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EFFECTS OF RUBIADIN-3-METHYL ETHER ON THE IMMUNE PROPERTIES OF HUC-MSCS

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SUMMARY

Mesenchymal stem cells (MSCs) have become an effective tool for treating immune-related diseases due to their multilineage potential and immunomodulatory capabilities. One of the main factors contributing to their immunomodulatory capabilities is the *IDO* cascade, which was chosen as the main subject in this research. The *IDO*-Kyn-AHR-*CYP* cascade was chosen to evaluate the immunomodulatory properties of treated MSCs, with expression levels of the key gene *IDO* (indoleamine 2,3-dioxygenase) selected as the screening criterion. Cultured human umbilical cord MSCs (hUC-MSCs) were treated with different natural bioactive compounds. Preliminary results indicated that Rubiadin-3-methyl ether, an anthraquinone derivative isolated from the roots of *Morinda longissima*, was able to significantly induce *IDO2* expression in UC-MSCs but not *CYPs*. This is the first study to show a link between Rubiadin-3-methyl ether and IDO2 in hUC-MSC. More research is needed to determine whether human UC-MSCs primed with Rubiadin-3-methyl ether have any significant benefits for treating immune-related diseases and disorders.

Keywords: MSCs, Immunomodulatory, IDO, CYPs, Rubiadin-3-methyl ether, UC-MSC

INTRODUCTION

MSCs are adult stem cells that possess multilineage potential and immunomodulatory capacity, which has made them an effective tool for treating immune-related disease (Pittenger *et al.*, 2019). Several strategies have been investigated to promote these properties. Pre-activation with inflammatory factors and cytokines is considered the most common solution to mimic the inflammatory microenvironment *in vivo*, and plays a significant role in regulating the immunomodulatory function of stem cells (Li *et al*., 2022). The gene *IDO* is an important component of the immune system that encodes a rate-limiting KP enzyme (isoforms IDO*1, IDO2*) that modulates cell behavior by catalyzing the pericellular breakdown of tryptophan (Trp) into the metabolite Kynurenine (Kyn), the start of the Kyn pathway in the tumor microenvironment, with the Trp reduction leading to immunosuppressive effects (Ball *et al*., 2007; Bilir *et al.*, 2017). In humans, MSCs respond to

pro-inflammatory cytokine production by producing *IDO1*, which suppresses this inflammatory response, leading to immunological homeostasis (Mbongue *et al*., 2015) Autoimmune disease, transplant rejection, and infection are a few pathologic conditions where the *IDO* pathway is activated (Bilir *et al*., 2017). Furthermore, *IDO1* (a gene belonging to the *IDO* family) also catalyzes the commitment and rate-limiting step of the Kyn metabolic pathway that produces Kyn, the endogenous agonist of transcription factor AHR (Cheong *et al.,* 2018), which plays an essential role in regulating a large number of gene expression patterns (Neavin *et al*., 2018). In particular, downstream of AHR are functionally linked *CYPs*, encoding enzymes that regulate cell growth, immune function (Fujii-Kuriyama *et al.* 2005; Effner *et al.*, 2017). Overall, the *IDO*-Kyn-AHR-*CYPs* cascade is of great significance in controlling the properties of MSCs (Lewis *et al.*, 2017).

Therefore, the goal of this study is to identify a prospective natural bioactive compound that is able to promote *IDO* expression in human umbilical cord-derived MSCs (hUC-MSCs). The effects of treatment were determined through measuring the expression levels of *IDO2* and *CYPs* using RT-PCR.

MATERIALS AND METHODS

The bioactive compounds collection

The natural compounds Morindone, Rezumbone, Chrysophanol, Chrysosplenol C, Lucidin-ω-ethyl ether, Rubiadin-3-methyl ether, 51A-PT-3B1, 51A-PT-3B2 were provided by the Institute of Natural Products Chemistry, VAST, Vietnam.

hUC-MSC collection and culture

hUC-MSCs were obtained from umbilical cords collected at the Vietnam Children's Hospital. The isolation and cell culture procedure (the data was not shown) was carried out according to a protocol by (Smith *et al*. 2017). At passage 3, day 6, hUC-MSCs were incubated with each of the 8 different natural compounds in 12-well plates at a density of 5 x 105 cells per well at 120 µg/mL for 24 hrs at 37^{o}C, 5 % CO_2, and 90 % humidity.

RNA extraction and real-time PCR

Total mRNA was isolated, and then qPCR was performed on the genes IDO2, AHR, CYP1A1, CYP1A2, and CYP1B2, using β-actin as the internal control gene (QIAGEN Kit, USA).

Statistics

A two-way ANOVA was used, and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Elevation of the *IDO2* expression levels by Rubiadin-3-methyl ether

In this study, 8 different natural bioactive compounds were tested for their ability to promote IDO2, with the anthraquinone derivative Rubiadin-3-methyl ether significantly increasing IDO2 expression levels by nearly threefold. Other stimulants had no discernible effect on expression levels when compared to the control sample Lucidin.

