171

Macrophage Inflammatory Protein (MIP)-3α/CCL20 and its receptor CCR6 are overexpressed in the bone microenvironment and involved in osteoclast formation in multiple myeloma patients: Potential relationship with the presence of osteolytic lesions

Nicola Giuliani [a], Gina Lisignoli [b], Sara Tagliaferri [a], Francesca Morandi [a], Vittorio Rizzoli [a]
[a] *Hematology, University of Parma, Parma, Italy*
[b] *Laboratorio di Immunologia e Genetica, Istituti Ortopedici Rizzoli, Bologna, Italy*

Recent evidences indicate that chemokines, small chemoattractant proteins involved in cancer cell homing, may contribute to osteoclast formation and activation. In this study, we evaluated the potential role of CCL20 namely also MIP-3α and its receptor CCR6 in the pathophysiology of osteoclast (OC) formation and osteolytic lesions in multiple myeloma (MM). First we found that CCL20 significantly increased both the number of multinucleated TRAP+ OCs and RANK+ OC progenitor cells in presence of RANKL. Following, the potential production of CCL20 by human MM cell lines (HMCLs) and fresh purified CD138+ MM cells was also checked. Significant levels of CCL20 were detected in one out of nine HMCLs tested and in about 10% of purified MM cells by ELISA and immunohystochemistry. On the other hand we found that MM cells up-regulated CCL20 secretion, in osteoblasts (OB) and bone marrow OB progenitor cells (PreOB) and its receptor CCR6 in OCs in co-culture system. Among potential soluble factors involved in the up-regulation of CCL20 by MM cells we found that IL-1β and TNFα together stimulate CCL20 production in both OB and PreOB. The role of CCL20 in OC activation by MM cells was finally demonstrated by finding that both blocking anti-CCL20 and anti-CCR6 Abs. but not anti-IgG control significantly decreased OC formation induced by the conditioned medium of MM cells co-cultured with OB and OC, respectively. This chemokine system was further studied in vivo in MM patients. CCL20 levels were detected in the BM plasma of MGUS subjects patients at the diagnosis in relationship with the presence of bone lesions. Significant higher CCL20 levels were detected in MM patients vs. MGUS and in MM osteolytic patients vs. non-osteolytic ones. Interestingly, no significant differences were observed between MGUS and non-osteolytic MM patients. By immunohystochemistry performed on BM biopsies, we consistently found that CCL20 was over-expressed in OBs in osteolytic MM patients as compared to non-osteolytic ones. In addition we found that OCs showed a strong CCR6 staining in the areas with an increased number of OCs. In conclusion our data indicate that CCL20 its receptor CCR6 are up-regulated in bone microenvironment by MM cells and involved in osteoclast formation and bone lesions in MM patients.

doi:10.1016/j.bone.2007.12.181

172

Renal function changes and resource use in patients with metastatic bone disease treated with IV zoledronic acid or oral ibandronic acid: Interim results

Stephen J. Houston, Robert J. Grieve, Fran Percival, Nicola J. Fisher, Liz Hamilton
St Lukes Cancer Centre, Guildford, United Kingdom
University Hospitals Coventry and Warwickshire, Coventry, United Kingdom
pH Associates, Marlow, United Kingdom
Roche Products Ltd, Welwyn Garden City, United Kingdom

Objectives: To describe changes in renal function and secondary care resources used in routine clinical practice in the management of metastatic bone disease with IV zoledronic acid (ZA) and oral ibandronic acid (IBA).

Methods: This ongoing six centre UK study will retrospectively review medical records of 200 patients with primary breast cancer and metastatic bone disease, from the first dose of bisphosphonate (BPN) until the end of therapy, focussing on renal function and adjustment of the BPN dose. A prospective time and motion study of resources involved in administering BPNs at the same centres will also be conducted.

Results: Interim renal function results from 129 patients, treated with IBA ($n=69$) and ZA ($n=60$) in 4 centres are available. Baseline creatinine clearance (CrCl) was recorded in medical notes for 41.7% (25/60) of ZA but no IBA patients. CrCl could be calculated from the notes of 90% (54/60) ZA and 10.1% (7/69) IBA patients. For patients with data available, 85% had a baseline CrCl >50 ml/min, i.e. normal or mildly impaired renal function. CrCl fell by at least one 10 ml/min band in 15/53 and 2/5 patients during treatment with ZA and IBA respectively. In patients where CrCl fell by at least one 10ml/min band, incorrect doses (according to SPC) were given in 89/186 occasions with ZA and 0/11 with IBA. Timings for dispensing prescriptions and patient treatments are shown below:

Staff Time (min)	IBA($n=10$)	ZA($n=16$)
Pharmacists	4.3	2.5*
Technician	1.3	0
Clerk	0.4	0
Nurses	0	27.0
Mean staff time	6.0	27.1
Median staff time	5.4	25.4
Patient Time (min)		
Median waiting time	25.5	62.6
Median infusion chair time	0	16.6

*1/16 pts.

Conclusion: Most patients had relatively normal renal function at baseline, but in almost 30% of patients CrCl fell during treatment with ZA. However, because in many patients CrCl is not calculated or monitored during treatment with BPNs, inappropriate doses were given in almost 50% of the ZA patients with falling renal function. With IBA, the small amount of data available suggests correct doses were given. Lack of renal function monitoring raises concerns regarding the safe administration of BPNs and current clinical practice may need

to be reviewed. The resource use results provide evidence that dispensing of oral IBA requires considerably less secondary care staff time, infusion chair use and patient waiting time than does the preparation and administration of IV ZA.

