Isfahan University of medical science

School of medicine – department of immunology

Ph.D. Thesis

Study of molecular mechanisms of TNF-α induced apoptosis in peripheral blood mono nuclear cell from patients with Beta thalassemia major

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Abstract

Background: Thalassemia major (TM) is a hereditary disease and it characterized by abnormal hemoglobin synthesis, which results in decreased oxygen delivery to the tissues, ineffective erythropoiesis and iron overload. There is increased susceptibility to infections in β-thalassemia. Changes in T- and B-lymphocyte subsets and Regulatory T cells (Treg cells) play a crucial role in the maintenance of immunological self-tolerance. Aryl hydrocarbon receptor (AhR) of these cells could be modulated by cytokines.

The purpose of this study:

The purpose of this study was to investigate the interaction of tumor necrosis factor alpha (TNF-α) with the tetrachlorodibenzo-p-dioxin (TCDD) and ligand of AhR on peripheral blood lymphocytes in patients with β-thalassemia major.

Material and method:

TM patients (n = ۳۲) who were on regular transfusion program were included in the study. Flow cytometry was used to determine the apoptosis, necrosis and tumor necrosis factor receptor1 (TNFR1). The expression of cysteine-aspartic proteases-1(Caspase-1), Caspase-3, Caspase-8, NOD-like receptor family, pyrin domain containing 3(NLRP3) and AhR were assessed by Realtime PCR. These results were compared with blood samples from healthy volunteers.

Result and conclusion:

Our data showed significant differences expression of TNFR1 in β-thalassemia major compare to control individuals ($P<0.05$).Treatment of control individual lymphocytes with TNF-α induced a significant increase in the rate of necrosis mediated by TNFR1 in compare to other

groups, whereas co-treatment of TCDD and TNF-α significantly decreased necrosis and cell death rather than control groups 1 ($P<0.05$). In contrast, co-administered of patient cells with TCDD and TNF-α (group 2) significantly increased cell death and necrosis rather than patient group 1 ($P<0.05$). Apoptosis was not significant between all groups. ($P>0.05$).

Collectively, our study showed that TCDD significantly inhibit effects of TNF-α on apoptosis and AhR expression of lymphocytes. These results showed that chronic inflammation in TM decreased Caspase-1, Caspase-3 and Caspase-8 expression and also exposure of human lymphocytes to TCDD could help to increase expression of Caspase-1, 3.

Keywords: Aryl hydrocarbon receptor, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, β-thalassemia major, tumor necrosis factor alpha, tumor necrosis factor receptor1, lymphocytes, apoptosis

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چکیده فارسی

ضرورت انجام مطالعه:

بتا تالاسمی ماژور یک بیماری ارثی است که با ساخت هموگلوبین غیر طبیعی مشخص می شود. خونسازی نا کارامد منجر به کاهش انتقال اکسیژن به بافتها و افزایش بار آهن می شود. احتمال ابتلا به عفونتها در این بیماری نیز افزایش می یابد که به دلیل تغییر در زیر مجموعه لنفوسیت های T و B بوده و همچنین سلول های T تنظیمی که نقش مهمی در تحمل به خود ایجاد می کنند. پذیرنده آریل هیدرو کربن (AhR) در این سلول ها می تواند تولید سیتوکین توسط این سلول ها را تنظیم کند.

هدف مطالعه:

بررسی اثر متقابل فاکتور نکروز دهنده تومور آلفا (TNF-α) با لیگاند رسپتور AhR (TCDD) در لنفوسیت های خون محیطی بیماران مبتلا به بتاتالاسمی ماژور است.

روش و مواد به کار رفته :

۳۲ بیمار مبتلا به بتا تالاسمی ماژور که تحت درمان دریافت خون مداوم بودند در این مطالعه شرکت داشتند. نمونه های خون به صورت تصادفی همسان شده با حجم ۱۵ سی سی از افراد شرکت کننده در این مطالعه جمع آوری شد. با استفاده از تکنیک فلوسیتومتری میزان آپاپتور و بیان سطحی TNFR-1 در این بیماران بررسی شد. میزان بیان ژن های کاسپاز ۱، کاسپاز ۳، کاسپاز ۸، آریل هیدروکربن رسپتور و NLRP-3 در این بیماران با استفاده از تکنیک Real time PCR در این بیماران ارزیابی شد. نتایج با افراد داوطلب شرکت کننده در گروه های کنترل مقایسه شد.

نتایج:

بر اساس مشاهدات این مطالعه میزان بیان TNFR-1 در افراد مبتلا به بتا تالاسمی ماژور افزایش معنی داری نسبت به گروه کنترل داشتند ($P<0.05$). تیمار لنفوسیت ها با TNF-α نشان داد که میزا مرگ سلولی در لنفوسیت های افراد سالم افزایش معنی داری ($P<0.05$) نسبت به سایر گروه ها داشت، در حالی که تیمار لنفوسیت ها با TCDD و TNF-α منجر به کاهش معنی دار ($P<0.05$) مرگ سلولی نسبت به گروه ۱ شده (گروهی که فقط با TNF-α تیمار شده). در مقابل تیمار لنفوسیت های بیماران با

TCDD و TNF-α منجر به افزایش معنی دار مرگ سلولی نسبت به گروه ۱ شد($P<0.05$). میزان آپاپتوز تفاوت معنی داری بین گروه ها نداشت.

بحث و نتیجه گیری :

مطالعه انجام شده نشان داد که TCDD به طور موثر باعث مهار آپاپتوز القا شده و بیان ژن AhR با واسطه TNF-α در لنفوسیت های بیماران مبتلا به بتا تالاسمی ماژر میشود. بر اساس نتایج به دست آمده به دلیل شرایط التهابی در بیماران مبتلا به بتا تالاسمی ماژور، میزان بیان ژن های کاسپاز ۱، کاسپاز ۳ و کاسپاز ۸ کاهش داشته و مجاورت لنفوسیت ها با TCDD می تواند میزان بیان کاسپاز ۳ و کاسپاز ۱ را افزایش دهد.

کلمات کلیدی : آریل هیدرو کربن رسپتور، ۲، ۳، ۷، ۸ تترا کلرو دی بنزو پی دیوکسین، بتا تالاسمی ماژور، فاکتور نکروز دهنده تومور آلفا، پذیرنده نوع ۱ فاکتور نکروز دهنده تومور ، لنفوسیت، آپاپتوز