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Dietary administration in rodent studies distorts the tissue deposition profile of lanthanum carbonate; brain deposition is a contamination artefact?

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ABSTRACT

Lanthanum carbonate is a non-calcium phosphate binder used to control hyperphosphataemia in patients with chronic kidney disease who are undergoing dialysis. Ultrastructurally, lanthanum ions are too large to traverse the tight junctions in the blood–brain barrier, yet tissue distribution studies using dietary administration have reported low concentrations in rodent brain, raising concern about accumulation. To investigate this, tissue lanthanum concentrations were measured in rats given the same lanthanum carbonate dose via powdered diet or oral gavage (838 and 863 mg/kg/day). Additional rats were dosed intravenously with lanthanum chloride (0.03 mg/kg/day), a route enabling much higher plasma lanthanum concentrations. After 28 days, median lanthanum concentrations in liver, bone, kidney and heart showed a direct relationship with those in plasma (highest after intravenous and lowest after dietary dosing). In contrast, brain concentrations were dramatically higher after dietary administration (≤500 ng/g), compared to the other routes (LLOQ of 11 ng/g). An identical skewed pattern was noted for skin, a tissue readily contaminated in powdered diet studies. These data indicate that brain deposition is a contamination artefact caused by transfer of lanthanum from cranial skin to brain as animals are manipulated during autopsy. Dietary administration should be avoided in distribution studies of trace elements due to the high contamination risk.

1. Introduction

Lanthanum carbonate (FOSRENOL®, Shire Pharmaceuticals, Basingstoke, UK) is an effective non-calcium, non-resin-based oral phosphate binder for the treatment of hyperphosphataemia in patients with chronic kidney disease (CKD) who are undergoing dialysis.

Lanthanum is a naturally occurring element that is found in trace levels in tap water (0.0–0.1 ng/mL) (Torres, 2005), and various foods (Ministry of Agriculture Fisheries and Food, 1998). It is very poorly absorbed from the intestinal tract, with an absolute bioavailability of 0.00127 ± 0.0008% in humans (Pennick et al., 2006). Plasma concentrations are typically less than 1 ng/mL in dialysis patients following long-term treatment with lanthanum carbonate (Hutchison et al., 2008). The kidney is not significantly involved in the clearance of lanthanum, the main excretion route for absorbed lanthanum being via the liver into bile (Damment and Pennick, 2007). Consistent with this hepatobiliary excretion, the plasma exposure and pharmacokinetics of lanthanum are similar in healthy individuals and patients undergoing dialysis (Damment and Pennick, 2008).

The safety of lanthanum carbonate was comprehensively evaluated in long-term preclinical and clinical studies prior to it being made available to patients (Damment, 2006). Like other cationic elements, deposition of lanthanum can occur in bone and liver (Damment and Pennick, 2008), but this process is limited for the carbonate salt because of its exceptionally low oral bioavailability (Pennick et al., 2006). The extent to which lanthanum deposits in other tissues, particularly the brain, is controversial (D'Haese et al., 2005; McLeod et al., 2005; Rambeck, 2005; Damment, 2006). In initial tissue deposition studies in animals, lanthanum was not found in the central nervous system (CNS) (Pennick et al., 2003), a finding that was consistent with several early electron microscopy studies showing that lanthanum ions are unable to pass through the tight junctions of the blood–brain barrier (Kato et al., 1989; Xu and Ling, 1994). However, two subsequent studies suggested more widespread tissue deposition of lanthanum.

In a preliminary report of a 28-day study in uraemic rats, Sacchiero et al. (2003) suggested that the highest tissue levels of lanthanum were in the brain (2499 ng/g), being 2–3-fold higher in this organ than in bone or liver (Sacchiero et al., 2003).

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Lacour et al. (2005) also reported elevated levels in the brain of uraemic rats (~110 ng/g), as well as in the heart (~130 ng/g), lung (~300–400 ng/g) and skeletal muscle (~180 ng/g), organs that had not previously been considered major sites of lanthanum deposition (Lacour et al., 2005). Both studies involved administration of lanthanum carbonate to rats via a powdered diet, whereas previous studies involved direct instillation of the drug into the stomach by gavage.

