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17- β Estradiol reduces atherosclerosis without exacerbating lupus in ovariectomized systemic lupus erythematosus-susceptible LDLr^{-/-} mice

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Abstract

Objective—To test the hypothesis that estrogen treatment in a radiation chimera mouse model of systemic lupus erythematosus (SLE) and atherosclerosis will increase SLE-associated atherosclerosis by increasing autoantibody production and inflammation.

Methods—We used a radiation chimera mouse model in which bone marrow from the polygenic B6.*Sle1.2.3* model of SLE was transferred to the low density lipoprotein receptor knock out (LDLr^{-/-}) model of atherosclerosis on a C57BL/6 background (Sle/LDLr^{-/-}). Ovariectomized chimeric mice were treated for 10 weeks with either 5.6 ug/day of 17 β -estradiol or placebo; outcomes included atherosclerosis plaque size, anti-dsDNA autoantibody production and renal pathology.

Results—Mean atherosclerosis plaque size was 67.4 $\pm$ 7.6% smaller in the estrogen treated group (p<0.0001). Estrogen treated Sle/LDLr^{-/-} mice had no significant difference in serum cholesterol concentration, lipoprotein distribution, anti-dsDNA autoantibody concentration, antibody isotype concentration and renal histopathology score compared to placebo. However, they had significantly lower mean urine protein to urine creatinine ratio (UP:UC). There was no correlation between atherosclerosis lesion size and either the renal histology score or UP:UC ratio in Sle/LDLr^{-/-} mice.

Conclusion—These results indicate that 17 β -estradiol is atheroprotective within the context of murine SLE independent of changes in serum cholesterol concentration, autoantibody concentration, or renal pathology. The SLE phenotype in Sle/LDLr^{-/-} mice is not exacerbated by exogenous 17 β -estradiol administration, and the reduced UP:UC ratio suggests a protective effect against lupus nephritis.

Keywords

Systemic lupus erythematosus; coronary artery disease; atherosclerosis; estrogen; autoimmune; nephritis

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Conflicts of Interest

The authors declare there is no conflict of interest.

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1. Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the production of pathogenic antinuclear autoantibodies [1]. These autoantibodies form immune complexes in various organs such as the skin, joints, and kidneys and elicit a chronic inflammatory reaction and eventual organ damage. Estrogen plays an important role in SLE development and activity. Disease incidence is 9 times greater in women than men [1], and in the NZB/NZW F₁ and MRL/lpr mouse models of SLE, estrogen treatment is a potent accelerator of disease activity resulting in increased autoantibody production, increased renal disease, and reduced lifespan [2, 3]. The use of estrogen or estrogen-containing drugs also puts women at increased risk for the development of SLE [4], and treatment of SLE patients with hormone replacement therapy significantly increases the rate of mild to moderate flares compared to placebo [5]. The SLE disease promoting effects of estrogen are thought to be due to its effects on B lymphocytes. Estrogen treatment in mice increases the number of splenic plasma cells [6], increases IgM and IgG production and promotes the rescue of autoreactive B cells [7]. Consistent with these findings, in vitro estrogen treatment of peripheral blood mononuclear cells and B cells from patients with SLE significantly increases the production of anti-dsDNA IgG [8]. Thus, within the context of SLE, estrogen is considered to be pro-inflammatory due to an increased production of pathogenic autoantibodies.

One of the most important causes of morbidity and mortality in patients with SLE is coronary artery disease (CAD). Women with SLE are 5–10 times more likely to develop CAD than women without SLE even after controlling for traditional CAD risk factors [9–12]. The onset of CAD in women with SLE is considered to be “premature” or “accelerated” because the most dramatic increases in relative risk are observed in young women of reproductive age. For example, Manzi et al. reported that 34 to 44 year-old women with SLE seen at the University of Pittsburgh Medical Center were over 50 times more likely to have a MI (rate ratio 52.43, 95% CI 21.6 to 98.5) compared to age-matched women in the Framingham Offspring Study [9]. The increased risk for CAD in young women with SLE is particularly striking because ovarian production of estrogen in women without SLE has been shown to provide atheroprotection via anti-inflammatory effects and improvements in the plasma lipid profile. Thus, the accelerated time course of cardiovascular events in women with SLE suggests that not only are the atheroprotective effects of ovarian estrogen lost, but that estrogen may be pro-atherogenic due to increased autoantibody production and inflammation.

