Intramuscular administration of estrogen may promote angiogenesis and perfusion in a rabbit model of chronic limb ischemia

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Abstract

Objective: Promoting angiogenesis may be an effective treatment for patients with diffuse peripheral vascular disease. This study investigated whether estrogen can promote angiogenesis and perfusion in a rabbit model of chronic limb ischemia. Methods and results: Ischemia was induced in one hindlimb of 24 oophorectomized New Zealand White rabbits. Ten days later (day 0), they were randomized into 4 groups for intramuscular treatment in the ischemic limb: controls receiving saline at day 0; Estrogen-1 group receiving estradiol valerate, modified release (EVMR), 1 mg/kg at day 0; Estrogen-2 group receiving EVMR 1 mg/kg at days 0 and 15; and Estrogen-3 group receiving EVMR 2 mg/kg at day 0. Revascularization was evaluated by clinical indexes, such as ischemic/normal limb systolic blood pressure (BPR), and capillary density/muscle fiber in the abductor muscle of the ischemic limb at the time of death (day 30). At day 30 the BPR was increased in all groups (0.39 ± 0.08 in the controls, 0.52 ± 0.11 in the Estrogen-1 group, 0.65 ± 0.13 in the Estrogen-2 group and 0.61 ± 0.16 in the Estrogen-3 group, \( F = 2.39, P = 0.04 \)). The capillary/muscle fiber at day 30 was 0.87 ± 0.09, 1.08 ± 0.15, 1.01 ± 0.14 and 1.10 ± 0.9 (\( F = 5.01, P = 0.01 \)), respectively, in the 4 groups. The capillary/muscle fiber was related to BPR (\( r = 0.48, P < 0.02 \)) and to 17β estradiol plasma levels of day 15 (\( r = 0.58, P = 0.003 \)) and of day 30 (\( r = 0.46, P < 0.02 \)). Conclusion: Administration of estrogen promotes angiogenesis and perfusion in ischemic rabbit hindlimbs. Thus, estrogen may represent a new therapeutic modality in the management of arterial insufficiency. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Circulatory physiology; Angiogenesis; Microcirculation; Collateral circulation; Hormones

1. Introduction

Despite the major advances in medical, surgical and percutaneous revascularization techniques, limb salvage and relief of ischemic pain cannot be achieved in many patients with diffuse peripheral vascular disease [1]. In such patients, promoting angiogenesis may be an effective treatment [2].

Angiogenic activity is generally low in the adult organism, except for the normal cyclical changes that occur in the female reproductive tract [3]. It is likely that neovascularization in reproductive tissues is under the control of steroid hormones, particularly 17β estradiol [4]. Several manifestations of vascular disease, such as lupus erythematosus and Takayasu’s arteritis, suggest that endothelial cell activity and formation of new vessels may be regulated by sex hormones. Both these diseases are associated with endothelial cell proliferation. A role for gonadal steroids in modulating endothelial cell behavior is supported by studies of blood vessel formation in the primate endometrium [5]. Gonadal steroids directly affect blood vessels by increasing the secretion of endothelium-derived relaxing factor (nitric oxide) [6].

Morales et al. [7] demonstrated in vitro and in vivo that estradiol enhances human umbilical vein endothelial cell activities (non-endometrial endothelial cells). These cells...
play an important role in neovascularization, suggesting a promoting influence of estrogens on angiogenesis. It has also been demonstrated recently that estrogen stimulates delayed mitogen-activated protein kinase activity in human endothelial cells via an autocrine loop that involves basic fibroblast growth factor [8]. Estradiol also enhances endothelial cell attachment, proliferation, migration, and differentiation in vitro and markedly increases the angiogenic effect of basic fibroblast growth factor in mice in vivo [7].

The aim of the present study was to test the hypothesis that exogenous estrogen can promote angiogenesis and perfusion in a rabbit model of chronic limb ischemia.

2. Methods

2.1. Animal model

The angiogenic response to intramuscular administration of estradiol in vascular insufficiency was investigated using a rabbit ischemic hindlimb model [9,10] which was designed to simulate ischemia characteristic of patients with lower extremity arterial occlusive disease. The Athens Animal-care Committee approved (no. 5007) all protocols. The care of the animals complied with the guidelines of the European Community and of the department of Experimental Surgery of Athens Medical School. The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (Publication No. 85–23, revised 1996).

