

HEPATOCELLULAR CARCINOMA: TREATMENT AND RECURRENCE MARKER

A phase one study of the hepatic arterial administration of 1,25-dihydroxyvitamin D₃ for liver cancers

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Abstract

Background and Aims: It is well established that exposure to 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) inhibits the proliferation of human colorectal cancer and hepatoma cell lines, both *in vitro* and *in vivo*. However, clinical trials of the administration of 1,25(OH)₂D₃ and analogs for the treatment of malignancy have been limited by the development of hypercalcemia. 1,25-dihydroxyvitamin D₃ is principally excreted in bile following hepatic catabolism. This suggested the hypothesis that hepatic regional administration may allow high doses of 1,25(OH)₂D₃ to be administered for the treatment of liver cancers without producing hypercalcemia, caused by a clinically significant first pass effect. This phase one study investigates the effect of hepatic regional administration of 1,25(OH)₂D₃ on serum calcium levels, together with other markers of renal and liver function.

Methods: Six subjects with hepatic colorectal cancer metastases and one with primary hepatocellular cancer were given continuous hepatic arterial infusions of 1,25(OH)₂D₃, for periods of 1–4 weeks. Blood samples were taken regularly and assayed for calcium levels, liver function tests and urea and electrolyte levels.

Results: Patients remained normocalcemic at dosages of up to 10 mcg/day. No patient experienced any side-effects from the treatment.

Conclusions: Administration of 1,25(OH)₂D₃ as a continuous hepatic arterial infusion allows a high dosage to be administered without inducing hypercalcemia. This route of administration may allow the potential of 1,25(OH)₂D₃ in the treatment of hepatic cancers to be realized.

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Key words: 1,25-dihydroxyvitamin D₃, colorectal cancer, hepatic arterial infusion, hepatocellular cancer, hypercalcemia.

INTRODUCTION

Colorectal carcinoma is responsible for more deaths in non-smokers than any other cancer in the developed world, with the liver being the most common site for metastases to occur.¹ Although hepatoma (hepatocellular carcinoma) is rare in the Western world, it is one of the commonest causes of cancer death throughout most of Asia.

Surgical resection or destruction offers the only potentially curative treatment for hepatic malignancy,

but is possible in only the minority of patients.^{2–4} The majority of patients are suitable only for cytotoxic chemotherapy. The delivery of cytotoxic chemotherapy as an infusion via the hepatic artery to treat patients with metastases from colorectal cancer is a comparatively recent strategy. Opinions are somewhat divided regarding its relative benefits over systemic chemotherapy, with enthusiasts claiming higher response rates,^{5–8} lower systemic concentrations,⁹ less side-effects and improved survival than intravenously administered chemotherapy or no treatment.^{10–13} However, overall

results are still poor and there remains a need for more effective and less toxic treatments for liver cancer.

In addition to its role in the control of calcium and phosphate homeostasis, it is well established that the hormonally active form of vitamin D—1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), has other important biologic effects. It is capable of inducing differentiation and controlling proliferation of both normal and malignant cells.^{14–20} The inhibition of the proliferation of colorectal cancer cells by 1,25(OH)₂D₃ was first reported *in vitro* by Lointer *et al.* in 1987,²¹ and *in vivo* by Eisman *et al.*²²

We have previously reported *in vitro* inhibition of the proliferation of several human colorectal cancer cell lines.²³ The 1,25(OH)₂D₃ analog EB 1089 has a similar effect *in vivo*.²⁴ This effect appeared to be mediated by the interaction of 1,25(OH)₂D₃ or its analogs with specific nuclear vitamin D₃ receptors. More recently we have demonstrated a more marked inhibition of the proliferation of hepatoma cell lines exposed to either 1,25(OH)₂D₃ or EB 1089.²⁵

The clinical use of 1,25(OH)₂D₃ and its less calcemic analogs to treat malignancy has been limited by hypercalcemic effects.^{26,27} The principal route of excretion of calcitriol is in bile.^{28–33} We hypothesized that administration of 1,25(OH)₂D₃ as a hepatic arterial infusion would allow high concentrations to be delivered to tumors within the liver while avoiding high systemic concentrations and hypercalcemia, caused by a significant hepatic first pass metabolism.

This is the first report of the intra-arterial administration of 1,25(OH)₂D₃. The aim of this study was to investigate the safety of intrahepatic arterial infusion of 1,25(OH)₂D₃ in patients with hepatic malignancy, and in particular to assess the risks of developing hypercalcemia.