Despite such a striking increase in CAD risk, the mechanisms of accelerated lupus-associated atherosclerosis (ALAA) are poorly understood. Currently, ALAA is thought to be related to the underlying immune dysregulation in SLE. The overarching hypothesis is that persistent SLE-induced activation of proinflammatory pathways increases inflammation in developing atheromas resulting in increased development of vulnerable plaques. This hypothesis has been bolstered by the finding that a variety of mouse models of lupus and atherosclerosis develop increased atherosclerotic lesion size compared to the non-autoimmune atherosclerosis-prone controls [13–16]. In addition to increased plaque size, these models also have increased numbers of plaque T cells and TUNEL-positive cells without an increase in plasma cholesterol concentration suggesting that inflammation is driving the increase in plaque size. Given that immune complexes are the primary source of inflammation in SLE and that estrogen has been shown to increase SLE disease activity through increased autoantibody production, we hypothesized that estrogen treatment would increase SLE-associated atherosclerosis by increasing autoantibody production and inflammation in a mouse model of SLE and atherosclerosis.

To address this hypothesis, we used a previously described radiation chimera mouse model in which bone marrow from SLE-susceptible B6.*Sle1.2.3* mice is transplanted into irradiated C57BL/6 mice that lack a functional low density lipoprotein receptor (LDLR^{-/-}) [14, 17]. The B6.*Sle1.2.3* strain is congenic for three lupus susceptibility loci (*Sle1*, *Sle2*, *Sle3*) derived from the NZM2410 mouse strain on a C57BL/6 background, and LDLR^{-/-} mice develop hyperlipidemia and atherosclerosis [18]. To determine whether estrogen exacerbates atherosclerosis in SLE, we measured aortic root atherosclerosis lesion area and plaque inflammation in ovariectomized mice after 10 weeks of treatment with either 17 β -estradiol or placebo. To determine the effect of estrogen on SLE in this model, we measured anti-dsDNA autoantibody concentrations, antibody isotype concentrations, renal histology scores, and urine protein to urine creatinine ratios (UP:UC).

2. Methods

2.1 Production of radiation chimeras

Female LDLR^{-/-} mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 5 weeks of age. One week after arrival, 900 rads of total body irradiation was administered in one dose using a cobalt gamma source followed by transplantation of either C57BL/6 (B6/LDLR^{-/-}) or B6.*Sle1.2.3* (Sle/LDLR^{-/-}) bone marrow cells as previously described [14, 17]. All mice were housed in ventilated racks and handled in accord with the Guide for the Care and Use of Laboratory Animals and with the approval of the Wake Forest University Institutional Animal Care and Use Committee.

2.2 Estradiol treatment and necropsy

Six weeks post-bone marrow transplant, the B6/LDLR^{-/-} and Sle/LDLR^{-/-} mice were ovariectomized and implanted subcutaneously with either a 90-day continuous release 5.6 μ g/day 17 β -estradiol (E) pellet (0.5 mg total dose) or a placebo (P) pellet (Innovative Research of America, Sarasota, FL) (Fig. 1A). This 2 $\times$ 2 experimental design resulted in the following groups: Group 1 = B6/LDLR^{-/-} + P (n=16), Group 2 = B6/LDLR^{-/-} + E (n=15), Group 3 = Sle/LDLR^{-/-} + P (n=18), and Group 4 = Sle/LDLR^{-/-} + E (n=34). Due to the higher mortality rate in Sle/LDLR^{-/-} mice [17], more of these mice were produced to ensure statistical power for our endpoints. The day after surgery, the diet was changed from Purina Prolab®RMH 3000 to a casein- and lactalbumin-based atherogenic diet prepared by the Wake Forest University Diet Laboratory [4.45% total fat (w/w) and 0.001% cholesterol (w/w)]. This diet was formulated to achieve moderate (~500 mg/dL) plasma cholesterol concentrations on the LDLR^{-/-} background. Saturated, monounsaturated, and polyunsaturated fat composed 48.9%, 36.1%, and 15.0% of total fat in the diet, respectively. At the end of the 10-week treatment period the mice were anesthetized, blood was collected via cardiac puncture, and the arterial system was flushed with normal saline at 100 mmHg for 10 minutes. In the absence of reliable serum estradiol assays for mice [19], estradiol bioavailability was confirmed by uterine weight as previously described [20].