A total of 24 oophorectomized female New Zealand White rabbits (weighing 3.6 to 4.2 kg, mean weight 3.9 kg, aged 7–9 months) were anesthetized with Ketamine (30 mg/kg) and occasionally sodium pentothal (25 mg/kg iv) was administered as needed. A longitudinal incision was then performed in the right thigh, extending inferiorly from the inguinal ligament. Through this incision, using surgical loops, the femoral artery was dissected free along its entire length, as were major branches of the femoral artery including the inferior epigastrial, deep femoral, lateral circumflex and superficial epigastric arteries. After the popliteal and saphenous arteries were further dissected distally, the external iliac artery and all of the above arteries were ligated. Finally, the femoral artery was completely excised, from its proximal origin as a branch of the external iliac artery to the point distally where it bifurcates into the saphenous and popliteal arteries.

Each animal received sodium chloride 0.9% (50 ml/h iv) during the surgery and Cephamandol (30 mg/kg bolus iv).

2.2. Study groups

Under anesthesia the peritoneal cavity was entered via a low middle incision and bilateral ovariectomy was performed after ligation of the vascular pedicle. The ovarian samples excised were analyzed and confirmed histologically. After oophorectomy the rabbits were left for 10 days for postoperative recovery and they then underwent femoral artery dissection and were randomly assigned to one of four groups:

- Control group. The rabbits received placebo (n=7).
- Estrogen-1 group. The rabbits received estradiol valerate, modified release (EVMR) 1 mg/kg at day 0 (n=6).
- Estrogen-2 group. The rabbits received EVMR 1 mg/kg at day 0 and day 15 (n=6).
- Estrogen-3 group. The rabbits received EVMR 2 mg/kg at day 0 (n=5).

The purpose of this grouping was to examine possible dose effects of estrogen therapy.

2.3. Administration of estrogen

EVMR (Progynon Depot 10 mg) was provided by Schering AG, Germany, and was stored in a cool and dry place. An interval of 10 days between the time of the second surgery (artery dissection) and administration of estrogen or placebo was allowed for the rabbits’ postoperative recovery and the development of endogenous collateral vessels. Beyond this time point, angiograms performed up to 40 days postoperatively have demonstrated no significant spontaneous collateral vessel augmentation [11]. The surgical preparation we used produce persistent hindlimb ischemia for at least 90 days, with decreased regional distribution of blood flow in rabbit calves [12]. For this reason, saline or estrogen was administered intramuscularly in three sites in the ischemic limb, as in other studies [9], in order to have high local concentrations.

Ten days after the induction of limb ischemia (day 0), saline or estrogen, 0.4 ml, was administered intramuscularly into the ischemic limb, except for the Estrogen-3 group in which 0.8 ml was administered. Each injection was given intramuscularly at three different sites in the thigh of the ischemic limb: 50% of the injection liquid in the medial thigh, 25% in the lateral thigh and 25% in the distal thigh near the knee. All of the above-described procedures were completed without the use of heparin. Revascularization in the ischemic limb was evaluated by clinical assessment and calf blood pressure measurement, and in terms of capillary density and capillary to muscle fiber ratio as described below.

2.4. Estradiol plasma level measurements

Blood samples from each rabbit were collected in tubes containing EDTA before oophorectomy and at day 0, 15 and 30 (the end of the experiment) for 17-β estradiol plasma measurements.
Estradiol plasma levels were measured by electrochemiluminescent technology (Elecsys 2010 Immunoassay system, Hitachi, USA).

2.5. Clinical assessment

At day 0, before initiation of estrogen or normal saline treatment, and at days 15 and 30, after initiation of treatment, all rabbits were clinically evaluated for incidence of distal limb necrosis by macroscopic examination; this was considered significant if skin necrosis was observed at the foot or ankle.

2.6. Calf blood pressure ratio

Calf blood pressure was measured serially in both hindlimbs using a Doppler flowmeter with an 8 MHz transducer (Direction volume meter, DVM 1200-Hadeco, USA) immediately before treatment (day 0) as well as on days 15 and 30 after initiation of the therapy. On each occasion, with the rabbits under sedation, the hindlimbs were shaved and cleaned, the pulse of the posterior tibial artery was identified using a Doppler probe and the systolic blood pressure in both limbs was determined using standard techniques. The calf blood pressure ratio (BPR) was defined for each rabbit as the ratio of the systolic pressure of the ischemic limb to that of the normal limb.

