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● *Original Contribution*

ENHANCED THERAPEUTIC ANTI-INFLAMMATORY EFFECT OF BETAMETHASONE ON TOPICAL ADMINISTRATION WITH LOW-FREQUENCY, LOW-INTENSITY (20 KHZ, 100 MW/CM^2) ULTRASOUND EXPOSURE ON CARRAGEENAN-INDUCED ARTHRITIS IN A MOUSE MODEL

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Abstract—The purpose of this work was to investigate whether low-frequency, low-intensity (20 kHz, <100 mW/cm^2, spatial-peak, temporal-peak intensity) ultrasound, delivered with a lightweight (<100 g), tether-free, fully wearable, battery-powered applicator, is capable of reducing inflammation in a mouse model of rheumatoid arthritis. The therapeutic, acute, anti-inflammatory effect was estimated from the relative swelling induced in mice hindlimb paws. In an independent, indirect approach, the inflammation was bio-imaged by measuring glycolytic activity with near-infrared labeled 2-deoxyglucose. The outcome of the experiments indicated that the combination of ultrasound exposure and topical application of 0.1% (w/w) betamethasone gel resulted in statistically significantly ($p < 0.05$) enhanced anti-inflammatory activity in comparison with drug or ultrasound treatment alone. The present study underscores the potential benefits of low-frequency, low-intensity ultrasound-assisted drug delivery. However, the proof of concept presented indicates the need for additional experiments to systematically evaluate and optimize the potential of, and the conditions for, tolerable low-frequency, low-intensity ultrasound-promoted non-invasive drug delivery. (E-mail: crb68@drexel.edu)

Key Words: **Low-frequency ultrasound, Betamethasone, Topical delivery, Carrageenan-induced arthritis, Anti-inflammatory effect, Near-infrared imaging.**

INTRODUCTION

The purpose of this work was to investigate whether low-frequency, low-intensity (defined here as 20 kHz, <100 mW/cm^2, spatial-peak, temporal-peak intensity) ultrasound (US), delivered with a lightweight (<100 g), tether-free, fully wearable, battery-powered applicator, is capable of providing a therapeutic effect in a mouse model of inflammatory arthritis.

The primary motivation for this work was to explore the possibility of syringe-free delivery of topically applied drug using 20-kHz tolerable (non-thermal and non-cavitational) levels of ultrasound. Such non-invasive delivery is important both societally and economically, because of e adverse reactions to injections, physical inability to administer drugs orally and costs of treatment associated with the delivery tools (no syringes needed and no need for controlled disposal of the syringes). Non-invasive delivery is also of interest because oral intake of a variety of drugs might be impeded as a result of such side effects as decreased white blood cell counts, increased risk of infections, fatigue, oral ulcers, hair loss, nausea, and diarrhea and liver toxicity. The pre-clinical evaluation was performed using a mouse model of rheumatoid arthritis (RA). RA was chosen because it is often encountered in children and young adults. Minimization of the side effects of pediatric rheumatology treatment usually requires the injection of drugs, which is painful. Also, many children develop such an intense psychologic aversion to these injections

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that they vomit before drug administration. Moreover, invasive delivery increases health care costs and reduces patient compliance.

Rheumatoid arthritis (RA) is a chronic, debilitating inflammatory, musculoskeletal disease that affects 0.5%–1.0% of the adult population in Western countries, with total treatment costs approaching $80 billion annually (Centers for Disease Control and Prevention [CDC] 2006; Lawrence et al. 2008). Its pediatric counterpart, juvenile idiopathic arthritis (JIA), affects 0.01%–0.4% of children (Manners and Bower 2002). The joint destruction and progressive functional disability associated with uncontrolled inflammation affect the ability to work and attend school, overall quality of life and mortality (Cheng et al. 2010; Yelin et al. 2007).

