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Microgravity and Bone Cell Mechanosensitivity

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Bone cells, in particular osteocytes, are extremely sensitive to mechanical stress, a quality that is probably linked to the process of mechanical adaptation (Wolff's law). The in vivo operating cell stress derived from bone loading is likely a flow of an interstitial fluid along the surface of the osteocytes and lining cells. The response of bone cells in culture to fluid flow includes prostaglandin synthesis and expression of inducible prostaglandin G/H synthase (PGHS-2 or inducible cyclooxygenase, COX-2), an enzyme that mediates the induction of bone formation by mechanical loading in vivo. Disruption of the actin-cytoskeleton abolishes the response to stress, suggesting that the cytoskeleton is involved in cellular mechanotransduction. Microgravity has catabolic effects on the skeleton of astronauts, as well as on mineral metabolism in bone organ cultures. This might be explained simply as resulting from an exceptional form of disuse under weightlessness conditions. However, under microgravity conditions, the assembly of cytoskeletal elements may be altered, as gravity has been shown to determine the pattern of microtubular orientation assembled in vitro. Therefore, it is possible that the mechanosensitivity of bone cells is altered under microgravity conditions, and that this abnormal mechanosensation contributes to the disturbed bone metabolism observed in astronauts. In vitro experiments on the International Space Station should test this hypothesis experimentally. (Bone 22:127S–130S; 1998)

Key Words: Microgravity; Mechanosensitivity; Bone cell; Prostaglandin; Fluid shear stress; Cytoskeleton.

Introduction

It has been well documented that bone tissue is sensitive to its mechanical environment. Subnormal mechanical stress as a result of bedrest or immobilization results in decreased bone mass and disuse osteoporosis.[10] Spaceflight produces a unique condition of skeletal unloading as a result of the near absence of gravity. Studies of animals and humans subjected to spaceflight agree that microgravity negatively affects the mass and mechanical properties of bone (for a review, see Reference 34). Although the exact mechanism whereby bone loss as a result of spaceflight occurs is still unknown, recent in vitro studies suggest that bone cells are directly sensitive to microgravity. Using organ cultures of living bone rudiments from embryonic mice, Van Loon et al.[33] showed that 4 days of spaceflight inhibited matrix mineralization, while stimulating osteoclastic resorption of mineralized matrix. Monolayer cultures of the human osteoblastic cell line MG-63 responded to 9 days of microgravity with reduced expression of osteocalcin, alkaline phosphatase, and collagen Iα1 messenger ribonucleic acid (mRNA) (Reference 3; see also this issue). In addition, microgravity induced prostaglandin E_2 and interleukin-6 production in rat bone marrow stroma cultures, an observation that may be related to alterations in bone resorption.[17] These results suggest that mineral metabolism and bone cell differentiation are modulated by microgravity, and that bone cells are directly responsive to micro-G conditions.

Direct responses of bone cells to mechanical stimuli have been studied using several methods to apply mechanical stress in vitro (for a review, see Reference 2). Stretching or bending of the cell substratum has been widely used, but recent evidence indicates that fluid flow over the cell surface may better simulate the cellular effect of mechanical loading of bone in vivo.[4,11,23,31,35] Strain (deformation) of the bone matrix as a result of mechanical stress in vivo causes flow of interstitial fluid through the network of osteocyte lacunae and canaliculi (**Figure 1**).[15,20] Weinbaum et al.[35] used Biot's porous media theory to relate loads applied to a whole bone to the flow of canalicular interstitial fluid. Their calculations predict fluid shear stresses of 0.8–3 Pa as a result of peak physiological loading regimes. Based on this hypothesis, we have recently tested whether osteocytes are sensitive to fluid shear stress in vitro, and which paracrine factors are produced in response to fluid flow. In the following, we will briefly review these studies. We will then discuss the hypothesis that microgravity may alter mechanotransduction in bone cells by interfering with the formation of the cytoskeleton.

Pulsatile Fluid Flow

For studies on cell mechanosensitivity, a pulsatile fluid shear stress was applied to monolayers of bone cells using the apparatus schematically shown in **Figure 2**. Essentially, a shear stress was applied by pumping culture medium through a flow chamber containing a monolayer of cultured cells. The flow chamber consisted of a machine-milled polycarbonate plate, a rectangular Dural (AlMgSi; 51ST) gasket, and a polylysine-coated [50 μg/mL; poly-l-lysine hydrobromide, molecular weight (MW) 15–30 $\times$ 10^4; Sigma, St. Louis, MO] glass slide containing the cell monolayer. A polycarbonate plate, gasket, and glass slide were assembled such that a channel was created above the cells that was 2–4 cm wide and 0.03 cm deep. The area of cells exposed to shear was 14 cm^2. The polycarbonate plate had two manifolds through which medium entered and left the channel. The entry port was larger than the exit port and served as a bubble trap. During an experiment, all components were placed in a 37°C