By combining coronary wedge pressure ($P_w$) with simultaneously recorded aortic pressure ($P_a$) and central venous pressure ($P_v$) at maximum arterial vasodilation, a quantitative index of collateral flow can be calculated, at least during artery occlusion by balloon inflation [13]. This index, called fractional collateral blood flow, expresses actual collateral flow ($Q_c$) as a ratio to normal maximum myocardial perfusion ($Q_N$).

The collateral circulation can be estimated according to the formula [13]:

$$Q_c/Q_N = (P_w - P_v)/(P_a - P_v)$$

where $P_w$ is the pressure in the occluded artery, $P_v$ is the right atrial pressure and $P_a$ is the mean aortic pressure.

As $P_v$ is low, the formula may be changed to:

$$Q_c/Q_N = P_w/P_a$$

This formula has been validated in animals and in humans [14,15]. The ischemic/normal limb arterial pressure has been used as an index for the estimation of ischemic limb perfusion [16]. The lower the ratio, the more the arterial perfusion of the ischemic limb is impaired.

2.7. Capillary density and capillary to muscle fiber ratio

The effect of estradiol on collateral microvascular formation was examined by measuring the number of capillaries in tissue sections under light microscopy. Tissue sections were obtained from the abductor striated muscle, from both the ischemic and the non-ischemic limb at the time of death (day 30). This muscle was chosen for light microscopic analysis because it is a major muscle of the medial thigh and it was originally perfused by the deep femoral artery, which was ligated at the same time that the femoral artery was excised.

The tissue samples were fixed in formaline and were subsequently paraffin embedded. Sections were cut at 3 μm onto silane coated slides with muscle fibers oriented in a transverse fashion. The slides were stained with the streptavidin method, using anti-factor VIII as a primary antibody (DAKO, UK) for the detection of the endothelial cells.

For immunohistochemistry, dewaxing was performed using citroclear for 15 min before passing through graded alcohols (100% ethanol, 100% ethanol, and 70% ethanol) into water. The sections were then placed into a tris buffer saline for 5 min before the application of antigen retrieval techniques. After dewaxing, sections were placed in a glass rack and put into a 0.1 M sodium citrate buffer. The solution with slides was heated by microwave (ProLine Powerwave 800) for 2 × 4 min at 750 watts and left to cool for 25 min. The slides were washed three times in tris buffer saline before application of the primary antibody.

After application of the primary antibody, a biotinylated goat antimouse antibody (DAKO, UK) at 1:40 was overlaid. The tertiary antibody complex of streptavidin-biotin-horse radish peroxidase (DAKO, UK) was then applied, diaminobenzidine was used as a chromogen and counterstained with hematoxylin. Blocking of endogenous peroxidase in paraffin-embedded tissues was performed by incubation of the section for 20 min in 0.5% H$_2$O$_2$ in methanol. The antibodies were incubated for 30 min with three washing steps of 5 min in tris buffer saline.

Ten different fields from one muscle section were randomly selected and the number of capillaries was counted using a 20 × objective to determine the capillary density (mean number of capillaries per square millimeter) (Fig. 1). The same procedure was followed in two additional muscle sections from different paraffin blocks of the same limb. Capillaries present in five square millimeters were counted in each limb (the range was approximately 800–1000 capillaries/limb). Arterioles were not included in the study. Most of them were located at the periphery of the muscle bundle and their number was not adequate. To ensure that the capillary density was not overestimated because of muscle atrophy, or underestimated because of interstitial edema, capillaries identified at necropsy were evaluated as a fraction of the muscle fibers in the histological section. The counting scheme used to compute the ratio of capillary to muscle fiber was otherwise identical to that used to compute capillary density. The observer was blinded to treatment in performing the
Fig. 1. Histologic examination of ischemic hindlimb muscle (abductor). (a, b) section from an animal treated with estrogens, (×200, ×400 respectively). The endothelial cells of the vessels are stained brown with this method. Note that the nuclei of the muscle fibers located at the periphery do not show immunoreactivity. (c, d) sections from an animal treated with normal saline (×200, ×400 respectively). Note that the capillary density is higher in the animal treated with estrogens.

2.8. Statistical Analysis

The values were analyzed using the 'Statistica' software program, (version 5, StatSoft Inc). All data were expressed as mean value ± standard deviation. Analysis of variance with repeated measures was used for the statistical analysis, followed by Tukey's honestly significant difference test for post-hoc comparisons. Linear regression analysis using the least square difference was used in order to examine possible correlations between the different variables studied. A $P$ value of $<0.05$ was considered as statistically significant.

