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1,25-Dihydroxyvitamin D_3: A Novel Agent for Enhancing Wound Healing

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Abstract 1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), has diverse effects in a variety of tissues and cell types, including skin. Since $1,25(OH)_2D_3$ affects both fibroblast and keratinocytes, we evalauated the effect of $1,25(OH)_2D_3$ on wound healing. We investigated the effect of the topically applied $1,25(OH)_2D_3$ or vehicle on the healing of cutaneous wounds in rats in a blinded manner. Wound areas were measured by planimetry technique. Healing was expressed as the percentage of the original wound area that was healed. $1,25(OH)_2D_3$ at concentrations between 5 and 50 ng/day caused a dose-dependent acceleration of healing. Time course and specificity studies indicated that $1,25(OH)_2D_3$ specifically promoted healing between 1-5 days after wounding as compared with vitamin D (0.5 μg/day), which showed no significant improvement over control. Our results suggest that $1,25(OH)_2D_3$ and its analogues may be a new class of compounds that could be developed to enhance wound healing.

Key words: 1,25-dihydroxyvitamin D_3, wound healing, vitamin D

INTRODUCTION

The major physiologic function of 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], the active form of the vitamin D endocrine system, is to maintain extracellular fluid concentration of calcium and phosphorus within the normal range. $1,25(OH)_2D_3$ carries out this function primarily by stimulating the intestinal absorption of dietary calcium and phosphorus as well as the mobilization of calcium and phosphorus stores from bone. Apart from these classic calciotropic tissues involved in maintaining calcium homeostasis, namely intestine, bone, and kidney, a wide variety of tissues appear to be targets for the vitamin D endocrine system, including skin, activated T cells, lymphocytes, monocytes, and the parathyroid gland [Stumpf et al., 1979; Narbaitz et al., 1981; Tanaka et al., 1982; Bikle and Pillai 1993; Chen et al., 1993]. Skin is not only the target tissue for $1,25(OH)_2D_3$; it is also the site for the photosynthesis of vitamin D_3 [Holick et al., 1980]. Epidermal skin is also able to metabolize 25-hydroxyvitamin D_3 (25-OH-D_3) to $1,25(OH)_2D_3$ and 24,25-dihydroxyvitamin D_3 [$24,25(OH)_2D_3$], a regulated process [Pillai et al., 1988; Bikle et al., 1989]. Recent studies of the cultured human keratinocytes and fibroblasts have demonstrated that they possess receptors for $1,25(OH)_2D_3$ and that the hormone has potent effects on their proliferation and differentiation [Smith et al., 1986; Bikle et al., 1993]. Since $1,25(OH)_2D_3$ affects both fibroblasts and keratinocytes, two primary cell types involved in the healing of cutaneous wounds, we evaluated the possible effect of topically applied $1,25(OH)_2D_3$ on healing of full-thickness cutaneous wounds in rats.

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MATERIALS AND METHODS

Eight-week-old male Sprague-Dawley rats (Charles River, Wilmington, MA) were used for the experiment. All experimental protocols were approved by the Animal Research Review Committee at Boston University Medical School. The wounding technique described by Danon et al. [1989] was used with minor modifications. In brief, rats were anesthetized, and their backs were shaved by using a hair clipper. Four rats were randomly assigned in each experimental

group. Four full-thickness cutaneous wounds were made on each rat by punching two holes on each side of the back with a 6-mm sterile biopsy punch (Baker Cummins Dermatologicals, Miami, FL). Each rat received daily topical application of with one of the different doses (10 μl per wound per day) of vitamin D_3 (Sigma Chemical Co., St. Louis, MO) or $1,25(OH)_2D_3$ (a kind gift from Dr. M. Uskokovic of Hoffmann-La Roche, Nutley, NJ) or vehicle alone immediately after the surgery for 4 days in a blinded manner. Vitamin D_3 and $1,25(OH)_2D_3$ were dissolved in a solution consisting of 4% ethanol in saline. All wounds were allowed to heal without dressings. Wound areas were measured by planimetry technique as previously described [Danon et al., 1989]. Healing was expressed as the percentage of the original wound area that was healed. The results were presented as means ± SEM. The statistical significance was determined by Student's *t*-test. *P*-values of < 0.05 were considered significant.

