Doxycycline and other tetracyclines in the treatment of bone metastasis

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The tetracycline family includes tetracycline, doxycycline and minocycline, all of which have been used as antibiotics effectively for decades. New uses emerged for these compounds after their effect on mitochondrial function was discovered. Cytostatic and cytotoxic activity of these compounds was shown against cell lines of various tumor origins. In addition, tetracyclines and chemically modified tetracyclines inhibit the activity of several matrix metalloproteinases (MMPs). Given the importance of these enzymes in tumor cell invasion and metastatic ability, the potential use of tetracyclines in cancer therapy needed to be investigated. Col-3, a chemically modified tetracycline, is now the subject of clinical trials in cancer patients. However, the potential of tetracyclines in cancer therapy takes on an added dimension in the bone. MMPs have been shown to be important mediators of metastasis formation in the bone, contributing largely to the morbidity of breast cancer and prostate cancer patients. The natural osteotropism of tetracyclines would allow them to be highly effective in the inhibition of MMPs produced by osteoclasts or tumor cells in the bone. This hypothesis has now been confirmed by experimental evidence showing that doxycycline reduces tumor burden in a mouse model of breast cancer-derived osteolytic bone metastasis. This effect is likely due to a combination of multiple roles of doxycycline, including MMP inhibition and a negative effect on osteoclast differentiation and survival. These encouraging results have now paved the way for an ongoing trial of doxycycline in early combination therapy for breast cancer and prostate cancer patients.
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Introduction: new functions for an old class of compounds

The tetracyclines are a well-known and widely used family of antibiotics. These compounds are particularly useful in several types of both common and rare infections including atypical pneumonias, rickettsial infections, chlamydial infections, Lyme disease and ehrlichiosis [reviewed in 1]. Doxycycline is the most widely used of the tetracyclines today, although minocycline is commonly applied to the treatment of acne vulgaris [2]. Doxycycline is frequently used in a first-line therapy, such as in the treatment of uncomplicated genital *Chlamydia trachomatis* infections [3] or acute Q fever [4]. In addition, doxycycline may be used after initial therapy has failed due to decreased antibiotic sensitivity or antibiotic resistance, as in the case of infections with penicillin-resistant *Streptococcus pneumoniae* [5].

The bacteriostatic activity of tetracyclines lies in their capacity to inhibit protein synthesis by preventing the binding of the aminoacyl t-RNA to the ribosome [6]. Because of the similarity between the prokaryotic protein synthesis machinery and that of eukaryotic mitochondria, tetracyclines are also able to interfere with mitochondrial protein synthesis in mammalian cells. The selective permeability of different mammalian cells to tetracyclines led to the hypothesis that these agents could be used to achieve cell proliferation arrest and applied to the treatment of malignancies [7]. As with many emerging new ideas, research into this hypothesis was slow to gain interest and for more than a decade would remain confined to a handful of research groups.

Cytotoxic effect of tetracyclines on cancer cells

Emerging from a research group's focus on mitochondrial function and the use of antibiotics for selective inhibition of mitochondrial processes was the realization that tetracyclines may have cytostatic activity, confirmed by initial studies showing inhibition of growth of carcinogen-induced tumors [8]. Specific cytostatic activity due to the selective permeability of different cells to tetracyclines was tested and confirmed by doxycycline inhibition of

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tumor cell proliferation in permeable T cell leukemia of the rat, but not in impermeable erythroid and B lymphoid cells [9]. These results in an animal model were subsequently tested in tumor systems of human origin; doxycycline was found to be cytostatic to human renal and prostate carcinoma cells, and even cytotoxic after prolonged treatment [10]. Cytostatic activity of tetracyclines was confirmed in fibroblasts and sarcoma cells, and the proliferation arrest in these cells was shown to be due to an accumulation of the cell population in the G_1 phase of the cell cycle [11]. Other *in vitro* studies have now confirmed the cytotoxic effects of tetracyclines on cultured human prostate cancer, breast cancer, osteosarcoma and mesothelioma cells [12–15].

Although tetracycline and at least two of its derivatives, doxycycline and minocycline, all show cytotoxic effects in these experiments, doxycycline was found to be the most effective at inhibiting survival in a side-by-side comparison of three human adenocarcinoma cell lines [12]. DNA fragmentation indicative of apoptosis was observed after doxycycline cytotoxic treatment of renal and prostate carcinoma cells [13,14]. In addition, doxycycline can induce apoptosis in Jurkat T lymphocytes [16], in HL60 leukemia cells [17] and in a T lymphoblastic human leukemia cell line by caspase-3 activation [18]. Conversely, doxycycline has a protective effect against apoptosis in polymorphonuclear neutrophils isolated from healthy donors, both in the presence and the absence of an infective agent [19], and a protective effect against apoptosis in neurons treated with ionizing radiation [20]. This apparent cell-specific selectivity of the apoptotic or cytotoxic effect of doxycycline does not appear to be a result of differences in experimental methodology, since a single study comparing doxycycline toxicity in different cell types showed a cytotoxic effect on cells of myeloid lineage but not on those of mesenchymal origin [21]. This apparent cytotoxic selectivity of doxycycline towards certain cells, in particular those of tumoral origin and not normal neutrophils, is an exciting find in the area of emerging cancer therapies, in particular in light of the known toxicity of many antineoplastic agents towards cells of the hematopoietic lineage.

