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Report

Bisphosphonates inhibit stromelysin-1 (MMP-3), matrix metalloelastase (MMP-12), collagenase-3 (MMP-13) and enamelysin (MMP-20), but not urokinase-type plasminogen activator, and diminish invasion and migration of human malignant and endothelial cell lines

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Bisphosphonates (clodronate, alendronate, pamidronate and zoledronate) at therapeutically attainable non-cytotoxic concentrations inhibited MMP-3, -12, -13 and -20 as well as MMP-1, -2, -8 and -9, but not urokinase-type plasminogen activator (uPA), a serine proteinase and a pro-MMP activator. Dose-dependent inhibition was shown by three independent MMP assays. The inhibition was reduced in the presence of an increased concentration of Ca^{2+} when compared to physiologic Ca^{2+} concentration. Alendronate inhibited the *in vitro* invasion (Matrigel) of human HT1080 fibrosarcoma and C8161 melanoma cells, and the random migration of these malignant and endothelial cell lines capable of expressing MMPs and uPA. The concentration of alendronate required to inhibit 50% of the activity (IC_{50}=40–70 μM) of MMPs corresponded to the IC_{50} of down-regulation of *in vitro* invasion and migration. The ability of bisphosphonates to down-regulate the *in vitro* invasion and random migration was comparable or slighty better in relation to the selective gelatinase inhibitor CTTHWGFTLC peptide. Alendronate but not CTTHWGFTLC peptide promoted the adhesion of HT1080 fibrosarcoma and C8161 melanoma cell lines on fibronectin. Bisphosphonates are broad-spectrum MMP inhibitors and this inhibition involves cation chelation. Bisphosphonates further exert antimetastatic, anti-invasive and cell adhesion-promoting properties, which may prevent metastases not only into hard tissues but also to soft tissues.

Key words: Bisphosphonates, inhibition, invasion, matrix metalloproteinases, migration.

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Introduction

Bisphosphonates have a profound effect on bone resorption, and are therefore used in the treatment of Paget's disease, osteolytic tumor metastases, osteoporosis and advanced multiple myeloma.[1,2] Pamidronate and clodronate have recently been successfully used to reduce the osteolytic and visceral metastases of breast cancer.[3–5] The use of clodronate adjunct improved several factors in women suffering from breast cancer in a large-scale study.[5] The mechanisms of action of bisphosphonates are not completely clarified, but they are thought to be

related to the high-affinity binding of these drugs to bone and their subsequent down-regulating effects on lytic bone cell functions.[6] Bisphosphonates have been suggested to inhibit recruitment and function of osteoclasts, to stimulate osteoblasts to produce an inhibitor of osteoclast formation, and to shorten the lifespan of osteoclasts.[6] These drugs have also been proposed to reduce the release of growth factors stored in bone matrix as well as to inhibit the adhesion of breast cancer cells to bone matrix.[6]

The precise molecular events, however, underlying the observed efficacy of bisphosphonates in the treatment of bone and other tissue destructive diseases remain essentially unknown. We observed that collagenase-1 (MMP-1) and collagenase-2 (MMP-8) activity can be inhibited by clodronate.[7–9] However, Stearns reported that bisphosphonates did not inhibit gelatinase-A (MMP-2).[10] Both up- and down-regulation by bisphosphonates have been reported regarding expression of distinct matrix metalloproteinases (MMP) proteins and RNA by malignant and non-malignant cell lines.[10–12] In this work, we have therefore further explored the possibility that bisphosphonates could directly inhibit a group of structurally closely related but genetically distinct MMPs involved in a number of conditions including tumor invasion and metastases, and inflammatory diseases such as rheumatoid arthritis and periodontitis.[13] Bisphosphonates have also been suggested to exert anti-angiogenic properties.[1] To evaluate these aspects more thoroughly, we examined the effects of alendronate, clodronate, pamidronate and zoledronate on purified human MMPs in the presence of low and high Ca^{2+} concentrations. We continued to study the effects of alendronate on the human HT1080 fibrosarcoma and C8161 melanoma cell lines for their ability to invade through a reconstituted basement membrane (Matrigel), and for random migration of these malignant and human endothelial cell lines.

We report here the direct inhibition of human MMPs by bisphosphonates, and compared this to the effects obtained with the selective gelatinase inhibitor and antagonist CTTHWGFTLC peptide.[21] The inhibition of MMPs by alendronate and clodronate was reduced with high Ca^{2+} levels present in the assay conditions. Additionally, alendronate proved to be an effective down-regulator of *in vitro* invasion and random migration of the human HT1080 fibrosarcoma, C8161 melanoma and endothelial [Eahy926 and human umbilical vein endothelial cell (HUVEC)] cell lines. Furthermore, alendronate promoted adhesion of human HT1080 fibrosarcoma and C8161 melanoma cell lines to fibronectin.

