한국산학기술학회논문지
Vol. 11, No. 11 pp. 4427-4432, 2010

Suppressive effect of electrolyzed reduced water on the paraben-induced DNA damage in human dermal fibroblast cells

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파라벤에 의한 피부섬유아세포의 DNA 손상과 환원전리수의 억제 효과

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Abstract Parabens have been widely used as preservatives in cosmetics due to the presumed low toxicity and long history of safe use. However, recent studies have shown the potent toxicity of parabens. In order to know if electrolyzed reduced water could suppress the oxidative DNA damage of HDF cell by methylparaben, one of the frequently used parabens, we performed comet assay in this study. As a result, interestingly, electrolyzed reduced water could suppress methylparaben-induced oxidative DNA damage in HDF cells.

요 약 파라벤은 낮은 독성과 안전성으로 인해 화장품 보존제로 널리 사용되어 왔다. 그러나 최근 파라벤의 잠재적 독성이 알려지고 있다. 본 연구에서는 환원전리수가 널리 사용되는 메틸파라벤에 의한 사람 피부섬유아 세포의 DNA 산화손상을 억제할 수 있는지 알아보기 위하여 코멧어세이를 실시하였다. 그 결과 흥미롭게도 환원전리수는 파라벤에 의한 피부섬유아세포 DNA의 산화손상을 억제할 수 있었다.

Key Words : Methylparaben, Electrolyzed-reduced water, Comet assay, HDF cell

1. Introduction

Parabens, stable methyl ester of 4-hydroxybenzoic acid [1], are widely used as antimicrobial agents [2] and preservatives [3,4] because they have broad activity spectrum against various microorganisms [5]. They have been widely used in cosmetic products such as body creams, skin moisturizers, deodorants, antiperspirants and sun care products, food ingredients, or in pharmaceutical preparations [2]. Methylparaben, ethylparaben, benzyl paraben, n-butylparaben, n-propylparaben and isobutylparaben are widely used esters of parabens[6,7]. Among them, methylparaben and propylparaben are the most used parabens [8]. Methylparaben has been known to be nontoxic and not mutagenic but it could cause contact dermatitis, drug hypersensitivity and potential estrogenic activity [8,9]. However, in some experiments methylparaben potentialized UV-induced damage of skin keratinocytes [10], and male reproductive disorders in animal models and humans [11]. Moreover, some reports addressed the estrogenic activity of p-hydroxybenzoic acid (common metabolite of paraben esters) and methylparaben in human breast cancer cell lines, which is estrogen-responsive [6]. Recent work also has shown p-hydroxybenzoic acid to give an estrogenic response in the rodent uterotrophic assay, and p-hydroxybenzoic acid

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Received October 5, 2010 Revised November 9, 2010 Accepted November 19, 2010

has been reported to possess estrogenic activity in a panel of assays in human breast cancer cell lines. There remains considerable controversy about the involvement of paraben in breast cancer. However, the information about protecting against the potential risk of paraben is not available.

Electrolysis of water produces electrolyzed reduced water (ERW) near the cathode and electrolyzed oxidized water (EOW) near the anode [12]. ERW has high pH, low oxidation-reduction potential, low dissolved oxygen and high dissolved hydrogen [13,14]. Electrolyzed-reduced water could scavenge active oxygen species like H_2O_2 and superoxide anions [12,13]. Moreover, this water has been reported to exert various biological effects such as anti-diabetic effect, growth-stimulating effect of anaerobic microflora in the human intestine and suppressive effect on the DNA, RNA and protein damage from reactive oxygen species [15].

In this study, the oxidative DNA damage by methylparaben and the suppressive effect of electrolyzed-reduced water on methylparaben-induced DNA damage of HDF-n cells were investigated by using single-cell gel electrophoresis (comet assay).

2. Material and Methods

2.1 HDF cell culture

Human dermal fibroblast, neonatal (HDF-n) cell was maintained in DMEM (Dulbecco's Modified Eagle Medium) medium (Hyclone) with 10% fetal bovine serum (FBS) and 1% penicillin at 37°C in a 5% CO_2 humidified environment.

2.2 Preparation of electrolyzed reduced water

The electrolyzed water-generating apparatus consisted of an anode, a cathode and middle chambers. Ultrapure water was supplied to each chamber, and the diluted electrolyte (NH_4Cl) was supplied to the middle chamber. The electrolyzed water was generated by the electrolysis of the water by adding electrolyte with a current of 9 A and a voltage of 10.5 V near the cathode using a Redox-water generator (Microbank). The electrolyzed reduced water (ERW) used had a pH of 10.6 and an oxidation-reduction potential of -800 mV.

2.3 Treatment of HDF cells with methylparaben

Methylparaben was dissolved in dimethylsulfoxide (DMSO) and diluted to 2 mM with DMEM medium containing 1% penicillin. 135 mg of powdered DMEM medium, 37 mg of $NaHCO_3$ and 100 μL of penicillin were dissolved in 10 mL of 100% ERW, and then sterilized with 0.4 μm syringe filter. For pre-treatment of HDF cells with ERW, the cells were incubated with DMEM medium dissolved in ERW for 1 hr at 37°C, and then 2 mM methylparaben was treated for 30 min at 37°C. For co-treatment of HDF cells with ERW and methylparaben, the cells were simultaneously mixed with DMEM dissolved in ERW and 2 mM methylparaben for 30 min at 37°C. 50% ERW was made by diluting the 100% ERW with same volume of deionized water(18 M Ω).

