RESEARCH ARTICLE

In Vitro and *in Vivo* Antitumor Evaluation of Berbamine for Lung Cancer Treatment

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Abstract

Purpose: Lung cancer, one of the most frequently diagnosed cancers in the world, is characterized by relatively high morbidity and mortality. Berbamine (BER) has been initially reported to exert anti-proliferative effects against a series of cancers. Methods: In this study the *in vitro* cytotoxicity of BER was measured by MTT assay. *In vivo* anti-cancer efficacy of BER was assessed in A549 xenografts. Results: Cytotoxicity tests showed dose-dependent cell growth inhibition effects of BER against A549 cells. Moreover, BER significantly reduced the growth of lung cancer in a dose-dependent manner in nude mice with prolonged survival time. Conclusion: Therefore, BER might be in herbal medicine for cancer therapy and further efforts are needed to explore therapeutic strategies.

Keywords: Berbamine - antitumor - lung cancer - therapy

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Introduction

Lung cancer, one of the most frequently diagnosed cancers in the world, is characterized with relatively high morbidity and mortality (Lu et al., 2013; Wang et al., 2013; Yan et al., 2013; Kim et al., 2014). Chemotherapy is recognized to be the main therapeutic way to delay tumor growth (Pikor et al., 2013). However, the overall survival remains poor (Pikor et al., 2013). Therefore, there is an urgent need to identify effective drugs for the treatment of lung cancer.

Recently herbal medicines have attracted more and more attention in the filed of cancer therapy. Alkaloids including tetrandrine, matrine, berbarine, etc. have been demonstrated their potential antitumor efficacy (Wang et al., 2009; Meng et al., 2013; Qin et al., 2013; Song et al., 2013; Kittakoop et al., 2014). Berbamine (BER), a kind of small-molecule compound extracted from Berberis amurensis, has been initially reported its anti proliferative effect against leukemia (Ji et al., 2002; Gu et al., 2012). Growing evidences then demonstrate that BER is capable to inhibit cell growth on human liver and breast cancer cells (Wang et al., 2009; Meng et al., 2013). The possible mechanisms underlying the antitumor effect of BER include regulating apoptotic proteins (Ji et al., 2002; Gu et al., 2012).

In the current study, anticancer efficiency of BER was evaluated *in vitro* and *in vivo*. The cytotoxicity of BER was studied by MTT assay. *In vivo* anti-tumor evaluation of BER was performed in A549 xenograft model. The growth curve of tumor volume and bodyweight of the mice were measured every two days. The overall survival of mice was then recorded.

Materials and Methods

Materials

Berbamine, was purchased from Sigma Chem. Co., (St. Louis, USA). All other chemicals were of analytical grade and used without further purification. Human lung cancer cell line A549 was obtained from Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China).

Male and female nude mice (nu/nu; 6–8 weeks old and weighing 18–22 g) were purchased from Model Animal Research Center of Nanjing University (Nanjing, China). The mice were housed and maintained in the animal facility of the Animal Center of Nanjing Medical University. The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Nanjing Medical University.

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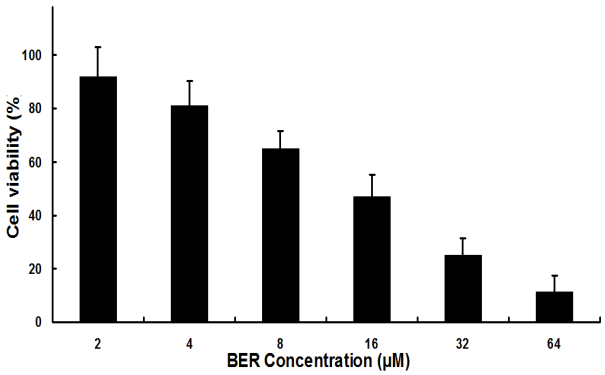

Figure 1. Effects of BER on A549 Cell Proliferation. (A) A549 cells were treated with BER at 2, 4, 8, 16, 32 and 64 μM for 48 hours

