Cytotherapy, 2018; ■■: ■■–■■

International Society for Cellular Therapy
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Functional organic cation transporters mediate osteogenic response to metformin in human umbilical cord mesenchymal stromal cells

FAISAL E. AL JOFI[1,2,⋆], TAO MA[1,⋆], DONG GUO[3], MONICA P. SCHNEIDER[4], YAN SHU[3,5], HOCKIN H. K. XU[5,6,7] & ABRAHAM SCHNEIDER[1,5]

[1]Department of Oncology and Diagnostic Sciences, School of Dentistry, University of Maryland, Baltimore, Maryland, USA, [2]Department of Preventive Dental Science, Division of Periodontics, Imam Abdulrahman Bin Faisal University, College of Dentistry, Dammam, Saudi Arabia, [3]Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, Maryland, USA, [4]Department of Orthodontics and Pediatric Dentistry, School of Dentistry, University of Maryland, Baltimore, Maryland, USA, [5]Greenebaum Comprehensive Cancer Center, Program in Oncology, School of Medicine, University of Maryland, Baltimore, Maryland, USA, [6]Biomaterials and Tissue Engineering Division, Department of Advanced Oral Sciences and Therapeutics, School of Dentistry, University of Maryland, Baltimore, Maryland, USA, and [7]Center for Stem Cell Biology and Regenerative Medicine, School of Medicine, University of Maryland, Baltimore, Maryland, USA

Abstract

Background. Compelling evidence indicates that metformin, a low-cost and safe orally administered biguanide prescribed to millions of type 2 diabetics worldwide, induces the osteoblastic differentiation of mesenchymal stromal cells (MSCs) through the 5' adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway. As a highly hydrophilic cationic compound, metformin uptake is facilitated by cell membrane organic cation transporters (OCTs) of the solute carrier 22A gene family. We hypothesized that to effectively enhance osteogenic differentiation, and ultimately bone regeneration, metformin must gain access into functional OCT-expressing MSCs. *Methods.* Data was obtained through immunoblotting, cellular uptake, mineralization and gene expression assays. *Results.* We demonstrate for the first time that functional OCTs are expressed in human-derived MSCs from umbilical cord Wharton's jelly, an inexhaustible source of nonembryonic MSCs with proven osteogenic potential. A clinically relevant concentration of metformin led to AMPK activation, enhanced mineralized nodule formation and increased expression of the osteogenic transcription factor Runt-related transcription factor 2 (RUNX2). Indeed, targeting OCT function through pharmacological and genetic approaches markedly blunted these responses. *Conclusions.* Our findings indicate that functional OCT expression in UC-MSCs is a biological prerequisite that facilitates the intracellular uptake of metformin to induce an osteogenic effect. Future pre-clinical studies are warranted to investigate whether the expression of functional OCTs may serve as a potential biomarker to predict osteogenic responses to metformin.

Key Words: *bone regeneration, mesenchymal stromal cells, metformin, organic cation transporters, osteogenesis, stem cells, umbilical cord*

Introduction

Compelling evidence suggests that a number of common drugs that were originally developed for specific chronic diseases can also be beneficial and potentially repurposed for other unrelated conditions. This seems to be the case with the anti-diabetic drug metformin [1]. As a member of the biguanide family, metformin was approved by the United States Food and Drug Administration in 1995 for treating type 2 diabetes, and is currently the most widely used oral anti-diabetic worldwide [2,3]. Intriguingly, recent studies indicate that metformin can also impact skeletal homeostasis and reduce the risk of bone fractures [4–7]. To this end, *in vitro* studies have reported that metformin enhances the proliferation and osteoblastic differentiation of bone marrow mesenchymal stromal cells (MSCs) and pre-osteoblastic cell lines [8–12].

Proposed mechanisms for the anti-diabetic effects of metformin have mainly focused on the role of the 5' adenosine monophosphate (AMP)-activated protein

⋆These authors contributed equally to this work.
Correspondence: **Abraham Schneider**, DDS, PhD, Department of Oncology and Diagnostic Sciences, University of Maryland School of Dentistry, 650 West Baltimore Street, Room 7265, Baltimore, MD 21201, USA. E-mail: schneider66@umaryland.edu

(Received 12 December 2017; accepted 11 February 2018)

