J. Rakuno Gakuen Univ., **25** (1) : 19～24 (2000)

Effects of Long-Wave Ultraviolet (UVA) Radiation on $MgCl_2$-Dependent Structural Transition of Chromatin in Isolated Chicken Liver Nuclei

Soichiro ARAI, Hiroshi NAKANISHI and Masanobu HAYASHI*

(May 2000)

Abstract

To investigate effects of long-wave ultraviolet (UVA) radiation on $MgCl_2$-dependent structural transition of chromatin in isolated chicken liver nuclei, the nuclei were aerobically irradiated with 200 kJ/m² of UVA at 5 mM $MgCl_2$ and the morphology of the irradiated nuclei was examined at various concentrations of $MgCl_2$. Phase-contrast micrographs showed that both sham- and UVA-irradiated nuclei were very granular in appearance at $MgCl_2$ concentrations of 2 to 50 mM. Sham-irradiated nuclei exhibited a homogeneous appearance with the exception of some prominent nucleoli at 1 and 100 mM $MgCl_2$. In contrast, UVA-irradiated nuclei retained a granular appearance at 1 and 100 mM $MgCl_2$. Scanning electron micrographs (SEM) of both sham- and UVA-irradiated nuclei fixed at 5 mM $MgCl_2$ revealed condensed chromatin of irregular size and shape which left empty spaces inside the nucleus. Structures of 30 to 60 nm in thickness were clearly observable in the condensed chromatin. Sham-irradiated nuclei fixed at 1 or 100 mM $MgCl_2$ revealed uniformly distributed granular-fibrillar structures, 30 to 60 nm in diameter. In contrast, even when nuclei were fixed at 1 or 100 mM $MgCl_2$, UVA-irradiated nuclei still exhibited condensed chromatin. In the present study, we showed the inhibitory effects of UVA radiation on the unfolding of condensed chromatin in isolated chiken liver nuclei using phase-contrast microscopy and SEM.

Key Words: chicken liver nuclei, chromatin structure, Mg^{2+}-dependent transition, UVA radiation

Introduction

In animals, prolonged sunlight exposure is associated with various pathological states, including erythemia, cataract, skin aging, and cancer. Long-wave ultraviolet radiation, ultraviolet A (UVA) radiation, of which the wavelength is from 320 to 400 nm, constitutes more than 90% of terrestrial UV solar energy on the earth's surface. UVA radiation causes cell death and mutation, although a much higher fluence of UVA raditation is necessary to induce these biological responses than that of ultraviolet B (UVB) radiation, from 290 to 320 nm, and ultraviolet C (UVC) radiation, from 200 to 290 nm[17,19,20]. UVA radiation has been reported to induce several types of DNA damage, such as strand breaks[10,12,16], base modification[8,9,15] and DNA-protein cross-links[11,13,14]. Because UVA rays are not directly absorbed by DNA and their biological effects depend strongly on the presence of oxygen, UV rays of this wavelength probably exert their effects through indirect mechanisms in which endogenous photosensitizers, such as NADH/NADPH and riboflavin, absorb UVA photons to generate reactive oxygen species (ROS)[7,18,20].

UVA radiation has also been shown to inhibit several biological processes, such as transcription in cultured human fibroblasts[5] and in isolated chicken liver nuclei[2], although few studies have focused on the effects of UVA radiation-induced DNA damage on biological processes. In the eukaryotic cell nucleus, DNA is complexed with histones and other proteins to form several hierarchical chromatin structures. It has been suggest-

Department of Veterinary Radiology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan
* Correspondence to Masanobu HAYASHI

ed that structural transition of chromatin from a compact inactive structure to a more extended open conformation is a prerequisite for a variety of biological processes, such as replication, transcription and DNA repair[6]. However, the effect of UVA radiation on the structural transition of chromatin remains unknown. We have previously shown in isolated chicken liver nuclei that while a significant fraction of chromatin exists as condensed chromatin at $MgCl_2$ concentrations of 2 to 50 mM, unfolding of condensed chromatin into the discrete 30-nm chromatin fibers occurs when $MgCl_2$ concentrations either decrease below 2 mM or rise above 50 mM[3]. This $MgCl_2$-dependent structural transition allows us to examine the effects of UVA radiation on the unfolding of condensed chromatin in isolated chicken liver nuclei. In the present study, we found that UVA radiation has inhibitory effects on the unfolding of condensed chromatin into the 30-nm chromatin fibers.

