Effect of 22-Oxa-1,25-Dihydroxyvitamin D₃ on Human Thyroid Cancer Cell Growth

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Abstract. To examine whether synthetic vitamin D₃ analog, 22-oxa-1,25(OH)₂D₃ (OCT) has an inhibitory effect on the growth of thyroid carcinoma, we tested the in vitro and in vivo effects of OCT on the growth of a well-differentiated thyroid cancer cell line, NPA. OCT bound to its receptor at the same rate as 1,25(OH)₂D₃, and inhibited the proliferation of NPA cells in vitro in a dose-dependent manner, similar to that observed with 1,25(OH)₂D₃. Northern blot analysis showed that steady-state and fetal bovine serum-stimulated levels of c-myc mRNA were suppressed after 0.5–4 hour treatment with OCT. Transfection studies with the deletion mutants of the 5'-up-stream flanking region of c-myc/chromamphenicol acetyltransferase chimera genes indicated the presence of an OCT responsive element between -410 and -106. Next, we examined OCT effects in implanted NPA tumor cells in nude mice. OCT showed no remarkable hypercalcemic effect compared to 1, 25 (OH₂)D₃, but OCT and 1, 25 (OH₂)D₃ had no significant inhibitory effect in vivo after either intra-tumor or intra-peritoneum injection. Our results demonstrate that OCT inhibits the proliferation of well-differentiated thyroid cancer in an in vitro system associated with the suppression of c-myc mRNA, but this inhibitory effect was not reproducible in in vivo model.

Key words: Thyroid cancer cell, Vitamin D₃, therapy.

1,25-DIHYDROXYVITAMIN D₃ (1,25(OH)₂D₃), a biologically active form of vitamin D₃, is a major calcium-regulating hormone that acts through binding to its intracellular receptor in bone, intestine, and kidney [1–4]. Vitamin D receptor (VDR) is widely expressed not only in these classic target organs but also in a variety of cell types [1–4]. VDR is a nuclear receptor family which functions as a transcriptional factor. VDR consist of a heterodimer with retinoid X receptor (RXR) which is a also partner of retinoic acid receptor (RAR). On the other hand, retinoic acid binding to RAR has been reported to be effective in acute promyelocytic leukemia [5]. PML-RAR fusion protein yielding caused by chromosomal translocation in acute promyelocytic leukemia inhibits Vit D₃/VDR signals associated with a reduction in cytosolic RXR. And retinoic acid is thought to rescue this inhibition [6]. This evidence leads to consider that vitamin D₃ might be a potential drug for some cancers instead of retionoic acid. 1,25(OH)₂D₃ induces cell differentiation and inhibits cell growth [7, 8]. In certain cancer cells, these actions are mediated in part by inhibition of c-myc gene expression [9, 10]. A number of vitamin D analogs have been developed recently in an effort to separate calcemic activity from the antiproliferative/differentiation-inducing activity [11]. Among these analogs, 22-oxa-1,25(OH)₂D₃ (OCT) is more potent than 1,25(OH)₂D₃ in inhibiting the proliferation of
HTLV-1 infected T cells in vitro and of breast cancer in vivo with little calcemic activity [12, 13].

Human papillary thyroid carcinoma accounts for about 85% of thyroid cancers [14]. Surgical excision is the primary treatment for differentiated thyroid carcinoma, and radiation and/or hormonal therapy remains an important component of therapy to suppress recurrence and metastasis after surgery. It is well known that serum levels of 1,25(OH)₂D₃ decreased after hemithyroidectomy [15]. Furthermore, thyroid hormone suppression therapy may occasionally cause osteoporosis, particularly in postmenopausal women [16]. Therefore, the use of vitamin D₃ analog that does not cause hypercalcemia may be essential in patients with thyroid cancer treated surgically.

Several abnormalities in oncogenes and/or tumor suppressive genes are present in thyroid papillary carcinoma, with c-myc overexpression reported in over 90% of cases [17]. We have demonstrated recently an inhibitory role for interleukin-1 and transforming growth factor β in human thyroid carcinoma cells accompanied by a decrease in c-myc mRNA [18, 19]. These findings suggested that the suppression of c-myc gene expression is involved in the antiproliferative action of these cytokines. To investigate possible OCT anti-tumor activity, we examined the in vitro effect of OCT on cell growth and c-myc gene expression in a human papillary thyroid carcinoma cell line, NPA. We also investigated the effect of OCT injection on thyroid cancer implanted in mice.

