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Review

Could transdermal estradiol + progesterone be a safer postmenopausal HRT? A review☆

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ABSTRACT

Hormone replacement therapy (HRT) in young postmenopausal women is a safe and effective tool to counteract climacteric symptoms and to prevent long-term degenerative diseases, such as osteoporotic fractures, cardiovascular disease, diabetes mellitus and possibly cognitive impairment. The different types of HRT offer to many extent comparable efficacies on symptoms control; however, the expert selection of specific compounds, doses or routes of administration can provide significant clinical advantages. This paper reviews the role of the non-oral route of administration of sex steroids in the clinical management of postmenopausal women. Non-orally administered estrogens, minimizing the hepatic induction of clotting factors and others proteins associated with the first-pass effect, are associated with potential advantages on the cardiovascular system. In particular, the risk of developing deep vein thrombosis or pulmonary thromboembolism is negligible in comparison to that associated with oral estrogens. In addition, recent indications suggest potential advantages for blood pressure control with non-oral estrogens. To the same extent, a growing literature suggests that the progestins used in association with estrogens may not be equivalent. Recent evidence indeed shows that natural progesterone displays a favorable action on the vessels and on the brain, while this might not be true for some synthetic progestins. Compelling indications also exist that differences might also be present for the risk of developing breast cancer, with recent trials indicating that the association of natural progesterone with estrogens confers less or even no risk of breast cancer as opposed to the use of other synthetic progestins. In conclusion, while all types of hormone replacement therapies are safe and effective and confer significant benefits in the long-term when initiated in young postmenopausal women, in specific clinical settings the choice of the transdermal route of administration of estrogens and the use of natural progesterone might offer significant benefits and added safety.

Contents

☆ Parts of this work were presented separately by L'Hermite, Simoncini and Genazzani at the 6th IMS symposium held in Pisa in December 2006; they can be consulted at www.6thimsworkshop.com. The present work has been, however, considerably extended and updated.

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1. Introduction

HERS [1] (Heart and Estrogen/Progestin Replacement Study), WHI [2,3] (Women's Health Initiative) and WISDOM [4] (Women's International Study of long Duration OESTROGEN after Menopause) studies, which all initiated hormone replacement therapy (HRT) in women mostly many years post menopause, failed to confirm the previously anticipated cardioprotective effect of exogenously administered estrogens (CEE = conjugated equine estrogens were used in these studies). It however remains very likely that endogenous estrogens do indeed protect the endothelium, as suggested by vast experimental data in animals as well as in humans. Furthermore, reanalysis of the WHI results [5] suggested a decreased arterial cardiovascular (CV) risk in young (50–60-year-old) postmenopausal (PM) women. As demonstrated in monkeys, estrogen treatment, when initiated far from the onset of estrogen deprivation, looses its protective effect and, in presence of advanced arteriosclerosis (unstable plaque), seems to become deleterious [6,7]. Therefore HRT needs to be started with the appropriate timing (i.e. around the menopausal transition) to exert beneficial CV effects [6,8,9].

Despite major criticisms [10–12] of the WHI estrogen (E) + progestin (P) study, it remains established that standard doses (i.e. 0.625 mg/day CEE or 2.0 mg/day micronized estradiol) of orally administered estrogens do indeed increase the incidence of thromboembolic events, mostly during the first year(s) of use [13]. Stroke incidence also appeared to be increased in older patients [3,14,15] by oral estrogen administration (whether combined or not to a P). In the Danish Nurse Study [16], a significantly increased risk of stroke was evident only in PM HRT users who were hypertensive (not in normotensive women) and used an E/P regimen with norethisterone acetate (NETA), compared to those receiving unopposed estradiol. This increased stroke incidence has once again been highlighted in a recent systematic review and meta-analysis by Magliano et al. [17]. These authors even claim that HRT does not significantly change the risk of all-cause mortality, CHD (coronary heart disease) death or non-fatal acute MI (myocardial infarction). Since it is presently admitted that stroke is most often related to advanced age and hypertension, it should be recalled that a high proportion of patients in the WHI trials had untreated or uncontrolled hypertension and that its prevalence was higher in current hormone users [18].

Oral estrogen administration, mainly through a pharmacological "first-pass" effect on liver metabolism, induces a variety of metabolic effects. Elevated triglycerides, decreased LDL particle size and increased production of some coagulation factors and C-reactive protein go along with increase in circulating HDL cholesterol and reduction of LDL cholesterol. On the contrary, non-oral estrogen administration is largely devoid of such changes, as reviewed by Modena et al. [19]. A recent large review on the pharmacology of estrogens and progestogens and the influence of different routes of administration can also be useful [20].

In the present paper, we will successively discuss:

- the influence of menopause and /or sex steroids on blood pressure (BP);
- the relationships between menopause, metabolic syndrome and diabetes mellitus (DM) and the effects of sex steroids on the occurrence of newly diagnosed DM, as well as on established DM;
- whether sex steroids exert any deleterious or protective effect on arterial vessels and on the heart;
- the effects of sex steroids on the incidence of thromboembolic events;
- the neuroprotective impact of progesterone (P4) versus medroxyprogesterone acetate (MPA) on the animal brain;
- and finally the relationships between HRT and the incidence of breast cancer (BC), with special reference to the type of progestin added, when required, to the estrogen;

- we will then discuss whether the use of specific sex steroids and/or route of administration could lead to optimization of CV risks and possibly even allow continued CV protection, in addition to prevention of osteoporosis and of postmenopausal fractures. The latter prevention is now universally admitted and has been definitively demonstrated by the WHI studies, even in old PM women who were not at high risk of osteoporosis [21].

2. Menopause, sex steroids and hypertension

2.1. Menopause and the development of hypertension

Climacteric symptoms such as hot flashes are associated with transient increases in BP [22] but it is not known whether this has any link to the later development of permanent hypertension. Nevertheless, the prevalence of elevated blood pressure in PM women increases from about 35% at the time of the menopausal transition to around 75% after the age of 75 [23]. As reviewed by Reckelhoff and Fortepiani [24], lack of estrogens and the resulting reduced arterial elasticity and compliance is likely to contribute to the age-related progressive increase in systolic blood pressure and eventually to the likelihood of developing hypertension. It has been suggested that impaired endothelial vasomotor function (measured as flow-mediated dilatation of the brachial artery) might precede and predict the future development of hypertension [25]. Furthermore, menopause is associated with over activity of the sympathetic nervous system [26] and activation of the renin–angiotensin–aldosterone system (RAAS): together with other factors such as obesity and oxidative stress, it can contribute to a high prevalence of hypertension in late PM women.

2.2. Biological effects of oral estrogens pertinent to hypertension development

There are specific biological actions of estrogens associated with its oral route of administration that might theoretically be associated with an impact on blood pressure control. The "first-pass" hepatic effects of oral estrogens (or, in case of ethinylestradiol, its higher potency and prolonged half-life, irrespective of its route of administration) could be relevant [27,28]. Oral estrogens induce an increase in hepatic production of angiotensinogen (the renin substrate) [29], with a subsequent rise in plasmatic angiotensins I and II, although this does not affect renin itself or aldosterone [30]. These effects, which might precipitate the development of hypertension or deteriorate it, do not occur with transdermal /percutaneous estrogen administration [30,31].

2.3. Oral estrogens and blood pressure

Clinical evidence that oral estrogens might in any way alter blood pressure is weak and inconsistent. Postmenopausal oral estrogen administration is generally not associated with elevations of blood pressure [30,32]. As cited in [29], old reports even suggested slight lowering of BP in PM women receiving oral HRT, including hypertensive subjects. Some other studies however suggested that PM women using HRT develop over time smaller increases in systolic BP than those not treated [33], especially at older ages. Overall, as reviewed [34], recent evidence from the WHI trials indicates that PM oral CEE and CEE + MPA treatments are associated with clinically (in)significant increases in systolic and diastolic BP [3]. Interestingly, the EPAT (estrogen in the prevention of atherosclerosis trial), recently indicated that estradiol (1 mg orally) may slightly increase systolic blood pressure in younger PM women but having the opposite effect in older PM subjects [35]. On the other hand, little is known on possible differences between different oral estrogens, particularly estradiol (E2) and CEE.

2.4. Transdermal estradiol and blood pressure

On the contrary, Vongpatanasin et al. reported that chronic (8 weeks) transdermal (but not oral) estradiol (E2) reduced by 30% the basal rate of sympathetic nerve discharge, associated with a small but statistically significant ambulatory diastolic BP decrease of about 5 mmHg [36]. As extensively reviewed by Ashraf and Vongpatanasin [34], descriptive evidence exists that transdermal (td) estrogen administration does not alter BP or even results in slightly reduced BP in normotensive PM women [32,36–38].