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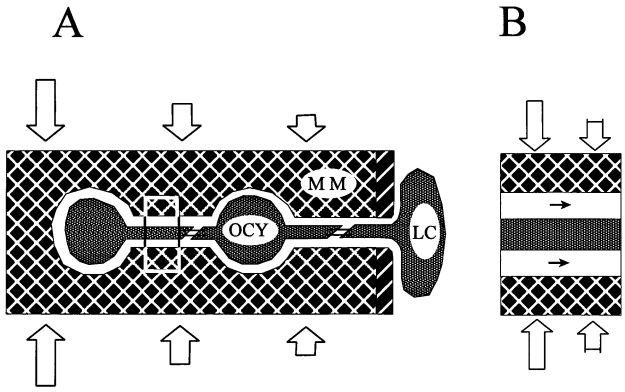

Figure 1. Schematic diagram of the transduction of mechanical stress to bone cells. (A) The osteocyte-lining cell network of a piece of bone under stress (large arrows). (B) The enlarged inset of (A). Bone tissue, because of its lacunar–canalicular network, may be considered as a hard sponge. Loading (large arrows) results in flow of interstitial fluid in the sheath of unmineralized matrix between cell membrane and mineralized wall. Osteocytes and the cell processes of bone lining cells are activated by the resulting fluid shear stress.

incubator, and the medium reservoir was connected to a gassing system that maintained a humidified atmosphere of 5% CO_2 in air. Pulsatile fluid flow (PFF) resulted from pumping the culture medium over the cells in a pulsatile (5 Hz) manner using a revolving pump. The flow rate was monitored using a flow probe (Figure 2). The wall shear stress on the cell monolayer was calculated using the momentum balance for a Newtonian fluid and assuming a parallel-plate geometry. In all studies discussed here, we subjected the cell monolayers to the same magnitude of shear stress, which was calculated to be 7.2 dyn/cm^2 (0.7 Pa). The flow profile was measured in the fluid circuit after the flow

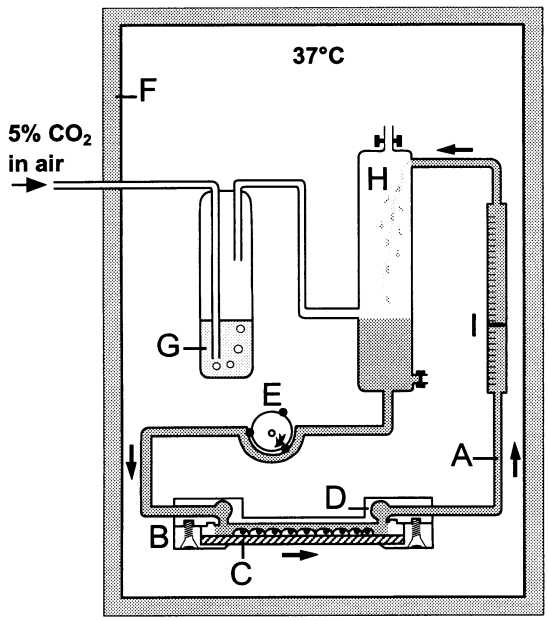

Figure 2. Diagram of application of pulsating fluid flow (PFF). Fluid culture medium (A) is pumped through a parallel-plate flow chamber (B) containing a monolayer of cells (C). The medium enters and exits the flow chamber through two slits (D) and is moved by a revolving pump (E). (F) 37°C incubator; (H) water reservoir; (G) reservoir for gassing the medium; (I) flow probe.

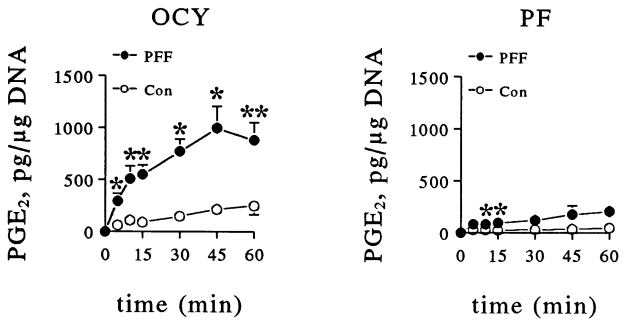

Figure 3. Effect of PFF on cumulative PGE_2 production by chicken calvarial osteocytes (OCY) and periosteal fibroblasts (PF). Values are means ± SE of four to eight separate experiments. Significant effect of PFF: $*p < 0.05$; $**p < 0.01$. Adapted from Ajubi et al.,[1] with permission.