Materials and Methods

Materials

1,25(OH)₂[26, 27-3H]D₃ (180 Ci/mmol) was obtained from Amersham (Tokyo, Japan), and unlabeled 1,25(OH)₂D₃, OCT, 24,25(OH)₂D₃ and [26-3H]OCT (86.3 Ci/mmol) were kindly provided by Chugai Pharmaceutical Inc. (Tokyo) [20].

Cell culture

A human papillary thyroid carcinoma cell line (NPA), kindly provided by Dr. G. Julliard (University of California Los Angeles Medical Center), was maintained in RPMI-1640 supplemented with 10% FBS (Gibco Oriental Co., Tokyo), 5.6% sodium bicarbonate, 100 IU/ml penicillin and 10 μg/ml streptomycin (Sigma Chemical Co., St. Louis, MO) in a 5% CO₂ environment at 37°C. Cells in the confluent stock plates were trypsined (0.05% trypsin and 0.05 M EDTA) and seeded on culture plates (Costar Co., Cambridge, MA) of the proper size for each experiment in a medium with 10% FBS.

Cell growth rate

NPA cells were seeded at a density of 10⁴ cells/well in 24-well tissue culture plates in 1.0 ml RPMI 1640 supplemented with 10% FBS and with or without OCT (10⁻⁷ M). After 1–14 days incubation in 37°C and changing the medium three times a week, the medium was removed, and 250 μl trypsin and EDTA were added to each well for 5 min at 37°C. The cells were counted in a hemocytometer (PC-602A, ERMA, Tokyo). The viability of cells was established by the trypan blue dye (0.4%) exclusion technique.

5-bromo-2′-deoxyuridine incorporation

NPA cells subplated in 96-well plates at a density of 5 × 10³ cells/well were cultured to subconfluence for 2 days. They were incubated in a medium supplemented with 0.3% BSA for a further 12 hours, and then exposed to various concentrations of OCT or 1,25(OH)₂D₃ (10⁻¹¹ – 10⁻⁷ M) for 21 hours in a medium containing 1% FBS or in the latter medium only for controls. To measure DNA synthesis, 5-bromo-2′-deoxyuridine (BrdU), a thymidine analog, was incorporated for another 3 hours. BrdU incorporation was assessed with a cell proliferation kit (RPN210, Amersham, Tokyo). Briefly, the cells were reacted with anti-BrdU antibody for 1 hour, followed by peroxidase-conjugated secondary antibodies for 30 min. The reaction product was color developed by incubation with a peroxidase substrate, and measured in a spectrophotometer (OD, 405 nm; ImmunoReader NJ-2001, Intermed, Tokyo).

Cytotoxic assay

1 × 10⁷ of NPA cells were labeled by incubation with 50 μCi [⁵¹Cr] sodium chromate for 12 hours in 500 μl culture medium. The cell concentration was
adjusted to $2.5 \times 10^4$ cells/well in 96-well V-bottomed tissue culture plates (Costar 3896), and incubated in 200 $\mu$l culture medium containing 10\% FBS with or without OCT or 1,25(OH)$_2$D$_3$ for 8 or 24 hours at 37$^\circ$C. After centrifugation of the plates at 400 $\times$ g for 5 min, aliquots (100 $\mu$l) of medium were collected from each well, and counted in a $\gamma$-counter. The percent release of $[^{51}Cr]$ was calculated accordingly to the following equation: (test medium cpm or spontaneous cpm)/(maximum cpm) $\times$ 100\%. Spontaneous release was defined as the radioactivity released from NPA cells incubated in medium only, and maximum release was defined as that released from NPA cells incubated in a medium containing 5\% (vol/vol) Triton X-100 (Sigma).

$[^{3}H]$ 1,25(OH)$_2$D$_3$ and $[^{3}H]$ OCT binding to NPA cells

The receptor was extracted from cell homogenates in TKD buffer (0.01 M Tris, 0.3 M KCl, 1 mM dithiothreitol, 5 mM diisopropyl fluorophosphate, pH 7.4) by 10,000 $\times$ g ultracentrifugation. Four hundred micrograms of extract was incubated overnight with 1.0 nM $[^{3}H]$ 1,25(OH)$_2$D$_3$ or $[^{3}H]$ OCT and graded concentrations of unlabeled vitamin D$_3$ derivatives at 4$^\circ$C. The hormone-receptor complex was separated by the hydroxyapatite method [19], and bound tritiated hormone was determined by scintillation spectrometry.

