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Geranylgeranyl transferase type II inhibition prevents myeloma bone disease

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ABSTRACT

Geranylgeranyl transferase II (GGTase II) is an enzyme that plays a key role in the isoprenylation of proteins. 3-PEHPC, a novel GGTase II inhibitor, blocks bone resorption and induces myeloma cell apoptosis *in vitro*. Its effect on bone resorption and tumor growth *in vivo* is unknown. We investigated the effect of 3-PEHPC on tumor burden and bone disease in the 5T2MM model of multiple myeloma *in vivo*. 3-PEHPC significantly reduced osteoclast numbers and osteoclast surface. 3-PEHPC prevented the bone loss and the development of osteolytic bone lesions induced by 5T2MM myeloma cells. Treatment with 3-PEHPC also significantly reduced myeloma burden in bone. The magnitude of response was similar to that seen with the bisphosphonate, risedronate. These data show that targeting GGTase II with 3-PEHPC can prevent osteolytic bone disease and reduce tumor burden *in vivo*, and represents a novel approach to treating tumors that grow in bone.

Protein prenylation is a biochemical process that transfers isoprenoid lipid moieties to key proteins, involved in important cellular signalling pathways. The process involves the covalent attachment of moieties to the C-terminal end of these proteins. These can be either C-15 isoprene farnesyl groups or C-20 isoprene geranylgeranyl groups. The addition of C-15 is performed by farnesyl transferase an enzyme in the mevalonate pathway (Fig. 1). The addition of C-20 geranylgeranyl groups to Rab superfamily proteins is performed by GGTase II, whereas, all other geranylgeranylation is carried out by GGTase I (Fig. 1) [1]. These post-translational modifications enable target proteins to localise to the correct site in the cell and undertake their normal biological function. Given its important role there is now considerable interest in determining whether targeting the enzymes in the mevalonate pathway that regulate prenylation can be used therapeutically to prevent uncontrolled cell proliferation and growth, particularly in cancers such multiple myeloma.

Multiple myeloma is a B-cell neoplasm characterised by the uncontrolled growth of malignant plasma cells in the bone marrow and the development of osteolytic bone disease. Currently, the only treatment for myeloma bone disease is with bisphosphonates (BPs) [2–5]. At the cellular level, BPs induce apoptosis of osteoclasts and inhibit bone resorption. The nitrogen containing bisphosphonates (N-BPs), such as risedronate, do this by inhibiting farnesyl diphosphate synthase (FPPS) (Fig. 1) [6–8]. These agents have been reported to be associated with anti-myeloma activity *in vivo*, although this is likely to be mediated via the inhibition of bone resorption, and the removal of a supportive microenvironment, rather than a direct effect [9,10]. However, we have also shown that it is geranylgeranylated proteins that are the dominant form of prenylated proteins required for inhibiting apoptosis of myeloma cells *in vitro* [11]. This raises the possibility that targeting other enzymes in the mevalonate pathway, such as the GGTases, may also have significant anti-myeloma effects.

Recently an inhibitor of GGTase II, known as 2-[3-pyridinyl]-1-hydroxyethylidene-1,1-phosphonocarboxylic acid (3-PEHPC, previously known as NE10790) has been described [12–14]. 3-PEHPC specifically prevents Rab prenylation and has been shown to inhibit bone resorption *in vitro* [13,15] and *ex vivo* [14]. In a similar manner to the bisphosphonate, risedronate, from which it was derived. 3-PEHPC has also been shown to cause apoptosis of human myeloma cells [12]. However, it is unclear whether 3-PEHPC will induce apoptosis of tumor cells *in vivo*. Since 3-PEHPC has a lower bone binding affinity than risedronate and other N-BPs [15], we hypothesised that tumor cells may be exposed to higher concentration of 3-PEHPC in bone than higher affinity compounds such as risedronate [10]. This may result in significant anti-tumor effects *in vivo*.

In the present study we have investigated the effects of 3-PEHPC and its parent bisphosphonate, risedronate, on the development of multiple myeloma, and the associated bone disease *in vivo*, in the 5T2MM murine model of myeloma.

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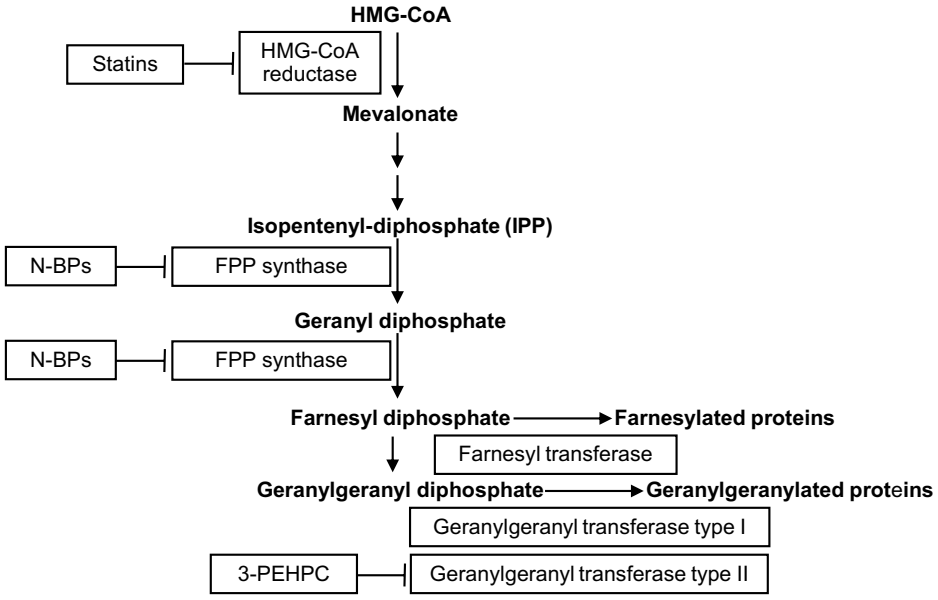

Fig. 1. The mevalonate pathway and known inhibitors. Statins block the action of HMG-CoA reductase, N-BPs inhibit FPP synthase and 3-PEHPC inhibits GGTase II.

