论著

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孕鼠补充1α,25-二羟维生素D3水平对仔鼠变应性气道炎症的影响

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[摘要] **目的** 观察孕鼠在孕期补充不同浓度1α,25-二羟维生素D3后,相应仔鼠经卵蛋白致敏与激发后变应性气道炎症的差异,探讨孕期孕鼠1α,25-二羟维生素D3(VitD3)水平对子代幼鼠气道变应性气道炎症的影响。**方法** 30只8周龄BALB/c小鼠按雌雄比例2:1分笼饲养,采用随机数字表法分为5组:空白组,哮喘组,50、100、150 ng 1α,25-二羟维生素D3组(50、100、150 ng组),每组6只。阴栓出现表明受孕,记为D0。50、100、150 ng组孕鼠从D0起隔日于皮下注射相应剂量1α,25-二羟维生素D3 0.1 mL。空白组、哮喘组孕鼠从受孕当天隔日于皮下注射生理盐水0.1 mL。孕鼠产仔后,从各组雌仔鼠中随机选取6只致敏/激发。仔鼠5周龄时哮喘组、50 ng组、100 ng组、150 ng组分别于D0和D2腹腔注射卵清白蛋白(ovalbumin,OVA)0.05 mg+氢氧化铝5 μg(0.5 mL),D7~12将致敏小鼠置于密闭容器中,用1%的OVA溶液15 mL超声雾化激发,每天1次,每次30 min。末次激发后24 h处理各组仔鼠,采用HE染色法观察肺组织病理变化,单盲法检测支气管肺泡灌洗液(bronchoalveolar lavage fluid,BALF)中细胞总数及分类,ELISA法检测BALF中IL-4、IL-5、IFN-γ水平和血清IgE水平。**结果** 50、100、150 ng组仔鼠变应性气道炎症较哮喘组明显减轻。与哮喘组相比,50、100、150 ng组仔鼠BALF中细胞总数和嗜酸性粒细胞(eosinophil,EOS)均明显减少($P<0.05$),且随VitD3剂量增加而减少,呈剂量依赖性,各组间差异有统计学意义($P<0.05$);BALF中IL-4、IL-5水平明显下降($P<0.05$),且随VitD3剂量增加而减少,呈剂量依赖性,各组间差异有统计学意义($P<0.05$);而IFN-γ水平明显增高,且随VitD3剂量增加而增加,呈剂量依赖性,各组间差异有统计学意义($P<0.05$);血清中IgE水平明显下降($P<0.05$),且随VitD3剂量增加而减少,呈剂量依赖性,各组间差异有统计学意义($P<0.05$)。**结论** 孕鼠补充VitD3可以明显减轻仔鼠变应性气道炎症,该作用呈剂量依赖性。

[关键词] 1α,25-二羟维生素D3;支气管哮喘;嗜酸性粒细胞;IL-4;IL-5;IFN-γ;IgE
[中图法分类号] R715.3;R725.62;R977.24 [文献标志码] A

Effect of maternal 1α, 25-dihydroxyvitamin D3 supplement on allergic airway inflammation of its offspring in mouse asthma model

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[**Abstract**] **Objective** To investigate the effects of 1α,25-dihydroxyvitamin D3 (VitD3) supplement during pregnancy on allergic airway inflammation of its offspring in a mouse allergic asthma model. **Methods** Thirty eight-week-old BALB/c mice were kept according to gender proportion (female/male: 2/1), and were randomly divided into 5 groups (6 mice per group): control, asthma group, 50 ng VitD3 group (50 ng group), 100 ng VitD3 group (100 ng group), and 150 ng VitD3 group (150 ng group). Observing the fertilization condition of female mice, day zero (D0) was recorded when vaginal plug was found. The pregnant mice in the 50 ng group, the 100 ng group and the 150 ng group were treated with subcutaneous injection of 0.1 mL VitD3 of corresponding dose from D0 to D20 (every other day). The pregnant mice in the control and the asthma group were treated in the same way but with 0.1 mL saline. The female offspring mice were selected to continue the study, 6 in each group. The asthma group, the 50 ng group, the 100 ng group and the 150 ng group mice were treated by intra-peritoneal injection of ovalbumin (OVA, 0.05 mg) and aluminum hydroxide (5 μg) as an adjuvant on D0 and D2 in order to establish the mouse allergic asthma model. These sensitized mice were kept in a closed container (25 cm×12 cm×12 cm), and challenged with 1% OVA 15 mL 30 min per day from D7 to D12. For analysis, all mice were sacrificed in 24 h after the last challenge. Histopathological

