Photoclick Reaction Programming Glutathione-Responsive System for Granuloma-Tracking and Anti-Tuberculosis

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Short Communication

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Abstract

Background:

Tuberculosis (TB) is a virulent form of infectious disease that causes a global burden due to its high infectivity and fatality rate, especially the irrepressible threats of the latent infection. Constructing an efficient strategy for the prevention and control of TB is of great significance. Fortunately, we found that granulomas are endowed with higher reducibility levels possibly caused by internal inflammation and a relatively enclosed microenvironment.

Results:

Therefore, we developed the first targeted glutathione (GSH)-responsive theranostic system (RIF@Cy5.5-HA-NG) for tuberculosis with a rifampicin (RIF)-loaded near-infrared emission carrier, which was constructed by photoclick reaction-actuated hydrophobic-hydrophobic interaction, enabling the early diagnosis of tuberculosis through granulomas-tracking. Furthermore, the loaded rifampicin was released through the dissociation of disulfide bond by the localized GSH in granulomas, realizing the targeted tuberculosis therapy and providing an especially accurate treatment mapping for tuberculosis.

Conclusions:

Thus, this targeted theranostic strategy for tuberculosis exhibits the potential to realize both granulomas-tracking and anti-infection of tuberculosis.

Introduction

Tuberculosis (TB) possessing highly contagious has placed a heavy burden on public health worldwide,¹⁻⁵ and this chronic disease caused by *Mycobacterium tuberculosis* (*M. tb*), most often affects the lungs.⁶⁻⁹ And tuberculosis also arises in other organs including bone and spine, then evolving one of the most typical forms of extrapulmonary tuberculosis.¹⁰⁻¹³ Recently, the surgical intervention accompanied by indispensable antitubercular drug therapy is the primary routine treatment.¹⁴⁻¹⁷ Although rifampicin (RIF) and isoniazid have been widely chosen as clinical anti-tubercular drugs due to their excellent effectiveness and reasonable price,¹⁸⁻²² their short plasma-life and relatively low concentration in tuberculosis granulomas, and the inescapable side effects of chemotherapeutic drugs have drawn growing attention from interdisciplinary and clinical medicine research circles.²³⁻²⁵ Thus, there is an urgent need to develop an efficient chemotherapy strategy for tuberculosis.

As the typical lesion core of tuberculosis, granuloma formation provides a relatively closed space that could prevent the entrance of anti-tuberculosis drugs.²⁶⁻²⁸ Few drugs can penetrate the central regions due to the compact structure of granulomas, and the nongrowing bacteria inside the granulomas are inherently recalcitrant to killing by most antibiotics. The infection of *M.tb* can remain 'silent' throughout an individual lifetime but can be reactivated by various conditions to stimulate new bacterial growth and

infect new patients, even after decades.^{29–30} And this is why patients with TB require lengthy multidrug therapy, which would increase the risk of multidrug resistance. As we reported in ACS Nano,²³ the granuloma formation possesses the effect of enhanced permeability and retention, which provides the possibility for targeted diagnosis and therapy.

Fortunately, we have found that granulomas are endowed with higher reducibility levels possibly caused by the internal inflammation and the relatively enclosed microenvironment.^{31–33} Therefore, we developed the first targeted glutathione (GSH)-responsive theranostic system (RIF@Cy5.5-HA-NG) for tuberculosis with a rifampicin (RIF)-loaded near-infrared emission carrier, which was constructed by photoclick reaction-actuated hydrophobic-hydrophobic interaction, enabling the early diagnosis of tuberculosis through granulomas-tracking (Scheme 1). The constructed GSH-activatable RIF@Cy5.5-HA-NG realized the *M. tb*-selective imaging, affording precise and effective inhibition of the localized tuberculosis via released RIF for the synergistic treatment of persistent bacteria. This work demonstrated that the rifampicin-loaded GSH-activatable hyaluronic acid (HA) system is a reliable tool for effective tuberculosis therapy.

In order to realize the theranostic effect on tuberculosis, the GSH-responsive nanoagent was rationally designed for targeted imaging and therapy of tuberculosis. RIF@Cy5.5-HA-NG was first synthesized between two types of extensively biocompatible hyaluronic acid (HA) as the host material and near-infrared dye Cy5.5 as a contrast agent via the photo-initiated bioorthogonal reaction and the hydrophilic-hydrophilic interaction. Furthermore, the loaded rifampicin was released through the dissociation of disulfide bonds by the original GSH in granulomas, realizing targeted tuberculosis therapy and providing especially accurate treatment mapping for tuberculosis. The resulting system was characterized for GSH-responsive rifampicin release, realtimely monitoring, and antibiosis properties. And then, the prolonged retention time of drug release in vitro and the in vivo was demonstrated using fluorescence imaging techniques.

