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ORIGINAL ARTICLE

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Hepatic arterial and intravenous administration of 1,25-dihydroxyvitamin D_3 – evidence of a clinically significant hepatic first-pass effect

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Abstract *Purpose*: We have previously shown that 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] inhibits the proliferation of a number of human cancers, including colorectal and hepatocellular carcinoma, both of which affect the liver and are major causes of cancer death. However, the clinical use of 1,25$(OH)_2D_3$ and analogues has been restricted by the development of hypercalcaemia upon systemic administration. We hypothesized that a clinically significant hepatic first-pass effect may exist upon the administration of 1,25$(OH)_2D_3$ as a hepatic arterial infusion, and that such an effect may allow high levels of 1,25$(OH)_2D_3$ to be delivered to the liver whilst avoiding high systemic levels. *Methods*: To examine this hypothesis, two groups of Landrace pigs were given identical doses of 1,25$(OH)_2D_3$ as continuous infusions, one group systemically, the other as a hepatic arterial infusion. Serum levels of 1,25$(OH)_2D_3$, calcium, phosphate and a number of liver and kidney function tests were performed regularly. *Results*: Concentrations of 1,25$(OH)_2D_3$ and calcium remained normal in the hepatic arterial infusion animals, in contrast to the intravenous infusion animals which developed elevated levels of 1,25$(OH)_2D_3$ and hypercalcaemia. Hepatic arterial infusion of 1,25$(OH)_2D_3$ did not produce any adverse effects upon renal or hepatic function. *Conclusion*: The present findings support the existence of a clinically significant hepatic first-pass effect when 1,25$(OH)_2D_3$ is administered as a continuous hepatic arterial infusion. Hepatic arterial infusion of 1,25$(OH)_2D_3$ has great potential in the treatment of hepatic cancers.

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Introduction

It is well established that in addition to its role in the control of calcium and phosphate homeostasis, 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] is capable of inhibiting the proliferation of a number of cancer cell lines via a specific nuclear receptor-mediated mechanism. The inhibition of colorectal cancer cell lines was first reported in vitro by Lointer et al. in 1987 [13], in vivo inhibition being later demonstrated by Eisman et al. [9]. We have recently demonstrated marked inhibition of the proliferation of colorectal and hepatocellular cancer cell lines exposed to 1,25$(OH)_2D_3$ under both in vitro and in vivo conditions [1, 2, 19, 20].

However, the clinical use of 1,25$(OH)_2D_3$ in the treatment of malignancy has been limited by the development of hypercalcaemia. In attempting to overcome this problem, several hundred analogues of 1,25$(OH)_2D_3$ have been synthesized, with the aim of developing analogues with a reduced hypercalcaemic effect but which retain an antiproliferative effect. Unfortunately, clinical trials of such analogues have also been limited by the development of hypercalcaemia in subjects [8].

1,25$(OH)_2D_3$ undergoes extensive metabolism and very little is excreted unchanged from the body. Following hepatic metabolism and conjugation, it is principally excreted in the bile. Its metabolism is complex and involves a number of metabolic pathways. The two main pathways have been defined as side-chain oxidative cleavage and 24-hydroxylation. Several other hydroxylases also take part in the metabolism of 1,25$(OH)_2D_3$. A large number of the metabolites formed are then conjugated and eliminated through the bile [3, 5, 11, 15, 16, 18]. This led us to hypothesize that regional delivery of 1,25$(OH)_2D_3$ as a continuous hepatic arterial infusion

may result in a clinically significant hepatic first-pass effect. The existence of such a first-pass effect could allow a large dose of $1,25(OH)_2D_3$ to be administered directly into the liver producing high intrahepatic levels whilst avoiding elevated systemic levels and resultant hypercalcaemia. By achieving high local hepatic levels, hepatic tumours sensitive to $1,25(OH)_2D_3$ could be treated. Colorectal cancer metastases and primary hepatocellular cancer are the two most common liver cancers, and both have been demonstrated to be sensitive to $1,25(OH)_2D_3$. By comparing the effects upon systemic levels of $1,25(OH)_2D_3$ and calcium of a hepatic arterial infusion of $1,25(OH)_2D_3$ with a systemic intravenous (IV) infusion of the same dose, this study aimed to determine whether a clinically significant hepatic first-pass effect exists.

