Hormone Replacement Therapy and Cardiovascular Disease: Lessons from a Monkey Model of Postmenopausal Women

J. Koudy Williams and Irma Suparto

Abstract

Concerns exist about the cardiovascular effects of hormone replacement therapy (HRT) in postmenopausal women because results from the Women’s Health Initiative (WHI) and the Heart and Estrogen/Progestin Replacement Study (HERS) are contradictory. In both of these studies, postmenopausal conjugated equine estrogens + medroxyprogesterone acetate did not reduce risk, and somewhat increased the risk of myocardial infarction in both primary (WHI) and secondary (HERS) prevention. These results appear to contradict numerous observational clinical trials and animal studies, which reported profound beneficial effects of HRT on cardiovascular disease risk. Results of both human and monkey studies indicate that estrogen replacement therapy (ERT)/HRT is effective in inhibiting progression of early stage (fatty streak) atherosclerosis but that ERT/HRT is much less effective in inhibiting progression of more advanced (established plaque) atherosclerosis. Results of these monkey studies are consistent with those of studies in women wherein ERT/HRT was initiated in postmenopausal women with different initial amounts of atherosclerosis. Based on these findings, it is speculated that ERT/HRT may be more cardioprotective in younger postmenopausal women with less coronary artery disease, and less effective in women with established coronary artery disease. Researchers are challenged to define the relative cardiovascular risk/benefit in different populations of postmenopausal women based on differences in age, amounts of pre-existing atherosclerosis, and risk factors.

Key Words: animal models; atherosclerosis; endothelial function; inflammation; plaque stability; risk factors

Introduction

Cardiovascular disease is the leading cause of death for both men and women in westernized countries. One of every two women will die of heart disease or stroke, compared with one of 28 women who will die of breast cancer. Coronary heart disease (CHD) rates in women after menopause are two to three times those of women the same age before menopause. The increased incidence of cardiovascular disease in women after the fifth decade of life coincides with the onset of menopause, which is associated with significant reduction in sex hormone, estrogen, progesterone, and androgen (AHA 2001; Bush et al. 1987). However, the increase in cardiovascular risk in the fifth decade may be due to aging effects on the arteries. In fact, both men and women have increased cardiovascular risk as they age. There is no change in the slope (age vs. cardiovascular disease) as women go through menopause. Thus, the increased cardiovascular risk in the fifth decade is age related and not related to changes in plasma hormone concentrations. This characteristic has led to speculation that postmenopausal hormone replacement therapy (HRT), which has been shown to reduce the risk of coronary heart disease by as much as 50% in observational studies (Bush et al. 1987), does not affect cardiovascular risk per se, but represents a “healthy women” phenomenon. In other words, healthier women are likely to take HRT and take care of themselves.

The healthy woman effect can only be examined in a randomized prospective double blind, placebo-controlled, clinical trial. Results of recent prospective, randomized, double blind, placebo-controlled trials have related HRT to adverse health outcomes in postmenopausal women, and have attracted considerable attention from health professionals, researchers, and the public. In particular, the Heart and Estrogen/Progestin Replacement Study (HERS) (Hulley et al. 1998) and the Women’s Health Initiative (WHI) (Writing Group for the WHI 2002) have generated concerns about the relative benefit/risk ratio of HRT in postmenopausal women. In contrast to observational studies, HRT did not decrease (and may have slightly increased) the risk of coronary heart disease among women with (HERS) or without (WHI) pre-existing CHD. Due to results of both HERS and WHI, many experts in women’s health do not currently

Volume 45, Number 2 2004 139
advocate the use of HRT in postmenopausal women except for short-term relief of postmenopausal symptoms.

The ovariectomized cynomolgus monkey model has been used extensively to examine the effects of HRT on surrogate markers of coronary heart disease in postmenopausal women. This monkey model develops dietary-induced atherosclerosis. Atherosclerotic coronary arteries of cynomolgus monkeys rupture, develop mural thrombosis, and produce myocardial infarctions when the animals are fed atherogenic diets for many years (Williams et al. 1991). The incidence of myocardial infarction is greater in males than in females (Williams et al. 1991); however, the incidence is small (as it is in a population of people with similar risk factors). For this reason, and the unpredictability of identifying exactly which monkeys will have a myocardial infarction (again, similar to people), surrogate endpoints (e.g., plasma lipids and lipoproteins, coronary vascular reactivity, and atherosclerosis extent) of clinical coronary heart disease (chest pain, death, heart failure) are routinely used to examine the effects of HRT on the risk of coronary heart disease. In the text below, monkeys and humans are compared in relation to cardiovascular risk factors and surrogate endpoints of coronary heart disease.