Dr Stephen J Houston; Roche Products Ltd, and Novartis Pharmaceuticals UK Limited; Dr Houston has received small honoraria from Novartis and Roche. Honoraria received for Chief Investigator role, advisory board and lecturing.

doi:10.1016/j.bone.2007.12.182

173

Effects of osteoblast-like cells on bone-related and invasive gene expressions in breast cancer cells

Hichame IdBoufker [a,b], Laurence Lagneaux [b], Tatiana Tondreau [b], Hugues Duvillier [b], Jean-Jacques Body [a], **Fabrice Journe** [a]

[a] *Laboratory of Endocrinology and Bone Diseases, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium*

[b] *Laboratory of Experimental Hematology, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium*

Breast cancer cells frequently metastasize to the skeleton, where they induce extensive osteoclast-mediated bone destruction. In this contexte, osteoblasts play a dual role in controlling bone formation and in mediating the effects of tumor cells on osteoclasts. Importantly, functions of osteoblasts are depending on their maturation stages. However, data on the influence of osteoblastic cells on metastatic cells are scarce: in vitro models using conditioned medium or membrane insert systems have been used to study the interactions between osteoblasts and breast cancer cells, but these systems do not address direct cell–cell contacts. Our objective was to investigate the effects of osteoprogenitor-like cells (MG-63) and mature osteoblast-like cells (SaOS-2) on luminal-like (MCF-7) and basal-like (MDA-MB-231) breast cancer cells, by examining mRNA expression of genes involved in osteoblast differentiation, osteoclastogenesis and tumor invasion. Breast cancer cells were first labelled with CellTrace CFSE (Eugene, OR, USA) and then cultured with osteoblastic cells during four days. Then, fluorescent tumor cells were separated from osteoblasts by flow cytometry and the purity of isolated cancer cells was controlled by assessing Epcam expression (purity = 98.9 ± 0.1% and 96.3 ± 2.5% for MCF-7 and MDA-MB-231 cells, respectively). The analyses by RT-PCR of gene expressions in breast cancer cells cocultured with osteoblastic cells revealed many changes. SaOS-2 cells weakly decreased the expression of osteopontin and RUNX2 mRNA in MDA-MB-231 cells, but increased their expressions in MCF-7 cells. They also increased the mRNA expression of osteolytic factors (RANKL and PTHrP) in MCF-7 cells, and they enhanced osteonectin mRNA expression in both cancer cell lines. By contrast, MG-63 cells only markedly increased gene expression of osteoprotegerin and osteonectin in the two tumor cell lines. A strong increase in MMP-2 mRNA expression was observed in both tumor cell lines cocultured with MG-63 cells and even more with SaOS-2 cells. Our results suggest that osteoprogenitor-like cells increase the invasive ability of luminal-like and basal-like breast cancer cell lines, while mature osteoblast-like cells enhance the osteoclastogenesis factor expression and the osteoblast phenotype mainly in the luminal-like tumor cells. Thus, cells from osteoblast lineage can modulate the ability of the breast cancer cells to invade the bone matrix and to adapt to the bone microenvironment.

doi:10.1016/j.bone.2007.12.183

174

The Src inhibitor dasatinib inhibits breast cancer cell growth in vitro and exerts synergistic effects with bisphosphonates

Fabrice Journe, Naima Kheddoumi, Carole Chaboteaux, Christos Sotiriou, Martine Piccart, Jean-Jacques Body

Laboratory of Endocrinology and Translational Research Unit, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium

Src is a nonreceptor tyrosine kinase, which is involved in proliferation, survival, adhesion, invasion and metastasis of cancer cells. Src kinase activity has been associated with the capacity of breast cancer to metastasize to bone by regulating cell growth and PTHrP production. It can cooperate with the receptor tyrosine kinases. Dasatinib (BMS-354825, Bristol Myers Squibb) is a new dual Src / Brc-Abl tyrosine kinase inhibitor. Recent data suggest that dasatinib can selectively inhibit the growth of basal-like breast cancer cell lines. On the other hand, bisphosphonates are potent inhibitors of osteoclasts but they can also suppress the mitogenic effects of growth factors on breast cancer cells (Fromigue et al., Br J Cancer 2003). We speculated that combination treatment of dasatinib with biphosphonates, by targeting growth factor pathways, might have additive or synergistic effects on breast cancer cells. The IC50 values of dasatinib for inhibition of cell proliferation were 10 μM, 200 nM and 50 nM for MCF-7, Hs578T and MDA-MB-231, respectively. The growth inhibitory effects of dasatinib were associated with induction of cell apoptosis. Total Src expression was similar in the three breast cancer cell lines and was not correlated with cell sensitivity to dasatinib. By contrast, 416-Tyr-Src was strongly expressed in MDA-MB-231 cells, weakly in Hs578T cells, and not at all in MCF-7 cells. This phosphotyrosine dramatically decreased in cells exposed to 100 μM dasatinib, confirming the efficacy of Src inhibition. We then assessed the effects of dasatinib in combination with bisphosphonates. Isobologram analysis to calculate combination index at 50% and 75% inhibition of cell growth identified synergistic interactions between dasatinib and ibandronate or zoledronic acid. There were also synergistic inductions of cell apoptosis. In conclusion, dasatinib reduced cell viability mainly in basal-type breast cancer cells, which are less sensitive to bisphosphonates. These effects were correlated with a decrease in Src autophosphorylation activity, especially in MDA-MB-231 cells. Furthermore, dasatinib inhibited breast cancer cells proliferation in synergy with bisphosphonates. Dasatinib is a