3. Results

3.1. Estrogen plasma levels

The 17-$\beta$ estradiol plasma levels were similar in all groups before oophorectomy and at day 0 (Table 1). In all groups 17-$\beta$ estradiol plasma levels decreased, although not statistically significantly, after oophorectomy and in the controls they did not change significantly by days 15 and 30. At day 15 these levels had increased by 130% ($P<0.05$ vs. day 0) in the Estrogen-1 group, by 90% ($P=ns$) in the Estrogen-2 group and by 242% ($P<0.05$ vs. day 0) in the Estrogen-3 group. At day 30 in the Estrogen-1 group 17-$\beta$ estradiol plasma levels had decreased to levels similar to those of day 0, in the Estrogen-3 group they remained at the same levels as day 15, whereas in the

| Table 1  |
|-----------------|--------|--------|--------|--------|--------|
| Group          | Before oophorectomy | Day 0 | Day 15 | Day 30 | $F$ value | $P$      |
| Controls (n = 7) | 18±3   | 11±4   | 15±4   | 13±6   | 10.74   | 0.000000 |
| Estrogen-1 (n = 6) | 20±2   | 10±4   | 23±7*  | 12±2   |         |         |
| Estrogen-2 (n = 6) | 18±3   | 10±4   | 19±3   | 26±12*†|         |         |
| Estrogen-3 (n = 5) | 17±3   | 11±2   | 41±4*† | 36±11*†|         |         |

* $P<0.05$ vs. day 0; † $P<0.05$ vs. Controls and Estrogen-1 group of the same day; ‡ $P<0.05$ vs. the other groups of the same day.
Estrogen-2 group they had increased by a further 37% ($P<0.05$ vs. day 0). The 17-β estradiol levels of day 30 in the Estrogen-2 and Estrogen-3 groups were higher than in the controls and the Estrogen-1 group on the same day.

3.2. Clinical assessment

Two rabbits from the controls, two from the Estrogen-1 and one from the Estrogen-3 group developed distal limb necrosis of the ischemic limb.

3.3. Calf blood pressure

The systolic blood pressure of the normal hind limb showed a tendency to increase in the Estrogen-1 and Estrogen-2 groups (Table 2). The systolic blood pressure of the ischemic hind limb at day 0, immediately before initiation of the therapy, was low and similar in all groups (Table 2). By day 30 the blood pressure increased statistically significantly ($P<0.05$) in all three treated groups of animals: in the Estrogen-1 group by 96%, in the Estrogen-2 group by 107% and in the Estrogen-3 group by 84%. The blood pressure of the ischemic hind limb at day 30 was related to the 17-β estradiol plasma levels of the same day ($r=0.43$, $P=0.03$).

At day 0, immediately before initiation of the therapy, ischemic/normal limb blood pressure demonstrated severe ischemia and was similar in all four groups (Table 2). By day 30 the calf BPR increased statistically significantly ($P<0.05$) in all three treated groups of animals: in the Estrogen-1 group by 100%, in the Estrogen-2 group by 110% and in the Estrogen-3 group by 103%.

The systolic blood pressure of the ischemic/normal limb at day 30 was related to the capillary/muscle fiber of the ischemic limb ($r=0.48$, $P=0.02$) and to the 17-β estradiol plasma levels of day 30 ($r=0.59$, $P=0.002$).

3.4. Capillary density and capillary to muscle fiber ratio

The capillary density was higher in the Estrogen-1, Estrogen-2 and Estrogen-3 groups than in the controls, but this difference was of marginal significance ($F=2.54$, $P=0.08$) (Table 3). The muscle fiber density was higher in the controls than in the treated animals, but this difference did not reach statistical significance. The capillary/muscle fiber ratio was higher in the treated animals ($F=5.01$, $P=0.01$).