MSCs are capable of exerting immunomodulatory effects and promoting tissue regeneration under certain conditions (Patel *et al*., 2013). Thus, they have become a forthcoming candidate in regenerative medicine and are being investigated for their therapeutic potential for inflammatory conditions (Cheung *et al.*, 2020; Yang *et al.*, 2021). MSCs immunosuppressive effects and inhibit the activation and proliferation of a variety of immune cells (Nauta *et al.*, 2007) was achieved through pro-inflammatory cytokines interferon-γ (IFN-γ) in combination with tumor necrosis factor-α (TNFα) (López-García *et al.*, 2021) and *IDO* enzyme. Furthermore, IDO has two isoforms (IDO1 and IDO2) (Orabona *et al.* 2011), and it has been determined that the IFN--IDO2 interaction results in direct suppression of T-cell proliferation and, indirectly, exerts

immunosuppressive ability in MSCs by assisting IDO1 in navigating T-cell regulation (Metz *et al.*, 2014). However, IFN- production is largely restricted to immune system cells, and the effect of IFN- exposure on IDO levels decreases as MSCs are cultured for longer periods of time (Ankrum *et al.*, 2015), necessitating the use of an alternative stimulant that mimics the effects of IFN- on IDO2 expression to further maximize the immunoregulatory effect of MSCs. Rubiadin-1-methyl ether significantly decreased the levels of all pro-inflammatory cytokines (IL-6, IL-12, and TNF-) while increasing the levels of anti-inflammatory cytokines IL-10 in an in vivo model of acute lung injury induced by LPS (Mohr *et al.*, 2019).

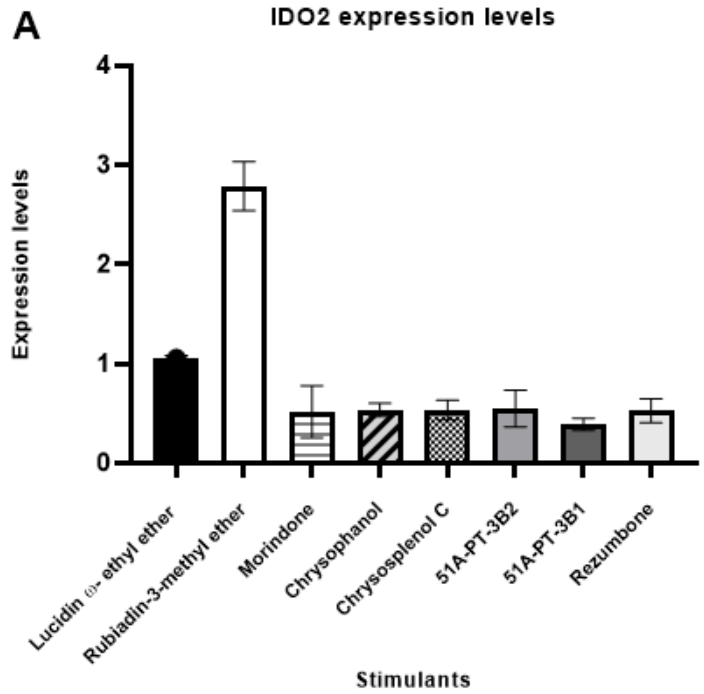

Figure 1. IDO2 gene expression levels in hUC-MSCs when treated with different types of stimulants measured using RT-PCR with Lucidin as the standard sample. $p < 0.05$.

The effects of Rubiadin-3-methyl ether on the target genes expression

When stimulated with IFN-, human MSCs produce IDO, which leads to the conversion of tryptophan into Kyn (Croitoru-Lamoury *et al.*, 2011).

This leads to a local deprivation of tryptophan along with the generation of Kyn metabolites, which in turn inhibits T cell proliferation and modulates the function of major cell populations involved in both the innate and adaptive immune systems, thus accounting for the immunosuppressive effect of MSCs (Orabona *et al.*, 2011; Tipnis *et al.*, 2010). IDO2 expression levels increased by 278.51 percent after treatment with Rubiadin-3-methyl ether compared to the -actin standard, whereas CYPs expression levels were much lower. In theory, the observed increase in *IDO2* levels would lead to increased Kyn production from Trp, followed by AHR activation and the induction of various downstream genes. However, the expression levels of all CYPs were not significantly changed, possibly due to the lack of IDO2 substrate, which led to no production of the potent AHR ligand Kyn and its metabolites and, subsequently, no induction of AHR and the genes that it regulates (*CYPs*).

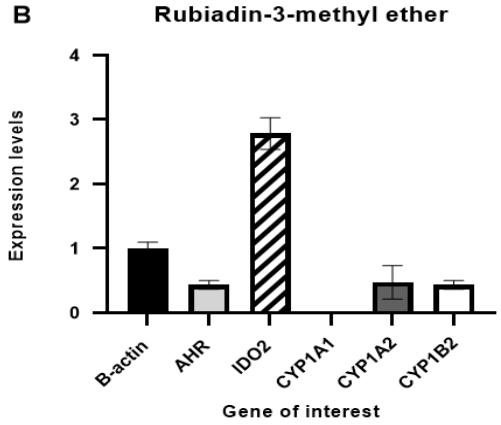

Figure 2. The effects of Rubiadin-3-methyl ether on the gene expression levels in treated hUC-MSCs measured using RT-PCR with B-actin as the standard sample, $p < 0.05$.

CONCLUSION

In summary, our findings show that Rubiadin-3-methyl ether can significantly increase IDO2 expression levels, implying that it could be used as an alternative approach to mimic the effects of IFN- on IDO2 *in vivo*. This is the first study to show a relationship between Rubiadin-3-methyl ether and IDO2 in hUC-MSC. Further investigation is required to determine the relationship between Rubiadin-3-methyl ether and the expression levels of AHR, along with its relevant downstream genes, such as the *CYPs*. Thus, whether human UC-MSCs primed with Rubiadin-3-methyl ether have any significant benefits for treating immune-related diseases or disorders needs to be investigated further.

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