Micron 68 (2015) 107–114

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Unwrapping microcomputed tomographic images for measuring cortical osteolytic lesions in the 5T2 murine model of myeloma treated by bisphosphonate

Mambaye N'Diaye[a], Hélène Libouban[a], Eric Aguado[a,b], Béatrice Bouvard[a,c], Maurice Audran[a,c], Daniel Chappard[a,*]

[a] GEROM Groupe Etudes Remodelage Osseux et bioMatériaux, LUNAM Université, IRIS-IBS Institut de Biologie en Santé, CHU d'Angers, 49933 Angers Cedex, France
[b] UPSP "Biologie et Biomatériaux du Tissus Osseux – Chirurgie Expérimentale", ONIRIS – National Veterinary School of Nantes, LUNAM Université, Route de Gâchet, BP 44706, F44307 Nantes cedex 03, France
[c] Service de Rhumatologie, CHU d'Angers, 49933 Angers Cedex, France

ARTICLE INFO

Article history:
Received 8 May 2014
Received in revised form
15 September 2014
Accepted 2 October 2014
Available online 13 October 2014

Keywords:
Myeloma
Bisphosphonate
Cortical bone
Osteolysis
MicroCT

ABSTRACT

Multiple myeloma is due to the proliferation of malignant plasma cells which increase the number of osteoclasts leading to trabecular and cortical bone osteolysis. The 5T2MM murine model reproduces the human disease and microcomputed tomography is a precise tool to investigate bone loss. Bisphosphonates (zoledronic acid or pamidronate) are used in preventing osteolysis. However, loss of cortical bone in not possible to quantify by histomorphometry on histological sections or microCT images.

Osteolysis was studied in mice grafted with the 5THL subline to see if one drug was more active after 10 weeks. Mice were distributed into 4 groups: control, untreated, treated with pamidronate or with zoledronic acid. The left femurs were embedded undecalcified and sectioned at 7 μm. The right tibias and femurs were analyzed by microCT and trabecular morphometric parameters were obtained. Cortical bone osteolysis was analyzed by developing a new algorithm to unwrap microCT sections of the cortices, allowing measurement of the number of perforations, porosity and mean perforation area.

The bisphosphonates had no significant effect on the tumor growth as evidence by the absence of effect on the M-protein level. Cortical perforations were evidenced on histological sections and their number seemed to be reduced by both bisphosphonates. MicroCT was used to quantify the trabecular bone: a bone loss was evidenced in the untreated myeloma group and both bisphosphonates appeared equal to preserve trabecular mass. However, the number and size of cortical perforations cannot be determined on 3D models. Unwrapping microCT images provided flat images allowing a precise determination of cortical perforations. Pamidronate did not reduce the number and size of cortical perforations but significantly reduced porosity. Zoledronic acid appeared significantly superior and considerably reduced all parameters.

Unwrapping microCT image is a new method allowing the measurement of cortical perforations in bone malignancies, a parameter that cannot be measured correctly on 2D histological sections.

Abbreviations: 2D, two dimensions; 3D, three dimensions; BM, bone marrow; BP, bisphosphonates; BV/TV, trabecular bone volume; Ec.Po, endosteal porosity; FPP, farnesyl pyrophosphate; IL-, interleukine; MicroCT, microcomputed tomography; MM, multiple myeloma; N.Po, number of pores; Pam, pamidronate; PamMM, mice treated with pamidronate; PBS, phosphate buffered saline; Po.Ar, mean area of a cortical perforation; Ps.Po, periosteal porosity; RANKL, receptor activator of NFκB ligand; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; UMM, mice with untreated myeloma; VOI, volume of interest; Zol, zoledronic acid; ZolMM, mice treated with zoledronic acid.

* Corresponding author at: GEROM–LHEA, IRIS IBS Institut de Biologie en Santé, LUNAM Université Nantes Angers Le Mans, CHU d'Angers, 49933 Angers Cedex, France. Tel.: +33 244 68 83 49; fax: +33 244 68 83 51.
E-mail address: daniel.chappard@univ-angers.fr (D. Chappard).