2.3 Artery histomorphometry

Serial 5 μ m sections of frozen Tissue-Tek® O.C.T.-embedded aortic root were mounted on glass slides. Section 1 was defined as the most proximal section in which all three mitral valve leaflets were present. Sections 8–12 were stained with Verhoeff van Gieson (VVG) to clearly delineate the internal elastic lamina, and atherosclerotic plaque size was measured using computer-assisted morphometric analysis (Image ProPlus v5.1, Media Cybernetics, Bethesda, MD). For each animal, plaque area was calculated as the mean of 5 sections measured.

2.4 Artery immunohistochemistry

Sections 14–15 were incubated with rat anti-mouse CD68 (1:75; AbDSerotec, Raleigh, NC) and sections 16–17 with Armenian hamster anti-mouse CD3- ϵ (1:100; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) overnight at 4°C. Lymph node and spleen were used as control tissue and the negative control tissues were incubated with either non-immune rat serum (BioGenex, Fremont, CA) or purified Armenian hamster IgG isotype control instead of primary antibody. Primary antibodies were localized with appropriate biotinylated secondary antibodies, streptavidin-alkaline phosphatase (BioGenex, Fremont, CA) and Vector® Red (Vector Laboratories, Burlingame, CA) substrate. Levamisole was used to block endogenous alkaline phosphatase. Sections were counterstained with Mayer's hematoxylin and examined by light microscopy. CD68 and CD3 staining were quantified using Image ProPlus v5.1 software (Media Cybernetics, Bethesda, MD). For each slide, a digital tiled image was created at 100X magnification and a 10² μ m grid overlay applied as previously described [21]. Each crosshatch of the grid was evaluated for positive or negative staining by an observer blinded to treatment group and genotype. Immunohistochemical positivity was expressed as the mean percentage of positive crosshatches within the entire atherosclerotic lesion area (tunica intima).

2.5 ELISAs

Serum concentrations of anti-dsDNA IgG antibodies, total IgG, IgM, IgG1, and IgG2a were determined by ELISA following the manufacturers' instructions (Alpha Diagnostic International, San Antonio, TX).

2.6 Kidney and spleen analysis

Formalin-fixed paraffin-embedded 5 μ m sections of kidney were stained with hematoxylin and eosin (H&E) and evaluated in a randomized, blinded fashion by a board-certified veterinary pathologist (JAC) using a histologic scoring system modified from the morphologic index for the evaluation of lupus renal biopsies developed by Hill et al [22]. Briefly, kidneys were scored on a 0–3 scale for glomerular activity (GA), including membranoproliferation, polymorphonuclear cell infiltration, karyorrhexis and hypertrophy of Bowman's epithelium; tubulointerstitial activity (TA), including tubular epithelial necrosis and regeneration, intraluminal cells, and interstitial inflammation; and chronicity (C), including glomerular scar formation and interstitial fibrosis. Results are expressed as a total histology score which was calculated as (GA/4 + TA/4 + C/2). Urine microalbumin was measured using an immunoturbidimetric assay (Kamiya Biomedical Company, Seattle, WA) and creatinine was measured by a colorimetric assay (AlfaWassermann, West Caldwell, NJ) following the manufacturer's instructions. The urine microalbumin and creatinine concentrations were used to calculate the UP:UC. The spleen was harvested, weighed, immersion fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with H&E for histopathologic evaluation.