Northern gel analysis

After treatment as indicated, the NPA cells were subjected to total ribonucleic acid (RNA) extraction by the acid-guanidinium thiocyanate phenol-chloroform method [22]. Total cellular RNA (30 $\mu$g) was denatured by incubation at 65$^\circ$C with 50\% formamide, and electrophoresed in a 1\% agarose gel with 6.0\% formaldehyde. After transferring the RNA from the gel to nylon membrane paper, the paper was prehybridized, and hybridized with 10$^7$ cpm $^{32}$P-labeled c-myc, or $\gamma$-actin complementary DNA (cDNA) before exposure to x-ray film for autoradiography as described previously [23]. The cDNA probes used in this study were human c-myc DNA [1.5-kilobase pair (kb) HindIII-EcoRI] provided by Dr. H. Ariga [24], and the $\gamma$-actin probe was provided by Dr. L. Kedes [25]. The probes were labeled with $\alpha$-$[^{32}P]$ dCTP by multiprime DNA-labeling systems to a specific activity of approximately $5 \times 10^7$ cpm/$\mu$g DNA.

c-myc/CAT expression vectors and CAT assay

To construct the chloramphenicol acetyltransferase (CAT) expression vector, plasmid containing c-myc 5'-untranslated region, a gift from Dr. H. Ariga [24], was digested with HindIII (position $\sim$2330) and XbaI (position 1177). The resulting 3.5-kb fragment was inserted into a pCAT-enhancer plasmid (Promega, Madison, WI). Deletion mutants, pCAT myc/-2330, -1160, -410, -106, and 70 were constructed by cutting each respective restriction enzyme-cutting site as described previously [19].

Three micrograms of plasmid DNA were transfected into subconfluent NPA cells in a 10-cm dish by the lipofection method [26]. Twenty-four hours after transfection, the cells were incubated with 1\% FBS for 24 hours in the presence or absence of 10$^{-7}$ M OCT, harvested with PBS, suspended in 150 $\mu$l Tris-HCl (pH 7.5), and then disrupted by five cycles of freeze-thawing. CAT assays were carried out as described previously [26].

Effect of OCT and 1,25(OH)$_2$D$_3$ on the in vivo growth of NPA cells

The study was performed in accordance with the animal care ethical guidelines of Nagasaki University. Five-week-old female nude mice [SPF/VAF Crj: CD-1 (ICR)-nu] (Charles River Breeding Laborato ries, Inc., Kanagawa, Japan) were housed in sterilized cages.

Single-cell suspension of NPA cells ($8 \times 10^6$ in 0.2 ml of RPMI medium) were injected subcutaneously into the right lower dorsal region of mice, and allowed to grow for 24 hours before treatment with OCT or 1,25(OH)$_2$D$_3$. The animals were divided randomly into three groups each containing 15 mice for intra-peritoneal treatment and another three groups of 10 mice each for intra-tumor treatment. Each group was treated three times a week with medium only, OCT or 1,25(OH)$_2$D$_3$ (1.0 $\mu$g/kg body weight) for 30 days. OCT and 1,25(OH)$_2$D$_3$ were dissolved in 100 $\mu$l RPMI medium just before injection. Five mice treated with intra-peritoneal 1,25?
(OH)$_2$D$_3$ died during the observation period. These animals were excluded from the analysis. At the end of the experiment, the mice were killed, and the tumor mass was weighed following careful resection from the surrounding tissue. Blood was also withdrawn and pooled. Serum calcium was measured by the orthocresolphthalein complex method in an autoanalyzer (Hitachi 736, Hitachi Co., Tokyo).

Statistical analysis

Data were expressed as the mean ± SD. The Kruskal-Wallis test was used to compare data for the three groups, and Mann-Whitney test for comparison of two groups. P values less than 0.05 were considered to indicate a statistically significant difference.

Results

Effect of OCT on number of cells

We first examined the effect of OCT on NPA cell proliferation. Cells were cultured in a medium containing 10% FBS with or without 10$^{-7}$ M OCT for 14 days. As shown in Fig. 1, OCT significantly suppressed the number of cells after seven days (p < 0.001). A viability test with 0.4% trypan blue showed that more than 95% of the cells were alive either in the absence or presence of OCT.

