Sustained Drug Delivery Device For Treatment Of Breast Cancer And Bone Metastases

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Disclosures:

Introduction: Up to 70% of the breast cancer patients have bone metastases at autopsy-based studies report [1] and the leading cause of death in the breast cancer patients is the development of distant metastases including bone. Until now, the treatment of bone metastases was mainly palliative therapies [2]. To effectively prolong the survival period and increase life quality of patients with breast cancer bone metastases, it is equally important to prevent primary tumor recurrence as well as to treat metastases.

In the case of bone metastases, after the tumor resection, patients need bone grafts or artificial substitutes in place of the resected tissue to provide immediate mechanical support. Thus, a strategy for bone metastasis treatment is to design a multifunctional implant to support the skeletal structure with local, controlled release of an effective drug to prevent cancer recurrence [3,4].

Methods: 1. In vitro. release profile of DOX from scaffolds: two concentrations of DOX 60ug/scaffold (group A) and 30ug/scaffold (group B). The release profile of DOX from scaffolds was monitored by incubating a scaffold in 1.0 ml of PBS (pH=7.4) at 37 °C (n=4) for 3 months and quantified with a Victor 1420 multilabel counter (Wallac, USA).
2. In vitro. cytotoxicity test of doxorubicin released from Desclaymr scaffolds
Human breast cancer cell line Mda-Mb-231 were seeded in 96-well plates at a density of 2,000 cells/well in each well with culture medium containing 10% fetal calf serum. After 2 days culture, 10 μL of doxorubicin released solutions from serious time points of 2h, 24h, 15 days, 30 days was added into the culture medium. After 3 days, cells were collected for viability test determined by XTT assay and cell numbers were estimated by Methylene Blue assay.
3. In vivo. anticancer effect of DESELYMR loaded with doxorubicin
8-11 week-old Female BALB/cATac-nude (C.Cg/AnBomTac-Foxn1nu N20 mice) mice with subcutaneous tumors induced by Mda-Mb-231-luc cells were used to evaluate the anticancer effect of DESLAYMR loaded with doxorubicin. Tumor volumes were measured twice a week after implantation in two dimensions and the tumor volumes (Vt) [(L * W2)/2] were calculated from caliper measurements.
4. Imaging and Quantification of bioluminescence and fluorescence data
Mice were imaged with an IVIS Imaging System. A region of interest (ROI) was manually selected according to the size of scaffold and the area of the ROI was kept constant within experiments.
6. Histologic and immunohistochemical examination
Formaldehyde fixed dissected tumors and other organs were were processed in a series of increasing ethanol concentrations and embedded in paraffin wax. Five-micrometer sections were cut and stained with H&E. Immunostaining for firefly luciferase was also done following the instructions.

Results: Our first experiments tested the release profile of DOX from scaffolds loading with different concentrations of DOX. The release profile of DOX rom Desclaymr scaffold showed an initial burst release in both groups. On day 3, DOX released 55.5% of the total amount of drug from the group A compared with 38.2% from group B (Figure 1). After that, it showed small amounted and sustained release in both groups up to 12 weeks.
Next we tested the anti-tumor effect of released solution from different time points in breast cancer cell lines. As expected, the MDA-MB-231-luc-D3H2LN cells responded in a dose-dependent manner to the DOX. There was a significant tumor inhibition effect in group A up to 4 weeks compared with control (p<0.05), while the effect period is shorten to 2 week in group B (Figure 2B).
Later we tested the effect of DESCALYMR_DOX treatment in a model of subcutaneous tumor. Human MDA-MB-231-luc-D3H2LN cells expressing luciferase were implanted subcutaneously into the flanks of BALB/cATac-nude mice and treated with locally implantation of DESCALYMR_DOX or locally injection of DOX (60ug/side) when average tumor volumes reached 200 mm3 (Figure 3; group A and B). A third group of mice received to treatment was set up as blank control (Figure 3; group C). Significant tumor growth delay was observed after 8 days of treatments either with subcutaneously implantation of DESCALYMR_DOX or
subcutaneously injection of DOX compared with blank control group (p=0.0033; 0.0385, respectively). Biweekly quantification of bioluminescence showed decelerated tumor growth in group A and B compared with group C. Mice receiving DESCLAYMR_DOX implantation showed greater tumor growth inhibition compared to mice receiving DOX injection (Figure 4). Decreased multi-organ metastases in mice with DESCLAYMR_DOX implantation compared with locally injection of same dosage of DOX and blank control group (Table 1) based on detection of multiorgan metastases by ex vivo bioluminescence. This result was further confirmed with immunohistochemistry (Figure 5).