Materials and methods

The 5T2MM syngeneic model of multiple myeloma. The 5T2MM murine model of multiple myeloma originated spontaneously in elderly C57BL/KaLwRij mice [16]. 5T2MM cells have been propagated since, *in vivo*, by the intravenous transfer of diseased bone marrow into young syngeneic mice [17]. Male 6-week-old C57BL/KaLwRijHsd mice were obtained from Harlan CPD (Horst, The Netherlands). 5T2MM cells were isolated from the bone marrow of disease-bearing animals, purified, and injected, via the tail vein, into young recipient mice as described previously [18].

Treatment of 5T2MM-bearing mice with 3-PEHPC or risedronate. Animals were grouped (10 per group) and treated as follows: group 1 = naive, group 2 = 5T2MM + vehicle (PBS), group 3 = 5T2MM + risedronate, group 4 = 5T2MM + 3-PEHPC. Animals treated with 3-PEHPC or risedronate received 125 μg/kg, subcutaneously, twice weekly, from the time of tumor cell injection (2×10^6 5T2MM cells) until sacrifice at 12 weeks. Both compounds were supplied as hydrated disodium salts by Proctor & Gamble Pharmaceuticals (OH, USA).

Radiographic analysis of osteolytic bone lesions. At sacrifice, femora and tibiae were radiographed using a Faxitron X-ray system (Hewlett Packard, OR, USA) and the numbers of osteolytic bone lesions were counted manually.

Histological and histochemical analysis of myeloma bone disease. Femora and tibiae were fixed in 10% formalin, decalcified in EDTA, and embedded in paraffin. 4 μm sections were cut and stained with haematoxylin and eosin (H&E). Cancellous bone area as a proportion of the total area (Cn.Ar/T.Ar%) was determined in the distal femoral metaphysis and proximal tibial metaphysis, in an area of 0.625 mm^2 starting 0.25 mm from the growth plate, using the dedicated OsteoMeasure Advanced Bone Histomorphometry Video System (Osteometrics, Inc., Decatur, GA, USA). Sections of both femora and tibiae were also stained for the presence of tartrate resistant acid phosphatase (TRAP) (Sigma, Poole, UK), to identify osteoclasts, and counterstained with Gills Haematoxylin. The number of osteoclasts present on a 3 mm length of each cortico-endosteal surface, beginning 0.25 mm from the growth plate, was counted. The data are expressed as the number of osteoclasts per mm of bone surface (N.Oc/Ec.Pm/mm) and as the proportion of bone surface covered by the osteoclasts (Oc.Pm/Ec.Pm%).

Analysis of the effect of 3-PEHPC or risedronate on tumor burden. After sacrifice the effects of 3-PEHPC and risedronate on indices of tumor burden were determined. These included the measurement of serum paraprotein levels as previously described [19], and the proportion of 5T2MM cells occupying the bone marrow. The latter was assessed by determining plasmacytosis on cytosmears, as described previously [19], and by examining H&E stained histological sections of the tibia, where the distinct morphology of the 5T2MM cells distinguishes them from normal marrow. The proportion of bone marrow occupied by 5T2MM cells was assessed in eight defined areas of 0.625 mm^2 starting 0.25 mm from the growth plate using the Leica QWin image analysis system (Leica Microsystems, Milton Keynes, UK).

Statistical analysis. All data were analysed using a Mann–Whitney *U* test or one-way analysis of variance with Tukey post hoc test. Data are presented as means ± standard error unless otherwise stated and were calculated using GraphPad Instat version 3.06 (CA, USA).

Results

Inhibiting GGTase II with 3-PEHPC decreases osteoclast numbers in vivo

We first examined the affects of 3-PEHPC on osteoclast number and the bone surface occupied by osteoclasts. TRAP-positive osteoclasts were seen lining the cortico-endosteal bone surfaces of naïve and 5T2MM-bearing mice (Fig. 2A). There was a significant increase in osteoclast surface in 5T2MM-bearing mice when compared to naive mice ($p < 0.01$) (Fig. 2B). In contrast, 5T2MM-bearing mice treated with either 3-PEHPC or risedronate had a reduction in osteoclast surface compared to 5T2MM-bearing mice treated with vehicle ($p < 0.001$ and $p < 0.001$, respectively) and naïve mice ($p < 0.05$ and $p < 0.05$, respectively).

5T2MM-bearing mice also had a significant increase in numbers of osteoclasts when compared to naive mice ($p < 0.05$) (Fig. 2C). In contrast, the 5T2MM-bearing mice treated with either 3-PEHPC or risedronate had significantly reduced osteoclast numbers compared to both 5T2MM-bearng mice treated with vehicle ($p < 0.001$ and $p < 0.001$, respectively) and naïve mice ($p < 0.05$ and $p < 0.05$, respectively).