2.7 Serum lipoproteins

Serum total cholesterol and triglyceride concentrations were determined using an enzymatic assay (Roche Diagnostics, Indianapolis, IN). To determine the lipoprotein cholesterol distribution, serum was fractionated into very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) fractions using a single Superose 6 (1 $\times$ 30cm) high performance column (GE Healthcare, Piscataway, NJ) and on-line mixing with an enzymatic total cholesterol reagent (Pointe Scientific, Inc., Canton, MI) [23]. The amount of cholesterol in each lipoprotein fraction was calculated as a percent of the total serum cholesterol concentration.

2.8 Statistical analysis

Significant differences were detected in normally distributed data using two-way ANOVA. A p-value of < 0.05 indicated significant differences. For data with an interaction effect, post hoc Student's t-tests with a Bonferroni correction for four comparisons were used to explore the relationship between the interacting variables. For data that could not be normalized, the Kruskal-Wallis test was used with a Mann-Whitney post-hoc analysis. Correlations were determined using Pearson's r correlation.

3. Results

3.1 Body weight and uterine weight

Ovariectomy is associated with relative body weight gain [20], and as expected, the placebo treated mice weighed significantly more than the estrogen treated mice after 10 weeks of treatment (Table 1). Estrogen administration also resulted in significantly higher uterine to body weight ratios in both B6/LDLr^{-/-} and Sle/LDLr^{-/-} mice as compared to placebo (Table 1).

3.2 Development of SLE disease

The SLE-like disease phenotype in mouse models of SLE is well established and consists of anti-nuclear autoantibody production, lymphoproliferation resulting in splenomegaly, and renal disease consistent with lupus nephritis resulting in protein losing nephropathy. We used the development of SLE-like disease as evidence that the bone marrow transplants were successful. We also quantified the degree of chimerism in a randomly selected group of mice using RT-PCR and found that approximately 85% of the blood cells were of donor origin six weeks after transplant (Supplemental Fig. 1). The Sle/LDLr^{-/-} mice had significantly higher renal histopathology scores when compared to B6/LDLr^{-/-} mice (Fig. 1B). Histopathologic changes included membranoproliferative glomerulonephritis and hypertrophy of Bowman's epithelium (Fig. 1C); and chronic interstitial nephritis and fibrosis (not shown). Anti-dsDNA antibody concentrations were also significantly higher in the Sle/LDLr^{-/-} mice compared to B6/LDLr^{-/-} mice (Fig. 1D). However, neither anti-dsDNA autoantibody concentrations nor renal histology scores differed by estrogen treatment within the Sle/LDLr^{-/-} or B6/LDLr^{-/-} groups. There was an interaction effect for the UP:UC ratio in which the placebo treated Sle/LDLr^{-/-} mice had significantly higher UP:UC ratio than both the estrogen treated Sle/LDLr^{-/-} mice and the placebo treated B6/LDLr^{-/-} mice (Fig. 1E). The Sle/LDLr^{-/-} mice also had splenomegaly compared to B6/LDLr^{-/-} mice irrespective of estrogen treatment (Fig. 1F and G).

