Angiogenetic and Anti-Angiogenetic Effects of Estradiol and its Metabolites

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A. O. Mueck, H. Seeger, C. Lippert, D. Wallwiener

Atherosclerotic plaques in later stages exhibit marked presence of new micro vessels. Thus angiogenesis may be important for the development of atherosclerotic plaques and long-term anti-angiogenetic therapy may present an effective new anti-atherosclerotic approach. 2-Methoxyestradiol, an endogenous estradiol metabolite, has already been shown to be an effective anti-angiogenetic substance. In the present study 14 endogenous estradiol metabolites were tested on their angiogenetic and anti-angiogenetic properties and compared to the effect of their parent substance, 17β-estradiol.

Endothelial cells from human umbilical veins were used for the experiments. 17β-estradiol showed a biphasic reaction on the proliferation of vascular endothelial cells. At low concentration it stimulated and at high concentrations it inhibited cell growth. The same pattern was observed for the hydroxylated A-ring metabolites. Methylation of these metabolites, however, completely abrogated the anti-proliferative effect at high concentrations, except for the metabolite 2-hydroxyestradiol. For the D-ring metabolites no marked changes were observed.

These results indicate that in addition to 2-methoxyestradiol other endogenous estradiol metabolites are potent anti-angiogenetic substances at high dosages. Since some of these metabolites are almost devoid of any estrogenic property, they may be useful for long-term anti-angiogenetic therapy in both men and women. This should be of interest to clinical pharmacological research since it points to potential new aspects in the treatment of cardiovascular diseases. J Clin Basic Cardiol 2001; 4: 153–155.

Key words: estradiol, estradiol metabolites, angiogenesis

Material and Methods

17β-estradiol and the A-ring metabolites 2-hydroxyestrone, 2-methoxyestrone, 2-hydroxyestradiol, 2-methoxyestradiol, 2-hydroxyestrinol, 2-methoxyestrinol, 4-hydroxyestrone, 4-methoxyestrone, 4-hydroxyestradiol, 4-methoxyestradiol, and the D-ring metabolites estrone, estriol, estetrol and 16α-hydroxyestrone were purchased from Steraloids, USA. The steroids were dissolved in ethanol and tested at the concentrations 10-8, 10-7, 10-6 and 10-5 mol/L. The experiments were carried out with cells at passage 7.

Endothelial cells from human umbilical veins were purchased from Biowhittaker, Germany. The cells were cultured in MCDB 131, 10 % foetal calf serum (FCS), 5 % endothelial cell growth factor and heparin, 0.3 mg/ml glutamine, 1 % amphotericin B and 1 % penicillin/streptomycin. Before reaching confluence 900 cells per well were transferred into 96-well plates and cultured in standard medium. The cells were preincubated for 3 days. The steroids were dissolved in ethanol and added to the medium while the controls were treated with the same concentration of ethanol used in the steroid solutions ie 0.1 %. Medium and test substances were changed every 48 h.

Proliferation of the cells was measured after 7 days incubation using a crystal violet staining technique according to Kueng et al. [9], which is based on the staining of the cell nuclei. Statistical analysis was performed by ANOVA and Dunnett’s-test from duplicates of three different experiments.

Results

Table 1 shows a summary of the changes in cell numbers after treatment with the test substances, expressed in percentages of the control values. At the lowest concentration used ie 10-8 mol/L
β-Estradiol exhibit proliferative effects on the endothelial cells at high concentrations. The parent substance estradiol caused a significant increase in the proliferation by 37.6%. This proliferation stimulating effect gradually diminishes with increasing concentration, even showing a reverse action at the highest concentration i.e. 10^{-5} mol/L inhibiting proliferation by 13.2%.

A significant increase in the proliferation of the endothelial cells at 10^{-5} mol/L is also seen for the 10 A-ring metabolites tested, the catechol estrogens. Of these 4-hydroxyestrone showed the strongest effect with a growth of 90% of the original cell number measured. In the case of 2-methoxyestrone, 2-methoxyestriol and 4-methoxyestrone the numbers returned to the values of the controls, i.e. they had no effect on cell proliferation when high concentrations were administered. The lowest cell numbers measured ie strongest inhibitions were 1.6% and 0.2% for the 4-catechol estrogens 4-hydroxyestrone and 4-hydroxyestradiol.

No marked changes were seen for the D-ring metabolites. At the lowest concentration, small increases in proliferation were observed for estrone, estradiol and estetrol. At 10^{-2} mol/L, the next highest concentration, the proliferation stimulating effect only remained for estrone. Higher concentrations showed no detectable effect on proliferation. 16α-Hydroxyestrone takes up a special position in the D-ring metabolites since it causes an inhibition of proliferation compared with the control value at the higher concentrations tested i.e. from 10^{-2} to 10^{-3} M.

**Discussion**

The results show the parent substance 17β-estradiol exhibiting a biphasic reaction on the proliferation of human umbilical vein cells having a stimulating effect at low concentrations and an antiproliferative effect at high concentrations.