The discovery of the cytotoxic activity of tetracyclines on tumor cells may help explain the molecular cellular mechanisms underlying the use of these compounds in an established treatment setting. A significant subset of metastatic cancer patients develop malignant pleural effusions, usually treated surgically by tube thoracostomy [reviewed in 22]. Doxycycline is often used in this type of treatment as an intrapleural sclerosing agent [23]. However, evidence from an experimental mouse model suggests that, besides the sclerotic effect, the suppression of malignant cell growth contributes significantly to the efficiency of this compound in the management of malignant pleural effusions [24]. Thus, doxycycline may have already found an effective but as-yet-unrecognized antineoplastic medical application.

Doxycycline and chemically modified tetracyclines inhibit the activities of matrix metalloproteinases (MMPs)

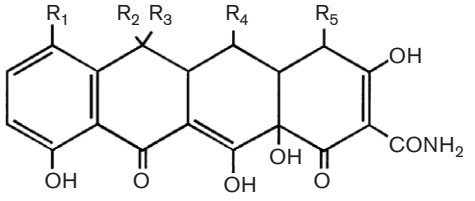
In parallel to the discoveries of the cytostatic and cytotoxic abilities of the tetracyclines was the mounting evidence that these compounds have the ability to inhibit the activities of various MMPs. Minocycline was fortuitously found to inhibit the collagenase activity of gingiva in diabetic rats [25] and this effect was determined to be independent of its antimicrobial activity. Evidence of collagenase inhibition was confirmed in samples of human synovial tissue or fluid from patients before and after minocycline treatment [26]. The collagenase activity produced by a wide variety of tumor cell lines *in vitro* was subsequently found to be inhibited by tetracyclines, including breast and prostate carcinoma, osteosarcoma and mesothelioma cells [12–15,27]. Comparisons between tetracycline, minocycline and doxycycline show doxycycline to possess the most potent collagenase inhibitory activity when tested in an immunoassay with gelatine as a substrate [28]. In line with the potency of its gelatinase inhibitory activity, doxycycline has been used consistently to inhibit the activity of MMPs from various cell types [12,14,15,29,30]. In addition to inhibiting the activity of these enzymes, tetracyclines have been shown in some cases to regulate the expression of MMPs at the RNA and protein levels, such as in the case of MMP-8 [29]. The confirmed activity of doxycycline in MMP inhibition has already found application in one of the many diseases that present pathological activation of these enzymes, specifically adult periodontitis. In this disease, a low-dose doxycycline treatment was found to be a valuable adjunct to instrumentation therapy [31,32] and is now an FDA-approved therapeutic option.

A few studies have gone further to identify the collagenolytic enzymes inhibited by tetracyclines, specifically MMP-1 [30], MMP-2, MMP-9 [12] and MMP-8 [29]. This list may not be exhaustive and the activity of other MMPs may also be found to be inhibited by tetracyclines. However, the identity of the MMPs identified so far adds to the interest that tetracyclines present in their potential use in the treatment of cancer. Positive or high expression of MMP-1, MMP-2 and MMP-9 is associated with poor prognosis or survival in almost every type of human cancer studied [reviewed in 33], partly due to the association of these enzymes with mechanisms of basement membrane degradation by cancer cells leading to invasion and metastasis. Therefore, MMP inhibition has emerged as a valid therapeutic goal in malignant disease treatment. To this effect, a number

of MMP inhibitors are in various stages of drug development and testing [reviewed in 34]. Doxycycline is, because of the data discussed above, a promising compound in this category. To add to its interest is the recent discovery that it is able to inhibit angiogenesis in an *in vitro* assay [35], a property which would only further contribute to its anticancer potential given the known contribution of angiogenesis to tumor growth and invasion. Doxycycline is currently in phase I trials for cancer therapy. One of the issues uncovered in these trials, however, is dose-limiting toxicity exhibited by symptoms such as fatigue and nausea [36]. This is likely due to the fact that many of the cytostatic, cytotoxic and anticollagenase effects of doxycycline leading to antitumoral behavior were observed at concentrations usually higher than those needed for bacteriostatic effects, and thus in an anticancer trial the effects of doxycycline at higher doses needed to be tested. Conclusions concerning the *in vivo* acceptability and efficacy of anticancer doxycycline doses in a first-line therapy will have to be suspended pending the final outcome of these trials.

