Journal of Cellular Biochemistry 56:323–330 (1994)

Blockade of Osteoclast-Mediated Bone Resorption Through Occupancy of the Integrin Receptor: A Potential Approach to the Therapy of Osteoporosis

Rivka Dresner-Pollak and Michael Rosenblatt

Divisions of Bone and Mineral Metabolism (R.D.-P., M.R.) and Gerontology (R.D.-P.), Thorndike and Charles A. Dana Laboratories, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215

Abstract Bone resorption requires the tight attachment of the bone-resorbing cells, the osteoclasts, to the bone mineralized matrix. Integrins, a class of cell surface adhesion glycoproteins, play a key role in the attachment process. Most integrins bind to their ligands via the arginyl-glycyl-aspartyl (R-G-D) tripeptide present within the ligand sequence. The interaction between integrins and ligands results in bidirectional transfer of signals across the plasma membrane. Tyrosine phosphorylation occurs within cells as a result of integrin binding to ligands and probably plays a role in the formation of the osteoclast clear zone, a specialized region of the osteoclast membrane maintained by cytoskeletal structure and involved in bone resorption.

Human osteoclasts express $\alpha_2\beta_1$ and $\alpha_v\beta_3$ integrins on their surface. Such signaling may also lead to "inside-out" effects, like increased expression of integrin receptors on the cell surface, or increased affinity of the integrin to its ligand. The $\alpha_v\beta_3$ integrin, a vitronectin receptor, plays an essential role in bone resorption. Antibodies to this integrin and short synthetic RGD-containing peptides are able to block bone resorption in vitro. Echistatin, an RGD-containing protein from a snake venom, binds to the $\alpha_v\beta_3$ integrin and blocks bone resorption both in vitro and in vivo. Peptides containing the RGD motif are potential competitive "antagonists" of the osteoclast integrins and may have utility in the blockade of bone resorption. Agonists may be identified by stimulation of intracellular signaling. In theory, tissue specificity can be achieved by 1) introducing specific amino acids in positions adjacent to the RGD sequence, 2) identifying non-RGD integrin binding domains, or 3) modulating the affinity of integrins for their endogenous ligands.

Key words: osteoclast, bone resorption, integrins, RGD-containing peptides

Bone resorption is carried out by osteoclasts, multinucleated cells of the monocyte/macrophage lineage. The process requires migration of osteoclasts to the future site of resorption and the subsequent formation of a tightly sealed compartment between the osteoclast convoluted basal membrane (the "ruffled border") and the mineralized bone matrix which demarcates an area of underlying bone to be resorbed. This compartment functions as an "extracellular lysosome" into which acid and enzymes are secreted by the osteoclast (Fig. 1). The acid solubilizes the mineral, allowing the proteolytic enzymes to digest the matrix proteins. After attachment of the osteoclast to the mineralized bone surface, the interfacial region of membrane forms not only the ruffled border but also the "clear zone," an organelle-free annular cytoplasmic area which is enriched in the contractile proteins vinculin and talin. The clear zone is thought to be the site of osteoclast attachment and tight-sealing to the bone surface.

Although the mechanism and the signal for the selection of the site of attachment are not known yet, it has become evident in the last few years that integrins, cell surface adhesion receptors, play a role in this process. The integrin superfamily of adhesion molecules were discovered a decade ago. The term integrin was coined to describe their presumed role in integrating the intracellular cytoskeleton with the extracellular matrix. Adhesion is of fundamental importance to a cell; it provides anchorage, cues for

Received April 19, 1994; accepted April 22, 1994.

Address reprint requests to Dr. Michael Rosenblatt, Division of Bone and Mineral Metabolism, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215.

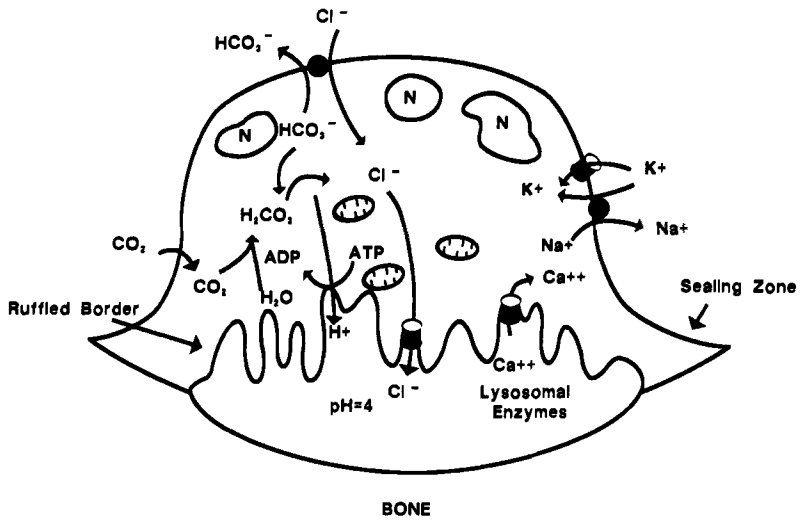

Fig. 1. Schematic of osteoclast structure and function. The drawing shows multiple nuclei, the acid-forming apparatus, calcium and chloride channels, the sealing zone, and the ruffled border.

