Fluid Shear Stress Synergizes with Insulin-like Growth Factor-I (IGF-I) on Osteoblast Proliferation through Integrin-dependent Activation of IGF-I Mitogenic Signaling Pathway*

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Sonia Kapur‡§, Subburaman Mohan‡§, David J. Baylink‡§, and K.-H. William Lau‡§¶
From the ‡Musculoskeletal Disease Center, Jerry L. Pettis Memorial Veterans Affairs Medical Center, and §Departments of Medicine and Biochemistry, Loma Linda University, Loma Linda, California 92357

This study tested the hypothesis that shear stress interacts with the insulin-like growth factor-I (IGF-I) pathway to stimulate osteoblast proliferation. Human TE65 osteosarcoma cells were subjected to a steady shear stress of 20 dynes/cm² for 30 min followed by 24-h incubation with IGF-I (0–50 ng/ml). IGF-I increased proliferation dose-dependently (1.5–2.5-fold). Shear stress alone increased proliferation by 70%. The combination of shear stress and IGF-I stimulated proliferation (3.5- to 5.5-fold) much greater than the additive effects of each treatment alone, indicating a synergistic interaction. IGF-I dose-dependently increased the phosphorylation level of Erk1/2 by 1.2–5.3-fold and that of IGF-I receptor (IGF-IR) by 2–4-fold. Shear stress alone increased Erk1/2 and IGF-IR phosphorylation by 2-fold each. The combination treatment also resulted in synergistic enhancements in both Erk1/2 and IGF-IR phosphorylation (up to 12- and 8-fold, respectively). Shear stress altered IGF-IR binding only slightly, suggesting that the synergy occurred primarily at the post-ligand binding level. Recent studies have implicated a role for integrin in the regulation of IGF-IR phosphorylation and IGF-I signaling. To test whether the synergy involves integrin-dependent mechanisms, the effect of echistatin (a disintegrin) on proliferation in response to shear stress ± IGF-I was measured. Echistatin reduced basal proliferation by ~60% and the shear stress-induced mitogenic response by ~20%. It completely abolished the mitogenic effect of IGF-I and that of the combination treatment. Shear stress also significantly reduced the amounts of co-immunoprecipitated SHP-2 and -1 with IGF-IR, suggesting that the synergy between shear stress and IGF-I in osteoblast proliferation involves integrin-dependent recruitment of SHP-2 and -1 away from IGF-IR.

Mechanical loading is essential for the maintenance of skeletal architectural integrity. Loading increased bone formation and inhibited bone resorption, leading to an increase in bone mass, whereas unloading decreased bone mass through an increase in resorption and a decrease in formation (1). Although the phenomenon of increased bone formation through an increase in osteoblast proliferation and activity in response to skeletal loading has been well described, the underlying mechanism(s) remains largely undefined. Loading produces strains in the bone that generate interstitial fluid flow through the lacunar-canalicular spaces (2). It is believed that this fluid flow exerts a shear stress at surfaces of bone cells lining the lacunar-canalicular spaces and that the shear stress generates biochemical signals in bone cells to stimulate osteoblast proliferation and activity. Shear stress stimulates bone cell proliferation and activity through multiple interacting signaling pathways (3).

Bone growth factors function as autocrine and paracrine mediators of bone formation (4, 5). The mechanism whereby mechanical loading stimulates osteoblast proliferation and activity could involve bone growth factors and corresponding signaling pathways. IGF-I is one of the most abundant growth factors in bone (4), produced by bone cells (4–7), and an important stimulator of bone formation (4–7). Loading increases bone cell production of IGF-I in vivo (8) and in vitro (9). The signaling pathway of IGF-I involves Erk1/2 activation, which is essential for mechanical stimulation of bone cell proliferation (10). It has been reported that the bone cell mitogenic response to mechanical strain is mediated by the IGF-IR (11). In addition, recent studies suggested that loading might have a permissive role in the IGF-I mitogenic action in bone, as skeletal unloading induces resistance to IGF-I with respect to bone formation. Accordingly, unloading blocked the ability of IGF-I to stimulate bone formation in the rat (12). IGF-I administration stimulates bone formation in the loaded bone, but not in unloaded bone in vivo (12) and in vitro (12, 13). There is evidence that unloading-related resistance to IGF-I is mediated by inhibiting the activation of IGF-I pathway through down-regulation of integrin expression (13). Because unloading blocked the osteogenic action of IGF-I, we postulated that increased loading enhances the osteogenic action of IGF-I. Accordingly, it has been suggested that loading enhances the anabolic effects of IGF-I on articular cartilage formation (14) and in nasopremaxillary growth (15).