METHODS

Patients were recruited from our hepatic oncology clinics. Patients with unresectable hepatic metastases from colorectal cancer or primary hepatoma were considered for entry into the trial if they were considered to have failed to have a response to conventional treatment. Hepatic lesions were considered to be from colorectal primary cancers if there was histological confirmation of the primary tumor, an elevated carcinoembryonic antigen (CEA) level and characteristic computerized tomography (CT) appearance of the lesions. Primary hepatocellular cancer was confirmed by liver biopsy histology. Treatment failure was defined as a rising tumor marker level, CEA or α -fetoprotein (AFP), or increasing tumor size on serial CT scanning. The trial protocol was approved by the South-eastern Sydney Area Health Service Ethics Committee.

Patients with high volume or symptomatic extrahepatic disease were excluded, as were patients with an expected survival period of less than 2 months, concomitant thiazide diuretic or calcium based antacid treatment, liver or renal failure, or known sensitivity to vitamin D compounds.

Some of the patients considered for entry into this trial already had a hepatic artery catheter (HAC) *in situ*, either connected to a subcutaneous port site or connected to an implanted 'Infusaid' pump (Intermedics, Infusaid, Norwood, MA, USA) for the delivery of hepatic arterial infusion chemotherapy. These patients had the patency and siting of their catheters confirmed prior to entry into the trial by radiologic contrast studies. Patients without hepatic arterial catheters already *in situ* were treated via a radiologically sited temporary HAC.

All patients received 1,25(OH)₂D₃—'Calciject' (Abbott Australasia, Kurnell, NSW, Australia) via the HAC as a continuous infusion delivered by an external 'Infusor' pump (Baxter Healthcare Corporation, Deerfield, IL, USA). In patients requiring a temporary HAC, the external Infusor pump was directly connected to the catheter at its exit point. Patients with indwelling HAC were connected via their subcutaneous port or the side port of their Infusaid implanted pump.

The duration of treatment depended on the type of HAC used. In patients requiring a temporary HAC, treatment was administered for only 1 week because of the necessary limitation of mobility and the need for hospitalization while these catheters were *in situ*. Patients with an indwelling HAC were initially treated for 4 weeks, and following a treatment free period of 4 weeks, these patients were then given a further period of treatment at higher doses.

All patients were examined twice daily for the first week, and three times a week subsequently. Blood was taken on each occasion for assessment of serum calcium, phosphate, urea and electrolytes, liver function tests, full blood count and coagulation studies.

An escalating dosage schedule was used as detailed in Table 1. The first two subjects (numbers 1 and 2) were treated with an infusion of 0.2 mcg/day 1,25(OH)₂D₃ for 4 days, and subsequently with 0.5 mcg/day. Subsequent subjects (numbers 3, 5, 6 and 7) were initially given 2 mcg/day, and then 4 weeks of 5 mcg/week. After a 4 week rest period they were restarted on treatment at a dose of 10 mcg/day. This was then increased to 15 mcg/day.

Patient number 4 was hypercalcemic prior to treatment because of a paraneoplastic syndrome, and therefore was given lower dosages (0.5 mcg/day increasing to 2 mcg/day and to 5 mcg/day at eight day intervals).

All patients adhered to a low calcium diet (estimated at < 350 mg/day) for 3 days prior to and throughout the treatment period.

RESULTS

Seven subjects (five males and two females) were entered into the trial. Six subjects had hepatic metastases from colorectal cancer and one had hepatoma. Subjects' ages ranged from 42 to 75 years with a median of 62 years. Five subjects had extrahepatic disease (Table 2).

Five subjects completed the intended period of treatment. One subject (Subject 2) withdrew from the trial after 14 days of treatment for personal reasons. Subject

Table 1 Treatment summary

Subject no.	Period 1		Period 2		Period 3		Period 4		Period 5	
	Dose	Days	Dose	Days	Dose	Days	Dose	Days	Dose	Days
1	0.02	1–4	0.05	4–7	—	—	—	—	—	—
2	0.02	1–4	0.05	4–7	—	—	—	—	—	—
3	2	1–4	5	4–24	0	24–59	10	59–67	15	67–71
4	0.5	1–8	2	8–16	5	16–26	—	—	—	—
5	2	1–5	5	5–26	0	26–61	10	61–65	15	65–75
6	2	1–4	5	4–27	0	27–52	10	52–57	15	57–60
7	2	1–3	5	3–29	—	—	—	—	—	—

Dose is in µg/day.

