Bone Remodelling in Space: A Review

Connor Keller and Shane Arsenault

Alzheimer's disease is the most common neurodegenerative disease in the world. Despite years of intense research, its pathogenesis remains quite controversial. Many different explanations have been proposed to describe its onset, the most established of which is the β-amyloid hypothesis. This hypothesis proposes that the disease is primarily caused by the formation of β-amyloid plaques in the brain. The presence of these plaques, it is suggested, ultimately leads to neuroinflammation, tau aggregation and, eventually, neuronal death and the often-cited neurocognitive sequelae observed in Alzheimer's patients. However, recent evidence suggests neuroinflammation may in fact be a root cause of the disease as opposed to acting as an eventual or coincidental manifestation. More specifically, it has been found that the activation of inflammasomes in microglia (the brains immune cells) contributes to the production of proinflammatory cytokines which then potentiates the neuroinflammatory response, with other downstream affects including increased β-amyloid plaque build-up, tau aggregation and a loss in cognitive function. Therefore, more and more studies are suggesting that neuroinflammation - and particularly the inflammasome - could be targeted therapeutically to prevent and treat Alzheimer's disease in patients.

Introduction

The homeostatic maintenance of human bone is an important process for conditions amenable to survival. Bone provides the skeleton for muscle attachment and support which enables motion as well as providing protection for vital organs such as the brain and heart. Bone also plays a necessary role in other basic homeostatic functions including maintaining constant levels of density and inorganic calcium. For example, human long bone must be preserved at around 60% inorganic calcium to maintain structural integrity while the ossicles of the middle ear must be kept at around 90% to allow for auditory proficiency (Seeman, 2006). Circulating calcium is also required in functional homeostasis as it is a central component in neurotransmitter release, muscle contraction and as a second messenger in cell signalling.

Considering the importance of bone in the maintenance of human physiology, it would follow logically that preserving adequate bone density and inorganic calcium levels would be critical in maintaining homeostasis during space flight. Understanding changes in bone mass and bone quality in the unique microgravity of the space environment is the first step to defining an osteoporosis risk, and establishing whether an intervention is required to mitigate that risk. This paper will examine the basics of bone remodelling and structural maintenance before delving into the past and current literature in homeostatic control of bone density in space.

Regulation of Bone Remodelling

To provide context for the review of bone density maintenance in space flight, we must look at what factors regulate physiological homeostasis. This centers around the balance between parathyroid hormone (PTH), activated 1,25-dihydroxycholecaliferol (1,25-(OH2)D3) and calcitonin. The parathyroid gland, specifically the chief cells, release PTH in response to a decrease in circulating plasma calcium levels. This increase in PTH is sensed in bone via the vascular network of the bone matrix, enabling upregulation of osteoclast activity. Osteoclasts mediate the breakdown of bone by carbonic anhydrase which causes an increase in circulating calcium levels by mobilizing calcium previously stored in bone.

The mechanism for this upregulation lacks a distinct physiological receptor which actively upregulates osteoclasts. Instead, osteoblasts secrete RANK-ligand, which in turn activates osteoclasts. PTH further increases circulating calcium by increasing reabsorption of calcium in the kidneys and increasing the secretion of phosphate in the urine. PTH also increases the conversion of

inactive Vitamin D to the active metabolite 1,25-(OH2)D3. Active 1,25-(OH2)D3 works similarly to PTH to increase serum calcium concentrations. Unlike PTH however, 1,25-(OH2)D3 exerts its influence by increasing the reabsorption of calcium in the kidney as well as increasing calcium absorption from the gut. Serum calcium self-regulates by decreasing levels of circulating PTH in a negative feedback loop.

Calcitonin disrupts osteoclast activity through binding and disabling osteoclast receptors that would normally recognize RANK-L for activation. This decreases calcium release and stabilizes bone density.

It is the combination of the aforementioned three factors, as well as peripheral influence by oestrogen, thyroid hormones and glucocorticoids, which allows the human body to maintain a delicate balance between trabecular and cortical bone turnover and serum calcium levels.

Bone Cell Types and Normal Bone Remodelling

In normal bone remodelling, a balance between bone resorption and bone formation is regulated and maintained to ensure there is no significant net changes in bone mass, and consequently, mechanical strength (Feng & McDonald, 2011). An imbalance between bone resorption and bone formation may occur under certain pathological conditions or in the absence of mechanical stimulation, leading to abnormal bone remodelling and the development of bone disorders.