Estrogen replacement therapy (ERT) and HRT have been shown to have beneficial cardiovascular effects on these surrogate markers in monkeys (Clarkson et al. 1996; Mikkola and Clarkson 2002). However, publication of the results from HERS and WHI provide results seemingly at odds with the outcomes of the observational studies in women as well as the prospective, randomized investigations conducted in monkeys. The question is not necessarily whether the monkey model is valid (no one believes that monkeys are exactly like women), but rather how these divergent outcomes can be reconciled. The overarching issue seems to be one of interpretation, which applies to the randomized human trials as well as the monkey studies and human cohort studies. A critical assessment of past studies in monkeys in the context of results of the WHI and HERS trials is presented in this review.

Characteristics of the Monkey Model

Cynomolgus monkeys share with women approximately 90% of their genome. Sexually mature females have regular, 28-day menstrual cycles and similar within-cycle gonadotropin and steroid sex hormone variations to those in women (Clarkson et al. 1996; Hamm et al. 1993; Kaplan et al. 1984). Additionally, cynomolgus monkeys share with humans a susceptibility to diet-induced artery atherosclerosis. Finally, the distribution, cellular characteristics, and extent of lesions closely resemble the pattern observed in humans (Clarkson et al. 1996).

Similar to their human counterparts, premenopausal female monkeys that consume a diet relatively high in saturated fat and cholesterol develop less atherosclerosis and have higher blood concentrations of high-density lipopro-


tein cholesterol (HDLC) than equivalently treated, age-matched males (Hamm et al. 1983; Kaplan et al. 1984). Furthermore, surgically postmenopausal (i.e., ovariectomized) monkeys fed an atherogenic diet develop more atherosclerosis and have lower HDLC concentrations than age-matched premenopausal females (Adams et al. 1985). Typically, adult ovariectomized monkeys are used as models of postmenopausal females. Natural menopause occurs in this species, but only near the end of life, and after most individuals have already died of other causes. It is therefore impractical to use naturally menopausal monkeys in most experiments, requiring experimenters to rely on the surgical removal of the ovaries to produce a model of postmenopausal woman.

ERT/HRT Effects on Lipids and Lipoproteins

Postmenopausal Women

At menopause, the plasma cholesterol profile of women changes to one of increased cardiovascular risk. Total plasma cholesterol (TPC) and low-density lipoprotein cholesterol (LDLC) concentrations increase, and HDLC concentrations decrease (Bush et al. 1987; Lobo et al. 2001; Walsh et al. 1991).

The cardiovascular effects of ERT/HRT on lipoproteins are among the most widely accepted beneficial effects (Bush et al. 1987). Although there is variability depending on the type, dose, and route of administration, oral ERT generally causes a decrease in LDLC and an increase in HDLC and triglyceride concentrations (Bush et al. 1987). In the Postmenopausal Estrogen/Progestin Intervention Trial (PEPI) (Writing Group for the PEPI Trial 1995), all hormone regimens reduced LDLC cholesterol level by 15 to 16 mg/dL, significantly reduced lipoprotein (a), but increased triglycerides. The most favorable effect on HDLC concentration was in women taking unopposed estrogens. The addition of either cyclic or continuous medroxyprogesterone acetate (MPA) resulted in 75 to 80% less increase in HDL compared with women taking estrogens alone.

HERS was the first large-scale randomized trial of HRT for prevention of CHD in postmenopausal women with established coronary disease (Hulley et al. 1998). Women were randomly assigned to conjugated equine estrogens (CEE, 0.625 mg) + MPA (2.5 mg), or placebo. After follow-up of 4.1 yr, the incidence of cardiac events was virtually identical in the two groups despite beneficial changes in plasma lipid concentrations analogous to those seen in the PEPI study.

The most consistent adverse effect of ERT/HRT is the increase of triglyceride levels. The elevated blood triglyceride levels are an important risk factor for both CHD and stroke (Hodis et al. 2003).