Materials and Methods

Isolation of Chicken liver nuclei

Chicken liver nuclei were isolated from 8- to 12-week-old White Leghorn chickens as described previously[2]. Briefly, small pieces of freshly resected chicken liver were homogenized in 2.15 M sucrose-TKMP buffer containing 10 mM Tris-HCl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$, and 0.2 mM phenylmethylsulphonyl fluoride (PMSF), in a Waring blender. The homogenate was mixed with an equal volume of 2.15 M sucrose-TKMP buffer, filtered through four layers of gauze, and then centrifuged at 35,000 X g for 60 min at 0℃. The pellets were suspended in 0.34 M sucrose-TKMP buffer, and the suspensions were centrifuged at 600 X g for 2 min. The nuclear pellets were washed twice with TM buffer (20 mM Tris-HCl, pH 7.5 and 5 mM $MgCl_2$) and resuspended in a small volume of TM buffer.

Irradiation of the nuclei

Nuclear suspensions were adjusted to 2 X 10^7 particles/ml in 2 mM Tris-HCl (pH 7.5) containing 5 mM $MgCl_2$ and exposed to UVA rays in glass vessels using a model 100A Blak Ray UV lamp (Ultraviolet Products, Inc., CA, USA) at a fluence rate of 65 J/m²/sec. The fluence rate was measured with a UVX radiometer equipped with a UVX-36 sensor (Ultraviolet Products, Inc., CA, USA). More than 95% of the ultraviolet emission from this lamp was at a wavelength of 365 nm. After being bubbled with air or nitrogen (N_2) gas for 5 min, the nuclear suspensions were irradiated through a continuous stream (about 4 l/min) of air or N_2 gas passed over the liquid surface. During irradiation, the suspensions were stirred gently with a small magnetic bar, and the vessel was maintained at 4℃ in a circulating bath. Sham-irradiated nuclei were treated in the same manner but were not irradiated.

Phase-contrast microscopy

After irradiation, the nuclei were collected by centrifugation at 600 X g for 5 min and resuspended in TM buffer to a concentration of approximately 1 X 10^8 particles/ml. The nuclear suspension was diluted 20-fold into 20 mM Tris-HCl (pH 7.5) -$MgCl_2$ solution prepared to give the desired concentration of $MgCl_2$, placed on a glass slide, and then observed with a phase-contrast microscope.

Scanning electron microscopy (SEM)

After irradiation, the nuclei were collected at 600 X g for 5 min and resuspended in 25 mM triethanolamine (TEA) buffer (pH 7.5) containing 5 mM $MgCl_2$. The nuclear suspension was diluted 20-fold into 25 mM TEA buffer prepared to give the final $MgCl_2$ concentraiton of 1, 5 or 100 mM. After incubation of the nuclear suspension on ice for 1 min, the nuclei were fixed with 0.2% glutaraldehyde for 30 min at 4℃, and then with 1.8% glutaraldehyde for 1 hr at 4℃. The fixed nuclei were encapsulated in gelatin to reduce the loss of nuclei during dehydration, freeze-fracturing, and then critical point drying as described previously[2]. The gelatin blocks containing the nuclei were conductive-stained with 2% tannic acid and 2% osmium tetroxide, dehydrated in a graded series of ethanol, and then freeze-fractured with a razor blade in liquid nitrogen to expose the nuclear interior. Finally they were

critical point dried with liquid carbon dioxide, and sputter-coated with gold (5–10 nm in thickness). The specimens were examined under a JEOL JSM-T300 scanning electron microscope.

Results and Discussion

Isolated chicken liver nuclei were aerobically irradiated with 200 kJ/m² of UVA in 2 mM Tris-HCl (pH 7.5) containing 5 mM $MgCl_2$, and the morphology of the irradiated nuclei was examined in 20 mM Tris-HCl (pH 7.5) containing the indicated concentrations of $MgCl_2$ by phase-contrast microscopy. As shown in Fig. 1, both sham- and UVA-irradiated nuclei were very granular in appearance at $MgCl_2$ concentrations of 2 to 50 mM. Sham-irradiated nuclei exhibited a homogeneous appearance with the exception of some prominent nucleoli at $MgCl_2$ concentrations of 1 and 100 mM. In contrast, UVA-irradiated nuclei retained a granular appearance at $MgCl_2$ concentrations of 1 and 100 mM.