There are two major types of bone in the human body; cortical and trabecular bone. Cortical bone provides mechanical support and leverage functions and protects vital organs, whereas trabecular bone provides strength, and is the major site of bone remodelling. Old or damaged bone is removed by osteoclasts, then replaced by new bone formed by osteoblasts at a rate of 25% turnover in trabecular bone and 3% in cortical bone annually (Clark, 2008). Bone composition is approximately 10% cells, 60% inorganic mineral crystals, and 30% organic matrix (Seeman & Delmas, 2006). Hydroxyapatite composes the majority of the inorganic mineral crystals and type one collagen represents approximately 88% of the organic matrix, while other non-collagenous proteins comprise approximately 10% and lipids and glycosaminoglycans the remaining 1-2% (Feng & McDonald, 2011).

The bone remodelling process is carried out by the bone multicellular unit (BMU) and requires the action of four major types of bone cells; bone-lining cells, osteocytes, osteoblasts and osteoclasts. The bone-lining cells cover the inner endosteum of the bone and are thought to be quiescent osteoblasts, connected to active osteoblasts via adherens junctions (Clarke, 2008). Pluripotent stem cells give rise to the osteoprogenitor cells which in turn differentiate into osteoblasts, osteocytes and bone-lining cells. (Partridge, 2010). Osteocytes differentiate from osteoblasts and are embedded within the bone matrix during skeletal development or during previous cycles of bone remodelling. The osteocytes maintain their connection with each other and the bone surface via filopodial cellular processes, forming a syncytium.

The osteocyte functions as a mechanosensor, as fluid may flow in response to external forces in osteocytic canaliculi causing fluxes in calcium across filopodial gap junctions and transmitting information from the osteocyte to the osteoblasts (Clarke, 2008). Furthermore, osteoclasts, the bone-resorbing cell, are multinucleate giant cells which differentiate from mononuclear cells of the monocyte/macrophage lineage when stimulated by the monocyte/macrophage stimulating factor (M-CSF) and the receptor activator of nuclear factor kappaB (NF-kappaB) (Feng & McDonald, 2011).

The bone remodelling process involves four major distinct yet overlapping phases. The activation phase begins when mononuclear/macrophage osteoclast precursors lift the endosteum containing the bone-lining cells off the bone surface (Clarke, 2008). This is followed by the fusion of the mononuclear cells to form the multinucleated pre-osteoclasts. These pre-osteoclasts then bind to the bone matrix via interactions between integrin receptors in their cell membranes and RGD-containing peptides in the extracellular

matrix (ECM) proteins, forming annular sealing zones around the bone-resorbing compartments beneath the multinucleate pre-osteoclasts.

The second phase of the bone remodelling process is resorption. The formation, activation, and resorption of osteoclasts are regulated by the ratio of receptor activator of NF-kappaB ligand (RANK-L) to osteoprotegerin (OPG), and by the concentrations of a number of factors includingparathyroid hormone (PTH), vitamin D and calcitonin (Arfat et al, 2014). Furthermore, sclerostin (SOST) also stimulates osteoclasts, and antagonizes the action of Wnts and bone morphogenetic proteins (BMPs), providing a potential target for monoclonal antibodies in diminishing osteoclastic activity (Qin et al, 2015) . Mature osteoclasts secrete hydrogen ions via proton pumps and chlorine ion channels to lower the pH of the resorbing compartment to 4.5. These osteoclasts also secrete tartrate-resistant acid phosphatase, cathepsin K (serine protease), matrix metalloproteinase-9 (MMP-9), and gelatinase from lysosomes to digest the organic matrix, forming Howship's lacunae in trabecular bone, and Haversian canals in cortical bone (Clarke, 2008). This phase is completed by mononuclear cells, once the multinucleate osteoclasts have undergone apoptosis.

The third phase refers to the reversal compomentof bone remodelling. Coupling signals have been proposed to link tresorption to formation including transforming growth factor-beta (TGF-beta), insulin growth factor-1 (IGF-1), IGF-2, BMPs and fibroblast growth factor (FGF) (Hock et al, 2004). TGF-beta released from bone matrix decreases osteoclast resorption by inhibiting RANK-L in a fashion variant on the strain gradient in the lacunae (Smit et al, 2002, Smit et al, 2002). The strain gradient refers to how osteoclasts resorb cortical bone in a cutting cone, with strain increased behind the osteoclasts and reduced in front. In trabecular bone lacunae, the strain is highest at the base and lowest in the surrounding edges, with osteoclasts activated by reduced strain and osteoblasts by increased strain.