LDL particle size is reduced in women with estrogen treatment. This reduction may be caused by a number of
metabolic changes, the most likely of which include changes in hepatic very-low-density lipoprotein (VLDL) particle production and subsequent LDL particle formation, a selective uptake of larger LDL particles by the liver, and changes in enzymes/proteins involved in the process of intravascular remodeling of lipoproteins. Reports from studies in women indicate that estrogen increases both the clearance and production of large LDL and small LDL particles, with the greatest effect on clearance of large LDL particles. (Wagner 2001)

Ovariectomized Monkeys

Female cynomolgus macaques, like human females, have higher plasma HDLC concentrations than their male counterparts when fed an atherogenic diet (Hamm et al. 1983; Kaplan et al. 1984). Like women, postmenopausal (ovariectomized) monkeys have an adverse plasma lipid profile (elevated TPC concentrations and reduced HDLC) compared with intact females. The addition of exogenous ERT is generally associated with beneficial changes in plasma lipoproteins (Wagner et al. 1997). However, plasma HDLC concentrations do not increase appreciably with ERT or HRT in this species of monkey. In this respect, monkeys differ from postmenopausal women (Wagner et al. 1997). Thus, the plasma lipid responses of surgically postmenopausal and postmenopausal women to hormone treatment are somewhat divergent. However, based on results from observational studies in women and in several monkey studies, ERT increases triglyceride levels primary by increasing the production of large, VLDL particles, most of which are cleared by the liver rather than being converted to small and more atherogenic VLDL particles or LDLC. In summary, female monkeys and women are similar with regard to their TPC, LDLC, VLDL, and plasma triglyceride response HRT. They differ in their HDLC response to HRT.

ERT/HRT Effects on the Artery Wall

Similar to postmenopausal women (Bush et al. 1987), the antiatherogenic effects of HRT appear to be somewhat independent of changes in TPC, HDLC and LDLC, apolipoprotein A1 and B concentrations, average LDL particle size, and HDLC subfractions (Adams et al. 1990). Adams and colleagues (1990) reported that both subcutaneously administered 17-ß estradiol (E2) and E2 + cyclically administered progesterone significantly inhibited progression of atherosclerosis in ovariectomized monkeys. The antiatherosclerosis effects of treatment were independent of variation in plasma lipoprotein concentration.

Another study conducted by Adams and colleagues (1997) was designed to test a commonly prescribed HRT in women. Ovariectomized monkeys were untreated (controls) or were treated with continuous oral CEE, MPA, or CEE + MPA. After 30 months, unopposed CEE resulted in a 72% reduction in average plaque size compared with control animals. In contrast, plaque size in animals receiving CEE + MPA was similar to that of controls. Clarkson and colleagues (2001) reported that addition of MPA did not antagonize the atheroinhibitory effects of CEE. Progestin issues are discussed below.

One major determinant of atherogenesis is the amount of accumulated LDLC in the atherosclerotic plaque. In 1991, Wagner and colleagues reported that HRT (subcutaneous estradiol and progesterone) significantly decreased (by 70%) the accumulation of LDLC and arterial LDLC degradation in coronary arteries of postmenopausal monkeys. These changes in arterial LDLC metabolism were independent of changes in plasma lipid, lipoprotein, and apoprotein concentrations, and they occurred without changes in indices of endothelial injury or changes in extra-arterial LDLC metabolism. The findings suggest that reduced arterial LDLC accumulation is one mechanism by which estrogen may inhibit atherogenesis.

Results from numerous experimental and clinical investigations indicate that estrogen treatment may act directly at the vascular wall level by affecting endothelial function (Mendelsohn and Karas 1999). The endothelium plays a key role in modulating vascular smooth muscle cell reactivity (Williams et al. 1997). Dilator and constrictor substances are released by endothelial cells that may modulate the vascular response to a wide variety of neurohumoral stimuli. Atherosclerosis impairs dilation and enhances constriction of coronary arteries (Ludmer et al. 1986; Williams et al. 1997). There is considerable evidence that estrogens improve dilation. The effect of estrogen on coronary artery reactivity has been evaluated by repeated quantitative angiography in ovariectomized monkeys. Changes in the coronary artery diameter have been measured after intracoronary infusion of acetylcholine, which induces endothelium-mediated vasodilatation in normal arteries and constriction in atherosclerotic arteries. After both short- and long-term estrogen treatments, the animals exhibited vasodilatation in response to acetylcholine, whereas estrogen-deficient controls exhibited vasoconstriction (Williams et al. 1997).

