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Oral nanotherapeutics: Redox nanoparticles attenuate ultraviolet B radiation-induced skin inflammatory disorders in Kud:Hr- hairless mice

Chitho P. Feliciano [a, b], Yukio Nagasaki [a, c, d, *]

[a] Department of Materials Science, Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8573, Japan
[b] Radiation Biology Research Group, Biomedical Research Section, Atomic Research Division, Philippine Nuclear Research Institute, Department of Science and Technology (PNRI-DOST), Commonwealth Avenue, Diliman, Quezon City, Philippines
[c] Master's School of Medical Sciences, Graduate School of Comprehensive Human Sciences, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8573, Japan
[d] Satellite Laboratory, International Center of Materials Nanoarchitectonics (WPI-MANA), National Institute for Materials Science (NIMS), University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8573, Japan

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ABSTRACT

The active participation of an anti-inflammatory drug in the biological pathways of inflammation is crucial for the achievement of beneficial and therapeutic effects. This study demonstrated the development of redox nanoparticles that can circulate in the blood at significantly high levels, thus increasing their efficacy as an oral treatment against the deleterious effects of reactive oxygen species (ROS) in an *in vivo* inflammatory skin model. To confirm the blood bioavailability of the nanoparticles, mice were injected with the nanoparticles solution (RNP^N) via oral gavage. Using electron spin resonance and radioactive labeling techniques, the blood circulation of the redox polymer that forms the nanoparticles was confirmed 24 h after oral administration. This contrasted with its low molecular weight counterpart (NH_2-TEMPO), which peaked 15 min post injection and was found to be cleared rapidly within minutes after the peak. We then tested its efficacy in the inflammatory skin model. Kud:Hr-hairless mice were irradiated with UVB (302 nm) to induce skin damage and inflammation. Throughout the entire period of UVB irradiation, RNP^N was administered to mice by free drinking. NH_2-TEMPO was used as the control. The results showed that oral supplementation of RNP^N significantly improved the therapeutic effects of the core nitroxide radical compared with its low molecular weight counterpart. Furthermore, RNP^N significantly reduced UVB-induced skin aging, epidermal thickening, edema, erythema, skin lesions, and various pathological skin inflammatory disorders *in vivo*. From the obtained data, we concluded that the use of long-circulating redox nanoparticles (RNP^N) provided an effective treatment against the damaging effects of excessive ROS in the body.

1. Introduction

Inflammation is attributed to many pathological processes in the body. Clinical and experimental results have suggested that inflammation plays a crucial role in the progression of various diseases. Acute and chronic inflammation can be induced by ultraviolet (UV) rays from the sun. Much of the mutagenic and carcinogenic actions of sunlight have been attributed to the UVB portion of the solar spectrum. Prolonged and frequent exposure to UVB radiation may cause inflammation of the skin, which is characterized by edema and erythema [9,17,23]. Additionally, the damaging effects of UVB exposure accelerate skin aging. Reactive oxygen species (ROS) generated by UVB exposure play a substantial role in these inflammatory responses. UVB is considered as a mutagen and cytotoxic to the skin cells that causes its damages and aging [9,10]. To prevent ROS-induced inflammation, versatile antioxidants such as vitamins C and E, synthetic compounds, and phytochemicals have been investigated. However, these low molecular weight (LMW) antioxidants internalize into healthy cells, which results in the dysfunction of important redox reactions, such

* Corresponding author. Department of Materials Science, Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8573, Japan.
E-mail address: happyhusband@nagalabo.jp (Y. Nagasaki).

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as the electron transport chain [22]. To prevent such undesired adverse effects, a novel strategy must be developed. In addition to the prevention of the adverse side effects of LMW compounds, antioxidants with a long-term effect are preferable to prevent the sunlight-induced skin damage, aging, and inflammation that occur because of excessive exposure to ultraviolet radiation. To meet this objective, we have designed and developed self-assembling polymer antioxidant nanotherapeutics. One of the most important aspects of this synthesis is the covalent conjugation of the nitroxide radical compounds, which have the capacity to effectively eliminate ROS, into the hydrophobic segment of the amphiphilic block copolymers. The nitroxide radical-containing polymer spontaneously forms a polymeric micelle in aqueous media, named redox nanoparticles (RNP^N), which has anti-inflammatory function that attenuates the damaging effects of UVB radiation. RNP^N were synthesized following the previously described methods [28, 24, 25]. Because the diameter of RNP^N was approximately 20–40 nm, as measured by dynamic light scattering (DLS) technique, the solution was completely transparent, and the mice could drink an amount equal to that of tap water when given free access to it. Kud:Hr-hairless mice were irradiated with UVB (302 nm) to induce skin damage and inflammation. Throughout the entire period of UVB irradiation, RNP^N were administered to mice via free access to drinking, and LMW-nitroxide NH_2-TEMPO was administered as control. The results showed that oral supplementation of RNP^N significantly improved the therapeutic effects of the core nitroxide radical compared with LMW nitroxide radicals. RNP^N significantly reduced UV-induced skin aging, edema, erythema, lesions, and various pathological skin inflammatory disorders *in vivo*. These results suggested that oral supplementation of RNP^N provided an effective systemic approach for the protection of skin against the harmful effects of ultraviolet radiation.