Systemic corticosteroids (CSs) are the drugs most often used to control disease in inflammatory arthritis, RA and JIA. However, if given for a prolonged period, CSs result in significant morbidity, including glaucoma, cataracts, weight gain, hypertension, diabetes and bone fragility (Gaujoux-Viala and Gossec 2014; Strehl and Buttgereit 2013). Therefore, alternate systemic agents are added to spare exposure to steroids. These include hydroxychloroquine, disease-modifying agents (*e.g.*, methotrexate), non-steroidal anti-inflammatory agents and biological agents (Grigor et al. 2004; Nam et al. 2014; Weinblatt et al. 2010). These non-steroidal agents have potential side effects, including cytopenias, increased risk of infection and pain (subcutaneous injections). Although biological agents may be helpful in recalcitrant disease, they typically cost 10 to 100 times more than older therapies, and there is global concern regarding their impact on health care budgets (Lawrence et al. 2008). These concerns have prompted interest in research into and development of novel, economically acceptable, tolerable methods for delivery of existing anti-inflammatory drugs, including CSs, to patients with inflammatory arthritis. The primary goal of these activities is to minimize the side effects of the drugs taken systemically or injected intra-articularly while maximizing their potency in alleviating arthritis.

Intra-articular CS injections (IACIs) are frequently used in the management of patients with inflammatory arthritis. They are preferred over systemic agents in the treatment of mono-articular/oligo-articular arthritis (Bigelow et al. 2011). Even in poly-articular disease, the addition of IACIs to systemic combination therapy helps achieve "tight control" of RA in a manner that may obviate the need for biologics (Grigor et al. 2004; Hetland et al. 2006; Proudman et al. 2000). Although studies have found, at least in children, that triamcinolone hexacetonide (with the lowest solubility) is the most effective (Zulian et al. 2004), clinical effects have been reported with methylprednisolone, betamethasone, triamcinolone acetonide and triamcinolone hexacetonide IACIs (Sherry et al. 1999). IACIs have rapid onset, often within the week. After an IACI, arthritis is controlled in 63%–82% of children at 6 mo, 45%–50% at 12 mo in JIA (Kovar et al. 2009; Ziskin 2010). However, IACIs are acutely painful; in children, but not adults, these injections often require sedation. Moreover, they can result in osteoporosis (Laan et al. 1999), skin hypopigmentation or subcutaneous atrophy and, rarely, introduce infection into the joint (Charalambous et al. 2003; Gilsanz and Bernstein 1984). Although intra-articular administration of CS derivatives can result in rapid onset of a durable anti-inflammatory response that may limit or obviate exposure to systemic medications (Conaghan et al. 2003; Grigor et al. 2004; Hetland et al. 2006; Proudman et al. 2000), alternative effective but non-invasive ways to deliver local CSs are needed. The fully wearable, battery-operated ultrasound applicator described here alleviates these issues. In particular, as already noted, it might provide a painless route of treatment, while minimizing side effects. It also offers a potential for home treatment, without the need to visit the physician's office. This, in turn, will potentially enhance the patient's quality of life and, by eliminating the need for transportation to the hospital, further lessen the cost of treatment.

Research in ultrasound-assisted drug delivery has gained increasing attention over the past two decades. A bevy of publications on the topic are available; however, here, for brevity and convenience, only one extensive and systematic review describing the current knowledge and state-of-the-art of US-assisted drug delivery is highlighted. This recent, well-documented (with 181 references) survey was published by the team of researchers from Ben Gurion University, Beersheba, Israel (Azagury et al. 2014). The information provided therein (Azagury et al. 2014) should be helpful in facilitating insight into the encouraging outcome of the *in vivo* animal study described.

In the Methods section, the ultrasound applicator used in this study and all relevant exposure parameters are described, as are the conditions of the *in vivo* mice experiments. Next, the *Results* section outlines the potential of LFLI ultrasound-assisted transdermal drug delivery and is followed by the Discussion and Conclusions.

METHODS

Before describing the applicator used in the experiments, it might be useful to elucidate the choice of US field parameters employed in this study.

The initial literature review suggested that US at frequencies in the tens of kilohertz range might be more effective for non-invasive, transdermal drug delivery in