http://dx.doi.org/10.1016/j.micron.2014.10.001
0968-4328/

1. Introduction

Multiple myeloma (MM) is a neoplastic hematological disease characterized by the production of a monoclonal gammopathy (M-protein) and plasma B cell infiltration of the bone marrow. Osteolytic lesions will develop in about 80–90% of MM patients; They are due to an increased osteoclastic activity resulting from interaction of plasma cells with the bone marrow environment in association with a decrease of the osteoblastic function (Bataille et al., 1989, 1991). Several cytokines have been shown to be responsible for an increase in osteoclast number and activity including upregulation of RANKL (receptor activator of NFκB ligand), repression of osteoprotegerin, overproduction of IL-6 and macrophage inflammatory protein MIP-1α by the plasma cells within the bone marrow microenvironment (Lentzsch et al., 2003; Pearse et al., 2001; Roodman, 2009). In parallel, there is an increased expression of Dickkopf-1 protein, IL-3 and secreted frizzled-related protein 2 which provoke an inhibition of the osteoblastic activity (Tian et al., 2003). This complex alteration in the cytokine network within bone marrow invaded by plasma cells induces a vicious circle between bone cells and the malignant cells. This is also responsible for the very specific aspect of bone lesions on X-ray images which appear as multiple "punched-out" holes (also coined cortical perforations) without reactive sign of reconstruction of the surrounding bone (Dimopoulos et al., 2009). However these lesions are only apparent when 30–50% of the bone mineral density has been lost (Dimopoulos et al., 2009; Dinter et al., 2009). This radiological finding corresponds to a complete perforation of the bone cortice developed in contact with nodules of plasma cell present in the bone marrow. Histological study, computed tomography or magnetic resonance imaging clearly illustrate that a perforation develops from the endosteal side and progresses to the periosteal surface of the cortices (Bauerle et al., 2009; Healy et al., 2011). In animal models of MM such as the 5T2MM model reported in the C57BL/KaLwRij mouse, similar cortical perforations (corresponding to osteolytic foci with disappearance of the whole cortical thickness from the endosteum to the periosteum) have been reported (Radl et al., 1985). These models are useful to understand the pathophysiology of the disease and to evaluate new pre-clinical therapeutic strategies and pathophysiological hypotheses (Benameur et al., 2013; Vanderkerken et al., 2003, 2005). As potent inhibitors of osteoclasts, bisphosphonates (BPs) have been proposed very early as bone-sparing agents in MM patients (Belch et al., 1991; Terpos et al., 2011). However, several generations of BPs have been developed with increasing anti-osteoclastic properties. Among them, amino BPs are the most active. Numerous clinical trials have shown the interest of the aminoBPs pamidronate (Pam) and zoledronic acid (Zol) in the prevention of osteolytic bone lesions and hypercalcemia in MM patients. Although these compounds are active in preventing trabecular bone loss, their activity on the cortical bone loss appears less pronounced (Chappard et al., 1991; Libouban et al., 2003). A single animal study conducted in osteosarcoma-induced osteolysis in the rat has raised the possibility that Zol could be active on cortical lesions but no quantitative evaluation of cortical perforations was done (Labrinidis et al., 2010).

The aim of the present study was to develop a new method to quantify, in 3D, the efficiency of two amino BPs currently used in the treatment of MM patients (Pam and Zol) in the 5T2MM model in the mouse. The trabecular and cortical bone loss were determined by microcomputed tomography (microCT). Because no algorithm exists to quantify the amount of cortical perforations, we developed a methodology of unwrapping images of cortical bone to evaluate the number, size and surface of these cortical osteolytic defects.

2. Materials and methods

2.1. Mice

C57BL/KaLwRij female mice 6–8-weeks-old were used for the study (Harlan, Gannat, France). They were acclimated for one week to the local vivarium conditions (24 °C and 12 h/12 h light dark cycle) where they were given standard laboratory food (UAR, Villemoison sur Orge, France) and water ad libitum. The Animal Care and Use committee at the University of Angers approved all procedures (experimentation #49028).

2.2. Culture cell line

We used the 5THL cell subline as previously characterized (Croese et al., 1987; Libouban et al., 2004). 5THL cells can be propagated into young syngeneic mice by intravenous transfer of the diseased bone marrow (BM). Progression of the disease in seven recipient mice was assessed by measuring the serum M-protein level using agar electrophoresis (Hydragel Protein, SEBIA, Issy les Moulineaux, France). Around 6 weeks post-injection of 5THL, mice had a detectable serum M-protein and were euthanized after 10–12 weeks by cervical dislocation. Femurs and tibias were dissected, cleaned of surrounding tissues and BM was flushed in Dulbecco's modified essential medium (DMEM.mod, GIBCO, Life Technologies, France) supplemented with penicillin-streptomycin, amphotericin-fungizone and pyruvate. BM cells were washed once in DMEM.mod. Mononuclear cells were isolated by a Lympholyte-M centrifugation gradient (Cedarlane, Hornby, Ontario, Canada) at 1250 g for 20 min. Mononuclear cells were then washed twice in DMEM.mod and counted.

2.3. Experimental design

As recommended in all papers concerned with the 5TMM model, mice of 6–8 weeks old were used (Vanderkerken et al., 2003, 2005). Sixty five mice were injected with 1.5×10^6 5THL cells in the tail vein and 8 additional mice were used as control and did not receive the malignant cells (Control group). The injected mice were divided in 3 groups according to the treatment received: 25 mice received a weekly injection of phosphate buffered saline (PBS) and constituted the untreated MM group (UMM) and were used to measure the importance of the loss of trabecular and cortical bone. Twenty mice were treated by subcutaneous injection of pamidronate (0.4 mg/Kg/day, 5 days per week) and constituted the PamMM group. Twenty mice were treated by subcutaneous injection of zoledronic acid (120 μg/Kg, 2 days per week) and constituted the ZolMM group (Croucher et al., 2003; Pataki et al., 1997). Pam and Zol (Novartis, Basel, Switzerland) were obtained from the hospital pharmacy. These dosages for Pam and Zol were similar to those used for the treatment of humans according to the supplier of the drugs (Green, J., Novartis Pharma, personal communication).

The success and progression of the graft was ascertained by quantitative electrophoretic as above at 6, 8 and 10 weeks. When electrophoresis did not evidence the M-protein at 6 weeks, the graft was found unsuccessful and the mouse was excluded. At the end stage of the disease (10–12 weeks), when osteolysis can be evidenced on X-ray images (Fig. 1), mice were euthanized for ethical reasons before the occurrence of fractures. They were bled before being sacrificed by cervical dislocation and the tibias and femurs were dissected and fixed in formalin.

2.4. Histological analysis

The left femurs were embedded undecalcified in poly (methylmethacrylate). They were cut-dry (7 μm in thickness) on a