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analysis was performed on each mouse per group with HE staining. The number of cells and their classification in bronchoalveolar lavage fluid (BALF) were assayed. Levels of IFN-γ, IL-4, and IL-5 in BALF as well as serum immunoglobulin E (IgE) were measured by ELISA. **Results** According to the pathological sections of lung, the inflammation conditions of the 50 ng group, the 100 ng group and the 150 ng group were eased as compared to the asthma group. Comparing with the asthma group, both total cells and the percentage of eosinophile (EOS) were decreased in BALF in the 50 ng group, the 100 ng group and the 150 ng group, showing statistical difference from those in the asthma group ($P<0.05$) and negative dose dependency with statistical difference ($P<0.05$). The levels of IL-4 and IL-5 in BALF of the 50 ng group, the 100 ng group and the 150 ng group were greatly decreased, showing statistical difference from those in the asthma group ($P<0.05$) and negative dose dependency with statistical difference ($P<0.05$). The levels of IFN-γ of the 50 ng group, the 100 ng group and the 150 ng group were increased, showing statistical difference from those in the asthma group ($P<0.05$) and negative dose dependency with statistical difference ($P<0.05$). The levels of serum IgE of the 50 ng group, the 100 ng group and the 150 ng group were greatly decreased, showing statistical difference from those in the asthma group ($P<0.05$) and negative dose dependency with statistical difference ($P<0.05$). **Conclusion** VitD3 supply during maternal pregnancy alleviates offspring allergic airway inflammation of a mouse allergic asthma model in a dose-dependent manner.

[**Key words**] 1α,25-dihydroxyvitamin D3; asthma; eosinophile; IL-4; IL-5; IFN-γ; IgE

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支气管哮喘是一种常见的慢性呼吸道疾病,是多种炎症细胞及炎症介质参与的气道慢性炎症,其发病机制极为复杂,目前尚未清楚。维生素D既是一种维生素,也是一种非甾体类激素。它的生物活化形式为1α,25-二羟维生素D3(1α,25-dihydroxyvitamin D3,简称VitD3),除了可以调节钙和骨的代谢外,在许多免疫细胞分化和发育中也起到重要作用[1]。随着生活方式的改变,室内活动时间增多,大量使用防紫外线用品,容易导致维生素D缺乏[2]。有研究[3-4]发现怀孕期间母亲增加维生素D摄入可降低幼儿发生喘息的危险性度。Camargo等[5]发现在美国东北部怀孕期间母亲较高的维生素D摄入可降低幼儿发生喘息的危险性度。这两项研究说明母亲孕期维生素D水平与儿童哮喘发病之间存在负相关。张巧玲等[6]发现大鼠生命早期适量的VitD3干预,可改善哮喘大鼠的肺功能,减少嗜酸性细胞浸润。本实验通过给孕鼠孕期补充不同剂量VitD3,仔鼠建立变态反应性哮喘模型,比较仔鼠激发后肺组织病理学形态变化,BALF中细胞总数及分类,BALF中IL-4、IL-5、IFN-γ水平和血清IgE水平变化,研究孕期补充VitD3对子代变应性气道炎症的影响,从而为孕期补充VitD3改善子代变应性气道炎症提供实验依据。