migration and signals for growth and differentiation. The importance of integrins in physiology is being rapidly recognized. Integrins play a role in platelet aggregation, immune function, and tumor invasion. The potential therapeutic utility of interfering with integrin-mediated events is illustrated in vivo by the inhibition of platelet-dependent coronary thrombus formation, or arrest of growth of malignant metastases by soluble integrin ligands or anti-integrin antibodies. Research in the above-listed areas is far advanced relative to studies of the role of integrins in bone biology.

INTEGRINS: GENERAL BACKGROUND

Integrins, a family of cell surface heterodimeric glycoproteins, are the primary mediators of cell-to-extracellular matrix adhesion (Fig. 2). All integrins are membranal receptors composed of a single α subunit and a single β subunit which are non-covalently linked [Hynes, 1992]. To date, 14 different α subunits and eight β subunits have been identified and sequenced at the cDNA level. Both integrin subunits have a single transmembrane and a short cytoplasmic domain, which lack conventionally recognized motifs for stimulation of kinase or phosphatase activities, for forming ion channels, or for acting through G-proteins. Thus, integrin structure per se does not predict intracellular signaling. However, increasing evidence indicates that the interaction between integrin and ligand does result in signal transduction and a series of intracellular events, including cytosolic alkalinization, increase in cytosolic calcium levels, tyrosine phosphorylation and dephosphorylation in proteins, early gene expression, and changes in the mechanical properties of the cell [Hynes, 1992; Wang et al., 1993]

Most integrins are not cell specific; they are expressed on more than one cell type. An exception is the $GPII_b/III_a$, a fibrinogen receptor, which is expressed solely on platelets. In addition, most cells express several integrins, and the profile of integrin display can be modulated by growth factors. Furthermore, individual types of integrins can often bind more than one ligand, and a given ligand is often recognized by more than one integrin. This overlap presents a challenge in targeting for therapeutic blockade a given integrin on a given cell. Fortunately for our objectives, the $\alpha_v\beta_3$ integrin displays a narrow distribution across cell types other than osteoclasts; it is expressed on platelets and endothelial cells. It binds vitronectin, fibrinogen, osteopontin, thrombospondin, von Willebrand's factor, and bone sialoprotein.

Most integrins bind ligands which contain an internal arginyl-glycyl-aspartyl (RGD) sequence. This three amino-acid motif is found in various extracellular matrix proteins, including fibronectin, laminin, vitronectin, fibrinogen, von Willebrand's factor, and osteopontin. Short synthetic peptides containing the RGD sequence can mimic the biological activity of the intact ligand and competitively inhibit cell adhesion to native ligands [Ruoslahti et al., 1987]. The essential role of the RGD motif for cell attachment

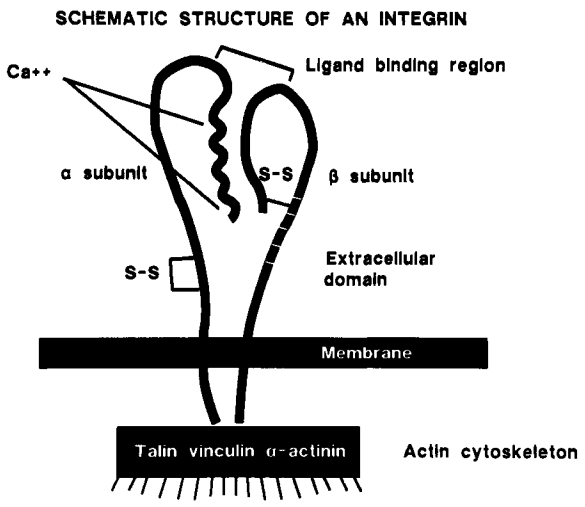

Fig. 2. Schematic structure of an integrin. Integrins are composed of two noncovalently associated subunits designated α and β. Both subunits are integral membrane glycoproteins. The extracellular domains contain the ligand-binding region. The α subunit contains areas thought to bind calcium (Ca^{++}). The cytoplasmic domains of both subunits are relatively small and contain regions capable of binding cytoskeletal elements that link the integrins to the actin cytoskeleton.

