学位論文内容の要旨

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学 位 論 文 題 名

Studies on developing a water-soluble polymer bearing adjacent cation-aromatic sequence as a novel embolic material

（新規塞栓物質としてのカチオン–アロマ配列隣接型水溶性高分子の開発に関する研究）

【Background and Objectives】Liquid Embolic Agents (LEAs) are widely used for endovascular embolization of cerebrovascular diseases such as cerebral Arteriovenous Malformations and cerebral Aneurysms. LEAs undergo embolization by forming a clot based on their phase transition. Despite the excellent performances of LEAs, all these classical liquid embolic materials are limited by the adhesion of microcatheter to the embolic agent, use of an organic solvent, unintentional catheter retention, and other complications. In mammals, blood clots formed by fibrin polymerization and platelet activation can act as hemostatic agents physiologically or pathologically obstruct vessels. As blood substance such as blood cells and proteins own negatively charged surface, we supposed that cationic polyelectrolytes can aggregate blood substance to achieve embolization. However, the electrostatic interaction between oppositely charged surfaces normally diminishes in saline water owing to the Debye screening effect, which prevents the formation of a blood gel. Therefore, we synthesized a series of water-soluble polymers bearing adjacent cation-aromatic sequences named poly(cation-*adj*-π) (adj is short for adjacent and π for aromatic monomer), which can show electrostatic adhesion with negatively charged blood substances in a physiological environment. In this study, we developed and evaluated the newly synthesized material through blood aggregation test, in vitro injection test, in vitro extraction test, biocompatibility test and in vivo injection test in order to examine its clinical utility.

【Materials and methods】Four different types of poly(cation-*adj*-π) aqueous solutions and poly(cation) aqueous solutions were synthesized in this research. In Vitro: First, four different poly(cation-*adj*-π) aqueous solutions as well as different concentrations of same polymer aqueous solutions were added into citrated blood and different contents of blood taken from Sprague-Dawley rats, poly(cation) aqueous solutions were set as control. Physical properties of formed blood gels were tested through Rheological tests. Second, injection force of injecting polymer solutions into clinical needles and catheters were recorded. Third, traction force of blood gel formed by polymer aqueous solutions and citrated blood after injection was also recorded. And Onyx, one of currently used commercial liquid embolic agents, was set as control. In Vivo: First, polymer aqueous solutions as well as a poorly flowing entangled polymer hydrogel disc were implanted under the dorsal skin for 30 days and 50 days. Peripheral blood was taken for biochemical test at 7^{th} day and 28^{th} day after implantation. Besides, Hematoxylin-Eosin (H&E) staining and immunohistochemical (IHC) staining of tissues around the incision were done for histological analyses. Second, quantified dose of poly(cation-*adj*-π) aqueous solutions were injected into rat's femoral artery. Skin color and tissues necrosis of injected hindlimb were observed and recorded at selected time points. Then, tissue H&E staining of injected hindlimbs were done for intravascular evaluation. Third, poly(cation-*adj*-π) aqueous solutions were mixed with tantalum powders for radiopaque ability. Next, different doses of mixture were injected into rat's femoral artery. Computed tomography (CT) scanning was conducted at selected points after injection. Finally, rat femoral artery injection was repeated by using 10 rat (20 hindlimbs in total) for verifying the reproducibility of this approach.

【Results】In Vitro: First, poly(cation-*adj*-π) aqueous solutions at different concentrations can rapidly interact with

blood within 1 min to form a stable blood gel. By contrast, the poly(cation) cannot. Also, poly(cation-*adj*-π) can glue different blood contents together respectively. The Rheological results showed that blood gel formed by poly(cation-*adj*-π) exhibited viscoelastic behavior and a strong frequency dependence of G′, which is larger than the loss modulus G″ over the tested frequency range. Second, poly(cation-*adj*-π) aqueous solutions can be smoothly injected through needles and catheters. Third, traction force of the poly(cation-*adj*-π)-based blood gel was much lower than that of Onyx 18 and was similar to that of non-agglomerate samples using normal saline. In Vivo: first, H&E staining and IHC staining results of subcutaneous tissues showed no obvious inflammation around the incision in either the polymer or hydrogel groups. Second, the results of in vivo injection test showed that right hindlimb with embolization gradually darkened and became unable to move, eventually leading to necrosis after surgery. Third, embolization conducted by polymer solutions was confirmed by CT scanning after mixing with tantalum powders. The CT results of the one-shot injection (2–3 μL) indicated that embolization only occurred near the injection site, while CT results of 0.05 mL injection showed that the mixture had good fluidity and could completely occlude the arteries of the right hindlimb. Besides, successful embolization was also confirmed on the 7th day after injection.

【Discussion】First, according to results of In vitro blood aggregation test, it was found that poly(cation-*adj*-π) aqueous solutions can rapidly interact with blood to form a stable blood gel, while the poly(cation) cannot. This result indicated that cationic monomers both in poly(cation) and poly(cation-*adj*-π) could react with negatively charged substances through electrostatic interactions, but the electrostatic interaction between oppositely charged surfaces normally diminishes in saline water owing to the Debye screening effect, which leading to unstable blood gel. Poly(cation-*adj*-π) formed a stable blood gel with blood due to the aromatic monomers, which enhance the electrostatic interactions of their adjacent cationic residues with the counter surfaces, even in a high ionic-strength medium. Besides, poly(cation-*adj*-π) aqueous solutions could also aggregate blood cell solution (mainly red blood cells) isolated from whole blood into a stable clot. This result further confirmed that poly(cation-*adj*-π) aqueous solutions can glue blood into gel through electrostatic interactions. Besides, Rheological results showed that blood gel formed by poly(cation-*adj*-π) exhibited viscoelastic behavior and a strong frequency dependence of G′, indicated that the blood gel was a solid-like gel. Second, the results of injection force test verified that poly(cation-*adj*-π) aqueous solutions could be injected smoothly. Then, the traction force test showed low traction force of poly(cation-*adj*-π)-based blood gel, which was much lower than that of Onyx 18 and similar to that of normal saline group. These results indicated that the blood gel formed by poly(cation-*adj*-π) showed no obvious adhesion to plastic tubes, thereby considerably reducing the risk of hemorrhage in the traction process. Third, results of subcutaneous implantation test showed poly(cation-*adj*-π) solutions are biologically well adapted. At the same time, result of 50-day hydrogel subcutaneous implantation showed the hydrogel volumes shrank but the shapes did not change, indicating good stability of the polymers in the body. Finally, in vivo embolization capacity of poly(cation-*adj*-π) aqueous solutions was confirmed by rat femoral artery injection model. poly(cation-*ad*j-π) aqueous solutions can occlude the vessel through forming blood gels with gluing blood contents together, which was verified by H&E staining of occluded vessels. Besides, after mixing with the tantalum powders, embolization conducted by poly(cation-*adj*-π) solutions cloud also be confirmed by post-surgery CT scanning even on the 7th day after injection.

【Conclusion】In summary, we have demonstrated a conceptual strategy for endovascular embolization. In contrast to existing LEAs, whose embolization is based on the solidification of exotic materials, the water-soluble polymers bearing adjacent cation-aromatic sequences can glue negatively charged blood substances into a gel-like material through electrostatic interactions in normal saline water, whereas common polycations cannot form these interactions. Owing to its easy delivery through a microcatheter with low injection force coupled with biocompatibility and controlled embolization, the poly(cation-*adj*-π) aqueous solution is a promising liquid embolic agent.