Preparation and evaluation of Pingyangmycin-loaded chitosan thermogels for vascular malformations therapy

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ABSTRACT SUMMARY

The purpose of our study was to investigate the potential of Pingyangmycin (PYM) -loaded chitosan (CS) thermogels for interventional embolization therapy. PYM-CS thermogels were prepared and evaluated by the rheological properties and in vitro release behaviors. Rheological studies proved that as the temperature raised to about 37 °C, the PYM thermogels transformed from solution phase to gel phase. It was showed that in vitro release of PYM from the thermogels could be delayed up to 10 days.

INTRODUCTION

Chemoeembolization, which connects the benefits of therapeutic drugs and embolic materials, has become a promising therapy for vascular malformations. However, few reports have been concerned about the connected use of sclerosants and embolic materials. The purpose of our study was to obtain an infarction effect and a sustained therapeutic effect by decrease of blood flow through the sclerosis effect and delayed release behavior of the chitosan (CS) thermogels. CS thermogels with suitable strength could decrease or even cut off the blood flow and the sclerosants release from thermogels can induce the proliferation and migration of vascular cells and thus thickening of the vessel wall which finally result in the occlusion of the vessels.

Pingyangmycin (PYM), which is a new type of cytotoxic glycopeptide antitumor antibiotic and was developed in China in the 1980’s, has been used extensively in the far East for treatment of head and neck tumors with a definite therapeutic effect was selected as a model sclerosant.¹ However, short half-life and lung-toxicity restrained its further application in clinic.² So, in present study, CS thermogels were chosen as carrier matrix due to its good biocompatibility, and then PYM-CS thermogels were prepared and played dual roles as sclerosants and embolic materials.

EXPERIMENTAL METHODS

Preparation of PYM-CS thermogels was as follows: typically, CS (1.8%-2.2%, w/v) was added slowly to 0.1 mol/l acetic acid with stirring, and then the mixture was continuously stirred overnight to make a clear solution. Then the CS solution was chilled to 4 °C in an ice bath. Glycerophosphate (GP) (6%-14%, w/v) and PYM (2mg/ml-4mg/ml) were dissolved in purified water separately, and chilled along with the CS solution to 4 °C. The GP solution was added dropwise into the CS solution with agitating and then they were mixed for 10 min. Finally, the formulation was obtained by adding PYM solution to the CS-GP solution under stirring for 6 min.

The rheology study was carried out on AR2000ex rheometer (TA. Co. Ltd., USA) equipped with a 40mm parallel plate rotor and 5ml formulation sample was needed. In variable temperature mode, the changes in elastic modulus (G’) and loss modulus (G’”) were recorded as a function of temperature. The oscillatory frequency was fixed at 1.0 Hz during the measurements. The temperature changed from 15 °C to 45 °C with a rate of 2 °C/min.

In the study of in vitro release, 3ml of the PYM-CS thermogels were injected into 20ml glass vials (diameter 20mm) and then kept warm in an 37 °C water bath for half an hour so
that they could be transformed into gel completely. Then 15ml phosphate buffer solution (pH7.4) (PBS) containing NaN₃ (0.05%, w/v) and lysozyme (1.0%, w/v) were added into the vials. The dissolution system was shaken in an incubator at 50rpm and 37 °C. The release medium was all collected at predetermined time intervals for analysis and replaced with same amount of fresh dissolution medium. The concentration of PYM in the release medium was assayed by a high performance liquid chromatography (HPLC) method. All the in vitro dissolution tests were carried out in triplicate, and the accumulation release amount of PYM from PYM-CS thermogels was calculated.

RESULTS AND DISCUSSION

The morphology of the PYM-CS thermogels before and after sol-gel transition was displayed in Fig.1. It was showed that the formulations, which were transformed from solution to gel with the increase of temperature, had good thermosensitive properties.

![Fig.1 Picture of the PYM-CS thermogels before and after sol-gel transition](image)

Generally, under the condition of constant frequency, the crossing point of G’-temperature profile and G’’-temperature profile is identified as phase inversion temperature (PIT). Fig.2 showed that both G’ and G’’ increased with temperature. When the temperature was raised to about 37 °C, the crossing point which was the PIT of PYM-CS thermogels was appeared.

As demonstrated in Fig.3, the PYM release from the PYM-CS thermogels could be extended up to 10 days. There was an initial burst with in the first day. But it was much lower than that of other in situ gel drug delivery systems for water-soluble drugs.

![Fig.2 Elastic modulus (G’) and loss modulus (G’’)](image)

![Fig.3 PM in vitro release profile from PYM-CS thermogels (n=6)](image)

CONCLUSION

The present research described preparation and evaluation of CS based thermogels for extended release of PYM. The results showed that by incorporation into the CS thermogels, a sustained release of PYM was obtained in vitro, which should be proved by in vivo pharmacokinetic study. In conclusion, the PYM-loaded injectable CS based thermogels are a promising formulation for interventional sclerosing embolization therapy of vascular malformations.

REFERENCES

2. Gao, Z. B.; Ding, P. T. Int. J. Pharm. 2007, 328, 57-46.

ACKNOWLEDGMENTS

We are thankful for the financial support of the Project Funds from Science and Technology Council of Liaoning Province (2010225034).