Another interesting MMP inhibitor also belongs to the tetracycline family, Col-3 (4-dedimethylaminosancycline, also known as CMT-3), a chemically modified tetracycline (Fig. 1). Chemically modified tetracyclines that lack antimicrobial activity, but still possess anticollagenase, activity have been developed and tested for various properties [reviewed in 37]. In the context of cancer research, they elicited a certain amount of interest because they effectively inhibit MMPs and possess some degree of selective cytotoxicity, similar to their antimicrobial tetracycline counterparts. Col-3 appears to be the most promising of the chemically modified tetracyclines, demonstrating inhibition of MMP-14 activity which leads to lack of activation of MMP-2 [38] and an antiangiogenic effect similar to doxycycline in an *in vitro* assay [35]. Col-3 also inhibits colon cancer cell invasiveness [39], and inhibits cell proliferation, invasion, tumor growth and metastasis in a metastatic prostate cancer model to the same extent as, and in some cases more efficiently, than doxycycline [40]. Once again, some degree of cell specificity is observed, since in leukemia cell lines the effects of Col-3 are not as strong and comparable to those generated by doxycycline [17]. Regardless of cell-specificity arguments, the potential of Col-3 was important enough to warrant clinical testing and was the subject of at least two phase I clinical trials. Emerging from one trial was the observed effect of disease stabilization in some patients with a non-epithelial form of malignancy [41], although concerns have also arisen due to drug-induced side-effects such as lupus and anemia [41–43]. Another phase I trial was conducted in a more homogeneous study population, eighteen patients with AIDS-related Kaposi's sarcoma. The results of this trial indicated antitumor activity in this population at well-tolerated doses, with adverse effects including photosensitivity and headache [44]. It is interesting to note that the variable response noted in this study may be partially explained by recent evidence from animal studies regarding factors that influence irregular absorption profiles of Col-3 after oral administration [45]. These factors include drug particle size, presence of food and levels of endogenous bile, and taking them into account in phase II trials may permit more homogeneous responses in the patient population.

Fig. 1



Name	R_1	R_2	R_3	R_4	R_5
Tetracycline	H	CH_3	OH	H	$N(CH_3)_2$
Minocycline	$N(CH_3)_2$	H	H	H	$N(CH_3)_2$
Doxycycline	H	CH_3	H	OH	$N(CH_3)_2$
Col-3	H	H	H	H	H

Chemical structures of the tetracycline family members discussed.

Effect of doxycycline and other tetracyclines on bone

The ability of tetracyclines to inhibit MMP activity of certain cancer cells points to other possible applications for this family of compounds. MMPs are known to be important mediators of metastasis formation in bone [reviewed in 46], contributing to significant morbidity in cancer patients, especially those with tumors of the prostate or breast. Formation of osteolytic lesions directly by cancer cells is partially mediated by MMPs [47,48]. Osteoclast-mediated osteolysis is also dependent on MMPs, not in the initial phase of bone mineral resorption but for unmineralized matrix degradation [46]. Thus, MMPs have a 2-fold role in osteolytic cancer lesion formation, being produced directly by the tumor cells and by osteoclasts after induction by tumor cells. The role of MMPs is likewise important in other osteolytic lesions such as osteoporosis. To this effect, MMP inhibitors are being studied in the treatment of osteoporosis, including the tetracycline family. In ovariectomized aged rats, a model for postmenopausal osteoporosis, minocycline was able to both increase bone formation and decrease bone loss in trabecular bone, comparatively to estrogen [49,50].

In a subsequent study, minocycline was found to stimulate the colony-forming efficiency of marrow stromal cells derived from ovariectomized rats, possibly explaining the effect of this compound on increased bone formation [51]. As for the decrease in bone resorption, it may be explained by the MMP inhibitory activity of minocycline and also by other data on osteoclasts. Doxycycline has been shown to reduce osteoclast numbers in rat models of surgically induced osteoclast recruitment [52,53]. A range of tetracyclines, including doxycycline and Col-3, were able to induce apoptosis in mature osteoclasts and inhibit *in vitro* osteoclastogenesis, possibly by functions independent of the antibiotic and anticollagenase properties of these products [54].

