ESRF	**Experiment title:** Study on the localisation and kinetics of efflux of lanthanum out of bone in chronic renal failure patients.	**Experiment number**: MD-101
Beamline: ID-21	**Date of experiment**: from: 24/11/2004 to: 29/11/2004	**Date of report**: 01/03/2005
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Background: In patients with chronic renal failure (CRF), serum phosphorus levels increase due to an insufficient renal excretion. To control serum phosphorus levels, phosphate binding agents are given orally, in order to reduce gastro-intestinal uptake of phosphate. A possible alternative for the currently used phosphate binders (aluminium hydroxide, calcium carbonate) would be lanthanum (La) carbonate. This compound has been shown to have excellent phosphate-binding capabilities, while it has a very low gastrointestinal absorption. We have previously shown however, that administration of La carbonate to CRF rats during 12 weeks, might induce a mineralisation defect in bone. Follow-up experiments have shown the mineralisation defect to be secondary to a severe phosphate depletion rather than beingthe consequence of a direct effect of La on bone. In previous experiments at the ESRF (LS-1709 and MD-53), we were able to localise La in rat bone. In CRF rats loaded orally with La at a 2 g/kg/day dose for 12 weeks, La was found to be localised at the edge of the calcified trabecular bone at ether quiescent and active sites, independent of the underlying type of renal osteodystrophy (Behets et al., 2005). Since La carbonate is currently being used in several clinical studies the aim of this proposal was to confirm these results in human bone.

Methods: Transiliacal bone biopsies of CRF patients taken in the frame of various clinical studies inverstigating the possible side-effects of La carbonate were examined. These were fixed in Burkhardt's fixative, and embedded in methyl-methacrylate. Samples from patients using La carbonate for up to 4 years were available, as well as follow-up samples of patients receiving the drug for 1 year, followed by a wash-out period of 2 years. Ten µm thick sections of these bone samples will be used for the analysis.
Ultrastructural mapping of La in bone samples was performed using the ID21 Scanning X-Ray Fluorescence Microscope. The primary excitation energy was set to 7.2 keV, above the La L-edges, using a double crystal fixed exit monochromator. The beam was focussed to a microprobe (1µm) using a Fresnel zone plate. Simultaneous mapping of Ca, Fe and La was performed by choosing appropriate energy windows (Ca: 3.53 to 4.12 keV; Fe: 6.39 to 6.42 keV; La: 4.94 to 5.16 keV) of the multichannel analyzer.

Results:

Figure 1: Analysis of a bone sample of a patiënt with secondary hyperparathyroidism with a bulk bone La concentration of 9.7µg/g . Left: indiviual element mappings for Ca, La and Fe. Right: Composite image of both mappings for Ca (Red) and La (Green). Lanthanum could be localised in the bone marrow as condensed particles often colocalised with iron.
Image size: 100 x 100 µm; Pixel size: 2 µm acquisition time: 2500 ms/pixel.

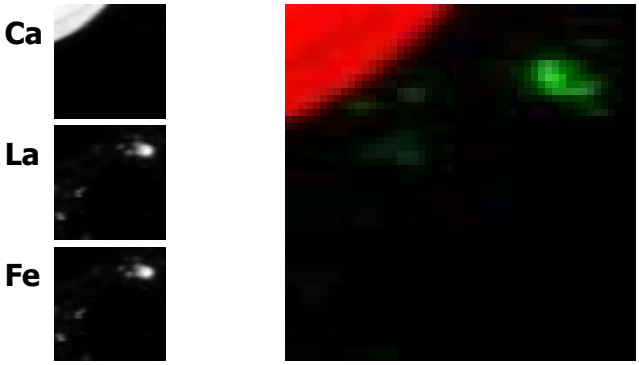


Figure 2-A: Lanthanum localisation in a first region of a bone sample of a patiënt with secondary hyperparathyroidism with a bulk bone La concentration of 9.06 µg/g. Left: Goldner stained adjacent section, for easy identification of region of interest. Middle: Composite image of both mappings for Ca (Red) and La (Green). Right: Indiviual element mappings for Ca, La and Fe. Lanthanum could be localised at the outer edge of the mineralised bone and in the bone marrow as condensed particles where it was colocalised with iron.
Image size: 400 x 280 µm; Pixel size: 4 µm acquisition time: 1000 ms/pixel.

