17β-estradiol, gender independently, reduces atheroma development but not neointimal proliferation after balloon injury in the rabbit aorta

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Abstract

The aim of the present study was to investigate anti-proliferative and anti-atherogenic properties of 17β-estradiol in balloon injured female and male rabbit aortae. Thirty-two female and 32 male New Zealand White rabbits where gonadectomised. Vascular injury was performed with a balloon catheter in the lower abdominal aorta. Male and female rabbits were randomised into four groups of eight animals each. Only two of four groups received a 0.5% cholesterol-enriched diet. One cholesterol-diet group and one normal-diet group received intramuscular injections of estradiol valerate (1 mg/kg body weight/week). After 28 days, the denuded part of the abdominal aorta was excised and analysed by morphometry and immunohistochemistry. Estrogen treatment did not show an inhibitory effect on neointimal proliferation in normo-cholesterolemic male or female rabbits. A gender independent inhibitory effect of 17β-estradiol was seen on atheroma development in cholesterol-fed female and male rabbits, while plasma total cholesterol levels were significantly reduced in male rabbits only. The 17β-estradiol treatment was associated with a significantly decreased number of luminal endothelial cells in normo- and hyper-cholesterolemic female rabbits, as evaluated by immunohistochemical staining for 'von Willebrand factor'. Staining for Ki-67-positive proliferating cells after 28 days showed a statistically significant increased proliferative activity in the neointima of hyper-cholesterolemic female rabbits. The neointimal content of macrophages increased significantly in all hyper-cholesterolemic rabbits. Under 17β-estradiol treatment, the number of macrophages was increased in female and decreased in male rabbits by tendency. Additionally, the 'classical' vascular estrogen receptor was present in both female and male rabbit aortae without statistically significant differences. In conclusion, 17β-estradiol did not reduce post-injury neointima formation in normo-cholesterolemic rabbits. However, in hyper-cholesterolemic rabbits, 17β-estradiol reduced atheroma development gender independently. This effect cannot be explained by lowering of plasma cholesterol levels or endothelium-mediated pathways, and requires further investigation on, for example, antioxidative, antiproliferative or estrogen receptor mediated effects. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: 17β-Estradiol; Vascular injury; Atherosclerosis; Endothelium; Estrogen receptor

1. Introduction

Cardiovascular diseases are still the main cause of deaths in western societies. Epidemiological, not randomised, cohort studies have shown that postmenopausal estrogen replacement therapy (ERT) is associated with up to 50% reduced risk of cardiac death, especially for women with present cardiovascular risk factors or pre-existing coronary artery disease [1,2]. In contrast, a first randomised, placebo-controlled and double-blinded clinical trial with postmenopausal women suffering from coronary artery disease did not support these findings: there was no cardiovascular benefit after 4.1 years of combined treatment with conjugated equine gestrogens and...
medroxyprogesterone acetate, although plasma cholesterol levels were altered beneficially [3].

The question how estrogen protects women from coronary artery disease came up when Ludden et al. demonstrated a sex-specific reduced lipid accumulation in female rabbit aortae under estrogen treatment [4]. Subsequently, a number of estrogen effects have been described in the cardiovascular system. These are lowering of serum cholesterol [5] or a reduced cholesterol uptake into the arterial wall [6], acute actions on endothelial function [7] and vasomotion [8–11], and calcium antagonistic [12,13], antiproliferative [14,15] and antioxidative [16,17] properties. Gender-specific antiatherogenic properties of 17β-estradiol have been found in rabbits [18]. In addition, Collins et al. demonstrated that estrogen’s acute vasodilating properties were exclusive for women as well [11]. These sex-specific effects have therefore been supposed to be mediated directly by vascular estrogen receptors that have been detected in the vessel walls of animals and humans [19–21]. Losordo et al. investigated an inverse correlation of the estrogen receptor content in vascular smooth muscle cells with atherosclerosis development in postmenopausal women [22].