3.3 Atherosclerosis

In the Sle/LDLr^{-/-} mice, 10 weeks of estrogen treatment resulted in a mean plaque size that was $67.4 \pm 7.6\%$ smaller compared to placebo ($p < 0.0001$; Fig. 2A and B). In B6/LDLr^{-/-} mice, estrogen treatment reduced plaque size by $82.5 \pm 5.7\%$ ($p < 0.0001$). We next investigated whether the atherosclerotic lesions of Sle/LDLr^{-/-} mice were more inflamed compared to B6/LDLr^{-/-} mice. However, we found no significant difference in the density of macrophages (CD68+ cells: B6/LDLr^{-/-} + P: $94.9 \pm 1.1\%$; B6/LDLr^{-/-} + E: $93.0 \pm 2.3\%$; Sle/LDLr^{-/-} + P: $96.1 \pm 1.0\%$; Sle/LDLr^{-/-} + E: $92.5 \pm 1.7\%$; $p = 0.33$, $n = 6-13$ per group) or T cells (CD3+ cells: B6/LDLr^{-/-} + P: $0.40 \pm 0.13\%$; B6/LDLr^{-/-} + E: $0.09 \pm 0.09\%$; Sle/LDLr^{-/-} + P: $1.48 \pm 0.56\%$; Sle/LDLr^{-/-} + E: $0.88 \pm 0.31\%$; $p = 0.03$, $n = 5-12$ per group) by immunohistochemistry. We also determined that there was no correlation between the renal histology score ($p = 0.288$, $n = 8-11$ per group) or the UP:UC ratio (Supplemental Fig. 2) with the atherosclerosis lesion area.

3.4 Serum lipids

We found that while the estrogen treated B6/LDLr^{-/-} mice had lower total serum cholesterol concentrations when compared to placebo treated B6/LDLr^{-/-} mice, there was no difference in cholesterol concentration between estrogen and placebo treated Sle/LDLr^{-/-} mice (Table 1). In the B6/LDLr^{-/-} mice, estrogen treatment significantly decreased VLDL and LDL cholesterol concentrations (Fig. 3A). However, in the Sle/LDLr^{-/-} group this change did not occur. Interestingly, although the placebo treated groups had no significant difference in atherosclerosis lesion size, there was a significant decrease in the LDL and VLDL cholesterol concentration in the Sle/LDLr^{-/-} placebo group compared to B6/LDLr^{-/-} placebo group. The Pearson's r correlation was calculated to further explore the relationship between hypercholesterolemia and atherosclerosis lesion size. There was a significant correlation between plaque size and VLDL + LDL cholesterol concentrations among the B6/LDLr^{-/-} mice, but no correlation among the Sle/LDLr^{-/-} mice (Fig. 3B and C).

3.5 Antibody isotype production

The Sle/LDLr^{-/-} mice had significantly increased serum total IgG, IgM, IgG1 and IgG2a antibody isotype concentrations versus B6/LDLr^{-/-} mice ($p < 0.005$; Table 2); however, no significant difference in the IgG1/IgG2a ratio was detected by genotype (Table 2).

4. Discussion

Considering the important role estrogen plays in the pathogenesis of atherosclerosis in women, the apparent loss of “female protection” against CAD in young women with SLE prompted us to question the role estrogen plays in ALAA. We hypothesized that estrogen contributes to the pathogenesis of ALAA by increasing autoantibody production and inflammation. Although the mechanisms of ALAA are not well understood, it is thought that the pro-inflammatory state of SLE patients contributes to inflammation in the atherosclerotic lesion [24]. Thus, an estrogen-driven increase in autoantibody production and inflammation could accelerate atherosclerosis development. We found that ovariectomized Sle/LDLr^{-/-} mice receiving 10 weeks of estrogen treatment developed SLE-like disease, but had no difference in autoantibody production, splenomegaly, renal histology score, and 67% less atherosclerosis than placebo treated Sle/LDLr^{-/-} mice. The estrogen-mediated decrease in atherosclerosis lesion size was similar to that found in estrogen treated non-autoimmune B6/LDLr^{-/-} mice.