2.5. Progestins and blood pressure

The progestin co administration may also be a variable, though most synthetic progestins are considered to be devoid per se of unfavorable effects on BP. However, progestins have recently been shown to exert distinct actions on vascular cells, such as differential alterations of nitric oxide synthesis [39], which may in some case contribute to impaired endothelium function and/or counteract, at least to some extent, the favorable effect of estradiol upon the endothelium [40]. This phenomenon could also, in the long-term, contribute to the development of hypertension. Nevertheless, many reports have suggested that the association of a synthetic progestin to oral estrogen has no effect on BP.

2.5.1. Progesterone and blood pressure

On the other hand, progesterone is clearly natriuretic and exhibits an anti-mineralocorticoid activity. Even within the low PM range of endogenous progesterone concentrations, the pressor and renovascular responses to angiotensin II are blunted with increasing P4 concentrations in low-sodium balance state [41].

In a small, short-term, placebo-controlled, double-blind crossover study, Rylance et al. [42] have shown that 200 and 300 mg/day oral micronized (mic) progesterone significantly reduced BP in hypertensive patients (men and PM women) deprived of their anti-hypertensive drugs. When added, usually cyclically, to oral [32,43,44] or transdermal [30,38,43] estrogens (CEE, estradiol or estradiol valerate), oral [30] or vaginal [43] progesterone did not induce any (further) modification in BP. Moreover, in the PEPI (Postmenopausal Estrogen/Progestin Interventions) study [45], an increase in BP after 1 year occurred in all CEE + medroxyprogesterone acetate (MPA) regimens but not in the CEE + mic P4 (200 mg/day for 12 days/month) group.

2.5.2. Drospirenone and blood pressure

A new progestin, drospirenone (DRSP), also exerts natriuretic and anti-mineralocorticoid effects similar to progesterone, but with greater potency. In a group of PM women with moderate hypertension, oral treatment with DRSP (0.5–3.0 mg/day) + 1.0 mg/day estradiol resulted in significant decreases in mean systolic and diastolic BP compared to baseline values. On the contrary, there was no statistically significant change in normotensive women [46]. Several randomized controlled studies have consistently demonstrated a significant BP lowering effect with DRSP + E2 (estradiol) in PM women. This favorable effect of DRSP on BP is most likely related to its potent anti-aldosterone activity. An additional benefit of E2 + DRSP was even found in hypertensive patients already treated with anti-hypertensive agents such as enalapril [47] or hydrochlorothiazide [48].

Thus, transdermal E2 administration, in association with micronized progesterone or drospirenone could be preferred in

hypertensive women and perhaps even in subjects with high normal blood pressure (systolic: 130–139 mmHg or diastolic: 85–89 mmHg), since these patients were recently reported, in a prospective cohort study [49], to be at higher CV risk. One can also speculate whether the use of td E2 + mic P4 might contribute to prevent to some extent stroke occurrence that is closely associated with untreated or uncontrolled hypertension.

3. Menopause, sex steroids, metabolic syndrome and diabetes mellitus

Metabolic syndrome and/or diabetes mellitus (DM) are important risk factors for CV (arterial and coronary) disease, especially when combined to hypertension. The incidence of DM increases with age and menopause. Estrogen deficiency resulting from menopause, through contributing to the development of abdominal obesity and insulin resistance, could represent a major step in the process of diabetogenesis in women. In a 2006 meta-analysis based on 107 trials, Salpeter et al. [50] concluded that HRT reduces abdominal obesity, insulin resistance, new-onset diabetes, lipids, pro-inflammatory adhesion molecules and pro-coagulant factors in women without diabetes. All these actions might be relevant in the long-term to reduce the risk of CV disease in PM women.

3.1. Effects of exogenous sex steroids on carbohydrate metabolism

In healthy postmenopausal women, hormone replacement with CEE does not substantially alter glucose tolerance, likewise oral or transdermal estradiol. However, slight differences on the effects of CEE versus estradiol (either administered orally or non-orally) have been reported on insulin response to glucose but, with CEE being associated with increases and estradiol instead inducing small decreases [51].

Progestins can also play a role in the modification of insulin/glucose metabolism, particularly related to mode of administration and type of progestin. Progestins with androgenic properties tend to increase insulin resistance. MPA can have a slight adverse effect on glucose and insulin, possibly because of its interference with glucocorticoid receptor signalling. On the contrary, progesterone and dydrogesterone do not exhibit adverse effects. A more comprehensive review of the relationships between diabetes, insulin resistance and progestins can be found in Rosano et al. [51].

Thus, glucose and insulin metabolism can be improved by estrogen replacement therapy but the addition of an androgenic progestin, such as NETA [52], may reduce the beneficial effect of estrogens on glucose metabolism and insulin sensitivity.

3.2. New occurrence of diabetes mellitus under HRT

Several studies as well as the meta-analysis of Salpeter et al. [50] suggest that HRT reduces the incidence of DM, irrespective of the hormonal combination used and despite the use of MPA (known to increase insulin resistance and impair glucose tolerance). In the PEPI study [45], administration of CEE + micronized progesterone did not deteriorate carbohydrate metabolism, in contrast to CEE + MPA, which did. Recent large randomized controlled trials investigating the use of CEE + MPA (HERS and WHI), have reported a significant reduction of new-onset of diabetes in treated women versus matched controls [53]. Particularly, WHI reported a 21% reduction of new cases of diabetes in the E + P arm while in the E-only arm, the reduction was not significant, possibly related to a higher proportion of obese subjects in this arm.

3.3. Effects of the type of HRT on established diabetes and metabolic syndrome

Although there is little information with respect to HRT-treated diabetic postmenopausal women, the meta-analysis of Salpeter et al. [50] shows reduced insulin resistance and fasting glucose in diabetic HRT-treated women.

Recently, low-dose HRT has attracted interest for the treatment of PM symptoms in diabetes, because of concerns about increased risk of coronary heart disease and stroke with conventional doses of HRT. In a double-blind, randomized placebo-controlled trial, Kernohan et al. [54] treated type 2 diabetic women with low-dose HRT (continuous oral 17β-estradiol 1 mg and norethisterone 0.5 mg). Conventional HRT with an androgenic progestin induce adverse effects on glucose clearance, triglycerides and hsCRP (highly sensitive C-reactive protein). On the contrary, with this low-dose combination, HRT showed decreased fasting glucose and total cholesterol without any other detectable adverse effect. Similarly, when estradiol (0.05 mg/day) and cyclical NETA (0.25 mg/day) were administered transdermally to type 2 diabetic patients, significant decreases in fasting glucose were observed [55]. Furthermore, in PM women with metabolic syndrome, Chu et al. [56] recently reported a worsening of insulin resistance and adipocytokine parameters with oral but not with transdermal estradiol.

Thus, menopause itself seems to be diabetogenic and HRT globally favorable, even preventing new-onset of diabetes. Transdermal estradiol administration could exhibit some additional metabolic advantage over the oral route in selected patients. Moreover, since micronized progesterone and non-androgenic progestins such as dydrogesterone, nomegestrol acetate or drospirenone have a more favorable metabolic profile, they should be preferred in overweight women, in women with insulin resistance, with metabolic syndrome or DM and in those in whom a long-term therapy is foreseen.

4. Sex steroids, arterial vessels and the heart: CV risk or protection?

After substantial evidence from experimental studies and observational clinical studies indicating a beneficial cardiovascular effect from HRT, the absence of a cardioprotective effect in randomized controlled trials was surprising [2,4,57]. One relevant question raised from the WHI trials is the impact that the type of progestin may have in altering estrogen's actions on the vessels. This seems especially relevant when comparing the E-only arm, showing protective effects in younger PM women [5] versus the CEE + MPA arm, where no protection was found in any age group.

Synthetic progestins are not completely equivalent to progesterone: Although synthetic progestins have been commonly assumed to exert analogous clinical effects, there are circumstantial indications that each progesterone receptor (PR) ligand may have specific cellular effects. The distinct pharmacokinetics of natural and synthetic progestogen, as well as the different affinities for the PR, may lead to recruit partially divergent signalling pathways in human vascular cells, due to differential PR modulation, as previously observed with estrogen receptor ligands. An additional level of complexity is added by the fact that some progestins are able to interact with other steroid receptors and may therefore activate non-PR-dependent signalling pathways or compete with the natural ligand for these receptors [58]. Synthetic progestins often interact with and transactivate androgen, mineralocorticoid, glucocorticoid or growth hormone receptors [59]. Due to this variety of actions, it can thus be foreseen that each progestin will have specific effects

and interferences with other steroid hormone signalling pathways, therefore resulting in unique clinical effects.

4.1. HRT effects on CV risk markers and on lipids

4.1.1. Effects of sex steroids on hsCRP

Although the meaning of minor elevations of hsCRP remains a matter of debate [60] and could just reflect tissue stress and injury, it is established that elevated basal levels of hsCRP are highly predictive of future CV events. Oral estrogens elevate hsCRP concentrations, which is not the case with transdermal estradiol [61–64]. Although this differential action on CRP levels between the non-oral versus the oral route of estrogen administration might suggest a better CV profile of non-oral therapies, it must be noted that oral HRT results in significant decreases of other relevant pro-inflammatory factors, including soluble endothelial-leucocyte adhesion molecules such as the vascular cell adhesion molecule-1 (VCAM-1) and E-selectin.

