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Review

Hormone therapy and asymmetrical dimethylarginine in postmenopausal women

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ABSTRACT

Women present an estradiol-dependent cardiovascular risk profile. Based on various studies, it was considered that estrogen therapy (ET) in postmenopausal women could probably reduce the higher cardiovascular risk in this group. Assymetric dimethylarginine (ADMA) is an endogenous methylated arginine which inhibits nitric oxide (NO) synthesis by competing with the substrate of NO, L-arginine, leading to endothelial dysfunction and, consequently, to atherosclerosis. Moreover, ADMA has been considered as an independent risk factor for cardiovascular disease. It has also been found that hormone therapy (HT), and mainly oral estrogen therapy, lowers ADMA concentrations in healthy postmenopausal women. The effect of estrogens on ADMA levels, although small, is considered important, as physiological variation of ADMA is limited. Nevertheless, larger randomized trials are necessary to establish that estrogens substantially lower ADMA levels and that these changes really reflect improved cardiovascular prognosis in postmenopausal women.

Key words: ADMA, Asymmetrical dimethylarginine, Estrogen therapy, Hormone therapy, HT, Menopause

INTRODUCTION

Estrogens have been known to exert various effects on the cardiovascular system.[1,2] It has thus been shown that sex steroids retard the atherosclerotic process and induce rapid vasodilatation through the production of an endothelium-derived vasoactive mediator, nitric oxide (NO).[2-4] Estrogen-induced vasodilatation via nitric oxide seems to be mediated by asymmetric dimethylarginine (ADMA) and ADMA serum concentrations are inversely related to endogenous estradiol levels.[5]

ADMA is an endogenous methylated arginine which inhibits NO synthesis, leading to endothelial dysfunction and consequent atherosclerosis.[6] Experi-

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mental data have shown that ADMA is negatively correlated with flow-mediated vasodilatation[7] and constitutes an important marker of carotid artery intima-media thickness.[8] Even slightly increased ADMA blood levels are associated with higher risk for acute coronary events.[9]

Estradiol also exerts an anti-inflammatory effect *in vitro*, as well as *in vivo*, and accelerates endothelial regrowth, thus promoting vascular healing.[10] Women present an age-dependent, and specifically an estradiol-dependent, cardiovascular disease risk pattern,[11] as is demonstrated by the rise in the number of cardiovascular events after menopause.

Estrogen therapy (ET) has been considered as a means of reducing cardiovascular risk in postmenopausal women.[12,13] Nevertheless, the validity of such intervention in cardiovascular disease (CVD) risk reduction remains controversial.[14-16] It has been shown, however, that hormone therapy (HT) affects ADMA levels and other independent risk factors.[17-20] Based on the observation that ADMA is linked to CVD, a review of available data on the impact of HT on ADMA and CV events was undertaken.[21-25]

METHODS

A comprehensive search was conducted via MEDLINE (http://www.ncbi. nlm.nih.gov/entrez/medline.html) employing the keywords: asymmetric dimethylarginine, ADMA, estrogen replacement therapy, cardiovascular disease, and postmenopausal women. Our search included all possible combinations of keywords without any limitation in language or date. Fifty-six articles were found. Relevant citations in the reference lists of selected articles were also reviewed. Inclusion or exclusion of any article was based on relevance.

MENOPAUSE

Menopause is defined as the permanent cessation of menstruation following the loss of ovarian activity. Menopausal status is considered to begin at the cessation of menstruation[26] and is characteristically accompanied by a 10-20-fold increase in FSH and a 3-fold increase in LH, with maximum values observed three years after menopause initiation. Following this stage, there is a gradual decline in both gonadotropins. However, estrogens still continue to circulate in postmenopausal women, these derived from the peripheral conversion of androgens[27,28] that are still produced by the ovaries and the adrenal glands.[29]

