Journal of Steroid Biochemistry & Molecular Biology 135 (2013) 24–29

Contents lists available at SciVerse ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Effects of glucocorticoid on human dermal papilla cells *in vitro*

Soon-Jin Choi[a,b,c,1], A-Ri Cho[a,b,c,1], Seong-Jin Jo[a,b,c], Sungjoo Tommy Hwang[d], Kyu Han Kim[a,b,c], Oh Sang Kwon[a,b,c,*]

[a] *Department of Dermatology, College of Medicine, Seoul National University, Seoul, Republic of Korea*
[b] *Institute of Dermatological Science, Medical Research Center, Seoul National University, Seoul, Republic of Korea*
[c] *Laboratory of Cutaneous Aging and Hair Research, Biomedical Research Institute, Seoul National University Hospital, Seoul, Republic of Korea*
[d] *Dr. Hwang's Hair-Hair Clinic, Seoul, Republic of Korea*

ARTICLE INFO

Article history:
Received 17 May 2012
Received in revised form
14 November 2012
Accepted 26 November 2012

Keywords:
Glucocorticoid
Dexamethasone
RU486
Dermal papilla cells
Growth factors

ABSTRACT

Glucocorticoid (GC) is synthesized mostly in the adrenal gland and is secreted in response to stressful conditions. The stress-induced increase in systemic GC may mediate diverse types of cellular damage. However, the specific effects of GC on the dermal papilla cells (DPCs) of hair follicles remain unknown, although stress-related hair loss has increased significantly in recent years.

The objective of this study was to determine the effect of a synthetic GC, dexamethasone (Dex), on human DPCs *in vitro*. We evaluated the effects of Dex on cell proliferation, survival, and the expression of growth factors in DPCs. Dex treatment (1 μM) significantly reduced the number of viable cells and the expression of the Ki-67 protein, VEGF and HGF were downregulated following treatment of DPCs with Dex. Taken together, we concluded that Dex inhibits human hair growth by inhibiting both the proliferation of, and growth factors expression by, DPCs.

1. Introduction

Glucocorticoids (GCs) are potent inhibitors of keratinocyte proliferation and are effective anti-inflammatory compounds [1]. GCs can induce a G1 cell cycle arrest and programmed cell death in immature thymocytes, several leukemic cell lines and mature peripheral T lymphocytes [2,3]. The GC signal is primarily mediated by the glucocorticoid receptor (GR), which acts as a ligand-dependent transcriptional factor. In the absence of ligand, GR is localized in the cytoplasm and is bound to the heat-shock protein complex. Upon interaction with its ligand, GR translocates into the nucleus where it mediates a variety of effector mechanisms [4]. These include binding as a homodimer to positive or negative regulatory elements in the promoter regions of GC-responsive genes (GRE), which results in the modulation of gene transcription [5].

GCs are produced and secreted by the adrenal cortex. The levels of GCs are regulated by adenocorticotrophic hormone (ACTH), largely under the control of the hypothalamic–pituitary–adrenal axis (HPA). Stressful environmental changes activate the HPA axis and elevate the secretion of GCs [6]. The stress induced increase in GC secretion is known to produce neuronal cell damage [7]. The skin and hair are targets of HPA axis activity, and they also produce GCs through their own neuroendocrine systems that have been called the "peripheral" HPA axis [8]. The stress-related neurohormones – such as those produced by the HPA axis (including corticotropin-releasing hormone, ACTH, and GC) have been shown to be secreted by human hair follicles [9].

Dermal papilla cells (DPCs) are a group of specialized fibroblasts within the hair follicle bulb that have an essential function in the control of hair growth, not only in the hair cycle but also in the pathogenesis of certain conditions [10]. Therefore, factors affecting the functions of DPCs are important from a therapeutic viewpoint. However, the specific effects of GC on DPCs in hair follicles remain largely unknown. To explore the effects of GC on DPCs, we treated human DPCs (hDPCs) with dexamethasone (Dex), a synthetic GC, and monitored the molecular and cellular changes *in vitro*. Our results suggest that Dex reduced the proliferation of hDPCs in hair follicles, thus demonstrating the inhibitory effects of GC (cortisol) on hair growth.

