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● *Original Contribution*

EFFICACY OF INDOCYANINE GREEN-MEDIATED SONODYNAMIC THERAPY ON RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIOCYTES

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Abstract—Sonodynamic therapy (SDT) has become a new therapeutic method because of its activation of certain sensitizers by ultrasound. Some studies have reported that indocyanine green (ICG) has the characteristics of a sonosensitizer and favorable fluorescence imaging in synovitis of early inflammatory arthritis. In this study, we aimed to investigate the cytotoxic effect of ICG-mediated SDT on MH7A cells *in vitro* and the potential mechanisms involved. ICG was found to be taken up mainly in cytoplasm, with maximal uptake in 4 h. Cell viability in ICG-mediated SDT (SDT-0.5 and SDT-1.0) groups decreased significantly to 73.09 ± 1.97% and 54.24 ± 4.66%, respectively; cell apoptosis increased significantly to 26.43 ± 0.91% and 45.93 ± 6.17%, respectively. Moreover, marked loss in mitochondrial membrane potential and greatly increased generation of reactive oxygen species were observed in ICG-mediated SDT groups. Interestingly, the loss in cell viability could be effectively rescued with pretreatment with the reactive oxygen species scavenger *N*-acetylcysteine. These results indicate that ICG-mediated SDT is cytotoxic to fibroblast-like synoviocytes and is a potential modality for targeted therapy of synovitis in rheumatoid arthritis. (E-mail: zhushenyin0486@sina.com) © **2017 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Sonodynamic therapy, Indocyanine green, Rheumatoid arthritis, Fibroblast-like synoviocytes, Reactive oxygen species, Apoptosis.**

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized mainly by synovial inflammation and hyperplasia, cartilage and bone destruction of joints and systemic complications (Firestein 2003; McInnes and Schett 2011). It is associated with progressive disability and socioeconomic costs, with 1.0% of the world population affected (Choy 2012; Zampeli et al. 2015).

Despite the significant progress made in the management of RA in recent decades, some patients do not respond well to the therapeutic agents presently available or suffer relapse after an initial response (Gabriel et al. 2012; O'Dell and Mikuls 2011; Polido-Pereira et al. 2011). In clinical practice, those whose joints have been destroyed by the invasively growing synovial tissue are considered for synovectomy to prevent further damage to vital joint structures. A new synovectomy technique, photodynamic therapy (PDT), has been proposed and intensively studied because of its minimal invasiveness and improved site selective action. The feasibility of using PDT-mediated synovectomy has been illustrated in different *in vitro* and *in vivo* studies in recent years (Gabriel et al. 2012; Hansch et al. 2008; Kirdaite et al. 2002; Torikai et al. 2008; Zhao et al. 2015). However, the translation of PDT-mediated synovectomy into the clinical setting in RA is impeded mainly by some limits, including the limited ability of light to penetrate deeply (Agostinis et al. 2011; Nowis et al. 2005). Compared with the light used in PDT, ultrasound can penetrate much deeper in biological tissue and easily act on deep lesions because of its low tissue attenuation coefficient

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(Mitragotri 2005); the corresponding strategy is known as sonodynamic therapy (SDT).

Sonodynamic therapy, which is derived from PDT (Wood and Sehgal 2015), has emerged as a new and promising modality for the treatment of many proliferative diseases, particularly those involving relatively deep proliferative lesions. It involves the sensitization of target tissues with a non-toxic sensitizer and subsequent exposure of the sensitized tissues to ultrasound. As a necessary element, the choice of sensitizer is a critical issue. Recent studies have reported that indocyanine green (ICG), which has been approved for use in clinical diagnosis for many years (Simal-Julian et al. 2015; Yamamichi et al. 2015), not only can be commonly applied in PDT investigations as a photosensitizer (Sheng et al. 2014), but also can be activated by ultrasound (Nomikou et al. 2012). Interestingly, it has also been reported that ICG-enhanced fluorescence optical imaging is a clinically applicable tool for the detection of synovitis in early inflammatory arthritis (Gemeinhardt et al. 2012; Licha and Resch-Genger 2011; Meier et al. 2010). Thus, ICG is a potential and promising theranostic agent in RA.