These results suggest that estrogen is atheroprotective in the presence of the SLE phenotype (autoantibody production, splenomegaly, and lupus nephritis); however, given that the extent of atherosclerosis did not differ between the SLE and non-SLE groups, it appears that the atheroprotective effect was mediated via general atherogenic mechanisms rather than lupus-specific accelerated atherogenesis mechanisms. In contrast to our findings in the placebo groups, previous studies using similar mouse models have found increased atherosclerosis in Sle/LDLr^{-/-} mice compared to B6/LDLr^{-/-} mice 24 weeks post-BMT [14, 17]; thus, it is possible that the post-BMT period in our model (16 weeks) was not of sufficient length to allow discernible differences in plaque size and development. Indeed, several studies of SLE patients have found that disease duration is an important risk factor for ALAA [9, 11, 25]. The lack of a difference in lesion size between placebo treated Sle/LDLr^{-/-} mice compared to B6/LDLr^{-/-} mice in our study may also be related to the finding that the fold change in plaque size in Sle/LDLr^{-/-} mice is not very dramatic under hyperlipidemic conditions. Braun et al found a 1.3-fold increase in lesion area and a mean TPC concentration of 767.1 ± 73.3 mg/dL after eight weeks of feeding an atherogenic diet (24 weeks post-BMT) [17]. In a parallel study in which the mice were fed a chow diet, they found a 2-fold increase and a TPC concentration of 239.4 ± 10.7 mg/dL. The mean TPC

concentration at the end of our study was moderately elevated at 492 ± 92 mg/dL which may have masked some of the athero-accelerative effects of the SLE phenotype. Thus, it will be important to determine in future studies how increased atherosclerosis develops in this mouse model over time and whether ALAA is a function of disease duration or other pathologic changes that occur during SLE pathogenesis.

Although we found no difference in atherosclerosis plaque size between B6/LDLr^{-/-} and Sle/LDLr^{-/-} groups, our finding of decreased serum cholesterol concentration in the placebo treated Sle/LDLr^{-/-} mice compared to placebo treated B6/LDLr^{-/-} mice is in agreement with earlier findings [14, 17]. While the mechanism for this decrease is unknown, the lack of correlation between atherosclerosis lesion size and serum cholesterol concentration in Sle/LDLr^{-/-} mice is important because it indicates that other mechanisms, such as inflammation, are driving atherosclerosis development in SLE. In contrast to Sle/LDLr^{-/-} mice, lesion size in the B6/LDLr^{-/-} mice was significantly correlated with serum cholesterol concentrations as expected for the well understood hyperlipidemia-driven mechanism of atherosclerosis lesion development in LDLr^{-/-} mice [26].

Our findings suggest that the mechanisms of estrogen atheroprotection in Sle/LDLr^{-/-} mice differ from the mechanisms found in non-autoimmune mice and people. In people and animals without autoimmune disease, estrogen reduces the development of atherosclerosis via improvements in the lipoprotein profile and anti-inflammatory effects on arterial cells [27]. Consistent with this, the estrogen treated B6/LDLr^{-/-} mice had decreased total serum cholesterol concentration compared to placebo. In contrast, we found that estrogen treated Sle/LDLr^{-/-} mice had no significant difference in total serum cholesterol, triglyceride, LDL, VLDL, or HDL cholesterol concentrations, despite having significantly decreased atherosclerosis lesion areas compared to placebo. This suggests that estrogen's anti-inflammatory effects on arterial cells, not improvement in the lipoprotein concentration and distribution, are a possible mechanism of atheroprotection in Sle/LDLr^{-/-} mice. Importantly, the arterial endothelial and smooth muscle cells are of LDLr^{-/-} origin. Thus, they would be expected to be responsive to the anti-inflammatory effects of exogenous estrogen and result in decreased migration of monocytes into the subendothelial space [28, 29]. Further studies are needed to determine whether estrogen treatment would be atheroprotective in mice with endothelium and smooth muscle cells of B6.*Sle1.2.3* origin.

One of the goals of our study was to investigate the potential SLE accelerating effects of estrogen treatment in Sle/LDLr^{-/-} mice. Previous studies in other mouse models of SLE such as the NZB $\times$ NZW F1 and MRL/lpr models have consistently shown that estrogen treatment increases autoantibody production and lupus nephritis, and can even promote the development of autoimmunity in otherwise non-autoimmune mice [2, 3, 7]. Thus, our finding that Sle/LDLr^{-/-} mice do not exhibit an increase in classic murine SLE disease parameters with estrogen treatment is unusual. This may be due to the genetic makeup of the B6.*Sle1.2.3* strain which has relatively little natural sex disparity in disease development [18]. It may also be that 10 weeks of estrogen treatment did not allow enough time for differences in SLE disease development to be detected. Interestingly, we did find that despite similar renal histology scores, estrogen treated Sle/LDLr^{-/-} mice were protected from the development of protein losing nephropathy compared to placebo. These results are consistent with an estrogen-mediated protective effect on the kidneys of Sle/LDLr^{-/-} mice. Estrogen is known to protect against a variety of chronic kidney diseases in both people and animals through a number of mechanisms including inhibition of TGF- β and improved hemodynamics [30]. Consistent with this, male SLE patients have more severe renal disease compared to females [31].

