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Review Article

The Use of Exosomes as Carriers for Inhibiting Breast Cancer through a Chitosan Base Hydrogel Scaffold

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Abstract:

Extracellular vesicles (EVs) are secreted from the cells to the extracellular space, which have the role of cellular communication both topically and systemically. EVs are divided into three categories: exosomes, micro vesicles and apoptotic body. Exosome particles are in the size of 30 to 100 nm. Exosomes, according to studies, include miRNAs that play a role in their biological effects and, after exosome entry into the target cell, has its own effects. On the other hand, exosomes can be used as drug carriers. Hydrogel scaffolds can be used as drug delivery systems for transferring cells and various factors. This hypothesis was designed with the aim of using a chitosan-based hydroxyl delivery system for the transfer of exosomes of anticancer drug carrier to inhibit breast cancer cells in mice.

Key words: Exosomes, Chitosan, Cancer, Hydrogel

Introduction

For many years, attention has been focused on the development of drug delivery systems, with the aim of designing these systems to reduce the frequency of intake, increase the effect of the drug and reduce its complications. Hydrogels are among the items that are used as a drug delivery system. Hydrogels used in this system are used as parts, microparticles, nanoparticles, coatings or films. Among other parameters that cause hydrogels to be widely used in the drug delivery system, their biocompatibility properties can be highlighted, which makes the hydrogel play an important role without harming adjacent cells(1-3). In recent years, chitosan hydrogels have been highly regarded in drug delivery systems. Among natural and synthetic polymers, chitosan is more biocompatible and biodegradable. The heat-sensitive chitosan hydrogels with the ability to inject at the desired location in many cases, eliminated the need for surgery or placement of the implant(4). Extracellular vesicles play the role of cellular communication both topically and systemically. Transmission of their contents between cells causes significant changes in cellular behavior. Extracellular vesicles are involved in regulating a large number of physiological and

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pathological processes, including developmental and neurological and cardiovascular diseases(5) (fig 1).

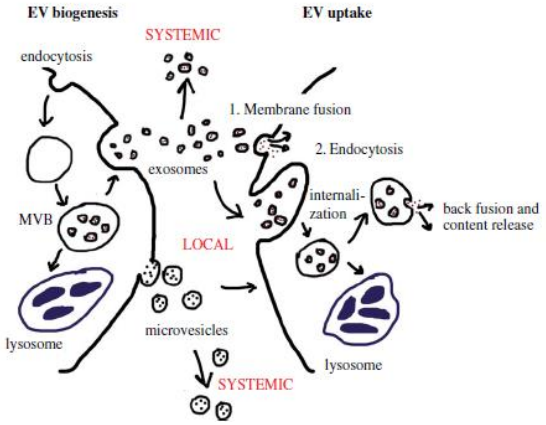

Fig 1. Extracellular vesicles(5).

Exosomes are secretive in physiologic and pathological conditions of various cells, such as tumor cells, hematopoietic cells including reticulocytes, dendritic cells, B and T lymphocytes, platelets and macrophages, and epithelial cells, fibroblasts, astrocytes and neurons. The roles of tumor-derived exosomes in cancer pathogenesis(6). Exosomes contain various compounds. Their fat compositions include cholesterol, Sphingomyelin, Phosphatidylserine, Hexosylceramides, and saturated fatty acids that exist in plasma membranes. The exosome proteins include related proteins in membrane and fusion transitions such as Rab GTPases and Annexins, as well as proteins involved in exogenous biogenesis, such as ESCRT complex, ALIX, TSG101. MiR and mRNA have also been identified in exosomes(7). Various studies have shown that exosomes can be used as nanoparticles in the drug delivery system. One of these studies has shown that exosomes can be released from a variety of cells and form a complex with curcumin. The goal is to treat mice infected with Lipopolysaccharides (*LPS*)septic shock. Curcumin has its anti-inflammatory effect in these mice. Application of exosomes as carriers increases the stability and solubility of curcumin in vitro and increases the bioavailability in vivo. The encapsulation of curcumin in the exosome increases the anti-inflammatory activity of curcumin(8). In another study, the role of exosomes as a carrier in the transmission of paclitaxel to lung cancer in mice was investigated. The results showed that inhibition of cancer cells in the exosoma encapsulated paclitaxel group was significantly more significant than paclitaxel alone(9).

