RESEARCH ARTICLE

# Evaluation of Efficacy, Safety and Tolerability of High Dose-Intermittent Calcitriol Supplementation to Advanced Intrahepatic Cholangiocarcinoma Patients - A Pilot Study

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## Abstract

**Antitumor activity (growth suppression) of vitamin D has been demonstrated using cholangiocarcinoma (CCA) cell lines, CCA cell-grafted animal models, and human CCA tissue cultures. The present study aimed to determine the toxicity and tolerability of intermittent-high dose calcitriol in advanced inoperable intrahepatic CCA patients and to evaluate the therapeutic efficacy of combinations of calcitriol and 5-fluorouracil-based chemotherapeutic drugs. The patients were divided into 3 groups: the first (n=2) received intermittent-high dose oral calcitriol 12 µg/day for 3 days, i.e. Monday-Wednesday, per week up to 3 months. The treatment did not cause any serious adverse events, except hypercalcemia grade I, once in 72 administrations. The second group (n=3) received chemotherapeutic drugs (5-fluorouracil, Mitomycin C and Leucovorin) for 3 cycles, one patient showing a partial response. The third group (n=4) received high dose calcitriol in combination with chemotherapeutic-drugs. All 4 patients encountered serious adverse events and two of them were withdrawn after the first drug cycle. This pilot study suggests that, although high dose-intermittent calcitriol appeared to be safe and tolerated well in advanced intrahepatic CCA patients, co-administration with 5-fluorouracil-based chemotherapeutic drugs caused unexpected potentiation of their toxicity. Adjustment of the doses of both drugs is required to avoid such toxicity and to optimize therapeutic efficacy of anticancer drugs when they were combined with high dose-intermittent calcitriol. USA Clinical Trial : NCT01039181.**

**Keywords:** Vitamin D - clinical trial - treatment - 5-fluorouracil

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## Introduction

Vitamin D is best known for its role in stimulating calcium absorption. Several epidemiological studies revealed the inverse relationship of sunlight exposure and/or dietary vitamin D intake with the incidence of breast, prostate and colon cancers in humans (Skowronski et al., 1993; Buras et al., 1994; Vandewalle et al., 1994; Evans et al., 1999; Narvaez et al., 2001). In the past two decades, the roles of vitamin D in the regulation of cell proliferation, apoptosis, metastasis and angiogenesis both *in vitro* and *in vivo* have been reviewed (Chen, et al., 2003; Grau et al., 2003; Hussain et al., 2003). Results from Phase I and phase II trials of calcitriol either alone or in combination with chemotherapeutic agents indicated its tumor suppression effects (Smith, et al., 1999; Beer, et al., 2001; 2003; 2005a; Muindi et al., 2002; Morris et al., 2004; Trump et al., 2006). However, therapeutic uses of calcitriol, 1,25-dihydroxyvitamin $D_3$, have been encountered with hypercalcemic side effect in most cases. Vitamin D analog with less calcemic effect were developed to solve this problem and have been used in the treatment of a variety of cancer, such as liver, pancreas, breast and colorectal cancers (Beer, et al., 2003; Grau et al., 2003; Hussain et al., 2003). Results from pre-clinical studies showed that these analog achieved therapeutic properties equal to calcitriol with lesser side effects. Nevertheless, clinical trial is rare and need more studies to determine the best fit dosages to cancer patients.

Cholangiocarcinoma (CCA) is a rare but highly lethal

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malignancy. Complete surgical resection offers the only chance for cure. However, majority of patients were diagnosed when diseases were at advanced stage. Patients with unresectable CCA have a very poor prognosis. Chemotherapy is the option left to treat these inoperable patients. However, data regarding chemotherapy are disappointing (Andre et al., 2004; Cho et al., 2005; Nehls et al., 2008). Searching for effective treatments is the hope to improve the survival of CCA patients. Our group have reported the anti-proliferative activity of calcitriol, ($1\alpha$,25$(OH)_2D_3$), on CCA cell lines (KKU-M213 and KKU-M214) (Seubwai, et al., 2007) and the effectiveness of intraperitoneal injection of Oxarol (22-oxa-1,25-dihydroxyvitamin $D_3$), a calcitriol analog, to suppress the growth of KKU-M213 and KKU-M214 tumor cells in xenografted mice (Seubwai et al., 2010). In addition, we also have shown the over-expression of vitamin D receptor (VDR) in CCA patients' tissues (Seubwai et al., 2007). These preclinical data suggest the potential of using vitamin D as an adjunct therapy in combination with classical chemotherapeutic agents for CCA.

