Nanomedicine: Nanotechnology, Biology, and Medicine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Nanomedicine: Nanotechnology, Biology, and Medicine

journal homepage: nanomedjournal.com

Silver nanoparticle treatment ameliorates biliary atresia syndrome in rhesus rotavirus inoculated mice

Ruizhong Zhang, MD, PhD [a], Zefeng Lin, Bsca [a], Vincent CH Lui, PhD [b], Kenneth KY Wong, MD, PhD [b], Paul KH Tam, MD [b], Puiyan Lee, PhD [c], Chun Nam Lok, PhD [c], Jonathan R Lamb, PhD [d], Yan Chen, PhD [a,b,*], Huimin Xia, MD [a,**]

[a] Department of Pediatric Surgery, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China
[b] Department of Surgery, The University of Hong Kong, Hong Kong, SAR, China
[c] Department of Chemistry, Faculty of Science, University of Hong Kong, Hong Kong, China
[d] Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, UK

ARTICLE INFO

Article history:
Received 17 August 2016
Accepted 20 November 2016
Available online xxxx

Key words:
Biliary atresia
Silver nanoparticles
NK cells, TGF-β, hepatic inflammation

ABSTRACT

Biliary atresia (BA) is a neonatal biliary system disease closely associated with viral infection and bile duct inflammation. Silver nanoparticles (AgNps) have previously revealed antiviral and anti-inflammatory properties. In this study, we have investigated the effects of AgNps in the treatment of the Rhesus rotavirus inoculation induced BA in mice. The morphology, liver histopathology, clinical biochemistry examination, and inflammatory cells were analyzed in BA mice. Results indicated that AgNps could significantly increase the survival rate of BA mice, and reduce jaundice and weight lost and the liver enzymes and bilirubin metabolism clinical parameters were close to the normal levels. Diminished numbers of NK cells were observed by flow cytometry analysis and immunohistochemical staining. Furthermore, the viral load was reduced and transcripts for TGF-β mRNA were augmented after AgNps treatment. Collectively, our results suggest that AgNps treatment has beneficial effects on the BA mouse model partially through upregulation of TGF-β.

Biliary atresia (BA) is the most common obstructive jaundice disease in pediatric patients with poor prognosis and high mortality. The incidence of the disease is approximately 1:10,000-15,000 live births. The pathogenesis of BA is not fully understood, but it is suggested that it is closely related to viral infection. BA may develop in the perinatal period between 28 weeks gestation and first 4 weeks after birth following hepatotropic viral infection, such as cytomegalovirus, reovirus or rotavirus. The infection can cause immune dysregulation resulting in immune reactivity against extra and intra hepatic bile ducts, with a series of pathological changes including inflammatory cell infiltration around bile duct, biliary epithelial cell apoptosis, biliary obstruction and liver fibrosis.[1–3] The process of BA is more aggressive than hepatobiliary disorders in adults. The outcome of BA can be improved by the Kasai Procedure, but in most cases the disease will progress and ultimately lead to life threatening liver cirrhosis, portal hypertension and liver failure.[4–7] Most BA patients cannot obtain liver transplantation within the first year of life and half of them die,[8] thus, there is an immediate and urgent need for developing new therapeutic drugs for BA.

The most commonly used animal models for BA was established by intraperitoneal injection of rhesus rotavirus (RRV) in mice within 24 h after birth.[9–11] The progressive accumulation of inflammatory cells damages the bile ducts and eventually induces biliary atresia in mice. NK cells, $CD4^+$ and $CD8^+$ T cells are the key cell types present in the inflammatory cell infiltrate.[12–15] Shortly after virus infection, NK cells accumulate around the bile ducts where they proliferate and are activated and through NKG2D ligation destroy biliary epithelial cells resulting in BA. The NK cells also release proinflammatory ligands which activate $CD4^+$ and $CD8^+$ T cells which cause further bile duct injury.[12] Therefore, reducing the number and inhibiting the activity of NK cells are thought to be key in the treatment of BA. The activation of $CD4^+$ and $CD8^+$ T cells also plays a central role in the pathogenesis of BA.[14,15] In IFN-γ (mainly produce by $CD4^+$ T cells) knockout mice or $CD8^+$ T cell depleted mice, the incidence of BA is significantly reduced and it has been demonstrated that the adoptive transfer of T cells from rotavirus infected rats to uninfected homologous SCID rats causes biliary inflammation.[16,17] This provides evidence that T

Abbreviations: BA, biliary atresia; AgNps, silver nanoparticles; RRV, rhesus rotavirus; CK19, cytokeratin 19; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, gamma glutamyl transpeptidase; TP, total protein; ALB, albumin; GLO, globulin; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TBA, total bile acids.