Methods

Ethical approval for this study was obtained from the University of New South Wales Animal Care and Ethics Committee. Two treatment groups of Landrace pigs (University of Sydney Farm, Camden, Australia) were used, into which animals were randomly assigned. One group received a continuous hepatic arterial infusion of $1,25(OH)_2D_3$, the other received the same dose of $1,25(OH)_2D_3$ as a continuous infusion via the femoral vein.

$1,25(OH)_2D_3$ was administered as the preparation Calcijex (Abbot Australasia, Cronulla, NSW, Australia), diluted to the required concentration with water for injection (Delta West, Bentley, WA, Australia). Each animal received a continuous infusion containing 0.267 μg/kg per 24 h of $1,25(OH)_2D_3$. The dose of the drug was chosen on the basis of pilot studies conducted in our laboratory in which IV administration of 0.267 μg/kg per 24 h led to development of hypercalcaemia in all animals. Infusions were delivered from an Infusaid 400 pump (Medical Specialities Australia, Willoughby, NSW, Australia) implanted subcutaneously on the right flank, via a tapered silicone catheter (Medical Specialities Australia) into either the hepatic arteries or femoral vein. Prior to implantation, pumps (which were reused) were sterilized in glutaraldehyde solution and tested to check flow rates. Infusions were delivered continuously for 14 days. Blood was taken at regular intervals and assayed for serum calcium and phosphate levels together with routine renal and liver function tests. Animals were killed on day 14 by IV injection of 20 ml pentobarbitone.

Anaesthetic details

All operations were performed under general anaesthesia administered by the same anaesthetist. Anaesthesia was induced by 5% halothane inhalation and animals underwent endotracheal intubation with a 5 mm cuffed endotracheal tube. Anaesthesia was maintained by 2–5% halothane with nitrous oxide 2 l/min and oxygen 3–5 l/min. Buprenorphine 0.1 mg/kg was given IV at the start of surgery. Postoperative analgesia was provided by 12-hourly doses of buprenorphine as required. Antibiotic prophylaxis was given as a single 500 mg IV dose of cephalothin (Eli Lilly, Ryde, NSW, Australia) on induction.

Operative details

Hepatic arterial infusion animals

All operative procedures were performed by the same surgeon. A midline incision was performed. The small bowel was packed inferiorly and the peritoneum reflected to display the hepatic arterial tree. The porcine hepatic arterial tree is rather variable and it was necessary to display the whole tree in order to plan the insertion of the hepatic arterial catheter (HAC). A tapered silicone rubber catheter was inserted retrogradely along either a side branch of the main hepatic artery or a minor hepatic artery until its end lay at the junction of the vessel with the main hepatic artery. The catheter was secured in place by ligating the vessel around it. Methylene blue 1% solution (5 ml) was injected with minimal force down the catheter in order to ensure that: (1) the bowel was not perfused from the hepatic arterial tree distal to the point of catheter insertion, demonstrated by blueing of the bowel wall; (2) that the liver was globally perfused by the hepatic arterial tree distal to the point of catheter insertion, demonstrated by blueing of the liver surface; and (3) that the infusion delivered from the catheter passed antegradely into the liver, and not retrogradely down the hepatic arterial tree to the bowel, again demonstrated by blueing of the bowel wall. In order for the operative intervention to be comparable with the IV-treated group a skin crease groin incision was made and the right femoral vein was ligated.

Femoral vein infusion animals

In order to be comparable to the hepatic arterial infusion group a "sham" laparotomy was performed: a midline incision was made and the peritoneum was reflected to display and mobilize the hepatic vessels as for the other treatment group. Via a right groin incision the femoral vein was identified, a venotomy was made and a tapered silicone rubber catheter was advanced proximally 3 cm into the vein. The catheter was held in place by ligating the femoral vein around it.

All animals

Via a transverse right flank incision a subcarnosal pocket was developed and the loaded and primed Infusaid pump was placed in the pocket. The pump was connected to either the hepatic arterial or femoral vein silicone rubber catheter. Via a longitudinal neck incision a Hickman catheter (Infusacath, Medical Specialities Australia) was inserted into the jugular vein and tunnelled through the skin. A specially constructed collar was applied to secure the catheter. All wounds were closed in two layers using 1.0 nylon sutures.

