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.
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:

\[
\text{Cell viability} \% = \frac{OD \text{ (test well)}}{OD \text{ (reference well)}} \times 100\%
\]

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 an 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).

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
might be a promising herbal medicine in cancer therapy and further efforts are needed to explore this therapeutic strategy.

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