was demonstrated by site-directed mutagenesis of the RGD motif in fibronectin and vitronectin to RGE (Arg-Gly-Glu), a conservative substitution which nevertheless resulted in the complete loss of cell attachment activity for these matrix proteins [Obara et al., 1988; Cherny et al., 1993]. Although the RGD motif represents a common recognition sequence in many adhesion proteins, other non RGD-containing cell-binding domains have been identified. For example, the sequences D-G-E-A in collagen type 1 [Staatz et al., 1991], L-D-V in alternatively spliced fibronectin [Humphries et al., 1986], and K-Q-A-G-D in the γ-chain of fibrinogen [Ginsberg et al., 1988] have been shown to competitively inhibit RGD-mediated cell attachment. Recently, a basic sequence in the viral protein *tat,* K-K-Q-R-R-R, and a related sequence, H-R-N-R-K-G-V, present in vitronectin, were found to bind to $\alpha_v\beta_3$ [Vogel et al., 1993]. Immobilized peptides bearing these sequences serve as cell attachment sites. The role (if any) of these non-RGD containing binding sequences, which appear in matrix proteins bearing the RGD motif, has not been completely elucidated. These auxiliary integrin-binding domains may play a role in modulating the affinity and specificity of the interaction between the integrin and the native ligand. They may also operate additively, or even synergistically, with the RGD motif in functions related to cell attachment or intracellular signalling.

Integrins not only stimulate signal transduction causing a cascade of intracellular events, but intracellular signals have been demonstrated, in turn, to modulate integrin function. This concept of "inside-out" signal transduction is illustrated in the platelet and polymorphonuclear cell. In circulation, the $GPII_b/III_a$ on the surface of the resting platelet does not bind to its ligand, soluble fibrinogen. However, when platelets are activated by an appropriate agonist, such as ADP, the affinity of the $GPII_b/III_a$ is markedly increased, probably due to a conformational change in the integrin. This mechanism of integrin activation may play a role in selectivity and specificity of integrin function.

INTEGRINS AND OSTEOCLAST-MEDIATED BONE RESORPTION

Osteoclasts express $\alpha_2\beta_1$ and $\alpha_v\beta_3$ integrins [Hughes et al., 1993]. $\alpha_2\beta_1$ associates preferentially with collagen, while the $\alpha_v\beta_3$ (vitronectin-type) receptor is relatively non-selective and binds vitronectin, fibronectin, fibrinogen, and von Willebrand's factor. Human osteoclastoma-derived osteoclasts, as well as rat and chicken osteoclasts, display abundant $\alpha_v\beta_3$ receptors on their surface [Zambonin-Zallone et al., 1989]. Conflicting data have been published as to the presence of the vitronectin receptor in the clear zone at the tip of osteoclast podosomes. The clear zone contains a high concentration of microfilaments in a specific arrangement consisting of a central axis of F-actin, surrounded by vinculin, α-actinin and talin. Zambonin-Zallone and colleagues [1989] have demonstrated the accumulation of a protein in the region of the podosomes which is immunoreactive with antibodies to β_3 integrin. Moreover, a role for $\alpha_v\beta_3$ in the tight attachment of the osteoclast to bone has been suggested by Reinholt et al. [1990] and Sato et al. [1990], who demonstrated co-localization at the osteoclast clear zone of the α_v subunit and the cytoskeletal proteins talin, α-actinin and vinculin.

Other studies have yielded discrepant findings. Davis et al. [1989] have found no indication of the presence of β_3-containing integrins in podosomes and Lakkakorpi et al. [1991] localized $\alpha_v\beta_3$ not to the clear zone, but to the ruffled border and the basolateral membrane of the osteoclast. Lakkakorpi and colleagues suggest that the vitronectin receptor is physically ex-

cluded from the area of closest attachment to the bone and may be involved in some other function, such as initial attachment to bone and/or cell spreading before clear zone formation. It is also possible that the lack of integrin staining reported by some is artificial: the clear zone may not be penetrated by multimeric molecules such as antibodies which were used to visual the presence of these integrins.