Micronized progesterone does not potentiate the effect of oral estrogen on hsCRP, while MPA does [45]. MPA and synthetic progestins per se exert different and even divergent *in vitro* inflammatory and anti-inflammatory effects on the endothelium, as compared to progesterone [39], As reviewed in [65], an elevation of plasmatic levels of interleukin 6 (IL-6) that seems related to the progestin [66], was seen in all oral CEE-treated groups of the PEPI study including those receiving oral micronized progesterone [67]. On the contrary, transdermal E2 (with or without any progestin, including oral micronized progesterone) failed to induce any increase in either hsCRP or IL-6 [65].

A very comprehensive systematic review of the effects of non-oral HRT on markers of CV risk is just in press by Hemelaar et al. [68]. It clearly emphasizes the advantage of non-oral over oral HRT with respect to CRP and resistance to activated protein C; changes in cell adhesion molecules and some fibrinolytic parameters tended to be smaller, whereas changes in other factors, including lipoprotein (a) and homocysteine, did not differ.

It should, however, be highlighted that detailed analysis of the WHI trial has indicated that although the subjects that received CEE + MPA did show a significant increase of serum hsCRP, this was not linked to any increase in CV risk, which was instead highly related to baseline levels [69].

4.1.2. Sex steroids and lipids

A major difference between the two routes of administration of sex steroid hormones is the effect on lipid metabolism. As reviewed by Modena et al. [19], oral estrogens result in complex modifications of the lipid profile, reducing total cholesterol and LDL cholesterol, increasing HDL cholesterol and triglycerides, decreasing the size of LDL particles, and possibly increasing fasting homocysteine [70]. These changes are largely absent in the case of transdermal administration of comparable doses of estradiol, due to the lack of the "first-pass" effect on the liver. Transdermal E2 actually results in decreased plasma levels of triglycerides [71], and larger LDL particles that are more resistant to oxidation, hence possibly preserving the antioxidant effect of estrogen. Indeed, transdermal E2 (alone or supplemented cyclically for 12 days with 5 mg MPA) diminished oxidative stress and increased anti-oxidative erythrocyte potency [72].

Elevated non-fasting triglycerides (or the associated lipoprotein disorder) have been recently shown [73] to be of predictive value for cardiovascular events (including ischemic stroke) and more so in women than in men [74]. In this respect, the baseline characteristics of the patient's lipid profile should be of guidance in choosing between the oral or transdermal administration of estrogens. However, there is no available indication that the changes of the lipid profile associated with oral estrogens result in major modifications of the CV risk. Actually, it is acknowledged that some of these effects, such as the reduction of LDL cholesterol, depend on changes in lipoprotein disposal because of increased hepatic expression of scavenger receptor [75,76], which might imply that the changes in LDL cholesterol are merely a bystander and therefore possibly irrelevant from a cardiovascular point of view.

As for the role of progestins, the addition, in the PEPI trial, of MPA to oral estrogen reversed the rise in HDL-C. On the contrary, the addition of micronized progesterone did not alter this estrogen-related increase in HDL-C [45]. Native progesterone also inhibits cholesteryl ester transformation by human macrophages, thus preventing macrophage accumulation of cholesterol involved in atherosclerotic process [77].

4.2. Sex steroids and endothelial function

Endothelial dysfunction was previously thought to represent a risk factor for the development of hypertension; this altered functional status of endothelial cells, associated with impaired vessel dilatation, is currently recognized as an important phenomenon on the road to atherosclerosis and a phenomenal predictor of future CV events. Dysfunctional endothelial cells, as during the early phases of atherosclerotic degeneration, are characterized by impaired synthesis of vasodilatory molecules, such as nitric oxide (NO), and by the expression on the cell membrane of adhesion molecules to circulating leukocytes [78].

A study showed that progesterone and MPA trigger significantly different signalling events in controlled *in vitro* systems: it was observed that progesterone stimulates NO synthesis via transcriptional and non-transcriptional pathways in human endothelial cells, as well as *in vivo* in ovariectomized rat abdominal aorta. In addition, when added to E2, progesterone does not impair the estrogen-dependent induction of eNOS expression, and it even potentiates the effects of estrogen during rapid stimulations. In contrast, MPA does not trigger eNOS expression either *in vitro* or *in vivo*, and it does not induce rapid increases of eNOS activity nor does it potentiate E2-dependent nongenomic eNOS activation. Even more interestingly, when endothelial cells were exposed to prolonged E2 + MPA, a reduction of estrogen-dependent eNOS over expression was observed, implying some sort of interference with estrogen receptor-dependent transcriptional signalling. These effects were confirmed *in vivo* in ovariectomized rats treated with clinically relevant doses of MPA or P4. The study then showed that P4 and MPA are not equivalent in terms of molecular signalling in human endothelial cells and in vascular tissues of ovariectomized rats. These two compounds have clearly distinct features and affect differently estrogen and glucocorticoid signalling [39]. A recent study on human endothelial NO synthesis reported a neutral effect of dydrogesterone, alone or in combination with estrogen, whereas the stable metabolite 20-α-dihydrodydrogesterone enhanced the expression of eNOS, similarly to natural progesterone [79]. Similar effects were found with nomegestrol acetate [80] as well as with drospirenone [81] that shows additional actions on endothelial nitric oxide synthesis because of its interference with the mineralocorticoid receptor.

Brachial artery flow-mediated vasodilatation (FMD) is a non-invasive assessment of endothelial function which correlates with invasive testing of coronary endothelial function. As recalled by Ho et al. [70], previous studies demonstrated the expression of endothelial NO synthase, the local increase of NO and the improvement of endothelial function by oral estrogens; Ho et al. [70] showed that flow-mediated vasodilatation in the brachial artery

is similarly and significantly increased from 5.9% to 13.9% after transdermal as well as oral E2 (6.0–14.7%) in healthy PM women. A similar effect of transdermal E2 has been reported in nine patients with a history of acute coronary syndrome, in whom basal post-ischemia FMD, though restricted to 1.2% (instead of 17.8% in healthy subjects), increased by 3.4% after 4 weeks of transdermal E2 [82].

Human endothelial cells are central for the function of human vessels in physiological and pathological conditions. Estrogens preserve endothelial function *in vitro* and *in vivo*. However, the addition of P4 or other synthetic progestins such as MPA has been suggested to interfere to different extents with estrogen's actions [83–85]. Detrimental effects of MPA on coronary vasomotion [86] and on arterial remodelling [87] have been described in female monkeys, where progesterone has neutral effects [88]. In agreement, Sorensen et al. [40] found that norethisterone acetate attenuated the favorable effect of oral E2 on FMD. As discussed by Adams et al. [89], although in some cases synthetic progestins do not antagonize estrogen's favorable effects, there are suggestions that specific progestins may do so in most cases: MPA (but not progesterone) interferes with estrogen protection against coronary vasospasm [86] and with the beneficial effect of estrogen on exercise-induced myocardial ischemia [90]; MPA (but not progesterone) inhibits the endothelium-dependent estrogen vasodilatation in the brachial artery FMD model [91,92].

4.3. Sex steroids and arteriosclerosis

Arterial compliance and stiffness can be evaluated by pulse wave velocity (PWV) that can be used as a marker of vascular damage. Vehkavaara et al. [93] reported that "physiological" E2 doses (2 mg orally or 50 mcg/day transdermally for 12 weeks) increased peripheral blood flow (forearm) in healthy PM women without any effect on large artery stiffness (aorta). In PM women with mild to moderate hypertension, Kawecka-Jaszcz et al. [94] found, on the contrary, a decreased PWV in carotid and femoral arteries (thus an improvement of the rigidity of these arteries) after 3–12 months of treatment with a transdermal patch delivering both E2 and norethisterone acetate. More recently, Sumino et al. [95] compared brachial PWV of normo- and hyper-tensive PM women at the end of a 12-month continuous treatment with estradiol (either oral CEE or an E2 patch) supplemented by cyclic (12 days/month) MPA (2.5 mg/day). They found that transdermal (but not oral) E2 therapy significantly decreased brachial PWV, improving arterial stiffness; there was, however, no significant correlation between changes in PWV and changes in vascular inflammatory markers.

A study performed on menopausal pre-atherosclerotic rhesus monkeys showed a protection of coronary arteries against hyper-reactivity, by low-dose transdermal progesterone compared with exaggerated vasoconstriction magnitude and duration in the placebo group. Salutary effects of sub-physiological blood levels of progesterone on coronary arteries [96–98] would thus extend to the much larger atherosclerotic population. Furthermore, treatment with progesterone but without E2 (and specifically excluding soy proteins and isoflavones that are found in monkey chow), was beneficial during an atherogenic diet.