To our best knowledge, no studies have been conducted to test the safety and tolerable dose of vitamin D supplementation in CCA. We therefore conducted a pilot study to examine the safety, tolerability, therapeutic and adverse effects of high dose-intermittent calcitriol supplementation to the chemotherapy for advanced intrahepatic CCA. In this study, the intermittent calcitriol treatment plan was chosen because it was better tolerated than continuous therapy in the prior studies of calcitriol (Smith et al., 1999). The results show that although high dose-intermittent calcitriol appeared to be safe and tolerated well in advanced intrahepatic CCA patients, co-administration with 5-fluorouracil-based chemotherapeutic drugs caused unexpected potentiation of the toxicity of chemotherapeutic drugs.

## Materials and Methods

*Patient eligibility criteria*

Patients eligible for this trial were CCA patients aged 30-65 years with histologically proven stage III-IV advanced CCA with measurable disease by CT scan. Performance status was required to be 0 or 1 according to the criteria of the Eastern Cooperative Oncology Group (ECOG). Patients were required to have normal hematologic and organ function parameters (white blood cell count ≥3,000 cells/$mm^3$, platelet count ≥100x$10^3$ cells/$mm^3$, hematocrit ≥30%, neutrophil ≥1,500 cells/$mm^3$, total bilirubin ≥1 mg/dL, creatinine <1.6 mg/dL, aspartate aminotransferase and alanine aminotransferase within 3 times of the normal limits). The corrected serum calcium was required to be <10.5 mg/dL. Patients with hyperparathyroid or any history of nephrolithiasis, kidney stone or on anticancer therapy were ineligible and all patients were required to have either a CT scan or ultrasound (US) examination of the kidneys and ureters that indicated no evidence of lithiasis within 30 days of study entry. A written informed consent was required before entry into the study and this study was approved by the Khon Kaen University Ethics Committee for Human Research (HE251139), and registered to the USA Clinical Trial : NCT01039181.

*Treatment plan*

This study was a clinical trial phase I/II of calcitriol in combination with 5-fluorouracil/mytomicin-c/leucovorin in an open label, non-randomized study to evaluate the tolerable dose, safety and objective tumor response in patients with advanced intrahepatic CCA. The patients were divided into 3 groups: calcitriol treatment group; chemotherapeutic drug treatment group and calcitriol in combination with chemotherapeutic drug treatment group.

Calcitriol (Decostriol®, Jesalis Pharmac GmbH, Germany) (0.25 $\mu$g caplet) was administered according to the following schedule: 12 $\mu$g in dividing doses in the morning and evening with meal given on three consecutive day (Monday, Tuesday and Wednesday) weekly for 4 consecutive weeks a cycle. Dietary calcium was not restricted during the study. The schedule for chemotherapeutic drug treatment was chosen based on our experience on Intention-to-treat for intrahepatic CCA. The regimen of chemotherapeutic agents for 1 cycle was composed of 5-Fluorouracil (5-FU) (600 mg/$m^2$) and Leucovorin (200 mg/$m^2$) x5 days, Mitomycin-C (7 mg/$m^2$) x1 day. Patients were given 6 cycles for the full course treatment. Supplementation of calcitriol was started on the first day of chemotherapy and continuously until 6 months.

*Patient monitoring and dose modification*

Serum calcium, phosphorus, creatinine, and electrolytes were measured on the first and third weeks of the first cycle and monthly thereafter. Liver function tests, hematologic values and urine analysis were performed to obtain base line data and followed up monthly. Tumor markers (CA 19-9, CEA and AFP), plain kidneys, ureters and bladder (KUB) x-ray for urolithiasis, chest x-ray for tumor metastasis, and upper abdominal CT evaluation to assess response to chemotherapy were performed at the base line and repeated every 3 months. Adverse events were monitored continuously throughout the study and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. In terms of calcitriol dose modification, if patients showed more than grade 2 hypercalcemia (serum calcium >11.5 mg/dL) on Monday, calcitriol supplementation was held and serum calcium measurement was repeated on the next Monday. If the serum calcium measurement on Monday were <11.5mg/dL, treatment was continued without modification of the dose or schedule. If the serum calcium remained >11.5 mg/dL, calcitriol supplementation was held and calcium measurement was repeated on Monday. Therapy was resumed at the same dose and schedule on the next Monday if the serum calcium were <11.5mg/dL. If a patient required 2 such dose interruptions, the calcitriol dose was to be reduced by 50%. Patients who experienced twice of hypercalcemia greater than grade 2 (>12.5 mg/dL) was withdrawn from the study. For the CCA chemotherapy, therapy dose was reduced to 25% if patients experienced more than grade 3 adverse events (http://cteo.cancer.gov). Treatment will be stopped

when patients have progressive disease or worsening of performance status.

