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Purinergic signaling mediates oxidative stress in UVA-exposed THP-1 cells

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ABSTRACT

Ultraviolet A (UVA) radiation, the major UV component of solar radiation, can penetrate easily to the dermis, where it causes significant damage to cellular components by inducing formation of reactive oxygen species (ROS). On the other hand, extracellular ATP is released in response to various stimuli, and activates purinergic P2X7 receptor, triggering ROS production and cell death. Here, we examined the hypothesis that ATP release followed by activation of P2X7 receptor plays a role in UVA-induced oxidative cell damage, using human acute monocytic leukemia cell line THP-1. Indeed, UVA irradiation of THP-1 cells induced ATP release and activation of P2X7 receptor. Irradiated cells showed a rapid increase of both $p67^{phox}$ in membrane fraction and intracellular ROS. Pretreatment with ecto-nucleotidase or P2X7 receptor antagonist blocked the UVA-initiated membrane translocation of $p67^{phox}$ and ROS production. Furthermore, pretreatment with antioxidant or P2X7 receptor antagonist efficiently protected UVA-irradiated cells from caspase-dependent cell death. These findings show that autocrine signaling through release of ATP and activation of P2X7 receptor is required for UVA-induced stimulation of oxidative stress in monocytes.

1. Introduction

Ultraviolet (UV) irradiation is divided into three wavelength ranges: UVA, UVB, and UVC. UVA covers the wavelength range 320–400 nm, and is the major component of UV exposure encountered in daily life [1]. Unlike UVB that cannot penetrate much deeper than the epidermis of the skin, it is estimated that about 50% of incident UVA penetrates Caucasian skin all the way to the dermis, and is capable of irradiating blood leukocytes passing through skin capillaries [2,3,41]. Polderman et al. [3] reported that UVA caused cell death of peripheral blood mononuclear cells, and monocytes seemed to be the most sensitive to UVA among white cells. However, it is still unclear how monocytes are impaired by UVA irradiation.

UVA-induced damage occurs mainly *via* oxidative stress at the cellular level, and UVA is considered as the most important oxidizing agent in human skin [42]. Exposure of skin to UVA results in the generation of large amounts of intracellular reactive oxygen species (ROS), which directly or indirectly affect various cell signaling pathways, as well as augmenting various UV-induced cutaneous reactions including inflammation, photosensitivity and carcinogenesis [4,5]. Singlet oxygen ($^1O_2$), the major ROS, is formed in cells by energy transfer to molecular oxygen from the triplet excited state of endogenous

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chromophores. The endogenous chromophores and their locations in mammalian cells have not been identified, but many chromophores such as porphyrins, NAD (P) H, flavins, and other enzyme cofactors have been considered as photobiologically active [6]. It is well known that $^1O_2$ contributes to UVA-induced responses [7,43]. However, other ROS are involved in UVA-induced responses, because the lifetime of $^1O_2$ in cells is very short [8,9], and ROS are detected long after the end of UVA exposure [10]. Among UVA-induced ROS, superoxide ($O_2\cdot^-$) and hydrogen peroxide can destroy normal cell structure and function, resulting in tissue injury [11]. Godar [43] reported that UVA radiation triggers two different apoptotic pathways, mediated by $^1O_2$ or $O_2\cdot^-$. Thus, ROS produced subsequent to $^1O_2$ appear to play a key role in UVA-induced cellular responses.

The purinergic P2X7 receptor belongs to the family of purinoreceptors for ATP, and is expressed in various immune cells, including monocytes [12]. Upon ATP stimulation, P2X7 receptor opens a cation channel, which permits K^+ influx, and gradually forms a larger pore on the membrane [13]. Since activation of P2X7 receptor results in membrane blebbing [14], ROS production *via* NADPH oxidase activation [15], and apoptotic and/or necrotic cell death [16], P2X7 receptor appears to have an important role in regulating inflammation. Cells injured at sites of inflammation can passively release ATP in amounts sufficient to active P2X7 receptor. It was recently reported that agonists of different pattern recognition receptors trigger release of endogenous ATP and stimulate P2X7 receptor in human monocytes [17]. However, it is unknown whether UVA irradiation of monocytes evokes ATP release and subsequent activation of P2X7 receptor.