Dietary administration is known to carry a high risk of sample contamination (McLeod et al., 2005), as drug particles can easily transfer onto the animals' skin and fur, and subsequently to tissue and biofluid samples during sampling procedures. This risk is elevated in tissue deposition studies with poorly bioavailable drugs like lanthanum, as high dietary concentrations (3% (w/w) in the Sacchiero et al. and Lacour et al. studies) must be given to achieve measurable systemic levels, resulting in a concentration difference of many orders of magnitude between the diet and the tissues of interest. The majority of a dose of lanthanum is excreted unabsorbed back into the animals' environment via the faeces, thus increasing the risk of contaminating skin and fur.

To investigate the extent to which contamination may have been a contributing factor leading to unexpected lanthanum deposition (especially in the brain) (Sacchiero et al., 2003; Lacour et al., 2005), we measured tissue lanthanum concentrations in rats treated with the same oral dose of lanthanum carbonate administered via a powdered diet or by gavage, as well as in rats dosed intravenously with lanthanum chloride, a route associated with a much higher plasma lanthanum concentrations and a much lower risk of sample contamination.

2. Methods

2.1. Animals and husbandry

Studies were performed by a contract research organization based in the United Kingdom. Crl:CD (SD) IGS BR VAF Plus rats (supplied by Charles River Ltd., Margate, UK), weighing 134–290 g were fed powdered (oral study, 2014 Teklad Global Laboratory Rodent Diet, Harlan Ltd., Bicester, UK) or pelleted diet (intravenous study, RM1 (E) SQC Rat and Mouse Maintenance Diet, Special Diets Services, Witham, UK) and allowed mains tap water *ad libitum*. To reduce the risk of transferring drug from food and faeces onto skin and fur, the rats were housed in grid-bottomed cages, 1 per cage in the oral study where the risk was greatest due to the high dose, and 3 per cage in the intravenous study. Each oral lanthanum carbonate group, along with their relevant control group, was housed in a separate room to further minimize transfer of drug between rats and across groups. The animal rooms were maintained at 22 ± 3 °C, and 50 ± 20% relative humidity, with a 12-h light–dark cycle. All procedures were performed in compliance with the Animals (Scientific Procedures) Act 1986.

2.2. Experimental procedures

In the oral study, groups of 10 male rats were fed lanthanum carbonate as 1% (w/w) of their diet or given a 10% (w/w) suspension of lanthanum carbonate in 0.5% carboxymethyl cellulose by gavage for 28 days. The concentration of lanthanum in the diet was analysed using inductively coupled plasma atomic emission spectrometry (ICP-AES) following acid digestion, and food consumption was recorded daily to enable accurate estimation of the dose received by each rat. The analytical method was linear over the concentration range 0.02–10 000 ng/mL, with accuracy and precision of 96.93–104.86%, and a lower limit of quantification (LLOQ) of 0.05 ng/g. The average dose consumed by the dietary lanthanum group on each day was calculated and the same dose was then administered by stomach intubation to the gavage lanthanum group, whose treatment was staggered by 1 day. This ensured that the dose levels for the two groups were closely matched. The average doses of lanthanum carbonate administered over the study period were 838 and 863 mg/kg/day for the diet and gavage groups, respectively. Control groups for each lanthanum-treated arm received either no treatment (diet control) or an equivalent volume of carboxymethyl cellulose vehicle (gavage control).

In the intravenous study, male and female rats ($n = 6$ per group) received saline (control) or lanthanum chloride at a dose of 0.03 mg/kg/day for 28 days by slow bolus injection into a tail vein. This dose was previously shown to produce peak plasma lanthanum concentrations about 20–30 times higher than the levels achievable after oral treatment of the carbonate salt and therefore would be expected to result in significantly higher systemic tissue concentrations.