Recent studies in smooth muscle cells (16–22) revealed that the ability of IGF-I to initiate its intracellular signals is regulated not only by its binding to its own transmembrane receptor (IGF-IR) but also by other transmembrane proteins, such as SHPS-1 and αvβ3 integrin, to recruit essential signaling pro-

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† To whom correspondence should be addressed: Musculoskeletal Disease Ctr., 1511, Jerry L. Pettis Memorial Veterans Affairs Medical Ctr., 11201 Benton St., Loma Linda, CA 92357. Tel.: 909-825-7084 (ext. 2536); Fax: 909-796-1680; E-mail: William.Lau@med.va.gov.

1 The abbreviations used are: IGF-I, insulin-like growth factor-I; IGF-IR, insulin-like growth factor-I receptor; pIGF-IR, phosphorylated IGF-IR; PGF-2, basic fibroblast growth factor; Erk1/2, extracellular regulated kinase 1/2; pErk1/2, phosphorylated Erk1/2; SHP-1, Src-homology 2 domain-containing protein-tyrosine phosphatase 1; SHP-2, Src-homology 2 domain-containing protein-tyrosine phosphatase 2; SHPS-1, SHP substrate 1; ANOVA, analysis of variance.
teins, such as SHP-2 and Shc. The integrin recruitment of SHP-2 is essential for regulation of the overall IGF-IR phosphorylation level (18) and the propagation of downstream signaling events (19). Accordingly, ligand occupancy of αβ3 integrin results in phosphorylation of the β3 integrin subunit, which leads to Downstream of tyrosine kinase 1 (DOK1)-mediated recruitment of SHP-2 (20). Blocking ligand occupancy of αβ3 integrin inhibited IGF-I-dependent downstream signaling events, membrane recruitment of SHP-2, and cell migration and proliferation (21, 22). Expression of a dominant negative mutant of the β3 integrin subunit in smooth muscle cells completely abolished the mitogenic activity of IGF-I (16). Thus, integrin activation may have a permissive action in the IGF-IR signaling pathway.

Integrins, which consist of a large family of heterodimers of α- and β-subunits, function as cell surface adhesion receptors for extracellular matrices (23) and link extracellular matrix components with various intracellular signaling mechanisms (24). It is believed that mechanical strains and shear stresses are distributed to cells through extracellular matrix scaffolds that hold the cells together and that mechanical signals that propagate from the extracellular matrix converge on integrins (25). The interaction between specific bone matrix ligands and corresponding integrin receptors has been suggested to be involved in the signal transduction process linking the extracellular mechanical signals to changes in gene expression, cytoskeletal reorganization, and DNA synthesis in osteoblasts and/or osteocytes (26). Specific antibodies for several integrins blocked mechanical strain-induced cellular responses (27). The integrin-β-catenin signal pathway has also been suggested to be involved in the cellular responses of human articular chondrocytes to mechanical stimulation (28). Thus, integrin activation has an important role in the transduction of mechanical signals. Consequently, we postulate that the integrin-dependent regulation of the IGF-I mitogenic signaling pathway could, in part, be involved in the mechanical stimulation of bone formation.

This study investigated the potential relationship between the signaling mechanism of mechanical stimulation of osteoblast proliferation and that of IGF-I-induced osteoblast proliferation by testing two hypotheses: 1) increased mechanical strain in the form of fluid shear stress could synergistically enhance the osteogenic action of IGF-I, and 2) the synergy between IGF-I and fluid shear stress involves the integrin-dependent up-regulation of IGF-IR phosphorylation through an inhibition of SHP-mediated IGF-IR dephosphorylation.