Sham- and UVA-irradiated nuclei were fixed with glutaraldehyde in the presence of 1, 5 and 100 mM $MgCl_2$ for SEM observation. Both sham- and UVA-irradiated nuclei fixed at 5 mM $MgCl_2$ revealed condensed chromatin of irregular size and shape which left empty spaces inside the nucleus (Fig. 2c and d). Structures of 30 to 60 nm in thickness were clearly observable in the condensed chromatin. Sham-irradiated nuclei fixed at 1 or 100 mM $MgCl_2$ revealed uniformly distributed granular-fibrillar structures, 30 to 60 nm in diameter (Fig. 2a and e). In contrast, even when fixed at 1 or 100 mM $MgCl_2$, UVA-irradiated nuclei still exhibited condensed chromatin (Fig. 2b and f). Because the measured diameters of these structures are probably thicker than those of their actual diameters because of a gold coating (5–10 nm in thickness), these structures most probably correspond to the 30-nm chromatin fibers.

A significant fraction of chromatin exists as condensed chromatin at $MgCl_2$ concentrations of 2 to 50 mM in the isolated nuclei and unfolding of condensed chromatin into discrete 30-nm chromatin fibers occurs when $MgCl_2$ concentrations either decrease below 2 mM or rise above 50 mM[3]. Thus, the $MgCl_2$-dependent transition of chromatin structure allows us to examine the effects of UVA radiation on the unfolding of

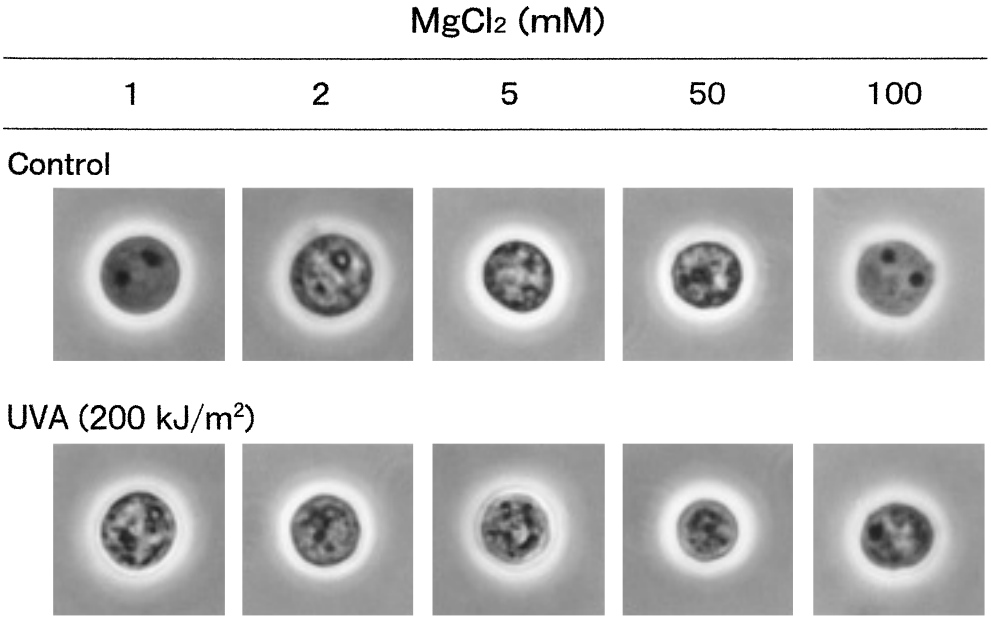

Fig. 1 Phase-contrast micrographs of UVA-irradiated chicken liver nuclei.
After isolation of the chicken liver nuclei, nuclei were sham-irradiated (control) and UVA-irradiated (200 kJ/m²) under aerobically at 5 mM $MgCl_2$ and observed at the indicated concentration of $MgCl_2$ by a phase-contrast microscope.