We then tested the in vivo release profile of DOX from Desclaymr scaffold. DOX in group A (Desclaymr_DOX) reaches peak on day4 post treatment in the surrounding tissue compared with day0 (4 hours after implantation) in group B (Injection_DOX). DOX in surrounding tissue mains high and can be detected on day 14 compared with released out in group B in the same time (Figure 6).

Finally, H&E staining of heart sections showed better myocardial structure preserved in mice receiving DESCLAYR_DOX implantation compared with Injection_DOX. Sirius-red staining showed more fibroses in mice receiving injection_DOX than DESCLAYMR_DOX imaplantation (Figure 7).

Discussion: Our goal was to test our DESCLAYMR scaffold loaded with doxorubicin in vitro. and in vivo. for the tumor inhibitory effect in order to take a further step in clinical use after tumor resection. Here we show DESCLAYR_DOX implantation has a greater tumor growth inhibition compared with non-treatment controls and more sustained anti-tumor effect compared with locally injection of DOX. Other than this, locally injection of DOX has side effects like skin irritation and a risk of infection for the animal while the same dosage of DOX loaded on DESCLAYMR scaffold has no such effect. This comparison indicates that it is safer as well as more effective in tumor inhibition using DESLAYMR_DOX implantation than locally injection of DOX. Although, the observation period is limited considering the aggressiveness of the implanted cell lines. Furthermore, the number of animals could be enlarged in the future.

Significance: This sustained drug delivery device had a prolonged anticancer effect in mice with subcutaneous tumor induced by human breast cancer cell lines. Later we will test it in animal model with breast cancer bone metastases. And we believe that it has a potential ability to prevent cancer recurrence after tumour resection as well as to provide a structure support meanwhile.

Acknowledgments:

Figure 1. Cumulative DOX release from group A (60μg/scaffold), group B (30μg/scaffold) at 37°C in PBS (pH 7.4) for 12 weeks.
Figure 2. Tumor inhibitory effect in vitro by XTT and Methylene blue assay.
Figure 3. Average tumor volumes before and after treatment (Day13). Group without any treatment reached tumor volume endpoint (1500 mm3) at 27 days, with comparative tumor volume significantly reduced in DESCLA/MR_DOX group (p=0.0007 by Student’s t test) and Injection_DOX group (p=0.0264 by Student’s t test) at the same time point. Group A, n=10; group B, n=6; group C, n=10. Data are presented as mean ± SEM.
Figure 4. Biweekly quantification of bioluminescence
Figure 5 Representative images of ex vivo. **bioluminescence**, with confirmation of metastases by hematoxylin and eosin (H&E) and anti-luciferase immunostaining.
Different release pattern of doxorubicin from DESCLAYMR scaffold and subcutaneous injection

Figure 6. DCX in group A reaches peak on day 4 post treatment in the surrounding tissue compared with day 0 (4 hours after implantation) in group B. DCX in surrounding tissue remains high and can be detected on day 14 compared with released out in group B in the same time. Data are presented as mean ± SEM. n=6 in all groups.
Figure 7: Representative micrograph of heart sections from mice with different treatments: better myocardial structure preserved in mice receiving DESCLAYR_DOX implantation compared with Injection_DOX.
Table 1. Multiorgan metastases detected by ex. vivo bioluminescence

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