Materials and methods

Chemicals

MMPs used in this study were purchased, prepared and/or kindly provided from the following sources: recombinant human MMP-1, -3 and -13 were from Drs Gillian Murphy and Vera Knäuper (Strangeways Research Laboratory, Cambridge, UK) and also purchased from Chemicon (Temecula, CA). MMP-2, -8 and -9 were purified as described.[8,15] Human recombinant MMP-20 was constructed, prepared and purified by the authors.[16] Human MMP-12 was from Elastin Products (Omnesville, MO). Urokinase-type plasminogen activator (uPA) was purchased from Chemicon. CTTHWGFTLC peptide, a selective gelatinase inhibitor and antagonist, was prepared as described.[21]

Measurement of β-caseinolytic activity

Autoactivated and APMA (1 mM)-pretreated pure human recombinant MMPs (100–150 ng) were pretreated with buffer or with different indicated concentrations of alendronate (Merck, Sharp & Dohme, West Point, AR), clodronate (Leiras, Turku, Finland) pamidronate and zoledronate (Novartis, Basel, Switzerland) for 60 min followed by incubation with 52 μM β-casein for 60 min at 37°C. The enzyme reactions were then halted, and the samples were electrophoresed and analyzed by sodium dodecylsulfate–polyacrylamide gel electrophoresis (8% SDS–PAGE) and quantitated by densitometry (model GS-700 imaging densitometer; BioRad, Richmond, CA).[15] The disappearance of the 21-kDa β-casein band resulted from MMP activity.[8,15,17]

Colorimetric assay for MMPs using modified pro-urokinase as substrate

The autoactivated and APMA (1 mM)-pretreated human MMPs were incubated with 0–400 μM alendronate and clodronate (1 h preincubation), and assayed for MMP activities according to Verheijen *et al.*[18] The high and low Ca^{2+} concentrations were 5 and 1 mM $CaCl_2$, respectively, in 50 mM Tris–HCl, 150 mM NaCl, 1 μM $ZnCl_2$, 0.01% Brij, pH 7.8 (TNC buffer). The activities were expressed as relative units. uPA activity was assayed with chromogenic S2444 substrate (Chromogenix, Molndal, Sweden).[15,17]

Gelatin degradation assay

The degradation of gelatin was also assayed using ^{125}I-labeled gelatin as substrate.[19] MMPs were treated as described above and samples to be tested were incubated with soluble ^{125}I-labeled gelatin (1.5 μM) for 1 h at 37°C. Undegraded gelatin was precipitated with 20% trichloroacetic acid. The radioactivity in the supernatants and precipitates were counted. Radioactivity in the supernatant reflected gelatinase activity.[19]

Cells and cell cultures

Human HT1080 fibrosarcoma and human umbilical vein endothelial cell (HUVEC) lines were commercially obtained from ATCC (Rockville, MD). The Eahy926 cell line is a HUVEC derivative. Human C8161 melanoma cell line was obtained from Dr Seftor.[20] The cells were cultured in 9-ml Nunclon flasks (Nunc, Roskilde, Denmark) and maintained in Dulbecco's modified Eagles medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 2 mM glutamine and penicillin–streptomycin (100 IU/ml–100 μg/ml) and hypoxanthine/aminopterin/thymidine additive with the Eahy926 cells. HUVECs were grown in RPMI 1640 medium containing penicillin–streptomycin (100 IU/ml–100 μg/ml), 10 mM HEPES, 30 μg/ml endothelial cell growth supplement (Biomedical Technologies, Stoughton, MA) and 20% fetal calf serum. Cultures of cells were harvested with 4 mM EDTA (no trypsin was used to avoid putative proteolytic degradation of relevant adhesion receptors by the enzyme) or trypsin–EDTA (endothelial cells), washed with PBS and resuspended in fresh serum containing media. Cells were cultured at 37°C in a humidified 5% CO_2 atmosphere.[21]

In vitro cell invasion and migration assays

Cell migration was studied using 8.0-μm pore size and 6.5-mm diameter Transwell inserts (Costar, Cambridge, MA) that were equilibrated in the serum containing medium for 2 h before use. Tumor cell invasion was studied using 6.4-mm diameter Boyden chambers precoated with Matrigel (Becton Dickinson, Bedford, MA). The layer of Matrigel matrix serves as a reconstituted basement membrane *in vitro*. This uniform layer occludes the pores of the membrane, blocking non-invasive cells from migrating through the membrane. By contrast, invasive cells are able to detach themselves from and migrate through the Matrigel matrix-treated membrane.

