Inhibitory effect on choroidal neovascularization using cyclic RGD tethered heparin-Pluronic nanogels carrying avastin

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ABSTRACT SUMMARY

Low molecular weight heparin-Pluronic (LHP) nanogels tethered with cyclic RGD (cRGD) and FITC were prepared for targeted delivery of avastin as an anti-angiogenic agent for inhibitory efficacy on choroidal neovascularization (CNV) induced region. Administered LHP nanogels via i.v. injection exhibited to localize into the CNV induced region. The obtained results demonstrated that LHP nanogels have remarkable potential for inhibiting CNV.

INTRODUCTION

Age-related macular degeneration (AMD) is a disease leading to severe visual loss in elderly populations, which is diagnosed as either dry or wet AMD. Particularly, CNV caused by growing new blood vessels on the macular region is the major mechanism of wet AMD. Current treatments for CNV are mainly focused on intravitreal injection of anti-angiogenic agents. However, it is reported that this approach has several limitations such as a complication occurred by glaucoma, vitreous hemorrhage, and detachment of retina. Several attempts have been made to overcome these limitations. Among them, targeted systemic delivery of anti-angiogenic agents using nano-carriers has been received a great deal of attention in the CNV treatment. Especially, nanogels have advantages for long-term circulation systems in the body and drug delivery systems due to having an interior network structure for the incorporation of drugs by non-covalent interaction. In this study, we prepared LHP nanogels tethered with cyclic cRGD and FITC for targeted delivery of avastin as an anti-angiogenic agent. The targeting efficacy of the avastin-loaded LHP nanogels to CNV was investigated by monitoring the CNV region in a laser induced rat model.

EXPERIMENTAL METHODS

LHP conjugate was synthesized as previously reported. Briefly, The hydroxyl group of Pluronic was modified to amine group with ethylene diamine by using p-nitrophenyl chloroformate as an activating agent. The aminate Pluronic was conjugated onto low molecular weight heparin backbone by using EDC and NHS as coupling agents. Cyclic RGD was conjugated to amino groups of LHP by using EDC/NHS chemistry, followed by FITC conjugation. The chemical structure of LHP conjugate was characterized by ¹H NMR. The bioactivity of LHP conjugate was measured by anti-Factor Xa assay. Avastin loaded LHP nanogels were prepared using a direct dissolution method. The average diameter and shape of the avastin loaded HP nanogels were measured by DLS and TEM, respectively. The encapsulating efficiency of avastin in the LHP nanogels was measured by MicroBCA kit. Cytotoxicity and intracellular uptake of avastin loaded LHP nanogels against RPE cells were evaluated by CCK analysis and CLSM, respectively. The targeting efficacy of the nanogels was investigated using a laser induced
rat model administrated via intravenous injection, which was visualized by fluorescence angiography.

![Image 1](image1)

**Fig. 2. The TEM image of LHP nanogels**

**RESULTS AND DISCUSSION**

In the $^1$H NMR spectrum of LHP conjugate, strong resonance signals at 3.6 and 1.1 ppm indicate the presence of Pluronic and heparin in LHP. The synthesized LHP conjugate exhibited reduced bioactivity of about 33% as compared to that of low molecular weight heparin. The average diameters of the cRGD and FITC tethered avastin loaded LHP nanogels were measured to be approximately 40 to 60 nm with an increase in the feed ratio of avastin. As shown in Fig. 2, the LHP nanogels exhibited spherical spheres with a narrow distribution in the size, similar to an average diameter determined by DLS. The encapsulating efficiencies of avastin were ranged from 70 to 80% depending on the feed ratio of avastin. The avastin loaded LHP nanogels showed no significant cytotoxic effect and they appear to stimulate the growth of RPE cells. The intracellular uptake studies revealed that the avastin loaded LHP nanogels were internalized into RPE cells after 6 h incubation and then exocytosed with further incubation of 6 h. As shown in Fig. 3, intravenous fluorescein angiography studies demonstrated that the cRGD and FITC tethered avastin loaded LHP nanogels could be localized into the CNV induced region within a few minutes upon intravenous administration as compared to the control.

![Image 2](image2)

**Fig. 3. Fluorescence angiography of CNV region treated with (a) dextran-FITC as a control and (b) LHP nanogel**

**CONCLUSION**

HP nanogels have exhibited high efficiency of avastin encapsulating and localizing on the target region. These nanogels have also shown significant non-cytotoxicity. Obtained results suggest that the HP nanogels can be promising carriers for inhibition of the CNV.

**REFERENCES**


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