Infusaid pump and catheter function

Prior to implantation, each pump was filled with the Calcijex solution and primed as per the manufacturer's instructions. Just prior to connection to the infusion catheter, pump function was confirmed, again as per the manufacturer's instructions. On days 3, 7 and 14 under halothane anaesthesia each pump was emptied, and the remaining volume measured in order to check that the pump was pumping at the predicted rate. Pumps were then immediately refilled. After the animals had been killed each infusion catheter was carefully dissected clear and examined in order to confirm that it had remained in the correct place, and that the catheter and distal vessels remained patent.

Blood samples

Blood (10 ml) was taken from each animal via the Hickman line on days 1, 3, 5 7, 8, 10, 12, and 14. The blood was placed in a Vacutainer SST, and centrifuged for 7.5 min at 3000 rpm, and the serum then decanted. The serum was assayed for calcium, phosphate, markers of renal function and hydration (urea and creatinine levels), and markers of hepatic function [aspartate amino transferase (AST) and alanine amino transferase (ALT)], using a Beckman LX 20 analyser and utilizing the manufacturer's reagents and protocols (Beckman-Coulter, Gladesville, NSW, Australia). Albumin was also assayed, and the calcium levels corrected accordingly.

$1,25(OH)_2D_3$ was assayed on days 0, 3, 7, 10, and 14 using an ^{125}I radioimmunoassay (Incstar Corporation, Stillwater, Minn.).

Statistical analysis

The mean levels of the assayed parameters were plotted and compared between the treatment groups at each sampling interval period using the Mann-Whitney *U*-test. In order to assess any change in assayed parameters between day 0 and day 14 within each treatment group the Wilcoxon Signed Ranks test was employed. All statistical analyses were performed using the SPSS for Windows computer package version 7.5.1. *P*-values <0.05 were considered to represent a significant difference.

Results

A total of 20 animals were entered into the trial. Nine animals were withdrawn from the trial and excluded from analysis: four developed blockages of either their HAC (two animals) or IV catheter (two animals), four animals developed adhesional small bowel obstructions, and one animal developed malignant hyperpyrexia on induction of anaesthesia. All withdrawn animals were killed immediately. The remaining 11 animals remained asymptomatic and healthy for the duration of the study and were included in the analysis, six in the hepatic arterial infusion group and five in the femoral vein infusion group. The weight of the animals ranged from 14.4 to 21.4 kg, with no significant difference between the treatment groups (Mann-Whitney *U*-test, $P=0.463$).

Pump and catheter function

All pumps functioned correctly for the duration of the study, the estimated pumping rate for each of the three measured periods being within 5% of that predicted for each pump. The HAC and IV catheters of all included animals remained patent for the duration of the study, and were found to be correctly sited post mortem.

Serum $1,25(OH)_2D_3$ levels

$1,25(OH)_2D_3$ levels in the animals given a hepatic arterial infusion of $1,25(OH)_2D_3$ remained constant for the duration of the study. $1,25(OH)_2D_3$ serum levels in these animals were 132.6 ± 12.9 and 135.8 ± 14.5 nmol/l on days 0 and 14, respectively (Fig. 1). In marked contrast, in the animals given an IV infusion, levels had increased rapidly by day 3 (444.8 ± 89.8 nmol/l) and continued to rise up to day 7 (480.6 ± 23.4 nmol/l), slowly decreasing thereafter to 448.0 ± 38.4 and 315.0 ± 72.3 nmol/l on days 10 and 14, respectively. In this group $1,25(OH)_2D_3$ levels were significantly different ($P<0.05$) between day 0 and days 3–14. More importantly, $1,25(OH)_2D_3$ levels differed significantly between the two groups on days 7 and 10 (Wilcoxon Signed Ranks test, $P=0.004$).

