EFFECTS OF DOXYCYCLINE ON CANCER CELLS IN VITRO AND IN VIVO

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Mortality from cancer is usually due to metastatic disease, which represents the uncontrolled proliferation of cells that no longer respond to the normal regulatory controls of the organism (Silverberg et al., 1990). There are several factors which may accelerate metastasis, including: increased extracellular matrix degradation, due to increased matrix metalloproteinase (MMP) activity, which can facilitate tumor spread (Liotta et al., 1980; DeClerck et al., 1992; Azzam et al., 1993); increased angiogenesis, which is related, at least in part, to elevated MMP activity, permitting the hematogenous spread of tumors (Moscatelli and Rifkin, 1988; Tamargo et al., 1991); and decreased apoptosis, or programmed cell death, in cancer cells, allowing the abnormal cells to escape one of the principal mechanisms for their removal and survive to spread through the body (Armstrong et al., 1992; Furuya et al., 1994).

We have been examining the effects of doxycycline, a synthetic tetracycline (TCN), on several human cancer cell lines in vitro, as well as in vivo in the athymic mouse/xenograft model of metastatic human breast cancer produced by orthotopic implantation of the human breast adenocarcinoma cell line, MDA-MB-435, into the inframammary fat pads of athymic mice. The ability of doxycycline and other TCNs to inhibit MMP activity has been described in the literature. We have shown that doxycycline suppresses MMP activity and cell proliferation in this and other cancer cell lines. We hypothesize that doxycycline inhibits tumor growth in breast and other cancers by a direct effect on the regulation of cell proliferation, which may be distinct from its ability to inhibit MMP activity.

Key words: Doxycycline, cancer cells, matrix metalloproteinases.

METHODS

Cell lines
MDA-MB-435 human breast adenocarcinoma cells were a generous gift from Dr. J. Price, M.D. Anderson Cancer Institute, Houston, TX. LNCaP human prostate adenocarcinoma cells and U20S human osteosarcoma cells were obtained from the American Type Culture Collection (ATCC).

Athymic mouse model of human breast cancer
The in vivo system that we have used to examine the effects of doxycycline is the MDA-MB-435 human breast cancer/athymic mouse xenograft model, originally described by Price et al. (Price et al., 1990a,b; Cornetta et al., 1994). Briefly, six-to-eight-week-old athymic (nude) mice were anesthetized with methoxyflurane, following which a small incision was made in the skin over the lateral thorax, exposing the inframammary fat pad. MDA-MB-435 cells (5 x 10^5 cells/mouse) were injected into the fat pad (“orthotopic” implantation), and the incision was closed with staples. Following tumor implantation, the animals were evaluated weekly with bi-dimensional caliper measurements of the primary tumor. Ten weeks later, the mice were reanesthetized, and the primary tumor was resected. Approximately 6-10 wks after resection, the mice were killed by CO2 narcosis (Cornetta et al., 1993; Sledge et al., 1995).

Twenty mice served as controls and 20 as the experimental group, which received 20 mg/day orally of doxycycline, which produced serum levels of 1-3 μg/mL. The doxycycline was solubilized in a small amount of alcohol and was placed in the animals’ water bottles, which were covered with aluminum foil to protect them from light. The animals were given doxycycline beginning immediately after tumor implantation.

All mice were evaluated for the tumor “take” rate at the primary tumor site, primary tumor growth rate over time (weeks 1-10), and primary tumor surface area at resection. The formula \((L/2) \times (W/2) \times \pi\) was used for the calculation of tumor surface area. Statistical analyses of data obtained from these mouse experiments were analyzed by means of the Statview statistical program on an Apple Macintosh Power Book Duo 230 computer.

Gelatin zymography
Type IV collagenase activity in tumor extracts was assayed by gelatin zymography in 9% gels containing gelatin, in which gelatinase produces a clear band in the gel (Davies et al., 1993).

RESULTS
In the in vitro studies, as reported elsewhere, doxycycline
inhibited gelatinolytic activity and cell proliferation in MDA-MB-435 (Fife and Sledge, 1995), LNCaP (Fife et al., 1998), and U20S (Fife et al., 1997) cells. Furthermore, doxycycline induced apoptosis in all of these cell lines (Fife et al., 1994, 1996, 1997, 1998).

In the in vivo studies described herein, doxycycline, when administered daily to mice following implantation of MDA-MB-435 cells into the inframammary fat pads, significantly inhibited tumor growth. Ten weeks after implantation, the mean surface area of the resected tumors was 81.6 ± 8.7 mm$^3$ in treated animals, while tumors from untreated animals had a mean surface area of 122.0 ± 11.0 mm$^3$ (p < 0.01) (Fig.). Doxycycline also inhibited gelatinolytic activity in tumor extracts.

**DISCUSSION**

Obviously, agents that can suppress MMP activity and/or synthesis have potential for use in therapeutic interventions in breast and other cancers. The TCNs are relatively well-tolerated antimicrobial agents that have been shown to suppress MMP activity in vitro and in vivo in a variety of tissues, including tumors. We have demonstrated that a synthetic TCN, doxycycline, inhibits gelatinolytic activity in the human breast cancer cell line, MDA-MB-435, as well as in the LNCaP and U20S cell lines. Doxycycline also inhibits cell proliferation and induces apoptosis in all three cell lines (Fife et al., 1994, 1996, 1997, 1998).

Furthermore, doxycycline inhibits tumor growth in vivo in athymic mice implanted with MDA-MB-435 cells in the inframammary fat pad, a model that mimics human metastatic breast cancer with involvement of axillary lymph nodes and lungs (Fife et al., 1994). We have demonstrated inhibition of primary tumor growth and, in other studies, regional regrowth by doxycycline in this athymic mouse/xenograft model of metastatic breast cancer (Fife et al., 1994; Sledge et al., 1996).

Other workers have also shown that TCNs inhibit tumor growth and metastasis in several model systems (Van den Bogert et al., 1985; Masumori et al., 1994; Teicher et al., 1994; Wakai et al., 1994). As has been noted, other data from our laboratory indicate that increases in doxycycline levels not only inhibit proliferation but actually have a cytotoxic effect in vitro (Fife et al., 1994, 1996, 1997). Thus, it appears that doxycycline may have direct cytotoxic effects on cancer cells in addition to its ability to block MMP activity.

Our data, and those of others, suggest that TCNs might offer a novel therapeutic approach to the management of human breast cancer. This potential clinical application is bolstered by the widespread, long-standing, safe use of TCNs for infectious diseases and, even more significantly, by their recent effective use in gingivitis (Lee et al., 1991) and rheumatoid arthritis (Tilley et al., 1995), two chronic disorders characterized by MMP-related dysregulation of extracellular matrix degradation.

**REFERENCES**


Cornetta K, Moore A, Johannesohn M, Sledge G (1994). Clonal dominance detected in metastases but not primary


