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Report

Combined effects of 1,25-dihydroxyvitamin D_3 and tamoxifen on the growth of MCF-7 and ZR-75-1 human breast cancer cells

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Key words: breast cancer, 1,25-dihydroxyvitamin D_3, growth inhibition, MCF-7, tamoxifen, ZR-75-1

Summary

In the present study we assessed the effect of combined treatment with 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) and tamoxifen (TAM) on the growth of estrogen-responsive (MCF-7) and estrogen-dependent (ZR-75-1) human breast cancer cells. Both basal and 17β-estradiol (17β-E_2)-stimulated growth were studied. 1,25-$(OH)_2D_3$ (10^{-10} – 10^{-7} M) time- and dose-dependently inhibited basal growth of MCF-7 cells, with growth arrest at 10^{-7} M. Also, 17β-E_2-stimulated growth of MCF-7 and ZR-75-1 cells was inhibited by 1,25-$(OH)_2D_3$ in a time- and dose-dependent manner. TAM inhibited 17β-E_2-stimulated growth of both cell lines and at high concentration (10^{-6} M) it also inhibited basal growth of MCF-7 cells. 10^{-6} M TAM together with 1,25-$(OH)_2D_3$ resulted in a further inhibition of basal (MCF-7 cells) as well as 17β-E_2-stimulated proliferation (MCF-7 and ZR-75-1 cells) compared to the inhibition by these agents alone. TAM in combination with 10^{-7} M 1,25-$(OH)_2D_3$ resulted in growth arrest of 17β-E_2-stimulated growth of MCF-7 cells. The inhibition of basal and 17β-E_2-stimulated growth of MCF-7 cells was additive at early time points (4 days), but less than additive at later time points (8–10 days). It was demonstrated that with co-treatment of MCF-7 cells an equipotent inhibition of basal growth could be reached with lower concentrations of 1,25-$(OH)_2D_3$, compared to treatment with 1,25-$(OH)_2D_3$ alone. Studies with low concentrations ($< 10^{-7}$ M) of TAM revealed a partial estrogenic effect, i.e. stimulation of MCF-7 proliferation in the absence of 17β-E_2. This effect, which may resemble TAM-induced tumor flare, was completely prevented by co-treatment with a low concentration of 1,25-$(OH)_2D_3$ (10^{-9} M). Together, these results demonstrate the potent inhibition of breast cancer cell proliferation by 1,25-$(OH)_2D_3$ combined with TAM and indicate a potential benefit of combining these agents for the treatment of breast cancer.

Introduction

The seco-steroid hormone 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) is the biologically most active form of vitamin D_3 and plays an important role in the regulation of calcium homeostasis and bone metabolism. The effects of 1,25-$(OH)_2D_3$ are mediated via the vitamin D receptor (VDR) in target tissues such as bone, intestine, and kidney [1]. VDRs are not confined to the classical target tissues, but have also been demonstrated in a variety of cells and tissues not directly related to calcium homeo-

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stasis. Several studies have indicated that 1,25-$(OH)_2D_3$ induces differentiation and inhibits proliferation of hemopoietic, epidermal, and many cancer cells [1–3].

The VDR is present in most breast cancer cell lines and tumors [2, 4–7] and 1,25-$(OH)_2D_3$ has been shown to inhibit proliferation of breast cancer cells *in vitro* irrespective of their estrogen dependence [2, 7–11]. Studies *in vivo* have shown that 1α-hydroxvitamin D_3, which is converted to 1,25-$(OH)_2D_3$ in the liver, suppressed the growth of carcinogen-induced rat mammary tumors [7, 12]. These findings suggest a potential use of 1,25-$(OH)_2D_3$ for the treatment of breast cancer. However, high doses of the sterol are needed and it remains to be established whether 1,25-$(OH)_2D_3$ can produce long-term antitumor effects without unacceptable side-effects, like the development of hypercalcemia.