These findings fill an important gap in knowledge regarding the effect of estrogen on atherosclerosis development in the presence of active SLE. Prospective, blinded, clinical trials investigating the effect of sex hormones on cardiovascular endpoints in SLE patients are lacking due to ethical constraints, and observational studies of SLE patients frequently have limited power for analysis of cardiovascular endpoints. Indeed, some studies have found male sex to be a risk factor for CAD in patients with SLE [32] while others have not [33, 34]. However, there is evidence that postmenopausal status is a risk factor for carotid atherosclerotic plaque in women with SLE suggesting that as ovarian function ceases, the atheroprotective effects of estrogen are lost [11, 35]. Taken together with our findings, these data support the alternate hypothesis that estrogen protects premenopausal women with SLE from accelerated atherosclerosis. This finding is of particular importance to women with SLE as they are at risk for premature ovarian failure due to the therapeutic use of alkylating agents [36, 37]. It also appears that women with SLE undergo menopause approximately 5 years earlier than women in the general population [38, 39]. Thus, early menopause itself may play a significant role in the pathogenesis of ALAA.

In summary, we show that exogenous estrogen reduces both atherosclerosis and protein losing nephropathy in a mouse model of lupus and atherosclerosis. Further studies are needed to determine whether preserving ovarian function in women with SLE reduces the risk of accelerated atherosclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- We report of the effects of estrogen on a mouse model of lupus and atherosclerosis
- Estrogen treatment was atheroprotective compared to placebo
- Estrogen also protected the lupus-prone mice from protein losing nephropathy
- Estrogen did not increase auto-antibody production compared to placebo

Fig.1A

Fig. 1B

Fig. 1C

Fig. 1D

Fig. 1E

Fig. 1F

Fig. 1G

Fig 1.

Study design and development of SLE disease. A) Six week old female mice were irradiated and received bone marrow transplants of either wild-type C57BL/6 or B6.*Sle.1.2.3* bone marrow. Six weeks post-transplant (day zero) the mice were ovariectomized and randomized into estrogen (5.6 μ g 17 β -estradiol/day pellet) or placebo (5.6 μ g placebo/day pellet) treatment groups and transferred to an atherogenic diet (0.001% cholesterol and 4.45% fat) for 10 weeks. B) Sle/LDLr^{-/-} mice have increased renal disease compared to B6/LDLr^{-/-} mice (n = 10–15 mice/group). Estrogen treatment did not affect the renal histopathology score when compared to placebo. C) Representative H&E sections of renal glomeruli (63X for all 4 frames). Glomeruli of the Sle/LDLr^{-/-} mice are enlarged and hypercellular with thickened capillary basement membranes and hypertrophic Bowman's epithelium. D) Serum anti-dsDNA antibody concentrations are increased in Sle/LDLr^{-/-} mice compared to B6/LDLr^{-/-} mice. Estrogen treatment did not alter anti-dsDNA antibody concentration when compared to placebo. E) Placebo treated Sle/LDLr^{-/-} mice have an increased UP:UC ratio compared to placebo treated B6/LDLr^{-/-} mice and estrogen treated Sle/LDLr^{-/-} mice. F) Sle/LDLr^{-/-} mice have splenomegaly when compared to B6/LDLr^{-/-} mice (n = 10–15 mice/group). Estrogen treatment did not affect spleen weight when compared to placebo. G) Representative images of post-treatment spleens (mm). Data expressed as mean $\pm$ SEM; * p < 0.05.

