**ABSTRACT SUMMARY**

Lipophilic superparamagnetic iron oxide (SPIO) nanoparticles were self-assembled with an amphiphilic chitosan derivative, carboxymethyl hexanoyl chitosan (CHC), to form superparamagnetic CHC/SPIO micelles and then loaded with camptothecin (a hydrophobic anti-cancer agent). The superparamagnetic micelles were then conjugated with albumin-based microbubbles (MBs) to form superparamagnetic-micelle-decorated MBs (CHC/SPIO-decorated MBs). The CHC/SPIO-decorated MBs demonstrated in vitro and in vivo MR/US imaging contrast.

**INTRODUCTION**

An imaging contrast agent combined with drug encapsulation and triggered release functions is expected to be helpful for precise determination of the position and trigger timing of vehicles accumulated at a specific site.

In general, nano-sized vehicles are suitable for extravascular targeted imaging and delivery due to the fact that the leaky vasculature (with fenestrations sizes of hundreds of nanometers varied with the tumor type) in tumor tissue allows nanoparticles to escape from blood capillaries and then access the capillary-surrounding tumor cells [1]. On the other hand, micron-sized vehicles are usually employed to enhance intravascular targeted image because the extravasation effect of micron-sized vehicles is restricted (i.e., vehicles remain in the vessel lumen). Microbubbles (MBs) conjugated with vascular endothelia growth factor (VEGF) have been used to demonstrate the US vascular image for monitoring the progression of angiogenesis of metastatic cancer [2]. Therefore, multifunctional vehicles with a combination of nano-sized liposome and micron-sized bubble have been reported [3]. It is known that albumin-based and lipid-based MBs have long been employed as commercial US contrast agents having good safety in both cardiac and abdominal ultrasound applications. However, very few studies have examined the albumin-based MBs exhibiting dual-modal (MR/US) imaging functionality and ultrasonically triggered behavior. These capabilities were realized by the novel vehicle proposed in the present study in which hydrophobic anti-tumor agent and lipophilic superparamagnetic iron oxide (SPIO) nanoparticles were self-assembled with an amphiphilic chitosan derivative developed by our group, carboxymethyl hexanoyl chitosan (CHC), to prepare superparamagnetic micelles (CHC/SPIO micelles). These drug-loaded micelles were then conjugated with albumin-based MBs to form superparamagnetic-micelle-decorated MBs (CHC/SPIO-decorated MBs). Studies of superparamagnetic-micelle-decorated albumin MBs have not been reported, which deserves systematic investigation.

The objective of this study was to prepare the CHC/SPIO-decorated albumin MBs and investigate the effects of the superparamagnetic micelles on the in vitro MR and US images. In addition, a preliminary investigation of the in vivo MR and US images was also conducted to demonstrate the dual-modal imaging capability of the CHC/SPIO-decorated MBs.

**EXPERIMENTAL METHODS**

Hydrophilic SPIO nanoparticles were prepared by following a well-known method using FeCl₂ as a precursor. Next, lipophilic SPIO nanoparticles were obtained by the phase inversion method. The as-prepared lipophilic SPIO nanoparticles were mixed with hexane and CHC aqueous solution. The mixture was placed in an ice bath and sonicated by probe sonication for 2 min, producing CHC/SPIO micelles. CHC/SPIO micelles loaded with a hydrophobic model drug were prepared by replacing hexane...
with a CPT/hexane solution during this assembly process. Drug carrying capacity was determined by extraction method.

RESULTS AND DISCUSSION

OM and TEM photographs of albumin MBs are shown in Figure 1(a) and 1(b), respectively. MBs with a hollow structure were clearly observed. Figure 1(c) shows a TEM micrograph of the CHC/SPIO micelles, which shows that Fe₃O₄ nanoparticles were successfully assembled in the micelles. The effect of CHC on the assembly process is shown in Figure 1(d); Fe₃O₄ nanoparticles could not be assembled in the absence of CHC. This was ascribed to the fact that CHC is an amphiphilic chitosan derivative which has been employed as a micelle forming material for encapsulating hydrophobic agents. The morphologies of albumin MBs decorated with differing amounts of CHC/SPIO micelles are shown in Figure 1(e) and 1(f), which illustrate that the MBs were successfully decorated with CHC/SPIO micelles.

In vivo MR imaging of drug vehicles in the mouse body was conducted to determine the vehicle distribution Figure 2. The T₂ contrast of the MR image was enhanced in the liver in the first 20 min after injection. According to the in vivo US experiment, the circulation lifetime of MBs in the mouse body was few minutes. Therefore, the increase in the MR T₂ contrast was attributed mainly to hydrophobic SPIO nanoparticles (6–10 nm) and/or CHC/SPIO micelles (80–200 nm) that detached from broken MBs.

In vitro and in vivo US images of the CHC/SPIO-decorated MBs were examined via a clinical US scanner (frequency range of 5 to 15 MHz). CHC/SPIO-decorated MBs are clearly visible in a vein and an artery in the US images (data not shown).

CONCLUSION

A novel drug vehicle with multimodal imaging functionality was successfully prepared by decorating albumin MBs with CHC/SPIO micelles. In an in vitro study, the CHC/SPIO-decorated MBs demonstrated significant US imaging contrast and MR T₂ imaging contrast.

REFERENCES

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