Recently, matrix metalloproteinases (MMP) and their tissue inhibitors have been reported to play an important role in the mechanisms of rupture of complicated atherosclerotic plaques: this could explain the late deleterious effect of estrogens, when started at a time at which the atherosclerotic process has reached this state [99]. Indeed, oral estrogens could up regulate the plaque inflammatory process with resulting plaque instability. Lewandowski et al. [100] have thus reported that oral CEE, but not transdermal E2, significantly increased plasma MMP-9 without an associated increase in its tissue inhibitor.

4.4. Clinical effects of HRT on arteriosclerosis

In addition to the many reports of less atherosclerotic processes in ever- and current HRT users, Le Gal et al. [101] showed, in a community-based cohort (the EVA study), that HRT use may prevent the development of atherosclerotic plaques in PM women, especially with estrogens administered by transdermal route: patients using transdermal E2 developed, over 4 years, less atherosclerotic plaques (as ultrasonographically assessed in the carotid arteries) than never users [101], however without changing intima-media thickness progression.

As recently reviewed [102], cardioprotection is likely in younger patients (less than 10 years since menopause or younger than 60), as suggested by the meta-analysis of Salpeter et al. [103]. Even the WHI investigators finally admitted, in a joint analysis of both their clinical trials and their observational studies [104], the hypothesis of some cardioprotection from estrogen alone for younger women. This is in agreement with the previous EPAT (Estrogen in the Prevention of Atherosclerosis) study [105], in which a lower average progression of sub clinical atherosclerosis had been evidenced in hyperlipidemic PM women after 2 years treatment with unopposed 1 mg/day micronized estradiol (versus placebo). More recently, Manson et al. [106] reported the results of an ancillary study of the WHI E-only arm. After a mean 7.4 years of CEE treatment followed by 1.3 years without treatment in 1064 women aged 50–59 years at randomization, the investigators evaluated, by computed heart tomography, coronary-artery calcium scores, a marker for atheromatous-plaque burden that is strongly predictive of future risk of CV events [107,108]. They found significantly lower scores in women having received CEE than placebo, even after adjustment for coronary risk factors and with even better scores among women with at least 80% adherence ($p < 0.004$).

It is thus now clear that the timing of HRT initiation is crucial and that CV protection from estrogen administration can be obtained by starting HRT around the time of the menopausal transition [5,109,110]. Since cardioprotection from estrogen is thus established for young PMN women in the E-only arm but not in the E/P arm, it suggests that MPA probably opposes the favorable effects of estrogen. Similar findings were reported in the observational Nurse's Health Study (NHS) by Grodstein et al. [8] for young PM women having started their HRT within 4 years of their menopause.

According to vast data comparing the effects of micronized progesterone to those of MPA, it is likely that, on the contrary, progesterone will not oppose the favorable CV effects of estrogens. The use of transdermal estradiol plus progesterone might thus have advantages over oral E plus other progestins administration, especially in the long-term; this has, however, to be confirmed in large randomized trials with clinical endpoints.

5. Sex steroids and thromboembolic disease

Venous thromboembolism (VTE), including deep venous thrombosis (DVT) and pulmonary embolism (PE), is a common disorder with an incidence of approximately 1 per 1000 person-years in PM women. VTE accounts for about one third of all potentially fatal cardiovascular events in PM women using HRT. Hence the importance of avoiding this increased risk.

5.1. Thromboembolic events and estrogens

It is well recognized that the risk of thromboembolism (TE) increases considerably with age, especially in women over 60 [111]. On the other hand, when given orally, all estrogenic products so far available (including combined hormonal contraceptives and

SERMs) are associated with an increased risk of thromboembolic events, mostly during the first (two) year(s) of use [112]. The WHI E/P study [113] clearly confirmed that oral HRT increases the risks of VTE associated with age, obesity and factor V Leiden. Furthermore, thrombotic stroke as well as acute myocardial infarction could also represent events where the activation of coagulation induced by oral estrogens likely plays a role.

5.2. Impact of the route of estrogen administration

This increased VTE risk seems to be attributable to the activation of the liver expression of some clotting factors by oral estrogens. As reviewed by Modena et al. [19], oral – but not transdermal – estrogens cause a rise in fragments 1 and 2 of prothrombin and a decrease in antithrombin III levels [114,115]. Although oral HRT also induces some pro-fibrinolytic changes, it is generally acknowledged that it results in an overall switch toward coagulation activation [114]. Furthermore, oral HRT induces (acquired) resistance to activated protein C [116], another prothrombotic contribution which does not occur with transdermal E2 [68,117]. To this extent, transdermal E2 elicits a decrease in blood biomarkers implicated in coagulation (factor VII-tissue factor complex VIIa-rTF, fibrinogen and plaminogen activator-1 PAI-1), without affecting protein C and protein S activities, plasminogen and antithrombin III [118].

For thromboembolic events occurring under estrogen treatment, there is no available prospective randomized controlled trial comparing the route (non-oral versus oral) of administration. However, solid observational trials, such as the ESTHER study help to sort out the existence of differences. In this case-control study of 155 consecutive cases with a first documented episode of venous thromboembolism versus 381 matched controls, Scarabin and Oger [119] found no increased risk with transdermal E2, as opposed to a relative risk of 3.5 (95% confidence interval (CI): 1.8–6.8) for users of oral estrogens. Extending their recruitment to, respectively, 235 and 253 cases versus 597 controls, the ESTHER investigators [120] reported:

- that, in contrast to oral use, transdermal estrogen use does not confer, in obese women, any additional risk of idiopathic TE, confirming in the mean time the increased risk from obesity [120];
- a massively (25-fold) increased risk of TE with oral estrogen in patients with factor V Leiden or prothrombin G20210A mutation (after adjustment for potential confounding factors), but a similar absence of further increases of TE events when estrogen is given transdermally [121] to these patients.

Fig. 1 shows the relative risks of VTE reported in the ESTHER study according to the route of administration (oral versus transdermal estradiol) and according to the type of progestin added (micronized progesterone, a pregnane derivative or a norpregnane derivative); the risks were adjusted for obesity, familial history of VTE, history of varicose veins, education, age at menopause, hysterectomy and cigarette smoking.

It should be stressed here that one cannot extend these results, obtained with estradiol, to those reported with synthetic estrogens (ethinylestradiol: EE) used in combined hormonal contraception. Indeed, Sitruk-Ware et al. [27,28] have conclusively shown the intrinsic potential of EE on estrogen-sensitive hepatic proteins (especially hemostatic parameters), irrespective of the route (oral or systemic through the vagina) of EE administration. This intrinsic EE activity explains probably why increased rates of TE are similar with the contraceptive patch than with the corresponding combined oral contraceptive [122].

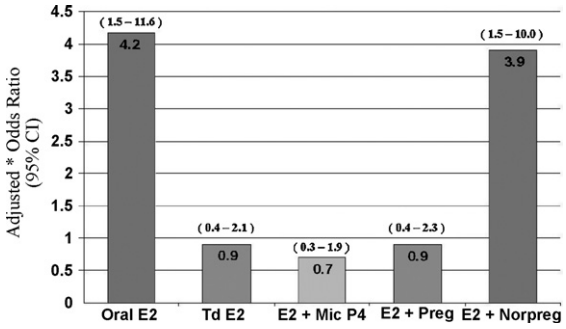

Fig. 1. Relative risks (95% confidence intervals) of HRT on VTE risk by route (oral versus transdermal) of estrogen administration and type of progestogen (combined to transdermal estradiol). Adapted from Canonico et al. (130). Td E2: transdermal estradiol; Mic P4: micronized progesterone; Preg: pregnane derivatives (dydrogesterone, medrogestone, chlormadinone acetate, cyprotérone acetate or MPA); Norpreg: norpregnanes derivatives (nomegestrol acetate or promegestone).

5.3. Modulation by the progestin of the estrogen-induced thromboembolic risk

As reviewed by Schindler [123], MPA and megestrol acetate, when used at very high doses in advanced gynecological cancer patients (with possible tumor-induced hypercoagulability), increase in a dose-dependent manner the occurrence of thromboembolic complications to 2–8%. Different progestins exert different effects on hemostasis parameters but it should be pointed out that most, especially MPA [124], decrease antithrombin III activity. When combined with EE in oral contraceptives, the increased TE risk (mainly attributed to EE) appeared somewhat greater (a 33% difference) [125] with non-androgenic than with androgenic progestins, as reflected by an increased resistance to activated protein C [126]. It can thus be anticipated that, in HRT, the progestogen might similarly influence the thrombotic effect of oral estrogen. Douketis et al. [127] reported, in a case-control study, that E/P HRT was associated with a significant 2.7-fold increased risk for deep venous thrombosis, while the 1.22 odd ratio for estrogen-only users was not significant. WHI studies gave relatively similar results since the TE risk was significantly elevated in the CEE + MPA arm [128] but not in the estrogen-only arm (pulmonary embolism) [3]. The detailed analysis of the latter study by Curb et al. [129] showed however that the venous TE risk was slightly higher (hazard ratio (HR), 1.47; 95% confidence interval: 1.06–2.06) in estrogen than in placebo users. Comparing these data to those of the WHI E/P arm, they found that the risk increase was significantly ($p = .03$) less for CEE users than for CEE + MPA users.