However, the idea of estrogen’s sex-specific atheroprotection has been restricted by experimental findings of Bourassa et al., who demonstrated an anti-atherogenic effect of 17β-estradiol in the aortas of female and male apolipoprotein E-deficient mice [23]. In the same animal model, Elhage et al., showed that a gender-specific effect was only dose dependent because, in this regard, female mice were twice as sensitive to 17β-estradiol treatment as male mice [24].

In cell and organ culture experiments, several authors were able to demonstrate inhibitory properties of 17β-estradiol on vascular smooth muscle cell proliferation [14,25–28]. On the other hand, Farhat et al. observed enhanced proliferation in vascular smooth muscle cells from rat pulmonary artery and in endothelium denuded rabbit pulmonary artery segments under 17β-estradiol treatment [29].

Animal experiments were designed to investigate whether 17β-estradiol’s presumed antiproliferative properties could be of clinical relevance, i.e. by reducing post-injury neointima development that is in part a proliferative response [30–32]. Indeed, several authors were able to demonstrate a reduction of post-injury neointima formation by estrogen treatment in carotid arteries from rats [15,33–36] and mice [37,38] or in aortas from rabbits [39]. Iafrati et al. [38] performed their experiments in estrogen receptor-α-deficient mice, suggesting that 17β-estradiol’s demonstrated antiproliferative effects were mediated by the very recently detected estrogen receptor-β [40].

In contrast, investigations in the iliac arteries of primates with pre-existing atherosclerosis could not support the idea of estrogen’s antiproliferative or antiatherogenic effects after vascular injury [41]. These experimental findings conform with investigations in postmenopausal women receiving coronary angioplasty: ERT did not result in a reduction of restenosis but had, however, a beneficial influence on survival and further cardiac events [42,43]. Only after atherectomy was the restenosis rate significantly decreased under ERT [42].

The cholesterol-fed rabbit has been a widely accepted model for atherosclerosis research since it was first introduced by Anitschkow [44,45]. Baumgartner and Studer established endothelial balloon denudation of the abdominal aorta in normo- and hypercholesterolemic rabbits as a model for vascular injury [46]. Because rats are resistant to dietary-induced hypercholesterolemia and atherogenesis, the rabbit model was used for our present investigations [44,45].

The purpose of this present in vivo study was to investigate anti-atherogenic and anti-proliferative properties of 17β-estradiol in balloon injured male and female rabbit abdominal aortae, and whether these effects are gender specific. The experiment was terminated after 28 days because it has been shown previously that post-injury proliferation in the rabbit carotid artery was terminated after this time [46,47].

2. Material and methods

2.1. Animals and study protocol (Table 1)

Thirty-two female and 32 male New Zealand White rabbits (Charles River Inc., Kisslegg, Germany), 12 weeks of age and 2.15–2.75 kg body weight, were randomised into four groups of eight animals each, and housed under constant conditions regarding temperature (22°C) and a 12 h day and night cycle. After 2 weeks of acclimatisation, the rabbits were gonadectomised under general anesthesia (Ketanest/Xylazinehydrochloride). Endothelial denudation and transmural injury of the abdominal aorta was performed by a 3 F Fogarty balloon catheter (Baxter Inc., Unterschleisheim, Germany) via the right femoral artery during the same session. The uninflated balloon was pushed forward into the abdominal aorta approximately as far as the renal arteries, inflated with 0.3 ml of 0.9% sodium chloride, and pulled down to the bifurcation of the iliac arteries three times. The muscular abdominal aorta was the target vessel for injury according to the model of Baumgartner and Studer [46]. The area of injury could be controlled quite easily under these circumstances and did not require X-ray control as would be necessary for distinct injury procedures in the thoracic aorta. A 0.5% cholesterol enriched diet (Altromin Inc., Lage, Germany) was started on the day of denudation in two female and two male groups. Intramuscular hormone
Table 1
Parameters for the eight animal groupsa