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### Table 2

The effects of estrogen on ischemic, normal and ischemic/normal hindlimb systolic blood pressure

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>F value</th>
<th>P value</th>
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<tr>
<td>Ischemic limb blood pressure</td>
<td>Controls ($n=7$)</td>
<td>25±8</td>
<td>32±10</td>
<td>36±11</td>
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<td></td>
<td>Estrogen-1 ($n=6$)</td>
<td>23±6</td>
<td>40±9*</td>
<td>55±13*</td>
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<td>Estrogen-2 ($n=6$)</td>
<td>29±18</td>
<td>53±19*</td>
<td>70±16*</td>
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<td>Estrogen-3 ($n=5$)</td>
<td>32±13</td>
<td>46±6</td>
<td>59±11*</td>
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</tr>
<tr>
<td>Normal limb blood pressure</td>
<td>Controls ($n=7$)</td>
<td>89±14</td>
<td>93±15</td>
<td>91±15</td>
<td>2.89</td>
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<td></td>
<td>Estrogen-1 ($n=6$)</td>
<td>90±15</td>
<td>105±10</td>
<td>105±6</td>
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<tr>
<td></td>
<td>Estrogen-2 ($n=6$)</td>
<td>90±16</td>
<td>115±16*</td>
<td>107±13</td>
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<tr>
<td></td>
<td>Estrogen-3 ($n=5$)</td>
<td>106±11</td>
<td>93±16</td>
<td>99±12</td>
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<tr>
<td>Ischemic/normal limb blood pressure</td>
<td>Controls ($n=7$)</td>
<td>0.29±0.09</td>
<td>0.35±0.13</td>
<td>0.39±0.08</td>
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<td>Estrogen-1 ($n=6$)</td>
<td>0.26±0.08</td>
<td>0.38±0.06</td>
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<td>Estrogen-2 ($n=6$)</td>
<td>0.31±0.16</td>
<td>0.46±0.13</td>
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<td></td>
<td>Estrogen-3 ($n=5$)</td>
<td>0.30±0.12</td>
<td>0.51±0.12*</td>
<td>0.61±0.16*</td>
<td></td>
</tr>
</tbody>
</table>

*, $P<0.05$ vs. Day 0.

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### Table 3

The effects of estrogen on capillary and muscle fiber density in the ischemic hindlimb

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 30</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary density</td>
<td>Controls ($n=7$)</td>
<td>151±20</td>
<td>2.54</td>
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<td></td>
<td>Estrogen-1 ($n=6$)</td>
<td>171±25</td>
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<tr>
<td></td>
<td>Estrogen-2 ($n=6$)</td>
<td>169±21</td>
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<td></td>
<td>Estrogen-3 ($n=5$)</td>
<td>182±7</td>
<td></td>
</tr>
<tr>
<td>Muscle fiber density</td>
<td>Controls ($n=7$)</td>
<td>177±15</td>
<td>1.55</td>
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<td></td>
<td>Estrogen-1 ($n=6$)</td>
<td>159±12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estrogen-2 ($n=6$)</td>
<td>167±12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estrogen-3 ($n=5$)</td>
<td>166±15</td>
<td></td>
</tr>
<tr>
<td>Capillary/muscle fiber ratio</td>
<td>Controls ($n=7$)</td>
<td>0.87±0.09</td>
<td>5.01</td>
</tr>
<tr>
<td></td>
<td>Estrogen-1 ($n=6$)</td>
<td>1.08±0.15*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estrogen-2 ($n=6$)</td>
<td>1.01±0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estrogen-3 ($n=5$)</td>
<td>1.10±0.9*</td>
<td></td>
</tr>
</tbody>
</table>

*, $P<0.05$ vs. Controls.
Fig. 2. Correlations between coronary/muscle fiber density and the 17-β estradiol plasma levels of Day 15 (upper panel) and Day 30 (lower panel).

Observational studies suggest that estrogen replacement therapy in postmenopausal women reduces cardiovascular events, and this cannot be fully explained by estrogen’s favorable effects on plasma lipoproteins [19]. To the best of our knowledge, the current study is the first to demonstrate in vivo that estrogen administration can produce rapid improvement in angiogenesis and limb perfusion. Our in vivo results are in agreement with those of in vitro experiments [7,8].

The BPR, which is a good index of collateral circulation [15], has been used in the same model for the estimation of ischemic hindlimb perfusion by other investigators [16]. Anatomic evidence of neovascularity was investigated by necropsy examination and documented an increase in vascularity at the capillary level, consistent with the classic definition of angiogenesis formulated by Klagsbrun and Folkman [20]. We know that in the rabbit hindlimb collateral arteries increase their lumen 20-fold after experimentally induced femoral artery ligation. The restoration of blood flow cannot be achieved only by an increased

$P=0.01$), even though in the Estrogen-2 group the difference did not reach statistical significance.

The capillary/muscle fiber density of the ischemic limb was related to the 17-β estradiol plasma levels of day 15 ($r=0.58$, $P=0.003$) and to those of day 30 ($r=0.46$, $P=0.02$) (Fig. 2).