An evolving hypothesis suggests that the $\alpha_v\beta_3$ integrin present on the osteoclast surface mediates the initial attachment of the osteoclast to the mineralized matrix. This interaction results in intracellular signaling and the phosphorylation of cytoskeletal proteins which induce cell shape changes and formation of the tight seal. There is circumstantial evidence suggesting that phosphorylation events may play an important role in the attachment of cells to their substrates (matrix) and may be involved in osteoclast activation. The transformation of fibroblasts with the src oncogene results in increased tyrosine phosphorylation of a number of cytoskeletal components, including two related proteins of 80 kD and 85 kD which colocalize with F-actin in the podosomes. Interestingly, transgenic mice in which the c-src gene was "knocked out," developed osteopetrosis, a phenotype resultant from impaired bone resorption. Osteoclasts were found have high levels of pp60c-src, comparable to the levels found in brain and platelets. Recent data indicate that pp60-src expression is not required for osteoclast formation but is required for their function, as evidenced by the observation that bone marrow cells from src-deficient animals are able to form multinucleated cells. However, these cells do not form ruffled borders or resorb bone. The absence of ruffled borders is probably due to defective phosphorylation of cytoskeletal proteins. Furthermore, other data suggest that two pp60c-src substrates, the cytoskeletal proteins cortactin (also called P80/85) and focal adhesion kinase (p125FAK) play a role in bone resorption. However, the exact cascade of intracellular events has not yet been elucidated (Fig. 3).

Although the different observations regarding the localization of the $\alpha_v\beta_3$ on the osteoclast surface mentioned above need to be reconciled, it is apparent that $\alpha_v\beta_3$ plays a key role in bone resorption. Antibodies to this integrin inhibit bone resorption by isolated osteoclasts in vitro [Horton et al., 1991; Ross et al., 1993]. Perhaps most convincing is the finding that ligands which bind to this integrin can block parathyroid hormone (PTH)-induced bone resorption in vivo.

INHIBITION OF OSTEOCLAST-MEDIATED BONE RESORPTION BY INTEGRIN ANTAGONISTS

The importance of the RGD motif to osteoclast-mediated bone resorption was first demon-

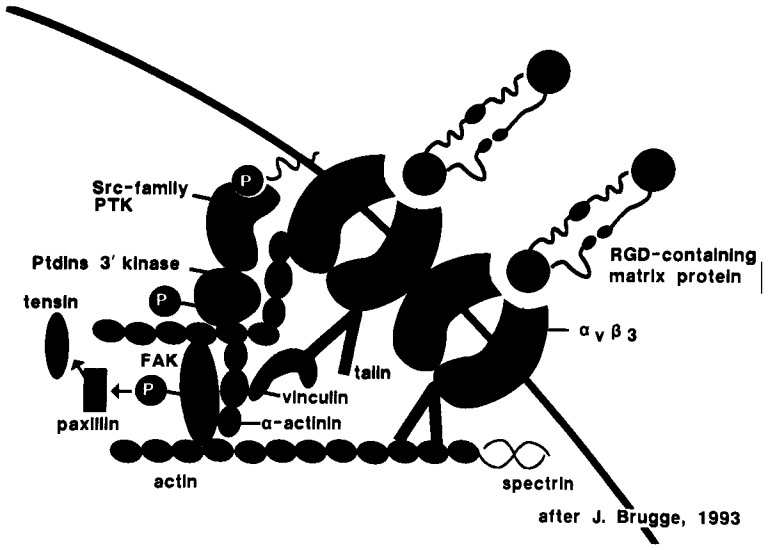

Fig. 3. Integrins and intracellular signaling: the cytoskeleton and protein tyrosine phosphorylation. The cytoplasmic domain of integrins is connected to the cytoskeleton which is composed of short actin filaments, spectrin, talin, vinculin, and actin-binding protein. Upon ligand binding to the extracellular domain of the integrin, redistribution of cytoskeletal components, and protein phosphorylation occur. The src-family of protein kinases is activated. Amongst phosphorylated proteins are cytoskeletal components like paxillin, a vinculin binding protein, and the protein tyrosine kinase focal adhesion kinase (FAK).

strated when short, synthetic RGD-containing peptides were shown to inhibit bone resorption in vitro by isolated osteoclasts [Horton et al., 1991]. The endogenous ligand within bone for the osteoclast $\alpha_v\beta_3$ integrin has not been established definitively. Various bone matrix proteins, such as osteopontin, bone sialoprotein, thrombospondin, collagen, and fibronectin, contain an RGD sequence and could serve as ligands for the osteoclast vitronectin-type receptor.

A role for osteopontin as an anchor within the bone matrix for osteoclast attachment has been postulated [Reinholt et al., 1990]. Osteopontin, a 357-amino acid glycoprotein, is biosynthesized by osteoblasts in their early stages of differentiation. There is also evidence that osteoclasts can synthesize osteopontin [Ikeda et al., 1992]. Electron microscopic immunohistochemistry studies of whole bone tissue have demonstrated an increased concentration of osteopontin in bone at a site opposite the clear zone of osteoclasts. In this locale, osteopontin and $\alpha_v\beta_3$ colocalize [Reinholt et al., 1990]. Recently, it has been demonstrated that RGD-containing peptides inhibit the attachment of isolated osteoclasts to osteopontin. In addition, a polyclonal antibody to osteopontin inhibits attachment of osteoclasts to whole bone particles [Reinholt et al., 1990].