Until now the most effective endocrine therapy for estrogen receptor (ER)-positive breast tumors is treatment with synthetic antiestrogens, e.g. tamoxifen (TAM) [13]. The effect of TAM on breast cancer cells is believed to be predominantly mediated through competition with estrogen for the ER thereby attenuating the proliferative effect of estrogen [14, 15]. Although antiestrogens are very effective in ER-positive tumors, not all ER-positive tumors respond favorably, and during prolonged antiestrogen therapy even patients with responsive tumors can be expected to become eventually resistant [16]. For ER-negative tumors therapeutic choices are limited and therefore treatment with 1,25-$(OH)_2D_3$ may offer a new approach.

In the present study we assessed the effects of combined treatment with 1,25-$(OH)_2D_3$ and TAM on the growth of the ER-positive and VDR-positive human breast cancer cell lines MCF-7 and ZR-75-1. The cell lines have different growth characteristics. MCF-7 cells have partially escaped from hormonal regulation and are called estrogen-responsive. These cells are able to grow in steroid-free culture medium without further additions and are growth stimulated by 17β-E_2. The proliferation of ZR-75-1 cells is dependent on the presence of estrogens. We have studied the effects of co-treatment on basal as well as 17β-E_2-stimulated proliferation.

Materials and methods

Materials

17β-E_2, TAM, RPMI-1640 culture medium, ethidium bromide, DNA (type I, highly polymerized), and Ribonuclease A were purchased from Sigma Chemical Co., St. Louis, MO. 1,25-$(OH)_2D_3$ was generously provided by LEO Pharmaceuticals BV, Weesp, The Netherlands. Glutamine, penicillin, streptomycin, and foetal calf serum (FCS) were obtained from Life Technologies, Breda, The Netherlands. Trypsin was from Boehringer, Mannheim, Germany, Hank's balanced salts solution was from Imperial Laboratories, Andover, UK, and heparin solution (5000 IU/ml) was from Organon, Boxtel, The Netherlands.

Cell culture and growth experiments

MCF-7 and ZR-75-1 cells were generously provided by Dr. J.A. Foekens (Department of Endocrine Oncology, Dr. Daniel den Hoed Cancer Center, Rotterdam, The Netherlands). For proliferation studies, cells were seeded in six-well dishes at a density of 16,000 cells/cm^2 for MCF-7 and 32,000 cells/cm^2 for ZR-75-1 cells in phenol red-free RPMI-1640 medium supplemented with 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 24 mM sodium bicarbonate, and 10% FCS. The cells were allowed to attach for 6–7 h. Next, medium was changed to medium with 2% charcoal-treated FCS (CT-FCS) and the agents to be tested or vehicle (0.1% ethanol) were added. For MCF-7 cells medium and agents were replaced every 24 h. For ZR-75-1 cells medium and agents were in initial experiments replaced every 24 h, in later experiments every 3 days. Similar results were obtained with both incubation procedures. At the end of the incubation, medium was aspirated and DNA content was measured according to the ethidium bromide method of Karsten and Wollenberger [17]. Cells were scraped in 200 μl trypsin solution (0.5 mg/ml in Hank's balanced salts solution) and suspended in 1.5 ml PBS containing 0.1% Triton X-100 (PBS-Triton). Cells were sonicated during 2 × 5 sec using a