The final phase of the bone remodelling cycle is bone formation as osteoblasts synthesize new collagenous organic matrix, and regulate the mineralization of this matrix by releasing small membrane-bound matrix vesicles that concentrate calcium and phosphate and destroy mineralization inhibitors such as pyrophosphate (Anderson, 2003).

Spaceflight

The influence of spaceflight on the regulation of calcium has been a central point in understanding the effects of space travel on human physiology. The lack of gravity in space means loss of one of the most important aspects in regulating levels of bone density - mechanical resistance or loading on bone. This has been an area of discussion and research from before the first manned space flights. Despite being an area with high levels of interest, research and development have proved less fruitful than one might expect, due in large part to a poor sample size and the importance of maintaining safety standards on manned spaceflights.

Given that observations and measurements, as well as potential interventions can only accurately be measured during missions, there have been less innovations made despite the time course of spaceflight and bloom of technology in the intervening years. There have however, been a number of seminal studies and recently developed methods used to study microgravity conditions outside of spaceflight.

History of Research

The initial studies revolved around pre- and post-flight measurements of bone density in the heel and wrist on the Gemini and Apollo missions in the 1960's and 1970's (Smith et al, 2014). This proved, first and foremost, the ability of human beings to withstand microgravity while remaining compatible with life. It also demonstrated a loss in bone density, even over short flights of up to a maximum of fourteen days. The 1970's were rounded out by the Skylab studies in which three missions went up for up to a maximum of eighty-four days, providing data that suggested increased levels of calcium loss through faecal and urinary excretions during flight

(Whedon et al, 2006). Samples were frozen from this flight and upon subsequent analysis in the 1990's demonstrated bone resorption resulting in losses of calcium and bone density.

Advances continued during the era of the space shuttle, though the Columbia mission, on which a highly specialized calcium kinetics study was designed, was ultimately ill fated as it crashed on landing. The majority of information gleaned from shuttle era studies was similar to that of early flights, in that pre- and post- flight measurements made up the majority of data, providing a limited data-set with which to observe in-flight changes.

The advent of the space stations proved more fruitful in furthering our knowledge of bone remodelling in microgravity, as well as providing evidence for effective interventions. First iRED and then aRED (interim and advanced resistance exercise device, respectively) (Zwart, 2007, Smith et al, 2012, Smith et al, 2014) were premiered on the international space station. These technologies replaced simple treadmills and stationary bicycles in providing resistance based exercise options to astronauts with the aim of increasing new bone formation, balancing microgravity based losses. Ultimately it was the aRED that proved to be effective in increasing bone formation as it provides greater loading capacity, a wider variety of exercises designed to target sites which traditionally display the highest degrees of bone mass decline and can simulate the inertia associated with gravitational loading (Smith, 2012). At the same time as the introduction of the aRED however, dietary changes such as an increase in omega-3 polyunsaturated fatty acids and supplementation with vitamin D, both factors important in calcium homeostasis, were improved in these missions, possibly confounding the findings (Smith et al, 2014). Other important nutritional components which can affect the excretion of calcium include diets high in animal protein and potassium and diets with a high dietary sodium intake, which is typical to an astronaut diet, as both have been shown to increase urinary calcium excretion (Arfat et al, 2014).

Finally, pharmacological interventions have been proposed and tested as a preventative course inhibiting bone resorption. Bisphosphonates including oral alendronate, etidronate and intravenous zoledronic acid, the first line treatment for osteoporosis according to the 2012 NICE guidelines, have been considered and tested. Though results are confounded by the conversion from iRED to aRED in the target population and dietary advances, the studies seem to suggest that the effects are beneficial beyond exercise and that side effects are outweighed by these benefits (Smith et al, 2014). Testosterone supplements have also been mooted as a potential preventive agent, though no benefits have been shown either in bed-rest or extended spaceflight studies.

