Control of Sulfatase Activity by Nomegestrol Acetate in Normal and Cancerous Human Breast Tissues

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Abstract. Nomegestrol acetate (NOMAC), a 17α-hydroxy-nor-progesterone derivative (17α-acetoxy-6-methyl-19-nor-4,6-pregnadiene-3,20-dione, the active substance in Lutenyl®), is a potent and useful clinical synthetic progestin for the treatment of menopausal complaints and is under current development for oral contraception. Previous studies in this laboratory demonstrated that NOMAC can block sulfatase and 17β-hydroxysteroid dehydrogenase, the enzymes involved in the biosynthesis and transformation of estradiol (E2) in hormone-dependent MCF-7 and T-47D breast cancer cells. In the present study, the effect of NOMAC on sulfatase activity using total breast cancer tissue, compared to the effect in normal breast tissue, was explored. Slices of tumoral or normal breast tissues (45-65 mg) were incubated in buffer (20 mM Tris-HCl, pH 7.2) with physiological concentrations of [3H]-estrone sulfate (5x10^-9 M), alone or in the presence of nomegestrol acetate (5x10^-5 - 5x10^-7 - 5x10^-9 M), for 4 h at 37°C. Estrone sulfate (E1S), estrone (E1) and E2 were characterized by thin layer chromatography and quantified using the corresponding standard. It was observed that [3H]-E1S was only converted to [3H]-E1 and not to [3H]-E2 in normal or cancerous breast tissues, which suggests a low or no 17β-HSD activity under these experimental conditions. The sulfatase activity was more intense with breast cancer tissue than normal tissue, since the concentrations of E1 were 42.5±3.4 and 27.2±2.5 pg/mg tissue, respectively. NOMAC, at the concentration of 5x10^-5 M, inhibited this conversion by 49.2% and 40.8% in cancerous and normal breast tissues, respectively. The sulfatase inhibition at low concentration (5x10^-7 M) was 32.5% and 22.8%, respectively. It is concluded that sulfatase activity is almost twice as potent in cancerous breast tissues than in normal tissues. Nomegestrol acetate is a strong anti-sulfatase agent, in particular with cancerous breast tissues. The inhibition of estrone sulfatase activity by NOMAC in total normal or cancerous breast tissues can open attractive perspectives for future clinical trials.

There is substantial information that mammary cancer tissue contains all the enzymes responsible for the local biosynthesis of estradiol (E2) from circulating precursors. Two principal pathways are implicated in the last steps of E2 formation in breast cancer tissues: the ‘aromatase pathway’, which transforms androgens into estrogens (1, 2) and the ‘sulfatase pathway’, which converts estrone sulfate (E1S) into estrone (E1) by estrone-sulfatase (3-5). The final step of steroidogenesis is the conversion of the weak E1 to the potent, biologically active E2 by the action of a reductive 17β-hydroxysteroid dehydrogenase Type 1 activity (17β-HSD-1) (6-8).

Quantitative evaluation indicates that, in human breast tumour, E1S ‘via sulfatase’ is a much more likely precursor for E2 than is androstenedione ‘via aromatase’ (9-11).

It is also well established that steroid sulfotransferases, which convert estrogens into their sulfates, are also present in breast cancer tissues (12-14). All this information extends the concept of ‘intracrinology’, where a hormone can have its biological response in the same organ in which it is produced.

In previous studies, it was observed that in the isolated MCF-7 and T-47D breast cancer cell lines, Nomegestrol acetate (NOMAC) can block estrone sulfatase activity (15).
in ethanol (final concentration <0.3%, v/v), at a range of concentrations of 5x10^-5, 5x10^-7 and 5x10^-9 mol/l, for 4 hours at 37°C in a shaking bath. Control breast tissues received ethanol vehicle only. After centrifugation, the supernatant and pellet were separated and each fraction treated with 80% ethanol (2 ml). The pellet fraction was homogenized using an ultraturrax apparatus (Ika-Werk, Janke & Kunkel, Staufen, Germany) and sonicated for 10 seconds. The radioactivity was extracted for at least 24 hours at -20°C and [14C]-E1 was added to monitor analytical losses. The cellular radioactivity uptake was determined in the ethanolic supernatant. After evaporation of the organic phase, the extracts were redissolved in 50 µl of ethanol and the qualitative analysis and quantitative evaluation of E1-E2 were carried out after isolation by TLC on silica gel 60F 254 plates (Merck, Darmstadt, Germany) developed with chloroform-ethylacetate (4:1, v/v). Unlabelled E2S, E1 and E2 (50 µg) were used as carriers and reference indicators. After visualization of the estrogens under U.V. at 254 nm, the appropriate areas were cut off into small pieces, placed in liquid scintillation vials with ethanol (0.5 ml) and allowed to extract for 30 minutes. Three ml of Opti-fluor (Packard Division - PerkinElmer Life Sciences) were added and the vials analyzed for 3H and 14C contents with quench correction by external standardization. The quantitative evaluation of the transformation of [3H]-E1S to [3H]-E1 or [3H]-E2, corresponding to the sulfatase activity at 4 hours, was calculated as a percentage of the total radioactivity associated with the tissue slices and then expressed as pg E1 or E2 formed/mg tissue.

Results

Effect of nomegestrol acetate on the conversion of estrone sulfate to estrone in human breast tissues

a) Normal breast tissues. Many studies of the sulfatase pathway, the main route for the intracellular biosynthesis of E2, have been done previously using breast cancer cells in culture. However, breast tissue slices are an interesting new experimental in vitro model, since the integrity of the organ is in part preserved and this represents a first approach to evaluate more physio- and pathological conditions before in vivo analyses. Normal breast tissues correspond to tissue distanced from the tumor in the same patient, allowing a more comparative study to evaluate differences between normal and tumor breast tissues.