Effect of OCT and 1,25(OH)$_2$D$_3$ on DNA synthesis

In the next step, we measured BrdU uptake in NPA cells treated with OCT or 1,25(OH)$_2$D$_3$ to further clarify the inhibitory effect on NPA cell proliferation. As shown in Fig. 2, both OCT and 1,25(OH)$_2$D$_3$ significantly reduced DNA synthesis after 24 hours.
treatment in a dose-dependent manner. The doses of OCT and 1,25(OH)_{2}D_{3} required to induce significant inhibition of DNA synthesis were 10^{-10} and 10^{-7} M, respectively. 10^{-8} M OCT and 10^{-7} M 1,25(OH)_{2}D_{3} produced the maximal suppression of DNA synthesis to approximately 38 and 41% of the control, respectively.

**Cytotoxic assay**

To determine whether the inhibitory effect of OCT and 1,25(OH)_{2}D_{3} on NPA cells represented a toxic effect, we examined the cytotoxic activity of OCT and 1,25(OH)_{2}D_{3} by [S1Cr] release assay (Table 1). After labeling with [51C] sodium chromate, the cells were treated with OCT or 1,25(OH)_{2}D_{3} for 8 or 24 hours. Neither OCT nor 1,25(OH)_{2}D_{3} (10^{-9}-10^{-7} M) showed any cytotoxic effect on NPA cells.

**Receptor binding assay**

To examine whether OCT mediated its effect through VDR, VDR binding assay was performed. Both OCT and 1,25(OH)_{2}D_{3} competed for the [3H] 1,25(OH)_{2}D_{3} binding with almost similar affinities (data not shown). The effective half-maximal concentration of OCT was 3.5 \times 10^{-9} M, and that of 1,25(OH)_{2}D_{3} was 4.0 \times 10^{-9} M. Binding affinity with [3H] OCT gave almost the same result as that with 1,25(OH)_{2}D_{3} (data not shown).

**Northern blot analysis**

The expression of c-myc mRNA in NPA cells treated with OCT, 1,25(OH)_{2}D_{3} or 24,25(OH)_{2}D_{3} was analyzed by Northern blot. Control NPA cells cultured with medium containing 1% FBS showed single species of c-myc mRNA approximately 2.4 kb in size. Fig. 3A illustrates the time-course effect of 10^{-7} M OCT. OCT suppression of c-myc mRNA expression was evident after 0.5 hour of incubation, and the maximal inhibitory effect was observed at 2 hours. Both OCT and 1,25(OH)_{2}D_{3} but not 24,25(OH)_{2}D_{3} significantly inhibited c-myc mRNA expression in a dose-dependent manner after 2 hours incubation (Fig. 3B).

We also examined the inhibitory effect of OCT and 1,25(OH)_{2}D_{3} on the 10% FBS-stimulated c-myc mRNA level. Incubation with FBS after preexposure to serum free medium for 24 hours resulted in a 6-fold increase in c-myc mRNA level at 2 hours. Preincubation with 10^{-7} M OCT for 10 min suppressed FBS-induced c-myc gene expression (data not shown).

**Transfection of c-myc/CAT gene into NPA cells**

To investigate whether OCT can directly influence transcriptional regulation of c-myc gene expression, transfection experiments with plasmid containing the

| Table 1. Cytotoxic effect of OCT and 1,25(OH)_{2}D_{3} in NPA cells |
|----------------------|----------|---------|----------|
| Treatment            | 8h       | 24h     |
|                      | cpm±SD   | % release | cpm±SD   | % release |
| Maximum              | 15320±510| 100.0   | 11250±502| 100.0     |
| Spontaneous OCT (M)  | 1651±312 | 10.8    | 1850±49  | 16.4      |
| 10^{-9}              | 1872±72  | 12.2    | 1922±55  | 17.0      |
| 10^{-8}              | 1592±117 | 10.4    | 1986±119 | 17.6      |
| 10^{-7}              | 1609±153 | 10.5    | 2056±299 | 18.2      |
| 1,25(OH)_{2}D_{3} (M) |          |         |          |           |
| 10^{-9}              | 1699±83  | 11.1    | 1993±101 | 17.7      |
| 10^{-8}              | 1721±196 | 11.2    | 1892±87  | 16.9      |
| 10^{-7}              | 1649±133 | 10.8    | 1852±99  | 16.5      |

Cytotoxic activity of OCT and 1,25(OH)_{2}D_{3} for NPA cells was measured by a [S1Cr] release assay as described in MATERIALS AND METHODS. Result are expressed as the mean CPM±SD of quadruplicate cultures. Similar results were observed in three independent experiments.
CAT gene fused to the 5'-up stream flanking region (UFR) of c-myc 2.3 kb were performed. Treatment with OCT for 24 hours resulted in a 53% decrease in CAT activity compared when treated with 1% FBS only (Fig. 4). To analyze the OCT-responsive element in c-myc 5'-UFR in NPA cells, deletion c-myc forms containing pCATmyc/ -610, -410, -106 and +70 were used. The CAT activity of the chimeras with upstream sequences from positions -2330 to -410 was reduced by 54-59% of the control by 10^-7 M OCT. In contrast, pCATmyc/70 and -106 activity was not influenced by OCT.