Further insight into the role of RGD-binding adhesion molecules was obtained through study of the "disintegrins," a group of relatively small RGD-containing proteins, such as echistatin [Gan et al., 1988], bitistatin [Musial et al., 1990], kistrin [Yasuda et al., 1991], trigrammin [Huang et al., 1987], and applaggin [Savage et al., 1990], which are present in snake venoms. These proteins are potent inhibitors of the integrin-mediated aggregation of platelets, via the fibrinogen receptor (the integrin $GPII_b/III_a$) present on the platelet surface. Echistatin, a 49-amino acid RGD-containing protein purified from the venom of the viper *Echis carinatus,* was first found to be a highly potent inhibitor of bone resorption in vitro. Echistatin inhibited both excavation of bone by rat osteoclasts (IC_{50} = 0.1 nM) and [^{3}H]proline-release from prelabeled bone particles by chicken osteoclasts (IC_{50} = 100 nM). In addition, exposure of chicken osteoclasts to low concentrations (14 nM) of synthetic echistatin significantly reduced osteoclast attachment to bone particles [Sato et al., 1990]. Furthermore, lamellipodia retraction and subsequent detachment of rat osteoclasts from glass could be induced by approximately 4 nM echistatin. These activities are dependent upon the RGD domain: substitution of the arginine of the RGD sequence in echistatin with alanine, as in [Ala^{24}]echistatin, resulted in the loss of inhibitory effect on bone resorption [Sato et al., 1990]. Echistatin displays an IC_{50} in the nanomolar range, compared to the micromolar inhibitory potency of short RGD-containing peptides [Sato et al., 1990]. Immunochemistry has shown colocalization of echistatin with an α_v-like subunit at the osteoclast clear zone, suggesting that echistatin blocks bone resorption by interacting with the osteoclast functional integrin [Sato et al., 1990] and prevention or disruption of tight seal formation.

Although the $\alpha_v\beta_3$ integrin shares a common β subunit with the platelet fibrinogen receptor (GP IIb/IIIa), echistatin is approximately 300-fold more potent in producing its anti-bone resorbing effects than its anti-platelet aggregation effects [Sato et al., 1990]. Independent studies with another series of "disintegrin" proteins also indicate that relative selectivity for a specific integrin ($\alpha_v\beta_3$ vs. GPIIa/IIIb) can be achieved and may be encoded in the amino acids adjacently positioned to RGD [Scarborough et al., 1993]. These observations have extremely important implications since they indicate that it is possible to identify RGD-containing peptides with relative specificity for the $\alpha_v\beta_3$ receptor in bone; hence, some degree of tissue selectivity can be achieved.

The therapeutic potential of an integrin antagonist in the treatment of diseases associated with bone loss was first demonstrated in an acute in vivo study using PTH-stimulated, bone-dependent increases in serum calcium in the rat [Fisher et al., 1993]. In this model, the effects of the endogenous calciotropic peptide hormones, PTH and calcitonin, are eliminated by surgical removal of the thyroid and parathyroid glands (TPTX). Serum calcium levels fall to 50–70% of basal levels approximately 20 h after surgery and fasting. Then anesthetized animals are infused with either PTH or the combination of PTH and echistatin. Infusion of PTH was found to reverse the decline and restored serum calcium to pre-TPTX levels. Echistatin, when coinfused with PTH, completely blocks the hormone-induced serum calcium rise. Under these experimental conditions, the calcium mobilized by exogenous PTH has been determined to be of skeletal origin and the result of hormone-induced osteoclast activation [Thompson et al., 1988; Horiuchi et al., 1987]. The effect of echistatin was dependent on the RGD sequence:

when alanine was substituted for Arg^{24}, [Ala^{24}]echistatin was ineffective at blocking the PTH-dependent serum calcium increase.