Normal breast tissues have the capacity to transform physiological concentrations (5x10^-9 mol/l) of [3H]-E1S, incubated for 4 hours at 37°C, to E1. This conversion corresponds to 44% of the initial [3H]-E1S substrate with a concentration ratio E1/E1S of 0.80. Under our conditions, no conversion to E2 was observed; thus, the measure reflects the sulfatase activity in the isolated tissues. The progestin NOMAC has a significant dose-dependent inhibitory effect.
on the sulfatase activity in normal breast tissues (Figure 2). At the concentrations of 5x10^{-5} mol/l and 5x10^{-7} mol/l, NOMAC exerted an inhibition of 40.8% and 22.8%, respectively (Table I).

Table I. Effect of NOMAC on the sulfatase activity by normal breast tissues.

<table>
<thead>
<tr>
<th>Estrone sulfate (E1S)</th>
<th>Estrone (E1)</th>
<th>Estradiol (E2)</th>
<th>R: E1/S</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>[3H]-E1S = 5 x 10^{-9}M</td>
<td>34.2±3.1</td>
<td>27.2±2.5</td>
<td>ND</td>
</tr>
<tr>
<td>+ NOMAC</td>
<td>5 x 10^{-7}M</td>
<td>35.6±2.5</td>
<td>24.8±2.4</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5 x 10^{-5}M</td>
<td>34.4±2.1</td>
<td>21.0±2.6*</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5 x 10^{-3}M</td>
<td>47.2±2.8</td>
<td>16.1±1.8*</td>
<td>ND</td>
</tr>
</tbody>
</table>

3[H]-E1S, 5 x 10^{-9} mol/l, was incubated with slices of normal breast tissues for 4 hours at 37°C in the absence (control) or presence of NOMAC in the range of 5 x 10^{-9} to 5 x 10^{-5} mol/l. The values of [H]-estrogens (E1, E2, E1S) were determined after isolation of the hormones, as indicated in Materials and Methods. The data represent the average±SEM of three independent duplicate determinations. R: ratio concentration of E1 to E1S in normal breast tissues. ND: not detectable. *p<0.05 vs E1 control value

Slices of cancer breast tissues (50-65 mg) were incubated for 4 hours at 37°C with a physiological concentration of estrone sulfate ([3H]-E1S: 5x10^{-9} mol/l) alone (control: non-treated cells) or in the presence of NOMAC at the range of concentrations 5x10^{-9} mol/l to 5x10^{-5} mol/l. Estrogens were calculated after isolation of the hormones, as indicated in Materials and Methods. The data are the mean±SEM of duplicate determinations of 3 independent experiments. *p<0.05 vs E1 control value.

Table II. Effect of NOMAC on the sulfatase activity by tumoral breast tissues.

<table>
<thead>
<tr>
<th>Estrone sulfate (E1S)</th>
<th>Estrone (E1)</th>
<th>Estradiol (E2)</th>
<th>R: E1/S</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>[3H]-E1S = 5 x 10^{-9}M</td>
<td>13.4±2.7</td>
<td>42.5±3.4</td>
<td>ND</td>
</tr>
<tr>
<td>+ NOMAC</td>
<td>5 x 10^{-7}M</td>
<td>15.1±2.4</td>
<td>36.8±3.1</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5 x 10^{-5}M</td>
<td>28.4±3.1</td>
<td>28.7±2.4*</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5 x 10^{-3}M</td>
<td>37.2±2.8</td>
<td>21.1±3.8*</td>
<td>ND</td>
</tr>
</tbody>
</table>

3[H]-E1S, 5 x 10^{-9} mol/l, was incubated with slices of tumoral breast tissues for 4 hours at 37°C in the absence (control) or presence of NOMAC in the range of 5 x 10^{-9} to 5 x 10^{-5} mol/l. The values of [H]-estrogens (E1, E2, E1S) were determined after isolation of the hormones, as indicated in Materials and Methods. The data represent the average±SEM of three independent duplicate determinations. R: ratio concentration of E1 to E1S in tumoral breast tissues. ND: not detectable. *p<0.05 vs E1 control value

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E1 / E2S of 3.18. NOMAC had a strong anti-sulfatase activity (Figure 3) and exerted an inhibition on the sulfatase activity of 50.2% and 32.5% at concentrations of 5x10^{-5} mol/l and 5x10^{-7} mol/l, respectively (Table II).

Discussion

In previous studies, it was demonstrated that NOMAC is a potent inhibitor of estrone sulfatase in the isolated MCF-7 and T-47D cell lines (15). The present study confirms and extends this blockage of sulfatase activity, using total breast tissues. The data are of interest because the effect obtained using the total tissue is closer to the physiopathological conditions.

The sulfatase activity was significantly more intense in the breast cancer tissue than in the area considered as normal (for a review see Ref. 16). The fact that NOMAC was more active as an anti-sulfatase agent in the breast cancer tissues than in the normal breast provides interesting information on the specific effect of this progestin. It is notable that, in the present data, only E1 was detected, indicating a very low 17'-hydroxysteroid dehydrogenase type I activity in the tissue used. This is of particular interest because, under the experimental conditions used, intact breast tissues can provide an interesting model to explore sulfatase activity, as only this enzyme is involved in the transformation of estrone sulfate to the unconjugated estrone.

In conclusion, NOMAC is an anti-sulfatase agent, not only in isolated breast cancer cells, but also in the intact tissue. The data can open attractive perspectives for future clinical trials with this progestin on patients with breast cancer.

References


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