These data from nonhuman primate and other animal models suggest that ERT and HRT have beneficial effects on several different risk factors and surrogate endpoints of coronary heart disease. These results conflict with the observations made in the HERS and WHI trials, which report little benefit of HRT on the incidence of coronary heart disease. Reconciliation of these apparent differences may lie in closer evaluations of the differences between these monkey studies and clinical trials.

What is Modeled in Monkeys?

To reconcile seemingly opposing effects of ERT or HRT on the prevention of coronary heart disease between monkey models and women, it is important first to define terms (Grodstein et al. 2003). Most animal studies begin treatment...
(HRT or ERT) in the absence of pre-existing atherosclerosis (Figure 1). Successful treatment (a positive effect of HRT) in this circumstance relates to inhibition of the earliest stages of atherosclerosis (Figure 1, upper panel). In contrast, postmenopausal women enrolled in a clinical study would, at the very least, start with some degree of atherosclerosis in their coronary arteries and, depending on age, could have well-established atherosclerotic plaques. Successful treatment in such circumstances is measured by the degree to which these more advanced plaques are prevented from rupturing and causing a clinical event (Figure 1, lower panel). Therefore, initiation of treatment begins at two very distinctly different stages of atherosclerosis when one compares monkey and human studies. The challenge then is to interpret more accurately the results of studies (in both animal models and women) that initiate HRT or ERT at these different stages of atherosclerosis to determine whether atherosclerosis extent is an important determinant of the success of treatment.

**Initiation of ERT with No Pre-existing Atherosclerosis**

In two separate studies, Clarkson and colleagues (1998) and Adams and coworkers (1997) initiated ERT (CEE given in the diet at the monkey equivalent of 0.625 mg/day) immediately after ovariectomy in monkeys with no pre-existing atherosclerosis. In both of these studies (Figure 2), CEE inhibited the very early progression of atherosclerosis by about 70% compared with untreated controls. These sorts of studies are the kind most commonly done in any animal species. It is important to note that ERT is rarely initiated in women with no pre-existing atherosclerosis.

**Initiation of CEE with Pre-existing Atherosclerosis**

In a more recent study, Clarkson and colleagues (2001) initiated CEE treatment immediately after ovariectomy in monkeys with pre-existing atherosclerosis. CEE inhibited further development of coronary artery atherosclerosis, but only by about 50% (compared with untreated controls, Figure 3). This treatment regimen would be equivalent to treating a postmenopausal woman with average amounts of pre-existing coronary artery atherosclerosis immediately after the onset of menopause. This population is still different from the study populations used in the HERS, ERA (Estrogen/Progestin Replacement and Atherosclerosis Trial; Herrington et al. 2000), and WHI trials, which did not receive HRT until several years after the onset of menopause.

**Additional Studies**

Two monkey studies closely model the situation in the HERS, ERA, and WHI trials. In Williams and colleagues’ (1995) study, monkeys were ovariectomized, but not started...
on ERT for another 2 yr. If 1 monkey year is roughly equivalent to 3 human years, then it would have been similar to starting ERT in women that had been menopausal for 6 yr. In this monkey study, CEE was ineffective in stopping further progression of coronary artery atherosclerosis (Figure 4). In another study (Anthony and Clarkson 2002), premenopausal monkeys were fed an atherogenic diet for 26 mo to develop a moderate amount of atherosclerosis and were then made surgically menopausal. At the same time, a segment of the common iliac artery was removed for measurement of baseline atherosclerosis extent. They were then given hormone treatment for 36 months. At the end of the treatment period, animals were divided into tertiles based on initial atherosclerosis extent, and the effect of estrogen treatment on atherosclerosis progression was evaluated. Estrogen treatment was most atheroprotective in the animals with small baseline lesions and was ineffective in those with larger baseline lesions.