Conflict of interest: There are no competing interests present in the study.

This work was supported by the Science and Technology Planning Project of Guangdong Province 2014, No: 2014A020212373 and National Natural Science Foundation of China (Grant No. 81600399).

* Correspondence to: Y. Chen, Department of Surgery, The University of Hong Kong, Hong Kong, SAR, PR China.
** Correspondence to: H. Xia, Guangzhou Women and Children's Medical Center, Guangzhou, PR China.
E-mail addresses: ychenc@hku.hk, ychenhk@gmail.com (Y. Chen), xia-huimin@foxmail.com (H. Xia).

http://dx.doi.org/10.1016/j.nano.2016.11.013
1549-9634/

cells can mount a specific immune response to the bile ducts suggesting that the disease is an autoimmune related phenomenon.[18]

Silver nanoparticles (AgNps) are silver particles measuring about 1-100 nm in diameter. Compared with the traditional silver particles, they have a much bigger surface area, greater quantum size effect and macroscopic quantum tunneling. Due to these characters, their potential biomedical applications in drug delivery, biological sensing and cancer treatment are rapidly developing.[19] Our previous studies on AgNps revealed that in a mouse model of peritonitis, AgNps could effectively inhibit the accumulation of inflammatory cells and reduce the production of inflammatory cytokines. Through increased expression of TGF-β1 they stimulated skin keratinocyte proliferation and the production of VEGF and IL-10.[20] Furthermore, AgNps promote local growth of blood vessels facilitating healing in a mouse model of burn wounds.[21] The anti-viral effects of AgNps have been studied in recent years. It is reported that AgNps can inhibit different viruses such as HIV,[22] hepatitis B[23] and influenza[24] through direct contact with viral surface proteins, binding to viral DNA/RNA and blocking viral replication or preventing their penetration into host cells.[25,26] But the the outcome of exposure to AgNps on enteric cytopathic human orphan viral infection is contradictory,[27] and to the best of our knowledge the effects of AgNps on RRV, a double strain RNA virus, have not been reported.

In light of the anti-virus and anti-inflammatory effects of AgNps and since BA is closely related to viral infection and bile duct inflammation, in this study, we have explored the potential of AgNps to modulate disease in a mouse model of BA. Our results demonstrated that AgNps could significantly ameliorate mouse BA syndrome, increasing the survival rate of mice by reducing jaundice, weight lost and hepatic inflammation which may occur partially through the inhibition of NK cells.

Methods

Reagents and antibodies

The antibodies used for immunohistochemical staining were rat anti-cytokeratin 19 (CK19, clone TROMA III) purchased from DSHB (Developmental Studies Hybridoma Bank, Iowa City, USA) and rat anti-mouse NKG2D (Clone:191004) obtained from R&D (R&D, MA, USA). For flow cytometric analysis, all antibodies were purchased from eBioscience (eBioscience Inc. San Diego, CA), including anti-mouse NKp46-FITC, anti-mouse CD4-PerCP-Cyanine5.5, anti-mouse CD3e-Alexa Fluor 488, anti-mouse CD8a-APC, anti-mouse CD11b-FITC, and anti-mouse F4/80-APC. For real time PCR quantification, RNeasy Mini Kit was purchased from Qiagen Company (Qiagen, Hilden, Germany) and the reverse transcription reagents were purchased from Invitrogen (Life Technologies Limited, N.T., Hong Kong) and Super Real PreMix was from Tiangen (Tiangen Biotech (Beijing) Co, Ltd., Beijing, China).

Synthesis of silver nanoparticles

Silver nanoparticles (AgNps) were prepared as previously described,[28] with final concentration of 1 mM and mean diameter of 10 nm (±5 nm) which was confirmed by electron microscopy.

Preparation of the AgNps-collagen mixture

The AgNps-collagen mixture was prepared as previously described.[28] Briefly, it was prepared by mixing 40% (v/v) collagen (4 mg/ml of type I collagen; Millipore, CA, USA), 10% (v/v) 10× PBS, 6.4% (v/v) 0.2 M NaOH, 3.6% H_2O, with 40% of 1 mM AgNps on ice. The mixture was prepared freshly before intraperitoneal injection. The AgNps-collagen mixture gelled inside the abdominal cavity where the environment temperature is about 37 °C, therefore the release of AgNps would be slowed.