CARDIOVASCULAR RISK AND MENOPAUSE

CVD represents the first cause of mortality and morbidity in both genders, with the onset established approximately ten years later in women than in men.[30] Aging and estrogen deficiency have been reported as the most important factors of pertinent morbidity in women.[31] The cessation of the ovarian function and the consequent reduction of sex steroid hormone levels have important metabolic and pathological consequences which adversely affect the cardiovascular system. Postmenopausal women have higher total cholesterol, LDL cholesterol, triglycerides, and a-lipoprotein levels and lower HDL cholesterol levels than premenopausal women.[32,33] Transition to postmenopause is associated with augmentation in total and LDL-cholesterol values and a 16% increase in triglycerides, thus exceeding men's levels.[34,35] Finally, in the Study of Women's Health Across the Nation (SWAN), transition to menopause, and specifically falling estrogen levels, were associated with changes in the peripheral vasculature indicative of an increased risk for CVD in the postmenopausal period.[36]

NON-GENOMIC ACTIONS OF ESTROGENS ON THE VASCULAR SYSTEM

Human endothelial cell plasma membrane[37] and vascular smooth muscle cells have estrogen receptors (ERs).[38] Estrogens bind to these receptors and promote the activation of several kinase cascades, all of which have a common action, namely vasodilatation.[1] Estradiol binds to ERα and activates the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Activated Akt catalyzes the phosphorylation of the endothelial isoform of nitric oxide synthase (eNOS), increasing its activity and leading to the increased production of NO.[39] NO is a potent vasodilator and mediates antiatherogenic and anti-inflammation actions. In the endothelial cells, the activation of PI3K/Akt modifies the expression of almost 250 genes, including cyclooxygenase, which mediates prostaglandin synthesis

resulting in long-term changes in cellular function.[40] Apart from this main pathway, there are several other actions on various pathways leading to rapid changes in calcium concentrations inside the endothelial cells and to induction of NO synthesis.[41]

Recent data have shown that estrogens also regulate cytoskeleton remodelling and endothelial cells migration to repair endothelial injuries.[42] Specifically, it has been found that non-transcriptional estrogen signals provoke instant re-arrangements of actin cytoskeleton and formation of membrane structures that promote movement to endothelial cells. Additionally, estrogens increase the permeability of junctions between endothelial cells and contribute to angiogenesis.[43] It must be stressed, however, that the function as well as the number of estrogen receptors decline with age.[44]

HORMONE THERAPY AND CARDIOVASCULAR DISEASE

Based upon the beneficial effects of the endogenous estrogens on the cardiovascular system and specifically on vasodilatation, it has been hypothesized that estrogen replacement therapy, alone or combined with progestins, might be capable of reducing the risk for cardiovascular disease in menopause. In fact, the positive effect of hormone therapy (HT) on endothelium-dependent vasodilatation was proven in healthy[45,46] or low CVD risk[47,48] postmenopausal women.

Publication of the estrogen-progestogen arm of the Women's Health Initiative (WHI) study in 2002 revealed that hormone therapy increased coronary heart disease, strokes, deep venous thrombosis, and breast cancer.[49] Subsequent results of the WHI study reversed the negative attitude concerning HT by showing that there was no significant difference between combined estrogen progestin therapy and placebo use in the risk for CVD.[50] Later, the ET arm of the WHI study also showed that there was no significant difference in cardiovascular events between the therapy and the placebo groups.[51] Both publications stated that there was no relation between the chronological and the menopausal age at therapy initiation and the observed effects. However, according to the final results of the ET arm of the study, there was a decrease in CVD risk when ET was initiated prior to the age of 60 years.[52] Subsequent publications supported the age-related favourable effect of ET in CVD events.[53,54] Currently, the use of HT is indicated as treatment of moderate to severe vasomotor symptoms.[55-57] Still, it is not recommended for prevention of cardiovascular events at any age, even though short-term HT, administered to women of 50-59 years old, lowered the risk of CVD and all-cause mortality.[58,59]

ASYMMETRIC DIMETHYLARGININE (ADMA)

ADMA is an aminoacid produced by the degradation of methylated nuclear proteins. ADMA is produced in all tissues and is released in the plasma. It mainly acts as an endogenous inhibitor of all three isoforms of nitric oxide (NO) synthase[60] and exerts important biological effects on the cardiovascular system. ADMA was first identified in 1992 and since then it has become the focus of great scientific interest. High serum levels of ADMA are found in pathological conditions leading to NO deficiency. Accumulation of ADMA results from increased methylation of proteins by methyltransferases (PRMT), precipitated proteolysis, decreased renal excretion, and impaired metabolism by N΄G-dimethylarginine dimethylaminohydrolase (DDAH).