2. Materials and methods

2.1. Chemicals and reagents

Dex, RU486 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma (St. Louis, MO). Dex and RU486 were dissolved in dimethylsulfoxide

* Corresponding author at: Department of Dermatology, Seoul National University, College of Medicine, 101 Daehak-ro, Chongno-Gu, Seoul 110-744, Republic of Korea. Tel.: +82 2 2072 1996; fax: +82 2 742 7344.
E-mail address: oskwon@snu.ac.kr (O.S. Kwon).
[1] These two authors contributed equally.

0960-0760/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jsbmb.2012.11.009

(DMSO) at 0.1 M and stored at −20 °C. For Western blot analysis, antibodies recognizing cyclin D1, GR and phosphorylated GR (pGR) were purchased from Cell Signaling Technology, Inc. (Beverly, MA). β-Actin antibody was purchased from Santa Cruz Biotech Inc. (Santa Cruz, CA).

2.2. Human dermal papilla cell culture

The methods used for isolating and culturing hDPCs have been described previously [11]. hDPCs were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Welgene, Daegu, Korea) containing 10% fetal bovine serum (FBS; Welgene), 10 ng/mL basic fibroblast growth factor (bFGF, R&D Systems, Minneapolis, MN, USA) and 1× antibiotic antimycotic solution (Gibco BRL, Gaithersburg, MD, USA) containing penicillin and streptomycin. Cells were incubated at 37 °C in a 5% CO_2 incubator. To study the effect of Dex, hDPCs were serum-starved for 24 h and then cells were exposed to Dex for the indicated time.

2.3. hDPC viability and proliferation assay

Cell viabilities were determined using MTT[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays. Briefly, DPCs at 1.5×10^4 cells/well were seeded into 96-wellplates, cultured for 24 h in serum-free DMEM, and then treated with vehicle (DMSO diluted 1:1000in serum-free DMEM) as a control, Dex(0.01–10 μM) for 2 days. Twenty microliters of MTT solution (5 mg/mL) was added per well and incubated for 3 h at 37 °C. The supernatant was removed, and the formazan crystals were dissolved in 200 mL of DMSO. The samples were incubated for 30 min at room temperature, and quantified by measuring optical density at 540 nm using an ELISA reader (TECAN, Salzburg, Austria) experiments were performed 3 times.

2.4. Immunofluorescence

For Ki67 labeling, hDPCs were incubated in 4 chamber slide dishes in the presence of 1 μM Dex for 24 h. hDPCs were fixed for 20 min with 4% paraformaldehyde, washed three times with PBS. The cells were incubated with anti-mouse Ki-67 (DAKO, Carpinteria, CA, USA) at 1:100. Secondary antibody used with Alexa Fluor 594-labeled goat anti-mouse (Invitrogen Japan, Tokyo, Japan). The nuclei were counterstained with DAPI. Pictures of four non-overlapping fields (200×) in each group were taken under a confocal microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY) and then Ki67(+) DPCs were counted. After normalizing the number of Ki67(+) cells to total number of DPCs (DAPI-positive), the proportion of Ki67 DPCs in each group is presented in Fig. 2B.

