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Advances in Brief

Protection from Chemotherapy-induced Alopecia by 1,25-Dihydroxyvitamin D_3

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Abstract

We have previously reported that several biological agents, when given simultaneously with cytosine arabinoside or cytoxan, will protect from cytosine arabinoside-induced but not from cytoxan-induced alopecia. In the present study we used the secosteroid 1,25-dihydroxyvitamin D_3 in a different timing schedule to protect from chemotherapy-induced alopecia. In three separate experiments, 0.2 μg of topical 1,25-dihydroxyvitamin D_3 protected rats from alopecia induced by etoposide, cytoxan, and an Adriamycin-cytoxan combination. In another experiment, 0.1 μg protected rats from etoposide-induced alopecia at the site of application. 1,25-Dihydroxyvitamin D_3 may offer a new and exciting approach to the prevention of chemotherapy-induced alopecia.

Introduction

Alopecia is often singled out as the most distressing side effect of cancer chemotherapy. In a recent study, 35 of 46 patients receiving chemotherapy ranked alopecia as a more important side effect than vomiting (1). Methods currently utilized to prevent chemotherapy-induced alopecia are unsatisfactory. Recently made observations in the young rat model have provided new insight into this problem and opened new avenues for further investigation. Thus we have demonstrated that ImuVert, a biological response modifier prepared from the bacterium *Serratia marcescens*, protected the animals from alopecia induced by cytosine arabinoside (2). In subsequent studies, similar protection from cytosine arabinoside-induced alopecia was observed with recombinant interleukin 1 β and more recently with epidermal growth factor and fibroblast growth factor (3–4). However, when used under similar conditions none of these agents offered protection from alopecia induced by cytoxan. In the clinical setting chemotherapy more often involves the use of combination regiments which frequently include alkylating agents. Accordingly, we have continued to explore in this model various compounds and ways to prevent alopecia from alkylating agents. In the work reported herein we demonstrate that pretreatment with the vitamin D_3 metabolite 1,25-$(OH)_2D_3$[2] protects rats from alopecia induced by cytoxan, VP-16, and cytoxan-Adriamycin combination.

Materials and Methods

Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA). Rats were fed and housed according to NIH guidelines. Daily weight gains were individually recorded, and the chemotherapy dose was adjusted accordingly. CTX was from Mead Johnson (Evansville, IN). VP-16 was from Bristol-Myers (Evansville, IN). ADM was from Adria Laboratories (Columbus, OH). Cholecalciferol (vitamin D_3) was purchased from Sigma (St. Louis, MO). 1,25-$(OH)_2D_3$ powder was a gift from Dr. Uskokovic (Hoffmann-La Roche, Nutley, NJ).

Topical Application of 1,25$(OH)_2D_3$. 1,25$(OH)_2D_3$ was dissolved in absolute ethanol and applied topically with an applicator. Control animals were similarly treated with the same amount of ethanol. Animals were then kept individually separated for a period of 3 h, following which the treated area was carefully washed with soap and water and dried. Treatment was given daily beginning on day 5 after birth and ending on day 10.

Chemotherapy. All chemotherapies were given i.p. and started at 11 days of age. CTX (35 mg/kg) was given for 1 day only. VP-16 (1.5 mg/kg) was given for 3 days. For the CTX plus ADM combination, CTX (25 mg/kg) was given for 1 day and ADM (2.5 mg/kg) for 3 days. At these doses neither CTX nor ADM alone will produce alopecia. Alopecia was recorded on the tenth day after the beginning of chemotherapy.

Results

A total of four experiments were carried out. In the first experiment, protection from cytoxan-induced alopecia was examined. The experimental group was pretreated with 0.2 μg of 1,25$(OH)_2D_3$ in 0.15 ml of absolute ethanol applied topically over the head and neck, and the control group received 0.15 ml of alcohol. All 10 rats in the control group became totally alopecic. In contrast, all animals in the experimental group were protected (Fig. 1*A*). The second experiment was carried out under similar conditions to examine protection from VP-16-induced alopecia. All 10 rats in the control group developed total body alopecia. In contrast, all rats in the experimental group were protected (Fig. 1*B*). The third experiment was designed to examine protection from alopecia induced by the cytoxan-Adriamycin combination. There were 11 rats in each group. Six rats in the control group developed alopecia over the head and neck, and five rats developed total body alopecia. In contrast, all rats in the experimental group were protected (Fig. 1*C*). In the fourth experiment, protection from VP-16-induced alopecia was similarly examined, except that the dose of 1,25$(OH)_2D_3$ was reduced to 0.1 μg in 0.1 ml absolute ethanol applied topically over the head area only. All 10 rats in the control group became completely alopecic. In contrast, all rats in the experimental group were protected primarily at the site of 1,25$(OH)_2D_3$ application (Fig. 2).