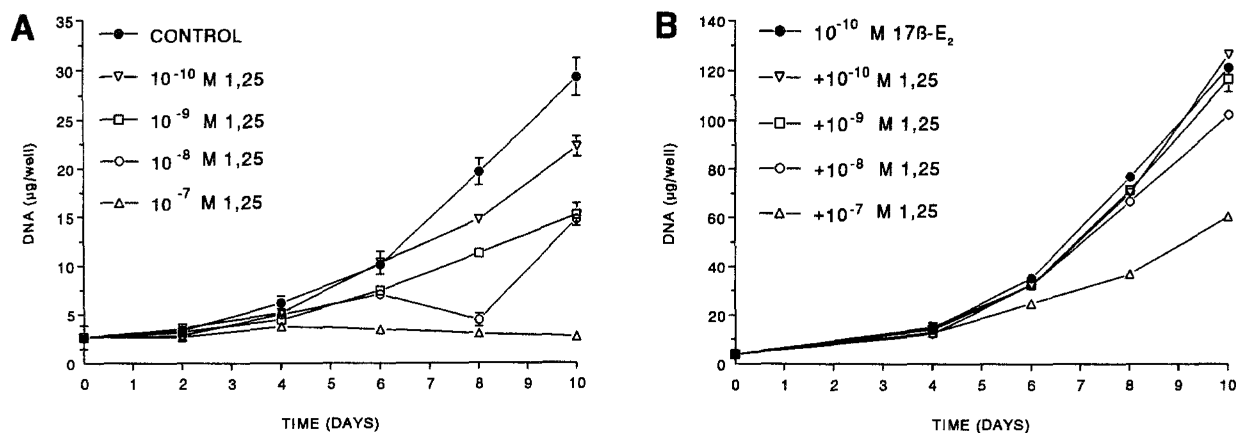

Fig. 1. Effect of $1,25\text{-}(OH)_2D_3$ on basal and $17\beta\text{-}E_2$-stimulated proliferation of MCF-7 cells. MCF-7 cells were cultured in 2% CT-FCS containing medium without (A) or with 10^{-10} M $17\beta\text{-}E_2$ (B), and a dose-range of $1,25\text{-}(OH)_2D_3$ (1,25). DNA was measured at the indicated times.

Soniprep 150 (Sanyo Gallenkamp PLC). Aliquots of the DNA samples were adjusted to 0.5 ml with PBS-Triton and incubated with 1 ml heparin solution (8.33 IU/ml in PBS) and 0.5 ml RNAse A solution (0.05 mg/ml in PBS) for 30 min at 37° C. Next, 0.5 ml ethidium bromide solution (0.025 mg/ml in PBS) was added and the samples were measured using a Perkin-Elmer LS-2B filterfluorimeter. Excitation and emission wavelength were 340 and 590 nm, respectively. A DNA stock solution (25 µg/ml inPBS-Triton) was used for a standard curve.

VDR levels of MCF-7 and ZR-75-1 cells, 28 ± 12 and 40 ± 9 fmol/mg protein respectively, were determined as described previously [18].

Data presented are representative for at least 2 independent experiments. All values are presented as mean ± SD of duplicate wells. Where no error bar appears the error is smaller than the symbol.

Results

Effect of $1,25\text{-}(OH)_2D_3$ on basal and $17\beta\text{-}E_2$-stimulated growth of MCF-7 cells

As shown in Fig. 1A, $1,25\text{-}(OH)_2D_3$ inhibited proliferation of MCF-7 cells in a time- and dose-dependent manner. The first significant effects were observed with 10^{-7} and 10^{-8} M $1,25\text{-}(OH)_2D_3$ after 4 days. After 6 days 10^{-9} M, and after 8 days 10^{-10} M $1,25\text{-}(OH)_2D_3$ also significantly inhibited cell growth. 10^{-7} M $1,25\text{-}(OH)_2D_3$ arrested cell growth, but after 4 and 10 days of incubation cell growth could be regained by adding fresh medium supplemented with 10% FCS (data not shown).

After evaluating the effect of $1,25\text{-}(OH)_2D_3$ on basal cell growth, we investigated whether $1,25\text{-}(OH)_2D_3$ was able to inhibit $17\beta\text{-}E_2$-stimulated proliferation. $17\beta\text{-}E_2$ stimulated cell growth very potently and maximal stimulation was already reached at 10^{-11} M. Comparison of Figs 1A and 1B demonstrates that $1,25\text{-}(OH)_2D_3$ inhibited basal growth more potently than $17\beta\text{-}E_2$-stimulated growth. With 10^{-7} M $1,25\text{-}(OH)_2D_3$ basal cell growth was arrested, whereas on day 10 this concentration inhibited $17\beta\text{-}E_2$-stimulated cells by only 50%. Also, with a lower concentration of $1,25\text{-}(OH)_2D_3$ (10^{-10} M) an inhibition of 23% of basal proliferation was observed, whereas it had no effect on $17\beta\text{-}E_2$-stimulated proliferation.