Hypothesis

Balb / c female mice between 4 to 6 weeks and 20 to 25 gr of with injection of 4T1 breast cancer cells in the right flank, will be diagnosed with breast cancer after two weeks. Exosomes in this study are extracted from rat bone marrow mesenchymal cells. Five rats will be used to extract mesenchymal cells. Rats are anesthetized with ketamine and xylazine. The surgical site is rinsed with iodine, sterilized in the surgical area, and skin and muscle will be opened with sterile surgical instruments. The femur and tibia bones are separated and carefully cleans muscles and soft tissues around them. The rats were then given an overdose of anesthesia and killed. The bones are located inside the DMEM medium and the falcon will be kept until it reaches the bottom of the hood on ice. Under the hood, cut off the two ends of the bone, and remove the bone marrow from the syringe and needle number 22 and place it in a DMEM medium containing the flask. The medium contains 15% FBS and antibiotics (streptomycin and penicillin). The incubation flask will be replaced after three hours of the medium. Then, 72 hours later, every 8 hours and after this time, the medium is changed every three days. After two weeks, mesenchymal cells sticking to the plate floor are ready for the passage(10). At this stage, when the cell density reaches about 90%, the FBS is removed from the environment and the supernatant of the cell will be collected after 48 hours. To remove dead cells and debris for 20 minutes, 2000 g at 4 ° C will be centrifuged. The supernatant is collected and the cell pellet is discarded. Re-centrifuge will be performed within 30 minutes, around 20000 and at 4 ° C. The supernatant is re-collected and the cell pellet is discarded. In the end for 90 minutes, a circle of 60,000 g will be centrifuged at 4 ° C. In this step, the supernatant is removed slowly and the pellet is suspended by 1ml, PBS(11).

After exosome extraction, evaluation of its confirmation methods will be performed with the electron microscope and western blot (CD81), and the extracted exosomal concentration will be calculated by BCA method(12). To load a docetaxel

chemotherapy drug in exosomes, the following procedure will work. First we mix 10^{11} exosomes in 1 ml of docetaxel. There are several ways to load the drug, which will be used here as an incubation method. The mixture should be shaker for 1 hour at 37 ° C(9). We prepare two different concentrations of exoDOX (docetaxel). Chitosan base hydrogel scaffold containing β-glycerol phosphate, chitosan and hydroxyethyl cellulose (HEC) will be prepared according to the formula. This scaffold is sensitive to temperature so that it is liquid at low temperature and after it enters the body, the liquid will become a gel. To prepare this scaffold, we first weighed 225 mg of chitosan powder and placed in 9 ml of 0.1 molar Hcl for a few hours on a shaker to completely dissolve and then autoclaved at 15 ° C for 12 minutes. Since the chitosan powder in 9 ml of Hcl adheres a lot to the wall, the solution may burn and grease during autoclave. So the chitosan powder was weighed and sterilized. The 0.1 molar acid was also sterilized separately. (After sterilizing the powder and the above solution in the autoclave, it was mixed under the hood). Then 2.25 g of β-GF was dissolved in 3.5 cc of deionized water and sterilized by filtering. The sterilized chitosan and β-GF solutions were each separately stored for 15 minutes on ice, and then β-GF was added dropwise and slowly added to the chitosan solution that was rotating with the magnet. 125 mg of HEC was dissolved in 10 ml in DMEM medium or in PBS. As the viscosity of this solution is high and it is not sterilized, the HEC powder should be autoclave(Chitosan according). Cold hydroxyethyl cellulose was added to chitosan-beta-glycerol phosphate 4.8: 0.8 (HEC: CH-GP). The HEC solution is added directly to the CH-GP just before the injection, and the CH-GP is then stored on the ice until it is injected before it can be prevented from gelling. Adding the cell simultaneously will be with HEC in a very small volume (10 million cells in 500 macrol)(13). The docetaxel loaded in the exosome was investigated by MTT on T1 cell lines and IC50 was obtained and two different concentrations were prepared and added to the hydrogel scaffold and then to the cancerous mice at two intervals of one week and two weeks Will be injected. Injection is carried out at the site of the tumor and also in a group through the dementia vein. The mice will be injected into three groups: control (cancerous mice receiving PBS), group 1 (exoDOX in the tumor area will be injected without hydrogel scaffold) and group 2 (exoDOX will be injected into the tumor region with a hydrogel scaffold) Are divided (fig 2). During and after the above time, the tumors are measured and then measured in size and weight after the end of the study. The tissue will be studied in terms of histological changes. P53 will be tested by RT-PCR.

