DEVELOPMENT OF A DRUG TARGETING APPROACH FOR CANCER THERAPY: Drug Carrier-Protein Conjugate

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ABSTRACT

Targeted delivery of anticancer drugs is one of the most actively pursued goals in anticancer chemotherapy. A major disadvantage of anticancer drugs is their lack of selectivity for tumor tissue, which causes severe side effects and results in low cure rates. Any strategy by which a cytotoxic drug is targeted to the tumor, thus increasing the therapeutic index of the drug, is a way of improving cancer chemotherapy and minimizing systematic toxicity. This study covers the preparation of the gelatin microsphere (GM)- albumin (BSA) conjugate for the development of a "drug targeting" approach in cancer therapy. Gelatin microspheres of 5% (w/v) gelatin content were prepared by crosslinking with glutaraldehyde (GTA) at 0.5% (v/v) concentration. The particle size and morphology of microspheres were analyzed by Particle Size Analyzer and Scanning Electron Microscopy (SEM). Gelatin micospheres were chemically conjugated to bovine serum albumin in PBS (0.01M, pH 7.4) at 4°C. The solution was then filtered and the GM-BSA conjugates were vacuum dried. The activity of BSA conjugated to GM was tested by using Immunodiffusion Techniques. The percent antigen-antibody binding between BSA and anti-BSA was evaluated by measuring the band widths of precipitates formed in the agar medium.

Keywords: Anticancer Chemotherapy, Drug Delivery Systems, Drug Targeting, GM-BSA Conjugate

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Abstract

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Introduction

Recently, microparticulate drug delivery devices have gained attention in targeting chemotherapeutic agents [1-3]. These systems have the advantage of being able to entrap relatively large amounts of pharmacologically active agents and are easy to prepare. Microspheres of natural materials such as gelatin [4], albumin [5], starch or synthetic polymers have been used for cancer chemotherapy.

Gelatin has wide applications in pharmacology and medicine since it is a biocompatible and biodegradable material. Gelatin is preferred in the production of particles [6] and microspheres [7] for the development of controlled release systems because it has a good film- and particle-forming property.

Microencapsulation of antineoplastic drugs appears to have special importance since many antineoplastic agents have several adverse side effects. The cuopling of microcarriers of anticancer drugs to suitable macromolecules is a promising approach of circumventing the toxic side effects of these agents to normal cells and of improving their efficacy towards malignant cells. These drug delivery systems were designed with the aim to selectively target antitumor agents and to obtain higher drug concentration at the tumor site [8]. Blood proteins (i.e. serum albumin, transferrin) have attracted the most interest regarding their potential as drug delivery systems for improved cancer chemotherapy. These proteins are suitable as drug carriers for a number of reasons: (a)they exhibit a preferential uptake in tumor tissue, (b) tumor cells express high amounts of specific receptors on their cell surface, (c) they are readily available in a pure form exhibiting good biological stability and (d) they are biodegradable, non-toxic and non-immunogenic [9].

Materials and Methods

Materials

Microspheres were prepared by using gelatin (Sigma) having the following specifications, approx. bloom 300; pH 6.3-9.2. Glutaraldehyde (47.53%, w/w), a product of GPR (General Purpose Reagent), 1-ethyl-3-(3-dimethylaminoprophyl) carbodiimide, EDAC (Sigma), bovine serum albumin (Sigma) and anti-bovine serum albumin (Sigma)

were used as obtained. All other materials and solvents were of the highest purity available.

Preparation of gelatin microspheres

Microspheres were prepared by using a (w/o) emulsion of the aqueous gelatin solution in paraffin oil at 30°C. The formed microparticles were washed with acetone and vacuum dried at 25°C.

Particle size and morphology analysis of gelatin microspheres

Average size and size distribution curves of microspheres are determined by using a particle size analyzer (Mastersizer S, Malvern Instruments, U.K). Scanning electron micrographs were obtained by using a Noran scanning electron microscopy (SEM, JSM-6400, Japan).

Preparation of GM-BSA conjugate

A known quantity of GM (5% w/v gelatin, 0.5% v/v glutaraldehyde) was put in PBS (0.01M, pH 7.4) containing EDAC and stirred gently at 25°C. Then, the GM was filtered, washed and put in the BSA solution, at 4°C. The resulting GM-BSA conjugates were used in the immunodiffusion tests.

Preparation of a calibration curve (for the evaluation of % antigen-antibody binding)

Five agar solutions having different concentrations of anti-BSA were prepared. A known quantity of BSA solution was put in the well which was punched in the middle of the solid agar. After 24 hrs of incubation at 25°C, clear bands were identified around the wells. The band diameters (D) were measured and a calibration curve was drawn for band diameter square (D^2) versus anti-BSA concentration in agar.

Testing the activity of BSA conjugated to GM4

New agar was prepared and the GM-BSA conjugates were put in the well punched in the middle of agar. After 1 week period of incubation at 25°C, 100% humid environment, a

band was formed around the well. The band diameter was measured and the percent antigen-antibody binding was evaluated by using the prepared calibration curve.

Results and Discussion

Effects of gelatin and glutaraldehyde concentration on microsphere properties

In this research, different types of microspheres were prepared. The variables were the concentration of gelatin and the croslinker (glutaraldehyde) and the stirring speed.

Particle size analysis of microspheres showed that as the concentration of gelatin was decreased, the mean particle diameter was also decreasing. The modification of gelatin concentration changes the viscosity of the gelatin solution. This in turn influences the mean diameter of particles produced. Indeed, when the concentration of gelatin was decreased to 50 mg/ml, smaller size microspheres with better surface characteristics were obtained.

On the other hand, decrease in glutaraldehyde concentration produced the reverse effect. Since the addition of glutaraldehyde to the aqueous gelatin phase produced the crosslinking between the protein chains, the matrix became stronger leading to a decrease in the mean diameter of the particles.

The stirring speed was another parameter which influenced the distribution of the dimensions of microspheres. It was found that increasing the stirring speed from 500 rpm to 1000rpm caused a decrease in the particle size of GM samples.

Percent (%) antigen-antibody binding

In this study, it is found that there is 80.26% binding between BSA conjugated to GM and the anti-BSA in agar. It could be concluded that by using the present experimental conditions it is possible to conjugate GM with BSA while reserving the biological activity of the molecule. Indeed, the 19.74 % amount of loss in the activity is suggested to be due to the differences in the diffusion abilities of the microparticles in the agar medium. Another reason is the possible hiding of the active groups of albumin (BSA) during the cunjugation process.

Serum proteins such as albumin and transferrin offer promise for the selective delivery of antineoplastic agents due to their accumulation in tumor tissue. Uptake of these proteins in solid tumors is mediated by a number of factors, including an increased metabolic activity of tumors, an enhanced vascular permeability of tumor blood vessels for circulating macromolecules, and a lack of a functional lympathic drainage system in tumor tissue. At the tumor site, albumin and transferrin are taken up by the tumor cell through receptor mediated and fluid phase endocytosis, respectively. Certainly, further studies are aimed in order to test the uptake mechanism of GM-BSA conjugate by tumor cells at the cellular level.

Future research

- preparation of anticancer drug loaded gelatin microspheres,
- studying the release characteristics of the anticancer drug from microspheres,
- examination of "growth inhibition" effect of GM [loaded with anticancer drug] *in vitro*,
- critical evaluation of GM-BSA conjugate with anticancer agents in animal models.

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