Effect of Tetracycline Derivatives on the Growth and Metastasis of a Mouse Mammary Tumor

LIBERTY UNIVERSITY

Anna King, Serratt Nong, Joshua Sparks, John Pryor, Jieyu Zhang, Savannah Higgins, Riley Smith, Tania Reginald, Jiayi Li, Stephen Christensen, and Yingguang Liu

Abstract

Background: Among cancer types, breast cancer is one of the most frequently diagnosed across the world. Once the cancer cells begin to metastasize from the original site and spread throughout the body, the survival rate is drastically reduced. **Methods**: We tested the effects of two tetracycline derivatives, doxycycline and incyclinide (CMT-3), on the growth and metastasis of a mouse mammary tumor induced by injection of 4T1 mammary cancer cells in the fat pad under a nipple. **Results:** Doxycycline did not have a statistically significant effect on tumor growth or metastasis, but incyclinde significantly inhibited metastasis and with no measurable toxicity at a dose of 8 mg/Kg. **Conclusions:** Incyclinide may be potentially used to prevent metastasis of breast cancer.

Introduction

Worldwide, breast cancer is the most prevalent cancer type with 2.3 million women diagnosed resulting in 685,000 deaths globally in 2020.[1] As the tumor progresses, it metastasizes in one-third of patients, resulting in a 5-year survival rate of 25%.[2] Doxycycline (Dox), a commonly used antibiotic, has been observed to inhibit growth of a mammary tumor induced by orthotopic injection of 4T1 mammary cancer cells.[3] Dox also inhibited cancer stem cells in a clinical pilot study.[4] Incyclinide, also known as CMT-3, is a newer tetracycline derivative that does not have antibiotic activity. Both Dox and CMT-3 inhibited the metastasis, but not the growth, of a rat prostate cancer.[5] CMT-3 was also found effective in treating Kaposi Sarcoma in a clinical trial.[6] In this study, we explored the effects of Dox and CMT-3 on mammary cancer growth and metastasis in the orthotopic 4T1 murine model.

Methods

For the first experiment, four groups of female BALB/c mice were inoculated with 30,000 4T1 mammary cancer cells in the fat pad of nipple number 9. The mice received daily injections of PBS, 5 mg/Kg or 50 mg/Kg Dox dissolved in PBS after palpable tumors formed. The fourth group received 5 mg/Kg of doxycycline two weeks after the other groups. Mice were sacrificed 30 days after inoculation. The right lung was minced, digested, and cultured to observe metastatic cell growth in the presence of 6-thioguanine, which selects for 4T1 cells. The second experiment followed the same methods except that Dox was dissolved in an organic vehicle (4% DMSO, 5% polyethylene glycol, 5% Tween 80 in saline). The third experiment used 8 mg/Kg of CMT-3 dissolved in PBS and 0.8% DMSO. The pH was raised to 7.79 with NaOH to facilitate solubilization. Control mice received the same alkaline solution. The Mann-Whitney U test was used in all comparisons. Statistical difference was considered at $p < 0.05$.

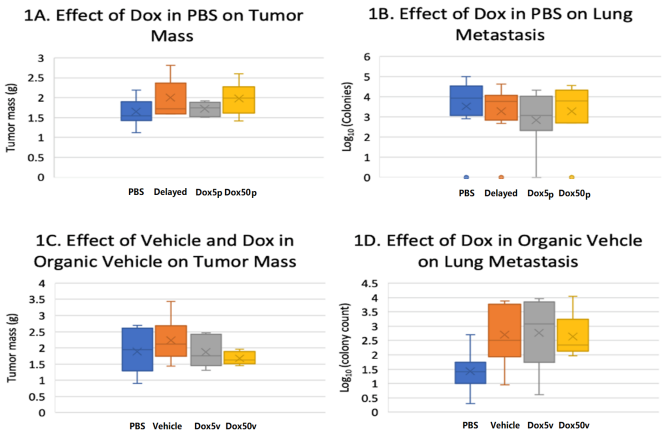

Figure 1. Effect of doxycycline dissolved in PBS and the organic vehicle on tumor growth and metastasis. A and B show the results of doxycycline dissolved in PBS. Results of doxycycline dissolved in the organic vehicle are shown in C and D. PBS: phosphate-buffered saline; Delayed: mice treated with Dox at 5 mg/Kg two weeks after initiation of treatment in other groups. Dox5p and Dox50p: Dox at 5 mg/Kg or 50 mg/Kg in PBS. Dox5v and Dox50v: Dox at 5 mg/Kg or 50 mg/Kg in organic vehicle (4% DMSO, 5% PEG, 5% Tween 80).