Effect of TAM on basal and $17\beta\text{-}E_2$-stimulated growth of MCF-7 cells

TAM had a biphasic effect on the proliferation of MCF-7 cells. 10^{-8} M TAM stimulated proliferation, whereas with 10^{-6} M an inhibition was observed (Figs 2, 3, 6). In contrast to the biphasic effect on basal growth, both concentrations of TAM inhibited $17\beta\text{-}E_2$-stimulated (10^{-10} M) growth, with 10^{-6} M being more potent than 10^{-8} M (Fig. 2). With a high-

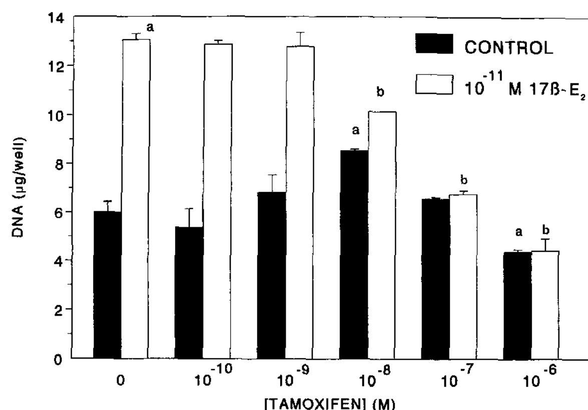

Fig. 2. Dose-response of TAM in the presence and absence of 17β-E_2. MCF-7 cells were cultured in 2% CT-FCS containing medium with the indicated concentrations of TAM in the presence or absence of 10^{-11} M 17β-E_2. After 4 days DNA content was measured. a, $p < 0.05$ versus control (no TAM); b, $p < 0.001$ versus 10^{-11} M 17β-E_2 (no TAM) as calculated with the Student's t-test.

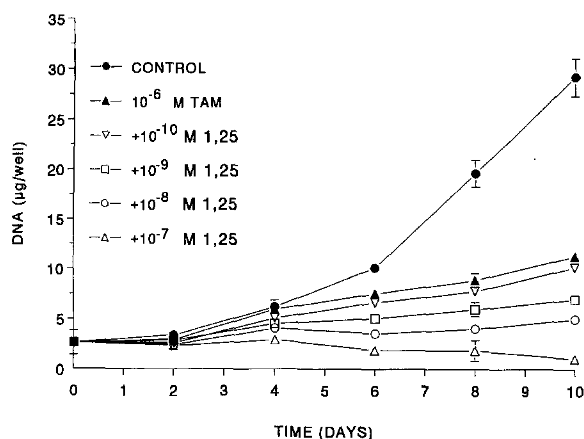

Fig. 3. Combined treatment with 1,25-$(OH)_2D_3$ and a growth-inhibitory dose of TAM. MCF-7 cells were cultured in 2% CT-FCS containing medium with 10^{-6} M TAM plus the indicated concentrations of 1,25-$(OH)_2D_3$ (1,25). Control cultures received vehicle only. DNA content was measured every two days.

er dose of 17β-E_2 (10^{-9} M) the effect of 10^{-6} M TAM could partially be reversed (data not shown). With ZR-75-1 cells a similar phenomenon was observed (Fig. 7).

Combined effects of 1,25-$(OH)_2D_3$ and TAM on MCF-7 proliferation

Subsequently, we investigated a possible interaction between 1,25-$(OH)_2D_3$ and TAM. First, we assessed the effect of co-treatment with 1,25-$(OH)_2D_3$ and a growth inhibitory dose of TAM (10^{-6} M). Figure 3 shows that 10^{-6} M TAM alone resulted in an inhibition of 68% on day 10. A further inhibition up to 100% could be achieved by co-treatment with 1,25-$(OH)_2D_3$ (10^{-10} – 10^{-7} M). At early time points (4 days) an additive effect could be observed, i.e. the reduction in DNA content, expressed in µg/well, by TAM and 1,25-$(OH)_2D_3$ alone adds up in the combined treatment. At later time points (8–10 days) the effect of combined treatment was, although not additive, stronger than the effect of either compound alone. The co-treatment was cytostatic rather than cytotoxic, since cell growth could be regained by adding fresh medium supplemented with 10% FCS after 4 and 10 days of incubation with 10^{-7} M 1,25-$(OH)_2D_3$ together with 10^{-6} M TAM (data not shown).