EXPERIMENTAL PROCEDURES

Materials—Tissue culture plasticware was obtained from Falcon (Oxnard, CA). Dulbecco’s modified Eagle’s medium was from Mediatech (Herndon, VA). Bovine calf serum was purchased from HyClone (Logan, UT). Trypsin and EDTA were products of Irvine Scientific (Santa Ana, CA). Bovine serum albumin was from United States Biochemical Corp. (Cleveland, OH). [3H]Thymidine (48 Ci/mmol) and [125I]NaI (2,215 Ci/mmol) were from ICN Biochemicals (Irvine, CA). Recombinant human IGF-I (or FGF-2) for 24 h, and [3H]Thymidine (1.5 μCi/ml) was added during the final 6 h of the incubation. Effects of a 2-h pretreatment with U0126 (10 μM) or a 24-h pretreatment with a disintegrin, ecastatin (100 nm), on shear stress and/or IGF-I-induced cell proliferation were also tested.

Western Immunoblots Analyses and Immunoprecipitation—Immediately following the 30-min shear stress and 10-min IGF-I treatments, the treated cells and corresponding controls were washed with phosphate-buffered saline and lysed in radioligand precipitation assay buffer as described previously (3). The protein concentration of each extract was assayed with the bicinchoninic acid method. Ten μg of extract protein from each extract was loaded onto 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membrane for Western immunoblot analysis. Erk1/2 activation was assessed by pErk1/2 level using the anti-pErk1/2 antibody normalized against the total Erk1/2 level. The pIGF-IR level was determined with an antibody against pIGF-IR, normalized against the level of total IGF-IR.

The relative level of IGF-IR-bound SHP-1 and -2 was each measured by co-immunoprecipitation followed by Western immunoblot analyses. Briefly, 1 mg of cell extract protein each from treated cells and corresponding controls was incubated with 2 μg of anti-IGF-IR or anti-integrin β3 antibodies for 2 h at 4 °C. A predetermined amount of anti-rabbit IgG beads (eBiosciences, San Diego, CA) was added for an additional 1 h at 4 °C. The bead-bound complex was washed three times with ice-cold lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 10 mg/ml phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 1 mM sodium orthovanadate). The washed complex was then resuspended in 40 μl of SDS sample buffer and boiled for 5 min. The relative amount of co-immunoprecipitated SHP-1 or SHP-2 were analyzed by Western analysis using anti-SHP-1 or anti-SHP-2 antibodies, respectively.

IGF-IR Binding Assays—Specific IGF-I binding to IGF-IR was measured by receptor-bound [125I]IGF-I in the presence of 100-fold “cold” IGF-I. Radio-lodination of IGF-I was performed by a modified chloramine T method (30). Aliquots were immediately stored at −70 °C until analysis. Assays were performed within 1 week of iodination. For the IGF-IR binding assay, TE85 cells were plated on glass slides and subjected to fluid shear stress as described above. Immediately after the shear stress, the treated and corresponding static control cells were rinsed with Dulbecco’s modified Eagle’s medium containing 20 mM HEPES, pH 7.4, and 1 mg/ml bovine serum albumin (binding medium). Fresh binding medium was then added and the cells were incubated at 37 °C for 2 h. Samples were performed within 1 week of iodination. The relative amount of bound [125I]labeled IGF-I in the absence or presence of 25–100 ng (i.e. 100-fold) of unlabeled IGF-I for total and specific binding, respectively. The cells were incubated at room temperature for 3 h, and the radioactive medium was removed and the slides rinsed five times with ice-cold binding medium. The cells were then lysed in the lysis buffer (10 mM Tris-HCl, pH 7.4, 5 mM EDTA, and 0.2% SDS). The amount of bound [125I]-labeled IGF-I was then quantified by γ-counter.

Statistical Analyses—Results are shown as mean ± S.D. with at least six replicates. The statistical significance of the differences between independent groups was determined with the two-tailed Student’s t test. The dose-dependent effects were assessed by one-way ANOVA, followed by Tukey post-hoc test. Interactions between two treatments (e.g. shear stress and IGF-I) were evaluated by two-way ANOVA. The difference was considered significant when p < 0.05.