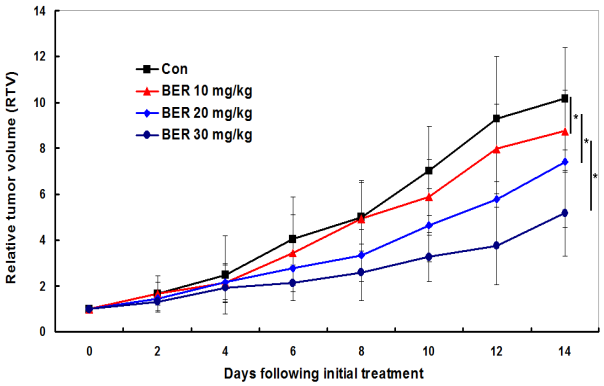

Figure 2. Antitumor Effect of BER in A549 Xenograft Models. (A) Tumor volume of established A549 xenografts in nude mice during therapy under different treatments. Mice were treated with different protocols on Day 0 as showed in the figure. Control: vehicle; BER was administered at the doses of 10, 20 and 30 mg/kg. Different agents were delivered through intravenous pathway when tumor volume measured 100 mm3. Data are presented as mean±SD (n = 6). *means $P < 0.05$

***In vitro** cytotoxicity*

The half maximal inhibitory concentration (IC50) of A549 cells were determined by the MTT assay. Briefly, cells were seeded in 96-well plates (1×10^4 cells per well) 24h prior to the assay. Then cells were exposed to a series of doses of BER. After 48 hrs of incubation, 20μl of 5 mg/mL MTT solution was added to each well and the plate was incubated for 4 h. Then, the media were removed and dimethylsulfoxide (DMSO) (150 μL) was added to each well. The optical density (OD) of each well was measured using a microplate reader at 560 nm (Bio-Rad, Hercules, USA).

Cell viability was determined by following formula:

Cell viability (%) = OD (test well)/OD (reference well) × 100% (4)

All the results obtained from MTT assays were confirmed by repeating the experiment on at least three independent occasions and testing in triplicate each time.

***In vivo** antitumor efficacy*

Nude mice implanted with A549 cell line were used to qualify the antitumor efficacy of BER through intravenous administration. The mice were raised under specific pathogen-free (SPF) circumstances and all of the animal experiments were performed in full compliance with guidelines approved by the Animal Care Committee of Nanjing Medical University. The mice were subcutaneously injected at the left axillary space with 0.1 ml of cell suspension containing $4–6*10^6$ A549 cells. Treatments were started after 7-8 days of implantation. The mice whose tumor reached a tumor volume of 100 mm^3 were selected and this day was designated as "Day 0".

On Day 0, the mice were randomly divided into four groups, with each group being composed of 6 mice. The mice were treated intravenously with saline and a series doses of BER, respectively. BER was administered at a equivalent dose of 10, 20, and 30 mg/kg. All mice were tagged, and tumors were measured every other day with calipers during the period of study. The tumor volume was calculated by the formula $(W^2*L)/2$, where W is the tumor measurement at the widest point, and L is the tumor dimension at the longest point. Each animal was weighed at the time of treatment so that dosages could be adjusted to achieve the mg/kg amounts reported. Animals also were weighed every other day throughout the experiments.

Relative tumor volume (RTV) was calculated by the formula (Vn/V0), where Vn is the tumor volume measured at the corresponding day, and V0 is the tumor volume measured at Day 0.

At the end of the experiment, survival curves were calculated by Kaplan-Meier analysis and comparisons were made with the log-rank test using SPSS® 13.0 software (IBM Corporation, Armonk, NY).

Statistical analysis and research experience

Results were presented as Mean±SD. Statistical comparisons were made by t test or ANOVA analysis. The accepted level of significance was P value < 0.05.

Results and Discussion

Dose-dependent cell inhibition effect of BER against A549 cells

As shown in Figure 1, BER inhibited the growth of A549 cells in a dose-dependent manner. Increase of drug concentration led to the reduction of cell viability. For instance, less than 10 % cells died under the treatment of 5μM BER, while more than 40% cell death was observed in cells exposed to 20 μM BER. As calculated from the survival curve, the IC50 value of BER against A549 cells was 11.2±2.1μM.

