Original Article

The efficacy of the inhalation of an aerosolized Group A streptococcal preparation in the treatment of lung cancer

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Objective: To observe the efficacy of the inhalation of an aerosolized group A streptococcal (GAS) preparation in treating orthotopic lung cancer in mouse models and assess the feasibility, safety, and effectiveness of this administration mode for lung cancer.

Methods: Lewis lung carcinoma (LLC) cell strains were administered via intrathoracic injection to establish orthotopic lung cancer mouse models. After the tumor-bearing models were successfully established, as confirmed by computed tomography, the mice were administered by inhalation with an aerosolized GAS preparation (GAS group) or aerosolized normal saline (control group). The anti-tumor effect of the aerosolized GAS preparation was evaluated histologically; meanwhile, the survival and quality of life were compared between these two groups.

Results: The aerosolized GAS preparation showed remarkably anti-tumor effect, causing the necrosis of the orthotopic lung cancer cells in tumor-bearing mice. Furthermore, mice in the GAS group had significantly better quality of life and longer survival than those in control group.

Conclusions: The inhalation of aerosolized GAS preparation may be a feasible, safe and effective solution for lung cancer.

Key Words: Group A streptococcal (GAS); lung cancer; Lewis lung carcinoma cell

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Introduction

In 2012, more than 1.6 million new cancer cases and close to 0.6 million deaths (about 35% of new cases) from cancer are projected to occur in the United States. Lung cancer represents the most common cause of cancer-related mortality in the United States and around the world. Despite medical advances, lung cancer still accounts for more than 150,000 deaths annually in the United States (1). For patients with advanced lung cancer, even after treatment with the third-generation platinum-based chemotherapy, the median survival time can only be prolonged from 4-6 months (2-4), and the prognosis is even worse for pulmonary metastases. Obviously, such prognosis cannot be satisfactory for both clinicians and patients, and new ideas and new ways for the treatment of lung cancer should be actively explored (5,6).

OK432 [a Group A streptococcal (GAS) preparation] is a bacteria-derived biological response modifier (BRM). During its preparation, human group A hemolytic streptococci were treated with penicillin in

the Berubeimer basic culture at 37 ℃ for 20 minutes and subsequently heat-treated at 45 ℃ for 30 minutes to yield low-virulence products (7). It can regulate the immune system by enhancing the activities of multiple immunocytes including natural killer (NK) cells and CD4+/CD8+ T cells (8). OK432 has been applied in clinical practices for over three decades and shown certain therapeutic effectiveness in controlling malignant effusions via serosal cavity injection (9). However, due to its side effects, the administration modes of OK432 only include intramuscular injection, subcutaneous injection, local injection directly into a tumor, and serosal cavity injection. In our current study, by establishing animal models of orthotopic lung cancer (10), we tried to observe the potential anti-tumor effect of the aerosolized OK432 (hereafter referred as a GAS preparation), which acts directly on lung tumors, and to evaluate the safety, effectiveness, and feasibility of this new administration mode in treating lung cancer.

Materials and methods

Cells and cell culture

The mouse Lewis lung carcinoma cell line was introduced from CAS Shanghai Institute of Cell Biology. It was cultured with DMEM medium containing 10% calf serum (Hyclone, USA) and grown in a 5% CO_2 incubator at 37 ℃ and routinely sub-cultured. The cells in the exponential growth stage were made into single cell suspensions.

Animals

Fifty C57BL/6 mice, 4-5 weeks of age and weighing (18.8±1.53) g, were purchased from the Experimental Animal Center of Guangdong Province. They were raised in SPF environment at room temperature (25±2) ℃ and given aseptic full-price nutritional pellet feed and sterile water.

Methods

These 50 C57BL/6 mice were intraperitoneally injected with pentobarbital sodium (10 mg/kg) to induce anesthesia and fixed in the left lateral decubitus position after anesthesia. Then 100 μL Lewis single cell suspension (5×10^6 mL^{-1}) prepared with the 1-mL injector was percutaneously inoculated into the upper margin of the sixth intercostal rib on the right anterior axillary line to a depth of about 5 mm rapidly and after that, the needle was promptly pulled out. The mice were maintained in the left lateral decubitus position after injection and observed until complete recovery.

Detection of survival time and gross observation

The survival status (body weight) and survival time were observed in the operated mice.

Spiral computed tomography (CT)

Spinal computed tomography (CT) was performed two weeks after the orthotopic implantation of tumor cells. Mice were fixed on a plane plate in a supine position following intraperitoneal injection of pentobarbital sodium (10 mg/kg) to induce anesthesia. The Toshiba Aquilion16-slice CT scanner was adopted to perform routine thin-slice plain CT scan from the mouse neck to abdomen, slice thickness 1 mm, reconstruction interval 0.5-0.8 mm, tube voltage 100 kV and tube current 90-110 mA.

Mice with CT-confirmed tumor cell implantation were divided into GAS group and control group (n=22 in each group)

In the GAS group, mice were put inside a 1000-mL glass container that was connected with a medical aerosolizer. The GAS preparation solution was dispensed with sterile water for injection to a concentration of 0.1 KE/mL, and then aerosolized continuously for 15 minutes. The procedure lasted 7 days. In the control group, mice were treated with equal volume of aerosolized normal saline.

Tissue embedding, pathological detection, and immunohistochemistry

After the aerosolization completed, two mice from each group were sacrificed by cutting off the neck under anesthesia to remove the intrathoracic heart, bilateral lungs, pleura, lymph nodes and mass etc, which were fixed in 10% formalin solution and embedded in paraffin to make into tissue sections, subjected to HE staining, and observed under a microscope. The remaining unstained tissue sections were stained using conventional S-P immunohistochemical method, with mouse CD3/CD4 as the primary and secondary antibodies; after having been added with diaminobenzidine (DAB) for color development and restained with hematoxylin, the sections were