The amount of ADMA produced in cells depends on the rate of the protein metabolism and the methylation of arginine, since there is no known pathway of ADMA biosynthesis from free arginine.[61] Its biosynthesis is mediated by PRMT. However, the exact mechanism regulating PRMT activity is unknown. Stress increases PRMT activity, and promotes ADMA synthesis in endothelial cells in vitro, through the activation of the transcription Nuclear Factor-B (NF-B).[62] Furthermore, low-density lipoproteins (LDL) stimulate PRMT activity and ADMA synthesis in the endothelium.[63] Protein methylation is increased in proliferating cells. Additionally, anti-DNA antibodies in systemic lupus erythematosus promote methylation of ribonucleoproteins and may be responsible for the increased synthesis of methylarginine.[64,65]

Precipitated proteolysis may contribute to elevation of ADMA in hypercatabolic states such as endotoxemia, hyperthyroidism, and muscular dystrophy.[66-68] The intracellular metabolism of ADMA to

L-citrulline and dimethylamine is mediated by the enzyme DDAH.[69] About 10% of total ADMA enters the plasma and is excreted by the kidneys. Thus, any severe renal insufficiency is liable to lead to increased ADMA blood levels.[70]

Interestingly, ADMA molecules produced in the cells of a specific tissue may act and inhibit NO synthase in other tissues. Such a phenomenon is observed between macrophage and endothelial cells[71] and possibly between smooth muscle and endothelial cells. It is speculated that this kind of action may comprise a trancellular signalling pathway.[72] ADMA levels determine the rate of NO production with a relevant effect on blood vessels.[73] It has been hypothesized that ADMA not only inhibits NO synthesis but its action as well.[69] Inhibition of NO synthesis by an increase in ADMA levels also leads to disturbances in the vascular homeostasis models, namely dilatation versus constriction, activation of platelets, and unfavourable changes in transcellular communication. Altogether, the aforementioned modifications accelerate atherogenesis.[74]

ADMA and cardiovascular disease

There is ample evidence that ADMA, apart from producing effects related to NO synthase inhibition such as elevation of blood pressure, vasoconstriction, increased renovascular resistance, reduced forearm blood flow, reduced heart rate, and reduced cardiac output, also constitutes a marker of endothelial function and of cardiovascular risk.[75-78] As already mentioned, ADMA levels are increased in various atherosclerotic conditions such as advanced age, hypertension, diabetes mellitus, hypercholesterolemia, and hyperhomocysteinemia.[69] Moreover, ADMA concentrations are high both in asymptomatic subjects with hypercholesterolemia[7] and in patients with cardiovascular or metabolic disease.[79]

Multivariate regression analyses of all known CDV risk factors showed that ADMA constitutes an independent risk factor for CDV. The practical value of such a marker becomes obvious in the group of patients of moderate risk, namely those who stand in the gray zone.[78]

It is relatively well established that ADMA not only reflects but also participates in the development of the atherosclerotic process. In a well designed prospective study, high ADMA levels were correlated with intima-media thickness (IMT), which is an independent prognostic factor of coronary disease.[80] In another study on the relation between cardiovascular risk and plasma ADMA concentrations in a cohort of haemodialysis patients followed for a mean period of 33.4 months, it was found that ADMA levels measured at start were correlated with the mortality observed throughout the study.[81] In another study in patients with end-stage renal disease followed for a year, it was shown that ADMA was correlated and, moreover, represented a prognostic factor of IMT. ADMA levels and age were the most powerful prognostic markers of cardiovascular morbidity and mortality[82] in this group.