Discussion

Since our initial observations of the prevention of Ara-C-induced alopecia by ImuVert in the young rat model (2), our efforts have been directed at the search for agents that are effective against commonly used chemotherapeutic drugs with a high propensity to produce alopecia in the clinical setting, *i.e.*, cytoxan and Adriamycin. Our initial work began with the

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[2] The abbreviations used are: 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; VP-16, etoposide; CTX, cytoxan; ADM, doxorubicin.

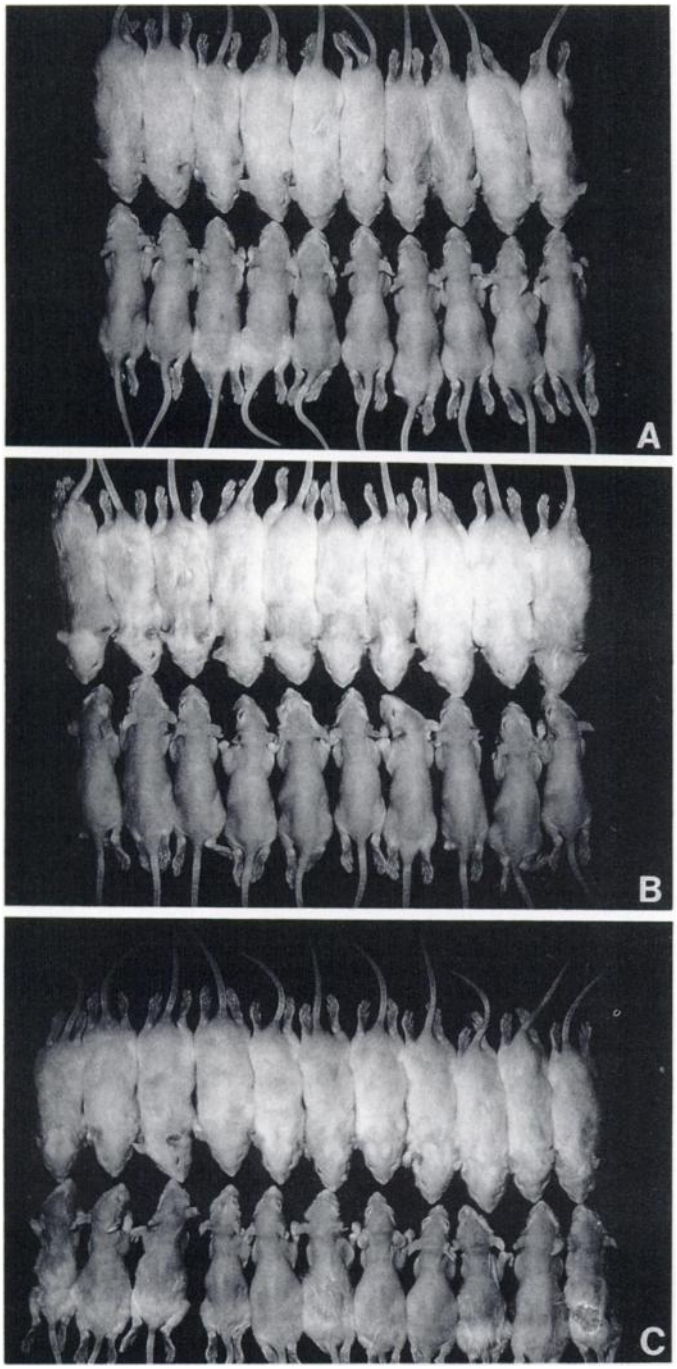

Fig. 1. For each experiment, 5-day-old rats were randomly divided into equal numbers. The experimental group of rats (*A*, *B*, and *C*, *top*) received 0.2 μg of $1,25(OH)_2D_3$ in 0.15 ml of absolute ethanol daily over the head and neck for 5 days. Control rats (*A*, *B*, and *C*, *bottom*) were similarly treated with 0.15 ml of absolute ethanol. One day after the last topical treatment, the rats from *A* were treated with CTX, rats from *B* with the VP-16 regimen, and rats from *C* with the CTX plus ADM regimen.