Table 2 Subject details

No.	Age	Sex	Tumor	Extrahepatic disease/ complications
1	62	F	Colorectal	Lung metastases
2	73	M	Hepatoma	Nil
3	42	M	Colorectal	Ascites and abdominal wall metastases
4	72	F	Colorectal	Obstructive jaundice, partially relieved by intrahepatic biliary stents
5	57	M	Colorectal	Nil
6	75	M	Colorectal	Lung metastases, obstructive jaundice relieved by intrahepatic biliary stents
7	57	M	Colorectal	Obstructive jaundice, partially relieved by extrahepatic biliary stents

7 was commenced on the same dosage regimen as patients 3, 5 and 6, but was withdrawn from the trial prior to starting 10 mcg/day because of progressive debilitation related to his disease.

The only complication that occurred during treatment was the transient elevation of the International Normalized Ratio (INR) of one subject (Subject 1). This patient had been treated with warfarin (Boots Health Care, North Ryde, NSW, Australia) for 6 months prior to entry because of multiple pulmonary emboli and the insertion of an Inferior Vena Caval Filter, after resection of her primary tumor. The INR returned to normal with temporary cessation of warfarin treatment, but was noted to rise once more some 3 weeks after the trial treatment finished.

No patient developed hypercalcemia during the treatment at doses up to and including 10 mcg/day, as shown in Fig. 1. Subject 4 had an elevated calcium level prior to treatment, and her calcium levels decreased during treatment. Three subjects were given 15 mcg/day dosage infusions. Of the three patients two, (subjects 5 and 6), became hypercalcemic after 10 and 3 days, respectively, and treatment was discontinued. Subject 3 did not become hypercalcemic prior to the discontinuation of treatment.

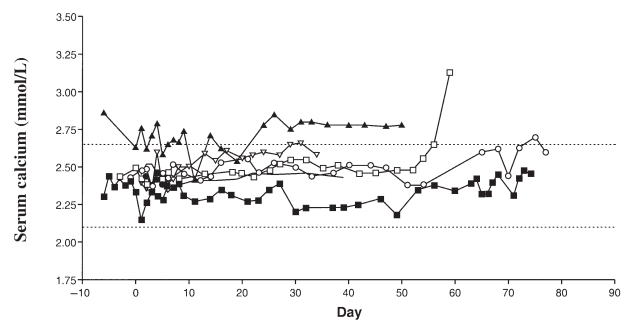


Figure 1 Serum calcium levels of all subjects. (.....) Denotes the normal range of serum calcium levels. (—) Patient 1; (●) patient 2; (■) patient 3; (▲) patient 4; (○) patient 5; (□) patient 6; (▽) patient 7.

Phosphate levels (Fig. 2) remained constant in all subjects except subject 7. This patient had a rise in the level of serum phosphate to above the normal range from day 20. The elevation persisted after treatment was discontinued on day 28 until the subject's withdrawal from follow-up on day 38.

In all patients, liver function tests and serum urea and electrolyte levels varied only within normal levels throughout the treatment period.

DISCUSSION

1,25-Dihydroxyvitamin D₃ and some of its analogs have been shown to inhibit the proliferation of many malignant cell lines, including colorectal cancer. We have previously reported inhibition of the proliferation of five out of seven human colorectal cancer cell lines by the 1,25(OH)₂D₃ analog EB 1089 *in vitro*,²³ and have also observed significant inhibition in the single line tested *in vivo*.²⁴ This effect appears to be mediated by the interaction of calcitriol or analogs and specific nuclear vitamin D₃ receptors. More recently we have demonstrated much more marked inhibition (of up to 70% over controls) of the proliferation of several hepatoma cell lines exposed to both calcitriol and its analog EB 1089.²⁵

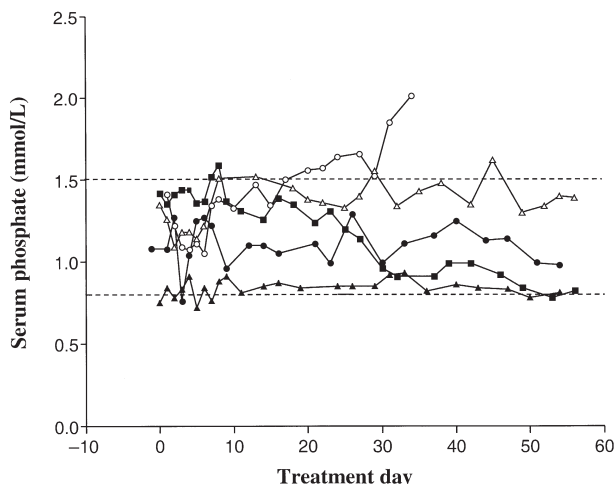


Figure 2 Serum phosphate levels in patients 3–7. (.....) Denotes the normal range for serum phosphate levels. (■) Patient 3; (▲) patient 2; (●) patient 5; (△) patient 6; (○) patient 7.