Quantification of migrating cells in varying concentrations of alendronate

Stock solutions of alendronate were prepared at a concentration of 1.0 mM in TNC buffer and 1.0 mM CTTHWGFTLC in dH_2O and further diluted to neutral pH buffers just before use. Cells were preincubated at 37°C in a humidified 5% CO_2 atmosphere for 2 h in the presence of 50, 100 or 500 μM alendronate or 500 μM CTTHWGFTLC peptide. For migration assays, 600 μl of the serum containing media was added to the lower compartments of the migration apparatus and 20 000 cells in a volume of 100 μl were plated in a Transwell. For Matrigel invasion, each well was plated with 10^5 cells in a 500 μl volume of fresh serum containing media, with or without alendronate or CTTHWGFTLC peptide. After culturing the cells for 16–24 h, cells were fixed in methanol, washed and stained in toluidene blue. Cells were removed from the upper surface of the membrane with a cotton swab and the cells that migrated to the underside of the membrane were counted under the microscope or quantitated by scanning with the BioRad scanner (model GS-700 imaging densitometer) and photographed. Experiments were repeated 5 times for the invasion assay and 3 times for the migration assay.[21]

Cell viability and adhesion

To assess the effect of alendronate on cell viability, 20 000 cells were plated in microtiter wells in 100 μl of medium containing 10% fetal bovine serum in the absence or presence of alendronate (50, 100 and 500 μM) or CTTHWGFTLC peptide (500 μM). After culturing for 20 or 40 h, the viability was determined with the MTT reagent according to the instructions of the manufacturer (Sigma). For cell adhesion studies microtiter wells were coated with fibronectin and blocked with BSA. Cells (100 000/well) were added together with alendronate (50, 100 and 500 μM) or CTTHWGFTLC peptide (500 μM) and cultured for 1 h in a serum-free medium. After washing twice with PBS the bound cells were assayed with the MTT reagent as above.[21]

Statistical analysis

Data are expressed as means $\pm$ SD and the significance of differences between group means was determined by the Student's *t*-test. Data were considered as significant when at least a $p=0.05$ was reached.

Results

Inhibition of human MMPs and uPA by bisphosphonates

Pamidronate and zoledronate inhibited dose-dependently human macrophage metalloelastase (MMP-12) and enamelysin (MMP-20) (Figure 1A and B). Alendronate, pamidronate and zoledronate at a concentration range 20–1000 μM inhibited the degradation of the 21-kDa β-casein band by human recombinant MMP-8, -3 and -13 in a dose-dependent manner (Figure 2). Alendronate and clodronate dose-dependently inhibited the activities of purified human MMP-1, -2, -3, -8, -9 and -13, but did not inhibit the serine proteinase uPA (Table 1). The ability of 400 μM alendronate and clodronate to inhibit MMP-1, -2, -3, -8, -9 and -13 was reduced in the presence of the high (5 mM) Ca^{2+} concentration in the assay buffer (Table 1). In the presence of the low or physiological (1 mM) Ca^{2+} concentration in the assay buffer the IC_{50} for MMP inhibition was 40–70 μM. Corresponding results were observed using β-casein and radioactive gelatin-degradation assays (not shown). When 50 μM alendronate, pamidronate or zoledronate were added together with substrate to enzyme reactions, a clear inhibition was observed after 20–40 min.