*Study design*

This is an open labeled, non randomized, prospective trial. There are 3 groups of patients enrolled into the study. First group (n=2) was allocated to high dose intermittent calcitriol. The second group (n=3) received chemotherapy alone. The third group (n=4) received the combination of high dose intermittent calcitriol and chemotherapy.

Two co-primary end points of this trial were toxicities and disease response rate. Tumor responses were evaluated according to RECIST criteria with the endpoint of this trial is objective response rate (complete response + partial response). Only patients who received at 3 cycles of allocated treatment are counted for response rate evaluation. Toxicities data were collected and reported in every patient who received at least 1 cycle of therapy.

## Results

*Patient characteristics*

Nine patients, 3 females and 6 males, were entered into this study from January 2010 to April 2011. All patients were eligible for toxicities assessment but only seven patients were eligible for response evaluation; two patients were excluded because of early worsening of hepatic failure after 1 course of treatment. The median age was 58.6 years (ranged 45-68 years). The baseline blood parameters are shown in Table 1. Mean hemoglobin value was 11.81 gm/dL, mean white blood cell count was 6600.78 cell/$mm^3$. All serum chemistries including serum calcium were within normal limits. Baseline vitamin D level was 33.88 ng/mL which was in normal limits. Normal vitamin D level in Thai people is 31.8 ng/mL according to the data collected in the year 2008. Baseline CA19-9 was 195.98 U/mL.

**Table 1. Hematological and Biochemical Parameters of Patients at Baseline**

| Parameters (Units) | Mean | SD | MAX | MIN |
|---|---|---|---|---|
| Blood paraneters | | | | |
| Hemoglobin (g/dL) | 11.81 | 1.97 | 14.8 | 7.5 |
| White blood count (cells/$mm^3$) | 6601 | 2912 | 12413 | 2827 |
| Platelet (/$mm^3$) | 255333 | 72085 | 342000 | 139000 |
| Serum parameters | | | | |
| Calcium (mg/dL) | 9.11 | 0.41 | 9.7 | 8.6 |
| Corrected Calcium (mg/dL) | 9.43 | 0.49 | 10.42 | 9.32 |
| Phosphorus (mg/dL) | 3.93 | 0.72 | 5.5 | 3.1 |
| Potassium (mEq/L) | 3.84 | 0.51 | 4.5 | 3 |
| BUN (mg/dL) | 10.40 | 3.42 | 17.7 | 7.4 |
| Creatinine (mg/dL) | 0.72 | 0.19 | 1.1 | 0.5 |
| Albumin (g/dL) | 3.71 | 0.52 | 4.6 | 3.1 |
| AST (U/L) | 48.89 | 29.62 | 117 | 22 |
| ALT (U/L) | 31.33 | 14.63 | 61 | 14 |
| ALP (U/L) | 265.67 | 223.29 | 769 | 46 |
| Vitamin D [1,25$(OH)_2D_3$] (ng/mL) | 33.88 | 7.41 | 44.11 | 22.82 |
| Tumor markers | | | | |
| CA19-9 (IU/mL) | 195.98 | 420.03 | 1000 | 0.6 |
| CEA (ng/mL) | 38.05 | 53.50 | 153.7 | 0.864 |
| AFP (ng/mL) | 42.08 | 107.92 | 309.1 | 1.66 |

*BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; CA19-9, carbohydrate antigen 19-9; CEA, carcino-embryonic antigen; AFP, alpha-fetoprotien, mEq, mili-equivalents

*Serum calcium and toxicity*

Six patients were received high dose-intermittent calcitriol, but only 4 patients could complete 3 cycles of administration (2 each in calcitriol treatment group and comibination treatment group). No hyperphosphatemia, signs of urolithiasis or renal dysfunction were observed in any patients who had high dose-intermittent calcitriol administration. During a total of 144 day-administrations in these 4 patients, only two hypercalcemia grade I (serum calcium values was >10.9-11.5 mg/dL) were detected within the first cycle of therapy. The criteria for dose reduction/interruption based on hypercalcemia were not seen in those 4 patients. Other 2 patients in drug combination treatment group were received only one cycle of calcitriol treatment because hypercalcemia grade II (serum calcium value >11.5-12.5 mg/dL) was detected once in one patient and hypercalcemia grade III (serum calcium values >12.5-13.5 mg/dL) was found once in another patients. These 2 patients with symptomatic hypercalcemia were to be ceased calcitriol therapy and were terminated from the study thereafter.

