Multi-functional micelles for the combination of chemotherapy and radiotherapy in hepatocellular carcinoma

Ying-Hsia Shih[*1,**], Cheng-Lian Peng[*2], Tsai-Yueh Luo[**,****] and Ming-Jium Shieh[*3, ***]

[*] Institute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University, Taipei 100, Taiwan.
[1]d00548025@ntu.edu.tw [2]d93548014@ntu.edu.tw [3]soloman@ntu.edu.tw
[**] Isotope Application Division, Institute of Nuclear Energy Research, Longtan, Taoyuan, Taiwan. tylo@iner.gov.tw
[***] Department of Oncology, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan.
[****] Institute of Radiological Science, Central University, Taichung, Taiwan.

ABSTRACT

In this study, multi-functional micelles loaded with doxorubicin (Dox) and labeled with the radionuclide rhenium-188 (^{188}Re) were developed to provide the combination therapy for liver cancer. BNL/Luc heptoma mice were randomly allocated to four groups. Including PBS control (n=4), ^{188}Re- perrhenate (n=4), Dox micelles (n=5) and ^{188}Re Dox-micelles (n=5). The biodsitribution and therapeutic efficacy were mesured. Bio-luminescence imaging of BNL/Luc heptoma showed the smallest tumor size in ^{188}Re-Dox micelles group ($p<0.05$). Moreover, ^{188}Re-Dox micelles group had more than thirty days survival, which was significantly different with control ($p<0.005$). Immunohistochemical staining of proliferating cell nuclear antigen (PCNA) shows that ^{188}Re-Dox micelles inhibited the proliferation of BNL/Luc cell comparing with control ($p<0.001$). We suggested the ^{188}Re Dox-micelles could apply for the combined therapies of hepatoma.

Keywords: rhenium-188, doxorubicin, micelles, PCNA.

1 INTRODUCTION

Hepatocellular carcinoma is the third leading cause of cancer deaths in worldwide [1]. The incidence of hepatocellular carcinoma is highest in Asia and Africa. In this study, multi-functional micelles loaded with anti-cancer drug doxorubicin (Dox) and labeled with the radionuclide rhenium-188 (^{188}Re) were developed to provide the combination of chemotherapy and radiotherapy for hepatoma.

Rhenium-188 (^{188}Re, $t_{1/2}$ =16.9 h) is emerging as a promising isotope for clinical use. ^{188}Re has several favorable characteristics, such as high beta-energy emission (2.1 MeV), suitable gamma-ray energy (159 keV), short half-life of 17 hours, deep tissue penetration for local treatment (maximum 11 mm, average 3.8 mm) [2], and in-house preparation using a ^{188}W/^{188}Re generator [3]. This unique property makes ^{188}Re a suitable radionuclide candidate for both therapeutic and diagnostic purposes.

We evaluated an intravenous injection drug delivery system, based on Methoxy poly (ethylene glycol)-*block*-poly(ε-caprolactone) (mPEG-*b*-PCL ; M510) micelles, containing ^{188}Re-perrhenate and doxorubicin (Dox) for local combined radiotherapy and chemotherapy for hepatocellular carcinoma.

2 METHODS

2.1 Prepared ^{188}Re Dox-micelles

We prepared Dox loaded DTPA-micelles (Dox-micelles) using DTPA-PEG-PCL by the co-solvent evaporation method [4]. The ^{188}Re-Dox micelles were prepared by reacting a mixture of 1 mL Dox DTPA-micelles, 1mL ^{188}Re-perrhenate ($^{188}ReO_4$, ~370MBq), and 5 mg stannous chloride for 1 hour at 37°C [5, 6, 7].

2.2 Cell Line and culture

The BNL/Luc cell line, a cancer cell line derived from chemically transformed hepatic epithelial cells of a BALB/c mouse, was used to generate liver tumors. The BNL/Luc cells were maintained in 5% CO_2 incubator at 37°C in DMEM supplemented with 10% FBS and 1% antibiotics.

2.3 BNL/Luc hepatoma model

Female BALB/c athymic (nut/nut) 5-6 weeks old mice were used as the orthotopic liver cancer model, using the BNL/Luc cell line. After cultured cells grew exponentially for 1 week, a concentration of approximately 10^5 cells per mL 20 μL per mouse was established. Using a 29-gauge needle, cell was injected slowly into one of the hepatic lobes under the liver capsule. After 14 days of cell inoculations, the tumor growth. Alone the mice with tumors in the liver which were included in the study.