Figure 4 shows the percentage inhibition by treatment with 1,25-$(OH)_2D_3$ alone and in combination with 10^{-6} M TAM on days 6 and 10. This figure illustrates for example that on day 6 an inhibition of 70% was achieved with 5.10^{-8} M 1,25-$(OH)_2D_3$, whereas a similar inhibition was achieved with a 50 times lower 1,25-$(OH)_2D_3$ concentration (10^{-9} M) when combined with TAM (Fig. 4A). In addition, on day 10, 80% inhibition was achieved with 3.10^{-8} M 1,25-$(OH)_2D_3$ alone and with 4.10^{-10} M 1,25-$(OH)_2D_3$ when combined with TAM (Fig. 4B). In this situation a 75 times lower concentration of 1,25-$(OH)_2D_3$ resulted in a similar inhibition when used in combination with TAM.

The effect of co-treatment with 1,25-$(OH)_2D_3$ and TAM (10^{-6} M) on 17β-E_2-stimulated proliferation is shown in Fig. 5. Although TAM was a very potent inhibitor of 17β-E_2-stimulated proliferation (Fig. 2), 10^{-6} M TAM did not completely inhibit the growth of MCF-7 cells. Co-treatment with 1,25-$(OH)_2D_3$ resulted in a further dose-dependent inhibition and growth arrest at 10^{-7} M 1,25-$(OH)_2D_3$.

Next we investigated the effect of co-treatment with 1,25-$(OH)_2D_3$ and a growth-stimulatory dose of TAM (10^{-8} M). As shown in Fig. 6, 1,25-$(OH)_2D_3$ inhibited TAM-stimulated growth in a time- and dose-dependent manner. 10^{-10} M 1,25-$(OH)_2D_3$ caused a small reduction of TAM-stimulated growth whereas 10^{-9} M 1,25-$(OH)_2D_3$ resulted in an

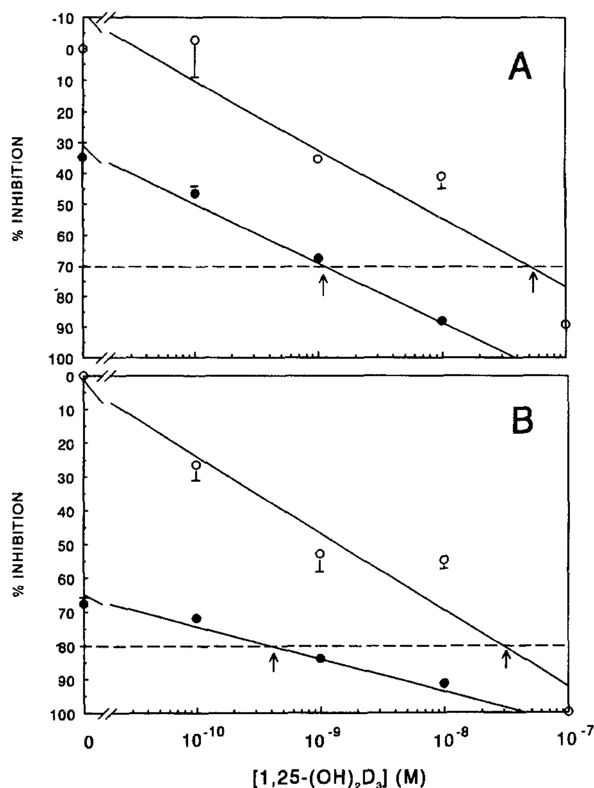

Fig. 4. Comparison of the effect of 1,25-(OH)$_2$D$_3$ alone and in combination with TAM on basal growth. MCF-7 cells were treated for 6 days (A) and 10 days (B) with 1,25-(OH)$_2$D$_3$ alone (open circles) or with 1,25-(OH)$_2$D$_3$ plus 10^{-6} M TAM (solid circles). DNA values were corrected for DNA values on day 0 and expressed as percentage inhibition relative to control (vehicle only).

inhibition to control level (no TAM), thereby completely preventing TAM-induced growth stimulation. 10^{-8} and 10^{-7} M 1,25-(OH)$_2$D$_3$ even suppressed TAM-stimulated growth to below control level.