<table>
<thead>
<tr>
<th></th>
<th>Female rabbits ($n = 32$)</th>
<th>Male rabbits ($n = 32$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal diet ($n = 8$)</td>
<td>Normal diet, 17β-estradiol ($n = 8$)</td>
</tr>
<tr>
<td>64 New Zealand White rabbits (endothelial denudation)</td>
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</tbody>
</table>

* Data presented as the median (1. Quartile / 3. Quartile).
treatment with 1 mg/kg body weight/week estradiol valerate (Progynon Depot-10; Schering Inc., Berlin, Germany) was started 1 week prior to endothelial denudation in two female and two male groups, one with normal and one with cholesterol-enriched diet.

2.2. Blood samples and determination of plasma parameters

Blood samples from each rabbit were drawn from the lateral ear vein and collected into tubes containing ethylenediamine tetraacetic acid before surgical or hormonal treatment and at the end of the experiment 28 days after vascular injury. The total plasma cholesterol concentration was measured by a standard enzymatic method (Boehringer Inc.) after centrifugation of the samples at 3000 rpm at 4°C for 15 min. The 17β-estradiol plasma levels were measured by radioimmunoassay with standard commercially available kits (Biermann and Diagnostic Products Corp.).

2.3. Fixation, immunohistochemistry and quantification

The rabbits were sacrificed with CO2 28 days after vascular injury. After opening the abdomen with a scalpel, connective tissue and fat were removed carefully with tweezers and swaps until the abdominal aorta was visible. Endothelium denuded areas from hypercholesterolemic animals could be distinguished macroscopically from those of normo-cholesterolemic rabbits by a light yellow atheroma formation. The abdominal aortae were excised and cut into four sections. Then they were fixed in 4% formaline, embedded in paraffin, and serially sectioned (4 µm slices) until the maximal thickness of the neointima was reached. As an indirect morphological marker for endothelial cells, immunohistochemical staining of cross-sections was performed using a specific monoclonal antibody against the human factor-VIII related antigen, the ‘von Willebrand factor (vWF)’ (Atlantic Antibodies — INCSTAR, Stillwater, MN). Using the avidin biotin peroxidase method, vWF was visualised with 3-amino-9-ethylcarbazole (Sigma, Deisenhofen, Germany). Finally, the sections were counterstained with hemalaune. Sections from human coronary arteries served as controls. For quantification, cells expressing the vWF and vWF-negative cells of the luminal layer of the aortic segments were counted out. The percentage of vWF-positive luminal cells was calculated by relating these cells to the number of all luminal cells. Luminal cells were considered to be vWF-positive only when a homogenous pattern of stained granula in the cytosol was present, as confirmed by microscopic observation (Fig. 1). Elastica van Gieson’s staining was performed for the morphometrical evaluation of the neointimal area (software package from Bilaney Consulting Inc., Düsseldorf, Germany). The neointima was defined as the area between luminal site and lamina elastica interna. Hemalaune and eosin staining was performed for neointimal cell count. Immunohistochemical staining with a monoclonal antibody against α-actin (Sigma-Aldrich, Deisenhofen, Germany) by the avidin biotin peroxidase method identified vascular smooth muscle cells and myofibroblasts. Staining of macrophages was performed with the monoclonal antibody RAM 11 (Dako, Denmark) by the APAAP-method (alkaline phosphatase and mouse monoclonal anti-alkaline phosphatase). For the detection of proliferating cells, the monoclonal antibody MIB 1 against Ki-67 (Dianova, Hamburg, Germany) was used, and staining performed with the avidin biotin peroxidase method. For staining of the ‘classical’ nuclear estrogen receptor (estrogen receptor-α), paraffin sections were incubated with the monoclonal estrogen receptor antibody Ab-1 (mouse IgG; Calbiochem, Bad Soden, Germany) and with the rhodamine (TRITC)-conjugated AffiniPure goat anti-mouse antibody (IgG + IgM; Dianova, Hamburg, Germany) for immunofluorescence staining. As a control, immunofluorescence staining of all cellular nuclei was performed by the DAPI method (4′,6-Diamidino-2-phenylindol 2HCl; Bioproducts, Boehringer Ingelheim, Germany).

