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Antimicrobial and non-antimicrobial tetracyclines in human cancer trials

Christopher Richards [a], Liron Pantanowitz [b], Bruce J. Dezube [a,*]

[a] Department of Hematology and Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States
[b] Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, United States

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ABSTRACT

Tetracyclines are capable of inhibiting mammalian collagenases by non-antimicrobial mechanisms. Because collagenases and other matrix metalloproteinases have been linked to cancer pathogenesis, this property of tetracycline's has led to speculation that these drugs could be used to slow tumor growth, invasion and metastasis in neoplasms that overly express these enzymes. The FDA has already approved two tetracycline derivates for treatment of chronic inflammatory periodontal disease and chronic inflammatory skin disease. Here we review the efforts to determine the efficacy of tetracyclines as chemotherapeutics in human cancer trials. While the majority of clinical trials have yielded disappointing results, tetracyclines have been shown to be generally well tolerated and have significant anti-proliferative effects in certain cancer types. In particular the chemically modified tetracycline derivative COL-3 (also known as CMT-3) has been shown to cause dramatic improvement in the tumor burden of patients with Kaposi Sarcoma. The experience using tetracyclines as chemotherapeutics is relatively limited, but further success is possible if future trials are focused on specific cancer subtypes that are known to rely heavily on collagenases and other matrix metalloproteinases for their pathogenesis.

1. Introduction

Based on the extensive literature describing the enzymatic inhibition and related anti-inflammatory properties of tetracycline's, we investigated the non-antibiotic properties of tetracyclines in human cancer trials. Tumorigenesis is a complex and multistep process by which neoplastic cells sequentially acquire the ability to proliferate, evade the immune system, invade local tissues, recruit and build a supportive blood supply, and finally metastasize and colonize distant tissues [1]. One of the most studied areas of tumor development and a target for potential chemotherapeutics has been the process of angiogenesis and invasion of tumors into surrounding tissues. Many preclinical and human trials have been aimed at inhibiting this process with varying success. It has long been understood that extracellular proteases, MMPs among them, are essential to the process of angiogenesis [2]. They have been shown to be up-regulated in virtually all human and animal tumors and have become a promising target of new therapies that have so far yielded mixed results in human trials. Among the variety of attempted synthetic and exogenous MMP inhibitors, tetracycline derivatives will be reviewed here.

2. Matrix metalloproteinases and their role in angiogenesis

The complex process of angiogenesis can be broken into two phases. The first is a period of activation that starts with a local increase in vascular permeability and deposition of a fibrin matrix that precedes endothelial cell dissociation, proliferation, migration and invasion through the ECM culminating in the formation of a new vessel lumen. The second phase of this process is one of resolution where endothelial cells stop proliferating and migrating, deposit a collagenous basement membrane, begin forming tight junctions between them and recruit pericytes to create a mature vasculature, which will feed the growing tumor [3]. Early understanding of the role that MMPs play in this process was limited to facilitating the migration of endothelial cells through the ECM. Further study has demonstrated that MMPs not only disrupt the ECM, but also create a tumor microenvironment at the cell surface via three distinct processes. First, MMP activity exposes the matricryptic domains on the collagenous basement membrane necessary for endothelial cell adherence [4]. Next, MMP degradation of the ECM releases tumor growth factors such as VEGF and BFGF [5]. Finally, MMP activity releases antiangiogenic factors

Abbreviations: MMP, Matrix metalloproteinase; ECM, Extracellular matrix; VEGF, Vascular endothelial growth factor; BFGF, Basic-fibroblast growth factor; LFTs, Liver function tests; AMC, AIDS Malignancy Consortium; NCI, National cancer institute; HIV, Human immunodeficiency virus; AIDS, Acquired immunodeficiency syndrome; HAART, Highly active antiretroviral therapy; KS, Kaposi Sarcoma; RCC, Renal cell cancer; OAL, Ocular adnexal lymphoma; IL-2, Interleukin-2; TNF-α, Tumor necrosis factor-α.

* Corresponding author at: Beth Israel Deaconess Medical Center, 330 Brookline Ave., Boston, MA 02215, United States. Tel.: +1 617 964 1126; fax: +1 617 632 9296.
E-mail address: brucedezube@gmail.com (B.J. Dezube).

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including MMP specific tissue inhibitors, the non-specific protease inhibitor alpha2-macroglobulin and the membrane anchored reversion-inducing cysteine-rich protein with Kazal motif (RECK), which significantly limit angiogenesis and tumor invasion through the ECM [6]. In order to successfully occur, the process of angiogenesis is now understood to require an appropriate balance between these competing forces. Too little MMP activity, seen either in knock out mice studies or with exogenous inhibitors, including tetracyclines, have demonstrated abnormal and non-viable tumor development [7].

MMPs are a class of 24 calcium-dependent proteases with a conserved catalytic motif consisting of three histidine residues that hold a Zn^{2+} ion and a nearby glutamic acid that is essential for peptide bond hydrolysis. Based largely on their substrate specificity, MMPs are divided into the collagenases (MMP-1 MMP-8, MMP-13), the gelatinases (MMP-2, MMP-9), stromelysins or proteoglycanases (MMP-3, MMP-7, MMP-10, MMP-11), elastase (MMP-12), the membrane type-MMPs (MT1-MMP, MT2-MMP, MT3-MMP, MT4-MMP) as well as a handful of unclassified MMPs [8]. The gelatinases (MMP-2 and MMP-9) are thought to be of greatest importance for angiogenesis as they degrade collagen IV, the major component of basement membranes [9]. Evidence for the roles of MMP-2 and MMP-9 role comes from experiments showing their increased expression in tissues exposed to VEGF and B-FGF, as well as to hypoxic conditions [10]. *In vitro* studies with MMP-2 and MMP-9 specific inhibitors, including tetracyclines have been shown to stop the process of endothelial cell invasion in collagenous stroma [11]. Evidence further supporting the importance of MMP-2 and MMP-9 comes from studies with MMP-2 and MMP-9 in knock out mice in which implanted human tumors develop abnormal angiogenesis as compared to normal controls [12]. Elevated levels of MMPs have also been found in the urine of cancer patients, serving as important surrogate markers of drug effect in therapeutic investigations [13,14].

