Short Communications

Protective effect of metallogluconates against UVA-induced cutaneous lesion in HR-1 hairless mice

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Abstract

UVA-induced reactive oxygen species (ROS) generation is well known to cause cutaneous lesion in the skin, therefore, it is important to prevent the skin from UV damage due to ROS generation. In 2000, we reported first the in vivo detection and imaging by chemiluminescence (CL) method of the generated ROS in the skin of live mice following UVA-irradiation [1]. Using this method, we found that superoxide anion radical (•O₂⁻) was intrinsically generated in the skin of live mice and singlet oxygen (¹O₂) was exclusively produced in the skin following UVA-irradiation. In our previously report, we found that topical application and oral administration of zinc compounds to the skin of live mice reduce the formation of UVA-induced ROS [2-3]. Then, in the present study, we examined whether metallogluconates (zinc gluconate (ZnGA) and copper gluconate (CuGA)) topically applied and orally administered reduce the intrinsic and UVA-induced ROS generations in the skin of live mice or not. We found that topical application and oral supplementation of CuGA reduced CL intensities in terms of the formation of UVA-induced ROS and ZnGA suppressed inflammation caused UVA-exposure.

Keywords: skin, hairless mouse, zinc gluconate, copper gluconate, UVA, reactive oxygen species (ROS), inflammation

Introduction

UV rays have been suggested to cause the various cutaneous inflammatory disorders such as the skin inflammation, photoaging and skin cancer. The free radical theory [4-6] have been proposed to explain aging in the skin, and received particular attention because the skin is constantly exposed to reactive oxygen species (ROS) from the environment such as air, solar radiation, ozone, and other air-borne pollutants, or from the normal metabolism [7]. Accumulated ROS have been suggested to play important roles in the intrinsic aging and photoaging in the skin of human [8], and to be responsible for various cutaneous inflammatory disorders and skin cancers [9-10].

In 2000, we found first the in vivo detection and imaging by chemiluminescence (CL) method of the generated ROS in the skin of live mice following UVA-exposure [1]. Using this method, we found that •O₂⁻ is intrinsically generated in the skin and ¹O₂ is produced in the skin following UVA-exposure. On the basis of the results, we tried to find potent scavengers for ¹O₂ with this method. In 2002, we found that topical application of zinc chloride (ZnCl₂) to the skin of live mice reduced the formation of UVA-induced ROS generation [2], and in 2003, we revealed that oral administration or intraperitoneal (i.p.) injection of ZnCl₂ and bis(picolinato)Zn(II) (Zn(pic)₂) complex reduced the formation of UVA-induced ROS generation [3]. Zinc and copper consist of ROS-related enzymes such as superoxide dismutase (SOD), and play important roles in growth, the reproductive function, the skin tissue maintenance in human body. ZnGA and CuGA are supplemented in the breastmilk as food additives for the purpose of the trace metals strengthening. Recently, it has been revealed that these trace elements are deficient in not only youth but also adult, application of these complexes in...
addition to the breastmilk substitute became possible. In the present study, we examined whether or not ZnGA and CuGA given by topically application or oral administration reduce both intrinsically generated and UVA-induced ROS generation in the skin of HR-1 hairless mice.

Materials and Methods

Materials

The chemiluminescent probe, CLA (2-methyl-6-phenyl-3,7-dihydroimidazolo-[1,2-a]pyrazin-3-one), was purchased from Tokyo Kasei Organic Chemicals (Tokyo Japan). ZnGA and CuGA were gift from Fuso Chemical Co. (Tokyo, Japan) All another chemicals used were of the highest analytical grade.

Animals

Female HR-1 hairless mice (4 and 6 weeks old) were purchased from Simizu Experiment Materials (Kyoto, Japan) and were maintained in a light/dark cycle in the central animal facility of Kyoto Pharmaceutical University for experimental periods. The animals were given free access to standard mice chow and water for these periods.

Application methods

The hairless mice was anesthetized by i.p. injection of pentobarbital (50 mg/kg body weight), fixed on a heating pad. The skin surface on the abdomen of hairless mice was carefully cleaned three times with 50% ethanol. ZnGA 100μL (1.0 and 2.0 μmol/5 cm²) or CuGA (0.5 and 1.0 and 2.0 μmol/5 cm²) was applied on the skin surface of abdomen for 30 min and then was removed with 50% ethanol. The mice was applied 50% ethanol as control.

Administration methods

The hairless mice were devided into 5 groups, and each group had 5 mice.

Group 1: Physiological saline was orally administrated to female hairless mice for 14 days.

Group 2: ZnGA was orally administrated to female hairless mice at a dose of 150 mg Zn/kg body weight/day for 14 days.

Group 3: CuGA was orally administrated to female hairless mice at a dose of 50 mg Cu/kg body weight/day for 14 days.

Group 4: Both ZnGA and CuGA were orally administrated to female hairless mice at doses of 150 mg Zn/kg body weight/day and 35 mg Cu/kg body weight/day for 14 days.

Group 5: GA was orally administrated to female hairless mice at a dose of 1480 mg GA/kg body weight/day for 14 days.