2.4 Determination of DNA Damage by comet assay

The single-cell gel electrophoresis (comet) assay was performed according to Singh et al. (1988) [16] for evaluation of DNA damage by methylparaben. Isolated HDF cells from 12 well plate were mixed with 75 μL of 0.7% low melting point agarose and then duplicated on slide glass which was precoated with 1% normal meting point agarose. And then agarose was solidified for 30 min at 4°C. After agarose solidification, 100 μL of 0.7% low melting point agarose was added and covered with 24×50 mm cover glass. After the agarose was solidified, lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium lauryl sarcosine, 1% Triton X-100, and 10% DMSO) was treated for 1 hr at 4°C. The slides were placed in an electrophoresis tank and electrophoresis buffer (300 mM NaOH and 10 mM Na_2EDTA, pH13) was added for 20 min to unwind DNA. After unwinding DNA, electrophoresis was performed for 20 min at 25 V/300 mA. The slides were neutralized with 0.4 M Tris buffer (pH 7.5) and fixed with crude ethanol for 5 min. Slides were dried for 15 min, and then stored in slide box at 4°C.

2.5 Image analysis

Slides were stained with ethidium bromide (50 μM) and measured using a fluorescence microscope (Leica, Wetzlar, Germany). Slides were viewed with a CCD camera (Hitachi, Japan) and the image was analyzed using Komet 5.5 soft ware (Kinetic Imaging, UK). To quantify DNA damage in the comet assay, the olive tail moment was calculated as (Tail.mean – Head.mean) × Tail% DNA/100 [17]. Total 100 cells were randomly captured and the comets were examined from each slide. The comet slides were codified to ensure the study was performed in a blind manner. Comet assay was performed from three independent experiments. All measurements were made in duplicate and presented as the mean±S.E.

2.6 Statistical analysis

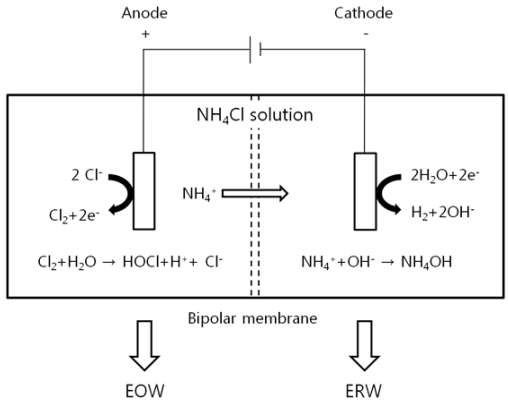
The comet assay data were analyzed using the SPSS package for Windows version 13 (SPSS Inc., Chicago, IL). The mean values of DNA damage (olive tail moment) for each treatment were compared using one way analysis of variance (ANOVA) followed by Duncan's multiple range test. $P<0.05$ was considered significant.

3. Results and Discussion

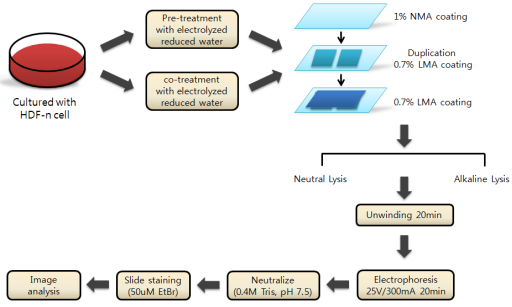
Parabens have been widely used as preservatives in cosmetics, toiletries, and pharmaceuticals due to their relatively low toxicity profile and a long history of safe use. Recently, however, the estrogenic activity and the potential risk of parabens were addressed.

The single-cell gel electrophoresis (comet) assay is widely used for assessment of DNA damage [18,19]. It is a sensitive and rapid assay for the detection of DNA damage at the individual cell level [20,21]. Also, this assay was used to assess the efficacy of enzymatic DNA repair processes such as subsequent rejoining of the damaged strands [21,22].

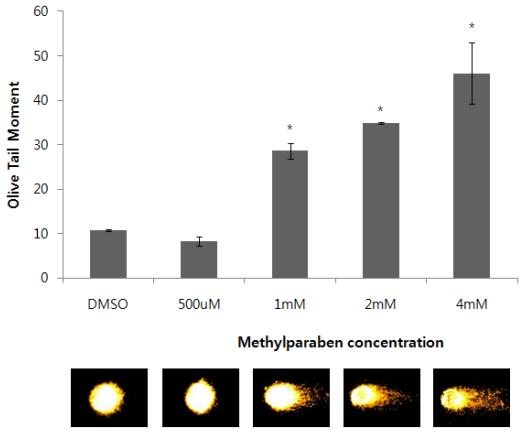
Fig. 1 shows the basic principle of the generation of electrolyzed water in electrolysis chamber. The electrolyzed-reduced water was generated at the cathode in this electrolysis chamber.



