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Estrogen actions and in situ synthesis in human vascular smooth muscle cells and their correlation with atherosclerosis☆

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Abstract

Various epidemiological studies have demonstrated a relatively low incidence of cardiovascular events in premenopausal women and its marked increment after menopause. In addition, estrogens have been postulated to exert direct anti-atherogenic effects via binding to estrogen receptors (ERs) in vascular smooth muscle cells (VSMCs). However, not all postmenopausal women develop atherosclerosis despite decreased levels of serum estrogen. Therefore, it is considered important to examine the status of estrogen metabolism in situ and of ER expression in the human cardiovascular system. Estrone sulfate (E_1S) is a major circulating plasma estrogen that is converted into the biologically active estrogen, estrone (E_1) by steroid sulfatase (STS). E_1 is also sulfated and reverted into E_1S by estrogen sulfotransferase (EST). These two enzymes have recently been shown to play important roles in the in situ estrogen actions of estrogen-dependent human tissues. STS and EST, however, have not been studied in detail in the human vascular system associated with atherosclerotic changes. Therefore, the relative abundance of STS- and EST-immunoreactive protein and mRNA expression in human aorta were evaluated using immunohistochemistry and reverse transcription followed by quantitative polymerase chain reaction in addition to enzyme activity. Furthermore, we evaluated the relative abundance of messenger RNA (mRNA) of both ER subtypes (ERα and ERβ) in the human aorta using reverse transcription followed by quantitative polymerase chain reaction (RT-qPCR), as well as the immunoreactivity of both ERs in VSMCs of human atherosclerotic lesions. STS expression levels were found to be significantly higher in the VSMCs obtained from female aortas with mild atherosclerotic changes than in those with severe atherosclerotic changes and in male aortas regardless of atherosclerotic changes. EST expression levels in the VSMCs of these aortas, however, were significantly higher in female aortas with severe atherosclerotic changes and in male aortas than in female aortas with mild atherosclerotic changes. In addition, the number of ERα and/or ERβ double positive cells in the neointima was higher in female aortas with a mild degree of atherosclerosis than in female aortas with severe atherosclerosis. They indicate that both abundance of these estrogen-metabolizing enzymes in female aorta and relative levels of ER in VSMCs of female neointima may be associated with the status of atherosclerotic changes.

Keywords: Estrogen; Sulfatase; Estrogen receptor; Sulfotransferase; Aorta

1. Introduction

Various epidemiological studies have reported a relatively low incidence of cardiovascular events in premenopausal women and its marked increment following menopause [1]. Estrogen has, therefore, been proposed as a cardioprotective agent, especially in women [2]. Estrogens are considered to exert direct anti-atherogenic effects through an initial interaction with the estrogen receptor (ER) in vascular smooth muscle cells (VSMCs). However, it is also true that not all post-menopausal women develop atherosclerosis despite decreased levels of serum estrogen [3]. In addition, the great majority of previous studies to date have failed to demonstrate a definitive correlation between the total levels of circulating estrogens or their metabolities and the degree of atherosclerosis both in men and post-menopausal women [3,4]. Therefore, it is important to examine the status of estrogen metabolism in situ and estrogenic effects in human atherosclerotic aorta.

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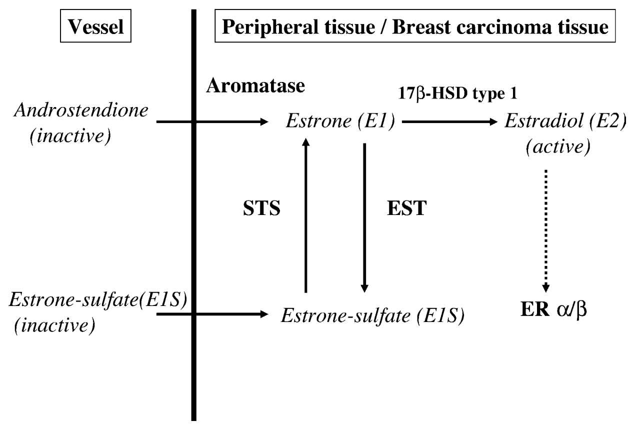

Fig. 1. Scheme representing local production of estrogens in human peripheral tissues and/or breast carcinoma. High concentration of circulating inactive steroids, androstendione and estrone-sulfate, are major precursor substrates of local estrogen production in those tissues. Aromatase catalyzes androstenedione into estrone, and STS hydrolyzes estrone-sulfate to estrone. Estrone is subsequently converted to potent estradiol by 17β-HSD type 1, and acts on target cells via ERα/β. EST sulfonates estrogens (predominantly estrone) to biologically inactive estrone-sulfates. STS; steroid sulfatase, 17β-HSD type 1; 17β-hydroxysteroid dehydrogenase type 1, EST; estrogen sulfotransferase, and ER; estrogen receptor. This figure refers to a previous report [27].

