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Different apoptotic patterns observed in tissues damaged by phenol and TCA peels

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KEYWORDS
Phenol;
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Apoptosis;
TUNEL;
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Summary Herein we intended to understand the histological differences in tissues treated with phenol and trichloroacetic acid (TCA) peels by study the expression of proteins involved in apoptotic cell death using immunohistochemical methods. Phenol penetrated into skin quickly and induced histological changes in endothelial cells in the dermis, which were detected by the TUNEL method and the expression of activated Caspase-3 molecules. However, we did not observe up-regulation of p53 and Fas antigens. Meanwhile degenerations from TCA peels were detected by the TUNEL method, but not observed with Caspase-3 activation. These findings suggest that skin degenerations from phenol peels undergo Caspase-3-mediated apoptosis from that of TCA peels in pathogenesis.
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1. Introduction

The number of patients with skin cancer has been increasing in Japan during the last two to three decades, possibly due to increase of aged population who spent a lot of time to take showers of sunlight in their life. One of the problem of theses aged patients is to refuse frequently operations for their skin lesions located in sites where surgical treatment is difficult. For these patients, non-invasive therapies such as topical therapies, photodynamic therapies, cryosurgeries [1] and laser therapies might be useful. Recently imiquimod (5%, w/v) cream has been used for topical therapy and may be a safe and effective non-invasive therapeutic option [2]. We recently used chemical peels with phenol and highly concentrated trichloroacetic acid (TCA) for non-invasive therapies to specifically treat elderly patients with skin tumors. Both agents injured skin tissues till the depth of the upper reticular dermis. Compared with other non-invasive therapies described above, chemical peel therapies with phenol and TCA have several advantages such as ease of use without requiring special equipment, speed, easy control of pain and easy post-treatment follow-up. However, detailed mechanisms of action and adequate techniques for phenol and TCA peels have not been completely elucidated.

In the present report, we investigate the mechanisms of the phenol and TCA peels in removing super-

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ficial epidermis by analyzing the expression of gene products which play a role in UV-induced tissue damages.

2. Subject and methods

2.1. Skin specimens

This study was performed in at least three healthy volunteers. Chemical agents used in this study were 40 and 60% (w/v) TCA (Wako Pure Chemical Industries Ltd., Osaka, Japan) and 100% pure phenol (Wako Pure Chemical Industries Ltd.). Skin application sites were carefully cleansed using 70% ethanol and acetone-soaked gauze to remove sebum. Three lines, each measuring 1 cm in width and 15 cm in length, were marked on the medial side of the upper arms, and the three chemical agents were applied to each marked region using cotton-tipped applicators until an even white frosting was recorded. Application sites were not dressed. Tissues were sampled, under local anesthesia using 1% lidocaine and 4 mm punch biopsies (Biopsy Punch; KAI Sterile Inc., Solingen, Germany) were taken from the central area of the applied regions, 1 cm apart from previously sampled sites, at either 2, 6 or 12 h and 1, 2, 3, 5 or 7 days after application. Each biopsy specimen was embedded in Tissue-Tek (Sakura Finetechnical, Tokyo, Japan; Code 4583) and snap-frozen in liquid nitrogen. Five micrometers thick cryostat sections were fixed in acetone for 10 min, air-dried and stored at −80 °C until further staining study.

2.2. Immunohistochemical studies

Cryostat sections, 5 μm thick, were prepared, air-dried and fixed in acetone for 5 min at 4 °C. After washing in phosphate-buffered saline (PBS), endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 5 min and washed in PBS.

For immunostaining, sections were air-dried and washed with 0.01 mol L^{-1} PBS, pH 7.2. Immunohistochemical measurements were performed using DAKO Envsion Plus HRP System (K4001; DakoCytomation, Kyoto, Japan). Primary antibodies used for immunostaining included mouse monoclonal antisera directed against human p53 (DO-7; DAKO, Glostrup, Denmark; Code M 7001, dilution ×50), Fas/APO-1 (APO-1; DAKO, Glostrup; Code M 3553, dilution ×10) and Caspase-3 (4-1-18; Chemicon, CA, USA; Code MAB4703, ×500). Bound primary antibodies

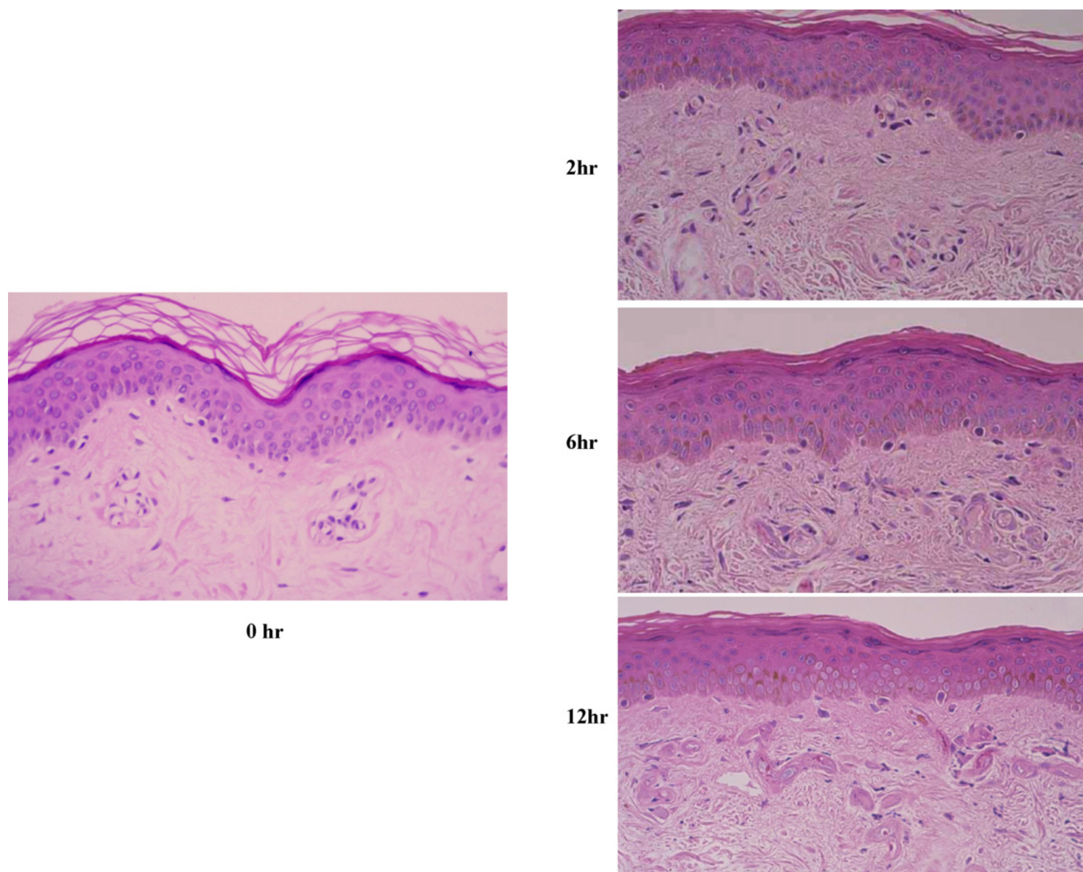

Fig. 1 Histopathological findings in phenol-treated skin (H.E. stain; original magnification ×400).

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