1 材料与方法

1.1 实验动物

8周龄清洁级BALB/c小鼠30只,体质量(18±3)g,雌雄比例2∶1,由上海西普尔-必凯实验动物有限公司提供,合格证号:SCXK(沪)2008-0016。

1.2 主要仪器与试剂

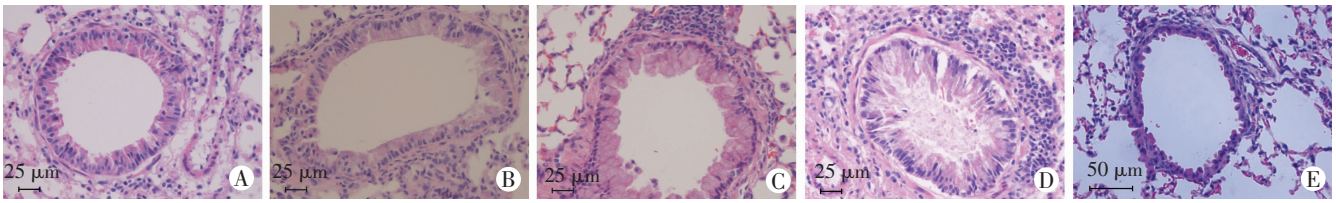
超声雾化器(上海四菱医疗器械厂),显微摄像显微镜(Feica公司),低温离心机(ZK-380 德国),酶标仪(美国Bio-Rad),卵清白蛋白(OVA,V级)、氢氧化铝凝胶、Vit D3均购自Sigma公司,小鼠IL-4、IL-5、IFN-γ、IgE ELISA试剂盒购自R&D Systems。

1.3 方法

1.3.1 实验分组与给药 30只8周龄BALB/c小鼠按雌雄比例2∶1分笼饲养,按随机数字表法分为5组:空白组,哮喘组,50、100、150 ng VitD3组(50、100、150 ng组[7]),每组6只。每日观察,发现阴栓即为受孕,记为D0。50、10、150 ng组孕鼠从受孕当天隔日于皮下注射相应剂量VitD3 0.1 mL。空白组、哮喘组孕鼠从受孕当天隔日于皮下注射生理盐水0.1 mL。

1.3.2 仔鼠致敏与激发 从各组雌仔鼠随机取6只继续试验。仔鼠至5周龄时开始致敏。哮喘组、50 ng组、100 ng组、150 ng组于D0和D2每只仔鼠分别腹腔注射致敏液0.2 mL(含OVA 0.05 mg及氢氧化铝5 μg)。于D7~D12将经过致敏的仔鼠置于密闭容器(25 cm×12 cm×12 cm)中,以1%的OVA溶液15 mL雾化吸入,每天1次,每次30 min。

1.3.3 仔鼠支气管肺泡灌洗和肺组织处理 末次激发后24 h处理5组仔鼠。小鼠腹腔注射10%水合氯醛(0.075 mL/只)麻醉后,采用摘除眼球取血法,采血1 mL,3 000 r/min离心5 min(4 ℃),收集上清液存储于-80 ℃冰箱检测IgE。颈椎脱臼法处死小鼠,将其固定,打开胸腔,结扎左肺。充分暴露小鼠颈部气管并将其断离,用1 mL注射器轻柔注入0.5 mL 4 ℃生理盐水,反复回抽3次后收集该液体,此即为BALF。以上操作



A:空白组;B:哮喘组;C:50 ng组;D:100 ng组;E:150 ng组

图1 HE染色观察各组仔鼠气道和肺组织病理学变化

重复复3次(回收率>80%),收集于1.5 mL Eppendoff管中。取100 μL细胞计数,余以3 000 r/min离心5 min(4 ℃),收集上清液存储于-80 ℃冰箱待测。细胞沉淀适当稀释,混匀后制备细胞涂片并行瑞氏-吉姆萨染色。取左肺放入4%多聚甲醛中固定12 h,用石蜡包埋肺组织,然后切片,再行HE染色。

1.3.4 ELASA法测定BALF中IL-4、IL-5和IFN-γ水平 取仔鼠BALF,按IL-4、IL-5和IFN-γ ELISA试剂盒使用说明书检测BALF中IL-4、IL-5和IFN-γ水平。

1.3.5 ELASA法测定血清中IgE水平 取仔鼠血清,按IgE ELISA试剂盒使用说明书检测血清中IgE水平。

1.4 统计学分析

数据以$\bar{x} \pm s$表示,采用SPSS 17.0统计软件,方差齐时采用单因素方差分析,并用SNK(Student-Newman-Keuls)法进行两两比较;方差不齐时,经过数据转换,方差齐后再行单因素方差分析(选用Games-Howel法)。

2 结果

2.1 各组仔鼠肺部病理改变

哮喘组仔鼠支气管、细支气管和肺泡腔缩小,肺泡间隔增宽,管壁及肺间质有大量炎症细胞浸润,以嗜酸性粒细胞(eosinophil, EOS)为主,支气管、细支气管及肺泡腔内可见渗出物,支气管和细支气管黏膜上皮部分坏死脱落伴杯状细胞增生。而其余各组未见上述肺组织的病理变化(图1)。

