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Preventive effect of chemical peeling on ultraviolet induced skin tumor formation

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ABSTRACT

Background: Chemical peeling is one of the dermatological treatments available for certain cutaneous diseases and conditions or improvement of cosmetic appearance of photoaged skin.
Objectives: We assessed the photochemopreventive effect of several clinically used chemical peeling agents on the ultraviolet (UV)-irradiated skin of hairless mice.
Methods: Chemical peeling was done using 35% glycolic acid dissolved in distilled water, 30% salicylic acid in ethanol, 10% or 35% trichloroacetic acid (TCA) in distilled water at the right back of UV-irradiated hairless mice every 2 weeks in case of glycolic acid, salicylic acid, and 10% TCA and every 4 weeks in case of 35% TCA for totally 18 weeks after the establishment of photoaged mice by irradiation with UVA + B range light three times a week for 10 weeks at a total dose of 420 J/cm^2 at UVA and 9.6 J/cm^2 at UVB. Tumor formation was assessed every week. Skin specimens were taken from treated and non-treated area for evaluation under microscopy, evaluation of P53 expression, and mRNA expression of cyclooxygenase (COX)-2. Serum level of prostaglandin E_2 was also evaluated.
Results: All types of chemical peeling reduced tumor formation in treated mice, mostly in the treated area but also non-treated area. Peeling suppressed clonal retention of p53 positive abnormal cells and reduced mRNA expression of COX-2 in treated skin. Further, serum prostaglandin E_2 level was decreased in chemical peeling treated mice.
Conclusions: These results indicate that chemical peeling with glycolic acid, salicylic acid, and TCA could serve tumor prevention by removing photodamaged cells.

1. Introduction

Chemical peeling is used for treating photoaged skin such as wrinkles and pigmented spots [1,2]. Glycolic acid, the most commonly used chemical peeling agent has been reported to contribute to the recovery of photodamaged skin through various actions depending on the skin cell type such as keratinocytes [3–6] melanocytes [7], and fibroblasts [3,5,6]. To achieve wrinkle removal by dermal remodeling, inflammatory process is accompanied [2]. Repeated tissue damage coupled with chronic inflammation can stimulate carcinogenesis [8]. Therefore, the safety of repeated chemical peeling, especially on tumor formation needs to be evaluated.

Extensive clinical trial of chemical peeling on precancerous actinic keratosis was done with histologic evaluation and superficial skin tumors could be recognized as good candidates for the therapy [9,10]. Using hairless mice, carcinogenic potential of chemical peeling after repetitive irradiation of UVB was studied with salicylic acid in polyethylene glycol (PEG) and 35% trichloroacetic acid (TCA). In contrast to salicylic acid in PEG, which reduced the number of UVB-induced skin tumors [11], 35% TCA was reported to enhance tumors in the treated area [12]. Therefore, the differential biological effect of each peeling agent such as glycolic acid, salicylic acid, and TCA on photocarcinogenesis needs to be elucidated.

In mouse study, UVB is the wave range mostly used to study photoaging. We have previously reported that UVA irradiation at a dose of 14 J/cm^2 using BL lamp (mainly emitting UVA) plus

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one-third of minimum erythema dose (MED) of UVB (20 mJ/cm^2) three times per week for 10 weeks causes fine wrinkles [13] by inducing changes in stratum corneum keratin [14]. UVA is now well known to induce a variety of damages to DNA, proteins and lipids accompanied with harmful consequences such as skin aging and carcinogenesis [15].

We conducted a murine study to investigate the photocarcinogenicity of four conditions of chemical peeling after repeatedly irradiated by UV using BLB lamps, which contain UVA and UVB. Four kinds of chemical peeling using 35% glycolic acid, 30% salicylic acid, 10% and 35% TCA were conducted. Photocarcinogenic potential as well as the distribution and status of photodamaged and mutated cells were evaluated in this study.

2. Materials and methods

2.1. Chemicals

Chemical peeling agents used; glycolic acid, salicylic acid, and TCA were purchased from Nacalai Tesque (Kyoto, Japan). Glycolic acid at a concentration of 35% (w/v) was prepared by dissolving in distilled water containing 0.7% (w/v) polyethylene glycol (PEG) 45 M. Salicylic acid at a concentration of 30% (w/v) was prepared by dissolving in ethanol. TCA at a concentration of 10% (w/v) and 35% (w/v) were prepared by dissolving in distilled water.

2.2. Mice

Female hairless albino mice (Hos, hr-1) aged 6 weeks were purchased from Japan SLC Inc. (Shizuoka, Japan). They were fed on standard diet and water ad libitum, and supported at controlled temperature and humidity with 12 h light/dark cycle in our University Animal Facility. This study was approved by the Institutional Animal Care and Use Committee and carried out according to the Kobe University Animal Experimentation Regulations.