The mechanism by which echistatin achieves blockade of bone resorption has not been definitively established. Extension of these in vivo studies provides direct evidence for osteoclast-mediated bone resorption in this calcium-mobilizing model based on histomorphometric analysis [Yamamoto et al., 1993]. Echistatin might act by one or more of several mechanisms: 1) disrupting the integrity of the osteoclast's tightly sealed extracellular compartment, thereby causing the escape or inactivation of lysosomal enzymes and acid (Fig. 4); 2) inhibiting osteoclast formation, recruitment, or de novo attachment to the bone surface, either at the time of initial attachment or subsequent formation of the tight-sealing zone [Sato et al., 1990; Reinholt et al., 1990; Lakkakorpi et al., 1991]; or 3) activating the $\alpha_v\beta_3$ integrin as a signal transducing receptor [Hynes, 1992]. Which of these, or other possible mechanisms, is responsible for the observed effects remains to be elucidated. These results, taken together with the in vitro data of Sato et al. [1990], suggest that echistatin interaction with the osteoclast $\alpha_v\beta_3$ integrin receptor reduces osteoclast activity.

The information cited above indicates an essential role for the RGD sequence in osteoclast-mediated bone resorption. The in vivo data serves as the impetus for identifying and designing shorter and more potent antagonists of the $\alpha_v\beta_3$ integrin. Until the last few years, progress in structure-activity studies for peptide ligands was limited by the number of peptides that could be prepared for biological evaluation. This constraint tends to be true particularly in the early stages of an analog design program, when an extensive, but empirical survey of peptide structure must be undertaken before clearer directions for analog design can be identified. Recently, it has been possible to generate collections of peptides which possess enormous numbers and diversity of peptides. Often, the array contains a wide spectrum of peptides, many of which differ from each other only in subtle, but potentially important, structural features. Novel methodologies have been devised for preparing large combinatorial peptide diversity libraries which include both natural and non-natural amino acids.

Once a critical functional domain within a peptide or protein sequence has been identified, the optimization of its structure in order to achieve selectivity, metabolic stability, and improved bioavailability become important goals in drug design. There are many instances in which modification of the native sequence of a ligand results in further improvement in affinity between the ligand and its macromolecular target. Specificity for a single target amongst a family of closely related structures can also be achieved with novel ligand analogs. This principal is illustrated in the platelet aggregation system where low molecular weight, selective, highly potent non-peptide antagonists of the fibrinogen receptor ($GPII_b/III_a$) were developed that mimic

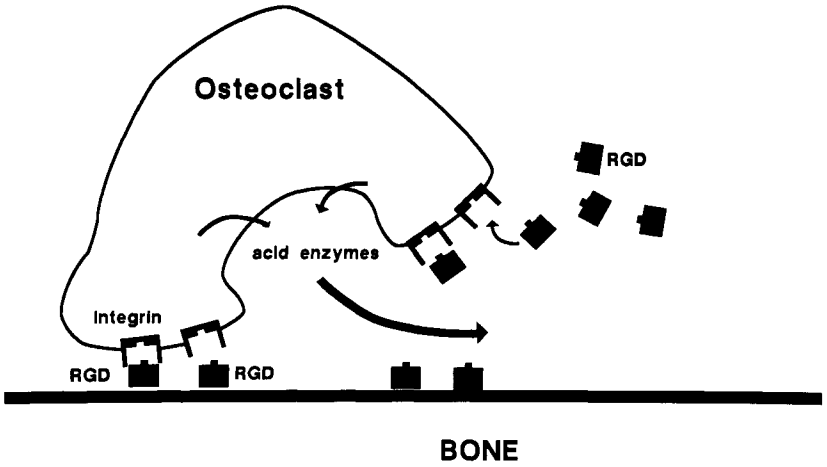

Fig. 4. Schematic model of the potential utility of RGD-containing peptides in the blockade of bone resorption. RGD-containing peptides compete with extracellular bone matrix proteins and occupy the osteoclast functional integrin $\alpha_v\beta_3$ leading to disruption of the tight seal between the osteoclast clear zone and the bone surface. This results in leakage of acid and proteolytic enzymes, which are essential in high concentrations for bone resorption.

the RGD sequence [Hartman et al., 1992]. In the case of extracellular matrix proteins, (e.g., vitronectin, fibrinogen, osteopontin, etc.) a range of affinity and selectivity for $\alpha_v\beta_3$ is displayed. Since these proteins share a common RGD motif, differences in binding properties most likely can be attributed to the amino acids flanking RGD. Recently we have been able to prepare such a library containing an estimated 360,000 peptides all of which contain RGD, but which vary in the sequences flanking the tripeptide motif. Availability of this and other libraries will facilitate efficient screening of large numbers of peptides based on their binding affinities and/or other functional properties. Constructing and screening combinatorial libraries of synthetic peptides based on defined endogenous sequences or random substitutions is likely to facilitate identification of osteoclast-specific integrin antagonists which may provide the leads for the development of a new class of inhibitors of bone resorption which will have clinical utility in osteoporosis and other related metabolic bone diseases.

