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8-Oxo-2′-deoxyguanosine ameliorates UVB-induced skin damage in hairless mice by scavenging reactive oxygen species and inhibiting MMP expression

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ABSTRACT

Background: Skin is uniquely vulnerable to damage caused by reactive oxygen species (ROS), which are most commonly produced in response to ultraviolet (UV) light. ROS generated at injury sites play an important role in modulating the inflammatory response. Besides inhibiting Rac, 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG) has also shown notable antioxidant action.
Objective: We tested whether 8-oxo-dG could protect skin from UVB-induced damage by scavenging ROS.
Methods: HaCaT cells and hairless mice were irradiated with 15 and 180 mJ/cm^2 narrow-spectrum UVB, respectively. ROS generation was detected through incubation with DCFDA and confocal microscopy. Western blot analyses and immunohistochemistry were performed to verify the activities of ERK, JNK, p38, ATF-2, and c-Jun, and the expression of matrix metalloproteinases (MMPs), in UVB-irradiated HaCaT cells and murine skin. Hydrogen peroxide production and protein carbonyl concentrations were measured in UVB-damaged mouse skin. *MMP-1* and *MMP-9* expression in UVB-irradiated HaCaT cells was measured by quantitative reverse-transcription polymerase chain reaction (qRT-PCR).
Results: In UVB-irradiated HaCaT cells, 8-oxo-dG inhibited ROS production, subsequent activation of mitogen-activated protein kinase (MAPK), ATF-2, and c-Jun, and MMP expression. It also prevented UV-induced skin reactions in hairless mice, inhibiting the increase in protein carbonyl content, activation of MAPKs, ATF-2, and c-Jun, the increases in MMP-9 and -13 expression, and epidermal hyperplasia.
Conclusion: 8-oxo-dG can be considered an endogenous antioxidant and its potent antioxidant activity might be a beneficial property that could be exploited to protect skin from ROS-associated photodamage.

1. Introduction

Reactive oxygen species (ROS) generated in response to UV irradiation play an important role in modulating the inflammatory response in the skin under acute and chronic injury conditions [1]. One of the primary events in ROS-mediated inflammation is the activation of transcription factors. Nuclear factor (NF)-κB and activator protein (AP)-1 are transcription factors that are regulated by cellular redox levels and that regulate gene expression [2]. These factors regulate the expression of a huge range of extracellular signalling molecules responsible for inflammation, tissue remodelling, oncogenesis and apoptosis, processes that orchestrate many of the degenerative processes associated with ageing [3].

Matrix metalloproteinases (MMPs) can be broadly classified into four major classes according to substrate specificity and structural and expression characteristics: collagenases, which break down collagens (MMP-1 and MMP-8); gelatinases, which degrade denatured collagens (MMP-2, MMP-9); stromelysins, which have broad specificity; and membrane-bound MMPs, which are located mainly on tumour cells [4]. MMP activity is regulated at multiple steps, from gene transcription to enzyme activation. Regulatory sites in MMP genes (5′-flanking regions) contain an AP-1 regulatory element. Several growth factors and cytokines stimulate the expression of AP-1 transcription factors (Jun and Fos), which form dimers, bind to the AP-1 binding sites

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in MMP genes, and activate their expression [5]. Mitogen-activated protein kinases (MAPKs) such as extracellular signal-regulated kinases (ERKs), and stress-activated protein kinase/Jun N-terminal kinases (SAPK/JNKs) are known to regulate AP-1 transcription factors [6]. Generation of ROS is necessary for the stimulation of MMPs expression through MAPKs signalling pathways [7,8].

Many biomarkers of DNA damage, including point mutations and DNA adducts, have been used to verify the effects of carcinogen exposure [9]. 7,8-Dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG), a well-known nucleoside with an oxidatively modified base, is released when chemically damaged DNA undergoes exonuclease repair [10,11]. 7,8-Dihydro-8-oxoguanine (8-oxo-Gua) in DNA is known to be promutagenic since it promotes the incorporation of dATP instead of dCTP opposite this lesion during replication, inducing a GC to TA transversion [12]. Thus, 8-oxodG is mainly repaired through the base excision repair pathway initiated by oxoguanine DNA glycosylase (OGG1) [13,14]. However, a recent study found that 8-oxo-dGMP was not phosphorylated into 8-oxo-dGDP, but instead, dephosphorylated to 8-oxo-dG and confirmed that exogenous 8-oxo-dG was not directly incorporated into DNA [15]. This indicated that 8-oxo-dG has no chance to be salvaged for DNA synthesis.

Instead, we found that 8-oxo-guanosine triphosphate [8-oxo-GTP; 8-hydroxy-guanosine triphosphate (oh^8 GTP)] inactivates the small GTP-binding protein Rac, while guanosine triphosphate (GTP) activates it [16,17]. These novel findings strongly suggest that 8-oxo-GTP can function as a biologically active molecule. In addition, because it has the lowest oxidation potential of the four DNA bases, guanine is preferentially attacked upon exposure to ROS and is mainly transformed into 8-oxo-Gua [18]. Interestingly, 8-oxo-Gua is more reactive towards free radicals (or more susceptible to radical attacks) than the unmodified base [19–21]. However, these phenomena suggest that free 8-oxo-Gua in solution can scavenge attacking ROS and thus act as an antioxidant. A recent study confirmed this by demonstrating the antioxidant action of 8-oxo-dG in a stress-induced gastritis model [22].