3. MMPs as therapeutic targets in oncology

Given their intimate role in the process of tumor angiogenesis, MMPs have become a target of potential chemotherapeutics [15,16]. At present, MMP inhibitors have had limited success in human trials. While this review focuses on the role of tetracyclines in human cancer trials, it is worth mentioning that MMP inhibitors from other drug classes have also been explored. The most promising result from non-tetracycline MMP inhibitors is Marimastat (BB-2516) which in a phase I trial for treatment of gastric cancer showed a modest survival benefit in treatment naive individuals ($p = 0.07$) and patients who had received prior therapy ($p = 0.045$) [17]. However, in the same study Marimastat failed to demonstrate any significant survival benefit in patients with glioblastoma or pancreatic, non-small-cell lung, small-cell lung, or ovarian cancer. The drug Tanomastat, a biphenyl MMP inhibitor, had less promising results, and trials in patients with small-cell lung and pancreatic cancer had to be terminated prematurely because of poorer survival compared to controls [18].

4. Tetracyclines as therapeutics in oncology: pre-clinical work

The initial finding that tetracyclines may have activity against MMPs came from a 1983 study by Golub et al. looking at the role collagenolytic enzymes played in the accelerated periodontitis during experimentally induced type I diabetes [19]. Their hypothesis was that Gram-negative bacteria induced the excessive expression of mammalian collagenase in the gingiva, which mediated pathologic collagen breakdown and periodontal bone resorption, and that treating the infection with minocycline would halt disease progression. The experiment compared the amount of collagenase activity in two groups of rats with streptozotocin-induced diabetes, one group colonized with disease-producing bacteria and the other germ free; both groups of diabetic rats received minocycline therapy. They found that minocycline had an almost identical beneficial effect in germfree (70% reduction) and colonized animals (62% reduction). They attributed this therapeutic improvement to the previously unrecognized ability of tetracyclines, such as minocycline, to inhibit collagenase activity. This finding has subsequently been confirmed *in vitro* [19,20]. They also developed the first of a series of chemically modified tetracyclines, in which the antimicrobial activity of the drug was eliminated but which retained its collagenase-inhibitory property, and described this compound, CMT-1 (4-dedimethylamino tetracycline) in 1987 [21,22]. One of the most potent chemically modified tetracyclines, CMT-3 or COL-3 (6-deoxy 6-demethyl 4-dedimethylamino tetracycline), was first developed and described by the same investigators in 1991 [23].

COL-3 is unique among MMP inhibitors, as it appears to inhibit MMP activity through three different mechanisms: by altering production, activation and activity [24]. COL-3 has been shown to inhibit MMP-2 and MMP-9 in C8161 melanoma cells and human neonatal foreskin fibroblasts and to have growth inhibitory effects on the human cancer cell lines BPH-1, DU-145, PC-3 and FHS-733 [18]. Interestingly, COL-3 has also been shown to have activity against phospholipase A_2 and is capable of inhibiting nitric oxide synthase [25]. In a study of the antitumor and anti-metastatic effects of COL-3 on a rat model of metastatic prostate cancer, COL-3 decreased primary tumor growth by 27–35% and reduced metastases by 58% compared to controls [26].

An alternate mechanism for the anti-oncogenic properties of tetracyclines has been proposed that relates to the compounds ability to inhibit mitochondrial protein synthesis. In prokaryotes, tetracyclines prevent the binding of aminoacyl t-RNA to the ribosome resulting in bacterostasis. This effect is also seen in mammalian cells due to the similarity of the protein synthesis machinery [27]. Tetracyclines have been shown to have cytostatic effects in human renal and prostate carcinoma cells *in vitro,* an effect that can become cytotoxic after prolonged treatment [28]. This finding lead to early speculation that tetracyclines may have clinical antineoplastic applications in addition to their well established use as antibiotics.

5. Tetracyclines as therapeutics in oncology: human trials

The first human clinical trial to look at tetracycline-facilitated inhibition of cancer was undertaken in 2001 by Rudek et al. using COL-3 [29]. This was a phase I clinical trial designed to determine the maximum-tolerable dose and the dose-limiting toxicities in patients with refractory solid tumors. The study's secondary outcomes were disease progression as well as changes in the circulating serum levels of MMP-2, MMP-9, VEGF and B-FGF. The trial recruited 35 patients with refractory solid cancers who were given escalating doses of COL-3 starting with 36 mg/m^2/day. The study found that the cutaneous photoxicity was dose-limiting at 98 mg/m^2/day. Patients were able to consistently tolerate doses of 70 mg/m^2/day by applying liberal amounts of sunscreen. The study also revealed a number of less frequent non-dose related toxicities including anemia, constipation, dizziness, elevated liver function tests (LFTs), fever, headache, nausea and vomiting, peripheral and central neurotoxicities, reversible sideroblastic anemia, and three cases of drug-induced lupus [20,30]. With regard to the secondary outcomes of the study, patients with hemangioendotheliomas, Sertoli-Leydig cell tumors and fibrosarcoma were observed to have stable disease for 26 months, 8 months, and 6 months,