Steroids and immunosuppressors agents potentiate the cytotoxicity of the EGFR inhibitor erlotinib (E) in human skin keratinocytes whereas menadione (Vit K3) exerts a protective effect: implications for the management of the skin rash.

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Abstract 9124

Background

Erlotinib and the EGFR inhibitors, cetuximab and panitumumab, are approved for the treatment of several tumors. The EGFR and its downstream pathways are required for cell proliferation and survival. However, these agents sometimes cause skin rashes that may necessitate the discontinuation of therapy. The use of potent immunosuppressive and corticosteroid drugs to treat skin rashes is empirical, and their use is associated with increased risk of infection and reduced efficacy for the tumor.

Objectives

1. To study the effects of different agents used to treat the skin toxicity secondary to EGFR inhibitors and their effect on EGFR-induced skin toxicity.

2. To confirm the protective effect of menadione on EGFR tyrosine kinase activity in human skin keratinocytes exposed to erlotinib.

3. To investigate the role of reactive oxygen species in the mechanism of action of menadione.

Materials and Methods

1. Cell lines

Human skin keratinocytes were obtained from Cell Line Service (Eppelheim, Germany). The human carcinoma cell line HaCat was obtained from the American Type Culture Collection.

2. Antibodies

Polyclonal anti-EGFR antibody and monoclonal anti-pEGFR were detected by western blot analysis using the corresponding antibodies. The EGFR and p-EGFR were detected by western blot analysis using the corresponding antibodies.

3. Measurement of ROS Generation

DCF-DA (2',7'-dichlorofluorescein diacetate) and DAPI (4',6-diamidino-2-phenylindole) were used to measure the intracellular ROS content. After exposure, cells were washed three times with cold PBS solution, and then suspended in lysis buffer containing 50 mM Tris-HCl (pH 7.5), 1% Triton X-100, 100 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, and 10% BSA. The intracellular ROS levels were detected by fluorescence microscopy. The fluorescence intensity of DCF in the cells was measured using a Perkin-Elmer LS55 luminescence spectrophotometer.

4. DAPI staining

Exponentially growing cells were exposed to varying concentrations of erlotinib alone or in combination with the indicated concentrations of NAC at 37°C for 1 h. After incubation, cells were harvested and the intracellular ROS content was measured using a Perkin-Elmer LS55 luminescence spectrophotometer.

Table 1 and Figure 1. Cytotoxicity of epidermal alone and in combination with other agents in HaCat cells

<table>
<thead>
<tr>
<th>Agents</th>
<th>IC50 (µM)</th>
<th>IC50 (µM)</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>0.9±0.5</td>
<td>1</td>
</tr>
<tr>
<td>E+100 µM Hydrozine</td>
<td>1±0.2</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>E+100 µM Diphenhydramine</td>
<td>1±0.2</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>E+100 µM Clidamycine</td>
<td>1±0.2</td>
<td>2.0±0.1</td>
</tr>
</tbody>
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Results

1. EGFR phosphorylation

EGFR phosphorylation was measured using a phospho-EGFR antibody. The phospho-EGFR was detected by western blot analysis using the corresponding antibodies.

Conclusion: Menadione activates EGFR tyrosine kinase in a concentration-dependent manner.

Figure 3. Effect of menadione on EGFR phosphorylation in HaCat cells as detected by immunofluorescence staining

Figure 4. Effect of menadione on the generation of reactive oxygen species (ROS) in HaCat cells

Figure 5. Effect of menadione on epidermal-induced inhibition of EGFR phosphorylation in HaCat cells

Figure 6. Effect of menadione on the generation of reactive oxygen species (ROS) in HaCat cells

Figure 7. Effect of antioxidant agent. NAC, on epidermal-induced inhibition of EGFR phosphorylation in HaCat cells

Conclusion: Menadione abolishes the inhibitory effect of erlotinib on EGFR tyrosine kinase activity.

Conclusions

1. The EGFR and its downstream pathways are required for cell proliferation and survival. However, these agents sometimes cause skin rashes that may necessitate the discontinuation of therapy. The use of potent immunosuppressive and corticosteroid drugs to treat skin rashes is empirical, and their use is associated with increased risk of infection and reduced efficacy for the tumor.

2. Menadione, an antioxidant drug, abolishes the inhibitory effect of erlotinib on EGFR tyrosine kinase activity and decreases by 5-fold the toxicity of erlotinib on human skin keratinocytes. Such observation may offer a rationale for the use of menadione in the treatment of the skin toxicity secondary to erlotinib.

3. Menadione mediates the generation of reactive oxygen species in human skin keratinocytes in a concentration-dependent manner.

4. The anti-inflammatory activity of menadione is likely due to the inhibition of the EGFR inhibitor mediated in human skin keratinocytes. Such observation may explain the mixed results observed with the clinical use of these agents in the treatment of the skin toxicity secondary to erlotinib.

5. Menadione, an antioxidant drug, abolishes the inhibitory effect of erlotinib on EGFR tyrosine kinase activity and decreases by 5-fold the toxicity of erlotinib on human skin keratinocytes. Such observation may offer a rationale for the use of menadione in the treatment of the skin toxicity secondary to erlotinib.