Effects of 1,25-(OH)$_2$D$_3$ and TAM on ZR-75-1 cells

Besides MCF-7 cells we tested the effects of 1,25-(OH)$_2$D$_3$ and TAM on the proliferation of another ER-positive breast cancer cell line. ZR-75-1 cells did not grow in the steroid-free culture medium we used for the proliferation experiments with MCF-7 cells. Addition of 17β-E$_2$ to the culture medium resulted in a dose-dependent stimulation of proliferation. TAM (10^{-6} M) caused a complete inhibition of 10^{-10} M 17β-E$_2$-stimulated growth whereas the effect of 10^{-9} M 17β-E$_2$ was partially inhibited (Fig. 7).

1,25-(OH)$_2$D$_3$ inhibited 17β-E$_2$-stimulated growth of ZR-75-1 cells in a dose-dependent manner (data not shown). 10^{-7} M 1,25-(OH)$_2$D$_3$ completely inhibited 10^{-10} M 17β-E$_2$-stimulated growth similar to TAM (10^{-6} M). Also, an almost complete inhibition of 10^{-9} M 17β-E$_2$-stimulated growth was observed using 10^{-7} M 1,25-(OH)$_2$D$_3$, whereas 10^{-6} M TAM was less potent (Fig. 7). Further, the inhibition of 17β-E$_2$-stimulated growth by 1,25-(OH)$_2$D$_3$ was more effective in ZR-75-1 cells compared to MCF-7 cells. Comparison of Figs 1B and 7 shows a partial inhibition of 10^{-10} M 17β-E$_2$-stimulated growth of MCF-7 cells by 10^{-7} M 1,25-(OH)$_2$D$_3$ and a complete inhibition of ZR-75-1 cells after 9 days. This may be directly related to the difference in response to 17β-E$_2$. However, in several experiments it was observed that an equipotent growth stimulation by 17β-E$_2$ of MCF-7 and ZR-75-1 cells was also inhibited more potently by 10^{-7} M 1,25-(OH)$_2$D$_3$ in ZR-75-1 cells (data not shown).

Although treatment with 1,25-(OH)$_2$D$_3$ (10^{-7} M) and TAM (10^{-6} M) alone resulted in a complete inhibition of 10^{-10} M 17β-E$_2$ stimulated growth, still a further inhibition to below control level was observed when used in combination. Also, at higher concentration 17β-E$_2$ (10^{-9} M), the effect of co-

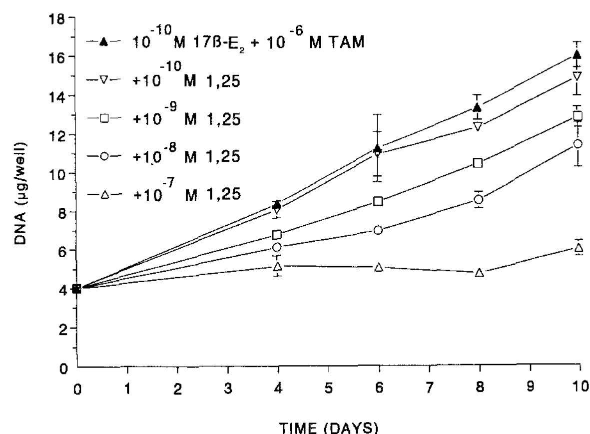

Fig. 5. Combined treatment with 1,25-(OH)$_2$D$_3$ and TAM of cells stimulated with 17β-E$_2$. MCF-7 cells were cultured in 2% CT-FCS containing medium with 10^{-10} M 17β-E$_2$ and 10^{-6} M TAM (solid triangles) and were co-treated with a dose range of 1,25-(OH)$_2$D$_3$ (1,25; open symbols). DNA was measured at the indicated times.

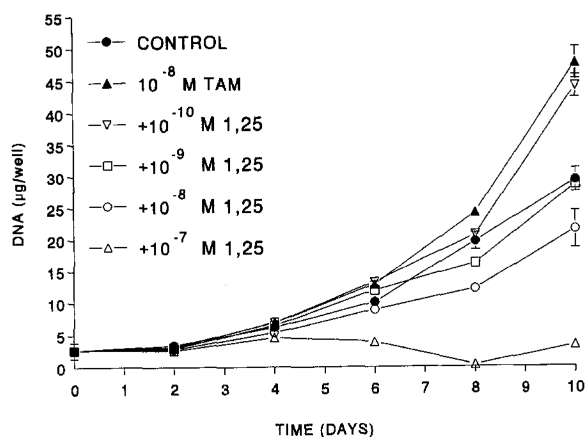

Fig. 6. Combined treatment with 1,25-$(OH)_2D_3$ and a growth-stimulatory dose of TAM. MCF-7 cells were cultured in 2% CT-FCS containing medium with 10^{-8} M TAM plus the indicated concentrations of 1,25-$(OH)_2D_3$ (1,25). Control cultures received vehicle only. DNA content was measured every two days.

treatment was more potent than the effect of either compound alone (Fig. 7).