***in Vivo** antitumor evaluation of BER against A549 xenograft*

Figure 2 indicates the dose-dependent tumor growth inhibition effect of BER in A549 xenograft. Significant differences were observed among the four groups ($p<0.05$, ANOVA). All of the doses of BER significantly delayed the tumor growth of A549 cells when compared to the control group since Day 6 ($p<0.05$). Most importantly, it is noted that dose-responsive growth inhibition of BER treatment decreases tumor enlargement effectively, indicating a promising therapeutic regimen. For example, 10 mg/kg BER treatment inhibited tumor growth moderately with

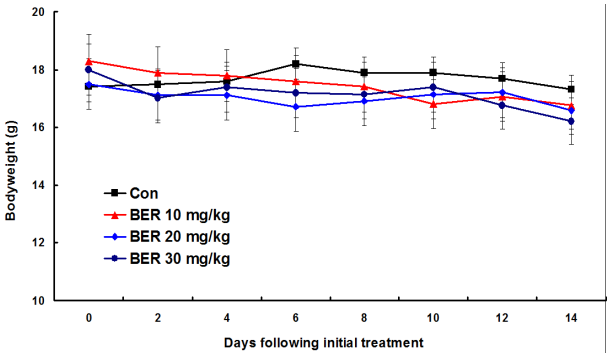

Figure 3. Bodyweight Change of Nude Mice Receiving Different Treatments During Therapy. Data are presented as mean±SD (n = 6)

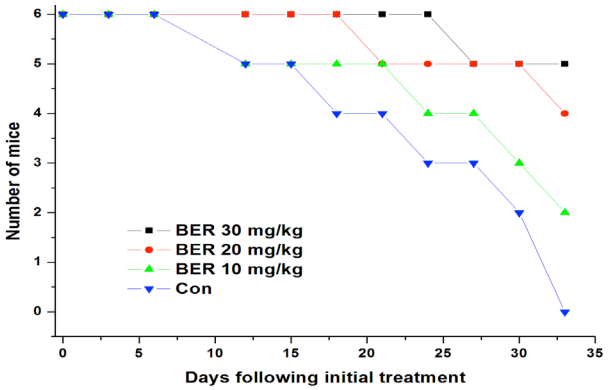

Figure 4. Survival Curves of Tumor-bearing Mice Treated with Different Doses of BER

the RTV of Day 14 being more than 8. A higher dose of 20 mg/kg BER intravenous injection significantly led to more tumor decline than 10 mg/kg BER. Moreover, the group receiving highest dose of 30 mg/kg BER was observed to maintain the greatest amount of antitumor activity. As shown in Figure 2, RTV of the group received high dose of BER (30 mg/kg) is the lowest among all the groups indicating the strongest tumor inhibition.

An analysis of body weight variations generally defined the adverse effects of the different therapy regiments (Figure 3). No significance was observed among the four groups. The mice receiving even high dose of BER were in a good state in the aspects of movement and spirit.

The overall survival of mice receiving different treatments is shown in Figure 4. It was noted that 80% of the mice receiving 30 mg/kg BER survived at the end of the experiment (day 33). However, none of the mice survived to the end in the control group. Therefore, *in vivo* evaluation demonstrated for the first time that BER exhibits significantly increased antitumor efficacy with longer survival time.

Conclusion

In conclusion, the present study demonstrates the antitumor effect of BER in the treatment of lung cancer. *In vitro* cytotoxicity evaluation indicates that BER possesses a dose-dependent cell inhibition effect against A549 cells. *In vivo* evaluation shows that BER effectively inhibits the growth of lung cancer in a dose-dependent manner in nude mice with prolonged survival time. Therefore, BER might be a promising herbal medicine in cancer therapy and further efforts are needed to explore this therapeutic strategy.

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The author (s) declare that they have no competing interests.

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