2.2 各组仔鼠BALF中细胞总数和细胞分类计数

哮喘组BALF中细胞总数、EOS均明显高于空白组,差异有统计学意义($P<0.05$)。与哮喘组比较,50 ng组、100 ng组、150 ng组仔鼠BALF中细胞总数、EOS均明显减少,差异有统计学意义($P<0.05$),且随剂量增加而减少,呈剂量依赖性,各组间差异有统计学意义($P<0.05$)。各剂量组BALF细胞总数和EOS虽较哮喘组下降,但仍高于空白组,差异有统计学意义($P<0.05$)。各组其他三类细胞分类计数百分比正常。见表1。

表1 各组仔鼠BALF中细胞总数和细胞分类计数

(10^3/mL, $n=6$, $\bar{x} \pm s$)

组别	细胞总数	EOS	巨噬细胞	淋巴细胞
空白组	176.84±13.43	0.19±0.05	161.68±16.32.	1.10±0.21
哮喘组	490.78±12.54[a]	68.24±10.21[a]	424.18±18.24[a]	1.68±0.20
50 ng组	374.53±10.08[ab]	42.18±11.25[ab]	328.15±16.47[a]	2.65±0.27[a]
100 ng组	339.64±12.24[ab]	20.32±10.54[ab]	310.46±20.34[a]	2.91±0.66[a]
150 ng组	318.62±18.94[ab]	10.38±8.54[abcd]	301.16±19.24[a]	3.41±0.88[a]

a:$P<0.05$,与空白组比较;b:$P<0.05$,与哮喘组比较;c:$P<0.05$,与50 ng组比较;d:$P<0.05$,与100 ng组比较

2.3 各组仔鼠BALF中IL-4、IL-5、IFN-γ水平与血清IgE水平

与空白组比较,哮喘组BALF中IL-4、IL-5水平均明显增高,差异有统计学意义($P<0.05$);IFN-γ水平明显下降,差异有统计学意义($P<0.05$)。与哮喘组比较,50 ng组、100 ng组、150 ng组BALF中IL-4、IL-5水平明显下降,差异有统计学意义($P<0.05$),且随剂量增加而减少,呈剂量依赖性,各组间差异有统计学意义($P<0.05$);而IFN-γ水平明显增高,且随剂量增加而增加,呈剂量依赖性,各组间差异有统计学意义($P<0.05$,表2)。与空白组比较,哮喘组血清中IgE水平明显增高,差异有统计学意义($P<0.05$)。与哮喘组比较,50、100、150 ng组血清中IgE水平明显下降,差异有统计学意义($P<0.05$),且随剂量增加而减少,呈剂量依赖性,各组间差异有统计学意义($P<0.05$,表2)。

表2 各组仔鼠BALF中IL-4、IL-5、IFN-γ与血清IgE水平

($n=6$, $\bar{x} \pm s$)

组别	IL-4(pg/mL)	IL-5(pg/mL)	IFN-γ(pg/mL)	IgE(IU/mL)
空白组	8.50±0.86	14.51±1.03	53.56±1.37	36.05±0.83
哮喘组	23.60±0.85[a]	55.84±1.21[a]	26.98±1.97[a]	366.83±3.07[a]
50 ng组	15.75±0.92[ab]	29.06±1.09[ab]	35.37±0.72[ab]	185.69±4.33[ab]
100 ng组	13.36±0.75[abc]	24.50±0.92[abc]	37.97±1.78[abc]	151.55±4.10[abc]
150 ng组	11.48±0.91[abcd]	20.18±1.05[abcd]	44.87±1.85[abcd]	131.57±3.36[abcd]

a:$P<0.05$,与空白组比较;b:$P<0.05$,与哮喘组比较;c:$P<0.05$,与50 ng组比较;d:$P<0.05$,与100 ng组比较

3 讨论

1α,25-二羟维生素D3除了可以调节钙和骨的代谢外,在许多免疫细胞分化和发育中也起到重要作用[1]。体外实验[5]已经证实维生素D可以促进单核细胞和巨噬细胞增殖和成熟,维生素D缺乏会导致巨噬细胞功能受损,影响其趋化性和吞噬性并导致炎症因子生成增多。维生素D受体(vitamin D receptor, VDR)介导VitD3的生物学作用,是核受体超家族成员,具有配体活化的转录因子作用。它与维生素D反应基因的特异性DNA反应元件(维生素D反应元件, VDRE)结合,影响RNA聚合酶Ⅱ介导的转录。