2.4 Treatment Group

The mice implanted with BNL/Luc hepatocellular carcinoma were were randomly assigned into the following four groups, including control (n=4), $^{188}ReO_4$ (n=4), Dox micelles (n=5), and ^{188}Re-Dox micelles (n=5). The ^{188}Re-Dox micelles were administered *via* tail vein triple intravenous injection on Days 0, 6 and 12, which equivalent to 22.2 MBq of ^{188}Re and 10 mg/kg of doxorubicin.

2.5 Biodsitribution and Therapeutic Efficacy

The tumor biodistribution image of the PBS control (n=4), ^{188}Re- perrhenate (n=4), Dox micelles (n=5) and ^{188}Re Dox-micelles (n=5) four treatments groups was studied by injecting intravenously through a tail vein of mice bearing with an IVIS imaging system (Xenogen, Alameda, CA, USA). Therapeutic efficacy was investigated by long-term therapeutic monitoring in BNL/Luc bearing orthotopic liver tumor model.

3 RESULTS AND DISCUSSION

This BNL/Luc bearing mice hepatocellular carcinoma model may be used to monitor lesion growth during treatment with ^{188}Re-Dox micelles teatment. Signals was detected after intraperitoneal injection of luciferin on day 0 (Figure : 1A). This imaged proved before treatment all groups had hepatocellular carcinoma tumors. The control, and alone $^{188}ReO_4$ and alone Dox micelles increased strongly start on day 13. Bioluminescence imaging of BNL/Luc bearing orthotopic hepatocellular carcinoma showed the smallest tumor size in ^{188}Re-Dox micelles group ($p<0.05$) comparing with control and $^{188}ReO_4$ alone treatments (Figure : 1B). Treatment with the ^{188}Re-labeled Dox micelles delayed the appearance and decreased the intensity of the signal on days 6, 13, 19 and 26. This suggests that treatment with ^{188}Re-Dox micelles may inhibit early events BNL/Luc cell growths.

Moreover, ^{188}Re-Dox micelles group had more than thirty days survival (Figure : 2), which was significantly different with control ($p<0.005$), ^{188}Re-perrhenate ($p<0.005$) and Dox micelles ($p<0.01$).

Immunohistochemical staining of proliferating cell nuclear antigen (PCNA) shows that ^{188}Re-Dox micelles inhibited the proliferation of BNL/Luc cell comparing with those in control ($p<0.001$) and ^{188}Re-perrhenate ($p<0.001$).

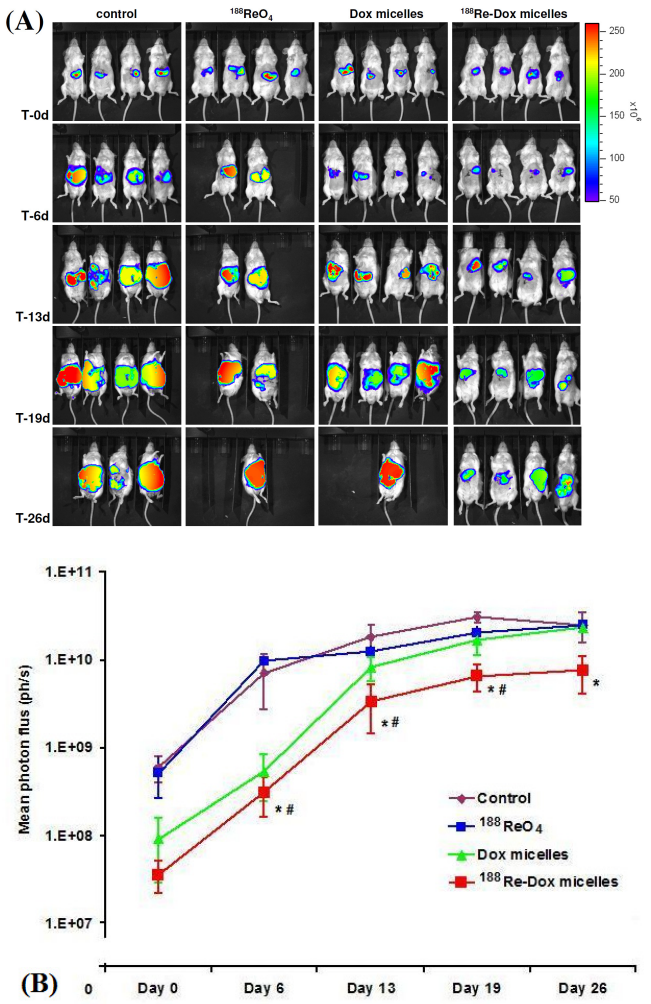

Fig 1 : The effects of ^{188}Re-Dox micelles in mice bearing BNL/Luc hepatocellular carcinoma were measured. (A) Time-lapse luciferins imaged mice bearing BNL/Luc hepatocellular carcinoma after intravenous injections of ^{188}Re-Dox micelles. (B) Mean photon flux as function of time after initiation of drug treatment. ^{188}Re-Dox micelles therapy had lowest photon collection, which represented best tumor growth suppression day 6, 13, 19 and 26 (compared within control and $^{188}ReO_4$ alone). All images were acquired under same experimental conditions and are displayed at same absolute scale. Data are expressed as mean 6 SE. Black arrow indicates time of drug injection. Experiments were repeated twice. (*) means $p< 0.05$ is significantly different from control by Student t test. ($^{\#}$) means $p< 0.05$ is significantly different from $^{188}ReO_4$ by Student t test.