Discussion

The present study describes for the first time effects of combined treatment with 1,25-$(OH)_2D_3$ and TAM on the growth of ER-positive and VDR-positive human breast cancer cells *in vitro*. As circulating levels of estrogens are believed to play an important role in promoting the growth of ER-positive breast tumors, we have studied the effects of both compounds on basal as well as 17β-E_2-stimulated proliferation. The current data show that basal growth of MCF-7 cells is inhibited by 1,25-$(OH)_2D_3$. ZR-75-1 cells did not grow in the absence of 17β-E_2 and therefore no effects on basal growth could be assessed. Our data are consistent with several reports describing an inhibitory effect of 1,25-$(OH)_2D_3$ on human breast cancer cells [2, 7–11]. In these studies the inhibition of cell proliferation was investigated using culture media supplemented with FCS or CT-FCS, but to our knowledge the effect of 1,25-$(OH)_2D_3$ on a specific growth stimulus like 17β-E_2 was not studied. Our data demonstrate that 1,25-$(OH)_2D_3$ inhibits 17β-E_2-stimulated proliferation of both MCF-7 and ZR-75-1 cells. Although the VDR levels were comparable, 17β-E_2 stimulated growth of ZR-75-1 cells is more sensitive to 1,25-$(OH)_2D_3$ than 17β-E_2-stimulated growth of MCF-7 cells. These results are suggestive for a difference in stimulation by 17β-E_2. It has been reported [19, 20] that 17β-E_2 acts synergistically with insulin and possibly insulin-like growth factors on MCF-7 cells, whereas others [21] did not find this synergistic action on ZR-75-1 cells. Therefore, the 17β-E_2-stimulated growth we observed in MCF-7 cells could be the result of a synergistic action of 17β-E_2 with serum-derived insulin-like growth factors, whereas the growth stimulation by 17β-E_2 in ZR-75-1 cells could be less sensitive to serum factors.

TAM antagonizes 17β-E_2-stimulated growth by competition with 17β-E_2 for the ER [14, 15]. Consistent with these findings, TAM dose-dependently inhibited 17β-E_2-stimulated growth of MCF-7 and ZR-75-1 cells. It was also observed that 10^{-6} M TAM inhibited the growth of MCF-7 cells in steroid-free culture medium. This might be explained by anti-growth factor activity of TAM, which has been demonstrated in several reports [20, 22, 23].

Both basal proliferation (MCF-7 cells) and 17β-E_2-stimulated proliferation (MCF-7 and ZR-75-1 cells) were inhibited more potently by the combination of 1,25-$(OH)_2D_3$ and TAM than by either compound alone. We have shown that an equipotent inhibition of basal growth of MCF-7 cells could be

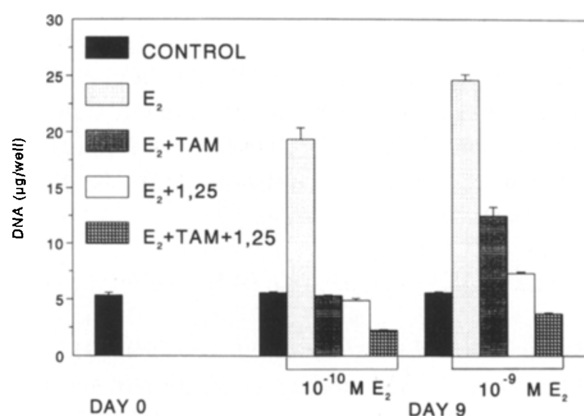

Fig. 7. Combined treatment with 1,25-$(OH)_2D_3$ and TAM of ZR-75-1 cells. ZR-75-1 cells were cultured in 2% CT-FCS containing medium with vehicle, 10^{-10} or 10^{-9} M 17β-E_2 (E_2) for 9 days. Cells were treated with 10^{-6} M TAM or 10^{-7} M 1,25-$(OH)_2D_3$ (1,25) or 10^{-6} M TAM together with 10^{-7} M 1,25-$(OH)_2D_3$. Medium and test agents were replaced every three days. DNA content was measured on days 0 and 9.

