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The preventive effect of vitamin D_3 on radiation-induced hair toxicity in a rat model

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Abstract

Our aim is to investigate the protective effect of vitamin D_3 especially from radiation-induced hair toxicity. A model of skin radiation injury was developed and a single fraction of 20 Gy Gamma irradiation was applied to the right dorsal skin of fourteen rats. All animals were randomly divided into 2 groups: Group I: irradiation alone ($n=7$) and Group II: irradiation and 0.2 μg vitamin D_3 given IM ($n=7$). Fifty days after post-irradiation rats were sacrificed. The outcomes were evaluated on the basis of histopathological findings and immunohistochemical staining for Vitamin D receptor (VDR) in skin and hair follicles. The number of hair follicles in the radiation field for the group of animals irradiated without pretreatment was significantly lower than outside of the irradiated area ($p=0.016$) as it is expected. Contrarily the number of hair follicles did not show significant difference in the pretreated group between the irradiated field and outside of the fields ($p=0,14$). Skin of the vitamin D_3 pretreated group demonstrated stronger immunoreactivity for VDR compared to irradiation alone group. These results indicate that administration of vitamin D_3 may protect hair follicles from radiation toxicity. Further clinical trials should be conducted to prove the preventive effect of vitamin D_3 as well as dosing and timing of the agent on radiation-induced alopecia.

Keywords: Calcitriol; Radiation toxicity; Hair

Introduction

Radiation-induced skin changes and associated hair loss are severe complications of radiotherapy. Unfortunately, in order to achieve a curative dose to the tumor, some degree of radiation damage in surrounding tissues is generally acceptable.

There are efforts to overcome these changes, especially for late skin toxicity but little is known to reduce hair loss. In some studies related with radioprotectors, systemic and topical applications of WR-2721 and PGE_2 enhanced hair regrowth following irradiation and provided some protection of hair follicles, and probably other tissues, lying under a radiation therapy field (Geng et al., 1992). In a study, water fraction of ginseng administration exerted a potent effect on the recovery of the hair follicles by its combined effects on proliferation and apoptosis of the cells in the hair follicle (Kim et al., 1998). Although these preclinical studies might suggest the clinicians to use these type of agents routinely, up to date no clinical data regarding favoring any of the agents were reported.

Chemotherapy-induced alopecia which is a result of damage of hair follicles is more extensively investigated than radiation-induced hair loss and some pharmacological compounds such as dexamethasone, immunophilic ligands, and cyclosporin are found to decrease the severity of toxicity in animal models (Paus et al., 1994). Furthermore there is also evidence that calcitriol prevents chemotherapy-induced alopecia (Paus et al., 1996; Schilli et al., 1998).

Vitamin D_3 which is pharmacologically known as calcitriol, is a steroid hormone best known for its activity in regulating calcium and bone metabolism (Smith et al., 1999). In addition, the active form of vitamin D_3 can act as a differentiating agent in normal tissues such as epidermis and hair follicles. It regulates epidermal keratinization by inducing terminal differ-

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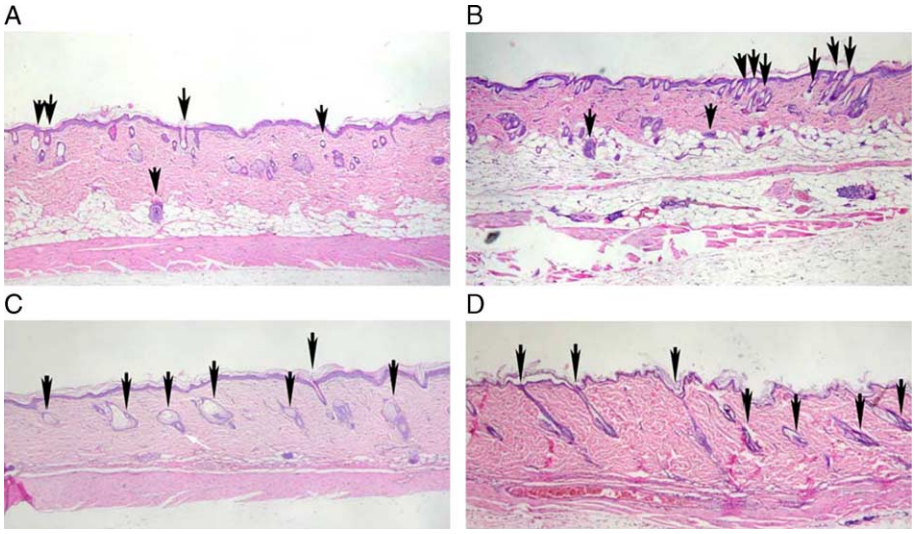