2. Materials and methods

2.1. Design, synthesis, and characterization of RNP^N

The design, synthesis, and characterization of RNP^N can be found in our previously published paper [5]. Here, we used the same RNP^N which was produced in the same production batch that we have used and analyzed previously.

2.2. Animals and treatment groups

Four-week-old female Kud:Hr-hairless mice were obtained from Kyudo Co. Ltd, Japan, quarantined for microbial inspection for 3 weeks, and earmarked and acclimatized for an additional week in the caging facility of the University of Tsukuba. All mice were maintained under a controlled environment (12-h light/dark cycle) in special enclosed cages supplied with clean air. The mice received a sterilized rodent diet and drinking water to prevent opportunistic microbial infections. All the animal experiments were performed following animal protocols approved by the Animal Ethics Committee of the University of Tsukuba (Animal Plan Number: 15–328). The mice were randomly divided into four groups (n = 5–6 mice/group): control; no irradiation; UVB-irradiation; UVB-irradiation + NH_2-TEMPO; and UVB-irradiation + RNP^N.

2.3. Dose mapping of the UVB irradiator

Mice irradiation was performed using a handheld UV lamp (UVM-57; 6 W, 302 nm, 0.20 A, 100 V, 50–60 Hz; UVP, LLC, Upland, CA, USA). The UV intensity was measured routinely using a UV meter (UVX-31 radiometer; UVP, LLC, Upland, CA, USA). To assure the uniformity of the UVB irradiation, the intensity of UV radiation in the chamber was mapped. Briefly, the irradiation platform was raised to 13.5 cm from the bottom of the chamber. The radiometer (2.2 cm high) was set at 20 mW/cm^2 and placed on top of the platform. The distance of the UV lamp from the surface of the radiometer was approximately 6.5 cm. UVX readings were recorded in triplicate after stabilization (a minimum of 1 min) at different specified points in the chamber. The irradiation dose was calculated following the standard formula: Dose (mJ/cm^2) = exposure time (seconds) × UV intensity (mW/cm^2). The dose mapping results are shown in Fig. 1.

2.4. RNP^N oral administration and UVB irradiation of Kud:Hr-hairless mice

For the first ten days, 0.5 mg/mL of RNP^N in sterile water were administered via free-drinking one day before the start of UVB irradiation. Subsequently, the concentration of RNP was increased to 1 mg/mL on day 11 and was continuously provided throughout the UVB treatment period. The total amount of water and RNP intake per mouse were calculated accordingly (~10 mL per mouse per day). An equivalent concentration of the low molecular weight nitroxide radicals 4-amino-TEMPO (NH_2-TEMPO) was administered as the control.

Mice irradiation was conducted following a previously published protocol [6] with some modifications. Mice were irradiated at the center of the irradiation chamber where the dose intensity was found to be uniform: ~1.59 mW/cm^2 at ~6.5 cm distance from the source. Kud:Hr-hairless mice were placed in a mice holder with a wire mesh cover. The mice heads were covered to provide protection from UVB exposure. The UV lamp was situated 6.5 cm above the dorsal skin of the mice. Then, the mice were exposed with 120 mJ/cm^2 (2 times of the MED, minimum edematous dose) three times for the first week. For the four succeeding weeks (2nd to 5th), mice were irradiated only once per week using the same UVB dose (2-MED). The final irradiation was performed at the end of the 5-week treatment period, 24 h before sacrificing the mice.

Mice skin were evaluated and scored before they were sacrificed. The total accumulated UVB dosage was 0.96 J/cm^2 per mouse. While the total RNP^N intake was 330 mg per mouse over the 5-week period or approximately 10 mg per day. A graphical illustration of the drug administration and UVB irradiation treatments are shown in Fig. 1.

2.5. Skin evaluation

The skin condition of all mice was evaluated at the end of the treatment period. The mice were anesthetized with isoflurane and the dorsal skin was examined and photographed. UVB-induced skin aging and wrinkling were assessed as previously described [13]. The number of skin lesions was also counted. Dorsal skin and ear erythema were scored by using previously described guidelines [18]. Skin edema, in terms of skin-fold and ear thickness, was determined using a digital Vernier caliper.

2.6. Histological examination

After macroscopic grading and evaluation, the dorsal skin of the mice was harvested. Skin samples were placed in 10% neutral-buffered formalin for 1–2 days, embedded in paraffin, and cut into 5-μm thick sections. The sections were then stained with hematoxylin and eosin (H&E), examined, and photographed under a bright field microscope (Keyence BZ-X700).