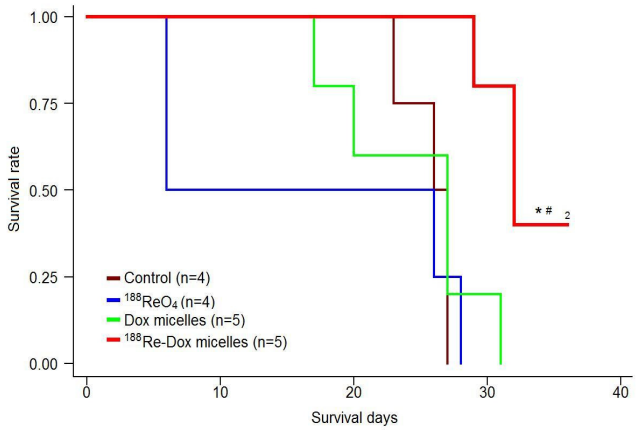

Fig 2 : Kaplan-Meier survival curves for BNL/Luc bearing hepatocellular carcinoma mice after administration of ^{188}Re-Dox micelles (contain ^{188}Re 22.2 MBq and Doxorubicin 10 mg/kg, 3 times interval 4 days), normal saline by single intravenous injection. Mice were treated 12 days after tumor inoculation. Each group means control (n=4), $^{188}ReO_4$ (n=4), Dox micelles (n=5), ^{188}Re-Dox micelles (n=5) respectively. (*) means compared within the control and the $^{188}ReO_4$ indicates $p<0.005$. ($^{\#}$) means compared within the Dox micelles indicates $p<0.01$.

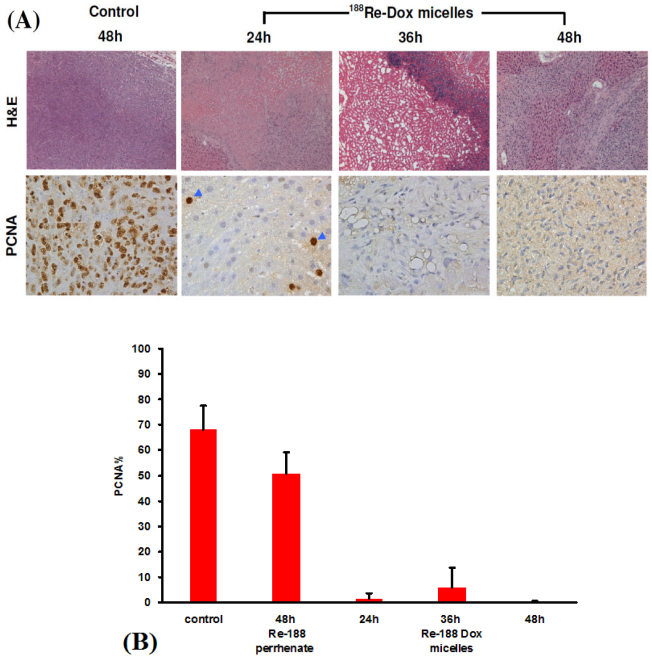

Fig 3 : Histological and immunohistochemical analysis in BNL/Luc hepatocellular carcinoma treated with ^{188}Re-Dox micelles dual therapy. (A) Tumor sections were analyzed by hematoxylin and eosin (H&E) stainingat 200× magnification. TUNEL and PCNA staining imaged at 400× magnification. The blue arrow indicates PCNA positive cell. (B) Cellular proliferation was quantified by assessing the number of PCNA-positive cells per field at 400× magnification. The results represent the mean ± SD in 10 distinct regions from examining 3 tumors per group. (*) means compare within the control indicates $p < 0.001$. ($^{\#}$) means compared within the $^{188}ReO_4$ indicates $p < 0.001$.

4 CONCLUSION

^{188}Re Dox-micelles shows a significant inhibitory effect on the tumor growth and extend the survival time in mice bearing BNL/Luc hepatoma. We suggested the multi-functional micelles could apply for the combined therapies of hepatocellular carcinoma.

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