Conclusion

Apoptosis is expected to occur in these cells in addition to inhibiting the growth of cancer cells, which will help to treat breast cancer with the preservation of the body's natural tissue. Current methods of treating breast cancer with chemotherapy drugs and ultimately mastectomy, in addition to many psychological complications, are imposed on the patient. The presence of hydrogel scaffolds is one of the important benefits of this study. This scaffold helps to keep the exosomes containing the drug in place of the tumor. A high dose of drug injection with this method will be reduced. Meanwhile, systemic side effects will be eliminated by this method. Meanwhile, the release of drugs containing exosomes from the hydrogel scaffold will help to improve the treatment over time. The proper porosity of the scaffold ensures the proper placement of exosomes. Exosome plays a role in the transfer of medicine as a nano-particle. As an exosome, as a carrier, you can easily carry the docetaxel chemotherapy that is specifically for breast cancer treatment. Due to the fact that exosomes are taken from the mesenchymal stem cells of the same mouse, there are no safety and rejection problems.

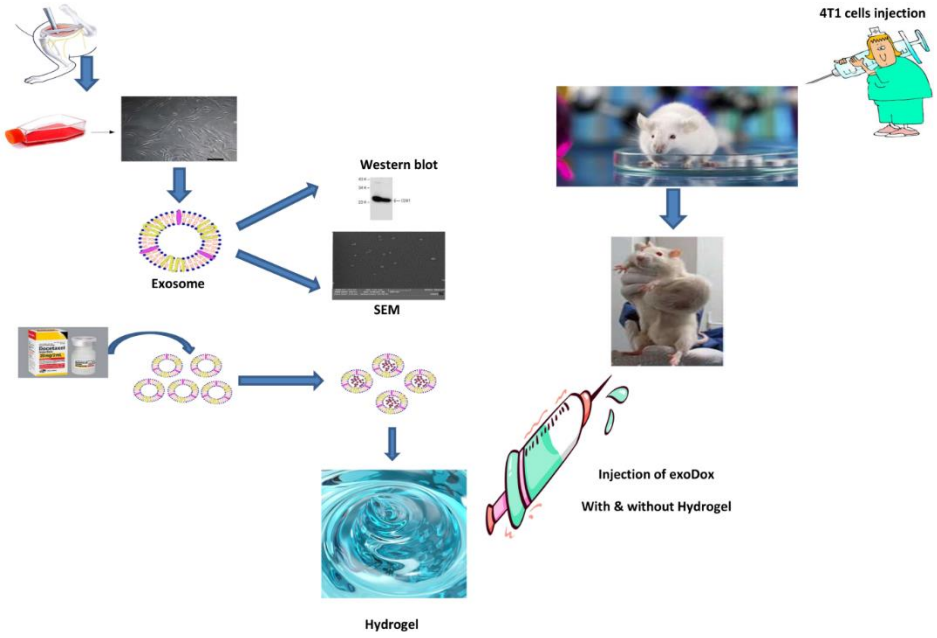

Fig 2. Exosomal extraction process from rat mesenchymal cells and injection of exosomal encapsulated drug into tumorized rat

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