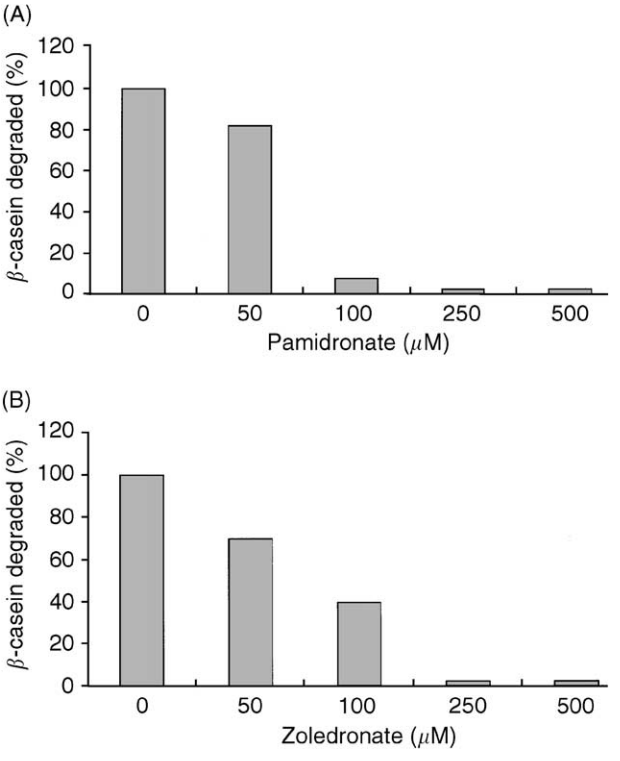

Figure 1. Inhibition of MMP-12 by pamidronate (A) and MMP-20 by zoledronate (B). Increasing concentrations of tested bisphosphonates were preincubated with the active forms of MMP-12 and -20. Subsequently, samples were incubated with 52 μM β-casein for 60 min at 37°C and analyzed by 10% SDS–PAGE with Coomassie brilliant blue staining. Block graphs represent quantitative densitometric analysis of intact β-casein and its degradation products within the gels.

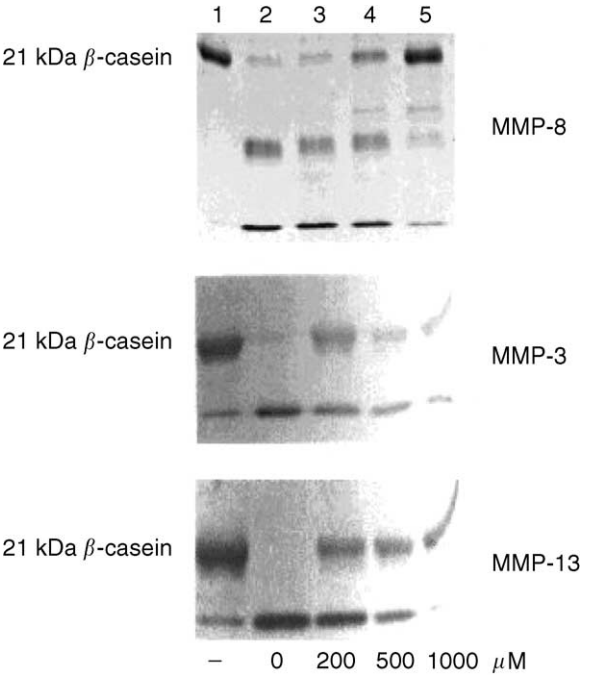

Figure 2. Dose-dependent inhibition of human MMP-8, -3 and -13 mediated degradation of 21 kD β-casein by pamidronate. Lane 1, 52 μM β-casein incubated with buffer and, lane 2, 500 ng of indicated activated MMP for 1 h at 22°C. Lanes 3–5 represent the effect of pamidronate (200, 500 and 1000 μM) pre-treatment (1 h, 37°C), respectively. Note the dose-dependent inhibition of MMP-catalyzed degradation of 21 kDa β-casein by pamidronate.

The effects of bisphosphonates on cell invasion, migration, viability and adhesion

Alendronate at 50, 100 and 500 μM concentrations efficiently and dose-dependently inhibited the invasion of HT1080 fibrosarcoma cells through type IV collagen-coated Matrigel cell culture inserts. The respective levels of dose-dependent inhibition of HT1080 cell and C8161 cell invasions mean were as follows: (i) 50 μM inhibited 72$\pm$30.10% (*t*-test, p=0.006) and 62$\pm$9.88% (p=0.002), (ii) 100 μM 84$\pm$15.02% (p=0.00) and 77$\pm$14.89% (p=0.025), (iii) 500 μM 98$\pm$4.47% (p=0.000) and 86$\pm$7.29%

Table 1. Inhibition of the activities of human MMPs and uPA by alendronate and clodronate in the presence of physiological or low (1 mM) and high (5 mM) Ca^{2+} concentrations

Enzyme assayed	Alendronate			Clodronate			Alendronate	Clodronate
	25 μM	100 μM	400 μM	25 μM	100 μM	400 μM	400 μM	400 μM
MMP-1	7.6	1.6	10	32.2	4.1	15	0	26.1
MMP-2	36.9	13.4	2	62.1	27.7	26	2.8	25.5
MMP-3			24			48	33	41.1
MMP-8	30.3	11.3	12	96.4	34.8	27	21.1	17.5
MMP-9	71.1	39.9	31	89.0	60.8	41	64.8	74.6
MMP-13	26.3	2.5	1	61.7	72.6	23	33	64.3
uPA		86.8	98.3		96.4	99.8		
	Physiological Ca^{2+}						High Ca^{2+}	

MMPs in active form were preincubated either with alendronate or clodronate and activity was measured using modified urokinase as a substrate for MMPs. uPA activity was measured with chromogenic S2444 substrate. Numbers represent percentage of activity as compared to the activity without bisphosphonates and they are the means of four determinations.