Ultra-Low chemiluminescence detecting system

A luminograph, NightOWL Molecular Light Imager (Luminograph LB 981, Wallack Berthold, Germany) was used as a high-performance low-light imaging system for detection of luminescent emission (400-600 nm) [1-2]. The NightOWL possesses a peltier-air-cooled CCD camera for ultra-low light imaging with a high sensitivity and is designed for macro imaging such as the tissue or whole organisms. Its analytical performance is sufficient to evaluate the quantitative detection of ultra-low light chemiluminescence [13]. The luminescent signals on a photocathode in the image intensifier were detected as photons. The system was controlled by a DOS/V PC provided with software for quantitative image analysis (WinLight 32).

Measurements of chemiluminescence in the skin of hairless mice

The skin surface on abdomen of a hairless mouse was carefully cleaned three times with 50% ethanol. The hairless mice was anesthetized by i.p. injection of pentobarbital (50 mg/kg body weight), fixed on a heating pad, covered with a black cloth in which two holes of the same area (10 mm diameter, 78.5 mm²) were cut. The measured area were divided into two parts (left and right side in the abdomen skin) for comparative investigation of intrinsic and UVA-induced ROS generation. One area was covered with black tape not to be exposured by UVA, and the other was exposed to UVA light (18 J/cm²). After UVA irradiation, CLA was immediately applied to both two areas on the skin of mice, and the CL was measured. The quantity of ROS generated in the skin of mice was determined, as reported previously [1-2].

Statistical analysis

All experimental results are presented as the mean values ± standard deviations. Statistical treatment was performed by analysis of variance (ANOVA) at a 0.1%, 1% or 5% significance of difference.
Results

Topical application

Metallogluconates were topically applied for 30 min, and CL due to ROS generation was measured and quantified with a NightOWL. On topical application of CuGA, the CL intensity due to UVA-induced ROS generation in the skin of mice was significantly reduced, but topical application of ZnGA was unchanged (Fig.1).

![Fig. 1](image)

**Fig. 1** Suppressive effect of ZnGA (a) and CuGA (b) application on UVA-induced ROS generation in the skin of HR-1 hairless mice for 30 min

- □50% EtOH, □ZnGA (1.0µmol), ■ZnGA (2.0µmol), □CuGA (0.5µmol), □CuGA (1.0µmol), ■CuGA (2.0µmol)

Significance : *p>0.05, **p>0.01 vs control

Oral administration

ZnGA, CuGA, ZnGA+CuGA, GA and saline were given by daily oral administrations for 14 days. Doses were determined by LD₅₀ values of metallogluconates. LD₅₀ value of ZnGA is reported to be 375 mg Zn/ kg body weight [14], and that of CuGA is 175 mg Cu/ kg body weight. After 1 day of the last administration of

![Fig. 2](image)

**Fig. 2** Suppressive effect of oral metallogluconates and GA supprementations for 14 days in the skin of HR-1 hairless mice under UVA irradiation (n=3-5)

- ■ZnGA, ▲CuGA, △ZnGA+CuGA, ◇GA

Significance : *p<0.05 vs control

ZnGA, CuGA and ZnGA+CuGA, CL intensity due to ROS generation under UVA irradiation was significantly reduced, but after 3 and 7 days the suppressive effects disappeared (Fig.2).

Discussion

In our previous research [3], we reported that on i. p. and oral administration of ZnCl₂ and Zn(pic)₂, the CL intensity due to UVA-induced ROS generation in the skin of mice was significantly reduced. Furthermore, Zn(pic)₂ complex exhibited a persistent suppression of UVA-induced ROS generation for longer-term periods rather than the ionic form (ZnCl₂) did. Zn has been used as an anti-inflammatory or antioxidative agent for many years [15]. In this research, we found that metallogluconates significantly reduced the CL intensity due to UVA-induced ROS generation in the skin of mice. At present, the zinc compounds used for supplementation are zinc oxide and zinc sulfate [16], however, their uses have several disadvantages. Zinc sulfate modifies food sensorial characteristics, rendering the flavor of food unpalatable. Zinc oxide is poorly absorbed [17], and it precipitates in the nutritional matrix when the compound is used for fortify liquid foods. While, gluconic acid as a ligand of metallogluconate is used as a food additive and improves taste and smell of foods. In oral administration of zinc sulfate in rats, the maximum blood level (Tmax) was reported to be one hr, but Tmax of ZnGA on oral administration in rats was 30 minutes [14]. Epidermal zinc concentration was reported to increase for 72 hr after oral supplementation of ZnGA in man [18]. Because ZnGA was absorbed earlier than the ionic Zn, we suggested that suppressive effect of oral supplementation of ZnGA disappeared at three days after the last administration. Metallogluconates are soluble in water, therefore, it is suggested that the excretion of metallogluconates is earlier than that of Zn(pic)₂, indicating the importance of daily intake of ZnGA.

At present, it is difficult to explain the reason why orally administration Zn and Cu compounds reduce ROS in the skin of mice under UVA exposure, where many factors contribute in the experimental systems. Zinc and copper has been known to induce the synthesis of metallothioneine (MT), which is a cystein-rich-protein, as a Zn-dependent antioxidant in several organs involving the skin of animals [19]. We try to clarify the mechanism for the antioxidative properties of Zn and Cu including the induction of MT in the skin.
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References