2. Estrogen metabolism in situ in human aorta

In pre-menopausal women, the ovary is the main source of circulating estrogen, but in men and post-menopausal women, estrogen is mainly synthesized in non-genital tissues via conversion of androstendione or C_{19} steroids derived from the adrenal cortex, ovary and/or testis [5]. In addition, a major circulating form of plasma estrogen is estrone sulfate (E_1S), a biologically inactive form of estrogen (Fig. 1). E_1S has a relatively long half-life in the peripheral blood [6], whereas serum levels of E_1S are known to be 10-fold higher than those of unconjugated estrone (E_1) or estradiol (E_2) [7] (Fig. 1). E_1 is sulfated into E_1S by the cytosolic enzyme, estrogen sulfotransferase (EST) [8]. EST, *SULT 1E1* or *STE* gene, is a member of the superfamily of cytosolic steroid sulfotransferases. EST enzyme activity has been also reported in several human tissues [9]. It is also well-known that marked differences in EST expression and/or activity exist in tissues depending on species, sex, age, development, and the physiological status of laboratory animals [10–12]. E_1S is transformed into a biologically active form, E_1, by steroid (estrone) sulfatase (STS) [13–15] (Fig. 1). STS expression has been examined in estrogen dependent neoplasia such as breast and endometrial carcinoma [13–15]. These two enzymes have recently been shown to play important roles in the in situ estrogen actions of estrogen dependent human tissues and various sex steroid-dependent tumors. Therefore, it is very important to evaluate levels of expression of these enzymes in human aorta and to correlate the findings with the degree of atherosclerosis. In addition, it is also important to examine the factors regulating the expression of these estrogenic metabolizing enzymes in human aorta. Various cytokines have been proposed as major regulators of these enzymes in other normal and malignant tissues. Reed and Purohit demonstrated that in situ estrogen synthesis in breast carcinoma cells were regulated by various cytokines, including IL-1, IL-6, TNF-α which were produced in situ by carcinoma or parenchymal cells and stromal cells [16]. IL-1β is well-known to exert effects on estrogenic enzyme expression in human tissues [17–20]. In advanced atherosclerotic lesions, the number of lymphocytes and/or macrophages producing various cytokines was, in general, more abundant than in early atherosclerotic lesions [21]. Among these cytokines, IL-1β has been shown to be produced in situ in many types of the cells present in human atherosclerotic lesions, including lymphocytes, macrophages, endothelial cells, and VSMCs [21–23]. When compared to other cytokines, IL-1β has been demonstrated to play very important roles in neointimal growth of atherosclerotic lesions [24–26].

We recently demonstrated that the presence of STS and EST and its possible biological significance in human aorta and its lesions [27]. In addition, we also demonstrated that STS expression levels were significantly higher in the VSMCs obtained from female aortas with mild atherosclerotic changes than in those with severe atherosclerotic changes and in male aortas regardless of atherosclerotic changes (Table 1). EST expression levels in the VSMCs of these aortas, however, were significantly higher in female aortas with severe atherosclerotic changes and in male aortas than in female aortas with mild atherosclerotic changes (Table 1).

Following the conversion of E_1S to E_1 by STS, E_1 was also converted to E_2 by 17β-HSD 1. Therefore, E_2 was postulated to be produced from E_1S and E_1 in human VSMCs, and may exert direct effects on vessels possibly in the anti-atherogenic manner. Therefore, in situ production of estrogens in VSMCs of female aorta via STS is considered to exert suppression of VSMC proliferation in the initial phase of atherosclerotic changes. In contrast, EST is known to be involved in metabolizing excessive amounts of biologically active estrogens to biologically inactive sulfonated forms with marked hydrophilic properties, with possible coordination

Table 1
Results of immunoreactivity for STS, EST and 17β HSD-1 in VSMCs of human aorta

Group	*n*	STS*	EST*	17β HSD-1
A	9	0.22 ± 0.15	1.11 ± 0.26	0.63 ± 0.32
B	8	0.25 ± 0.16	1.50 ± 0.27	0.38 ± 0.18
C	6	1.83 ± 0.17	0.83 ± 0.17	1.60 ± 0.25
D	9	0.22 ± 0.15	1.78 ± 0.15	0.40 ± 0.25
E	7	1.57 ± 0.30	1.00 ± 0.31	1.00 ± 0.19
Total	39	0.64 ± 0.14	1.28 ± 0.12	0.77 ± 0.13

Data are the mean ± S.E.M. Relative immunoreactivity for each sample was classified into the following groups: 2, strongly positive; 1, weakly positive, and 0, negative. Statistical significance was evaluated among the groups using Kruskal–Wallis test. This table refers to a previous report [27].
* $P < 0.05$.