Effects of Microgravity on Bone Remodelling

Space and the simulated microgravity environment alters the cellular physiology of bone MSCs, osteoblasts, osteocytes and osteoclasts. MSC differentiation is redirected from osteogenesis to adipogenesis in those who have experienced a brain or spinal cord injury and subsequent withdrawal of mechanical loading on the bones (Arfat et al, 2014). This withdrawal of mechanical loading makes these injuries a convenient terrestrial model for research into unloading in spaceflight. Cytoskeletal changes affecting MSCs include alterations in the F-actin filaments that mimic those seen in cellular apoptosis. In addition to this, microgravity induced reduction in MSC differentiation to osteoblasts is mediated by a decrease in the mitogen-activated protein kinase (MAPK) pathway (Huang, 2009).

Microgravity-induced bone loss has been attributed to the reduced proliferation and differentiation of osteoblasts, as well as their decreased responsiveness to bone-related factors. Actual or simulated weightlessness disrupts osteoblast microfilaments, resulting in defective bone formation, and a decrease in the expression of the transcription factor alkaline phosphatase (ALP), which is important for osteoblast differentiation (Carmeliet et al, 1997). Regarding

the signalling pathways which affect osteoblast differentiation, integrin B1, a microgravity sensitive BMP2 regulator, causes inhibition of focal adhesion kinase (FAK) as well as ERK 1 and 2 activation when blocked by antibodies(Arfat et al, 2014). BMP2 is responsible for starting osteoblast cytoskeletal rearrangement during osteoblast induction, affecting osteoblast migration and adhesion (Huang, 2011). Osteocytes regulate bone resorption and formation in the context of both bone remodelling. Under normal gravity, mechanical loading can lead to strain and deformation of the bone matrix and disturb the interstitial fluid surrounding the lacunar-canalicular network of osteocytes. This fluid shear stress is one of the major mechanical stimuli acting on osteocytes.

In microgravity, there is an absence of mechanical loading, resulting in a dramatic reduction in fluid shear stress, and subsequent activation of the osteocytes and release of the physiologically important second messenger molecules. In addition to this, microgravity also induces alterations in the cytoskeletal architecture of the osteocytes and suppresses the gap junctions connecting these cells to one another (Di, 2011). Apoptosis of the osteocytes will negatively impact the bone remodelling events which maintain weakened bone. This loss detrimentally affects the areal bone mineral density (aBMD), the current standard in the measurement of bone density, by up to 10% according to Sibonga (2013). LeBlanc et al (2000) have reported aBMD losses of between 1-1.5% per month, exceeding the aBMD decline observed with primary osteoporosis in older individuals. When one considers the increased risks of mortality associated with osteoporotic fractures, this provides a very real marker for the requirement of management of aBMD decline. Lastly, osteoclast differentiation is enhanced in the microgravity environment, likely due at least in part to elevated levels of specific growth factors in pre-osteoclasts, possibly leading to an alteration in the RANKL:OPG ratio (Sambandam, 2010).

Concerns

Unfortunately, there remain a number of concerns with regards to the current breadth of research in bone remodelling during spaceflight. With the small number of missions and limited number of crew members on each mission it is difficult to produce satisfactory experimental design to confirm or update hypotheses.

Furthermore, due to the intense nature of spaceflight missions cannot be compromised to craft studies in which astronauts were exposed to different conditions or experimental measures. Safety requires that each are subjected to conditions which maximize their likelihood of returning home intact. Also due to the nature of the astronaut selection process it is impossible to truly randomize or control for a number of selection variables, ensuring that the studied sample is often quite homogenous.

With further regards to the safety concerns, the fact that diet, nutrition and pre-screenings have been improved has meant that any data gleaned from studies of exercise in the microgravity environment may have been altered by the dietary changes. Thus comparing missions or forms of exercise is difficult due to a high degree of variability in underlying conditions.

Conclusions

In summary, we see extraterrestrial research into bone resorption as a field which has made significant advances, but one which still invites discovery. While countermeasures have been derived, they remain imperfect, with room for improvements to aid in maintenance of skeletal integrity. Optimizing nutrition to aid in maintaining a healthy balance has also progressed, specifically in regulation of Calcium, Vitamin D and caloric intake, with enhanced Omega-3 polyunsaturated fatty acids. However, there remains significant room for an expansion of knowledge when considering the potential requirements of longer spaceflight missions currently under development.

Conflict of Interest

Neither author has any conflict of interest to declare.

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