4. Discussion

Our results indicate that intramuscular administration of estrogen promotes angiogenesis and perfusion in a rabbit model of chronic limb ischemia.

The therapeutic potential of administering various angiogenic growth factors to augment revascularization of the ischemic limb, as well as the myocardium, has been demonstrated in animals [9,11,17] and in humans [2,18]. Estradiol enhances, in vitro and in vivo, endothelial cell activities important in neovascularization, suggesting a promoting influence of estrogens on angiogenesis [7].
number of capillaries, the result of stimulated angiogenesis, but rather through structural enlargement by growth of preexisting arteriolar connections into true collateral arteries, that is, arteriogenesis [21]. In our study we did not examine arteriogenesis by angiography. This is a limitation of our study, and because of that we could not distinguish precisely if the limb perfusion augmentation was the result of arteriogenesis per se, the direct effect of estrogens on the vasculature or a combination of the two. However, in the Estrogen-1 group of animals in which the 17-β estradiol blood levels at day 30 had returned to the baseline (Day 0) levels, their BPR was elevated: an indication that the increased perfusion was possibly the result of increased neovascularization and not a direct effect of estrogens on the vasculature. Also, the finding that BPR and the other clinical assessment indexes of Day 30 were related to the capillary/muscle fiber ratio is an indication that the limb perfusion and other possible beneficial effects of estrogen were related to the degree of neovascularization.

The finding of lower muscle fiber density in the hindlimbs of the animals treated with estrogen, even though not statistically significant, is possibly the result of local edema developed via the estrogen receptor activation [22]. In the treated groups estradiol levels after treatment were higher than the pre-oophorectomy, pre-treatment levels and we therefore consider them to be satisfactory. It is, however, possible that local levels are higher than the serum ones and this may be associated with the effects we observed. The capillary/muscle fiber density was related to the 17-β estradiol plasma levels of day 15 and of day 30. Systemic administration of basic fibroblast growth factor enhances collateral conductance in dogs with progressive single-vessel coronary occlusion [17]. The beneficial effect of this substance occurs primarily between the 10th and 17th days after ischemia. No additional effect of this therapy was apparent after this period. On the other hand it has been demonstrated that basic fibroblast growth factor does not induce further collateralization in dogs with mature collateral vessels [23]. The present study demonstrates that exogenous estrogen promotes angiogenesis, not only during the early period after ischemia, but also more than 15 days later. The question whether estrogen would produce greater increases in collateral vessel development if administered at an earlier time point has not been answered in this study. In a group of 5 non-oophorectomized rabbits, however, the neovascularization was the same as in the oophorectomized animals treated with estrogen, but their BPR was quite high from day 0 and stayed the same until day 30 (data not shown).

The potential therapeutic benefit of our model is suggested by the hemodynamic evidence of increased downstream perfusion pressure, documented on serial measurements of the lower limb BPR. These clinical indexes were correlated with the capillary/muscle fiber density and with the 17-β estradiol plasma levels of Day 30, but not of Day 15. Physiological levels of 17-β estradiol acutely and selectively potentiate endothelium-dependent vasodilation in both large coronary conductance arteries and coronary microvascular resistance arteries in postmenopausal women [24]. In a previous study we showed that acute intracoronary estradiol administration decreases coronary blood flow velocity during angioplasty in patients with stable angina [25], possibly by promoting collateral artery vasodilation. This could explain our finding that the Estrogen-2 and Estrogen-3 groups had higher, though not statistically significantly so, BPR measurements than the Estrogen-1 group, while having the same degree of neovascularization; it seems, therefore, that estrogen administration may not only promote angiogenesis but might also directly increase ischemic limb perfusion. The finding that capillary/muscle fiber density was correlated to the 17-β estradiol means that there is a dose-dependent relation between estradiol blood levels and neovascularization, even though no greater neovascularization occurred in the Estrogen 2 and Estrogen-3 groups compared to the Estrogen-1 group. Mechanisms for the increase in collateral growth with estrogen were not examined. This is a limitation of the study and a field for future research.

In conclusion, this study demonstrates evidence of increased angiogenesis and limb perfusion after intramuscular estradiol administration in rabbit ischemic hindlimbs. The study thus supports the hypothesis that angiogetic stimulation may represent a new therapeutic modality in the management of arterial insufficiency. Further investigation should clarify the extent to which the use of estrogen may be appropriate for treatment of patients with lower-extremity arterial occlusive disease.

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References