2.4. Statistical evaluation

The results of all groups are expressed as medians (1. Quartile/3. Quartile). The U-test (Mann–Whitney–Wilcoxon) was used to determine statistical significance at a level of \( P < 0.05 \). The Spearman test was performed to evaluate correlations between parameters. The percentage of vWF-positive luminal cells was calculated by relating these cells to the number of all luminal cells. The number of estrogen receptor positive cells in the neointima was expressed by a semi-quantitative...
Table 2: Study design: the different study groups at the experiment’s start

<table>
<thead>
<tr>
<th></th>
<th>Plasma cholesterol, 4 weeks (mg/dl)</th>
<th>Plasma estradiol, 4 weeks (pg/ml)</th>
<th>Neointimal cells (absolute)</th>
<th>Macrophages (%) in neointimal cells</th>
<th>Ki-67-positive cells (%) in neointimal cells</th>
<th>ER-positive cells (%) in neointimal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, normal diet</td>
<td>82 (72/91)</td>
<td>&lt;20</td>
<td>1646 (1545/1711)</td>
<td>0 (0/3.19)</td>
<td>0.69 (0.45/1.335)</td>
<td>50 (50/50)</td>
</tr>
<tr>
<td>Female, cholesterol</td>
<td>1938a (1665/2318)</td>
<td>23.1 (22.7/23.3)</td>
<td>2910a (2411/3232)</td>
<td>18.41a (8.82/23.78)</td>
<td>2.73a (2.27/3.87)</td>
<td>50 (40/50)</td>
</tr>
<tr>
<td>Female, normal diet + estrogen</td>
<td>70 (56/74)</td>
<td>111.7 (68.8/189.3)</td>
<td>1909 (1749/2185)</td>
<td>0 (0/0)</td>
<td>0.36 (0.26/0.44)</td>
<td>50 (40/57.5)</td>
</tr>
<tr>
<td>Female, cholesterol + estrogen</td>
<td>1500a (1250/1685)</td>
<td>177.4 (159.1/359.7)</td>
<td>1874b (1453/2241)</td>
<td>33.56abc (17.5/42.66)</td>
<td>1.13b (0.68/1.65)</td>
<td>65 (50/80)</td>
</tr>
<tr>
<td>Male, normal diet</td>
<td>87 (86/90)</td>
<td>&lt;20</td>
<td>1684 (1426/2217)</td>
<td>0 (0/0.07)</td>
<td>0.2 (0.12/0.41)</td>
<td>30 (10/50)</td>
</tr>
<tr>
<td>Male, cholesterol</td>
<td>2352a (2099/2830)</td>
<td>&lt;20</td>
<td>3087a (2710/3446)</td>
<td>18.35a (11.27/26.38)</td>
<td>0.62 (0.16/0.94)</td>
<td>50 (50/50)</td>
</tr>
<tr>
<td>Male, normal diet + estrogen</td>
<td>59 (53/78)</td>
<td>139.9 (61.5/187.2)</td>
<td>1922 (1488/2068)</td>
<td>0 (0/0)</td>
<td>0.85 (0.75/0.92)</td>
<td>50 (10/50)</td>
</tr>
<tr>
<td>Male, cholesterol + estrogen</td>
<td>1296b (1248/1541)</td>
<td>139.4 (116.4/195.9)</td>
<td>2054b (1833/2754)</td>
<td>6.48a (5.47/13.81)</td>
<td>0.54 (0.25/1.10)</td>
<td>50 (50/50)</td>
</tr>
</tbody>
</table>

*Significantly (P<0.05) increased compared with normal diet groups.

b Significantly (P<0.05) decreased compared with the hyper-cholesterolemic group.

c Significantly (P<0.05) increased compared with the male, cholesterol + estrogen group.

score: < 10, < 50, < 80, and < 100% of neointimal cells.

