Hyaluronate - Gold Nanoparticle / Tocilizumab Complex for Combination Therapy of Rheumatoid Arthritis

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ABSTRACT SUMMARY

Tocilizumab (TCZ) is a humanized monoclonal antibody against interleukin-6 (IL-6) receptor, interfering IL-6 in the pathogenesis of rheumatoid arthritis (RA). Gold nanoparticle (AuNP) has an anti-angiogenic effect and has been used for the treatment of RA. HA is known to have cartilage-protective and lubricant effect. Taking advantages of all these components, we developed HA-AuNP/TCZ complex for the treatment of RA. HA was modified with cystamine via reductive amidation, which was reduced with dithiothreitol (DTT) to prepare end-group thiolated HA (HA-SH). AuNP was chemically modified with HA-SH and physically modified with TCZ. The formation of HA-AuNP/TCZ complex was confirmed by UV-Vis spectroscopy, dynamic light scattering (DLS), and transmission electron microscopy (TEM). The therapeutic effect of HA-AuNP/TCZ complex on RA was confirmed by ELISA, histological, and immunohistochemical analyses in RA model mice.

INTRODUCTION

RA is a chronic inflammatory immune disease with the inflammation of synovial membrane and the resulting articular cartilage destruction. AuNPs have been widely used in a variety of biomedical applications due to its biocompatibility, safety, simple synthesis, facile surface modification, versatile conjugation with biomolecules, and tunable optical properties. Moreover, AuNPs have been known to have an anti-angiogenic effect by binding vascular endothelial growth factor (VEGF) which plays a crucial role in the pathogenesis of RA. TCZ is the first monoclonal therapeutic antibody against IL-6, binding IL-6R and antagonizing the interaction of IL-6 with IL-6R. TCZ has gained its popularity for rapidly suppressing inflammation and preventing joint destruction.

In this work, we developed a new RA treatment system using HA-AuNP/TCZ complex. HA is a linear biopolymer which is biocompatible, biodegradable, non-immunogenic, and nontoxic. In addition, HA has unique rheological property, showing cartilage protective and lubricant effect in synovial fluid. Moreover, HA has a positive effect on the stability of HA-AuNP/TCZ complex by reducing its non-specific interaction with the serum components in the body. The pathogenesis of RA is so complicated that completely alleviating the events of RA is very difficult by interfering with one molecule. After in vitro characterization, we assessed therapeutic efficacy of the dual targeting HA-AuNP/TCZ complex to VEGF and IL-6R for the treatment of RA and discussed the feasibility for further clinical applications (Figure 1).

Figure 1. Schematic illustration for the dual targeting HA-AuNP/TCZ complex to VEGF and IL-6R for the treatment of RA.

EXPERIMENTAL METHODS

HA-SH was prepared by reductive amidation with cystamine and the following reduction with DTT. HA-SH was immobilized onto the surface of AuNP via Au-thiol chemistry. After that, HA-AuNP solution was centrifuged and resuspended to remove unbound HA-SH. TCZ was mixed with HA-AuNP solution for physical binding of TCZ to AuNP. The resulting HA-AuNP/TCZ complex was characterized by DLS and TEM. The anti-angiogenic effect of HA-AuNP/TCZ complex was assessed by MTT assay for the inhibition of VEGF induced proliferation of HUVECs. Arthritis model mice were prepared by the treatment of 8 weeks old DBA/1j mice with the mixture of complete Freund adjuvant and type 2 collagen. Then, the RA model mice (n = 3) were treated at one arthritic ankle joint per mouse with four types of samples at 5 weeks after initial immunization: PBS for group 1, HA-AuNP for group 2, TCZ for group 3, and HA-AuNP/TCZ complex for group 4. The treated mice were monitored to assess the therapeutic effect of the samples on RA for a month and then sacrificed for the histological and immunohistochemical analyses.
RESULTS AND DISCUSSION

According to the analysis by DLS, the hydrodynamic size of AuNP was ca. 19.02 nm with a narrow PDI of 0.18 and that of HA-AuNP was ca. 46.54 nm with a PDI of 0.17. The diameter of HA-AuNP/TCZ complex was ca. 64.83 nm with a PDI of 0.183 (Figure 2a). The monodisperse formation of the HA-AuNP/TCZ complex was also confirmed by TEM. The size of AuNP was ca. 15 nm and the layer of TCZ surrounding AuNP was observed on the TEM image (Figure 2b). The anti-angiogenic effect of HA-AuNP/TCZ complex with increasing concentration of AuNP from 5 nM to 50 nM on the proliferation of HUVECs treated with VEGF was confirmed by MTT assay (Figure 3).

![Figure 2](image1.jpg)

Figure 2. (a) The hydrodynamic diameters of AuNP, HA-AuNP, and HA-AuNP/TCZ complex. (b) TEM image of HA-AuNP/TCZ complex.

![Figure 3](image2.jpg)

Figure 3. Effect of AuNPs (5 nM, 10 nM, and 50 nM) on the VEGF induced proliferation of HUVECs.

The therapeutic effect of HA-AuNP/TCZ complex was further assessed by investigating the inflammation, and the degeneration levels of cartilage and bone. According to the histological analysis with H&E staining, while normal mice without RA induction showed no signs of inflammation, and the degeneration of cartilage and bone, RA model mice showed severe inflammation, and the cartilage and bone degeneration (Figures 4a, 4b). In contrast, TCZ and HA-AuNP/TCZ complex treated groups showed drastically reduced levels of inflammatory cell infiltration, and the cartilage and bone degeneration (Figures 4c, 4d). Especially, the treatment group with HA-AuNP/TCZ complex showed the comparable clear interface between cartilage and bone to the normal control group (Figure 4d). Western blot analysis also confirmed the therapeutic effect of HA-AuNP/TCZ complex (Figure 5). While the negative control group showed significantly up-regulated expression levels of IL-6 and CD68, a marker for the various cells of the macrophage lineage, the treatment with HA-AuNP/TCZ complex resulted in significantly reduced levels of IL-6 and CD68.

![Figure 4](image3.jpg)

Figure 4. Histological analysis of 4 groups treated with (a) positive control, (b) negative control, (c) TCZ, and (d) HA-AuNP/TCZ complex.

![Figure 5](image4.jpg)

Figure 5. Densitometric analysis of the Western blot bands of (a) IL-6 and (b) CD68.

CONCLUSION

HA-AuNP/TCZ complex was successfully prepared for the treatment of RA. The formation of HA-AuNP/TCZ complex was clearly confirmed by DLS and TEM analysis. In vitro biological activity of HA-AuNP/TCZ complex was corroborated for the binding to VEGF. The therapeutic effect of HA-AuNP/TCZ complex on RA in model mice was verified by ELISA, histological, and immunohistochemical analyses. Taken together, HA-AuNP/TCZ complex might be developed as a dual targeting drug candidate to VEGF and IL-6R for the treatment of RA. In addition, the novel platform of HA-AuNP/protein complex can be exploited for various therapeutic applications.

REFERENCES