Serum calcium and phosphate levels

Details of the serum calcium levels of each treatment group are given in Fig. 2. The changes in calcium levels were similar to those for $1,25(OH)_2D_3$ levels. The hepatic arterial infusion group remained normocalcaemic throughout the treatment period, whilst from day 3 onwards there was a statistically significant increase (Mann-Whitney *U*-test, $P<0.05$) in serum calcium levels of the IV-treated animals. Whilst this increase peaked on day 7 as did $1,25(OH)_2D_3$ levels, calcium levels remained significantly elevated up to day 14 (Wilcoxon Signed Ranks test, $P=0.043$), in contrast to the subsequent fall in $1,25(OH)_2D_3$ levels of the IV infusion group.

Details of the serum phosphate levels of each treatment group are presented in Fig. 3. From day 5 on-

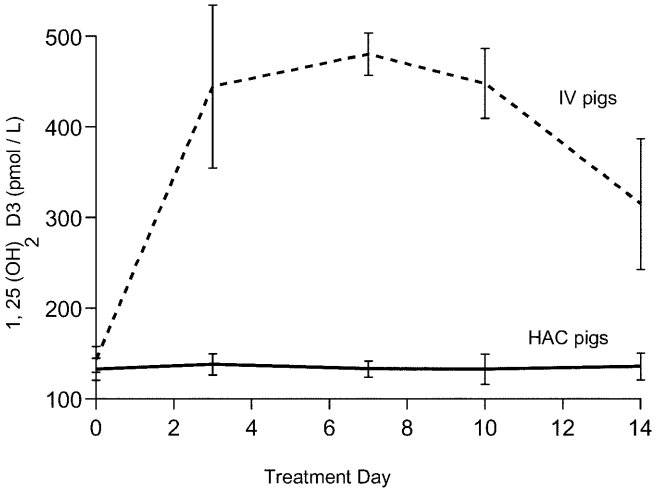

Fig. 1 Serum $1,25(OH)_2D_3$ levels for each treatment group (*error bars* standard error of the mean). For hepatic arterially treated pigs (*HAC*, hepatic arterial catheter) $Z=-0.949$, $P=0.343$; for IV-treated pigs (*IV*) pigs $Z=-0.182$, $P=0.068$; Wilcoxon Signed Ranks test, day 0 vs day 14 mean levels

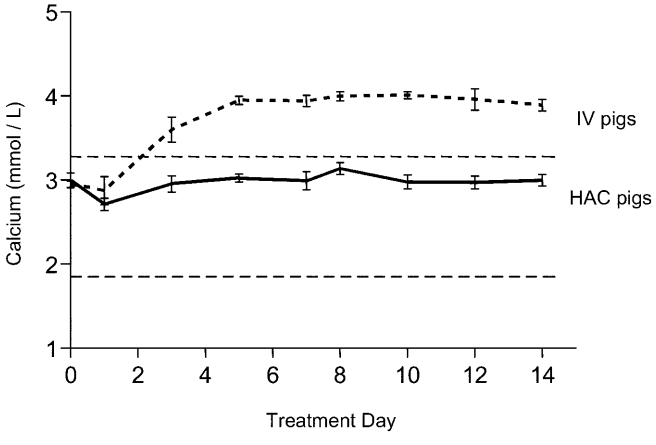

Fig. 2 Serum calcium levels for each treatment group (*dotted horizontal lines* normal ranges, *error bars* standard error of the mean). For hepatic arterially treated pigs (*HAC*, hepatic arterial catheter) $Z=-0.105$, $P=0.917$; for IV-treated pigs (*IV*) pigs $Z=-2.023$, $P=0.043$; Wilcoxon Signed Ranks test, day 0 vs day 14 mean levels

wards there was a statistically significant decrease in serum phosphate levels of the IV-treated animals, when compared to the hepatic arterial infusion group (Mann-Whitney *U*-tests, $P < 0.05$). However, this fall was not statistically significant when compared to the pretreatment level (Wilcoxon Signed Ranks test, $P = 0.680$).

Measures of renal function

Serial mean urea levels are shown in Fig. 4 for each treatment group. There was no significant difference in urea levels between the groups at any time-point (Mann-Whitney *U*-test), nor was there any significant change between days 0 and 14 in either treatment group (Wilcoxon Signed Ranks test). Similar results were obtained for serum creatinine levels.