In this study, HA-Cys-MA and HA-Lys-Tet, which could first form nanocages via UV-induced click reaction, were mixed with rifampicin (RIF) to create a RIF-loaded carrier (RIF@HA-NG). Photo-inducible click chemistry has been widely applied to functionalize and investigate the dynamics and roles of biomolecules in living systems.^{34–37} The fluorescent imaging contrast Cy5.5, was then modified on the RIF@HA-NG through amidation in the existence of carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) to obtain the aimed nanosystem which combined the diagnosis and therapy of tuberculosis (Scheme 1). Among them, the synthetic routes of HA-Cys-MA and HA-Lys-Tet are summarized in Figure S1 and S2, respectively. On the one hand, GSH plays an important role in many diseases including cancer and tuberculosis^{38–39}. And the cysteine (Cys) containing disulfide bond^{40–41} was reasonably chosen to possess the GSH-responsive peculiarity. On the other hand, a polymer pre-monomer containing the photoclick functional groups including methacryloyl (MA)⁴² and tetrazolium (Tet)^{35,43} was designed and synthesized to obtain a controllable nano-delivery system.

The synthesis of RIF@HA-NG was first characterized by the dynamic light scattering (DLS) analyzer. As shown in the Fig. 1A, RIF@HA-NG nanoagent had a hydrodynamic diameter of approximately 120 nm, which was slightly larger than that of HA-NG owing to the RIF-loading (Fig. 1A). To further confirm the successfully loading of RIF, the zeta potential was measured, showing the zeta potential change from -27.5 mV to -31.3 mV. In other words, the negative RIF obviously decreased the potential of the nanoagent RIF@HA-NG (Fig. 1B). Transmission electron microscopy (TEM) revealed that RIF@HA-NG exhibited a uniform morphology and size with a diameter of 100 nm, indicating that no obvious changes were recorded in the size and shape of the nanoprobe after loading RIF (Fig. 1C). Meanwhile, the drug-release ability of nanocarriers was assessed, so the RIF release analysis was explored (Fig. 1D). Obviously, the ~55% of the total release was observed within 10 h, and the total release reached ~70% upon GSH-treating for 70 h. Whereas, the total release reached only ~20% in PBS solution up to 70 h. In vitro drug release analysis with GSH-triggered indicated that a greater amount of RIF can be released in *Mycobacterium*-infected macrophage cells. Collectively, these results confirmed that the GSH-responsive water-soluble RIF@HA-NG was successfully synthesized.

With the nanoagent in hand, we then aimed to estimate the deliverability of RIF in targeting cells. To determine whether the RIF-loaded nanoagent were endocytosed by the macrophage cell, the RIF@HA-NG was modified by the near-infrared fluorescence dye Cy5.5, simultaneously realizing the imaging of tuberculosis. As shown in the Fig. 2, the obviously red fluorescence was been detected in the RIF@Cy5.5-HA-NG-treated group compared to the Cy5.5-treated group, indicating that RIF@HA-NG can accumulate in the granuloma. That is to say, the RIF@HA-NG was successfully modified by Cy5.5, and thus it can be rationally used to monitor tuberculosis.

Owing to the outstanding drug-release properties of RIF@Cy5.5-HA-NG toward GSH-enriched tuberculosis, we then investigated its antibacterial performance. To investigate the antibacterial activity of RIF and RIF@HA-NG *in vitro*, *Mycobacterium*-infected M1 macrophage cells were first incubated with RIF and RIF@Cy5.5-HA-NG at different times. Survival analysis was performed to confirm the antibacterial effects of RIF and RIF@Cy5.5-HA-NG. As displayed in Fig. 3A, the survival rate of the group treated with RIF@Cy5.5-HA-NG for 1 to 3 h was lower than that of the RIF-treated group. In particular, RIF@Cy5.5-HA-NG or RIF was co-incubated with cells for 3h, and the survival rate of bacteria decreased to 28% and 63%, respectively. When the processing time was extended, the bacterial damage caused RIF@Cy5.5-HA-NG to increase, but the survival rate changed gently, which may have been caused by the phytocytosis of macrophages. Meanwhile, the survival analysis of RIF@Cy5.5-HA-NG and RIF treated with *Mycobacterium*-infected M2 macrophage cells also showed a similar antibacterial tendency, indicating the antibacterial activity of NPs was superior to that of pure RIF. Taken together, these results performed that RIF@Cy5.5-HA-NG NPs have clipping high antibacterial efficiency against cellular bacteria *in vitro* and therefore hold potential for tuberculosis treatment.