RESULTS

Figure 1 demonstrates the effect of $1,25(OH)_2D_3$ at three different dosages, 0.5, 5, and 50 ng per rat per day, on the healing of full-thickness wounds. The most significant improvement in the rate of healing was observed on day 1. The percentage healing was 2 ± 6% in control rats and 14 ± 5%, 17 ± 5%, and 17 ± 4% in animals treated with a daily dose of 0.5 ng, 5 ng, and 50 ng $1,25(OH)_2D_3$, respectively. On days 2–4, $1,25(OH)_2D_3$ at both 5- and 50-ng dose levels continued to enhance the percentage healing as compared to the controls. Although the effect of $1,25(OH)_2D_3$ at 0.5-ng dose was greater than the controls over the treatment period, it did not reach statistically significant values.

To investigate the specificity of $1,25(OH)_2D_3$ effect on wound healing, we examined whether vitamin D_3 itself would promote the wound healing. Two different doses of vitamin D_3 (50 and 500 ng/rat/day) and one dose of $1,25(OH)_2D_3$ (5 ng/rat/day) were applied to wounded rats. As shown in Figure 2, vitamin D_3 at either concentration, had no significant effect on the healing of full-thickness wounds when compared to the control group, whereas $1,25(OH)_2D_3$-treated rats showed a 75% increase on the first day compared to the control group and remained significantly higher thereafter ($P < 0.05$).

DISCUSSION

$1,25(OH)_2D_3$ has diverse biological effects in a variety of cell types and tissues. The mechanism of action of $1,25(OH)_2D_3$ is believed to follow the classic steroid hormone mainly by regulating the activity of specific genes in the target tissues [Darwish and DeLuca, 1993]. The presence of the $1,25(OH)_2D_3$ receptor has been demonstrated in the skin cells, and a role of the $1,25(OH)_2D_3$ in regulating skin cell proliferation and differentiation has been suggested [Bikle and Pillai, 1993]. $1,25(OH)_2D_3$ is generally de-

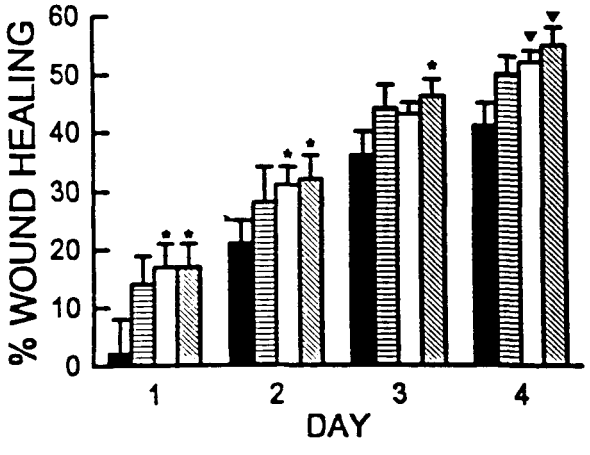

Fig. 1. Dose-response effects of $1,25(OH)_2D_3$ on wound healing in rats. Wounds were induced and measured as described under Materials and Methods. Values represent the means ± SEM. ▨, 50 ng of $1,25(OH)_2D_3$ per rat per day; □, 5 ng of $1,25(OH)_2D_3$ per rat per day; ▤, 0.5 ng of $1,25(OH)_2D_3$ per rat per day; ■, controls. *$P < 0.025$; ▼$P < 0.05$.