As far as progesterone itself is concerned, alone or combined to estrogen, there are few data available. However, in a recent report considering the impact of the route of estrogen administration and of the type of progestin added, Canonico et al. [130], showed that, in association with transdermal estradiol, progesterone does not modify the VTE risk (OR: 0.9–95% CI: 0.3–1.9), while an odds ratio of 3.9 (confidence interval: 1.5–10) is reached in patients using transdermal E2 combined with 19-norpregnane derivatives (nomegestrol acetate or promegestone). Micronized progesterone appears thus to be quite safe with respect to thrombotic risk.

6. Progesterone (versus MPA) and the brain (animal data)

Progesterone and synthetic progestins play an important role in the modulation of brain activity and responses to environmental stimuli. These effects have been attributed to the direct interaction of progestogens with their receptor in the central nervous system (CNS) and their metabolism into neuroactive 3α–5α

reduced metabolites. In addiction, the profound effects on the serotoninergic and opioid systems explain the meaningful influence of progesterone in the modulation of affective behaviour, cognition and measures of quality of life.

6.1. Neurotransmitters, sex steroids and stress

Experimental studies in ovariectomized rats (OVX) showed an impairment of the catecholaminergic neurons with an increase in noradrenaline release and a decrease in dopamine. Estrogen administration was able to revert these effects, decreasing the release of noradrenaline, increasing the dopaminergic neuronal activity and the dopamine release from the medio-basal hypothalamus. The effect of estrogens in modulating adrenergic receptors appears to be bimodal by up-regulating the α1-adrenergic and down-regulating the β-adrenergic receptor activity [131,132]. In ovariectomized rats, progesterone increased serotonine turnover in the limbic structures [133]. Following stress, progesterone increased 5-hydroxytryptamine (5-HT) concentrations in several regions of rat brain: 5-HT is significantly higher when compared to that of not treated stressed rats [134]. Moreover, the activity of MAO and catechol-*O*-methyl transferase (COMT) in rat brain is increased by progesterone treatment [135–138].

6.2. Progesterone synthesis and metabolism in the CNS

Neurons and glial cells possess enzymes necessary for sex steroid hormones metabolism (aromatase, 5α-reductase (5α-*R*), mainly in neurons; 3α-hydroxysteroid dehydrogenase (3α-HSD), mainly in type 1 astrocytes) [139]. The CNS is also able to synthesize steroids independently of peripheral steroidogenic glands secretion [140], leading to the production of a series of potent steroidal compounds. These brain-produced steroids have been named "neurosteroids", and have been found to exert important regulatory actions on neurons and glial cells [141,142].

Several studies have shown that some psychological functions and symptoms such as depression, anxiety, irritability and affectivity can be related to the fluctuation of the synthesis and the release of such neurosteroids, especially allopregnanolone and dehydroepiandrosterone (DHEA). Allopregnanolone, a 3α,5α reduced metabolite of progesterone, acts as an agonist on γ-aminobutyric acid A receptor ($GABA_A$), modulating stress, mood and behaviour, with anxiolytic, sedative and antiepileptic effects [143].

Ovariectomy significantly decreases allopregnanolone levels both in serum and in the central nervous system while it increases the adrenal content of allopregnanolone. In castrated female rats administered 17-β-E2, allopregnanolone levels increase in the hippocampus, hypothalamus, pituitary and serum, but decreases in adrenals. In OVX rats, progesterone increased, in a dose-dependent manner, allopregnanolone levels in the parietal lobe, hippocampus, hypothalamus and anterior pituitary. This is in agreement with preliminary reports that progesterone administration is correlated with an increase in allopregnanolone in blood and cortical areas in rats, which is probably responsible for the anxiolytic effect of this compound [144,145]. It was recently [144] described that brain concentrations of allopregnanolone are higher in animals receiving estradiol benzoate (EB) plus P4 than in those treated with EB alone or EB plus P4 in combination with an inhibitor of 5α-reductase or of P4 metabolism. The positive effect of the combined P and EB treatment on allopregnanolone levels may be due to the estrogen effect (via the modulation of 5α-reductase and 3α-hydroxysteroid oxydoreductase) [145,146] and on the higher availability of the progesterone substrate for metabolization into allopregnanolone [147].

In OVX rats, MPA administration, with or without estradiol treatment seems to behave somewhat differently in modulating central and peripheral allopregnanolone levels. MPA does not affect neither circulating nor adrenal allopregnanolone levels, indicating that the central effect observed depends on a direct impact of MPA, independently of the eventual peripheral contributions to circulating levels. [148]. Thus, a differential interaction of synthetic progestins with the 5α*R*–3αHSD enzymatic systems, can be hypothesized as a direct consequence of their different chemical structure.

6.3. Neuroprotective actions of progesterone

In the nervous system, progesterone is a pleiotropic hormone that exerts neuroprotective effects [149]. In the rat spinal cord, progesterone promotes neurological and functional recovery after contusion injury [150]; in the wobbler mouse, it promotes the survival of ventral motoneurons [151], and it also increases the survival of facial motoneurons after axotomy [152]. Very interesting protective effects of progesterone have been documented in the rat brain after traumatic injury, which could explain why female rats have significantly less edema and show better cognitive recovery than males [153]. P4 was still effective in reducing edema when treatment was delayed for 24 h after injury [154]. In addition to the reduction in edema, treatment with P4 also prevented secondary neuronal degeneration and reduced the behavioural impairments resulting from contusion of the medial frontal cortex [155]. Progesterone has also been shown to offer neuroprotection after axotomy, contusion injury of the spinal cord and cerebral ischemia [137,151,152].

The neuroprotective role of progesterone has also been described in a study by Nilsen and Brinton [156] using primary hippocampal neuron cultures treated with 17β-E2 and progestin, alone and in combination, before glutamate insult. Progesterone also has a role in neuroprotection mediated by activation of intracellular signals, such as MAP-kinase, which promotes the expression of anti-apoptotic genes thus preventing cell-death. This study shows that estrogen, progesterone, and 19-norprogesterone, alone or in combination, protected against glutamate toxicity. In contrast, medroxyprogesterone acetate failed to protect against glutamate toxicity. Not only was MPA ineffective as a neuroprotective drug, but, when coadministered, it attenuated the estrogen-induced neuroprotection.

Another positive central role of progesterone is in myelinisation. It was first observed in peripheral nerves, where progesterone synthesized by Schwann cells promotes the formation of new myelin sheaths after lesion. This function of progesterone is of significance for the aging brain and peripheral nerves, characterized by the loss of myelin [157]. Progestins regulate myelinisation in peripheral nerves via two distinct signalling mechanisms, involving either the intracellular progesterone receptor or membrane $GABA_A$ receptors, both of which are expressed by Schwann cells. The fact that progesterone also stimulates myelinisation by oligodendrocytes in the CNS has been recently demonstrated in a study by Ghoumari et al. [158] using organotypic cerebellar slice cultures of 7-day-old rats and mice. When added to the culture medium, P4 accelerated myelin formation in cultures of both males and females. P4 promoted myelinisation via the classical receptor, as its action could be blocked by mifepristone and was not observed in cerebellar slice cultures from PR knockout mice. In cerebellar explants, 3α,5α-TH progesterone also promoted myelinisation in a bicuculline-sensitive manner involving $GABA_A$ receptors but it remains unknown whether 3α,5α-TH progesterone acted on neuronal or glial $GABA_A$ receptors [158].

There is a complete clinical dearth of any indication that there might be any advantage of the route of administration of estrogen, as well as of the nature of the progestin used. However, progesterone (the "Nature's choice"), behaves thus also differently in

the brain than synthetic progestins (particularly MPA), through direct effects, as well as indirectly through effects on the vascular endothelium. This may have important implications for the effective use of hormone replacement therapy in the maintenance of neuronal function during menopause and aging and for protection against neurodegenerative diseases [156]. As far as prevention of Alzheimer disease by HRT is concerned, it remains a matter of debate, although there are data suggesting that, in this respect, the important seems also to start HRT around the menopause [159].

7. Impact of HRT on breast cancer (BC)

Understanding the effects of estrogen versus estrogen + progesterone (or + progestin) on the postmenopausal breast is essential. It can help understand how these hormones may play a role in the genesis (initiation and/or promotion) of cancer, although their role in development and maintenance of an established breast cancer (BC) can differ.

The breast is a hormone-responsive organ by excellence. Its development is influenced by a myriad of hormones and growth factors, responding selectively to given hormonal stimuli with either cell proliferation or differentiation. Among all of the complex hormonal influences, estrogens are considered to play a major role in promoting the proliferation of both the normal and the neoplastic breast epithelium [160]. Progesterone is another major, although controversial, player in mammary gland biology: it also acts, in conjunction with estrogens, in the regulation of breast development. The respective roles of estrogens and progesterone on breast epithelial proliferation remain a subject of controversy. Animal *in vitro* experimental data indicate that estrogens stimulate the proliferation of cultured breast cells implanted in athymic nude mice; P4, on the contrary, has no effect or even inhibits cell growth [160]. However, the clinical data in humans are quite controversial.