2.3. Ultraviolet irradiation

For UV irradiation, a bank of four fluorescent UVA lamps (FL20SBLB/DMR, Toshiba, Tokyo, Japan) emitting 300–430 nm was used. Emission range was calculated as follows; 300–320 nm: 2.2%, 320–400 nm: 96.2%, longer than 400 nm: 1.6%. Flux intensity was measured with a UVR-305/365D digital radiometer (Opto-Electronic Measuring Instruments). The irradiation dose was 14 J/cm^2 for UVA and 320 mJ/cm^2 for UVB.

Forty hairless mice were irradiated thrice a week for 10 weeks. The total cumulative dose energy of irradiated UVA was 420 J/cm^2, and that of UVB was 9.6 J/cm^2.

2.4. Chemical peeling

The UV-irradiated mice were divided into five groups of eight mice each. The first group received treatment of 35% (w/v) glycolic acid for 5 min once every 2 weeks. The second group received treatment of 30% (w/v) salicylic acid for 5 min once every 2 weeks. The third group received treatment of 10% (w/v) TCA for 5 min once every 2 weeks. The fourth group received treatment of 35% (w/v) TCA for 20 min once every 4 weeks. The last group served as UV-irradiated non-chemical-peeled control. On each treatment, 0.1 ml of specific peeling agent was applied on surface area of 2 cm × 2 cm at the right side of the back of mice (Fig. 1a) starting three days after termination of UV irradiation (Fig. 1b). After specific peeling treatment, the skin was thoroughly rinsed with distilled water and gently wiped dry with cotton gauze. For non-peeled control group (sham-peeled group), only rinsing with distilled water and wiping process was done on the right back area of 2 cm × 2 cm. Treatment with chemical peeling agents lasted 18 weeks. Two weeks after the last treatment (30 weeks from the beginning of UV irradiation), skin specimens from the dorsum of the mice in treated site (right back) and non-treated site (left back), and serum were obtained after sacrifice of four out of eight mice from each group (Fig. 1b).

2.5. Experimental observation and data analysis

Mice were checked weekly for any visible skin abnormalities, and findings such as redness, scratching, scaling and tumors were recorded. The day of the first UV irradiation was defined as $t = 0$ and chemical peeling was done from 10th to 28th week (Fig. 1b). The number of developing tumors with diameters larger than 2 mm and lasted more than 3 weeks was counted as tumor. Tumors <2 mm in diameter, or regressed, were not counted.

2.6. Histological analysis

Biopsied skin was fixed in 10% neutral buffered formalin, embedded in paraffin, and then stained using hematoxylin and eosin. Expression of P53 protein was examined using the polyclonal antibody CM-5 (NCL-p53-CM5p, Novocastra, Newcastle upon Tyne, UK) at a dilution of 1:100 and binding of antibody was detected using a Nichirei kit (Nichirei, Tokyo, Japan).

2.7. Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted using the ISOGEN solution (Nippon Gene, Toyama, Japan) following the manufacturer's kit. For the reverse transcription – PCR assays 5 μg of total RNAs were reverse transcribed using oligo (dT) as primers and Moloney Murine Leukemia Virus ReverseTranscriptase (GIBCO-BRL, Life Technologies, NY). Only samples that proved to be free from DNA contamination by running PCR amplification with GAPDH primers without prior reverse transcription were used for the experiments. Reaction products were electrophoresed in 2% agarose gel and visualized with ethidium bromide (EtBr). Each band was densitometrically quantified by image analysis and normalized versus GAPDH intensity. The primer sequence used was: murine COX-2 (sense, 5′-CGGATCCATCCTTGCTGTTCCAATC-CATGTCAA-3′; antisense, 5′-CGAATTCCCAGGTCCTCGCTTAT-GATCTGTCTT-3′) [16], and murine GAPDH (sense, 5′-CTTCATTGACCTCAACTACAT-3′; antisense, 5′-CCAAAGTTGT-CATGGATGACC-3′) [16].

2.8. PGE_2 immunoassay

PGE_2 concentration in the serum was determined by using a monoclonal antibody/enzyme immunoassay kit from Cayman Chemical (Ann Arbor, MI, USA), according to the manufacturer's instruction. Bloods were harvested from hearts under anesthetics. The bloods were allowed to clot for 2 h at room temperature and then centrifuged for sera. All measurements were carried out in triplicate.

2.9. Statistical analysis

All values were expressed as the mean ± standard deviation (S.D.) obtained from four or eight samples in each group. Two-way analysis of variance (ANOVA) followed by the Tukey test was used to evaluate differences between more than three groups. Differences were considered to be significant for values of $p < 0.05$