[Fig. 1] The principle of electrolysis of water.



[Fig. 2] The scheme of comet assay.



[Fig. 3] Methylparaben-induced oxidative damage of DNA in human dermal fibroblast (HDF) cells. *$P<0.05$, significantly different from the DMSO control value.

Fig. 3 shows that methylparaben induced oxidative DNA damage in HDF cells evaluated by the olive tail moment in a comet assay. The low dose of paraben did not induce the oxidative DNA damages. Even the olive tail moment at 500 μM was about 8.34±1.06, indicating similar value as control. However, the olive tail moment at 2 mM and 4 mM methylparaben increased up to about 34.82±0.22 and 46.07±6.88, respectively.

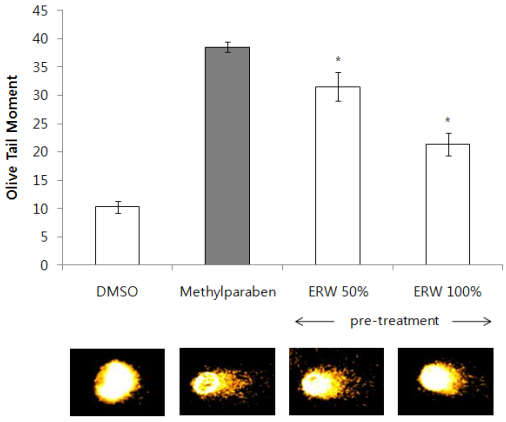
However, interestingly, the oxidative DNA damage by methylparaben was notably reduced by electrolyzed reduced water (ERW) treatment as shown in Fig 4. The addition of ERW ,prior to adding methylparaben, inhibited the oxidative DNA damage caused by 2 mM methylparaben as demonstrated by the reduction of the olive tail moment. The extent of the suppression by ERW was dependent on the ERW quantity in the DMEM media.

The olive tail moment at 2 mM methylparaben was about 38.53±0.87, compared with 10.29±1.02 in the DMSO-treated control, indicating an approximately 4-fold increase in DNA damage with 2 mM methylparaben. However, the treatment of the DMEM media with 100% ERW and 50% ERW notably suppressed the oxidative DNA damage by methylparaben. Olive tail moment of 100% ERW was approximately 21.36±2.05, showing more protective effects than 31.51±2.5 seen at 50% ERW.

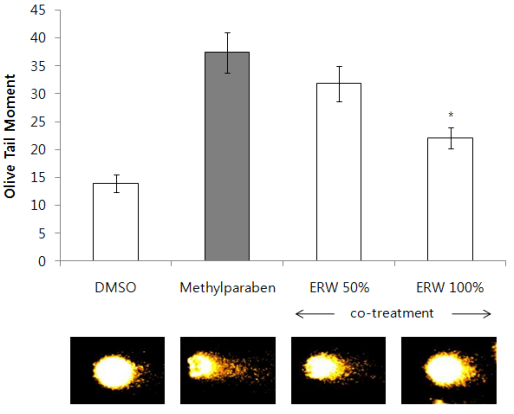
Fig. 5 shows the inhibitory effect of ERW on HDF cells treated simultaneously with methylparaben and ERW. The olive tail moment at 2 mM methylparaben was about 37.36±3.64, approximately 3-fold more damaged than DMSO control. However, the olive tail moment of HDF cells treated with DMEM media with 100% ERW were about 22.02±1.89, indicating notable inhibitory effect against oxidative DNA damage. The olive tail moment at 50% ERW was about 31.84±3.15, showing less inhibitory effect than that at 100% ERW.

This result shows that ERW could suppress the oxidative DNA damage by methylparaben probably due to the antioxidative activity of ERW. Several reports have shown the antioxidative activity of ERW as a powerful radical scavenger[13,17]. ERW could protect against the oxidative DNA damage by paraquat[23] and melamine [17] in our earlier reports.

DNA is a significant target of oxidative damage in the cell, and it is widely known that chronic oxidative DNA damage might be a significant contributor to many diseases. A lot of antioxidative agents such as vatamins and polyphenolics have been shown to protect the DNA damage through acting as scavengers of reactive oxygen species. In this study, ERW seems to be helpful to suppress the oxidative DNA damage by methylparaben in HDF cells. Thus, ERW could be used as a preventive water against potential toxicity of parabens in cosmetic products.



[Fig. 4] Protective effect of electrolyzed reduced water (ERW) pre-treatment on methylparaben-induced oxidative damage of DNA in human dermal fibroblast (HDF) cells. $^{*}P<0.05$, significantly different from the methylparaben-induced oxidative DNA damage.



[Fig. 5] Suppressive effect of electrolyzed reduced water (ERW) co-treatment on methylparaben-induced oxidative damage of DNA in human dermal fibroblast (HDF) cells. $^{*}P<0.05$, significantly different from the methylparaben-induced oxidative DNA damage.

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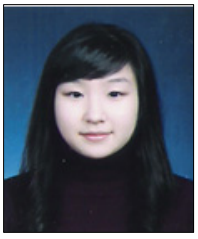

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