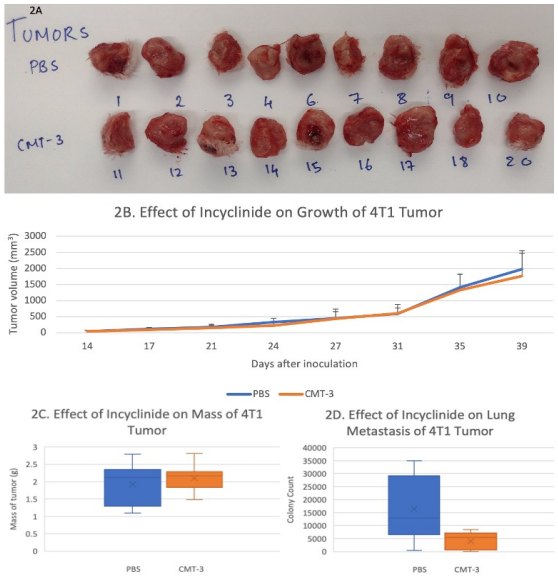

Figure 2. Effect of incyclinide on tumor growth and metastasis. CMT-3: incyclinide at 8 mg/Kg

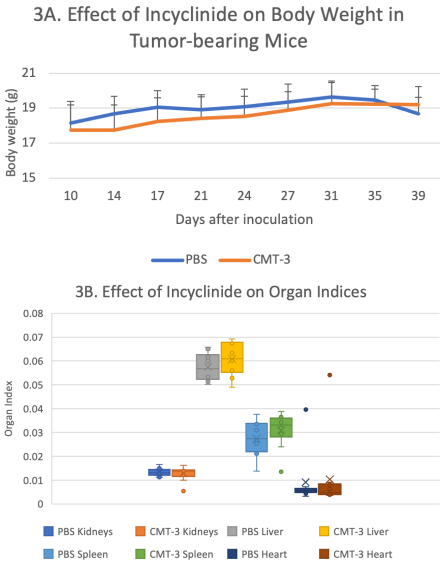

Figure 3. Effect of incyclinide on body weight and organ indices.

Results and Conclusion

1. Doxycycline might have slight effects on tumor growth and metastasis.
Dox dissolved in PBS at 5 mg/Kg seemed to have a slight effect in suppressing lung metastasis, and Dox dissolved in the organic vehicle at 50 mg/Kg seemed to have a slight effect on the growth of the primary tumor, although neither effects reached statistical significance. Interestingly, the organic vehicle itself appeared to promote tumor growth and metastasis to the lungs.

2. Incyclinide did not inhibit tumor growth but inhibits metastasis.
There was not a significant difference in tumor growth between mice treated with vehicle or CMT-3 at 8 mg/Kg. Metastasis was inhibited by CMT-3 as shown by the decreased colony count from lung tissue cultures. The difference in metastasis between vehicle and CMT-3 groups was statistically significant.

3. Incyclinide showed no measurable toxicity in treated mice.
After 39 days, mice treated with CMT-3 maintained a steady body weight while the body weight of the control mice began to decrease. There was no statistical difference in organ indices between treatment groups.

Conclusion

- Doxycycline did not have a significant effect in preventing tumor growth or metastasis in our murine mammary cancer model.
- An organic vehicle commonly used to dissolve lipophilic drugs may promote tumor growth and metastasis.
- Incyclinide did not have an effect in mitigating tumor growth but did have a statistically significant effect in preventing metastasis.
- Incyclinide administered at 8 mg/Kg did not result in measurable toxicity.

Future Work

1. Repeat treatment with CMT-3 by dissolving it in high-pH PBS without the introduction of DMSO.
2. Investigate the mechanisms of action of CMT-3 especially in regard to cytotoxicity and inhibition of matrix metalloproteinases.[5]
3. Explore the possibility of a clinical trial with CMT-3 in preventing metastasis of breast cancer.

References

1. DeSantis CE, et al. International Variation in Female Breast Cancer Incidence and Mortality Rates. *Cancer Epidemiol Biomarkers Prev*. 2015; 24(10): 1495-506.
2. Howlader N, et al. (eds). SEER Cancer Statistics Review, 1975–2018. National Cancer Institute, posted to the SEER website, April 2021. Last accessed April 19, 2021.
3. Li Y, et al. Targeting myeloid-derived suppressor cells to attenuate vasculogenic mimicry and synergistically enhance the anti-tumor effect of PD-1 inhibitor. *iScience*. 2021; 24(12):103392.
4. Scatena C, et al. Doxycycline, an Inhibitor of Mitochondrial Biogenesis, Effectively Reduces Cancer Stem Cells (CSCs) in Early Breast Cancer Patients: A Clinical Pilot Study. *Front Oncol*. 2018; 8:452.
5. Lokeshwar BL, Selzer MG, Zhu BQ, Block NL, Golub LM. Inhibition of cell proliferation, invasion, tumor growth and metastasis by an oral non-antimicrobial tetracycline analog (COL-3) in a metastatic prostate cancer model. *Int J Cancer*. 2002; 98: 297-309. doi:10.1002/ijc.10168
6. Dezube BJ, et al. Randomized phase II trial of matrix metalloproteinase inhibitor COL-3 in AIDS-related Kaposi's sarcoma: an AIDS Malignancy Consortium Study. *J Clin Oncol*. 2006; 24(9):1389-94.