Inhibition by an Alkyl-Lysophospholipid of the Uptake of Epidermal Growth Factor in Human Breast Cancer Cell Lines in Relation to Epidermal Growth Factor Internalization

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ABSTRACT

The effects of 1-O-octadecyl-2-O-methyl-sn-glycerol-3-phosphocholine (ET-18-OCH₃), an alkyl-lysophospholipid derivative, on the binding and uptake of labeled epidermal growth factor (EGF) in hormone-dependent (MCF-7 and ZR-75-1) and hormone-independent (BT-20) breast cancer cell lines were investigated at 4°C and 37°C. The total (bound and intracellular) EGF associated with breast cancer cells tested were largely temperature dependent. By pretreatment of the cells with ET-18-OCH₃ (10 μg/ml) for 12 h, the EGF uptake at 37°C was greatly reduced in both MCF-7 and ZR-75-1 (ET-18-OCH₃-susceptible) but not in BT-20 (ET-18-OCH₃-resistant) cell lines. The ET-18-OCH₃ pretreatment slightly decreased the EGF uptake at 4°C in MCF-7 and ZR-75-1 and had little effect on that in BT-20. The EGF binding at 37°C was unaffected by ET-18-OCH₃ in MCF-7 and BT-20 and slightly decreased in ZR-75-1. The EGF binding at 4°C was not changed by ET-18-OCH₃ in all cell lines tested. These results suggest that labeled EGF is taken up by the cells in a temperature-dependent manner and ET-18-OCH₃ may inhibit this internalization process only in ET-18-OCH₃-sensitive human breast cancer cell lines. It is inferred that the inhibition of the internalization process for EGF may be one of the modes of antitumoral action of ET-18-OCH₃.

INTRODUCTION

It has been known that ALPs² are more cytotoxic to certain tumor cells than to normal cells (1). Antitumoral phospholipids of this type are structurally related to a recently identified group of novel and native bioactive phospholipids known as platelet activating factor (2). Because of their cytotoxic effects on malignant cells in vivo and in vitro, ALPs have recently been attracting much attention in their biological functions (3, 4).

Several mechanisms may mediate the antitumoral effect of ALPs, i.e., enhancement of the tumoricidal capacity of macrophages (5, 6), induction of tumor cell differentiation (7), or direct cytotoxic effect on tumor cells (8–12).

Recently, we reported that 1-O-alkyl-2-O-methyl-sn-glycerol-3-phosphocholine, an alkyl-lysophospholipid derivative, inhibited the growth of hormone-dependent breast cancer cell lines (MCF-7 and ZR-75-1) by reducing EGF binding, while it did not affect both EGF binding and cell growth of the hormone-independent cell line BT-20 (13).

In the present study, we have investigated the effect of ET-18-OCH₃ on the uptake of labeled EGF into the intracellular fraction of ET-18-OCH₃-susceptible (MCF-7 and ZR-75-1) and ET-18-OCH₃-resistant (BT-20) human breast cancer cell lines.

MATERIALS AND METHODS

Chemicals. ¹²⁵I-EGF (168 Ci/g) was obtained from New England Nuclear (Boston, MA). ET-18-OCH₃ was purchased from BACHEM Feinchemikalien AG (Bubendorf, Switzerland). ET-18-OCH₃ as a stock solution (0.5 mg/ml) was added to a culture medium to the desired concentrations. Unlabeled mouse submaxillary EGF was purchased from Takara Shuzou (Kyoto, Japan), and all culture media used were from Flow Laboratories (McLean, VA). Other chemicals used were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Cell Lines. The breast cancer cell lines MCF-7, ZR-75-1, and BT-20 were subcultured as described previously (13).

EGF Binding Assay. EGF binding assay was carried out as described previously (13, 14). For the binding assay, the cells were incubated for a given period at 4°C or 37°C with 1 ml of Hanks' balanced salt solution containing 0.1% bovine serum albumin containing 0.1 ng/ml of ¹²⁵I-EGF.

Distribution of ¹²⁵I-EGF in Breast Cancer Cells. The amounts of surface-bound EGF and intracellular EGF were measured as described previously (15) with minor modifications. After the binding incubation, the medium was removed and the cells were washed three times at 4°C each with 1 ml of ice-cold Hanks' balanced salt solution containing 0.1% bovine serum albumin to remove nonspecifically adhered EGF. The cells were then treated with 1 ml of 0.2 M acetic acid containing 0.5 M NaCl (final pH 2.5) for 6 min at 4°C and rinsed with 0.5 ml of the acetic acid solution, and the remaining radioactivity was released from the cells by overnight incubation at room temperature with 1 N NaOH/0.5% Triton X-100. The radioactivities recovered by acetic acid (surface-bound EGF) and NaOH (intracellular EGF) were determined separately by gamma counter (ARC-605; Aloka, Tokyo, Japan).