An association between BC and estrogens is reasonable, supported by a variety of epidemiological, clinical and experimental evidence, although paradoxes and contradictions exist [161]. Beyond the evidence of an association between lifetime exposure to estrogens and BC risk (early menarche, late menopause), BC incidence continues to rise with age throughout the postmenopausal years despite the fall in estrogen levels. Deprivation of ovarian hormones could increase the mammary sensitivity to sex steroids and, when dietary or other lifestyle habits are rapidly altered, changes to BC incidence follow relatively quickly. Epidemiological trends point out that estrogen would act as promoters when a carcinogenic agent is present or when a protective agent is absent: not as carcinogens. Thus sex steroids are possibly able to enhance the growth of existing, ER + breast cancers, but not to determine their development, which is primarily due to an accumulation of a large number of mutations and chromosomal abnormalities [162]. Sex hormones control the rate of mitosis and therefore influence the rate that mutations can occur.

7.1. Effects of estrogen, progesterone and progestins on breast proliferation: indirect in vitro/in vivo experimental human and primate data

Although estrogens are commonly considered to be responsible for cell proliferation, the breast epithelium of sexually mature and normally cycling women does not exhibit maximal proliferation during the follicular phase of the menstrual cycle, when estrogens reach peak levels (with very low levels of progesterone). Instead, the maximal proliferative activity occurs during the luteal phase, when progesterone reaches its maximal concentrations: this has been shown by evaluation of the proportion of Ki67 positive cells in fine needle aspiration biopsies [163]. Using the same technique in PM women, Conner et al. [164] reported a more than 4-fold increase in proliferation (from 2.2% to 9.1%) of Ki67 positive cells after 3 months (and even up to 25% in some individual women) with continuous E/P treatment (E2 + dienogest or norethisterone acetate). But the effect of progesterone itself appears quite different from that of these synthetic progestins. Thus, Foidart et al. [165] assessed histologically the proliferative activity in the terminal ducto-lobular unit of PM women who had applied for 14 days on both breasts a gel containing either E2, progesterone, E2 + progesterone or a placebo. They concluded that progesterone does not result in any mitogenic activity of the breast epithelium and that, moreover, progesterone is able to counteract the estrogen-induced proliferation of human mammary epithelial cells, in conformity with the results of a double-blind randomized study in PM women [166]. Nevertheless, it should be emphasized that the activity of sex steroids can clearly differ between postmenopausal and cycling women. Furthermore, applying simultaneously both sex steroids can be different (such as on the endometrium) than applying progesterone after a preliminary estrogen priming.

In contrast, in HRT-treated PM women utilizing MPA, Hofseth et al. [167] reported that cell proliferation and density, mostly in the terminal ducto-lobular units of the breast (the site of development of most cancers), were significantly higher than in women treated with E-only or receiving no treatment. There was also a positive association between higher levels of proliferation and increasing length of time on HRT.

Desreux et al. [168] studied both *in vivo* and *in vitro* the withdrawal effect (apoptosis) from the synthetic progestin nomegestrol acetate: it appeared to be specific to normal breast cells, being absent in tumoral *in vitro* T47D cells, as well as in fibroadenoma cells. Thus the effects of progestins can also differ in malignant than in benign conditions.

In a postmenopausal monkey model, Wood et al. [169] found that E2 + MPA resulted in significant proliferation in lobular and ductal epithelium while E2 + progesterone (P4) did not. Intramammary gene expression of the proliferation markers Ki67 and cyclin B1 was also higher after treatment with E2 + MPA but not with E2 + P4. Furthermore, results obtained with vaginal P4 were similar to those with oral micronized P4 [170]. Though *in vitro* studies on established cell lines cannot truly reflect *in vivo* effects, the study of Seeger et al. [171] on human BC MCF-7 cells indicates that the effects of simultaneous E/P can highly differ from combined cyclic E/P treatment: when P4 was added sequentially after E2, there occurred little inhibitory effect on cell proliferation and only at high concentrations; on the contrary, a very prominent inhibitory effect was observed when P4 was continuously combined to E2. The *net* inhibitory *in vitro* effect of the different progestins tested appeared however rather minimal at clinically relevant dosages. Franke and Vermes [172] observed that some progestins (MPA, NETA and dienogest), alone or combined with E2, stimulate the proliferation of MCF7 cells; on the contrary, progesterone and dihydrodydrogesterone, alone or combined with E2, induce apoptosis. Seeger et al. [173] also compared the effects of MPA and norethisterone on the ratio of apoptosis to proliferation of normal and cancerous epithelial breast cells incubated in presence of E2 alone, growth factors or E2 + growth factors. Their results emphasize that normal and malignant breast cells do not behave similarly in response to different progestins. Progesterone and progestins can thus exhibit quite divergent effects as far as carcinogenesis or development /maintenance of an established BC is concerned. In the breast, cancerous cells locally produce their own estradiol [174] which is found in much greater concentrations than in the general circulation. The importance of this phenomenon in the promotion of breast cancers, as well as that of growth factors, is also presently

emphasized. As reviewed by Druckmann [175], progestins without androgenic activity have been found, in breast tissue, to markedly inhibit the enzymes that are responsible for the local synthesis of estradiol. In this respect, Xu et al. [176] reported, *in vitro* in cultured hormone-dependent BC cell lines, that MPA + E2 stimulated the mRNA levels and activities of estrogen-activating enzymes (aromatase, 17β-hydroxysteroid dehydrogenase type 1 and sulfatase); progesterone also stimulated enzyme activity, but to a lower extent.

In addition, Wiebe et al. [177] showed that breast tissue is able to convert progesterone into two classes of steroids: the 4-pregnenes (greater in normal breast tissue) and the 5α-pregnanes (greater in tumoral breast tissue). The 4-pregnenes significantly inhibited whereas the 5α-pregnanes stimulated *in vitro* proliferation and detachment of breast cell lines, thus exhibiting potent opposing actions on breast cells.

7.2. Epidemiology of BC in HRT-treated postmenopausal women

7.2.1. Combined estrogen/progestogen treatment

HERS and WHI E/P studies, being randomized controlled double-blind versus placebo (RCT), are considered as the gold standard, providing level 1 evidence. Both used CEE combined to MPA and observed a slight increase in invasive BC incidence. For the entire duration of the HERS/HERS II studies (6.8 years), the 1.27 hazard ratio was not statistically significant (95% confidence intervals: 0.84–1.94) and it became even smaller in the "as treated" analysis (HR = 1.11; 95% CI: 0.62–2.03) [57].

On the contrary, WHI investigators concluded that their HR of 1.26 (95% nominal CI: 1.00–1.59) was clinically relevant (an absolute risk of 0.4%, giving an excess of eight cases of BC per 10,000 women per year), despite the fact that their trial was not a single trial for a single occurrence. Since they evaluated in fact multiple unrelated outcomes, adjusted CI had to be used, accounting for a Bonferroni correction. It should be highlighted that the published Bonferroni CI (0.83–1.92) were not significant. This goes along with the very high drop-out rates (42% in the HRT and 38% in the placebo group) and differential unblinding (40.5% in the HRT and 6.8% in the placebo group) in the cohort, which is likely to have heavily influenced the detection of breast cancers in the active-treatment group. Several authors even consider, for a number of reasons, that the WHI E/P results must in fact be interpreted like those of an observational study. Nevertheless the publication of this trial in 2002 led to a world-wide collective hysteria, entertained by the lay press and often incoherently followed-up by the regulatory authorities, leading many women in the world to quit their hormonal treatment (or not to initiate one), even in the presence of clear and proper indications.

In none of these trials was ever reported an increased risk of death from BC. Furthermore, the incidence of *in situ* BC was not increased [178]. Assuming a temporal sequence from *in situ* to invasive BC, the rate of *in situ* cases should have risen, especially in the first years, before the rate of invasive disease.

Right from the initial WHI publication, it was apparent that BC incidence was not increased in women who had never used any HRT before entering the WHI E/P study (HR = 1.06–95% CI: 0.81–1.38). It is however only at the end of 2006 that Anderson et al. [179] published a detailed analysis of the relationship between prior HRT and BC risk in E/P users. It confirmed, over an average 5.6 years of follow-up, a significantly greater risk (adjusted HR = 1.96–95% CI: 1.17–3.27) in E/P users among prior HRT users, but not among never users (HR = 1.02–95% CI: 0.77–1.36). Sensitivity analysis, to take into account non-adherence to study medications, was even more impressive for prior users but still not significant over placebo for never users (HR = 1.23–95% CI: 0.90–1.67). The Kaplan–Meier estimate of cumulative incidence over time appears visually to cross that of placebo users at about 3 years for prior HRT users and after 5 years for never users. From this study however, a safe interval for combined E/P use cannot be reliably defined and it is similarly impossible to predict precisely after exactly how many years of E/P use the risk for BC becomes significantly greater than without E/P treatment. Some authors, such as Clark [180] even negate most of the WHI E/P conclusions, including the increased risk of invasive BC from combined E/P.