ISSN 1465-3249
https://doi.org/10.1016/j.jcyt.2018.02.369

kinase (AMPK) pathway, a conserved signaling cascade that acts as master sensor of cellular energetics [13,14]. Most studies performed in bone marrow MSCs and pre-osteoblasts have reported that metformin enhances osteoblastic differentiation via AMPK signaling in doses ranging from 0.5–500 μmol/L. These responses include a dose-dependent effect on cell proliferation together with increases in extracellular mineral nodule formation and well-known osteoblastic markers, such as type I collagen, osteocalcin, alkaline phosphatase and transcription factor runt-related transcription factor 2 (RUNX2) [11,15,16]. Recently, our group reported similar responses to metformin in induced pluripotent stem cell-MSCs (iPSC-MSCs). We found that metformin when used at a clinically relevant concentration of 10 μmol/L significantly stimulated alkaline phosphatase activity, enhanced mineralized nodule formation and increased expression of the osteogenic markers RUNX2 and Osterix. Furthermore, inhibition of LKB1 activity, a key upstream serine/threonine kinase responsible for activating AMPK, markedly reversed metformin-induced AMPK activation as well as the expression and nuclear localization of RUNX2 [17,18]. These findings underscore the significance of using therapeutically relevant doses when trying to extrapolate *in vitro* results to the clinical setting in humans. Plasma concentrations of metformin found in patients within 2–4 h after an oral dose of 500–1500 mg range between 2.7 and 20 μmol/L [11,19].

Pre-clinical animal studies designed to evaluate the effects of metformin on bone mass and on quality following traumatic bone fracture or bone loss associated with estrogen deficiency have been overall encouraging, but some conveyed ambiguous results. In general, metformin significantly increases total body bone mineral density, bone trabecular volume, osteocyte density and osteoblast number and exerts protective effects against bone loss [6,15,20–22]. In contrast, others report that metformin has no effect on bone mass or fracture healing [23]. Although discrepancies in experimental findings may reflect variability in experimental design and methodology, responses to metformin may also differ relative to its effects in endochondral or intramembranous bones, rodent strain, gender, age, treatment dose and duration, or glycemic status. Yet, a major gap in knowledge that remains elusive is the role played by organic cation transporters (OCTs) on the intracellular uptake of metformin and their contribution to the osteogenic action in MSCs.

As a highly hydrophilic cationic drug, metformin uptake relies on tissue-specific mechanisms facilitated by a group of polyspecific cell membrane transporters of the solute carrier 22A (*SLC22A*) gene family. OCT-1, OCT-2 and OCT-3 mediate transport of structurally diverse, small hydrophilic organic cationic endogenous compounds, toxins and drugs, including metformin [24–28]. Despite the critical involvement of OCTs in hepatic and renal cellular transport [29,30], little is known about the impact of these transporters in metformin-induced osteoblastic differentiation. By elucidating OCT expression and function in MSCs we would afford a better understanding of the intervening cellular determinants mediating the osteogenic action of metformin. Furthermore, as a low-cost drug with well-accepted tolerance after long-term use, metformin could emerge as an attractive systemic or locally delivered option to pharmacologically potentiate MSC-based bone regeneration. To this end, we hypothesized that to effectively enhance osteogenic differentiation and ultimately bone regeneration metformin must gain access into functional OCT-expressing MSCs.

As multipotent cells capable of differentiating into osteoblasts, human bone marrow MSCs are regarded as the "gold standard" for autogenous MSC-based bone tissue engineering [31]. However, bone marrow MSCs are harvested through an invasive procedure, have lower self-renewal ability and aging and underlying chronic conditions negatively impact their proliferative and differentiating capabilities [32–35]. Other alternative MSC sources are being studied to regenerate skeletal tissues in a more predictable fashion. Recent reports have pointed to the human Wharton's jelly of the umbilical cord (UC) as an attractive extra-embryonic, inexpensive and inexhaustible source of perinatal MSCs for regenerative medicine, with neither donor site morbidity nor ethical issues associated with their use [36–38]. UC-MSC osteogenic capacity has been demonstrated both *in vitro* and in experimental pre-clinical models when seeded in three-dimensional scaffolds [39–44]. Notably, UC-MSCs are able to match the bone regenerative efficacy of bone marrow MSCs in critical size cranial defects in athymic rats [45]. Moreover, to mimic their regenerative potential in congenital oral and craniofacial bone defects in pediatric patients, novel pre-clinical tissue engineering strategies have been recently proposed to repair maxillary alveolar bone defects with nanomicrofiber scaffolds seeded with UC-MSCs in juvenile swine [46,47].

The present *in vitro* study was conducted to investigate (i) whether OCTs are expressed and functional in UC-MSCs, and (ii) whether they play a key role in mediating the osteogenic action of metformin. This work may not only offer new insight into critical cellular determinants driving the osteogenic capacity of metformin, but also provide a significant step forward in the area of perinatal MSC research and bone regeneration, especially if further translated into novel autogenous, locally delivered