chamber using an animal research flow meter (Transonic Systems, Ithaca, NJ). We observed a sinusoidal flow profile with a minimum and maximum shear stress of, respectively, 2.1 and 9.9 dyn/cm^2 (0.2 and 1.0 Pa), and an estimated peak stress rate of 12.2 Pa/sec.[26]

Response of Monolayer Bone Cell Cultures to PFF

The theory of fluid flow-dependent mechanosensing in bone tissue assumes that osteocytes, bone lining cells, and osteoblasts, but not osteoblast precursors or osteoclasts, are the "professional" mechanosensor cells of bone. This is because the flow of interstitial fluid resulting from load-induced strain is only important in the lacunar–canalicular network, and is negligible in the Haversion and Volkman channels. These latter channels are much wider (about 30,000 times wider than canaliculi) and the fluid pressure in them is more uniform as it must be almost the same as the blood pressure. To test this theory, the mechanosensitivity of osteocytes was compared with that of osteoblasts and periosteal fibroblasts.[11] Cells were isolated from chicken embryo calvariae and separated in three fractions. One fraction consisted for more than 95% of osteocytes as a result of immunoseparation based on the osteocyte specific antibody OB 7.3. A second fraction consisted of more than 90% of osteoblasts, and the third fraction contained periosteal fibroblasts.[32] The three cell types were submitted to PFF as well as to intermittent (0.3 Hz) hydrostatic compression of 13 kPa.[12] Osteocytes, but not osteoblasts or periosteal fibroblasts, reacted to 1 h PFF with a sustained release of prostaglandin E_2 (PGE_2).[11] Intermittent hydrostatic compression stimulated prostaglandin production to a lesser extent, i.e., after 6 and 24 h continuous treatment in osteocytes, and after 6 h in osteoblasts. These data provided evidence that osteocytes, at least in chickens, are the most mechanosensitive cells in bone, and that a fluid flow of 0.7 Pa was more effective than hydrostatic compression of 13,000 Pa. The results therefore supported the hypothesis that strain-derived fluid flow in the lacunar–canalicular system provides the stimulus for an adaptive response in bone.

In another study,[13] it was shown that chicken osteocytes but not periosteal fibroblasts responded to PFF with a rapid and transient two- to threefold upregulation of nitric oxide (NO) release. The effect was transient, reaching a maximum after 5 min and leveling off thereafter. A similar effect was observed in the late-released fraction of mouse calvarial bone cells obtained by sequential digestion.[13] PFF also acutely stimulated PGE_2 release by mouse[13] and chicken[1] bone cells (**Figure 3**). This effect was significant after 5–10 min and continued throughout

60 min of PFF treatment. Importantly, inhibition of NO release by the competitive NO synthase inhibitor Na-monomethyl-L-arginine prevented the effect of PFF on NO release *as well as* on PGE_2 release.[13] These results suggested that NO is another mediator of mechanical effects on bone, and that NO release is critical for the PFF-mediated PGE_2 release. In addition, these results suggest that the response of bone cells to mechanical stress resembles that of endothelial cells to blood flow.[7–9] In the vascular system, changes in arterial diameter occur in response to changes in blood flow rate, in order to ensure a constant vessel tone, and endothelial cells are widely recognized as the mechanosensory cells of this response. The early response of endothelial cells to fluid flow in vitro includes the release of NO and prostaglandins.[9] Surprisingly, therefore, bone tissue seems to use a similar sensory mechanism to detect and amplify mechanical information as the vascular system.

PGE_2 upregulation continued throughout the 1 h PFF treatment, and also at least 1 h after PFF treatment,[14] suggesting an autoamplification mechanism whereby a short-lived stimulus such as mechanical stress is transduced into a sustained cellular response. A major step in prostaglandin production is the formation of prostaglandin G_2 (PGG_2) and subsequently prostaglandin H_2 (PGH_2) through the action of prostaglandin G/H synthase [PGHS or cyclooxygenase (COX)] on arachidonic acid.[25] There are two distinct enzymes for PGHS, encoded by separate genes.[16,24] PGHS-1 (or COX-1) is expressed constitutively in many tissues but can be upregulated by serum and growth factors.[5] In contrast, the expression of mRNA for PGHS-2 (or COX-2) is not constitutive in most tissues, including bone,[21] but can be induced rapidly and transiently by a variety of acute cell stresses, such as inflammatory mediators[16] and growth factors.[21] We examined the effect of mechanical stress on the expression of PGHS-1 and PGHS-2 in mouse calvarial bone cells. PFF treatment induced the expression of PGHS-2 within 1 h.[14] In the presence of 2% freshly added fetal bovine serum (FBS), which by itself induces PGHS-2 expression, the stimulating effect of PFF was about twofold. When the serum was reduced to 0.1%, the inductive effect of PFF on PGHS-2 was eight- to ninefold, relative to static controls. No effect was found on PGHS-1 expression. PFF treatment also increased the production of PGE_2 as well as prostaglandin I_2 (PGI_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), both acutely during PFF and for at least 1 h after PFF treatment.[14] The enhanced expression of PGHS-2 continued also for at least 1 h after PFF treatment. These results suggest that the mechanical stress had no effect on PGHS-1, but selectively upregulated PGHS-2 synthesis.