**In vivo effect of OCT and 1,25(OH)_2D_3 on NPA cells**

To test a possible anti-tumor in vivo effect of OCT on thyroid cancer, NPA cells were implanted in nude mice, and OCT or 1,25(OH)_2D_3 was injected directly into the NPA tumor or into the peritoneum. As shown in Table 2, although intra-peritoneal administration of OCT and 1,25(OH)_2D_3 for 30 days failed to have any apparent anti-tumor effect, intra-tumor injection of them reduced the mean weight of the tumor mass but, there was no significant difference

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**Fig. 3.** Effect of OCT and 1,25(OH)_2D_3 on c-myc mRNA. **A**, Time course effect of 10^-7 M OCT on the c-myc mRNA level at 0, 0.5, 1, 2, 4, 8 and 12 hours. **B**, Dose-response effect of 24, 25(OH)_2D_3, OCT and 1,25(OH)_2D_3 (10^-8 - 10^-7 M) after 2 hours of treatment. 32P-labeled c-myc cDNA probe was hybridized to 30 μg total RNA isolated from NPA cells at the indicated times and concentrations, as shown in the upper panel. The same membrane reprobed with 32P labeled γ-actin cDNA after washing with 0.1% SDS at 95°C for 10 min (middle panel). The relative amounts of c-myc mRNA measured by densitometry are shown in the lower panel.
between OCT, 1,25(OH)₂D₃ and vehicle-treated groups in tumor weight (intra-tumor injection; p=0.10, intra-peritoneal injection; p=0.87). The use of a higher dose (10 μg/kg) also had no significant effect (data not shown). The mean tumor weight in animals receiving intra-tumor injection was significantly lower than that of intra-peritoneal injection group (OCT; p=0.023, 1,25(OH)₂D₃; p=0.0006, Vehicle; p=0.0006 ). The histopathologic features of implanted NPA cells in all six groups were similar. Serum calcium levels of OCT-treated animals were only slightly higher than those of the control group (OCT vs Vehicle: intra-tumor; p=0.056, intra-peritoneum; p=0.085). In contrast, treatment with 1,25(OH)₂D₃ significantly increased the serum calcium level (OCT vs 1,25(OH)₂D₃: intra-tumor; p=0.0001, intra-peritoneum; p=0.0035).

**Table 2. In vivo effect of OCT and 1,25(OH)₂D₃ on the growth of NPA cells**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor weight (g)</th>
<th>Relative tumor weight (%)</th>
<th>Serum Ca (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-tumor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.98±0.35</td>
<td>100</td>
<td>4.2±0.17</td>
</tr>
<tr>
<td>OCT</td>
<td>0.68±0.26</td>
<td>69±26</td>
<td>4.5±0.16</td>
</tr>
<tr>
<td>1,25(OH)₂D₃</td>
<td>0.76±0.19</td>
<td>78±19</td>
<td>5.6±0.26*</td>
</tr>
<tr>
<td><strong>Intra-peritoneum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.39±0.30</td>
<td>100</td>
<td>4.3±0.26</td>
</tr>
<tr>
<td>OCT</td>
<td>0.40±0.21</td>
<td>103±53</td>
<td>4.6±0.25</td>
</tr>
<tr>
<td>1,25(OH)₂D₃</td>
<td>0.36±0.19</td>
<td>92±48</td>
<td>5.3±0.58*</td>
</tr>
</tbody>
</table>

OCT or 1,25(OH)₂D₃ (1 μg/kg BW) was given three times a week by intra-tumor or intra-peritoneum injection. On the 30th day, the animals were killed, and the weight of each tumor was measured. Data are expressed as the mean±SD. Blood was also drawn and pooled for each group, and serum calcium levels also were measured, as described in MATERIALS AND METHODS.

*p<0.01 vs OCT and vehicle.