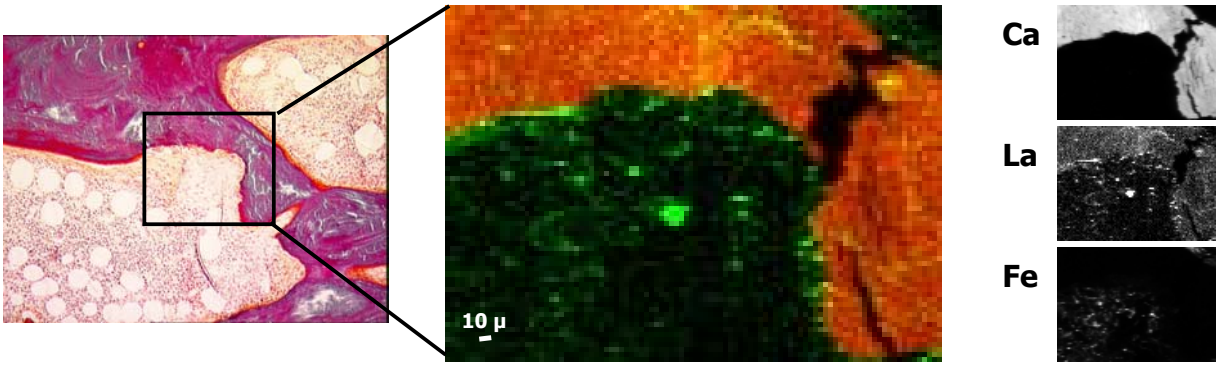
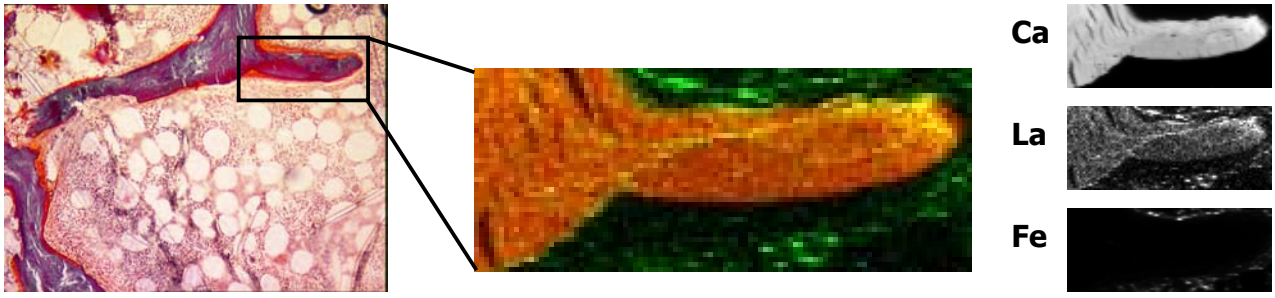


Figure 2-B: Lanthanum localisation in a second region of the same bone sample from Figure 2-A. Left: Goldner stained adjacent section. Middle: Composite image of both mappings for Ca (Red) and La (Green). Right: Indiviual element mappings for Ca, La and Fe. In this section Lacould be localised in the middle of the mineralised bone, concentrated in a thin band. This particular localisation might indicate a the presence of La on a cement line. In the bone marrow La was found as condensed particles where it was again colocalised with iron.
Image size: 400 x 160 µm; Pixel size: 4 µm acquisition time: 1000 ms/pixel.



The results obtained in this proposal will be the subject of a new publication.

References:

Behets,G.J. al., Ultrastructural localisation of lanthanum in bone of chronic renal failure rats after oral dosing with lanthanum carbonate. Kidney Int. In press