Effects of doxycycline on human prostate cancer cells in vitro

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Abstract

Prostate cancer is the most common form of cancer in older men and the major cause of death from prostate cancer is metastatic disease. The matrix metalloproteinases (MMPs) play a significant role in the growth, invasion and metastasis of many tumors, including those of the prostate. We previously demonstrated that doxycycline, a synthetic tetracycline, inhibits MMPs and cell proliferation and induces apoptosis in several cancer cell lines. We also demonstrated that in an in vivo model of metastatic breast cancer in athymic mice doxycycline inhibits tumor size and regrowth after resection. In the present study, gelatinolytic activity in the human prostate cancer cell line, LNCaP, was suppressed and significant inhibition of cell growth occurred after exposure to 5 or 10 μg/ml of doxycycline, while cell growth was normal in untreated cells. Radioisotope incorporation into proteins was reduced by doxycycline. DNA fragmentation, consistent with apoptosis, was demonstrated in cells treated with doxycycline. These data suggest that doxycycline may have potential utility in the management of prostate cancer.

Keywords: Doxycycline; Human prostate cancer cells; In vitro

1. Introduction

Prostate cancer is the most common cancer in American men over the age of 65 years and is the second leading cause of cancer deaths in these individuals. Most deaths from prostate cancer, as from other malignancies, result from metastatic disease. Numerous mechanisms are involved in metastasis, including increased activity of the matrix metalloproteinase (MMP) family of enzymes. MMPs degrade the extracellular matrix surrounding cancer cells, allowing them to spread beyond the primary site [1–3] and MMPs also enhance angiogenesis, or new blood vessel formation, a crucial phenomenon in metastasis [4]. Another factor contributing to metastasis is the relatively unrestrained growth characteristic of most tumors. Many cancer cells exhibit a diminution in apoptosis, or programmed cell death, which is normally involved in the removal of damaged or senescent cells and which appears to be regulated in part by oncogenes and tumor suppressor genes and their protein products [5,6]. This suppression of apoptosis probably plays a role in the unchecked growth and spread of tumors.

The tetracyclines (TCNs) are a group of antibiotics that have an inhibitory effect on MMP activity [7–11]. The mechanism of this inhibition remains
unclear, but it has been postulated to result from at least one of the following: (a) binding of calcium at the active site of the enzyme, which is critical to MMP function; (b) steric hindrance in the interaction of the enzyme with TCN; or (c) binding of TCN to the enzyme substrate [12]. We recently have obtained evidence that the synthetic TCN, doxycycline, also selectively inhibits mRNA synthesis of types I and IV collagenase and stromelysin, thus suggesting yet another possible mechanism for its inhibitory action [13]. We now present data indicating that doxycycline kills human prostate cancer cells in vitro and that at least some of the observed cell death is due to enhanced apoptosis.

2. Materials and methods

2.1. Cells

LNCaP human prostate adenocarcinoma cells were purchased from the American Type Culture Collection (ATCC).

2.2. Cell proliferation

Cells were grown at a concentration of 10⁶ cells/ml in six-well Costar plates in MEM at 37°C for 6 days in a 95% O₂/5% CO₂ incubator. Aliquots of cells and medium were removed at 2-day intervals [12]. The following culture conditions were examined: control cultures without doxycycline and cultures containing 5 or 10 μg/ml of doxycycline. Cells were counted at each time-point.

2.3. Protein synthesis

To assess the synthesis of new proteins, portions of each culture were incubated with [³H]leucine for 2 h, followed by the removal of labeled medium and addition of fresh medium [14]. Cultures were maintained for 6 days and aliquots of medium were removed at 2-day intervals and counted in a scintillation counter after dialysis to remove free radioisotope.

2.4. Gelatin zymography

Equal aliquots of medium from each time-point in each culture condition were placed in wells of 9% SDS-polyacrylamide gels containing 1% gelatin. After electrophoresis, the gels were stained with Coomassie Blue and destained with 30% methanol and acetic acid [15,16]. Gels without gelatin were also prepared and proteins were stained with Coomassie Blue after electrophoresis.

2.5. DNA laddering

DNA was extracted from cells in each experimental condition after 2, 4 and 6 days in culture and electrophoresed in 1.2% agarose gels that were then stained with ethidium bromide to identify fragmentation (laddering) indicative of apoptosis [17,18].

3. Results

3.1. Cell proliferation

Growth curves for human prostate adenocarcinoma cells demonstrated that in the presence of 10 μg/ml of doxycycline, the cells did not grow and cell death was evident, as determined by Trypan Blue exclusion, as early as 2 days after the experiment began.
was initiated (Fig. 1). Cells treated with 5 μg/ml of doxycycline showed approximately a three-fold reduction in proliferation (Fig. 1). In contrast, untreated cells appeared healthy and demonstrated 90–95% viability by Trypan Blue exclusion at the end of the 6-day period.

3.2. Protein synthesis

The radiolabeling studies demonstrated that doxycycline inhibited general cell protein synthesis by approximately two- to four-fold compared with controls prior to cell death (Fig. 2).

3.3. Gelatin zymography

We examined the gelatinolytic activity of the prostate cancer cells in the presence and absence of 10 μg/ml of doxycycline using gelatin zymography. We demonstrated a 50–75% reduction in gelatinolytic activity in conditioned medium from doxycycline-treated cells compared with untreated cells (Fig. 3).

3.4. DNA laddering

The DNA gels demonstrated laddering of DNA most prominently in samples from cultures treated with 5 μg/ml of doxycycline for 2 days and 10 μg/ml of doxycycline for 2, 4 and 6 days in culture, indicating the occurrence of apoptosis, or programmed cell death (Fig. 4).

4. Discussion

We have been studying the effects of doxycycline on several types of cancer cells, specifically human breast cancer cells (MDA-MB-435), human prostate adenocarcinoma cells (LNCaP) and human osteosarcoma cells (U2OS), and have found inhibition of cell proliferation, gelatinolytic (MMP) activity, MMP mRNA synthesis and Matrigel invasion (an in vitro surrogate for metastasis) [12]. We previously conducted a trial of doxycycline administered either before implantation of breast cancer cells into athymic mice or at the time of resection of the primary tumors and showed significant reduction in the size of the primary tumors in the pretreated animals [19] and in the number of lung metastases in all animals [20].

In the present study, we have demonstrated that doxycycline kills prostate cancer cells and that at least some of this cell death is due to apoptosis, which is demonstrated by DNA laddering. Apoptosis is a normal mechanism for eliminating senescent or diseased cells and is suppressed in many cancers by
various factors, including oncogene and tumor suppressor proteins. The ability to ‘turn on’ apoptosis in cancer cells has great potential for inducing tumor remission. It is unclear at present whether there is any relationship between the effects of doxycycline on MMP activity and the induction of apoptosis; indeed, it is likely that these represent two discrete aspects of doxycycline activity. We also have shown elsewhere that doxycycline induces apoptosis in vitro in human osteosarcoma cells \[13\] and in human breast cancer cells \[21\].

The potential ramifications of these data are significant. If a relatively safe well-tolerated agent such as doxycycline can inhibit cell proliferation and induce apoptosis, it could represent a major addition to the treatment options available for patients with prostate cancer, especially for adjuvant therapy, and could reduce the metastatic burden in animals and, ultimately, in humans. We have begun a phase I clinical trial in humans to determine the best tolerated dose of doxycycline in patients with prostate cancer. Preliminary results indicate that serum levels of 5–10 $\mu$g/ml can be safely achieved with doses that are tolerated by most patients \[22\].

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