Hepatic arterial and intravenous administration of 1,25-dihydroxyvitamin D₃ – evidence of a clinically significant hepatic first-pass effect

Abstract  Purpose: We have previously shown that 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] 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)₂D₃ 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)₂D₃ as a hepatic arterial infusion, and that such an effect may allow high levels of 1,25(OH)₂D₃ 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)₂D₃ as continuous infusions, one group systemically, the other as a hepatic arterial infusion. Serum levels of 1,25(OH)₂D₃, calcium, phosphate and a number of liver and kidney function tests were performed regularly. Results: Concentrations of 1,25(OH)₂D₃ 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)₂D₃ and hypercalcaemia. Hepatic arterial infusion of 1,25(OH)₂D₃ 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)₂D₃ is administered as a continuous hepatic arterial infusion. Hepatic arterial infusion of 1,25(OH)₂D₃ has great potential in the treatment of hepatic cancers.

Keywords  1,25-Dihydroxyvitamin D₃ · Cancer · Hepatic arterial infusion · First-pass effect

Introduction

It is well established that in addition to its role in the control of calcium and phosphate homeostasis, 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] 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 Lointier 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)₂D₃ under both in vitro and in vivo conditions [1, 2, 19, 20].

However, the clinical use of 1,25(OH)₂D₃ 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)₂D₃ 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)₂D₃ 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)₂D₃. 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)₂D₃ 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)\(_2\)D\(_3\) 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)\(_2\)D\(_3\) 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)\(_2\)D\(_3\). By comparing the effects upon systemic levels of 1,25(OH)\(_2\)D\(_3\) and calcium of a hepatic arterial infusion of 1,25(OH)\(_2\)D\(_3\) 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)\(_2\)D\(_3\), the other received the same dose of 1,25(OH)\(_2\)D\(_3\) as a continuous infusion via the femoral vein. 1,25(OH)\(_2\)D\(_3\) was administered as the preparation Calcijex (Abbott 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)\(_2\)D\(_3\). 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 Specialties Australia, Willoughby, NSW, Australia) implanted subcutaneously on the right flank, via a tapered silicone catheter (Medical Specialties 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 bluing 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 bluing of the liver surface; and (3) that the infusion delivered from the catheter passed antegrade into the liver, and not retrogradely down the hepatic arterial tree to the bowel, again demonstrated by bluing 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 subcapsular 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 Specialties Australia) was inserted into the jugular vein and tunneled 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.