Citation: IBMS BoneKEy **10**, Article number: 406 (2013) | doi:10.1038/bonekey.2013.140

www.nature.com/bonekey

NEWS

Imaging the bone vasculature: what can it tell us?

Neil A Andrews

International Bone and Mineral Society, Chicago, IL, USA.

IBMS BoneKEy **10,** Article number: 406 (2013) | doi:10.1038/bonekey.2013.140; published online 4 September 2013

Recent *IBMS BoneKEy* webinar highlighted the potential of techniques that combine barium sulfate infusion with CT analysis

Emerging imaging techniques that assess bone vascularization have great potential to illuminate the relationships between skeletal blood vessels and bone remodeling processes. Such was the underlying theme of Bone Vascularization: Assessment and Role in Bone Remodeling, a recent *IBMS BoneKEy* webinar presented by Marie-Hélène Lafage-Proust (INSERM U1059, Université de Lyon, Saint-Etienne, France). The techniques are already helping to decipher the role of the bone vasculature in bone loading and unloading conditions, as well as the effects of osteoporosis drugs on the bone vasculature. They also have promise to reveal the importance of the bone vasculature in bone healing and in a number of pathogenetic processes, including osteoarthritis and bone metastasis.

The webinar was moderated by Larry Suva (University of Arkansas for Medical Sciences, Little Rock, AR, USA), and is available for viewing here: http://www.nature.com/bonekey/webinars/index.html?key=webinar28

Dr Lafage-Proust began her presentation by reviewing the many important functions that blood vessels in bone are known to play. They facilitate the disposal of metabolic waste products, allow hematopoietic cells to leave the bone marrow and enter the circulation, and are also an important component of the hematopoietic niche. The skeletal vasculature also delivers circulating and local growth factors to bone, carries bone cell precursors to remodeling sites, and brings oxygen and nutrients to bone.

The coupling of angiogenesis to bone formation during modeling situations, including endochondral ossification, bone growth and fracture repair, is well documented and is being further studied in bone tissue engineering. In contrast, the relationships between blood vessels and bone during bone remodeling remain unclear. Sprouting of a new blood vessel at the center of the cutting cone is thought to have a role in cortical bone remodeling, while in trabecular bone remodeling, it is unclear whether sprouting of a new blood vessel or rather a change in the position of an existing blood vessel is the key event. In her introduction, Dr Lafage-Proust also emphasized the heterogeneity of bone marrow microvessels, both in terms of their structure as well as the proteins they express.

Considering the importance of the skeletal vasculature, one aim of Dr Lafage-Proust's research is to examine the functional and spatial relationships between bone and blood vessels, during situations of bone remodeling in mice and in rats. To do so, she and her colleagues have developed tools that allow for a combined quantitative assessment of bone vascularization and bone remodeling. In rats, her general approach is to infuse barium sulfate, a contrast agent, into the vena cava and then to use conventional micro-computed tomography (micro-CT) or synchrotron radiation micro-CT, to image and quantify bone blood vessels. Validation of three-dimensional quantification of blood vessels is performed using two-dimensional histology.

Use of this strategy has already yielded valuable insight into the changes in the bone vasculature that occur when bone is presented with a mechanical challenge, and in that regard Dr Lafage-Proust first described results from studies using conventional micro-CT. For instance, using a tail suspension unloading model, which induces bone loss, Dr Lafage-Proust and colleagues found that unloading resulted in a statistically significant decrease in vessel number[1] in the tibia in rats, after 2 weeks of unloading. 'Interestingly, we also found in this work that there was a negative correlation between bone formation rate and vessel number only in the unloaded animals, while there was no such correlation in the unchallenged animals,' Dr Lafage-Proust said.

Conversely, in another study using conventional micro-CT[2] in rats subjected to loading in the form of running, along with the expected increase in bone mass and bone formation in the tibia was an increase in vessel number as well as expression of vascular endothelial growth factor (VEGF) messenger RNA, compared to sedentary control animals, after 3 weeks. Furthermore, the increase in vessel number could be prevented by administration of an anti-VEGF antibody. In this study, 'as far as mechanical strain is concerned, there is a direct and proportional relationship between mechanical strain and vessel number: the lower the mechanical strain, the lower the vessel number, and the higher the mechanical strain, the higher the vessel number,' Dr Lafage-Proust emphasized.

However, work submitted for publication by Dr Lafage-Proust's group suggests an uncoupling between bone formation and bone marrow angiogenesis in rats challenged with chronic normobaric hypoxia. Such animals exhibited decreased bone mass, and a dramatic increase in bone vessel number. In this experimental scenario, 'bone angiogenesis is probably related to an increase in hematopoiesis, which was illustrated by a dramatic increase in hematocrit after three weeks of chronic hypoxia,' Dr Lafage-Proust emphasized.

