Report on Study Leave, INSERM Unit 831, Lyon 2007-2008.
Christine and T Jack Martin Research Travel Grant 2006,
Mark R. Forwood

Introduction

Like the decline of bone mass with age, my study leave had its antecedents 20 years earlier. In 1987, studying the development of microdamage in bone during doctoral studies in Brisbane was akin to being a scientific castaway. Frost's work on microdamage, circa 1960, was still equated with artifact in mainstream skeletal science, Carter and Hayes had undertaken classical fatigue studies of cortical bone and David Burr had recently published original observations on the association between microcracks and resorption cavities. Even our own attempts to engender microdamage in rat bone seemed somewhat arcane. One of few studies of microdamage in bone tissue had been published in French, and I needed education in methods of studying bone tissue. Under the guise of scientific discovery, I therefore undertook a four-week tour of France and visited the laboratory of Professor Pierre Meunier. Unfortunately, Professor Meunier was absent on conference leave, but Pierre Delmas, recently appointed Professor of Medicine at the University of Lyon, introduced me to Dr Monique Arlot, who gave me a tour of the laboratories at the Faculté de Médecine Alexis Carrel (now Faculté de Médecine Laennec), and then shared with me their methods of bone histomorphometry. Twenty years later, motivated by new techniques for investigating bone quality, and funding from the Christine and T Jack Martin Research Travel Grant, I returned, déja vu, to Lyon to work with Pierre Delmas and Monique Arlot. Compared to the long-term professional relationships he held with his clinical colleagues and mentors, my interactions with Pierre exposed the tip of an iceberg. But that tip had clarity of purpose and insight to elucidate the pathogenesis of fragility fracture. The collaboration was aimed at studying fragility in vertebral bodies, but also sadly witnessed the departure of these two mentors; Monique to retirement and Pierre to the ravages of disease.

INSERM Unit 831

In 2007, the program grant for INSERM unit 831 (previously Unit 403) had just been renewed for a 7-year period. Pierre took the bold step of putting bone quality on the map and called the new program "Qualité Osseuse dans L'Ostéoporose " or bone quality in osteoporosis. The mission was to investigate 3 principle themes: in vitro and ex vivo study of the role of matrix properties, microarchitecture and mineralization on bone fragility; clinical evaluation of the determinants of fracture risk; and, effects of therapeutic agents on bone quality. In addition, the unit manages numerous industry contracts, and Pierre also managed a separate institute, Association Prévention des Maladies Osseuses, principally charged with management of the longitudinal cohort studies:

The OFELY cohort (recruited 1992) is a representative sample of the female population, comprising 1000 women from 30-90 years of age;

The MINOS cohort (recruited 1995) comprises of 1040 old men of 19-85 years of age of which 750 old men of more than 50 years of age were followed over 7.5 years; and,

The STRAMBO cohort (recruited 2005), a monocentric prospective epidemiologic study of bone fragility in approximately 1000 men from 20 to 85 years of age.

The laboratory (Faculté de Médecine Laennec) is multidisciplinary enabling, in the one location, use of leading edge technologies for analyses of collagen biochemistry and in vitro study of its resorption, quantitative bone histomorphometry integrating new measurement techniques of microcracks (confocal microscopy) and osteocytic apoptosis, analysis of the degree of mineralization by quantitative microradiography and of its hardness by microindentation, analysis

of maturation of mineral substance and bone matrix by Fourier transformed infrared microspectroscopy (FTIRM), analysis of bone architecture by high resolution computed tomography (micro CT and micro MRI applied in vitro and in vivo), biological markers, and biomechanical analyses of bone tissue. At Hôpital Edouard Herriot there are the clinical assessment tools for densitometry, QCT (Xtreme CT), pQCT, clinical biochemistry, and cell and molecular biology.

The Project

The principal aim of the study leave was to use these technologies to investigate the presence of microcracks and diffuse damage within the anterior cortical bone of human vertebrae, and to relate these data to the physical, biochemical and architectural characteristics of the cortex, adjacent trabeculae, and to the age and sex of the subjects from which samples were obtained. Other projects related to work in Brisbane were also undertaken concurrently.