(p=0.020) compared to buffer alone (Figure 3). In the migration test dose-dependent inhibition of HT1080 cell and C8161 cell migrations were as follows, respectively: (i) 50 μM inhibited 29$\pm$4.58% (p=0.001) and 31$\pm$10.60% (p=0.025), (ii) 100 μM 53$\pm$6.03% (p=0.001) and 41$\pm$10.07% (p=0.010), (iii) 500 μM 87$\pm$4.04% (p=0.000) and 65$\pm$2.52% (p=0.000) compared to buffer alone (Figure 4A and B). Alendronate dose-dependently and significantly reduced the migration of human endothelial cell lines (Eahy926 and HUVEC) (Figure 4C and D). Similar to the IC_{50} for inhibition of MMP activity, the IC_{50} for *in vitro* basement membrane (Matrigel) invasion and random migration of the HT1080 fibrosarcoma, C8161 melanoma and endothelial cell lines were in the range of 40–70 μM. As a positive control for the cell invasion and random migration assays we used CTTHWGFTLC decapeptide (500 μM), which significantly reduced the Matrigel invasion and random migration of the HT1080 fibrosarcoma, C8161 melanoma and endothelial cell lines (Figure 4A–D).[21]

To study whether alendronate affected cell viability, we examined the effect of alendronate on cell growth and cell adhesion for a fibronectin substratum. Alendronate did not decrease the growth of C8161 melanoma cells at any concentrations studied (not shown). At the 500 μM concentration, alendronate partially decreased the growth of HT1080 fibrosarcoma cells during a 24-h culture, but did not affect the cells at the 50 or 100 μM concentrations, which are the concentrations that significantly inhibited cell invasion and random migration. Furthermore, the cell adhesion was promoted by alendronate; during a 1-h assay, alendronate (50, 100 and 500 μM) significantly promoted the adherence of both HT1080 fibrosarcoma and C8161 melanoma cell lines to fibronectin (Figure 5) and Matrigel (not shown) substrata when compared to untreated cell lines. The CTTHWGFTLC peptide did not affect cell adhesion for fibronectin, was not toxic to cells and did not affect cell viability (not shown).[21]

Discussion

Bisphosphonates have been found to decrease the collagen degradation as measured by the serum and urine levels of collagen degradation products in osteoporosis and advanced malignancies with bone metastases.[6] We provided evidence that bisphosphonates at therapeutically attainable non-cytotoxic concentrations can directly inhibit various human MMPs. This was shown by use of three distinct MMP activity assays: (i) β-casein assay, (ii) degradation of radioactive gelatin and (iii) colorimetric assay using modified pro-urokinase as substrate.

MMP-3 has been found to promote tumor burden, and MMP-13 was originally isolated from breast carcinomas and subsequently found in conditions involving bone destruction.[24–27] MMP-13 is synthesized by cancer cells as an inactive latent proform that can be activated by another MMP member, stromelysin-1 (MMP-3).[26,27] Osteoclastic bone cells and malignant cells have recently been found to express macrophage metalloelastase (MMP-12).[28] Enamelysin (MMP-20), in addition to its expression during tooth development, has also been found to be expressed and activated by oral squamous cancer cells.[16,29] Thus, the MMPs can act in concert by forming a cascade to degrade extracellular matrix and basement membrane proteins as well