However, several US groups [181] recently reported a sharp decline in ER + breast cancer incidence in 2003, following a major drop of HRT use in the general population after the publication of the WHI E/P results in the JAMA as well as by Time's magazine. Although other explanations can be possible (including a reduction in breast cancer screening that apparently did not parallel), Berry and Ravdin [182] consider that the most plausible explanation is that stopping HRT (at least in the USA where CEE with or without MPA are mostly used) removed the fuel that was promoting the growth of some tumors.

7.2.2. Estrogen-only treatment

It is mostly in the 1980s that, due to the fear of increased incidence of endometrial cancer from unopposed estrogen, the standard unopposed estrogen replacement therapy became a combined estrogen + progestin, either cyclic or continuous, HRT. A possible increase in BC incidence from E-only could never be firmly established [183] but had already been suggested in 1997 by the reanalysis performed by a Collaborative Group on Hormonal Factors in BC [184]. For women who had used HRT for 5 years or longer (average use = 11 years), the calculated relative risk was 1.35 (95% CI: 1.21–1.49), while 80% of these women had mostly used preparations containing estrogens alone.

Nevertheless, many – but not all – studies indicated very little (or no) increased BC risk from unopposed estrogens (mostly CEE) but pointed out a possibly greater effect when androgenic progestins had been added. In the study by Ross et al. [185], CEE replacement was not associated with a greater risk of BC, except in women using it for 15 years or longer (OR = 1.24). According to a very long follow-up, up to 25 years, Schairer et al. [186] evidenced a slightly increased relative risk (RR = 1.2–95% CI: 1.0–1.4; increased RR by 0.01 with each year of use) in E-only users, with a greater effect in lean women with a BMI < 25 (0.03 increased RR with each year of use). On the contrary, Li et al. [187] did not find any appreciable increase in BC risk in exclusive estrogen users, even for 25 years or longer, though they mention that the associated odds ratios are not inconsistent with a possible small effect.

In a prospective cohort of hysterectomized PM women from the Nurses' Health Study, Chen et al. [188] now report a significant trend ($p < 0.001$) for increased beast cancer risk correlated to duration of unopposed estrogen use among longer-term users, despite the fact that this increased risk became statistically significant only after 20 years of use (RR = 1.42–95% CI: 1.13–1.77) but, for hormone receptors positive BC, already after 15 years (RR = 1.48–95% CI: 1.05–2.07).

The WHI E-only arm results (in PM women with prior hysterectomy) are based on the best available study methodology; they are much stronger (unblinding of only 1.7%) than the E/P results. First published in 2004 [3], they showed, over an average 6.8 years of treatment with CEE alone, an unexpectedly decreased risk of BC, decrease that was almost statistically significant (HR = 0.77; nominal 95% CI: 0.59–1.01; $p < 0.06$). In an updated and detailed analysis [189] after a mean follow-up of 7.1 years, adherence-adjusted analysis showed a large and significant protection against invasive (but not *in situ*) BC in patients who adhered very well to their CEE therapy (HR = 0.67; 95% CI: 0.47–0.97; $p = 0.03$). This protective effect was mostly observed in patients with no first-degree relatives with BC, in patients with no history of benign

breast disease and in patients with a low Gail's score of BC risk. Furthermore it was also suggested that this protective effect was concentrated in women without prior hormone exposure of any type. In this E-only arm, the mean BMI (30.1 kg/m^2) of participating women was higher than in the E/P arm and was even in the range of obesity [20]. Thus, taking in consideration that obesity, especially when gained after menopause, appeared as a major risk factor for BC [190], one can hardly imagine how E-only treatment could reduce BC incidence in these women at higher basal risk, though a possible impact of several factors has been evoked. To our mind, the best hypothesis to explain this estrogen paradox comes from Santen and Allred [191]: they suggest that a "short-term" reduction of BC would result from estrogen-induced tumor cell apoptosis in the pool of occult malignant tumors. This is parallel to their *in vitro* observations: long-term (6 months to 2 years) estrogen-deprived hormone-dependent BC cells undergo adaptative changes that allow estrogen to stimulate apoptosis [192]. This could indeed explain this paradoxical protection observed in the WHI E-only arm, since most of their enrolled subjects were indeed "estrogen-deprived" at baseline (95% never used E + P, 50% to 55% never used estrogen and >50% did not use hormones in the last 5 years; 40% to 45% had been oophorectomized, of which 80% before 50 years of age). It could also possibly explain why no other study conclusively reported any such protection: indeed, premenopausal women that are castrated at the time of hysterectomy almost always and immediately require HRT for severe climacteric symptoms.

Lack of effect of unopposed estrogen treatment on BC risk (but not protection) has been confirmed recently [193], even using the UK General Practice Research Database [194]. As reviewed by Kenemans [195], there could, however, be a transatlantic difference since more European studies (none being a RCT) reported a significant increase in BC risk from unopposed estrogen, which is usually micronized estradiol rather than CEE. Thus, crossing the Finnish Cancer Registry with the medical reimbursement register, Lyytinen et al. [196] reported, among estrogen-only users, no increased risk (RR: 0.93–95% CI: 080–1.04) for less than 5 years use but a higher RR (1.44–95% CI: 1.29–159), including an increased incidence of carcinoma *in situ* (RR: 2.43–95% CI: 1.66–3.42), for use for more than 5 years. It is true that obesity is less prevalent in Europe and that lean European women could thus be more sensitive to estrogenic promotion of BC. Among other possibilities, one should also investigate whether the combination of the various steroids found in CEE could not behave as a selective modulator of estrogen receptors. Nevertheless, in the prospective E3N-EPIC cohort study, there was no effect on BC incidence (RR: 1.1–95% CI: 0.8–1.6) in estradiol-only users followed for a mean 5.8 years [197] but a slight and significantly increased risk (RR: 1.29; 95% CI: 1.02–1.65) after further follow-up, up to 8.1 years [198].

As far as the route of estrogen administration is concerned, the highly biased and thus controversial MWS [199] did not report any difference between oral and transdermal administration of estradiol, similarly to the Finnish report [196], but, in both studies, transdermal E2 users were very few compared to oral users. In a population-based case-control study, Opatrny et al. [200] found that the BC rate was not increased among exclusive users of unopposed estrogens; on the contrary, they reported an increased incidence with the use of opposed estrogens in oral form (adjusted RR: 1.38; 95% CI: 1.27–1.49), in contrast to patch form (RR: 1.08; 95% CI: 0.81–1.43). Though the potential for biases and erroneous inferences in such studies is immense, one could relate the latter finding to the work of Mueck and Seeger [201]. These authors report indeed that oral estradiol led to 10-fold greater urinary concentrations of possibly toxic, carcinogenic, metabolites (16-α-hydroxyestrone) than transdermal therapy.

7.2.3. Impact of the (type of) progestin added to estrogen

In the E/P arm of the WHI, it was thus observed an increased invasive BC incidence but paradoxically a decreased incidence in the E-only arm. This decrease became statistically significant in a subgroup [178] and was qualified as protection. Though the characteristics of the populations in the two arms are not quite comparable, it is intriguing to speculate that the addition of medroxyprogesterone acetate could be a reason for this striking difference.

As reviewed by Kenemans in 2005 [195], it seems presently well established that the addition to estrogen, of MPA or of an androgenic, testosterone-derived, progestin does indeed slightly increase BC incidence. Furthermore there are indications, such as those from Schairer et al. [186], that this increased risk might concern mainly lean patients (BMI < 24.5 kg/m^2).

One should also recall the similar difference in the effects of combined E/P versus E-only treatment, on breast mammographic density, presently considered as an independent risk factor (or an indicator of risk) for BC, at least among subjects with a family history [202]. PM women under E-only develop seldom (3.9%) increased mammographic density, while women on E/P develop it much more often (31.1%) [203]. In a follow-up of the PEPI study, Greendale et al. [204] reported similarly much increased, statistically significant, odds ratios for increased breast density under continuous as well as cyclic combination of MPA + CEE. Even the cyclic addition of 200 mg/day of micronized progesterone resulted in a significant increase in mammographic density, though of lower magnitude.