A.

Fig. 2B

Fig 2.

Post-treatment (10wk) aortic root atherosclerosis lesion area. A) Estrogen treatment significantly decreased the size of atherosclerotic lesions in the aortic root of both B6/LDLr^{-/-} and Sle/LDLr^{-/-} mice (n = 7–14 mice/group). B) Representative VVG sections of

aortic roots (10x). Expansion of the intimal area is apparent in the placebo treated groups. Data expressed as mean $\pm$ SEM; * $p < 0.0001$.

Fig. 3A

Fig. 3B

Fig. 3C

Fig 3.

Cholesterol distribution among serum lipoproteins and correlation with atherosclerosis lesion area. A) Placebo treated Sle/LDLr^{-/-} mice had decreased serum VLDL-cholesterol (VLDL-C) and LDL-C concentration compared to placebo treated B6/LDLr^{-/-} mice. Estrogen treatment decreased VLDL-C and LDL-C in B6/LDLr^{-/-} mice, but not in Sle/LDLr^{-/-} mice ($p < 0.0007$ for a vs. b within the respective lipoprotein category; $n = 9-11$ mice/group). There was no difference in HDL-C. After 10 weeks of treatment, there is a significant correlation between serum VLDL-C + LDL-C concentration with atherosclerosis lesion area in B6/LDLr^{-/-} mice (B), but not in Sle/LDLr^{-/-} mice (C). Data expressed as the mean $\pm$ SEM.

Table 1
Body weight, uterine to body weight ratio, and serum cholesterol and triglyceride concentrations

Experimental Group	Treatment	n	Week 0		Week 10		
			Body Weight (g)	Body Weight (g)	Uterine to Body Weight Ratio (ratio $\times 10^{-3}$)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
B6/LDLr ^{-/-}	Placebo	12	18.39 $\pm$ 0.39	21.94 $\pm$ 0.60	0.68 $\pm$ 0.06	768 $\pm$ 154 [*]	274 $\pm$ 76
B6/LDLr ^{-/-}	Estrogen	9	18.59 $\pm$ 0.46	18.33 $\pm$ 0.80 [*]	3.08 $\pm$ 0.46 [*]	414 $\pm$ 78	161 $\pm$ 92
Sle/LDLr ^{-/-}	Placebo	10	19.55 $\pm$ 0.43	20.48 $\pm$ 0.44	0.69 $\pm$ 0.11	492 $\pm$ 92	203 $\pm$ 63
Sle/LDLr ^{-/-}	Estrogen	11	18.67 $\pm$ 0.47	19.08 $\pm$ 0.40 [*]	4.21 $\pm$ 0.48 [*]	428 $\pm$ 90	169 $\pm$ 57

Results are expressed as mean ± SEM

^{*} p < 0.0001

Table 2

Serum antibody isotype titers

Experimental Group	Treatment	Total IgG ($\pm$ SEM)	IgM ($\pm$ SEM)	IgG ₁ ($\pm$ SEM)	IgG _{2a} ($\pm$ SEM)	IgG ₁ /IgG _{2a} ($\pm$ SEM)
B6/LDLr ^{-/-}	Placebo	0.53 (0.06)	0.85 (0.10)	0.74 (0.15)	0.03 (0.00)	26.71 (7.54)
B6/LDLr ^{-/-}	Estrogen	0.62 (0.06)	0.67 (0.11)	0.91 (0.07)	0.03 (0.00)	29.51 (3.94)
Sle/LDLr ^{-/-}	Placebo	1.00 (0.12) [*]	1.30 (0.2) [*]	1.86 (0.16) [*]	1.06 (0.43) [*]	27.08 (6.96)
Sle/LDLr ^{-/-}	Estrogen	0.79 (0.04) [*]	1.19 (0.13) [*]	1.56 (0.18) [*]	1.46 (0.39) [*]	19.72 (7.16)

Results are expressed as mean OD450 $\pm$ SEM^{*} p < 0.005