3. Results

3.1. Plasma cholesterol and 17β-estradiol levels

Plasma cholesterol levels were between 25 and 119 mg/dl in all animals at the beginning of the experiment. Due to the 0.5% cholesterol-enriched diet, cholesterol levels rose significantly (more than tenfold) in all these groups over 28 days (Table 2). Under 17β-estradiol treatment, plasma cholesterol levels were significantly decreased in hyper-cholesterolemic male rabbits (P < 0.05). The decrease in cholesterol levels correlated positively (r = 0.87) with the significantly reduced (P < 0.05) neointima formation in this group (Table 2 and Fig. 3).

Basic plasma 17β-estradiol levels were below 20 pg/ml in all animals and were raised significantly under estradiol valerate treatment, without statistically significant differences between the estradiol treated groups (Table 2).

3.2. Neointimal area, neointimal cell count, and endothelial cell layer

No morphological alterations were seen in undenuded parts of the aorta, neither of normo- or hyper-cholesterolemic rabbits after 28 days. The luminal site consisted of the endothelial monolayer. In the endothelium denuded parts of the aortae of all animals, strong neointima formation was found. Using morphological criteria, the cell nuclei were homogeneously arranged with a high density in normo-cholesterolemic animals. In hyper-cholesterolemic rabbits, the neointima morphology was inhomogeneous with frequent vacuoles as typical for atheroma (Fig. 2). The neointimal area was significantly (P < 0.05) increased in hyper-cholesterolemic compared with normo-cholesterolemic animals, with no gender-specific differences (Table 2). Estradiol valerate treatment resulted in a significantly (P < 0.05) reduced neointima formation without significant differences between female and male hyper-cholesterolemic rabbits (Fig. 3). However, estradiol valerate treatment had no influence on neointima formation in normo-cholesterolemic rabbits. The neointimal cell count in all groups led to the same statistically significant findings (Table 2).

There were no statistically significant differences between all male groups when comparing the relative amount of endothelial cells in the denuded aortae (vWf-positive luminal cells in percent of all luminal cells) (Fig. 4). The relative amount of vWf-positive cells was significantly (P < 0.05) less in the denuded aortic sections of estradiol valerate treated normo- and hyper-cholesterolemic female rabbits (Fig. 4).
3.3. Macrophages, actin, proliferating cells, estrogen receptor protein

Macrophages could be detected sporadically in the neointima of normo-cholesterolemic animals, but in hyper-cholesterolemic rabbits, neointima macrophages were seen frequently. The relative number (percent of neointimal cells) of macrophages was, not significantly, increased in the neointima of hyper-cholesterolemic female rabbits under 17β-estradiol treatment (Table 2). However, in hyper-cholesterolemic male rabbits, the relative number of neointimal macrophages was, not

Fig. 2. Neointima formation in normo-cholesterolemic female (a) and male (d) rabbits, compared with hyper-cholesterolemic female (b) and male (e) rabbits, and hyper-cholesterolemic female (c) and male (f) rabbits with 17β-estradiol treatment. The cholesterol diet (0.5%) resulted in a significantly increased (P < 0.05) (atheromatous) neointima development in female and male rabbits. This neointima formation was significantly decreased without gender differences under 17β-estradiol treatment (1 mg/kg body weight/week) after 28 days. HE staining. N, Neointima; M, media. Magnification: bar = 10 μm.
statistically significant, decreased under 17β-estradiol treatment. Staining for α-actin (Fig. 5) as a marker for vascular smooth muscle cells and myofibroblasts was positive in medial and neointimal tissue of all animals. As evaluated semi-quantitatively, no statistically significant differences were seen between the eight groups while the amount of α-actin positive cells in the neointimal tissue was always between 50 and 80%. The relative number (in percent of neointimal cells) of Ki-67-positive proliferating cells was statistically significantly (P < 0.05) increased in the neointima of hyper-cholesterolemic female rabbits compared with normo-cholesterolemic female rabbits, and was statistically significantly decreased in hyper-cholesterolemic female rabbits.