These findings are not inconsistent with results of human trials, which show little beneficial cardiovascular effect of HRT or ERT in women with significant pre-existing atherosclerosis. Importantly, there is precedence that ERT inhibits progression of atherosclerosis in women with minimal to moderate amounts of atherosclerosis (Hodis et al. 2003). In that study, women were purposely chosen to have small baseline lesions and then were given estrogen and progression atherosclerosis measured by ultrasound. In an earlier, contrasting study, Angerer and colleagues (2001) administered HRT to women with more advanced baseline atherosclerosis. In that study, HRT was not effective in slowing progression of subclinical atherosclerosis. Results of these two studies (Angerer et al. 2001; Hodis et al. 2003) are similar to those of the monkey studies and may indicate that hormone treatment more effectively inhibits atherosclerosis in early stages.

Thus, it is possible that the apparent discrepancy between monkey studies and human trials may lie in the degree of the initial lesion in the coronary arteries. In Figure 5, one possible scenario is presented, by which estrogen has beneficial effects on the coronary artery at early stages of atherosclerosis (left side of Figure 5), but adverse effects on the coronary artery at later stages of atherosclerosis (right side of Figure 5). The hypothesis bears exploration because it is indeed a critical clinical issue if one is to identify a population of women (if one exists) that might derive beneficial cardiovascular effects of ERT/HRT. The potential for ERT/HRT to have beneficial effects early in the atherogenic process has been discussed. The potential for ERT/HRT to have harmful effects late in the atherogenic process is discussed in the text that follows.

Can We Make Animal Models More Predictive?

Animal models are, of course, not women, and results of animal studies should be interpreted as such. In the text below, several differences between the human and nonhuman primate model of postmenopausal women are described. As noted above, results of monkey studies have
been predictive (when properly interpreted) of studies done in women. However, improved modeling may improve interpretation.

Plasma Hormone Concentrations

Naturally postmenopausal women still produce significant amounts of androgens and varying amounts of estradiol. Surgically postmenopausal women and ovariectomized animals produce very little androgen or estradiol. Therefore, one approach to make animals more predictive of postmenopausal women might be to have the ovariectomized animals receive a certain amount of androgen and estradiol.

Pre-existing Atherosclerosis

Women become menopausal between 40 and 50 yr of age. Thus, most recently postmenopausal women would have been exposed to varying amounts of cardiovascular risk factors and have (on average) fatty streaks to small atherosclerotic plaques in their coronary arteries by this age. As described above, the amount of pre-existing atherosclerosis may be an important determinant of the cardioprotective effects of ERT/HRT. Furthermore, women who have been postmenopausal for several years are likely to have fairly advanced coronary artery lesions. Arteries (in both human and nonhuman primates) remodel in the outward direction as atherosclerosis progresses (Clarkson et al. 1994). This atherosclerosis may not be apparent as measured by angiography, but may be advanced, nonetheless.

For these reasons, it may be important to create the animal model of interest depending on the amount of pre-existing atherosclerosis. It is also important to keep in mind the age of menopause being modeled when interpreting the data.

Cardiovascular Endpoint Considerations

Traditionally, the endpoints measured in animal studies include plasma cholesterol concentrations, coronary artery reactivity, and atherosclerosis extent. Although these endpoints have been associated with cardiovascular risk, there is current interest in atherosclerosis as an inflammatory disease. The role of inflammation in plaque stability/instability is an emerging area of research (Ross 1999), and data related to the effects of ERT/HRT on measures of inflammation are now available.

Researchers have examined plasma markers of inflammation (e.g., C-reactive protein [CRP]) and plasma markers of plaque disruption (e.g., matrix metalloproteinase-9 [MMP-9]). Zanger and colleagues (2000) report that HRT (Prempro®, Wyeth, Radner, PA) increases plasma concentrations of MMP-9 in postmenopausal women (average, 66 yr of age). Similarly, treatment with CEE (Premarin®, Wyeth) increases plasma concentrations of CRP (Koh et al. 2001) in postmenopausal women of similar age. In contrast, potent cardioprotective drugs such as statins reduce plasma concentrations of CRP and MMP-9 (Koh et al. 2001) and reduce plaque measurements of inflammatory products such as interleukin -6, ICAM-1, and MMP-9 (Sukhova et al. 2002). The effects of statins on these markers are independent of their effects on plasma lipids and atherosclerosis, which emphasizes the importance of adding inflammatory markers as endpoints to HRT studies. Although it appears that HRT/ERT increases plasma markers of inflammation in postmenopausal women with advanced coronary artery disease, the effects of ERT/HRT on inflammation at the earliest stages of atherosclerosis have not been explored.