2.3. Measurements and observations

The health of the animals was assessed twice daily and body weight was measured at least weekly. Blood (~1 mL) was obtained under isoflurane anaesthetic by orbital sinus puncture at the mid-point of the dark cycle in the oral study (assumed T_{max}) and, for the intravenous study, before and 0.25, 1, 2, 4, 8 and 24 h after dosing. The animals were killed by inhalation of carbon dioxide and exsanguinated from the caudal vena cava prior to post-mortem procedures.

Stringent precautions were taken to reduce sample contamination during collection and processing. All reagents were of high purity and were screened for lanthanum content prior to use, as were all plastic ware and glassware used to store samples. At autopsy in the oral study, tissues for each group were weighed on separate balances and placed on a cleaned surface, clear of residue from other samples. Gloves, instruments and cork boards were washed between animals or if they came into contact with intestinal contents. The abdominal cavity was opened and a syringe used to cleanly exsanguinate from the vena cava. Tissues were removed in strict sequence. Mid-ventral, mid-dorsal and cranial skin samples were extracted; equipment and instruments were cleaned and the scalpel blade changed. The brain was removed and sections cut from the left cerebrum, left mid-brain and left cerebellum. Remaining skin was removed and discarded. Used equipment was cleaned and the scalpel blade changed. Skeletal muscle (left hind limb) and left femur (head, shaft and growth plate) were removed. Equipment was cleaned and the scalpel blade was changed. The thoracic cavity was opened and heart (left ventricle) and lung (left lobe) samples removed. Used equipment was cleaned and the scalpel blade was changed. Liver (margin of left lobe) and kidney (section of left kidney) samples were obtained. Finally, the GI tract was removed taking care not to transfer any contents into the abdominal cavity.

Organs were weighed, sampled for lanthanum analysis and examined histologically. For lanthanum analysis, approximately 250 mg of soft tissues and 60 mg of bone were immediately frozen. Plasma and tissues were analysed for lanthanum by inductively coupled plasma mass spectrometry (ICP-MS, Hewlett Packard 4500) using praseodymium as an internal standard. For plasma, the analytical method was linear over the concentration range 0.05–100 ng/mL with accuracy and precision in the range 88.5–101.3%, and a LLOQ of 0.05 ng/mL. For digested tissues, the method was linear over the range 0.021–10 ng/mL with accuracy and precision in the range 98.5–103.5%. The LLOQ depended on the weight of sample, and varied in the range 1.5–13.7 ng/g wet weight for soft tissues (see Tables and Figures) and 33 ng/g wet weight for bone.

2.4. Statistical analysis

Descriptive statistics of plasma and tissue lanthanum data were obtained, including the median, 25th and 75th percentiles, and range. These values are reported in preference to mean ± standard error (as published for other studies (Lacour et al., 2005)), as means may be skewed greatly by the presence of occasional outliers. Presentation of the median and range gives an indication of the variability of the data, which may be influenced by contamination. The mean and standard deviation were calculated for body weight, food intake and organ weight data.

3. Results

All animals survived treatment in good health and showed normal weight gain during the studies. Mean brain, heart, lung, liver, kidney and femur weights were unaffected by treatments, except in the intravenous study, where mean liver weight was 10% lower than the control for males only; there were no associated histopathological findings.

The median plasma lanthanum concentration in the dietary lanthanum group was marginally elevated compared with controls (0.06 ng/mL vs. 0.05 ng/mL, respectively), but over 6-fold higher in rats given the same dose by gavage (0.34 ng/mL). In rats administered intravenous lanthanum, plasma concentrations were >300-fold higher than controls (18.83 ng/mL vs. 0.05 ng/mL, respectively; Fig. 1).

Following dietary and gavage administration of lanthanum, tissue concentrations in internal organs were highest in the liver and bone (Table 1), but median levels remained below 500 ng/g. Levels were several-fold lower in the kidney and heart. Following intravenous administration of lanthanum, tissue concentrations were again highest in the liver and bone (Table 1); median levels (up to 2500 ng/g) were several-fold higher than observed following dietary or gavage administration of lanthanum. The rank order for lanthanum concentration in these organs was the same as the rank order for plasma concentration, i.e. lowest following