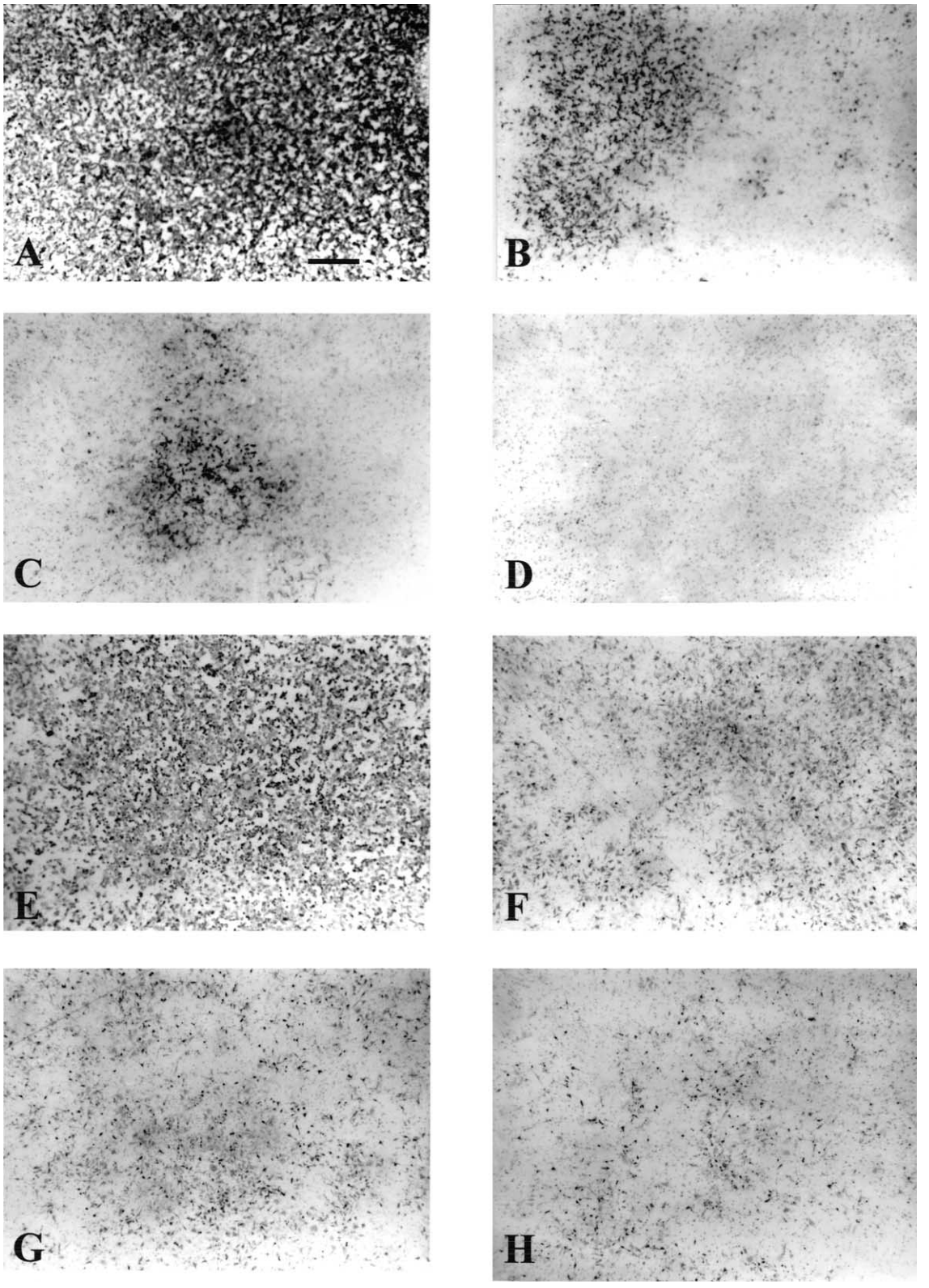

Figure 3. Down-regulation of *in vitro* Matrigel invasion of HT1080 fibrosarcoma and C8161 melanoma cells by alendronate. The cells were plated on Matrigel filter in the absence (A and E) or presence of 50 (B and F),100 (C and G) or 500 (D and H) μM alendronate and allowed to invade for 16–24 h. Cells that invaded to the underside of the filter were stained. Scale bar=10 μm.