Interestingly, a recent study by Forwood[6] suggests that induction of PGHS-2 (or COX-2) is important for the induction of adaptive bone formation in vivo. In that study, rats were treated with a specific PGHS-2 inhibitor (NS-398), or indomethacin, which primarily inhibits PGHS-1, before loading one tibia by four-point bending.[31] Endocortical bone formation was significantly increased 5–8 days after a single bout of loading (300 cycles, 65 N) but not by sham loading. The increase in endocortical bone formation caused by bending was completely prevented by NS-398, but only partially by indomethacin, even at very high doses.[6] These results suggest that induction of PGHS-2 (or COX-2) is important for lamellar bone formation elicited by mechanical strain. Therefore, the in vitro induction of PGHS-2 by fluid flow treatment mimics a critical event in the adaptive response to loading in vivo. This suggests that fluid flow treatment of bone cells in vitro is indeed a meaningful way to mimic the effect of mechanical loading of bone tissue in vivo.

Microgravity and the Response of Bone Cells to Mechanical Stress

As stated earlier in this paper, microgravity negatively affects the skeleton, and there is evidence that bone cells are directly influenced by micro-G conditions.[3,17,33] The loss of bone mineral during spaceflight could be solely the effect of an unusual form of unloading of the skeleton as a result of weightlessness. In that case, countermeasures developed on earth against disuse osteoporosis should also be effective against spaceflight-related osteoporosis. However, recent observations on the nonlinear behavior of in vitro preparations of microtubules[27,29,30] suggest an alternative explanation that seems worthwhile to consider.

Experiments performed on earth on in vitro microtubule formation have shown that the self-assembly of microtubules from tubulin is to a certain degree regulated by gravity. In vitro solutions of microtubules that contain guanosine triphosphate (GTP) as an energy source show macroscopic self-ordering when brought to 37°C.[29] The morphology of the structures that form depends upon the orientation of the sample with respect to gravity.[30] The sensitivity for gravity is only present during a critical moment prior to the appearance of the self-organized state,[27,28] and an important condition for the effect of gravity is that the microtubule preparation is chemically and structurally far from equilibrium.[30] These results provide an explanation for biological gravitropism, and also fit a theory about direct action of gravity on single cells as proposed by Mesland.[18] Based on the work of Prigogine and Stengers,[22] Mesland applied nonlinear nonequilibrium thermodynamics to living cells under changing gravity conditions. His approach predicted gravitational effects in chemical reactions that are far from equilibrium.[18] In thermodynamic terms, this is precisely the principle characteristic of living cells, in particular, the cytoskeleton.

Microtubules are an important part of the cytoskeleton, and several observations on plant and animal cells indicate that the effects of microgravity are likely established via the cytoskeleton, either via the actin filament part or via the microtubular part or both (for a review, see Reference 19). We recently found that the transduction of mechanical signals in bone cells also involves the cytoskeleton.[1] Therefore, an alternative explanation of the interference of microgravity with bone cell function may be that, under microgravity conditions, the mechanosensitivity of bone cells is impaired as a result of alterations in the bone cell's cytoskeleton. Impaired bone cell mechanosensitivity might subsequently lead to a negative bone balance, even when countermeasures such as strenuous exercise are taken by astronauts. The experiments on microtubule assembly[27,29,30] as well as bone cell mechanosensitivity[1] were performed on earth and not during spaceflight. It seems worthwhile to further explore the hypothesis of a direct interaction of microgravity with cytoskeleton-mediated cellular processes in well-controlled studies under microgravity conditions. The International Space Station will offer a unique possibility to perform such sophisticated experiments. They will doubtlessly make a significant contribution to furthering our understanding of the role of gravity in living cells, and could shed new light on the phenomenon of microgravity-related osteopenia.

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