DNA Assay. DNA was measured by the method of Burton as modified by Taylor et al. (17).

Statistics. The statistical significance of the difference between two data groups shown in Table 1 was tested by Student's t test and those shown in Figs. 1, 2, and 3 were tested by two-way analysis of variance.

RESULTS

Table 1 shows the total (surface-bound plus intracellular) amount of ¹²⁵I-EGF recovered from the breast cancer cell lines incubated at 37°C and 4°C with and without 12-h pretreatment with 10 μg/ml of ET-18-OCH₃. Control values in Table 1 indicate that the incorporation of labeled EGF into each cell line was largely temperature dependent; i.e., the amounts of labeled EGF recovered from the cells after 4°C incubation were about 22, 43, and 20% of those after 37°C incubation in MCF-7, ZR-75-1, and BT-20, respectively. The amounts of labeled EGF in MCF-7 and ZR-75-1 were reduced by ET-18-OCH₃ by 36–37% (P < 0.01 in MCF-7 and P < 0.001 in ZR-75-1) at 37°C and by 10–13% (not significant in MCF-7 and P < 0.02 in ZR-75-1) at 4°C as compared to corresponding controls. On the other hand, the amounts of labeled EGF in BT-20 at 37°C and 4°C were not significantly changed by pretreatment with 10 μg/ml of ET-18-OCH₃.

Changes in the amounts of surface-bound and intracellular labeled EGFs during 37°C and 4°C incubations in MCF-7 and ZR-75-1 with and without 12-h pretreatment with 10 μg/ml of ET-18-OCH₃ are shown in Figs. 1 and 2, respectively. In MCF-7 at 37°C, the amount of intracellular labeled EGF was markedly reduced, while the amount of surface-bound EGF was not changed by ET-18-OCH₃ pretreatment (Fig. 1A). In ZR-75-1
Table 1  Effects of incubation temperature and ET-18-OCH₃ on the total (bound and intracellular) amount of ¹²⁵I-EGF associated with breast cancer cell lines

The cells cultured for 12 h with 10 μg/ml of ET-18-OCH₃ at 37°C were washed, equilibrated with 37°C or 4°C, and incubated with ¹²⁵I-EGF (0.1 ng/ml) in 1 ml of Hanks’ balanced salt solution containing 0.1% bovine serum albumin for 1 h. The values shown are the specific ¹²⁵I-EGF binding and the mean ± SD from triplicate observations. Numbers in parentheses are the percentages of corresponding controls.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Control</th>
<th>ET-18-OCH₃</th>
<th>Control</th>
<th>ET-18-OCH₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37°C</td>
<td>4°C</td>
<td>37°C</td>
<td>4°C</td>
</tr>
<tr>
<td>MCF-7</td>
<td>30.0 ± 2.0</td>
<td>19.2 ± 1.3*</td>
<td>6.5 ± 0.9</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(64.0)</td>
<td>(100)</td>
<td>(89.7)</td>
</tr>
<tr>
<td>ZR-75-1</td>
<td>561.6 ± 34.8</td>
<td>351.6 ± 9.1*</td>
<td>241.0 ± 10.3</td>
<td>209.0 ± 8.9*</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(62.6)</td>
<td>(100)</td>
<td>(86.4)</td>
</tr>
<tr>
<td>BT-20</td>
<td>7719.0 ± 142.1</td>
<td>7803.2 ± 131.4</td>
<td>1541.9 ± 35.2</td>
<td>1657.2 ± 88.1</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(101.1)</td>
<td>(100)</td>
<td>(107.4)</td>
</tr>
</tbody>
</table>

* P < 0.01.
+ P < 0.001.
# P < 0.02 (Students’ t Test).

at 37°C, ET-18-OCH₃ pretreatment reduced markedly the amount of intracellular labeled EGF and slightly the amount of surface-bound labeled EGF (Fig. 2A). At 4°C, the amounts of intracellular EGF both in MCF-7 and ZR-75-1 were slightly affected by pretreatment with ET-18-OCH₃, while the amounts of surface-bound EGF in both cell lines were little or slightly affected by ET-18-OCH₃ (Figs. 1B and 2B). The amounts of intracellular and surface-bound ¹²⁵I-EGF during incubations at 37°C and 4°C with BT-20 with and without 12-h pretreatment with 10 μg/ml of ET-18-OCH₃ are shown in Fig. 3. In this ET-18-OCH₃-resistant cell line, the amounts of both intracellular and surface-bound EGFs were slightly affected by pretreatment with ET-18-OCH₃.