Dr Lafage-Proust then moved on to discuss the information that synchrotron radiation micro-CT can bring. In particular, she described work investigating the effect of intermittent parathyroid hormone (iPTH) 1–84, at a dose of 100 μg per kg, on bone vessels in male rats.[3,4] This research found that, while as expected, iPTH 1–84 was anabolic, unexpectedly vessel imaging and quantification revealed that bone blood vessel number was lower at 15 and 30 days, in the tibia, while there was no change in vessel number in the femur. These were unexpected findings since it is known that PTH increases VEGF expression. Further analysis, though, using three-dimensional synchrotron radiation micro-CT and two-dimensional bone histology, revealed that PTH affects the smallest vessels by relocating them closer to bone-forming sites.

In addition to using barium sulfate infusion and micro-CT to image bone vasculature in rats, Dr Lafage-Proust is doing the same in mice, and it was to those animals that she then turned. 'After having done this work in rats, we were interested in looking at and testing some molecular hypotheses, and of course we needed genetically modified mice' to do so, Dr Lafage-Proust said. Her group has used these imaging techniques to study the effects of iPTH 1–84, zoledronate, and propranolol on vessel density in 4-month-old ovariectomized mice, and she presented some preliminary data from that work during the webinar. As expected, zoledronate and propranolol decreased the bone formation rate, while iPTH increased it. Meanwhile, iPTH prevented the decrease in vessel number induced by ovariectomy, while zoledronate and propranolol had no impact on that parameter. Further studies comparing continuous PTH to iPTH 1–84 treatment revealed that vessel size was smaller with continuous PTH, compared to iPTH. Dr Lafage-Proust noted that these results could account for the differing effects of iPTH, which is anabolic, and continuous PTH, which is not, on bone remodeling, but cautioned that this hypothesis, based on preliminary findings, must be tested in additional studies.

For use in mice, another promising technique is intravital microscopy, which enables researchers to examine cellular processes in living animals, by attaching a window on a particular area, such as the femur or calvarium, and looking through the window using a microscope. Other investigators have used intravital microscopy, along with cell fluorescence techniques, to examine processes such as homing of osteoclasts, and homing of hematopoietic stem and progenitor cells. Dr Lafage-Proust has used intravital microscopy in mice to image the vasculature in the calvaria, and in the tibia, though there are many important factors to be worked out, such as the resolution of the imaging, as well as the position of the mouse during the imaging.

All of the work that Dr Lafage-Proust described, even at this relatively early stage of research, has already produced a surprising finding. 'We were expecting to find a coupling between angiogenesis and bone formation, and we were able to show, after having developed all of these tools, that sometimes they are coupled, but sometimes they are not,' she said. 'The relationship between bone formation and the blood vessel, especially in trabecular bone, remains to be further studied. The mechanisms [underlying that relationship] are very important because blood vessels in bone might be a therapeutic target for treating metabolic bone diseases,' she concluded. During the discussion after the presentation, Dr Suva suggested that imaging the bone vasculature in genetic models characterized by excessive bone formation, for instance, could be a feasible way to make progress in this regard. The challenge, Dr Lafage-Proust said, is that the relationship between bone and the vasculature changes over time, and so assessing that relationship at just one point in time may provide an erroneous impression of what is actually occurring; making observations at a number of different time points is a necessity, she stressed, for a true understanding.

In the bone field, what are the other potential uses of imaging the bone vasculature? One that Dr Suva suggested is the possibility of using imaging to help define the skeletal phenotype of the animal models that investigators use to better understand bone biology. In terms of specific conditions upon which imaging may be brought to bear, Dr Lafage-Proust is currently imaging the bone vasculature to better understand its role in the progression of osteoarthritis. She also said that imaging the bone vasculature has promise to illuminate the process of bone metastasis, the progression of primary tumors in bone and bone healing.

Conflict of Interest

The author declares no conflict of interest.

References

1. Fei J, Peyrin F, Malaval L, Vico L, Lafage-Proust MH. Imaging and quantitative assessment of long bone vascularization in the adult rat using microcomputed tomography. *Anat Rec (Hoboken)* 2010;**293**:215–224.
2. Yao Z, Lafage-Proust MH, Plouët J, Bloomfield S, Alexandre C, Vico L. Increase of both angiogenesis and bone mass in response to exercise depends on VEGF. *J Bone Miner Res* 2004;**19**:1471–1480.
3. Prisby R, Guignandon A, Vanden-Bossche A, Mac-Way F, Linossier MT, Thomas M *et al.* Intermittent PTH(1-84) is osteoanabolic but not osteoangiogenic and relocates bone marrow blood vessels closer to bone-forming sites. *J Bone Miner Res* 2011;**26**:2583–2596.
4. Langer M, Prisby R, Peter Z, Boistel R, Lafage-Proust MH, Peyrin F. Quantitative investigation of bone microvascularization from 3D synchrotron micro-computed tomography in a rat model. *Conf Proc IEEE Eng Med Biol Soc* 2009;**2009**:1004–1007.