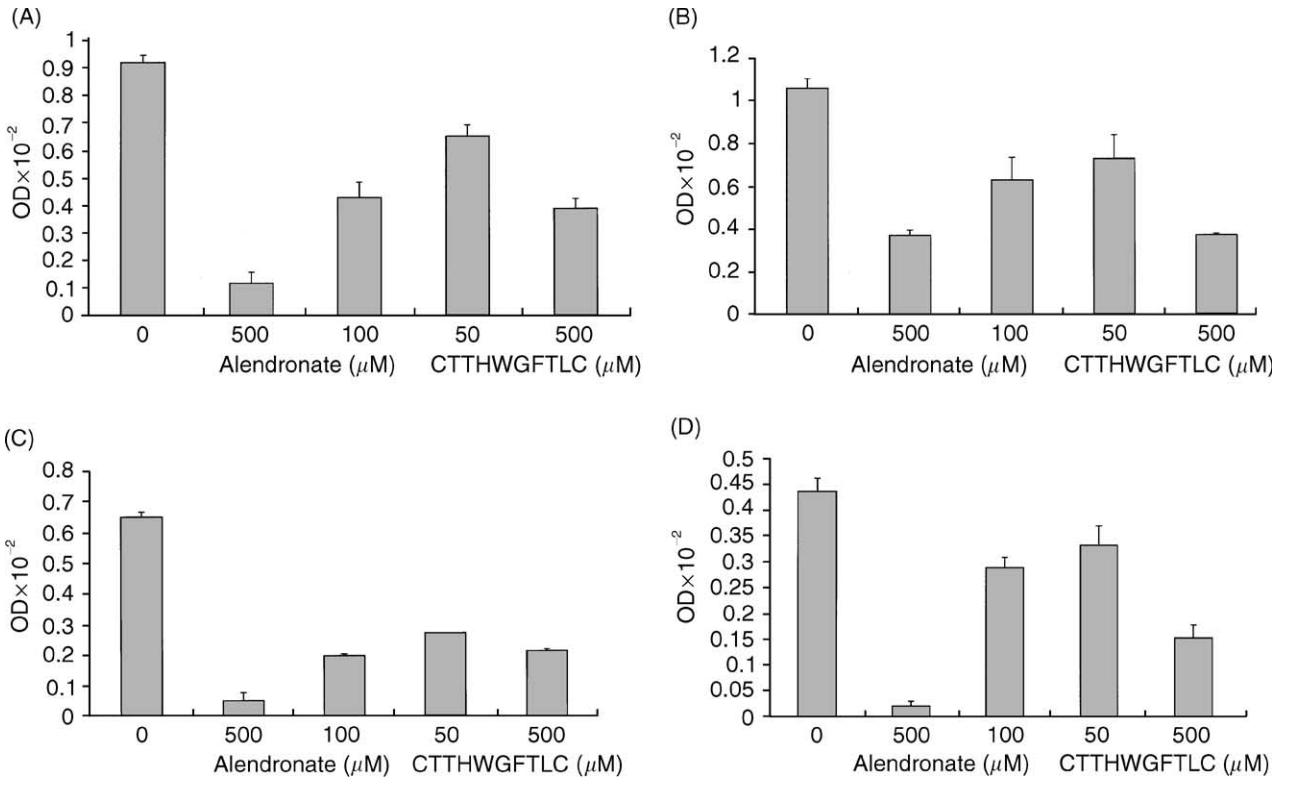

Figure 4. Down-regulation of the random cell migration by alendronate and the CTTHWGFTLC peptide. Cells were plated on Transwell filters in the absence or presence of alendronate (50, 100 and 500 μM) or CTTHMWGFTLC (500 μM) and allowed to migrate for 24 h. Transwell migration is shown for the following human cell lines: (A) HT1080 fibrosarcoma, (B) C8161 melanoma, (C) Eahy926 and (D) HUVEC. Cells that migrated to the underside of the filter were stained. In each case, data represent means $\pm$ SD; n=3.

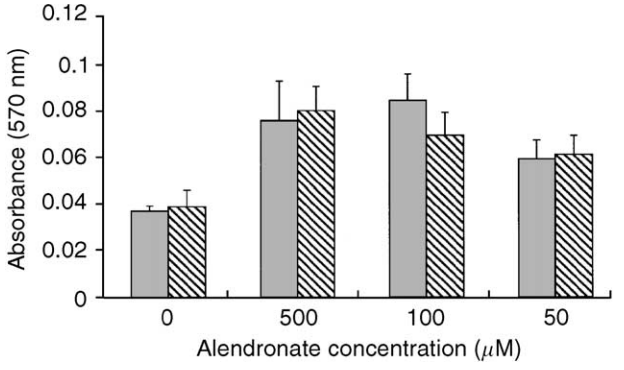

Figure 5. The effect of alendronate on cell adhesion of human HT1080 fibrosarcoma and C8161 melanoma cell lines. The cells were transferred to microtiter wells coated with fibronectin and blocked with BSA in the absence of serum. After 60 min the bound cells were quantified with the MTT reagent. In each case, data represent means $\pm$ SD; n=4–5.

as activate each other.[13–17,24–27] Present findings confirmed and further extended our previous results describing that therapeutically attainable concentrations of bisphosphonates can directly inhibit the activities of certain human MMPs.[7–9] Our present and previous results differ from Stearns who showed that gelatinase-A (MMP-2) could not be directly inhibited by bisphosphonates.[10] In agreement with our data, Boissier *et al.* recently showed that MMP-2, -9 and -12 activities can be inhibited by bisphosphonates.[7–9,30] The mechanism(s) of MMP inhibition evidently involve the ability of bisphosphonates to act as cation chelators and to support this we show that cations (Ca^{2+}) can revert the MMP inhibition by bisphosphonates. Overall, these data indicate that differences exist among MMPs in respect to their sensitivity to inhibition by bisphosphonates, which together with the involvement of cation chelation resemble the inhibition of MMPs by tetracyclines and their chemically modified non-antimicrobial derivatives (chemically modified tetracyclines).[31,32]

MMPs are known to act in multiple activation cascades promoting angiogenesis, tumor progression and metastases.[33] Therefore, the broad-spectrum inhibition of various genetically distinct MMPs may down-regulate, at least partially, the