RESULTS

Effects of Fluid Shear Stress on the Bone Cell Mitogenic Action of IGF-I in TE85 Cells—IGF-I at 10–50 ng/ml concentrations significantly and dose-dependently (p < 0.01, one-way ANOVA) increased the proliferation (i.e. [3H]thymidine incorporation) of TE85 cells by ~1.5–2.5-fold (Fig. 1A). The 30-min steady shear stress of 20 dynes/cm² also significantly (p < 0.05) increased [3H]thymidine incorporation in TE85 cells by 70% compared with the corresponding static control cells. The combination of the 30-min shear stress and IGF-I treatment produced much greater than additive stimulations (3.5–5.5-fold) of each treatment alone (Fig. 1A). Two-way ANOVA indicates a highly significant (p < 0.01) interaction between the two treatments, suggesting a synergistic interaction between shear
stress and IGF-I on bone cell proliferation.

To test whether the synergistic interaction between shear stress and IGF-I on human bone cell proliferation is a general feature between bone cell growth factors and shear stress, we evaluated whether shear stress would also synergistically enhance the mitogenic activity of FGF-2 (another potent bone cell growth factor) in TE85 cells. Fig. 1A shows that FGF-2 alone significantly and dose-dependently (p < 0.01) stimulated the TE85 cell proliferation (by ~1.5–2.0-fold). The combined treatment of the shear stress and FGF-2 yielded no further enhancement (not significant, two-way ANOVA) than FGF-2 alone, indicating that the synergistic interaction between shear stress and IGF-I is not universal to all bone growth factors.

Effects of Fluid Shear Stress on the IGF-I-mediated Activation of the Erk1/2 Mitogenic Signaling Pathway in Human TE85 Cells—Because the mitogenic action of IGF-I is mediated through Erk1/2 activation and fluid shear stress also activates Erk1/2 in osteoblasts (3, 10), we investigated the effect of shear stress and/or IGF-I (or FGF-2) on Erk1/2 phosphorylation (an index of Erk1/2 activation). Fig. 2A confirms that IGF-I alone, at the test doses, significantly and dose-dependently (p < 0.01, one-way ANOVA) increased the pErk1/2 level (by ~1.2–5-fold) in TE85 cells. The 30-min steady shear stress alone also significantly (p < 0.01) increased the pErk1/2 level (by ~2.5-fold).

The combination of shear stress and IGF-I (treatment produced a synergistic (p < 0.01, two-way ANOVA) enhancement (up to 12-fold) in Erk1/2 phosphorylation. Fig. 2B indicates that the mitogenic doses of FGF-2 (0.1 and 1 ng/ml) alone also markedly and significantly increased the pErk1/2 levels in TE85 cells (p < 0.01, one-way ANOVA). In contrast to IGF-I, the combination treatment of shear stress and FGF-2 did not result in a further increase in the pErk1/2 level compared with the FGF-2 treatment alone (not significant, two-way ANOVA). These findings further support the conclusions that the synergistic interaction between shear stress and IGF-I on bone cell proliferation is mediated through synergistic enhancement of IGF-I-dependent activation of the Erk1/2 mitogenic signaling pathway and that the synergy between shear stress and IGF-I on human bone cell proliferation is not shared by FGF-2.

To further evaluate whether activation of the Erk1/2 mitogenic signaling pathway is essential for the synergy, we tested the effect of U0126 (a specific inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1) on the stimulation of cell proliferation and Erk1/2 phosphorylation induced by IGF-I with or without the shear stress. Fig. 3A shows that pretreatment with U0126 at 10 μM completely blocked the IGF-I-mediated as well as the shear stress-induced TE85 cell proliferation. It also completely abolished the synergistic enhancement of IGF-I and shear stress. Fig. 3B reveals that the U0126 pretreatment also completely eliminated the synergistic enhancement on Erk1/2 activation by shear stress and IGF-I. Thus, these results are consistent with the conclusion that the synergistic activation of Erk1/2 by IGF-I and shear stress is associated with the synergistic enhancement on osteoblast proliferation. These findings indicate that the synergy between shear stress and IGF-I leading to activation of bone cell proliferation occurs upstream to the Erk1/2 activation. Consistent with previous findings (3, 10), U0216 had no inhibitory effect on either basal proliferation or basal Erk1/2 activation, indicating that basal TE85 cell proliferation is mediated primarily through Erk1/2-independent pathways.