*Treatment and response assessment*

Calcitriol group: Two patients in the calcitriol group received 3 cycles (3 months) of calcitriol. Both of them had stable disease as a best response. Because of the lack of the anti-cancer effect of calcitriol alone, both of them received chemotherapy after the completion of the calcitriol treatment. The first patient had 27% tumor shrinkage (stable disease by RECIST definition) and the second patient had decrease pleural effusion after 3 cycles of chemotherapy. The first patient achieved stable disease after completion of 6 cycles of chemotherapy and alive without disease progression at the end of the follow-up study (15 months after diagnosis). The other patient with the decrease of pleural effusion had progressive disease after the fifth cycle of treatment.

Chemotherapy group: There were 3 patients in this group. To ensure patient's safety, chemotherapy was started at 75% of maximum doses and escalated to 100% in the next cycle if patient had no grade 3 or 4 adverse events. Partial response (33% objective response rate) was observed in one patient after 3 cycles of chemotherapy treatment. The other 2 patients had progressive disease. In term of toxicities, there was no serious adverse event with the use of chemotherapy only even with the maximum dose of 100%.

Combination of calcitriol and chemotherapy group: There were 4 patients in this group, 2 patients had 3 cycles and 2 patients had only one cycle of the calcitriol treatment. Among 2 patients received 3 cycles of calcitriol treatment, the first patient received 100% dose of both calcitriol and chemotherapy at the beginning. Grade 4 febrile neutropenia, grade 3 stomatitis, grade 3 hypokalemia and grade 1 hypercalcemia occurred after first cycle of full dose treatment. Chemotherapy was reduced to 75% in the next cycle. Febrile neutropenia, grade 2 stomatitis and grade 1 hypokalemia was observed. Then chemotherapy

**Table 2. Characteristic, Adverse Effect and Response of Each Patient Under Study**

| Sex | | Age (year) | Cycle | | | Outcome |
|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | |
| Calcitriol | F | 64 | No chemotherapy, hypercalcemia grade I, No SAE | No chemotherapy, No SAE | No chemotherapy, No SAE | Receive chemotherapy after 3 cycles of calcitriol, tumor size reduced 27%, stable disease after 6 cycles. Patient was alive at the end of program. |
| | M | 54 | No chemotherapy, No SAE | No chemotherapy, No SAE | No chemotherapy, No SAE | Receive chemotherapy after 3 cycles of calcitriol, plural effusion reduced after 3 cycles, tumor progress after the 5th cycle, terminated |
| Chemotherapy | M | 52 | Chemotherapy 75%, No SAE | Chemotherapy 75%, No SAE | Chemotherapy 75%, No SAE | progressive disease by RICST |
| | F | 59 | Chemotherapy 75%, No SAE | Chemotherapy 100%, No SAE | Chemotherapy 100%, No SAE | progressive disease by RICST |
| | M | 45 | Chemotherapy 75%, No SAE | Chemotherapy 100%, No SAE | Chemotherapy 100%, No SAE | Partial response after 3 cycles, received full course (6 cycles), progressive disease by RICST thereafter |
| Chemotherapy+ Calcitriol | F | 60 | Chemotherapy 100%, febrile neutropenia grade III, hypokalemia grade IV, stomatitis grade III, hypercalcemia grade I | Chemotherapy 75%, febrile neutropenia grade III, hypokalemia grade I, stomatitis grade II, | Chemotherapy 50%, hypokalemia grade I | progressive disease by RICST |
| | M | 68 | Chemotherapy 100%, febrile neutropenia grade II, | Chemotherapy 75%, No SAE | Chemotherapy 75%,No SAE | progressive disease by RICST |
| | M | 66 | Chemotherapy 75%, hepatic encephalopathy hypercalcemia grade II | withdrawn | withdrawn | progressive disease |
| | M | 60 | Chemotherapy 75%, hypercalcemia (grade III), hepatic encephalopathy (grade IV), jaundice (grade III), anemia and thrombocytopenia (grade I) | Terminated | Terminated | hepatic encephalopathy |