Approaches to the treatment of osteoporosis have focused on restoring the balance between bone formation and resorption by either decreasing the overall rate of bone turnover through direct inhibition of bone resorption or stimulating bone formation. While promising agents such as the bisphosphonates, synthetic PTH analogs, and synthetic estrogen analogs are in clinical trials, currently available medications for the treatment and prevention of osteoporosis are few, their efficacy is limited and side-effects are problematic. These include estrogen, calcitonin, calcium and vitamin D. The efficacy of combination therapy composed of an anti-resorptive agent and a bone formation stimulator, such as PTH and estrogen, or bisphosphonate and estrogen, is currently being studied. Future research will reveal if osteoclast integrin antagonists may have synergistic or additive effects with any of the agents mentioned above.

Tissue specificity and toxicity are major concerns in drug design. Bone specific integrin antagonists may be developed by identifying specific amino acids adjacent to the RGD motif, non-RGD binding domains in bone matrix proteins, and by modulating the affinity of the osteoclast $\alpha_v\beta_3$ integrin for its endogenous ligand. Coagulopathy and bleeding secondary to interference with platelet aggregation is one of the potential risks associated with osteoclast integrin antagonists. Although this side-effect was not found in animals treated with echistatin for a number of hours, this potential side-effect of chronic administration should be further studied.

Finally, drug delivery remains an important issue for this line of investigation. Peptides are generally not bioavailable orally and usually require parenteral administration. However, orally active non-peptide fibrinogen ($GPII_b/III_a$) receptor antagonists have been designed. This precedent provides encouragement for those undertaking initiatives to block bone resorption and treat osteoporosis through use of integrin ($\alpha_v\beta_3$) antagonists.

ACKNOWLEDGMENTS

Rivka Dresner-Pollak M.D., is a recipient of the American Federation of Aging Research Award for 1993–1994.

REFERENCES

Cherny RC, Honan MA, Thiagarajan P (1993): Site-directed mutagenesis of the arginine-glycine-aspartic acid in vitronectin abolishes cell adhesion. J Biol Chem 268:9725–9729.

Davies J (1989): The osteoclast functional antigen, implicated in the regulation of bone resorption, is biochemically related to the vitronectin receptor. J Cell Biol 109:1817–1826.

Fisher JE, Caulfield MP, Sato M, Quartuccio HA, Gould RJ, Garsky VM, Rodan GA, Rosenblatt M (1993): Inhibition of osteoclastic bone resorption in vivo by echistatin, an "arginyl-glycyl-aspartyl" (RGD)-containing protein. Endocrinology 132:1411–1413.

Gan Z-R, Gould RJ, Jacobs JW, Friedman PA, Polokoff MA (1988): Echistatin: A potent platelet aggregation inhibitor from the venom of the viper, *Echis carinatus*. J Biol Chem 263:19827–19832.

Ginsberg MH, Loftus JC, Plow EF (1988): Cytoadhesions, integrins, and platelets. Thromb Haemost 59:1–6.

Hartman GD, Egbertson MS, Halczenko W, Laswell WL, Duggan ME, Smith RL, Naylor AM, Manno PD, Lynch RJ, Zhang G, Chang CT-C, Gould RJ (1992): Non-peptide fibrinogen receptor antagonists. 1. Discovery and design of exosite inhibitors. J Med Chem 35:4640–4642.

Horiuchi N, Caulfield MP, Fisher JE, Goldman ME, McKee RL, Reagan JE, Levy JJ, Nutt RF, Rodan SB, Schofield TL, Clemens TL, Rosenblatt M (1987): Similarity of synthetic peptide from human tumor to parathyroid hormone in vivo and in vitro. Science 238:1566–1568.

Horton MA, Taylor ML, Arnett TR, Helfrich MH (1991): Arg-Gly-Asp (RGD) peptides and the anti-vitronectin receptor antibody 23C6 inhibit dentine resorption and cell spreading by osteoclasts. Exp Cell Res 195:368–375.

Huang T-F, Holt JC, Lukasiewicz H, Niewiarowski S (1987): Trigramin: a low molecular weight peptide inhibiting fibrinogen interaction with platelet receptors expressed on glycoprotein IIb-IIIa complex. J Biol Chem 262:16157–16163.

Hughes DE, Salter DM, Dedhar S, Simpson R (1993): Integrin expression in human bone. J Bone Miner Res 8:527–533.

Humphries MJ, Akiyama SK, Komoriya A, Olden K, Yamada KM (1986): Identification of an alternatively spliced site in human plasma fibronectin that mediates cell type-specific adhesion. J Cell Biol 103:2637–2647.