Effects of Fluid Shear Stress on the Specific Binding of IGF-I to IGF-IR in TE85 Cells—We next tested whether the synergy between IGF-I and shear stress occurs prior to or after the phosphorylation of IGF-IR receptor. As expected, IGF-I at the test mitogenic doses significantly increased the IGF-IR phosphorylation level in a dose-dependent manner by 2–3.5-fold (Fig. 4). The 30-min steady shear stress alone also significantly (p < 0.01) increased the IGF-IR phosphorylation by 2.5-fold. The combination treatment of shear stress and IGF-I yielded a highly significant synergistic (p < 0.01, two-way ANOVA) enhancement in IGF-IR phosphorylation level (up to 8-fold).

Effects of Echistatin on the IGF-I- and/or Shear Stress-induced Proliferation of TE85 Cells—Because shear stress synergistically enhanced IGF-IR phosphorylation, which is initiated by the binding of IGF-I to IGF-IR, we next assessed whether the synergistic enhancement between IGF-I and shear stress was because of an increase in IGF-I binding to IGF-IR. Fig. 5 shows that the application of a 30-min fluid shear stress at 20 dyne/cm² led to a relatively small, but statistically significant (p < 0.05, one-way ANOVA) enhancement in the specific binding of IGF-I to IGF-IR in TE85 cells. However, this increase appeared to be of additive nature, as the two binding curves (i.e., with or without shear stress) were parallel to each other.

Effects of Echistatin on the IGF-I- and/or Shear Stress-induced Proliferation of TE85 Cells—Because shear stress involves integrin activation in bone cells (3, 26, 27), we evaluated whether integrin activation is involved in the synergy between IGF-I and shear stress in TE85 cells by determining the effect of the disintegrin echistatin (a competitive integrin receptor antagonist) on IGF-I- and/or shear stress-mediated cell proliferation and IGF-IR phosphorylation. Fig. 6A shows that echistatin, not only reduced the basal (by ~60%) and shear stress-induced TE85 cell proliferation (by ~20%), but also com-
pletely abolished the increase in cell proliferation induced by IGF-I alone as well as that by the combination treatment. Similarly, echistatin also completely abolished the basal, shear-stress, or IGF-I-induced IGF-IR phosphorylation (Fig. 6B). These findings suggest that the synergy between IGF-I and shear stress but not between FGF-2 and shear stress on $[^{3}H]$thymidine incorporation. C, control; St, stressed; Pan-Erk, the anti-pan Erk antibody recognized all forms of Erks.

**DISCUSSION**

Mechanical loading is essential and required for normal bone physiology. Defective cellular responses to mechanical loading has been implicated as the etiology and progression of a number of musculoskeletal diseases, including disuse osteoporosis, senile osteoporosis, and osteoarthritis (32, 33). Appropriate
mechanical loading is also required for fracture healing (34). Thus, it is not surprising that the molecular mechanism of this important physiological process to regulate bone formation is complex and involves multiple interacting signal transduction pathways (3, 35). Information about the nature and molecular mechanism of the interaction among these various pathways should provide, not only a better understanding of the mechanical regulation of bone formation, but also important insights into the etiology of various musculoskeletal diseases. In this regard, this investigation addresses the potential mechanism of a cross-talk between the IGF-I and integrin signaling pathways in the stimulation of bone cell proliferation in response to a steady shear stress.

In this study, we demonstrated for the first time that a 30-min steady fluid shear stress of 20 dynes/cm² in human TE85 osteosarcoma cells enhanced synergistically the mitogenic action of IGF-I through an up-regulation of the Erk1/2-mediated IGF-I mitogenic signaling pathway. Our findings that the disintegrin echistatin completely abolished the synergy on IGF-IR and bone cell proliferation raise the strong possibility that the synergy between shear stress and IGF-I on bone cell proliferation involves integrin activation. Bikle and co-workers (12, 13) have recently reported that skeletal unloading by hind limb suspension induced a resistance to IGF-I with respect to bone formation in the rat. They also concluded that unloading-induced resistance to IGF-I was caused by inhibition of the IGF-I signaling pathway through down-regulation of the integrin pathway. This conclusion was based on the findings that 1) skeletal unloading down-regulated integrin expression and blocked the ability of IGF-I to stimulate cell proliferation in osteoblasts and 2) echistatin also blocked the IGF-I-mediated stimulation of bone cell proliferation in vitro (13). Consistent with the results of Bikle and co-workers, our findings demonstrated that shear stress interacts synergistically with the IGF-I signaling pathway to promote bone cell proliferation and that this interaction involves integrin β3 signaling. Consequently, it appears that mechanical loading not only plays a permissive role in the osteogenic actions of IGF-I, but also...
interacts synergistically with the IGF-I signaling pathway to promote bone formation.