was reduced further to 50% along with the full dose of calcitriol without any serious adverse event. This patient had progressive disease evaluated by CT scan after 3 cycles. The second patient received full dose of both drugs in the first cycle. Grade 4 febrile neutropenia occurred. After the reduction of chemotherapy to 75%, patient can tolerate treatment without any serious adverse events for the next 2 cycles. This patient had progressive disease after 3 cycles of treatment. Another 2 patients in this group received 75% of chemotherapy combined with full dose of calcitriol at the beginning but both of them had fulminant hepatic failure after only one cycle of treatment and one of them showed grade 3 hypercalcemia and the other showed grade 2 hypercalcemia. Because of rapidly worsening hepatic failure, CT scan could not be done. Thus, it remains unclear whether the hepatic failure was due to the progress of the disease or due to the adverse effect of treatment. The summary of all the patient's treatment and outcome are shown in Table 2

## Discussion

Accumulated preclinical data suggests that vitamin D may be useful as an antiproliferative agent in clinical cancer management. Our preclinical studies showed the antitumor effects of vitamin D on CCA cell lines (Seubwai et al., 2007), xenografted CCA cells in mice and histoculture of CCA tissues from patients (Seubwai et al., 2010). In the present study, we planned to evaluate the safety and tolerability of high dose-intermittent calcitriol supplementation to the advanced intrahepatic CCA patients. A causal relationship between vitamin D supplementation and chemotherapy toxicities was also assessed as the secondary objective.

Several clinical trials have evaluated high dose-intermittent vitamin D analog in cancer therapy (Beer et al., 2001, 2003, 2005a; Muindi et al., 2002; Morris et al., 2004b). Morris (2004) noted that calcitriol 30 $\mu$g given three consecutive days per week plus zoledronic acid in progressive prostate carcinoma was safe and feasible. In addition, the clinical phase II trial in androgen-independent prostate cancer patients with a high dose-intermittent calcitriol (12 $\mu$g three consecutive days per week) plus dexamethasone appeared to be safe, feasible, and had antitumor activity (Trump et al., 2006). These administration protocols yielded the average blood calcitriol of 2 nM without hypercalemia greater than grade II (Beer et al., 2004; Trump et al., 2006).

To our knowledge, the high dose-intermittent calcitriol has never been tested in advanced CCA patients. The dose and schedule employed in this study (12 $\mu$g x3 consecutive days weekly) were chosen. This is because in the previous reports of the doses >14-18 $\mu$g, the correlation between the administered dose and the plasma concentration was no longer linear, suggesting a limitation of bioavailability at higher doses (Muindi et al., 2002; 2005). Secondly, the daily dose of 12 $\mu$g calcitriol yielded 0.5-1.0 $\mu$M of calcitriol in blood (Muindi et al., 2002; Morioka et al., 2002; Beer et al., 2005b) and this level was equivalent to the optimal dose used for the cytotoxicity test in the CCA cell lines (Seubwai et al., 2007). In the present study, the safety and tolerability of high dose-intermittent calcitriol were examined in two advanced intrahepatic CCA patients. The patients could tolerate with calcitriol treatment without obvious toxicity. Having high dose calcitriol alone for 3 cycles, one patient had experienced only once hypercalcemia grade I (>10.9-11.5 mg/dL) without any sign, symptom or serious side effects. The calcitriol treatment regimen described in this study was extremely well tolerated. In addition, both patients had stable disease as a best response after having received 3 cycles of chemotherapy. Such a beneficial result was not observed in any patients of other groups. With the limitation of the small sample size, the observed response to chemotherapy in these two patients might be due to the schedule-dependent sequential exposure effects.

Two of the three patients in chemotherapy treatment group had a progressive disease and one of them exhibited partial response. The third patient had a progressive disease after receiving a full course of therapeutic drug (6 cycles). In general, the response rate of CCA patients to the treatment regimen regardless of the chemotherapeutic drugs (Uttaravichien et al., 1999; Charoentum, et al., 2007; Demols et al., 2007; Marechal et al., 2007). In our pilot study, even a low number of patients, we found one of the three patients (33%) had partial response to the designed treatment of the current study.