Equally important was the study of patients hospitalized in an intensive care unit, in which it was shown that the mortality among patients with elevated ADMA concentrations was 17-fold higher, compared to the mortality of patients with lower values.[81] In a prospective study of 153 patients with angina pectoris, subjected to selective coronary angioplasty and divided into groups according to the ADMA values prior to intervention and followed for an average period of 16 months, 51 cardiovascular events were noted. Coxs multivariate regression analysis revealed that the risk was positively correlated with ADMA incensement. Additionally, ADMA was an independent risk factor among age, smoking, hypercholesterolemia, and stent placing.[82]

It is worth mentioning that ADMA levels are higher in patients who manifest an acute coronary event compared to patients diagnosed with angina pectoris.[83] There are many other prospective and well designed studies demonstrating the important role of ADMA as an independent prognostic marker of cardiovascular risk.

ADMA and estrogens

The favourable action of estrogens on the endothelium is mediated through stimulation of transcription of the endothelial isoform of the nitric oxide synthase (eNOs) gene[41] and, furthermore, through the activation of the eNOs, during the binding with ERa, and through the PI3-kinase pathway.[84] The end result of the estrogenic action (genomic and non genomic) is

the maintenance of NO at normal or higher levels. Additionally, estrogens inhibit the accumulation of ADMA by increasing the activation (but not expression) of DDAH and by protecting the DDAH, which is sensitive to oxidative stress.[25] Finally, estrogenic action per se is beneficial in the biochemical profile of classic CV risk factors.

The fact that ADMA levels are lower in premenopausal women than in men of the same age and rise in the postmenopausal period indicates an effect of estrogens in ADMA metabolism *in vivo*.[85] Estrogens also inhibit the accumulation of ADMA in endothelial cells cultures,[86] and in ovariectomised animals *in vivo*[87] and can counteract the changes induced by oxLDL on the DDAH/ADMA/NO pathway. Specifically, estradiol counteracts oxidized LDL-induced ADMA production by cultured human endothelial cells.[88]

Furthermore, ADMA levels are significantly decreased a in hyperestrogenemic conditions such as pregnancy[89] and ovulation induction with gonadotropins.[86] By contrast, endothelial dysfunction in preeclampsia is characterised by high ADMA levels.[90,91] Finally, women with polycystic ovary syndrome present higher ADMA concentrations than controls, while treatment with combined estrogens and antiandrogens significantly decrease ADMA.[92]

ADMA and hormone therapy

ADMA having been established as an independent marker of endothelial function and cardiovascular risk is currently used in the assessment of the cardiovascular effects of hormone therapy. Following a two-week subcutaneous implantation of 100mg ethynylestradiol, Holden et al showed a significant reduction (around 18%) of plasma ADMA concentration.[25] In the same experiment, it was found that human and murine endothelial cell lines exposed to 17β-estradiol expressed a dose-dependent decrease, in ADMA production.[25] This study supported retrospective and cross-sectional studies indicating that HT acts beneficially on the cardiovascular system.[93]

Teerlink et al demonstrated that conjugated estrogens, and raloxifene to a lesser extent, decrease ADMA levels in healthy postmenopausal women.[21] The study included hysterectomized women who received either conjugated equine estrogens (0.625mg/d), raloxifene or placebo. During the two-year treatment, there was a consistent reduction in ADMA levels only in women taking estrogens. The average post-baseline difference in ADMA was decreased by 8% (p=0.003). Interestingly, there was a trend towards a slight rise in ADMA concentrations in the placebo group throughout the two-year period, probably reflecting an effect of aging. Finally, reductions were also observed in the raloxifene group, although non-significant.