Samples of the anterior cortex and adjacent trabeculae were bulk-stained in xylenol orange for determination of *in vivo* microcracks (Mdx) and then embedded in plastic and scanned using a Skyscan 1076 at a resolution of 18 um, and sections cut for histology, Mdx and FTIRM analysis in the sagittal and transverse planes. Thick sections of this region were used for analysis of hardness, and the mean degree of mineralisation. Preliminary studies showed that the concept of a cortical shell composed of a wall of dense osteonal bone at a thickness of about 300-400 um, is misleading. Among a range of vertebrae collected, only a small proportion demonstrated this typical characteristic, and there is a wide range from classical cortical bone, to highly fenestrated structures in which the “cortical bone” elements are indistinguishable from the adjacent trabeculae. We are investigating if this phenomenon is similar to the increase in endocortical porosity seen at other skeletal sites. Further, we demonstrated that, contrary to our hypothesis, the mean degree of mineralisation (DMB) and the Vickers micro-hardness (Hv) of the cortical bone was significantly lower than that of the adjacent trabeculae, and its mineral heterogeneity index (HI) higher. This difference between cortical and trabecular bone is not observed for the iliac crest (Boivin et al., JCEM 2003; Bone 2008) which also has a relatively low microdamage (Mdx) burden (Chapurlat et al JBMR, 2007). These differences could be the result of the different loading at the two sites. It also strongly suggests that remodelling in the cortical bone of vertebral bodies is higher than that of the trabeculae, which could also be a result of load sharing by the cortex and its associated Mdx burden, which was observed and is being quantified.

Outcomes

Based on the laboratory projects, one paper has been submitted to Osteoporosis International (Forwood M and Vashishth D. Translational Aspects of Bone Quality: Vertebral Fractures, Cortical Shell, Microdamage and Glycation), and one abstract was presented at the ASBMR Annual Meeting in Montreal. Papers are in preparation for projects on the vertebral cortex (2), and trabecular bone (1), influence of bisphosphonate treatment on bone quality of the iliac crest (1); influence of radiation sterilisation on collagen cross-links in allograft bone (1), influence of loading mode on microdamage morphology (1).

<u>Other outcomes include</u>:

Competitive appointment as a Visiting Professor in the University of Lyon in 2007-2008, the only appointment made in Lyon out of 16 National appointments in France by the French Minister for Higher Education.

Invited consultant to European Commission evaluation and audit of the ERA Mobility portal for researchers, Deloitte Consultants, Paris.

Invited member of PhD Defense Jury, Dorina Ianc, Université Blaise Pascale, Clermont Ferrand.

Arthritis Australia Grant-In-Aid "Contribution of the Cortical Shell to Vertebral Fracture". Forwood MR and Delmas PD. $15,000

Invited Lectures:

Université Jean Monnet, St Etienne. INSERM Unité 890 – Laurence Vico Director. "Si Galilée était vivant aujourd'hui: Les adaptations du tissu osseux de Pise à Brisbane"

Nestlé Research Centre, Physical Performance and Mobility Laboratories, Lausanne Switzerland. "Effective application of pre-clinical models for skeletal research".

Conclusion

The period of study leave from April 2007 to May 2008 could not have been undertaken without the support of the ANZBMS Christine and T Jack Martin Research Travel Grant. This grant, and the flexibility it affords for contribution to travel and maintenance, is a unique funding vehicle not accessible from traditional sources. The objectives of the program have largely been met, and the opportunity to spend 12 months back in the laboratory provided long-needed training in techniques for assessment of bone quality (and R & R!). The opportunity to undertake this program with a wonderful group of investigators in Pierre's laboratory, to work with them to create new collaborations and friendships in a beautiful French city was a rare opportunity. I am also full of gratitude to have the privilege of working with Pierre for the past year, and to experience at close hand his brilliance and influence, and was humbled by his strength of character and courage as he raged against the dying of the light and continued to do so until the end. It was with immense sadness that I left Lyon having watched a man so full of life, activity and intellect be gradually eroded by lung cancer.

Monique Arlot, PDD and yours truly at the retirement party for Monique, March 2008.