与哮喘慢性气道炎症有关的树突状细胞、肥大细胞、支气管平滑肌细胞和气道上皮细胞等均组成性表达VDR。体外实验发现树突状细胞、巨噬细胞和单核细胞表达1α羟化酶,促进VitD3合成[8],提示维生素

D与哮喘的发病存在联系。VDR基因的多样性与哮喘的发病密切相关,不同的基因型对哮喘易感性不同,这一点在女性中尤为显著。Yu等[9]证实VDR基因敲除的小鼠无法产生AHR;Wittke等[10]也证实VDR基因敲除的小鼠不能形成实验性过敏性哮喘,提示维生素D在Th2型炎症发展过程中的重要作用。VitD3还能提高正常人及激素抵抗型哮喘患者$CD4^+$ T细胞在地塞米松作用下分泌IL-10的能力[11],目前维生素D及其活性成分已被推荐为临床上治疗激素抵抗型哮喘的重要辅助药物。此外,Clifford等[12]的研究还揭示了VitD3可抑制支气管平滑肌细胞过度增殖,改善哮喘气道重构现象。

维生素D在肺脏免疫系统和呼吸系统相关疾病上有重要作用[13],多项研究显示母亲孕期维生素D水平与胎儿出生后呼吸道感染和儿童时期的喘鸣有关[14-15]。维生素D对于胎儿免疫系统和肺脏发育有重要作用,而胎儿血清维生素D水平依赖母体水平[16],故孕鼠外源性补给维生素D活性成分1α,25-二羟维生素D3,可以影响仔鼠的发育。本实验通过给孕鼠外源性补充维生素D,提高其血清维生素D水平,观察并评估仔鼠激发后气道变应性炎症情况,研究孕鼠血清维生素D水平对仔鼠激发后气道变应性炎症的影响。结果显示孕鼠补充维生素D可以明显减轻仔鼠致敏后的气道炎症,使仔鼠BALF中炎症细胞总数和EOS比哮喘组明显减少($P<0.05$);BALF中IL-4、IL-5水平比哮喘组明显降低($P<0.05$),IFN-γ水平比哮喘组明显升高($P<0.05$);血清IgE水平比哮喘组明显降低($P<0.05$)。说明孕鼠补充维生素D对仔鼠致敏后的气道炎症具有保护作用,且该作用具有剂量依赖性。孕鼠孕期高维生素D水平有利于降低仔鼠变应性气道炎症。

本研究按照Matheu等[7]的报道,以50 ng为初始剂量,同时累加补充,结果发现,孕鼠补充1α,25-二羟维生素D3的剂量与子代幼鼠气道炎症呈现剂量依赖关系。150 ng组子代幼鼠气道炎症最轻,且未发现孕鼠流产,胎儿畸形等情况。因此,我们推荐孕鼠孕期可隔日补充150 ng 1α,25-二羟维生素D3。

本研究发现孕鼠孕期补充1α,25-二羟维生素D3可以减轻气道炎症细胞浸润,特别是嗜酸性粒细胞浸润,减少IL-4、IL-5等细胞因子分泌,降低血清中IgE水平,增加IFN-γ分泌。总之,孕期补充1α,25-二羟维生素D3可以减轻仔鼠变应性气道炎症。

上述结果提示在孕期补充维生素D对子代哮喘发病可能具有保护作用。为预防和减轻哮喘发病,因此应主动为孕妇特别是有哮喘家族史者补充一定剂量的维生素D。但是过大剂量的维生素D对机体存在危害,血清维生素D水平过高会损害心脏,增加房颤的几率,影响钙磷代谢,导致转移性钙质沉积。故为寻找合适的剂量还需更进一步的研究。虽然本实验未发现孕鼠出现畸胎和流产情况,但大剂量维生素D对孕妇的安全性还需要进一步验证。

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