Fig. 3. Neointima development (mm$^2$) in the different study groups. * Statistically significant (P < 0.05) increase compared with normo-cholesterolemic groups. ** Statistically significant (P < 0.05) decrease compared with hyper-cholesterolemic nonestrogen treated groups.
Fig. 4. Immunohistochemical staining for vWF-positive endothelial cells (which were related to the total number of luminal cells) demonstrated that in female animals, estradiol valerate treatment reduced the percentage of vWF-positive endothelial cells significantly ($P < 0.05$): * compared with normo-cholesterolemic female rabbits; ** compared with hyper-cholesterolemic female rabbits. No differences could be seen between the male rabbit groups. Values given as the median (1. Quartile: 3. Quartile).

animals under 17β-estradiol treatment (Table 2). In male rabbits, the neointimal proliferative activity did not reach statistically significant differences between the four groups. Estrogen receptor staining in the abdominal aorta was positive for female and male rabbits. Semi-quantitative evaluation of estrogen receptor positive cells in the neointima did not lead to statistically significant differences between female or male rabbits or gender differences (Table 2).

4. Discussion

The present experiment did not show an inhibitory effect of 17β-estradiol on neointima development in the balloon injured abdominal aortas of normo-cholesterolemic female or male rabbits. These findings are in contrast to experiments of several authors who were able to demonstrate a significantly reduced neointima formation under estrogen treatment in carotid arteries from rats [15,33–36] and mice [37,38] or in aortas from rabbits [39]. Our contrast findings in the rabbit model may essentially be explained by the differences of species or the different target vessel. However, Foegh et al. could support the idea of estrogen’s anti-proliferative vascular effects in an experiment with injured rabbit aortas (running over 22–24 days) [39]. According to the results presented here, Geary et al. did not find any effect on neointimal area or indexes of arterial remodeling in balloon injured iliac arteries from primates after 28 days of estrogen treatment [41]. Together with Geary et al., our data suggest that, beside differences in species or target vessels, time points of measurements may be most important and sometimes misleading because ‘estrogen treatment may simply delay the proliferative response while having no lasting effect on clinically relevant end points’ [41]. Focusing on the clinical dimension of such experiments, we have to note that postmenopausal women under ERT did not have significantly reduced restenosis rates after coronary angioplasty [42,43].

By the way, in our present experiment, Ki-67 staining for proliferating cells [48] did not show statistically significant differences in the neointima of any normo-cholesterolemic group after 28 days. However, our data are limited since no 7- or 14-day groups were included to show possible differences in the proliferative response early after vessel wall injury.

Fig. 5. Combined staining of α-actin and macrophages in a hyper-cholesterolemic female rabbit abdominal aorta. N, Neointima; M, media. Short arrow, vascular smooth muscle cell (dark brown); long arrow, macrophages (red). Magnification: bar = 10 μm.
In contrast to our own previous findings in hypercholesterolemic rabbits (experiments over 84 days) [18], the additionally performed vascular injury resulted in a gender independent anti-atherogenic estrogen effect after 28 days of treatment. In female rabbits, this anti-atherogenic effect was independent from alterations to plasma cholesterol levels, while male rabbits had significantly decreased plasma cholesterol levels under estradiol treatment that may contribute to the anti-atherogenic effect.