In all cell lines tested, the amounts of surface-bound and intracellular EGFs in the ET-18-OCH₃-pretreated group were significantly (P < 0.01) different from those in the control group.

DISCUSSION

Recently, we reported that ET-18-OCH₃ suppressed EGF binding prior to the onset of its inhibitory action on cell growth in hormone-dependent human breast cancer cell lines MCF-7 and ZR-75-1 (13), although the mechanism of this EGF action was not understood. The ¹²⁵I-EGF binding was measured only at 20°C and no intracellular compartmentation of incorporated EGF was attempted in the previous study (13). In the present study, the binding and uptake of labeled EGF were measured separately and at two incubation temperatures, 4°C and 37°C, in order to examine not only the temperature dependence of the binding and uptake of labeled EGF but also the effect of ET-18-OCH₃ on the endocytosis of labeled EGF.
In the previous study, we found that the half-maximal inhibitory concentration of ET-18-OCH₃ was approximately 10 μg/ml and the growth of either MCF-7 or ZR-75-1 was unaffected by 10 μg/ml of ET-18-OCH₃ in 12 h incubation (13). Therefore, we used a 12-h time point and a ET-18-OCH₃ concentration of 10 μg/ml in this study.

It was found in the present study that the total EGF binding in all cell lines tested was greatly decreased by a temperature fall from 37°C to 4°C, although no essential change in the EGF binding was noted between incubation temperatures of 20°C and 37°C. In 4 h incubation at 20°C with labeled EGF in our previous study (13), the percentage of control of the total binding of labeled EGF after pretreatment with ET-18-OCH₃ was 56.8 ± 3.5% (SD) for MCF-7 and 66.1 ± 4.0% for ZR-75-1.

The amount of surface-bound labeled EGF at 4°C (Figs. 1A and 2A) but not in BT-20 (cell line resistant to ET-18-OCH₃) was markedly reduced by 12-h pretreatment with ET-18-OCH₃, which was 56.8 ± 3.5% (SD) for MCF-7 and 66.1 ± 4.0% for ZR-75-1. In 1 h incubation at 37°C with labeled EGF in the present study, the total EGF binding was 64.0 ± 6.8% of control for MCF-7 and 62.6 ± 2.6% of control for ZR-75-1 (Table 1).

In either MCF-7 or ZR-75-1 (cell lines susceptible to ET-18-OCH₃ (13)), the amount of intracellular labeled EGF at 37°C was markedly reduced by 12-h pretreatment with ET-18-OCH₃ (Figs. 1A and 2A) but not in BT-20 (cell line resistant to ET-18-OCH₃ (13)) (Fig. 3A). The amount of intracellular labeled EGF at 4°C, which was much smaller than that at 37°C, was decreased by ET-18-OCH₃ in MCF-7 and ZR-75-1 but not in BT-20 (Figs. 1B, 2B, and 3B). The amount of surface-bound labeled EGF at 37°C was affected only slightly by ET-18-OCH₃, but in a variable fashion, with no change in MCF-7 (Fig. 1A), mild decreases in ZR-75-1 (Fig. 2A), and a small increase in BT-20 (Fig. 3A). Whether these changes are cell specific is to be studied. The amount of surface-bound labeled EGF at 4°C was little changed by ET-18-OCH₃ in all cell lines tested (Figs. 1B, 2B, and 3B).

Although the amounts of both surface-bound and intracellular EGFs were significantly (P < 0.01) different between the ET-18-OCH₃-pretreated group and the corresponding control group in all cell lines tested, ET-18-OCH₃ was found to be extremely potent in decreasing the amount of intracellular EGF in both MCF-7 and ZR-75-1 as compared with other changes induced by ET-18-OCH₃. Therefore, we consider that ET-18-OCH₃-induced decreases of the total amount of labeled EGF in MCF-7 and ZR-75-1 were predominantly due to a decrease in the amount of intracellular EGF.

According to the Scatchard plot obtained by saturation binding experiments in our previous study (13), the number of binding sites for labeled EGF after pretreatment with ET-18-OCH₃ in ET-18-OCH₃-susceptible breast cancer cell lines was 56.8 ± 3.5% (SD) for MCF-7 and 66.1 ± 4.0% for ZR-75-1 (Table 1).

The temperature-dependent uptake of labeled EGF may reflect the existence of the process of receptor-mediated endocytosis, i.e., internalization of EGF in human breast cancer cell lines. It is concluded that one of the modes of antitumoral action of ET-18-OCH₃ in hormone-dependent breast cancer cell lines appears to be the inhibition of the internalization of EGF.

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REFERENCES

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