At low concentrations the A-ring metabolites, the catechol estrogens, respond similarly to the parent substance while substantial differences can be seen at the highest concentration i.e 10^{-5} mol/L. Methylation of the catechol estrogens appears to be of great importance regarding their biological activity, an exception being the metabolite 2-hydroxyestradiol. Methylation completely abolishes the very strong anti-

**Table 1.** Changes in cell number of endothelial cells from human umbilical veins after treatment with estradiol and its metabolites. The values are expressed in percent of cell counts compared to cell counts of the controls = 100% (means ± SD, n = 6)

<table>
<thead>
<tr>
<th>Compound</th>
<th>10^{-8} mol/L</th>
<th>10^{-7} mol/L</th>
<th>10^{-6} mol/L</th>
<th>10^{-5} mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>137.6 ± 12.7**</td>
<td>127.9 ± 11.5**</td>
<td>108.8 ± 5.6**</td>
<td>87.8 ± 6.7**</td>
</tr>
<tr>
<td>A-ring metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Hydroxyestrone</td>
<td>110.7 ± 4.2**</td>
<td>100.3 ± 3.4</td>
<td>86.5 ± 9.3**</td>
<td>8.5 ± 2.2**</td>
</tr>
<tr>
<td>2-Methoxyestrone</td>
<td>124.5 ± 9.5**</td>
<td>116.9 ± 7.0**</td>
<td>99.1 ± 8.0</td>
<td>94.6 ± 6.0</td>
</tr>
<tr>
<td>2-Hydroxyestradiol</td>
<td>124.2 ± 12.5**</td>
<td>112.5 ± 11.3*</td>
<td>72.6 ± 0.2**</td>
<td>9.0 ± 2.7**</td>
</tr>
<tr>
<td>2-Methoxyestradiol</td>
<td>116.5 ± 7.9**</td>
<td>102.2 ± 10.3</td>
<td>46.9 ± 7.0**</td>
<td>6.4 ± 4.0**</td>
</tr>
<tr>
<td>2-Hydroxyestrone</td>
<td>113.0 ± 6.8**</td>
<td>108.8 ± 6.2**</td>
<td>100.0 ± 6.3</td>
<td>83.2 ± 6.1**</td>
</tr>
<tr>
<td>2-Methoxyestriol</td>
<td>128.8 ± 7.6**</td>
<td>124.4 ± 6.9**</td>
<td>113.4 ± 10.6</td>
<td>95.4 ± 7.0</td>
</tr>
<tr>
<td>4-Hydroxyestrone</td>
<td>129.2 ± 7.4**</td>
<td>115.4 ± 9.7**</td>
<td>86.2 ± 18.1</td>
<td>0.2 ± 0.6**</td>
</tr>
<tr>
<td>4-Methoxyestrone</td>
<td>124.4 ± 10.3**</td>
<td>113.5 ± 5.2**</td>
<td>108.3 ± 13.5</td>
<td>97.5 ± 7.7</td>
</tr>
<tr>
<td>4-Hydroxyestradiol</td>
<td>130.4 ± 7.5**</td>
<td>127.3 ± 7.0**</td>
<td>103.2 ± 18.2</td>
<td>1.6 ± 2.4**</td>
</tr>
<tr>
<td>4-Methoxyestriol</td>
<td>118.2 ± 5.5**</td>
<td>114.6 ± 9.9**</td>
<td>105.2 ± 12.9</td>
<td>84.8 ± 9.3**</td>
</tr>
<tr>
<td>D-ring metabolites</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td>117.0 ± 14.8*</td>
<td>120.1 ± 14.6**</td>
<td>103.2 ± 13.4</td>
<td>102.7 ± 9.7</td>
</tr>
<tr>
<td>Estradiol</td>
<td>110.8 ± 10.0*</td>
<td>114.2 ± 4.2</td>
<td>99.6 ± 6.8</td>
<td>100.3 ± 3.0</td>
</tr>
<tr>
<td>Estriol</td>
<td>115.0 ± 17.1*</td>
<td>104.9 ± 5.6</td>
<td>97.6 ± 9.3</td>
<td>93.8 ± 7.7</td>
</tr>
<tr>
<td>16α-Hydroxyestrone</td>
<td>92.8 ± 15.3</td>
<td>77.8 ± 5.2**</td>
<td>76.7 ± 9.6**</td>
<td>66.1 ± 6.9**</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01
cardioprotective. These effects were, at least in part, more pronounced than those of their parent substance, 17β-estradiol.

The recent results of Moulton et al. [3], who found an anti-atherosclerotic effect of anti-angiogenic substances in an animal experiment, open up new avenues for anti-angiogenic compounds for the prevention of coronary artery diseases. Since 2-methoxyestradiol and other potent anti-angiogenic estradiol metabolites possess only little estrogenic activity, these compounds may be of interest for therapeutic approaches in women as well as in men. Thus estradiol metabolites at high dosages might be an exciting option for clinical use in the future.

In summary it can be concluded that estrogen metabolites as well as the parent substance estradiol, are able to exert effects on the endothelium of the vascular system in vitro. However, the effects of the metabolites on the endothelium are substantially different from estradiol, which can be seen from the observation that the catechol estrogens exhibit much stronger proliferation inhibiting effects at high concentrations. This should be of interest to clinical pharmacological research since it points to potential new aspects in the treatment of cardiovascular diseases.

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