An intact endothelium has been suggested to be essential to protect the vessel wall from thrombocyte [49] or monocyte [50] adhesion, inflammatory responses [51] and cholesterol invasion [52]. The endothelial cell layer is directly involved into acute vasomotor function. 17β-Estradiol mediates the endothelial production of nitric oxide [7] or prostacyclin [53]. Moreover, 17β-estradiol treatment has been found to accelerate functional endothelial recovery after balloon injury of rat carotid arteries [34,36]. One possible mechanism was demonstrated in cell culture experiments recently: 17β-estradiol reduced hypoxia induced programmed cell death (apoptosis) in endothelial cells [54].

In this present experiment, immunohistochemical staining was performed for vWf as an indirectly morphological marker of endothelial cells, and a significantly decreased relative amount of vWf-positive luminal cells (related to the absolute number of luminal cells) found in found normo- and hyper-cholesterolemic female rabbits under 17β-estradiol treatment. So far, our data does not support the idea of 17β-estradiol’s beneficial effect on the (morphological) endothelial recovery. Once again, the cited authors made their observations in rat carotid arteries after 2 weeks of estradiol treatment, thus limiting their finding to one distinct section of the vessel tree of one distinct species at an early state after vascular injury. However, we were able to demonstrate the variability of the endothelial layer morphology within the arterial vessel tree of one identical rabbit recently [55]. In both hyper-cholesterolemic and normo-cholesterolomic rabbits, the highest (morphological) endothelial integrity was found in the lower abdominal aorta and the carotid arteries, while the integrity was mostly disturbed in the aortic arch, i.e. by shear stress. This observation corresponds with findings of Garbarsch et al. on a typical distribution of spontaneous atherosclerotic lesions in the arterial vessel tree of rabbits [56]. However, in this present experiment, the percentage of vWf-positive stained luminal cells was between 51.6 and 60.3 (median) in hypercholesterolemic male and female rabbits, suggesting that the endothelial cell layer may only play a minor role in 17β-estradiol’s demonstrated anti-atherogenic effect.

Other mechanisms, i.e. antioxidative [16,17] or calcium antagonistic [12,13] properties of 17β-estradiol have to be taken into consideration. They may possibly interact with neointimal kinetics or macrophages removing cholesterol from the vessel wall [57,58]. While endothelium mediated estradiol effects on atherogenesis [18,59] and vasomotion [11] have been found to be sex specific for females only, antioxidative and calcium antagonistic properties could explain gender independent findings.

In the present experiment, 17β-estradiol treatment of hypercholesterolemic rabbits was associated with a relative but not statistically significant increase of neointimal macrophages in female animals. However, in male rabbits, the neointimal amount of macrophages was relatively but not statistically significantly decreased. So far, this finding cannot explain 17β-estradiol’s gender-independent anti-atherogenic effect.

Whether estrogen receptor mediated pathways are involved in the demonstrated anti-atherogenic effects requires further investigation. The ‘classical’ estrogen receptor protein was detected in female and male rabbit aortic tissue with no statistically significant differences in this experiment. Two subtypes of estrogen receptors (ER-α, which is identical with the ‘classical’ estrogen receptor, and ER-β) have been identified recently [40,60]. Experiments with ER-α-deficient mice suggested that 17β-estradiol’s anti-proliferative effects after vascular injury could be mediated by ER-β [38]. Interestingly, vascular injury in male [61] and female [62] rats resulted in a significantly increased distribution of the ER-β in vascular smooth muscle and endothelial cells. Whether this may contribute to 17β-estradiol’s anti-atherogenic effect after vascular injury needs to be clarified.

In conclusion, our in vivo data does not support the idea of 17β-estradiol’s anti-proliferative properties after vascular injury and cannot demonstrate a relevant reduction of post-injury neointima formation after 28 days of treatment. Concerning the present experimental setting, the anti-atherogenic properties of 17β-estradiol were seen gender independently in female and male rabbits after vascular injury, suggesting that an intact endothelium is not required for the mediation of these estrogenic effects.

References


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