Progestogen Effects on Cardiovascular Risk

The most common HRT prescribed in the United States is CEE + MPA. It has been speculated that MPA may diminish the beneficial effects of CEE on cardiovascular risk (Adams et al. 1997; Williams et al. 1997), which would likely depend on the endpoint and animal model. Results of the ERA trial (Herrington et al. 2000) clearly show that MPA added to CEE does not differ from unopposed CEE on angiographically measured atherosclerosis. It is not possible...
to reach conclusions about MPA from the HERS trial because it comprised only two treatment arms (placebo vs. CEE + MPA). Similarly, the WHI trial contrasted CEE + MPA to placebo, but also had a CEE-only treatment group for the women who did not have a uterus. The CEE + MPA group of the WHI was stopped, and the CEE-only arm was allowed to continue. Although the final report of the CEE group had not been published when this article went to press, the report should provide insight into the potential, harmful cardiovascular effects of unopposed estrogens on cardiovascular risk.

Results of animal studies vary regarding progestogen effects on cardiovascular risks. Adams and colleagues (1997) clearly documented that MPA diminished the athero-inhibitory effects of CEE in ovariectomized monkeys. This result is in contrast to results of Clarkson and colleagues (2001), who report that MPA does not diminish the effects of CEE on progression of atherosclerosis. Yet our laboratory had documented earlier (Williams et al. 1997) that MPA consistently diminishes the effects of CEE on endothelium-mediated dilation of atherosclerotic coronary arteries of ovariectomized monkeys. Differences in methodologies (e.g., dose, administration schedule) among studies might explain such opposing findings about the cardiovascular effects of MPA.

Nevertheless, it is important not to lose sight of the most important issue for researchers, clinicians, and women, in light of the findings from HERS, ERA, and WHI. This issue focuses on defining the effects of ERT/HRT on the cardiovascular health of women at various ages and with different amounts of cardiovascular risk. We know that CEE + MPA has no beneficial effects on the risk of myocardial infarction in women who are, on average, 63 yr of age (HERS, WHI). Whether this knowledge applies to women who are younger, or women with smaller baseline amounts of atherosclerosis, is not known and bears additional attention.

### Different HRT Regimens

Not all HRT regimens are the same. Less commonly prescribed HRTs such as ethinyl estradiol (EE) or norethindrone acetate (NETA) contain different estrogens and progestins than CEE + MPA. As Suparto and colleagues (2003) have reported, some cardiovascular differences may exist between CEE + MPA and EE + NETA. Results of the PEPI trial support the concept that not all HRTs have similar effects on cardiovascular risk factors. Additionally, results of studies using nomogestrol acetate and estradiol (Paris et al. 2000) have different effects on vascular reactivity in monkeys than the effects of CEE + MPA (Williams et al. 1995, 1997).

Much attention is now being given to alternatives to traditional HRT. These alternatives include the selective estrogen receptor modulator class of drugs, nutritional approaches such as soy, and non-sex steroid compounds such as tibolone. However, the looming issue remains—to define the effects of ERT/HRT regimens on different populations of postmenopausal women and to determine whether HRT/ERT has any beneficial cardiovascular effects on any of these populations.

### Conclusions

Animal models remain a potentially important tool for examining the effects of ERT/HRT on cardiovascular risk. However, as the recent results of human trials have underlined, it is critical to interpret the results of animal studies correctly. Most studies, particularly those using nonhuman primates, model the potential of ERT/HRT to improve/worsen the risk of coronary heart disease individuals having the equivalent of early atherosclerotic plaques, as might characterize peri- and recently postmenopausal women. This population of women would be, on average, 45 to 55 yr of age. To model the women in HERS, ERA, or WHI, either the studies must be longer in duration (which would impose significant logistical impediments), or other animal models must be considered (e.g., Rosenfeld and colleagues [2002] recently published a study using transgenic mice as models of early and advanced atherosclerosis). Regardless, more questions remain unanswered than have been answered. Women will continue to request ERT/HRT products. Studies are needed to obtain comprehensive knowledge about the effect of ERT/HRT regimens on cardiovascular risk.

### References