Having established that RIF@Cy5.5-HA-NG NPs were able to efficiently kill *Mycobacterium*, our next goal was to validate its potential to monitor the mycobacterium-infected mice. First, we established the tuberculosis model by injecting *Mycobacterium marinum* into the tail vein of mice according to our

previously reported methods. The mice were imaged under a 633 nm laser using the fluorescence in vivo imaging system at different times (0, 6, 12 and 24 h). As exhibited in Fig. 4A, the fluorescence intensity at 690 nm in the granuloma region was found to reach the maximum at 24 h, and it is to say that the concentration of RIF@Cy5.5-HA-NG increased gradually over time and were maintained at a relatively high level even at 24 h post-injection. To further investigate the distribution of nanoagent in various organs at 2, 4 and 24 h time point, ex vivo fluorescence imaging was also studied. Intense fluorescence signals at 690 nm were observed in the liver (Fig. 4B), which indicated that the nanoagent was preferred to selectively accumulate in liver. The selective accumulation may be own to reticuloendothelial system^{44–45}, which indicated that these nanoagents could be metabolized through the liver. Furthermore, hematoxylin and eosin (HE) staining of various important organs revealed no pathological changes after RIF@Cy5.5-HA-NG nanoagents injection at different timepoints (Fig. 4C). Taken together, these results firmly demonstrate that RIF@Cy5.5-HA-NG is capable of directly reflecting the tuberculosis, revealing the feasibility of our nanoagent for monitoring the mycobacterium in vivo as an excellent biomaterial.

Conclusions

In summary, the GSH-activatable theranostic nanoagent RIF@Cy5.5-HA-NG, which is a rifampicin(RIF)-loaded near-infrared emission carrier, was constructed via photoclick reaction-actuated hydrophobic-hydrophobic interaction, realizing the early diagnosis of tuberculosis through granulomas-tracking. Under the trigger of GSH, RIF@CY5.5-HA-NG exhibited not only excellent *Mycobacterium tuberculosis* targeting selectivity and biocompatibility but also a high anti-tuberculosis effect. Therefore, this study exploited an original access to develop the tuberculosis-specific degradable theranostic strategy for targeted imaging and therapy of tuberculosis.

Abbreviations

TB: tuberculosis; GSH: glutathione; RIF: rifampicin; HA: hyaluronic acid; M. tb: Mycobacterium tuberculosis; HE: hematoxylin and eosin; MA: methacryloyl; Tet: tetrazolium; EDC: 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; NHS: N-hydroxysuccinimide; Cys: cysteine; MA: methacryloyl; Tet: tetrazolium; DLS: dynamic light scattering; TEM: transmission electron microscopy.

Declarations

Associated Content

Not applicable.

Authors' contributions

JDZ, XL, YL and YHL is responsible for the design of the experiment and the writing of the thesis. HC, and ZSJ are responsible for data collation and draft the manuscript. ZSJ and KQ is responsible for the animal

experiments. JDZ, BWS and RY, YCL edited the manuscript draft. YL and YHL are responsible for providing funds and unified management of work. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional file 1.

Ethics approval and consent to participate

All procedures involving experimental animals and clinical samples were carried out under guidelines approved by the Ethics Committee of Southern Medical University.

Consent for publication

All authors agree to be published.

Competing interests

The authors declare no conflict of interest, financial or otherwise.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/>

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Additional File

Additional File is not available with this version.

Figures

Figure 1

Characterizations of the RIF@HA-NG. (A) The dynamic light scattering (DLS) analysis of HA-NG and RIF@HA-NG. (B) The zeta potential results of HA-NG and RIF@HA-NG. (C) The transmission electron microscopy (TEM) images of RIF@HA-NG, scale bars are 200 nm. (D) The RIF release of RIF@HA-NG (PBS) in the absence or presence of GSH.

Figure 2

Uptake of RIF@Cy5.5-HA-NG. Blue fluorescent image indicates image of DAPI stained cells; Red fluorescence indicates uptake of Cy5.5 and RIF@Cy5.5-HA-NG and Green fluorescent image indicates the image of Actin.

Figure 3

Antibacterial activity of RIF and RIF@Cy5.5-HA-NG in vitro. Survival analysis of (A) macrophage cells (M1) and (B) macrophage cells (M2) with RIF and RIF@Cy5.5-HA-NG.

Figure 4

Evaluation of the Antibacterial Activity and Toxicity of the RIF@Cy5.5-HA-NG. (A) Fluorescence imaging of mice at 0, 6, 12, 24 hours postinjection. (B) Fluorescence imaging of major organs (liver, spleen, lung, kidney) collected from animals at 0, 6, 12, 24 hours postinjection. (C) Tissue damage analysis of different important organs in mice after intravenous injection of RIF@HA-NG in different times.

Supplementary Files

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