Safety and efficacy of high dose calcitriol supplementation to 5FU/mitomycin-C/leucovorin were also assessed in this pilot study. Higher grade (grade II and III) of hypercalcemia was frequently observed in this group. Three of four patients in this group had one observation of hypercalcemia of grades I, II, or III. All hypercalcemia were manageable by stopping calcitriol supplementation, but one case of hypercalcemia grade III received intravenous fluid and bisphosphonate. Only 2 patients could complete full 3 cycles of treatment allocated and were included in the response evaluation. No response was found. The other 2 patients had rapid worsening of hepatic failure after only 1 cycle of treatment and were terminated the treatment earlier. Of note no patient could tolerate full dose of chemotherapy combined with high dose calcitriol. The full dose treatment was ended up with severe grade 3 and 4 toxicities which include febrile neutropenia, stomatitis, hypokalemia and hypercalcemia. The reduction of chemotherapy to 75% combined with high dose calcitriol seemed to be more tolerable but still patients were suffered with very high incidence of grade 3 and 4 toxicities of 50% (3 in 6 cycles). These adverse events were not observed in the patients having chemotherapy alone. Even though this is a limited pilot study, it is clear that combination of high dose calcitriol plus chemotherapy did not improve the treatment outcome. Conversely, this regimen potentiated the chemotherapy toxicity as indicated by febrile neutropenia found in 3 out of 4 patients. Severity of the adverse events described above was reduced by reduction of chemotherapy dose to 50-75%. Calcitriol seems to potentiate the cytotoxic effects of 5-FU-based chemotherapy. Unfortunately, the enhanced cytotoxic effects of 5-FU based agents did not reflect to anti-tumor effect, as all patients in the combination group had progress of the disease.

The present study failed to demonstrate the positive effect of calcitriol supplementation on anti-tumor activity. Alternatively, calcitriol potentiated the adverse effect of chemotherapy because the patients who received chemotherapy alone could tolerate maximum dose of 100% without any observed adverse events in terms of both hematologic and non-hematologic parameters. Toxicities described above were decreased by reducing the dose of chemotherapy. Why high dose calcitriol enhanced adverse effects without enhancing anti-tumor activity remains to be clarified. One interesting observation from our study is that the schedule between high dose calcitriol and chemotherapy may be critical on the therapeutic effects, because 2 patients who received high dose calcitriol only at the beginning achieved tumor response of 27% reduction of the tumor after switching to chemotherapy in one patient and the disappearance of pleural effusion in another. The effects of drug combination is schedule-dependent. As was reported by Kano et al. (1998), simultaneous exposure to paclitaxel and irinotecan produced antagonistic (sub-additive and protective) effects on the human cancer cell lines of lung, breast, and colon. On the other hand, sequential exposure to paclitaxel followed by irinotecan, and also the reverse sequence, produced additive effects on the all cell lines tested. These findings suggest that sequential administration, not simultaneous administration, may be the appropriate schedule for the therapeutic combination of paclitaxel and irinotecan. The similar observation was reported by Akutsu et al. (2002) on cytotoxic effects of methotrexate and cytarabine in combination against human leukemia cell lines. In our study, simultaneous administration of high dose calcitriol and chemotherapeutic drugs resulted in the adverse effects without potentiation of anti-cancer effects. However, the

sequential exposure to calcitriol for 3 cycles followed by chemotherapeutic drugs suggested to have synergistic effects as the response signs to chemotherapy were observed in 2 of 2 patients who received the complete schedule of calcitriol. Further preclinical and clinical studies should be continued to determine the optimal schedule and doses for this combination towards the clinical use.

In some studies (Munker et al., 1996; Mellibovsky et al., 1998), calcitriol administrations have been limited because of the occurrence of hypercalcemia. In our study, 4 CCA patients who received 3 cycles of intermittent-high dose of calcitriol either alone or in combination with chemotherapy had no severe hyperphosphatemia, no adverse effect on kidney function and most of them showed manageable hypercalcemia. The present results revealed that intermittent high-dose calcitriol is safe and can be tolerated in advanced CCA when it were given alone. For the safety of using calcitriol at a higher dose, Morris et al. (2004) used 30 $\mu$g every day x 3 weekly for the treatment of progressive prostate carcinoma without serious adverse effects. However, a recent systematic review on the efficacy of vitamin D supplementation in cancer patients indicated that hypovitaminosis D seemed to be associated with a worse prognosis in some cancers but vitamin D supplementation failed to prevent the progression of prostate cancer (Buttigliero et al., 2011). Moreover, the association between vitamin D depletion and chemotherapy toxicity was reported (Kitchen et al., 2011).

The available evidence is not strong enough to recommend vitamin D supplementation for cancer chemotherapy in clinical practice. Conclusion regarding the benefits or harms of vitamin D supplementation for the treatment of CCA is insufficient at this stage. The optimal approach to calcitriol supplementation for CCA patients in combination with chemotherapeutic drugs has to be evaluated with caution. Further study is required for the determination of appropriate dose and dosing regimens, safety as well as the efficacy.

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