The objective of the present study was to examine whether autocrine signaling through ATP-P2X7 receptor is involved in UVA-induced ROS production and caspase-dependent death of human monocytes. Our results suggest that ATP released from cells activates P2X7 receptor, and this leads to formation of ROS. These findings indicated that autocrine ATP signaling contributes to UVA-induced cellular injury of monocytes.

2. Materials and methods

2.1. Reagents and antibodies

L-Ascorbic acid and probenecid were purchased from Wako Pure Chemical Industries (Osaka, Japan). MnTMPyP was purchased from Merck (Darmstadt, Germany). Caspase-3 antibody, caspase-9 antibody, goat horseradish peroxidase (HRP)-conjugated anti-rabbit IgG antibody and goat HRP-conjugated anti-mouse IgG antibody were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Purified mouse anti-p67 and purified mouse anti-flotillin-1 were purchased from BD Biosciences (San Jose, CA). Mouse anti-β-actin antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Unless otherwise stated, all other reagents were obtained from Sigma Aldrich (St. Louis, MO). All chemicals used were of the highest purity available.

2.2. Cell culture and irradiation

THP-1 human acute monocytic leukemia cells (American Type Culture Collection) were grown in RPMI 1640 medium (Wako), supplemented with 10% fetal bovine serum (HyClone Laboratories, South Logan, UT), penicillin (100 units/mL) and streptomycin (100 μg/mL) (Sigma–Aldrich) in a humidified atmosphere of 5% CO_2 in air at 37 °C.

The medium was removed, and cells were washed twice with phosphate-buffered saline (PBS) before UVA irradiation. The medium was replaced with RPMI Medium 1640 (without Phenol Red) (Life Technologies, Carlsbad, CA), and then UVA irradiation was performed. The UVA irradiation source was a black light (UVA) lamp (Sankyo Denki, Tokyo, Japan) with peak energy emission at 360 nm. The emitted dose was measured with a radiometer (UVX-36; UVP, Inc., San Gabriel, CA). No UVB component was detected with a UVX-31 sensor (UVP, Inc.). The irradiance at the sample level was about 2.5 mW/cm^2. The cooling control device was used to prevent excessive temperature rise by the generation of heat (Preset temperature is 21 °C).

2.3. Detection of ROS level

ROS were assayed with ROS indicator 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H_2DCFDA) (Molecular Probes, Eugene, OR). Cells were loaded with 10 μM carboxy-H_2DCFDA for 30 min at 37 °C and washed twice with PBS. After UVA irradiation, fluorescence was detected using a dual-scanning microplate spectrofluorometer (SpectraMax M5, Molecular Devices, Orleans, CA) with 485 nm excitation and 538 nm emission, or visualized with a fluorescence microscope (Olympus, Tokyo, Japan).

2.4. Immunoblotting

Aliquots of samples (10 μg per lane) were separated by means of 10% SDS-PAGE and transferred onto a PVDF membrane. Blots were incubated for 2 h at room temperature in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) (10 mM Tris–HCl, 100 mM NaCl, 0.1% Tween 20, pH 7.5) with 1% BSA and incubated at 4 °C overnight with purified mouse anti-p67 (1:1000), caspase-3 antibody (1:1000), or caspase-9 antibody (1:1000). Purified mouse anti-flotillin-1 (1:1000) and anti-β-actin antibody (1:1000) were used to confirm equal loading. Blots were washed with TBS-T, incubated with goat HRP-conjugated anti-rabbit IgG antibody (1:20,000) or goat HRP-conjugated anti-mouse IgG antibody (1:20,000) for 30 min at room temperature, and washed again with TBS-T. Specific proteins were visualized using ECL Western blotting detection reagents (GE Healthcare, Buckinghamshire, UK).

2.5. Cell fractionation

Cellular membrane fraction was prepared with the Mem-PER™ Eukaryotic Membrane Protein Extraction Reagent kit (Pierce Chemical, Rockford, IL) according to the manufacturer's instructions. Cells were washed with PBS twice and the cell pellet was lysed at room temperature