parent compound vitamin D_3. In experiments involving over 180 rats, the injection of 25–50 μg vitamin D_3 daily for 5 days prior to chemotherapy yielded excellent protection from alopecia induced by cytoxan, etoposide, or cytoxan-Adriamycin combination. However, since vitamin D_3 itself is biologically inactive and requires a two-step hydroxylation for activity, we directed further experiments to the study of the active metabolite $1,25(OH)_2D_3$ (5, 6). The topical route was chosen for its potential applicability to the clinical setting. Our data clearly demonstrate the ability of this metabolite, administered topically, to protect rats from alopecia induced by CTX, VP-16, and a CTX-ADM combination. It is of interest that protection from 0.2 μg $1,25(OH)_2D_3$ was not limited to the site of application but involved the entire body, suggesting systemic absorption. Thus, when the dose was reduced to 0.1 μg applied to the head area only, protection from VP-16-induced alopecia was less generalized and was more limited to the site of application.

The mechanism of protection by $1,25(OH)_2D_3$ remains uncertain at present. It could act by modulating the effects of other factors. For example, $1,25(OH)_2D_3$ has been shown to increase epidermal growth factor receptors on breast cancer cells and on a cell line established from rat calvaria (7, 8). We have demonstrated that pretreatment with epidermal growth factor partially protected from CTX- and CTX plus ADM-induced alopecia (9). On the other hand, $1,25(OH)_2D_3$ might protect hair follicles directly. Although $1,25(OH)_2D_3$ has been reported to be the principal vitamin D_3 metabolite in bone and mineral metabolism (10–11), recently a wide spectrum of biological effects have been described in several tissues (5, 6). Among these, the skin has been demonstrated to be a target for $1,25(OH)_2D_3$ (12). Specific receptors for $1,25(OH)_2D_3$ have been demonstrated in rat, murine, and human skin cells (13–15). Radioactively labeled hormone has been detected in layers of rat epidermis and associated hair sheaths (16). $1,25(OH)_2D_3$ has been shown to induce differentiation of murine epidermal keratinocytes (17). When cultured human keratinocytes are incubated with $1,25(OH)_2D_3$, there is a time- and dose-dependent stimulation of differentiation and inhibition of DNA synthesis (18). These latter findings led to clinical trials of topical $1,25(OH)_2D_3$ for psoriasis (12, 19–20).

These observations suggest that protection from chemotherapy-induced alopecia by $1,25(OH)_2D_3$ is related to its ability to stimulate differentiation of hair follicles, somehow rendering them resistant to drug-induced injury. Elucidation of the underlying mechanism should be of great interest. Whatever the mechanism, the observations recorded herein should offer a new and exciting potential approach to chemotherapy-induced alopecia in the clinical setting.

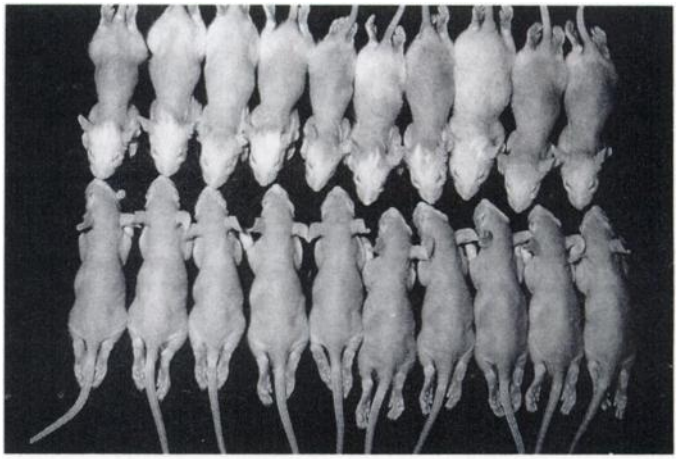

Fig. 2. Twenty 5-day-old rats were randomly divided into two groups of 10 rats each. The experimental group of rats (*top*) received 0.1 μg of $1,25(OH)_2D_3$ in 0.1 ml of absolute ethanol daily over the head only for 5 days. Control rats (*bottom*) were similarly treated with 0.1 ml of absolute ethanol. One day after the last topical treatment, all rats were treated with the VP-16 regimen.

The fact that topical $1,25(OH)_2D_3$ has already been used for psoriasis without significant side effects should facilitate early clinical trials.

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