Infection of neonatal mice with Rhesus rotavirus

The Rhesus rotavirus (RRV) strain MMU 18006 was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The virus was amplified in MA104 cells and virus quantification measured by a plaque assay method as described previously.[29] Day 12.5 pregnant Balb/c mice aged between 10 and 12 weeks were purchased from Guangdong Animal Experimental Center and maintained under specific pathogen-free conditions and housed in a room with a 12-h dark–light cycle. All animal protocols were approved by The Institutional Animal Care and Use Committee of Sun Yat-Sen University Laboratory Animal Center where all the animal experiments were performed (#IACUC-DB-16-0602).

In order to establish an experimental model of BA, the neonatal mice were injected with 20 μl of 1.2×10^5 pfu/ml RRV or supernatant of MA104 cell culture medium as controls intraperitoneally within 24 h of birth. Infected mice that died within the first 2 days or that were not fed by their mothers were excluded from further analysis. All mice were weighed and examined daily, and in general, the development of icterus on the skin not covered with fur and acholic stools appeared on day 5 to 6

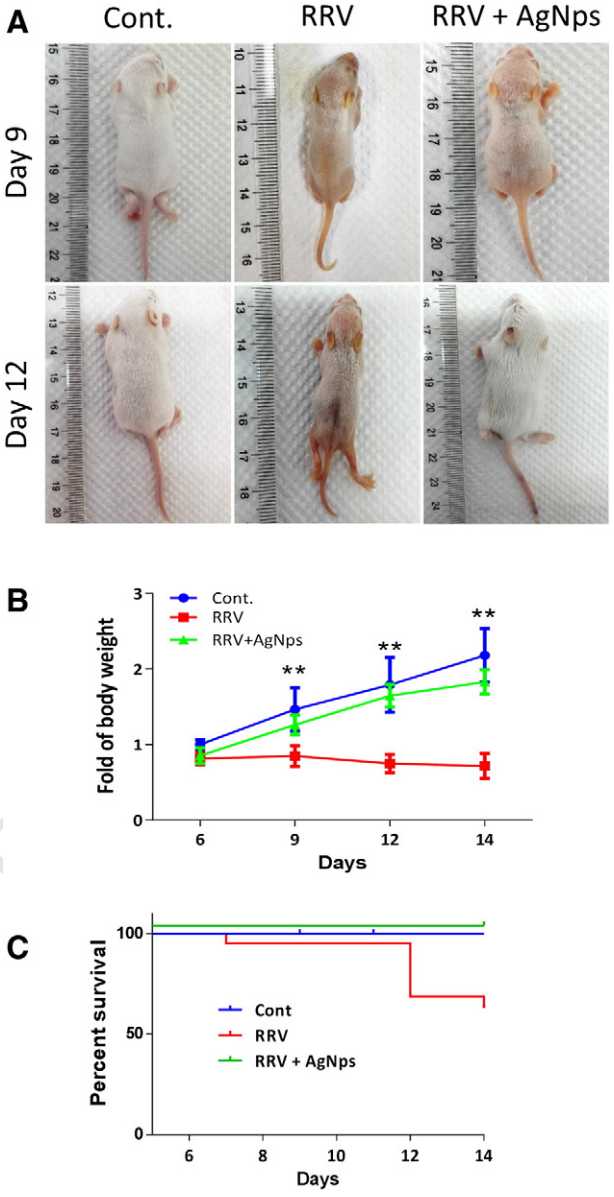

Figure 1. Effect of AgNps on biliary atresia (BA) syndrome in rhesus rotavirus-induced BA mouse model. (**A**) The physical appearance of mice at days 9 and 12 after virus inoculation (RRV) alone and at days 3 and 6 after injection with AgNps (RRV + AgNps). **(B)** Weight of each group at different time points after injection with AgNps was recorded; y-axis indicates the fold increase of weight, which was calculated relative to the weight of the control group at day 6. $^{**}P < 0.01$, n = 16, 18 and 17 in Cont, RRV and RRV + AgNps group. **(C)** Survival curve of each group at different time points were recorded.

Please cite this article as: Zhang R, et al. Silver nanoparticle treatment ameliorates biliary atresia syndrome in rhesus rotavirus inoculated mice. *Nanomedicine: Nanotechnology, Biology, and Medicine* (2016), http://dx.doi.org/10.1016/j.nano.2016.11.013