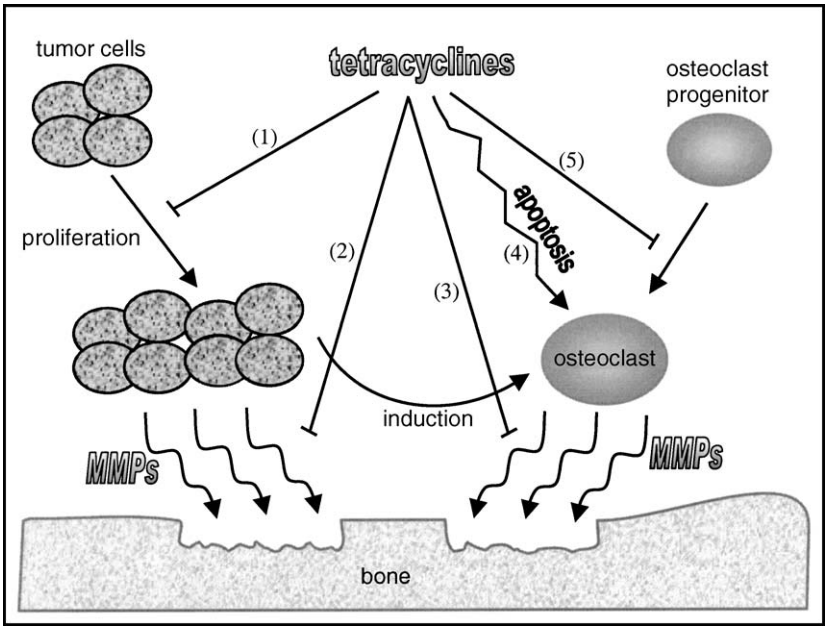
Multiple effects of doxycycline combine to reduce tumor burden in bone

It is now clear that doxycycline and other tetracyclines have many functions that make them of potential interest in anticancer therapy (Fig. 2). However, the observed effects of tumor cytotoxicity, MMP inhibition and inhibition of osteoclast viability take on an added dimension in the bone, in particular in light of the fact that tetracyclines are highly osteotropic. Thus, the concentrations of these drugs needed to achieve the desired effects are present in the bone even while serum concentrations are below toxic levels, and the undesirable toxic side-effects observed in clinical trials are not likely to be encountered. This hypothesis was validated recently in a mouse bone metastasis model of human breast cancer. The model, first described by Sasaki *et al.* [55], is produced by intracardiac injection of MDA-MB-231 human breast carcinoma cells, leading to bone metastases. Doxycycline treatment of mice prior to injection, reminiscent of early metastatic disease, effectively and significantly reduced tumor burden [56]. In addition, osteolytic lesions were reduced in the treated group and the bone resorption parameters and number of osteoclasts were lower, while bone formation parameters were higher. This study confirms the potential of doxycycline and possibly other tetracyclines in lowering tumor burden and osteolytic lesions due to cancer cells *in vivo*. It is especially interesting in light of the fact that similar animal studies with the bisphosphonate risedronate resulted in comparable though less effective outcomes [55], in particular knowing that bisphosphonates are accepted therapeutic options in the prevention of bone lesions in breast cancer. It remains to be seen whether other tetracyclines, in particular the chemically modified tetracyclines such as Col-3, have similar or greater effects and whether this therapy is beneficial in patients with established bone metastases.

The near future: advantages, trials and concerns

Among the many advantages of doxycycline is the fact that it has been prescribed for years without major side-effects being experienced. Therefore, at the doses used

Fig. 2



Representative scheme of the putative modes of action of tetracyclines in the bone: (1) inhibition of tumor cell proliferation, (2) inhibition of tumor cell MMP production, (3) inhibition of osteoclast MMP production, (4) induction of osteoclast apoptosis and (5) inhibition of osteoclastogenesis. MMPs are depicted as non-exclusive contributors to bone degradation by tumor cells and osteoclasts.

for reduction of bone metastases, it should not present any important toxicity. This may be especially true if it is prescribed at normal antimicrobial doses early on in a therapy regimen, as preventive therapy against bone metastases. The potential of such combination therapy is now being tested at our institute with a phase II trial of doxycycline in early-stage breast and prostate cancer patients. The major aims of this trial are to verify the potential of doxycycline in affecting serum bone markers in these patients. Preliminary results for this trial should be available in late fall of 2003.

Among the existing concerns surrounding the long-term use of doxycycline for non-antimicrobial purposes is the potential for development of antibiotic resistance. Although the concern is justified, it may not necessarily outweigh the potential for efficacy in the reduction of bone metastases. However, it is important enough to justify continuing research into the potential of non-antimicrobial tetracyclines, in particular Col-3. In this regard, it would be important to determine whether the activity of Col-3 in the inhibition of bone metastases is as important as that of doxycycline, and whether the toxicity and adverse effects of the former are comparable or lower to that of the latter. Either inadequate efficacy or too high a toxicity may negate the advantage of using a non-antimicrobial drug, in particular in the kind of patient where toxicity and adverse effects are already important considerations.

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