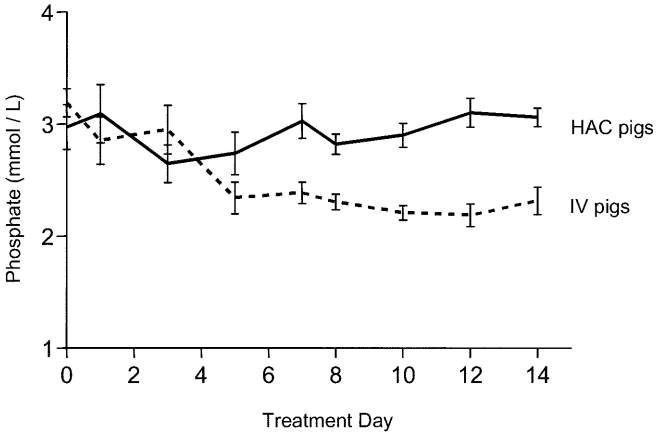

Fig. 3 Serum phosphate levels for the two treatment groups (*error bars* standard error of the mean). For hepatic arterially treated pigs (*HAC*, hepatic arterial catheter) $Z = -0.524$, $P = 0.600$; for IV-treated pigs (*IV*) pigs $Z = -1.826$, $P = 0.680$; Wilcoxon Signed Ranks test, day 0 vs day 14 mean levels

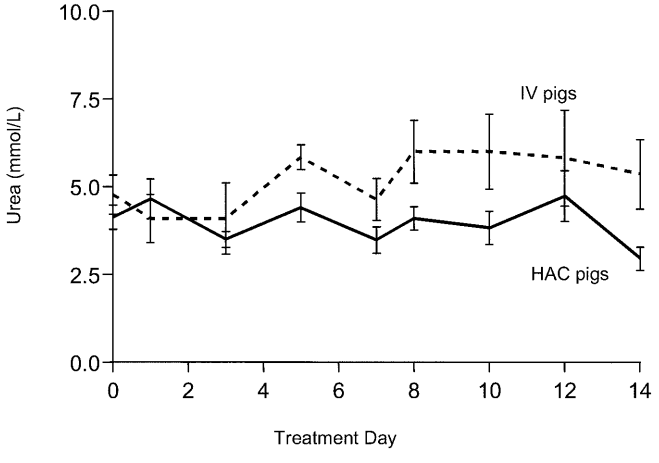

Fig. 4 Serum urea levels of each treatment group (*error bars* standard error of the mean). For hepatic arterially treated pigs (*HAC*, hepatic arterial catheter) $Z = -1.572$, $P = 0.116$; for IV-treated pigs (*IV*) pigs $Z = -0.674$, $P = 0.500$; Wilcoxon Signed Ranks test, day 0 vs day 14 mean levels

Measures of hepatic function

There was no difference between the levels of AST or ALT between the treatment groups at any time-point. ALT levels were comparable between days 0 and 14 for each treatment group. There was a statistically significant difference ($P = 0.046$) in AST levels between days 0 and 14 in the hepatic arterially treated animals, the mean day-14 level being lower than that prior to treatment. Serum alkaline phosphatase levels did not vary between treatment groups at any point, nor was there a difference between pretreatment and day-14 levels.

Discussion

Biliary secretion has been shown to be the main route for the excretion of $1,25(OH)_2D_3$ metabolites [3, 4, 5, 6, 7, 12, 15, 18] with between 12% and 65% of an administered dose of $1,25(OH)_2D_3$ appearing as metabolites in the bile within 24 h. Whilst in the rat urinary excretion of metabolites following C24 hydroxylation in the kidney appears to be considerable [10, 14], tracer experiments in humans indicate urinary excretion to be of little importance, with only 2.4% of an administered dose appearing in the urine over 48 h [3].

Considerable work has been performed to identify the water-soluble biliary metabolites, which represent 77–91% of the total biliary metabolites [18]. Calcitroic acid has been identified, having been produced from the hepatic P450 cytochrome C24 hydroxylation of $1,25(OH)_2D_3$ [17, 18]. In addition to this, many conjugates have been identified, the first being a monoglucuronide [5, 6] and others including taurine, glycine, and mixed conjugates. $1,25(OH)_2D_3$ monosulphides, disulphides and other carboxylic acids are also present [3]. The knowledge that hepatic catabolism and biliary excretion predominates in the breakdown of $1,25(OH)_2D_3$ led us to hypothesize that there may be a clinically significant first-pass effect when $1,25(OH)_2D_3$ is administered as a hepatic arterial infusion. Such an effect may allow $1,25(OH)_2D_3$ to be used in the treatment of liver cancers.