Sonodynamic therapy has been used successfully in pre-clinical studies for cancer, atherosclerosis, coronary restenosis and inflammation (Dan et al. 2015; McHale et al. 2016; Sun et al. 2015), but it has not yet been tried in joint diseases. We hypothesized that ICG-mediated SDT may be a promising and more applicable modality for RA therapy on the basis of the following facts: RA fibroblast-like synoviocytes (FLSs) and tumor cells share some pathophysiological characteristics (*i.e.,* abnormal proliferation, angiogenesis, locally aggressive phenotype); SDT has an advantage over PDT for relatively deep proliferative lesions; and ICG has the characteristics of a sonosensitizer and favorable fluorescence imaging in synovitis of early inflammatory arthritis.

In this study, our aim was to investigate the cytotoxic effect of ICG-mediated SDT on RA fibroblast-like synoviocytes *in vitro* and to explore the potential mechanisms involved. To the best of the authors' knowledge, this is the first effort to use the promising SDT modality in an anti-arthritis investigation, and it is believed that the results of this study will pave the way for an in-depth study of ICG-mediated SDT in RA.

METHODS

Chemicals

Indocyanine green and Rhodamine 123 (RHO123) were purchased from Sigma-Aldrich (St. Louis, MO, USA). A Hoechst 33342 staining kit was obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). *N*-Acetylcysteine (NAC), (2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride) (DAPI), (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide) (MTT) and 2′,7′-dichlorofluorescein diacetate (DCFH-DA) were all supplied by Beyotime Biotechnology (Shanghai, China). Singlet oxygen Sensor Green (SOSG) was purchased from Thermo Fisher Invitrogen. All other reagents used in this study were commercial products of analytical grade.

Cell culture

The human fibroblast-like synoviocyte MH7A cell line was purchased from Biovector Science Lab, NTCC (Beijing, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 15% fetal bovine serum under a humidified atmosphere in 5% CO_2 at 37°C. Cells in the exponential phase of growth were used in all experiments.

Ultrasound exposure setup

Figure 1 is a schematic of the ultrasound exposure apparatus. The ultrasonic generator (T&C Power Conversion, Rochester, NY, USA), power amplifier and focused ultrasound transducer (diameter: 25 mm; resonance frequency: 1.0 MHz, duty cycle: 10%, pulse repetition frequency: 100 Hz, focus length: 15 mm, focus area: 0.4 cm^2) used in this study were assembled by National Engineering Research Centre of Ultrasound Medicine (Chongqing, China). The surface of the transducer was placed 15 mm under the upper surface of the plates through a water bath (room temperature). Intensities of 0.5 and 1.0 W/cm^2 (as measured with a hydrophone, Onda, Sunnyvale, CA, USA) and a duration of 2 min were used in our experiments. An infrared thermal system (FLIR TG165, Portland, OR, USA) was used to measure temperature increases before and after ultrasound exposure, and no significant variation was detected.

Experimental protocol

Cells were divided into six groups: (1) control group (control); (2) ICG alone (ICG); (3) ultrasound exposure alone, 0.5 W/cm^2 (US-0.5); (4) ultrasound exposure alone, 1.0 W/cm^2 (US-1.0); (5) ICG plus ultrasound

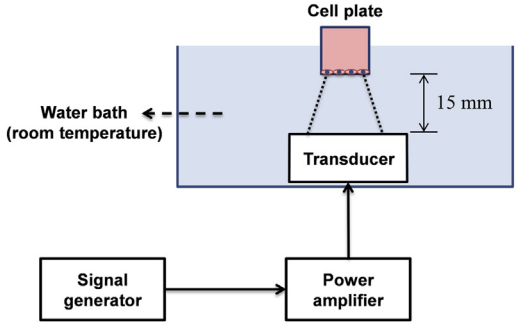

Fig. 1. Schematic of the ultrasound exposure system.