Fig. 1. Skin samples (original magnification ×40H&E). (A) Upper left—irradiated right dorsal skin; (B) upper right—irradiated right dorsal skin of the rats administered with vitamin D_3; (C) lower right—non-irradiated skin of the rat irradiated without pretreatment; (D) lower left—non-irradiated skin of the rat administered with vitamin D_3 (original magnification ×40H&E). The amount of terminal and/or vellus hair follicles in irradiated field of the rats administered with vitamin D_3 was not decreased.

entiation as well as keratinocyte proliferation (Reichrath et al., 1994). A specific receptor of vitamin D (VDR) belonging to the nuclear receptors family of ligand-activated transcription factors that coordinate physiological and developmental processes by regulating specific changes in gene expression (Mangelsdorf et al., 1995; Mangelsdorf and Evans, 1995) mediates the terminal differentiation (Haussler et al., 1998).

Vitamin D receptor (VDR) mediates the signaling by 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and is a member of the thyroid hormone/retinoic acid receptor subfamily of nuclear receptors that heterodimerize with the retinoid X receptor (RXR) on repeat hormone-responsive elements in the promoters of regulated genes (Mangelsdorf et al., 1995; Haussler et al., 1998). VDR is required primarily for the stimulation of calcium and phosphate absorption from the intestine to prevent rickets, the induction of the CYP24 enzyme that initiates the catabolism of $1,25(OH)_2D_3$, and progression of the normal hair cycle in mammalian skin (Haussler et al., 1998). Furthermore it was shown that vitamin D_3 receptor knockout mice display defects leading to postnatal alopecia (Reichrath et al., 1994; Hsieh et al., 2003; Alonso and Rosenfield, 2003).

Regarding the data concerning about the efficacy of vitamin D_3 and its analogs' regulatory effects on keratinocytes leading to hair growth and activity on chemotherapy-induced alopecia, we conducted this study to evaluate the protective effect of vitamin D_3 on hair follicle in an in-vivo model of skin radiation injury.

Material and methods

Animals

A total of 14 female, 12–14 weeks old and weighting 250–300 g Wistar albino rats were used in the study. All rats were housed 5 rats per cage in a specific pathogen-free environment in a temperature-controlled room (21 °C, relative humidity 50%–70%) with a 12-h light–dark cycle and were fed standard rat food and water ad libitum until evaluation. All rats were subjected to regular veterinary care. Fourteen rats were divided into two groups as Group I and II. Before irradiation, Group I did not receive any drug, whereas Group II received 0.2 μg of vitamin D_3 (calcitriol) (Calcijex amp, Abbott, Lot# 13021NJ) intramuscularly 2 h prior to irradiation. The rats were sacrificed 50 days after irradiation to evaluate the skin and especially hair toxicity. The study protocol was approved by the ethical council of the hospital.

Radiation therapy

All the rats with a 250 g of mean body weight were irradiated under general anesthesia provided with 10 mg/kg body weight xylazine hydrochlorate and 15 mg/kg of body weight ketamine hydrochloride diluted in sodium chloride injection. Irradiation was delivered by a Theratron ^{60}Co teletherapy unit. Rats were irradiated in supine position individually using an right anterior 3 × 2 cm single field with a depth of 2 cm with a single 20 Gy fraction dose. Radiation field was shielded with lead blocks to reduce the dose to the left hemithorax and left side of the thorax skin. Group II consisting of seven rats was injected with intramuscularly 0.2 μg of vitamin D_3 2 h before irradiation and was irradiated with the same radiation dose protocol as in Group I.

Table 1
The statistical correlation of the data obtained, between the irradiated and non-irradiated areas

	Number of hair follicles counted in a high power unit significance (p)
Group1a and 1b	0.01
Group2a and 2b (vitamin D treatment)	0.4
Group1a and 2a	0.15