Preparation of PLGA microspheres loaded with 10-hydroxycamptothecin and arsenic trioxide and their treatment for rabbit hepatocellular carcinoma

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Objective. This study aims to study the preparation method of arsenic trioxide (As$_2$O$_3$) polylactic-co-glycolic acid (PLGA) microspheres and 10-hydroxycamptothecin (HCPT) PLGA microspheres and explore their therapeutic effects as embolic agents for VX2 hepatocellular carcinoma in rabbits.

Methods. As$_2$O$_3$ and HCPT PLGA microspheres were prepared by multiple emulsion solvent evaporation method. Scanning electron microscopy (SEM) and particle size distribution were used to analyze the morphology, the drug sustained release ability was observed by the release of microspheres in vitro. The rabbit model of VX2 hepatocellular carcinoma was established and the hepatocellular carcinoma was treated with combined microspheres. The therapeutic effects were detected by qPCR, western blotting, HE staining and immunohistochemical methods.

Results. The PLGA microspheres loaded with As$_2$O$_3$ and HCPT were successfully prepared by optimizing the ratio. The particle size was between 30 and 50 μm. In vitro release results showed that PLGA microspheres loaded with As$_2$O$_3$ released completely in 10 days and PLGA microspheres loaded with HCPT released completely in 12 days. Western blotting and qPCR results showed that the expression of ALDH1A1 and Nanog decreased significantly in treatment group. HE staining and immunohistochemical analysis showed that the expression of CD31, HIF and VEGF decreased significantly and the apoptosis of tissues was obvious.

Conclusion. The combination of As$_2$O$_3$ and HCPT PLGA microspheres as embolization for VX2 hepatocellular carcinoma in rabbits has significant therapeutic effect.

Key words: polylactic-co-glycolic acid (PLGA), arsenic trioxide (As$_2$O$_3$), 10-hydroxycamptothecin (HCPT), western blotting; Immunohistochemistry

INTRODUCTION

Primary liver cancer is one of the most common malignant tumors. Surgical resection is usually used in the early stage, but most patients with liver cancer are in the middle and advanced stage when they are diagnosed. At this time, non-surgical therapy is generally used$^{1,2}$. Drug chemotherapy is a routine treatment for hepatocellular carcinoma. Many anticancer drugs have significant inhibitory effects on hepatocellular carcinoma cells, but they are easy to spread and have strong side effects on normal tissues, which limiting the development and application of drug therapy for hepatocellular carcinoma$^{3,4}$.

Arsenic trioxide (As$_2$O$_3$) was first used as an adjuvant drug in the treatment of malaria, tuberculosis and syphilis, it was later used in the treatment of acute promyelocytic leukemia and achieved certain therapeutic effects. Then it was found that As$_2$O$_3$ could also inhibit hepatocellular carcinoma and lung cancer, and As$_2$O$_3$ injection was developed for clinical treatment$^5$. However, there are also many problems. Firstly, As$_2$O$_3$ has strong toxicity and side effects, it can kill tumor cells and damage normal cells at the same time. Secondly, As$_2$O$_3$ is less water-soluble and easy to precipitate when administered$^6$. Hydroxycamptothecin (HCPT) is a kind of alkaloids extracted from plants with anti-cancer effect. Its disadvantage is that it is insoluble in water and slightly soluble in most organic solvents with short half-life. At present, the forms used in clinic are sodium salt injection, powder injection and capsule, but it is easy to oxidize and hydrolyze when exposed to light and heat, its curative effect is low$^7$.

In order to overcome these shortcomings, we developed a new treatment technology of hepatic artery embolization of hepatocellular carcinoma using polylactic-co-glycolic acid (PLGA) microspheres as drug carriers to embolize As$_2$O$_3$ and HCPT. PLGA has good biocompatibility and biodegradability. It can be metabolized by human body, and eventually degraded to H$_2$O and CO$_2$. PLGA is widely used in drug carrier research. PLGA has the advantages of protecting the embedding drug, targeting the lesion, controlling drug release, prolonging drug action time, reducing drug toxicity and irritation$^{5,10}$. Therefore, this study explored the preparation method of PLGA microspheres, and successfully embedded As$_2$O$_3$ and HCPT into PLGA microspheres. At the same time, the related characterization in vitro was car-