

EFFECTS OF DOXYCYCLINE ON CANCER CELLS *IN VITRO* AND *IN VIVO*

R.S. FIFE
G.W. SLEDGE, JR.

Indiana University School of Medicine, Indianapolis, Indiana

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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.

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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 (5×10^5 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 re-anesthetized, 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 $\mu\text{g/mL}$. 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 U2OS (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 $81.6 \pm 8.7 \text{ mm}^3$ in treated animals, while tumors from untreated animals had a mean surface area of $122.0 \pm 11.0 \text{ mm}^3$ ($p < 0.01$) (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 U2OS 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 also have 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

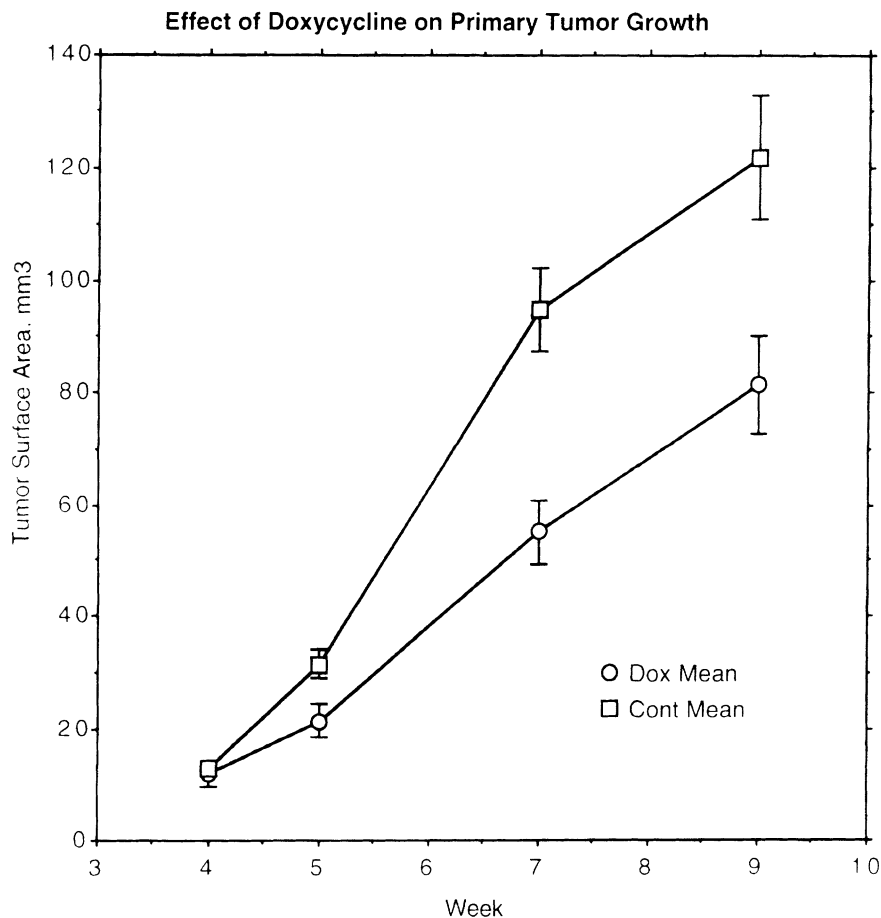


Fig.—Graph comparing growth of primary inframammary fat pad tumor in the absence of doxycycline (squares) and in the presence of doxycycline (circles). Note that the difference between the two curves is statistically significant ($p < 0.01$).

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.

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