Research has also been focused on the type of estrogen therapy, the dose, the route of administration, and progestogens addition as to their effect on the cardiovascular system. Post et al conducted a study on 65 women who randomly received unopposed micronized 17β-estradiol (2 mg/d), or micronized 17β-estradiol (2 mg/d) plus either dydrogesterone (10 mg/d), or trimegestone (0.5 mg/d), or placebo during a 12-week period.[22] The results showed reduction in ADMA levels in all treatment groups, but not in the placebo group. Compared to baseline and placebo, the greater reduction in ADMA concentration was noted in the estrogen plus trimegestone group (approximately 19%) and less in the estrogen plus dydrogesterone group (almost 6.5%), while in the unopposed estrogen group the reduction was ≈4%. The same research team had shown 8% reduction with conjugated equine estrogens.[21] The difference in ADMA changes between the dydrogesterone and trimegestone was attributed to the stronger antiestrogenic and antiandrogenic action of the latter.

In 2006, Verhoeven et al investigated the changes in ADMA levels according to the route of administration.[23] The authors randomly assigned 152 women to receive either transdermal 17β-estradiol (50μg/d), or oral micronized 17β-estradiol (1mg/d) unopposed, or oral micronized 17β-estradiol (1mg/d) plus gestodene (25μg/d), for thirteen 28-day cycles. They found significant reductions in all treatment groups. In fact, oral treatment groups presented greater reduction in ADMA levels than transdermal estrogen group. Compared to baseline, a 4.4% reduction was noted in the transdermal estrogen group 6.8% in the unopposed oral estrogen group and 8.5% in the oral estrogen plus gestodene group. The differences from baseline were similar to those found in two previous studies of the same research team.[21,22] Gestodene in

contrast to trimegestone seems to minimally influence the lowering effect of estrogens. The difference between the transdermal and oral administration may be attributed to the fact that ADMA is metabolized in the liver.[94,95]

Verhoeven et al randomly assigned 90 healthy postmenopausal women to receive either intranasal 17β-estradiol (175μg/d) plus norethisterone (275μg/d), or oral 17β-estradiol (1mg/d) plus norethisterone (0.5mg/d), for 52 weeks.[24] They found that there was a significant reduction in ADMA levels only in the oral treatment group ($p<0.001$). Specifically, the mean percentage decrease in ADMA levels, compared to baseline, was approximately 1.6% in the intranasal treatment group and 6.7% in the oral treatment group. It should be underlined that there was a consistent decrease of around 8% in ADMA concentrations by oral estrogens in the last and in the three previous studies, conducted by the same research team. The ADMA lowering effect is considered important, though statistically moderate, considering the fact that biological variation of ADMA in plasma concentration does not exceed 12%.[96]

A recent study suggested that transdermal estrogen treatment had a modulating effect on ADMA plasma levels in patients who had undergone surgery in the early premenopausal period;[97] after six months of treatment, women who received oral 17β-estradiol did not present significant reduction in ADMA concentrations, while controls (no treatment) had significantly higher ADMA levels.

CONCLUSIONS

Hormone therapy has been traditionally prescribed for women complaining of climacteric symptoms rather than postmenopausal cardiovascular disease prevention. However, there has been growing interest in trying to additionally improve metabolic and cardiovascular risk in postmenopausal women through HT. Nevertheless, the report of the initial results of two large-scale randomized clinical trials, namely WHI and HERS, raised considerable scepticism and serious concerns regarding not only effectiveness but also adverse events of the hormonal therapy. Critical evaluation of these controversial results, in accordance with other observational and mostly cross-sectional studies, raised the question as to whether or not younger, early postmenopausal women with non-established vascular disease could be candidates for HT. For this reason, many cardiovascular risk markers are being sought and applied in pertinent studies in perimenopausal women.

ADMA, a NO synthase inhibitor, has proven to be an independent cardiovascular disease risk marker. Overall, HT, and particularly oral estrogen therapy, has been shown to lower ADMA concentrations, even within two weeks, in healthy postmenopausal women. The effect of estrogens on ADMA levels, although small, is considered important, as physiological variation is limited. Nevertheless, larger randomized trials are necessary to substantiate the notion that estrogens lower ADMA levels and that these changes really reflect improved cardiovascular prognosis in postmenopausal women.

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