The conclusion that the loading-mediated activation of integrin signaling pathways may not only allow the IGF-I signaling pathway to function (16–22) but also cross-talks with the IGF-I signaling pathway to synergistically enhance the mitogenic effects of IGF-I in bone cells is consistent with the findings of several previous studies in fibroblasts (36–39) and smooth muscle cells (16–22), showing that integrin activation has an essential regulatory role in the mediation of the signal transduction pathways and cellular responses of a number of growth factors, including platelet-derived growth factor, epidermal growth factor, FGF-2, and IGF-I. Consistent with an important role for integrin signaling pathways in mediating mechanical stimulation of bone cell proliferation and activity (23–28, 40), we found synergy between the shear stress and IGF-I on bone cell proliferation and the IGF-IR-Erk1/2 signaling pathway. In contrast to fibroblasts, which show an enhancing interaction between FGF-2 and integrin activation (36, 39), our study did not find a synergistic enhancement of shear stress on the bone cell mitogenic activity of FGF-2 and FGF-2-mediated stimulation of Erk1/2 activation in TE85 cells. This would suggest that the synergistic interaction between IGF-I and shear stress is not shared by FGF-2 in osteoblastic cells. Future work is needed to confirm whether similar synergy occurs between shear stress and platelet-derived growth factor or epidermal growth factor in bone cells to determine whether the synergy is unique to IGF-I in bone cells.

Bikle and co-workers (13) have also reported that mechanical unloading markedly diminished the ability of IGF-I to activate several members of its mitogenic signaling pathway (i.e. IGF-IR, Ras, Erk1/2) in osteoblasts. Accordingly, we found that shear stress potentiates the IGF-I-mediated Erk1/2 activation. To gain insights into the molecular mechanism whereby shear stress interacts with the IGF-I signaling pathway to promote osteoblast proliferation, we examined whether synergy between shear stress and IGF-I also occurred at Erk1/2 activation and IGF-IR phosphorylation, two important steps of the IGF-I mitogenic signaling pathway. We reasoned that if the point of interaction (i.e. cross-talk) occurs prior to a given step in a pathway, a synergy would be evident at and after that particular step of the pathway. Conversely, if the point of interaction happens after a given step, no synergy would be expected at or prior to that given step. Accordingly, our findings that shear stress also synergized with IGF-I on Erk1/2 activation and IGF-IR phosphorylation strongly suggest that the synergy between shear stress and IGF-I to promote bone cell proliferation occurs prior to or at the step of IGF-IR phosphorylation.

Although Bikle and co-workers (13) report that skeletal unloading or the blocking of integrin activation by echistatin has no effect on the binding of IGF-I to IGF-IR in osteoblasts, our study showed that the shear stress slightly but significantly enhanced the IGF-I binding to IGF-IR. However, the increase in ligand binding was too small to explain the large synergic enhancement of shear stress in the stimulatory action of IGF-I on IGF-IR phosphorylation (8-fold), Erk1/2 activation (12-fold), and cell proliferation (5-fold). In addition, we noted that the ligand binding curves in the absence or presence of the shear stress were parallel to each other. This would argue against a synergy between shear stress and IGF-I on IGF-IR ligand binding.

The relatively low amounts of receptor-bound IGF-I (i.e. <2%) compared with the total amounts of added IGF-I, presumably because of the fact that bone cells (including TE85...
cells) release a large amount of IGF binding proteins (41) that compete with IGF-IR for IGF-I binding, has precluded an accurate determination of receptor number and/or binding affinity by Scatchard analysis. Thus, it is not known whether the small increase in IGF-I binding in response to shear stress was because of an increase in the number of IGF-IR or to an increase in ligand binding affinity. However, the parallel binding curves in the absence or presence of shear stress suggest that the increase in IGF-I binding could be due to a small increase in IGF-IR number. The reason for shear stress to increase the IGF-IR number is not clear, but the main point of the concept of our work is that the synergy between shear stress and IGF-I in the stimulation of bone cell proliferation occurs after the ligand binding, but prior to or at the step of IGF-IR phosphorylation, and that activation of the integrin signaling is essential for the synergy.