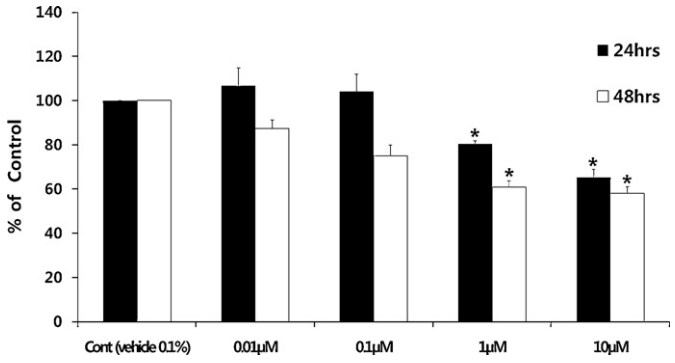

Fig. 1. Effect of dexamethasone (Dex) on cell proliferation in cultured human dermal papilla cells (hDPCs). Treatment of hDPCs with Dex (0.1–10 μM) resulted in a significant reduction in viable cells, as measured by an MTT assay. *$p < 0.05$, compared with the vehicle (0.1% DMSO) treated control.

2.5. Immunocytochemistry

For GR immunocytochemistry, the primary antibody was used with an anti-mouse GR (R&D systems, Minneapolis, MN, 1:100) and secondary antibody was used with a goat anti-rabbit biotinylated antibody (Vector Laboratories, Burlingame, CA, 1:200). AEC (Invitrogen, Carlsbad, CA) was used as the chromogen to visualize biotin/streptavidin-peroxidase complex. Cells were counterstained with Mayer's hematoxylin (Dako, Carpinteria, CA), mounted using aqueous mounting solution (Dako), and examined under an optical microscope at 100×.

2.6. Quantitative real time PCR and Western blot analysis

Total RNA was isolated from hDPCs using RNA iso Plus (Takara Bio Inc., Otsu, Shiga, Japan). We used 1 μg of total RNA for the cDNA synthesis reaction. The following primers for human genes were

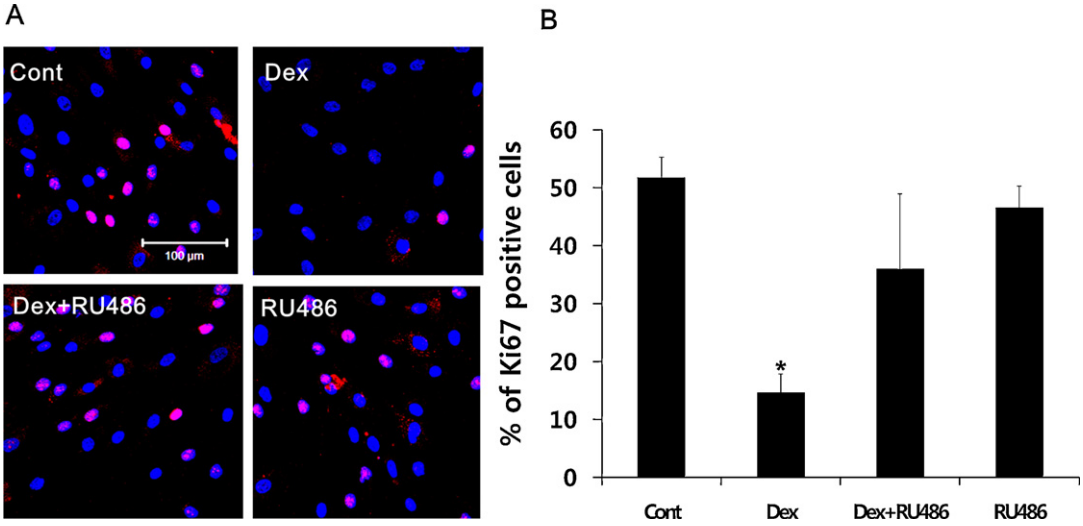

Fig. 2. Dex treatment decreased the number of Ki67-positive cells in hDPCs. (A) The effect of 1 μM Dex on proliferation for 24 h was measured by Ki67 expression and (B) The graph represents the percentage of Ki67(+) cells (red) in the total cell population. Nuclei are stained with DAPI. The GR antagonist RU486 (5 μM) blocks the antiproliferative activity of Dex. *$p < 0.05$ *vs.* vehicle-treated control. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)