achieved with lower concentrations of 1,25-$(OH)_2D_3$ when combined with TAM, compared to treatment with 1,25-$(OH)_2D_3$ alone. This is an interesting observation since it is of clinical importance to use 1,25-$(OH)_2D_3$ as an antiproliferative compound at the lowest possible doses in order to prevent the development of hypercalcemia.

In MCF-7 cells we observed a growth stimulation by TAM at low concentrations ($< 10^{-7}$ M) in the absence of estrogens. This effect is consistent with previous work [20, 24] and is thought to be due to a partial estrogen agonistic action mediated via the ER. It is hypothesized that TAM-induced tumor flare, which is often observed in patients, is the result of this estrogenic effect of TAM [25]. We observed that growth stimulation by TAM was already completely prevented by a low concentration (10^{-9} M) of 1,25-$(OH)_2D_3$. Thereby these data are pointing to a possible role of 1,25-$(OH)_2D_3$ in the prevention of tumor flare. A further interesting observation was that a growth-stimulatory concentration of TAM (10^{-8} M) was nevertheless able to inhibit 17β-E_2-stimulated growth. This phenomenon agrees with previous studies [20, 24]; however, the underlying mechanism is not yet clear.

The observation that at early time points the inhibitory effects of 1,25-$(OH)_2D_3$ and TAM on the proliferation of MCF-7 cells are additive suggests that they inhibit cell growth via different mechanisms. This could indicate that 1,25-$(OH)_2D_3$ acts independent of the 17β-E_2-induced pathway leading to proliferation. Further support for this 17β-E_2-independent action comes from observations in previous reports that both ER-positive and ER-negative breast cancer cell lines are growth-inhibited by 1,25-$(OH)_2D_3$ [2, 7–11]. Further, in MCF-7 cells 1,25-$(OH)_2D_3$ inhibited basal growth more potently than 17β-E_2-stimulated growth. However, several observations do point to an interference of 1,25-$(OH)_2D_3$ with the 17β-E_2-induced pathway. Firstly, in ZR-75-1 cells, 1,25-$(OH)_2D_3$ caused a complete blockade of 17β-E_2-induced growth. Secondly, TAM-stimulated growth of MCF-7 cells, which is probably mediated via the ER, was potently inhibited by 1,25-$(OH)_2D_3$. Moreover, a recent report about a synergistic growth inhibition of MCF-7 and ZR-75-1 cells by a vitamin D_3 analog and TAM [26] is suggestive for an interaction. More experiments are needed to define precisely whether 1,25-$(OH)_2D_3$ interferes with the 17β-E_2-mediated pathway and presently we are investigating the role of 1,25-$(OH)_2D_3$ in other 17β-E_2-mediated responses.

In conclusion, the current results demonstrate a potent inhibition of breast cancer cell proliferation by combined treatment with 1,25-$(OH)_2D_3$ and TAM. The combined treatment may provide the advantages that a) tumors positive for both ER and VDR have a more beneficial response, b) lower doses of 1,25-$(OH)_2D_3$ can be used which do not cause hypercalcemia, and c) in tumors heterogeneous for the ER, both ER-positive and ER-negative cells can be inhibited. In addition, TAM may diminish the stimulatory side effect of 1,25-$(OH)_2D_3$ on bone resorption since several reports have indicated that TAM exerts positive estrogenic effects on bone and protects against steroid-induced bone loss [27, 28]. An important drawback for the clinical use of 1,25-$(OH)_2D_3$ as an antiproliferative compound is the development of hypercalcemia at high doses. At the moment, numerous attempts are being made to develop vitamin D_3 analogs with potent growth inhibitory and reduced calcemic activity. In the future co-treatment with these vitamin D_3 analogs and TAM may provide an even greater benefit and studies on this subject are currently in progress.

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