1,25-Dihydroxyvitamin D₃ is routinely used in the treatment of osteoporosis and renal failure-associated hyperparathyroidism, the usual dose being 0.25 mcg, twice daily.³⁴ However, clinical use of 1,25(OH)₂D₃ in patients for the treatment of malignancy has previously been limited by the development of hypercalcemia, an unsurprising complication considering the central role that 1,25(OH)₂D₃ plays in calcium homeostasis, with elevated systemic levels stimulating enhanced calcium absorption from the gut and bones. In 1985, Keoffler *et al.* administered 2 mg/day of 1,25(OH)₂D₃ orally to patients with myelodysplastic syndrome and found that 50% of patients developed symptomatic hypercalcemia.²⁶ Over 400 analogs of 1,25(OH)₂D₃ have been synthesized and tested in the hope of finding a compound capable of inhibiting proliferation without hypercalcemic effects. However, one such analog, EB 1089, which has much greater antiproliferative than hypercalcemic effects, has recently been shown to still induce hypercalcemia when given to human subjects in potentially therapeutic dosages.²⁷

Hepatic metastases from colorectal cancer and primary hepatic malignancies offer a unique situation in which 1,25(OH)₂D₃ may be therapeutically useful. The regional delivery of chemotherapy via the hepatic artery is an established technique for the treatment of liver cancers. High concentrations of the therapeutic agent can be achieved in the liver with low concentrations in the systemic circulation because of hepatic extraction and catabolism of the agent on its first pass through the liver.³⁵ Within the liver, chemotherapy delivered via the hepatic artery is preferentially distributed to tumors, as they derive 75% of their blood supply from the hepatic artery in contrast to a normal liver which receives only 25% from the hepatic artery.^{36–38}

The principal route of excretion of calcitriol is in bile, as calcitroic acid, glucuronides, taurine and glycine conjugates, mono- and disulfides, and carboxylic acids.^{28–33} We hypothesized that regional delivery via a hepatic

arterial infusion would result not only in high concentrations within hepatic tumors, but that a significant first pass hepatic extraction would result in only a small amount of calcitriol reaching the systemic circulation thereby avoiding the development of hypercalcemia.

The dosage selection was made on a pragmatic and literature guided approach. The doses given to the first two subjects (0.2 and 0.5 mcg/day) were chosen to be comparable with the relatively low doses commonly administered in clinical practice. Subsequent subjects received increasing dosages, intended to be comparable to and later exceed those given systemically by previous researchers. In the present study, we have demonstrated that it is possible to safely administer up to 10 mcg/day of 1,25(OH)₂D₃ as a hepatic artery infusion without producing hypercalcemia or other side-effects. This is 20-fold the dose administered clinically³⁴ and fivefold greater than the maximum dosage that previous researchers have been able to administer without producing hypercalcemia.²⁶ While these results may lend support to our hypothesis that regional administration via the hepatic artery results in a significant first pass effect, this hypothesis cannot be further commented upon in the absence of 1,25(OH)₂D₃ levels. This high dose of 1,25(OH)₂D₃ was administered safely, without the occurrence of renal or hepatic toxicity. The preferential distribution of hepatic arterial blood flow to tumors over normal liver may suggest that hepatic arterial infusion will also result in higher concentrations of calcitriol within hepatic tumors than would result from systemic administration, although we were not able to examine this effect in the present study.

In conclusion, we accept that the data in the present study is preliminary because of the small patient numbers involved; however, to our knowledge this is the first reported use of hepatic arterial administration of 1,25(OH)₂D₃, and we believe that it indicates that the administration of high dosages of 1,25(OH)₂D₃ by hepatic artery infusion is safe. This novel treatment approach has potential as a non-cytotoxic therapy for liver cancers and warrants further research; this study will prove extremely useful in the initiation and planning of such. Further studies could be justified in administering dosages approximately in the 10 mcg/day range, and concentrating upon the therapeutic effects produced without undue concerns regarding hypercalcemia.

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