In this study, we demonstrated that 8-oxo-dG potently inhibited ROS generation induced by UVB radiation in HaCaT cells, and accordingly diminished UVB-induced MAPK activation and AP-1 transcription. The antioxidant potential of 8-oxo-dG was also evaluated *in vivo* by determining its protective effect against UV-induced skin damage in mice.

2. Materials and methods

2.1. Antioxidant activity assay (oxidation of DCHF by Fenton reagent)

The antioxidant activities of 8-oxo-guanoisine (8-oxo-G), 8-oxo-dG and other antioxidants (Trolox, ascorbic acid, NAC and uric acid) (Sigma–Aldrich, St. Louis, MO, USA) were assessed by their ability to inhibit oxidation of 2′,7′-dichlorofluorescein (DCHF; Sigma–Aldrich) by Fenton reagent (H_2O_2/Fe^{2+}). In a 96-well plate, DCHF (50 μM) was incubated with 200 μl of PBS (pH 7.0) containing 6 mM H_2O_2 and 0.75 mM $FeCl_2$ in the absence or presence of each of the above antioxidants (concentration 1 mM) at 37 °C. The reactions were initiated by the addition of H_2O_2. During incubation in the dark (to prevent DCHF oxidation), fluorescence from oxidised DCHF was measured at 5 min intervals for 30 min at 485 nm (excitation) and 535 nm (emission) using an F-MAX 0200-1300 spectrofluorometer (Molecular Devices, Sunnyvale, CA, USA). Data were calculated as % of a control, which was measured in the absence of the antioxidants, and are presented as means ± SD ($n = 6$).

2.2. Antioxidant activity assay (oxidation of DCHF by peroxynitrite)

In a 96-well plate, DCHF (50 μM) was incubated for 60 min in a reaction (200 μl) containing 0.5 mM sodium peroxynitrite and 0.1 M sodium phosphate (pH 7) in the absence or presence of each of the antioxidants (concentration 1 mM) at 37 °C. The reaction was initiated by the addition of peroxynitrite. Data were calculated as % of a control, which was measured in the absence of the antioxidants, and are presented as means ± SD ($n = 6$).

2.3. Antioxidant activity assay (oxidation of LDL by H_2O_2/NaClO (hypochlorite))

Human low-density lipoprotein (LDL) is oxidised by singlet oxygen ($^1O_2$), which is generated by the reaction of H_2O_2 with NaClO (the Mallet reaction) [23]. Human LDL ($d = 1.019 - 1.063$) was supplied as a lyophilised powder, which was dialysed overnight at 4 °C against PBS containing EDTA (200 μmol/L). The dialysed LDL (200 μg protein/ml) was incubated for 20 h in a reaction (1 ml) comprising 90% D_2O containing 150 mM NaCl, 2.4 mM hydrogen peroxide, 2 mM sodium hypochlorite and 10 mM sodium phosphate (pH 7.4) at 37 °C in the absence or presence of 8-oxo-dG and other antioxidants. D_2O was used to enhance the lifetime of $^1O_2$. The extent of LDL oxidation was determined by fluorometry (excitation at 350 nm and emission at 433 nm) [24] using a spectrofluorometer (Varian AT/Cary Eclipse, USA). The fluorescence intensity of each reaction was compared to the fluorescence of LDL incubated with 100 μM EDTA and 20 μM butylated hydroxytoluene. Experiments were performed in triplicate and the results are presented as means ± SD.

2.4. Superoxide inhibition assay

Superoxide (O_2^-) generated by the oxidation of hypoxanthine by xanthine oxidase was supplied as a free radical source. Tris–HCl (50 mM; pH 8.0) containing 0.1 mM diethylenetriamine-pentaacetic acid (DTPA), 0.1 mM hypoxanthine, and 0.1 mM tetrazolium salt, plus various chemicals (concentration 1 mM) or enzymes (*e.g.* 8-oxo-dG, 2-dG and superoxide dismutase (SOD)), was incubated with 5 nM xanthine oxidase at 37 °C for 20 min. Then, absorbances at 450 nm were monitored using a spectrophotometer. Percent inhibition of superoxide production was determined using the following equation: % inhibition = [(corrected absorbance of sample) × 100/corrected absorbance of negative control] [25].

2.5. Cell culture and UV radiation

HaCaT, an immortal human keratinocyte cell line, was obtained from the Korean Cell Line Bank (Seoul, Republic of Korea). Cells were grown in Dulbecco's modified Eagle's medium (Lonza, Walkersville, MD, USA) containing 10% heat-inactivated foetal bovine serum (Lonza), and 100 mg/ml of penicillin–streptomycin (GIBCO, Grand Island, NY, USA). The cells were incubated in serum-free medium for 12 h before exposure to UV radiation. The medium was removed and the cells were then irradiated using a Vilber-Lourmat Bio-Link-BLX-E crosslinker (Vilber-Lourmat Inc., France). 8-Oxo-dG or 2-deoxyguanosine (dG) was applied for 1 h before exposure to UV radiation, and was then further applied to cells in serum-free medium until they were harvested.

2.6. Measurement of ROS generation

ROS generated as a result of exposure to UV radiation were visualised as described previously [22]. Briefly, UV-irradiated cells