Hynes RO (1992): Integrins: Versatility, modulation, and signaling in cell adhesion. Cell 69:11–25.

Ikeda T, Nomura S, Yamaguchi A, Suda T, Yoshiki S (1992): In situ hybridization of bone matrix proteins in undecalcified adult rat bone sections. J Histochem Cytochem 40: 1079–1088.

Lakkakorpi R, Horton MA, Helfrich MH, Karhukorpi E-K, Vaananen HK (1991): Vitronectin receptor has a role in bone resorption but does not mediate tight sealing zone attachment of osteoclasts to the bone surface. J Cell Biol 115:1179–1186.

Musial J, Niewiarowski S, Rucinski B, Stewart GJ, Cook JJ, Williams JA, Edmunds Jr. LH (1990): Inhibition of platelet adhesion to surfaces of extracorporeal circuits by disintegrins: RGD-containing peptides from viper venoms. Circulation 82:261–273.

Obara M, Kang MS, Yamada KM (1988): Site-directed mutagenesis of the cell-binding domain of human fibronectin: Separable, synergistic sites mediate adhesive function. Cell 53:649–657.

Reinholt FP, Hultenby K, Oldberg A, Heinegard D (1990): Osteopontin: a possible anchor of osteoclasts to bone. Proc Natl Acad Sci USA 87:4473–4475.

Ross FP, Chappel J, Alvarez JI, Sander D, Butler WT, Farach-Carson MC, Mintz KA, Gehron Robey P, Teitelbaum SL, Cheresh DA (1993): Interactions between the bone matrix proteins osteopontin and bone sialoprotein and the osteoclast integrin alpha$_v$beta$_3$ potentiate bone resorption. J Biol Chem 268:9901–9907.

Ruoslahti E, Pierschbacher MD (1987): New perspectives in cell adhesion: RGD and integrins. Science 238:491–497.

Sato M, Sardana MK, Grasser WA, Garsky VM, Murray JM, Gould RJ (1990): Echistatin is a potent inhibitor of bone resorption in culture. J Cell Biol 111:1713–1723.

Savage B, Marzec UM, Chao BH, Harker LA, Maraganore JM, Ruggeri ZM (1990): Binding of the snake venom-derived proteins applaggin and echistatin to the arginine-glycine-aspartic acid recognition site(s) on platelet glycoprotein IIb.IIIa complex inhibits receptor function. J Biol Chem 265:11766–11772.

Scarborough RM, Rose JW, Naughton MA, Phillips DR, Nannizzi L, Arfsten A, Campbell AM, Charo IF (1993): Characterization of the integrin specificities of disintegrins isolated from American pit viper venoms. J Biol Chem 268:1058–1065.

Staatz WD, Fok KF, Zutter MM, Adams SP, Rodriguez BA, Santoro SA (1991): Identification of a tetrapeptide recognition sequence for the alpha-2,beta-1 integrin in collagen. J Biol Chem 266:7363–7367.

Thompson DD, Seedor JG, Fisher JE, Rosenblatt M, Rodan GA (1988): Direct action of the parathyroid hormone-like human hypercalcemic factor on bone. Proc Natl Acad Sci USA 85:5673–5677.

Vogel BE, Lee S-J, Hildebrand A, Craig W, Pierschbacher MD, Wong-Staal F, Ruoslahti E (1993): A novel integrin specificity exemplified by binding of the alpha$_v$beta$_5$ integrin to the basic domain of the HIV tat protein and vitronectin. J Cell Biol 121:461–468.

Wang N, Butler JP, Ingber DE (1993): Mechanotransduction across the cell surface and through the cytoskeleton. Science 260:1124–1127.

Yamamoto M, Balena R, Markatos A, Gentile M, Garsky VM (1993): Direct demonstration of inhibitory effect of echistatin on PTH-induced bone resorption in vivo in mice. J Bone Miner Res 8:S123 (abstract).

Yasuda T, Gold HK, Leinbach RC, Yaoita H, Fallon JT, Guerrero L, Napiez MA, Bunting S, Collen D (1991): Kistrin, a polypeptide platelet GPIIb/IIIa receptor antagonist, enhances and sustains coronary arterial thrombolysis with recombinant tissue-type plasminogen activator in a canine preparation. Circulation 83:1038–1047.

Zambonin-Zallone A, Teti A, Grano M, Rubinacci A, Abbadini M, Gaboli M, Marchisio PC (1989): Immunocytochemical distribution of extracellular matrix receptors in human osteoclasts: a beta-3 integrin is colocalized with vinculin and talin in the podosomes of osteoclastoma giant cells. Exp Cell Res 182:645–652.