Recent studies from Clemmons and co-workers (16–22) in smooth muscle cells have disclosed important information about the nature of the cross-talk between the integrin signaling pathway and the IGF-I signaling pathway. Specifically, they found that activated integrin β3 serves to recruit SHP-2 from the cytosol and subsequently to transfer SHP-2 to SHPS-1 and IGF-IR for activating and terminating, respectively, the IGF-I signaling pathway. These findings have provided the basis that integrin activation is potentially relevant to the molecular mechanism whereby shear stress interacts with IGF-I to enhance the IGF-I signaling mechanism in bone cells. The IGF-I signaling pathway is initiated, not only by IGF-IR autophosphorylation induced by ligand binding, but also by the recruitment of activated SHP-2 to SHPS-1 from activated integrins. The transfer of activated SHP-2 to IGF-IR is responsible for the dephosphorylation of IGF-IR and the termination of the IGF-I signaling pathway (16–22). Accordingly, we postulate that mechanical strains or shear stresses, which activate the integrin signaling pathways in bone cells, enhance SHP-2 recruitment to activated integrins and also to SHPS-1. At the same time, the integrin activation in response to shear stresses inhibits the transfer of activated SHP-2 to IGF-IR, resulting in a reduction in the dephosphorylation of IGF-IR and an overall increase in IGF-IR phosphorylation level. This model would explain the synergy between shear stress and IGF-I on the IGF-I receptor phosphorylation level, Erk1/2 activation, and bone cell proliferation.

Our findings that shear stress, IGF-I, and the combination treatment each increased the relative amount of SHP-2 that was associated with integrin β3 and that each also reduced the relative amount of SHP-2 co-immunoprecipitated with IGF-IR in TES5 cells are consistent with our hypothesis that the shear stress-mediated recruitment of SHP-2 to activated integrins and away from IGF-IR may be responsible for the synergy between shear stress and IGF-IR to promote bone cell proliferation. It should be noted that the IGF-I-mediated and shear stress-mediated recruitment of SHP-2 to integrin β3 induced by IGF-I and/or shear stress was relatively low. More importantly, we found that shear stress, IGF-I, and the combination treatment each also increased the relative amount of the integrin β3-associated SHP-1 and reduced the relative amount of IGF-IR-associated SHP-1. This suggests that SHP-2 and the related SHP-1 are both involved in the IGF-I signaling mechanism as well as in the synergy between shear stress and IGF-I in enhancing the overall IGF-IR phosphorylation level.

The effect of unloading on recruitment of SHP-2 (or SHP-1) to integrins has not been assessed previously (13). Thus, it is unclear at this time whether or not the unloading-induced resistance to IGF-I may also involve a reduction of SHP-2 (and/or SHP-1) recruitment to integrins. However, because unloading down-regulated integrin expression in osteoblasts (13) and because SHP-2 recruitment to integrins is essential for IGF-I signaling (16–22), it is likely that the reduced integrin recruitment of SHP-2 and/or SHP-1 in response to unloading-mediated down-regulation of the integrin pathway could also play a pivotal role in the permissive effect of mechanical loading on the IGF-I anabolic action in bone (12, 13).

In conclusion, this study provides the first evidence for a synergistic interaction between shear stress and IGF-I in the stimulation of osteoblastic proliferation. This study also provides strong circumstantial evidence that the synergy involves an integrin-dependent up-regulation of IGF-IR phosphorylation level through an inhibition of the recruitment of SHP-1 and/or SHP-2 to IGF-IR as well as an inhibition of the SHP-1 and/or SHP-2-mediated IGF-I dephosphorylation. These findings not only confirm that the integrin activation is essential for the IGF-I mitogenic pathway, but also provide mechanistic insights into the cross-talk between the integrin and IGF-I signaling pathways in the underlying molecular mechanisms of enhanced bone formation in response to mechanical loading.

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Sonia Kapur, Subburaman Mohan, David J. Baylink and K.-H. William Lau

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