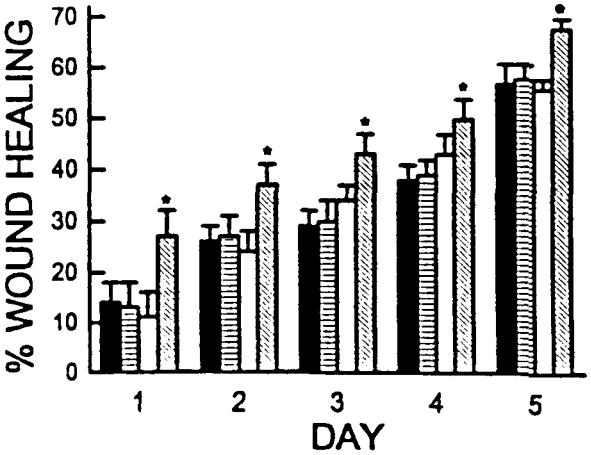

Fig. 2. Effects of vitamin D_3 and $1,25(OH)_2D_3$ on wound healing in rats. Values represent the means ± SEM. ▨, 5 ng of $1,25(OH)_2D_3$ per rat per day; □, 500 ng of vitamin D_3 per rat per day; ▤, 50 ng of vitamin D_3 per rat per day; ■, controls. *$P < 0.05$.

scribed as a potent inducer of cell differentiation and an inhibitor of proliferation. $1,25(OH)_2D_3$ and its analogues have been used as an effective treatment of psoriasis [Holick, 1994]. However, numerous reports in the literature revealed that the responses of keratinocytes to $1,25(OH)_2D_3$ differed significantly in different media and/or in different stages of cell confluence. The inhibitory effect of $1,25(OH)_2D_3$ on cellular proliferation can be even reversed [McLane and Katz, 1988]. Several reports have suggested that mitogenic effect of $1,25(OH)_2D_3$ depends on the presence of serum or growth factors [Dosquet-Bernard et al., 1986; Darwish and DeLuca 1993]. It has also been reported that in confluent cultures, $1,25(OH)_2D_3$ stimulated cell growth at all doses tested, ranging from 10^{-10} to 10^{-6} M [McLane and Katz, 1988].

$1,25(OH)_2D_3$ has also been shown to stimulate the synthesis of fibronectin, a prominent component of wound healing [Fransson and Hammar, 1992; Brown et al., 1993]. Using similar in vivo wound models, Nagelschmidt et al. [1987] and Cheng et al. [1988] reported that topical fibronectin treatment enhanced wound healing in rats. Thus, it is reasonable to suggest that at least some of the beneficial effects of $1,25(OH)_2D_3$ on wound healing may be the result of a direct effect of the hormone on extracellular matrix synthesis. In vivo, the beneficial effect of $1,25(OH)_2D_3$ on the regeneration of the liver has been reported [Ethier et al., 1990]. It has been demonstrated that vitamin D depletion retarded the normal liver regeneration process, while $1,25(OH)_2D_3$ treatment significantly promoted normal liver recovery in two-thirds of hepatectomized rats [Ethier et al., 1990]. The cellular and molecular basis for the effects of $1,25(OH)_2D_3$ on liver regeneration has not been established. It has been reported [Abe et al., 1984] that $1,25(OH)_2D_3$ (1) activates the maturation of macrophages, cells actively involved in wound healing process, and (2) increases the chemotactic and phagocytic responses of macrophages. It is also known that macrophages produce growth factors, such as interleukin-1 (IL-1) [Bhalla et al., 1986], that stimulate the proliferation of fibroblasts in vitro and that activated macrophages induce vascular proliferation. In addition, defect in macrophage function has been shown to be one of the cause of impaired wound healing and local injection of macrophages into wound areas promotes wound repair in an old mouse cutaneous wound model [Danon et al., 1989]. Furthermore, topical application of IL-1 has been shown to be effective in enhancing wound healing in vivo in some animal models. Thus, macrophages may also play an important role in the $1,25(OH)_2D_3$-mediated wound healing process. Alternatively, it is possible that the $1,25(OH)_2D_3$-induced wound repair may be mediated by the increased production of activated soluble tissue transglutaminase in the wounded inner layer of the skin, since it has been suggested that $1,25(OH)_2D_3$ stimulated both the soluble and particulate transglutaminases in cultured human keratinocytes [Lee et al., 1989; Bowness et al., 1988]. In summary, the time course and specificity studies indicated that $1,25(OH)_2D_3$ specifically promoted healing at 1–5 days after wounding as compared with vitamin D_3, which showed no significant improvement over control. Our results clearly demonstrate that $1,25(OH)_2D_3$ significantly accelerates wound healing.

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