In this study, animals given a continuous systemic IV infusion of $1,25(OH)_2D_3$ at a dosage of 0.267 μg/kg per 24 h experienced a statistically significant rise in systemic $1,25(OH)_2D_3$ concentrations, whilst animals that received the same dosage as a continuous hepatic arterial infusion did not experience any change in $1,25(OH)_2D_3$ concentrations. The increase in levels of $1,25(OH)_2D_3$ in the IV group confirms that the dose delivered was sufficient to produce a measurable increase in $1,25(OH)_2D_3$. The absence of any increase in $1,25(OH)_2D_3$ levels in the

hepatic arterial infusion group supports our hypothesis that a hepatic first-pass effect may exist.

As would be expected, serum calcium levels reflected $1,25(OH)_2D_3$ concentrations. The rise in calcium levels seen in the IV-treated group, and the normocalcaemia seen in the hepatic arterial infusion group confirm that the hypothesized hepatic first-pass effect is clinically significant. It is possible to deliver a high dose of $1,25(OH)_2D_3$ via the hepatic artery and avoid the development of hypercalcaemia produced by the same dosage administered systemically. Similar effects upon serum phosphate levels were also seen. Hepatic arterial infusion animals remained normophosphataemic throughout, and IV-treated animals rapidly became hypophosphataemic. Why serum phosphate levels should fall with IV treatment is not clear. Conventional physiology teaching is that serum phosphate levels rise and fall in parallel with calcium. However, this unexpected finding is of little relevance to this study, other than to describe another side effect of IV treatment with $1,25(OH)_2D_3$. The fact that the serum phosphate levels of hepatic arterially treated animals did not alter with treatment is further support of the existence of a clinically significant first-pass effect.

A secondary aim of this study was to examine whether $1,25(OH)_2D_3$ hepatic arterial infusion resulted in any unwanted renal or hepatic effects. Serial urea and creatinine levels were assayed in order to assess whether renal function was impaired and whether any dehydration developed in the animals, as this could affect serum calcium results as well as have implications for the wellbeing of the animals. There were no significant changes in the urea levels of either treatment group. Similar results were obtained for serum creatinine levels, although there was a significant fall in the creatinine levels of hepatic arterially treated animals. This result would appear to be due to unexplained elevated mean creatinine levels in these animals preoperatively, and not to have been a result of treatment. Renal function was not impaired as a result of either hepatic arterial or IV infusion of $1,25(OH)_2D_3$, and animals did not become dehydrated.

Hepatic function was assessed by considering serial serum transaminase levels (markers of hepatocellular damage), and alkaline phosphate levels (a marker of biliary canalicular obstruction). ALT and AST levels did not vary significantly between the two treatment groups at any time-point. There was no significant rise in ALT levels with treatment, although AST levels in the hepatic arterially treated group on day 14 were slightly but significantly elevated over pretreatment levels. However, this result would appear to be a statistical aberration rather than an indicator of hepatocellular damage. Were hepatocellular damage occurring one would expect both AST and ALT levels to progressively rise to the range of several thousand units per litre. Neither IV nor hepatic arterial infusion of $1,25(OH)_2D_3$ resulted in untoward hepatocellular effects. Alkaline phosphatase levels did not vary between the two groups at any time, nor was there any significant change in mean levels of either group with treatment. $1,25(OH)_2D_3$ infusion did not result in any biliary obstructive effects.

In conclusion, this study demonstrated that by administering a high dose of $1,25(OH)_2D_3$ as a continuous hepatic arterial infusion, it is possible to avoid the elevated $1,25(OH)_2D_3$ concentrations and resultant hypercalcaemia that is produced upon administering the same dosage as a systemic infusion. These findings support our hypothesis that there is a clinically significant hepatic first-pass effect when $1,25(OH)_2D_3$ is administered via the hepatic artery. There did not appear to be any renal or hepatic side effects from this method of administration of $1,25(OH)_2D_3$, and we believe that it holds great potential in the treatment of liver cancers.

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