MMP-activation cascade.[13,17] However, uPA, a serine proteinase, expressed by HT1080 fibrosarcoma cells and C8161 melanoma cells capable of activating latent proMMPs via generation of plasmin was not inhibited by alendronate and clodronate.[13,14] However, bisphosphonates may abolish the ability of tissue inhibitors of matrix metalloproteinases (TIMP) to protect MMPs from degradation by serine proteinase, such as plasmin.[34]

The IC_{50}s for inhibition of MMPs in the presence of a physiologic Ca^{2+} concentration (1 mM) and for the *in vitro* basement membrane (Matrigel) invasion of HT1080 fibrosarcoma cells and C8161 melanoma cells were in the range 40–70 μM indicating that a direct inhibition of MMP activity is eventually responsible for the down-regulation of malignant cell invasion.

When bisphosphonate (clodronate) is used in the treatment of metastatic breast cancer, up to 300 mg can be given i.v. during 5 successive days (total dose 1500 mg) and at least 20% taken up by bone with total volume of 1.75 l.[35] This gives a 593 μM concentration in bone. Bisphosphonates are further concentrated in the extracellular fluid of Howship's lacunae, in which the concentration of alendronate has been shown to vary from 100 μM to 1 mM.[36] These estimation and animal studies show that 40–100 μM concentrations of bisphosphonates are eventually relevant *in vivo*.[35,36]

Regarding the down-regulation of random migration by bisphosphonates in the human fibrosarcoma, melanoma and endothelial cell lines, our present findings differ to a certain extent from Boissner *et al.*, who were not able to find bisphosphonate-induced down-regulation of human breast cancer and prostate carcinoma cell migration.[30] We demonstrated down-regulation of random migration of both malignant and endothelial (Eahy926 and HUVEC) cell lines with an IC_{50} corresponding to that of the down-regulation for *in vitro* invasion and MMP inhibition. Similar to alendronate, the recently discovered selective gelatinase inhibitor (CTTHWGFTLC peptide) also inhibited the *in vitro* invasion and random migration of the studied human malignant and endothelial cell lines.[21] Of note was the ability of alendronate to down-regulate both the *in vitro* invasion and random migration of the human malignant and endothelial cell lines in a way that was comparable or slightly better than the CTTHWGFTLC peptide.

In addition to their effects on cell invasion, the anti-tumor activity of bisphosphonates is also likely to depend on their ability to inhibit migration and eventually angiogenesis in tumors.[1] Tumors require blood vessels to support their progressive growth and spread, and MMP inhibitors and antagonists can inhibit angiogenesis.[13,14,21] We further suggest that MMP inhibition by bisphosphonates may be closely related to their observed ability to promote adhesion of certain tumor cell lines to the extracellular matrix. Therefore, we suggest that since the bisphosphonates inhibit MMPs, they may interfere with the initial or early spread of malignant cells by promoting their attachment to each other or the extracellular matrix or basement membrane molecules (this study) and may also prevent adhesion of already escaped metastasizing malignant cells to bony structures.[37,38] Regarding the latter point, Boissier *et al.* and van der Pluijm *et al.* showed that bisphosphonates can reduce human prostate and breast carcinoma (but not human fibroblast cell line) adhesion to bony extracellular matrices.[37,38]

Overall, the detected decrease of not only bony but also visceral metastases in the treatment of breast cancer with bisphosphonate (clodronate) strongly suggest that in addition to the effect of clodronate on bone resorbing cells, there must be another mechanism of action, which explains the favorable effects in preventing soft tissue destruction and metastases.[5,39] The present results strongly suggest that bisphosphonates, by virtue of their potent broad-spectrum inhibitory action on MMPs, cell invasion and migration together with their safety of administration, low toxicity and long action might be beneficial in decreasing extracellular matrix and basement membrane degradation both in malignancies and inflammatory soft tissue and bone destructive diseases. Recent studies have further demonstrated that a combination medication of a bone-specific (bisphosphonate and/or chemically modified tetracycline derivative) and common metastases inhibitor (MMP inhibitor), such as TIMP-2, is much more effective in synergistically inhibiting both the spread of osteolytic bone metastases and the progression of bone destructive inflammatory disease (periodontitis) than a single inhibitor alone.[22,23,40] Thus, in the future bisphosphonates may prove to be especially useful down-regulators of MMP-dependent hard and soft tissue destruction when used in combination with other drugs or other MMP inhibitors.[21–23,40,41]

Conclusion

Bisphosphonates are broad-spectrum MMP inhibitors and this inhibition involves cation chelation. Bisphosphonates further exert anti-metastatic, anti-invasive

and cell adhesion-promoting properties, which may prevent metastases not only into hard tissues but also to soft tissues.

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