**Fig. 4.** Schematic representation of c-myc promoter/CAT forms and the effect of OCT on CAT protein production in transfected NPA cells. NPA cells were transfected with the indicated pCAT-myc forms, and the effect of 10⁻⁷ M OCT treatment (black bar) was determined. White bars represent the respective control experiments. The remaining data are expressed as the percent inhibition of CAT activity compared with the control value. Each value represents the mean±SD of three determinations. P1 and P2 are the starting sites of transcription of human c-myc gene.
Discussion

The major finding of the present study was that OCT, a vitamin D3 analog, significantly reduced the number of cells and DNA synthesis of the human thyroid papillary adenocarcinoma cell line NPA to levels similar to those produced by 1,25(OH)2D3, the biologically active form of vitamin D3. These in vitro effects were not associated with cytotoxicity. In addition, receptor binding of [3H]-OCT and [3H]-1,25(OH)2D3 was completely replaced by OCT and 1,25(OH)2D3, respectively, with the same affinity. Our results suggest that OCT has a potent in vitro anti-proliferative effect in NPA cells by binding to 1,25(OH)2D3 receptor. There is some controversy concerning the OCT receptor. It has been demonstrated that the binding of OCT to its receptor is lower than that of 1,25(OH)2D3 [27, 28] although OCT is more potent than 1,25(OH)2D3 [13, 29]. The reason for this discrepancy is unclear. We consider that the in vitro anti-proliferative effect of OCT on NPA cells was mediated by binding to 1,25(OH)2D3 receptor, although the existence of a specific OCT receptor in certain cell types cannot be ruled out.

We also demonstrated in this study that OCT and 1,25(OH)2D3 inhibited c-myc mRNA expression under basal (1% FBS) and FBS-stimulated conditions with a very short latency. We also found an OCT-response element in human c-myc upstream sequence between −106 and −410. It is well known that vitamin D response element (VDRE) belongs to a direct repeat motif [30], and that VDR forms a heterodimer with retinoid X receptor, and binds to the consensus sequence (AGGTCA) [31]. In contrast, it has also been demonstrated that human PTH gene which is suppressed by 1,25(OH)2D3, has a single motif (AGGTTCA) for VDR [32]. VDR-specific direct repeat motif is not present within human c-myc −106 to −410 fragment, but his fragment contains the sequence (AGGACA) at −325 similar to the VDRE present in the PTH gene. We therefore suggest that regulation of the c-myc gene by 1,25(OH)2D3 may be mediated directly through this element.

OCT has been demonstrated to suppress the growth of several types of cells in in vitro systems [12, 27]. A suppressive in vivo effect of OCT on breast carcinoma has also been reported [11]. Although local injection of OCT in the tumor slightly decreased the average weight of the tumor mass, neither OCT nor 1,25(OH)2D3 significantly suppressed tumor growth in nude mice. The observed effect after intra-tumor injection and its absence after intraperitoneal injection suggest the use of an insufficient dose in the latter therapy. The mechanism underlying the failure of OCT to reduce tumor size compared with the in vitro effect and the effects observed in breast cancer are not fully understood. It is possible that this phenomenon may not be directly related to the action of the OCT but is rather due to circulatory factors related to the nature of NPA tumors. For example, injected OCT or 1,25(OH)2D3 may be catalyzed or flushed out immediately after administration in the presence of a high blood supply. Further studies including in vivo metabolism of OCT would be necessary to investigate this phenomenon. In addition, tumor size in the intratumor group was larger than that in the intraperitoneal group. Repeated direct injection into the tumor may spread NPA cells and mechanically or reactively stimulate the growth of the tumor. Although the established NPA cell line is reflected by only some of the characteristics of human papillary thyroid carcinoma, the use of OCT as an adjunct therapy in postoperative thyroid cancer may be limited in view of our in vivo results. Recently, combination of a potent Vit D3 analogue with 9 cis-retinoic acid or tumor necrosis factor alpha has been reported to be effective in leukemia and renal carcinoma cells [33, 34]. In this regard, it is noteworthy that OCT did not change serum calcium levels. This property may warrant clinical use of OCT in combination with chemotherapy or radiation therapy, besides a role in preventing osteoporosis insted of vitamin D3 during treatment.

In summary, proliferation of well-differentiated thyroid cancer cell in in vitro conditions was inhibited by OCT associated with inhibition of transcription of c-myc gene expression, but this inhibitory effect was not present in vivo.

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References


