to activation by adenosine-5′-diphosphate (ADP), arachidonic acid (AA), epinephrine (EPI) and collagen (COL) [23]. Using flow cytometry, Faraday and collaborators [24] quantitated gender differences in the number of binding sites for fibrinogen and pro-caspase activating compound (PAC-1), an activation marker, in platelets from men and women. These authors showed that, in response to ADP, the number of glycoprotein IIb/IIIa complex (GPIIb–IIIa) receptors capable of binding fibrinogen was significantly higher in platelets from women than men. Similarly, the number of GPIIb–IIIa receptors (capable of binding PAC-1 in response to ADP) and thrombin receptor-activating peptide (TRAP) was 50% to 80% higher in women than in men. Further analysis of data from female subgroups demonstrated an association of GPIIb–IIIa reactivity with luteal rather than follicular phase, suggesting that hormones may regulate the activation of these receptors [25]. This is in accord with data showing an increased platelet activity during luteal phase [26]. It was suggested that GPIIb–IIIa receptors on platelets from premenopausal women are more “activable” than those on platelets from young men, depending on serum concentrations of estrogens and/or progestins [27]. In addition, Roshan and collaborators [28] showed significantly higher values of the activation-related receptor CD62P and PAC-1 in post-menopausal women as compared to the pre-menopausal group, further suggesting the role of platelets in the increased incidence of thrombotic events in association with estradiol decrease.

4. Estrogen and blood coagulation

4.1. Hormones and coagulation

Hormone levels can affect the levels of clotting factors and favor an acquired resistance to the actions of activated protein C, leading to an increased risk of VTE [29]. Decreased levels of von Willebrand factor (VWF), fibrinogen, and activated factor VII have been measured during the menstrual cycle [29]. Conversely, pregnancy is a procoagulant state with progressive increase in levels of factors VII, VIII, X, and XII, fibrinogen, and VWF, as well as increased resistance to activated protein C [29]. During post-menopausal period, genetic and environmental factors “negatively” modulate the expression of proteins involved in the hemostatic process leading to modifications of the hemostatic system at different levels (blood coagulation, fibrinolysis, platelet activity, vascular endothelium) [30]. This may also explain the higher incidence of arterial and venous thrombosis in the elderly compared to young people.

4.2. Estrogen therapy

Therapy with estrogen is of great importance in the clinical management of a variety of clinical settings. Oral estrogen therapy is in fact clinically effective but, depending on the route of administration and the type and dose of estrogen, may also be accompanied by severe cardiovascular side effects that are associated with alterations in liver metabolism [31]. In particular, oral administration of synthetic estrogens has profound effects on liver-derived plasma proteins, coagulation