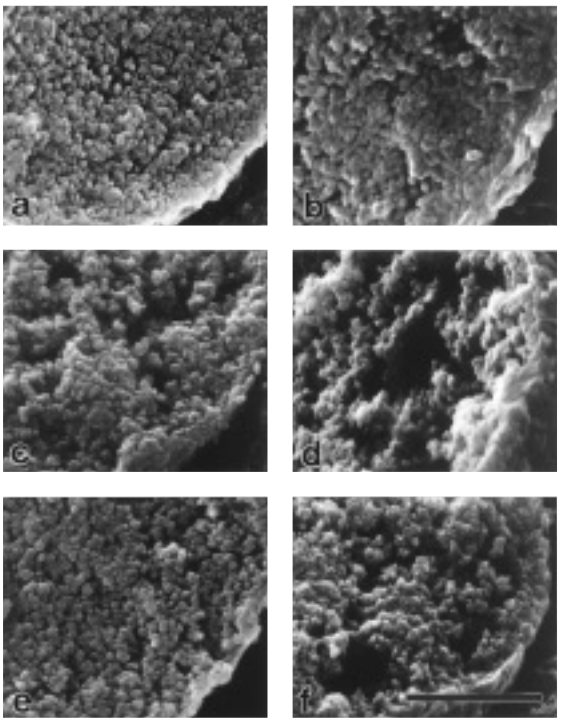

Fig. 2 Scanning electron micrographs of the surfaces of freeze-fractured UVA-irradiated chicken liver nuclei.
After isolation of the chicken liver nuclei, nuclei were sham-irradiated (a, c and e) or UVA-irradiated at 200 kJ/m² under aerobic conditions (b, d and f) in the presence of 5 mM $MgCl_2$. Nuclei were fixed with glutaraldehyde in the presence of $MgCl_2$ at concentrations of 1 mM (a and b), 5 mM (c and d) and 100 mM (e and f). All electron micrographs are taken at the same magnification. The scale bar denotes 1 μm.

condensed chromatin in isolated nuclei. Recently, we reported that UVA radiation of isolated nuclei inhibits the $MgCl_2$-dependent unfolding of condensed chromatin by measuring the $MgCl_2$-dependent changes in the relative turbidity of nuclear suspensions[4], since the turbidity of nuclear suspensions is correlated with the nuclear morphology[1,3]. In the present study, we confirmed the inhibitory effects of UVA radiation on the unfolding of condensed chromatin by morphological analysis of isolated nuclei using phase contrast microscopy and scanning electron microscopy.

Several types of DNA damage, such as single- and double-strand breaks[17,20,21], base modification[10,12,16], and DNA-protein crosslinks[11,13,14] were induced in DNA by UVA radiation. It is thought that such DNA damage is produced by ROS, such as $^1O_2$ and hydroxyl radicals, generated by UVA radiation[20,21]. In the recent paper, we showed that DNA-protein crosslinks, not DNA strand breaks, are the main type of DNA damage responsible for the inhibition of structural transition of chromatin and that $^1O_2$ may be a primary ROS involved in the UVA radiation-induced inhibition of $MgCl_2$-dependent transition of chromatin structure[4].

It has been suggested that structural transition of chromatin from a compact inactive structure to a more extended open conformation is a prerequisite for a variety of biological processes such as replication, transcription and DNA repair[6]. In the present study, we found that UVA radiation has inhibitory effects on the unfolding of condensed chromatin. These inhibitory effects may be responsible for deleterious UVA radiation-induced effects on several biological processes.

Acknowledgement

This work was supported in part by a Grant-in-Aid to Cooperative Research from Rakuno Gakuen University, 1998-1.

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要　　約

ニワトリ肝臓単離核中におけるクロマチンの $MgCl_2$ 依存性の構造遷移に対する長波長紫外線（UVA）照射の影響を検討するために核を空気存在下，5 mM の $MgCl_2$ 条件下で 200 kJ/m² の UVA

線量で照射し，照射核の形態を種々の $MgCl_2$ 濃度条件下で解析した。$MgCl_2$ が 2〜50 mM の濃度範囲では偽照射核も UVA 照射核も核内に非常に粒子状の構造が存在することが位相差顕微鏡で観察された。偽照射核では 1 および 100 mM の $MgCl_2$ 存在下では，核小体を除いて核は均一な状態で観察されたが，UVA 照射核では 1 および 100 mM の $MgCl_2$ 存在下でも粒子状の状態が維持された。5 mM の $MgCl_2$ 条件下で固定された偽照射核ならびに UVA 照射核は共に走査型電子顕微鏡像で核内に空間が存在した状態で不規則な形と大きさの凝縮クロマチンとして観察された。この場合，太さ 30 から 60 nm の線維が明らかに凝縮クロマチン内に認められた。 1 および 100 mM の $MgCl_2$ 存在下で固定された偽照射核では直径が 30 から 60 nm の線維が均一に分散した粒子状あるいは線維状の構造として観察された。一方，UVA 照射核の場合は 1 および 100 mM の $MgCl_2$ 存在下で固定された場合でも凝縮クロマチン状態を示した。本研究で私共は位相差顕微鏡と走査型電子顕微鏡を用いてニワトリ単離核中における凝縮クロマチンの巻き戻しに対する UVA 照射の阻害効果を示した。