MPA is the progestin most often used in the USA. The various biological activities and affinities for different receptors of all the progestins available makes it clearly impossible to admit a class-effect solely based on the effects of MPA. Therefore, results obtained in France (where other progestins, mostly micronized progesterone, are widely used) are quite interesting. In a large cohort followed for a mean of 8.9 years, de Lignières et al. [205] found an adjusted RR of 0.98 (95% CI: 0.97–1.05) among the HRT users. The majority of the patients were combined HRT users (89%); the progestins were mainly oral micronized progesterone (58%) or dydrogesterone (10%), while fewer than 3% used MPA. Another interesting study coming from France is the MISSION study, with a historical-prospective design with case randomization, of which the methodology has been described by Chevallier et al. [206]. The results clearly illustrate the bias of selective HRT prescription by the physician, as well as of selective acceptance /request from their patients: the treated group had significantly less overweight, fewer late menopauses and less first-degree family history of BC. Mean HRT duration was 7.9 years and 30.5% of women had been treated

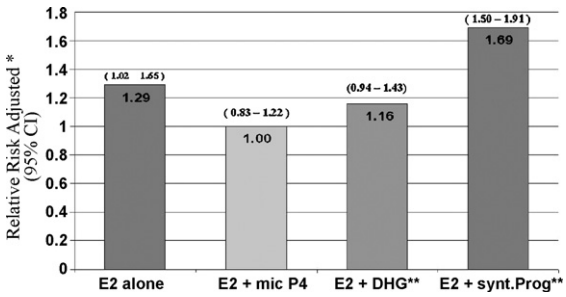

Fig. 2. Relative risks (95% confidence intervals) for invasive breast cancer by type of HRT and type of progestagen, compared with HRT never-use (E3N cohort study) Adapted from Fournier et al. (198) E2: estradiol; mic P4: micronized progesterone; DHG: dydrogesterone; synt. Prog.: synthetic progestins (mainly nomegestrol acetate, promegestone, chlormadinone acetate, cyproterone acetate, medrogestone).

for 10 years or more. In these conditions, BC prevalence was six times greater ($p < 0.0001$) in the untreated than in the treated group [207].

Later on, in the E3N-EPIC cohort study, Fournier et al. [197,198] assessed prospectively the risk of BC associated with HRT use in PM women: the risk was significantly increased by the use of HRT containing synthetic progestins while there was no increase with HRT containing micronized progesterone [82]. It should be emphasized that, after a mean 8.1 years follow-up, the RR for estradiol + progesterone users was still unchanged at 1.0 (95% CI: 0.83–1.22), while the RR had reached 1.29 for estradiol alone and 1.69 (95% CI: 1.50–1.91) for estradiol + synthetic progestins (other than progesterone and dydrogesterone) users.

Fig. 2 shows the relative risks for invasive breast cancer in this E3N-EPIC cohort, according to the use of estradiol alone or to the use of estradiol combined with either micronized progesterone, dydrogesterone or with other synthetic progestins; risks were adjusted for time since menopause, age at menarche, parity and age at first full-term pregnancy, breastfeeding, age at menopause, type of menopause, personal history of benign breast disease, family history of breast cancer, body mass index, physical activity, previous mammography and further stratified on year of birth.

It should additionally be reported that Campagnoli et al. [208,209] suggest that synthetic androgenic (testosterone-derived) progestins might, when combined to estrogens, increase BC risk through non-progesterone-like effects, such as by contributing to an increased IGF-1 activity, which exerts potent mitogenic and anti-apoptotic effects on BC cells, in synergy with estrogens. In this respect, it should be emphasized that continuous administration of P4 (100 mg/day), together with td E2, failed to increase IGF-1 concentrations over a 6 months period [210].

Thus the choice of the progestin to be used, when required for endometrial protection, can be crucial in relation to the possible influence of HRT on breast cancer incidence. Until now, progesterone and dydrogesterone seem to be neutral and thus the safest. It will however be necessary to investigate whether such modulation of breast cancer incidence might be also related to the doses of progestin used and not solely to the type of progestin.

8. Conclusions

Postmenopausal hormone replacement therapy with estrogen alone or estrogen plus progestin is the first line therapy to alleviate menopausal symptoms. HRT also reduces the risk of osteoporosis, coronary-artery disease (if initiated early after the menopause), and possibly Alzheimer's disease [159,211]. Relatively long-term HRT prescription for osteoporosis prevention seems more and more to become again a valid option, as shown by the WHI investigators, especially in osteopenic women. This is supported by the additional evidence that the quality of bone (and of intervertebral discs), beyond the simple prevention of bone mineral content, is rapidly altered after the menopause. Bone quality is maintained by HRT [212] but cannot be restored later on by bone-specific therapies which, furthermore, have no benefit on other tissues (such as the skin and the eye), menopausal symptoms and quality of life (including sleep). The majority of fractures (>50%) occur in the osteopenic patients rather than in the osteoporotic patients and, as reviewed by Torgerson and Bell-Syer [213], HRT is more effective in preventing non-vertebral fractures when started before 60 years than later on. Furthermore, this protection against fractures can also result from an improved postural balance that is rapidly restored by early HRT to what is normally seen in young women [214]. In short, HRT remains the best means of fracture prevention, especially in osteopenic women, provided its use could be extended without major harm.

Women's protection against CV diseases, as opposed to men, is evident until the menopause and is logically attributed to their endogenous estrogens, which vanish with menopause together with this relative CV protection. The total duration of menstrual cyclicity is inversely correlated to the number of myocardial infarctions [215]. Both the time since menopause and the age at menopause are aggravating factors for MI, independently of age [215]. Furthermore, in women referred for coronary angiography, the severity of CHD is correlated with measures of exposure to endogenous estrogen [215]. It is also possible that this CV protection from endogenous estradiol might be mediated, at least partially, through non-genomic mechanisms and/or E2 metabolization in hydroxyestradiols and methoxyestradiols [216]. Thus these effects could vary according to genetic variations, responsible for individual differences in E2 metabolization, diet, environmental factors and the use of progestins. It has recently been reported that the urinary ratio of 2–OH estradiol to 16–OH estradiol, is a significant predictor of systolic blood pressure [217] among PMN women; this ratio may reflect the effects of 2-hydroxyestradiol, known to be a potent inhibitor of vascular smooth muscle cell proliferation. However, a large set of evidence stands for a clear CV protection (around 50%) for those women who start HRT around the time of menopause. The timing of HRT initiation could also be relevant for ischemic stroke since, in the observational Nurse's Health Study, Grodstein et al. [218] reported a non-significant risk (RR = 0.94–95% CI: 0.58–1.53) for women younger than 55 years, especially for those within 4 years of their menopause and not taking hormones for less than 5 years (RR: 2–95% CI: 0.95–1.80).

The present review has highlighted that, on some CV end-points, selected advantages are associated with the transdermal route of estradiol administration. MPA (possibly due to its glucocorticoid activity) may counteract some of the favorable effects of estradiol, which is not the case of progesterone. The KEEPS (Kronos Early Estrogen Prevention Study) will in the future give us a partial answer, as it evaluates the progression of carotid intima-media thickness or coronary calcium in early PM women treated with transdermal E2 + progesterone [219].

Since conjugated equine estrogens are a mixture of at least 10 potent estrogenic steroids, of which most are not natural to humans [220] and since CEE also contains equine androgens and progestins [221], it remains to be established whether they are associated with any major biological difference leading to clinical advantages or disadvantages over estradiol itself.

The addition of a progestin to estrogens is required for endometrium protection and the PEPI trial has demonstrated that micronized progesterone works [222] as well as MPA. Another study with progesterone applied as a vaginal gel led similarly to prevention of endometrial hyperplasia in all women tested [223]. In addition, there is good observational data [198] to suggest that HRT combining micronized progesterone to estrogens will not result in any increased incidence of breast cancer, in contrast to most synthetic progestins [128]. Overall very long-term use of unopposed estrogens might still induce a very slight increase in BC risk.

Another area where the choice of the compound may make a difference is the nervous system. A very extensive and comprehensive review of the effects of progesterone at this level was recently published [224], highlighting the neuroprotective effects of progesterone, not only for preventing but also for reverting age-dependent changes and dysfunctions. Some of these actions, particularly those mediated by conversion to neurosteroids such as allopregnanolone, may not be shared by synthetic progestins. Although it remains to be established whether HRT does really

prevent or retard Alzheimer's disease, the impact of the timing of HRT initiation is likely to be similar [159] to that admitted for cardiovascular protection.

HRT started at the menopausal transition and optimized through expert personalization (for example combining low doses of E2 given transdermally and micronized progesterone), will be cardioprotective and avoid an increased incidence of thromboembolic events as well as of breast cancer; it could eventually prevent to some extent the development of diabetes mellitus and possibly protect cognition.

Proper use of HRT in older patients might also contribute to minimize the development of hypertension, of events related to plaque rupture and of ischemic stroke. The latter event appeared to be also related to the dose of oral estrogen, since no increased stroke risk occurred in the NHS in women using the ultra-low dose of 0.3 mg CEE [218].

Although some of these statements need to be substantiated by large, long-term, controlled, randomized trials, this is not a reason to deny access to HRT regimens that have prospectively proven their beneficial effects, at least the way they are usually prescribed in Europe, i.e. starting around the time of menopause with careful consideration for possible contraindications. Indeed, all the available evidence indicates that patient selection, with a careful risk/benefit assessment, and adaptation of the therapy, including the choice of compounds, the dose, the route of administration and the tailoring throughout time, result in significant benefits associated with a clearly safe profile.

In this respect, in order to replace efficiently "Nature", low doses of physiologic hormones, as well as their systemic administration, exert probably the least risk and, as a precaution, might be preferred.

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