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Relatório final

Efeito da vitamina D sobre diferenciação de linfócitos T reguladores induzida por interação entre receptor TNFR2 e TNF de membrana presente em monócitos M2 de gestantes portadoras de pré-eclâmpsia

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EFEITO DA VITAMINA D SOBRE DIFERENCIAÇÃO DE LINFÓCITOS T REGULADORES INDUZIDA POR INTERAÇÃO ENTRE RECEPTOR TNFR2 E TNF DE MEMBRANA PRESENTE EM MONÓCITOS M2 DE GESTANTES PORTADORAS DE PRÉ-ECLÂMPSIA

Relatório de Pós-doutorado realizado na Universidade Estadual Paulista (UNESP), Instituto de Biociências de Botucatu.

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RESUMO

Introdução: A pré-eclâmpsia (PE) é uma síndrome específica da gravidez caracterizada por ativação de monócitos com perfil inflamatório (M1) em detrimento do perfil anti-inflamatório M2, resultando em níveis elevados de citocinas pró-inflamatórias, como o fator de necrose tumoral (TNF-α). O TNF-α é produzido como uma proteína de transmembrana (mTNF-α) que pode ser proteoliticamente clivada pela enzima conversora de TNF (TACE) dando origem ao TNF solúvel (TNF-α). A ligação de TNF-α com os receptores TNFR1 e TNFR2 na superfície celular ativa diferentes vias de sinalização. TNFR1 pode ser ativado por ligação com ambos mTNF-α e TNF-α, enquanto TNFR2 é principalmente ativado por mTNF-α. O TNFR2 é predominantemente expresso por células T reguladoras $CD4^{+}/FoxP3^{+}$ e, a co-expressão de CD25 com TNFR2 identifica células com maior capacidade supressora. Em vista disso, o uso de moduladores da resposta inflamatória como a vitamina D, no tratamento de células de gestantes com PE pode ser importante para melhorar a resposta inflamatória através da ativação de células com perfil M2 e Treg, capazes de modular e suprimir a inflamação presente nessa síndrome da gestação. **Objetivo:** O presente projeto tem como objetivo avaliar se o tratamento com vitamina D aumenta a expressão de monócitos M2 em gestantes portadoras de PE e, consequentemente, induz aumento de linfócitos Treg por essas gestantes. **Sujeitos e Métodos:** O sangue periférico de 10 gestantes portadoras de PE e 10 mulheres saudáveis, não-grávidas (NP) foi obtido e as células mononucleares (PBMCs) foram cultivadas na presença ou ausência de vitamina D por 24h, sendo a expressão por monócitos dos receptores CD14, CD163, CD206, TNFR2 e de mTNF-α e a expressão de linfócitos T reguladores CD4, CD25, TNFR2 e FoxP3 determinada por citometria de fluxo. Os resultados foram analisados por testes não paramétricos com nível de significância de 5%. **Resultados:** Monócitos e linfócitos cultivados juntos na presença ou ausência de VD apresentaram diferença significativa em relação a mulheres NP para os parâmetros, mTNF-α, CD163, FoxP3 e FoxP3+TNFR2. Além disso, o tratamento com VD aumentou a percentagem de linfócitos expressando FoxP3 e FoxP3+TNFR2. **Conclusão:** Os resultados preliminares indicam que a VD tem potencial de melhorar a resposta inflamatória em gestantes pré-eclâmpticas através do aumento da expressão de células com perfil anti-inflamatório, sendo elas, tanto monócitos M2 como linfócitos Treg. Sendo assim, o uso da vitamina D, poderá induzir a modulação da resposta inflamatória observada na PE, e possivelmente, poderá, futuramente, ser associada ao tratamento dessa importante síndrome da gestação.

Palavras-chave: vitamina D, monócitos M2, citometria de fluxo, células T reguladoras, pré-eclâmpsia.

ABSTRACT

Introduction: Preeclampsia (PE) is a pregnancy-specific syndrome characterized by the activation of monocytes with a pro-inflammatory (M1) profile at the expense of the anti-inflammatory M2 phenotype, resulting in elevated levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α). TNF-α is initially produced as a transmembrane protein (mTNF-α) that can be proteolytically cleaved by TNF-α converting enzyme (TACE), generating soluble TNF-α (sTNF-α). Binding of TNF-α to its receptors, TNFR1 and TNFR2, on the cell surface activates distinct signaling pathways. TNFR1 can be activated by both mTNF-α and sTNF-α, whereas TNFR2 is primarily activated by mTNF-α. TNFR2 is predominantly expressed by $CD4^+/FoxP3^+$ regulatory T cells (Tregs), and the co-expression of CD25 with TNFR2 identifies cells with greater suppressive capacity. In this context, the use of inflammatory response modulators such as vitamin D in the treatment of cells from pregnant women with PE may be important for improving the inflammatory response by promoting the activation of M2-like monocytes and Treg cells capable of modulating and suppressing the inflammation associated with this pregnancy syndrome. **Objective:** This study aims to evaluate whether treatment with vitamin D enhances the expression of M2 monocytes in pregnant women with PE and consequently induces an increase in Treg lymphocytes in these individuals. **Subjects and Methods:** Peripheral blood was collected from 10 pregnant women diagnosed with PE and 10 healthy, non-pregnant (NP) women. Peripheral blood mononuclear cells (PBMCs) were cultured in the presence or absence of vitamin D for 24 hours. Monocyte expression of CD14, CD163, CD206, TNFR2, and mTNF-α, as well as Treg lymphocyte expression of CD4, CD25, TNFR2, and FoxP3, were evaluated by flow cytometry. Data were analyzed using non-parametric statistical tests with a significance level of 5%. **Results:** Monocytes and lymphocytes co-cultured with or without vitamin D showed significant differences compared to NP women for the markers mTNF-α, CD163, FoxP3, and $FoxP3^+$TNFR2. Additionally, treatment with vitamin D increased the percentage of lymphocytes expressing FoxP3 and $FoxP3^+$TNFR2. **Conclusion:** Preliminary results suggest that vitamin D has the potential to improve the inflammatory response in preeclamptic pregnant women by enhancing the expression of anti-inflammatory cell profiles, including both M2 monocytes and Treg lymphocytes. Therefore, vitamin D may contribute to modulating the inflammatory response observed in PE and, potentially, could be considered as an adjunctive therapeutic strategy for this significant pregnancy-related syndrome.

Keywords: vitamin D, M2 monocytes, flow cytometry, regulatory T cells, preeclampsia.

SUMÁRIO

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