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【基础研究】

维生素 B_6 对动脉粥样硬化小鼠血管内皮损伤的影响及作用机制

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摘要: 目的 探讨维生素 B_6(VB_6)对动脉粥样硬化(AS)小鼠血管内皮损伤的影响及作用机制。**方法** 将 36 只 $ApoE^{-/-}$ 小鼠随机分为对照组、AS 组、VB_6 组、AS + LiCl 组、AS + VB_6 组和 AS + VB_6 + LiCl 组,每组 6 只。AS 组、AS + LiCl 组、AS + VB_6 组和 AS + VB_6 + LiCl 组小鼠高脂饮食 12 周建立 AS 模型;对照组和 VB_6 组小鼠常规饮食、正常饮水 12 周。12 周后,对照组小鼠常规饮食,每日给予和 VB_6 组等体积的生理盐水灌胃;VB_6 组小鼠常规饮食,每日灌胃给予 VB_6($50\ mg \cdot kg^{-1}$);AS + LiCl 组小鼠继续给予高脂饮食,每日灌胃给予 LiCl($1\ mg \cdot kg^{-1}$);AS + VB_6 组小鼠继续给予高脂饮食,每日灌胃给予 VB_6($50\ mg \cdot kg^{-1}$);AS + VB_6 + LiCl 组小鼠继续给予高脂饮食,每日灌胃给予 VB_6($50\ mg \cdot kg^{-1}$)和 LiCl($1\ mg \cdot kg^{-1}$);6 组小鼠均干预 4 周。干预结束后,采用酶联免疫吸附试验检测各组小鼠血清中一氧化氮(NO)、丙二醛(MDA)水平和超氧化物歧化酶(SOD)活性。苏木精-伊红染色观察各组小鼠胸主动脉组织形态,并计算 AS 斑块面积占血管总面积的百分比。离体血管环实验检测胸主动脉舒张率。免疫组织化学法检测胸主动脉中钠氢交换蛋白 1(NHE1)表达。**结果** 与对照组相比,AS 组小鼠血清中 NO 水平和 SOD 活性显著下降,MDA 水平显著升高($P<0.05$);VB_6 组与对照组小鼠血清中 NO、MDA 水平和 SOD 活性比较差异无统计学意义($P>0.05$)。与 AS 组相比,AS + VB_6 组小鼠血清中 NO 水平和 SOD 活性显著上升,MDA 水平显著下降($P<0.05$);AS + LiCl 组、AS + VB_6 + LiCl 组与 AS 组小鼠血清中 NO、MDA 水平和 SOD 活性比较差异无统计学意义($P>0.05$)。与 AS + VB_6 组相比,AS + VB_6 + LiCl 组小鼠血清中 NO 水平和 SOD 活性显著下降,MDA 水平显著升高($P<0.05$)。AS 组小鼠 AS 斑块面积占血管总面积的百分比显著高于对照组($P<0.05$);VB_6 组与对照组小鼠 AS 斑块面积占血管总面积的百分比比较差异无统计学意义($P<0.05$)。AS + VB_6 组小鼠斑块面积占血管总面积的百分比显著低于 AS 组($P<0.05$);AS + LiCl 组、AS + VB_6 + LiCl 组与 AS 组小鼠 AS 斑块面积占血管总面积的百分比比较差异无统计学意义($P<0.05$)。AS + VB_6 + LiCl 组小鼠 AS 斑块面积占血管总面积的百分比显著高于 AS + VB_6 组($P<0.05$)。对照组小鼠血管内皮光滑平整,细胞排列整齐有序;AS 组、AS + LiCl 组和 AS + VB_6 + LiCl 组小鼠的血管组织结构紊乱、血管内皮粗糙;VB_6 组和 AS + VB_6 组小鼠的血管壁结构正常、血管内皮光滑、细胞排列有序。AS 组小鼠乙酰胆碱(Ach)诱导的胸主动脉舒张率显著低于对照组($P<0.05$);VB_6 组与对照组小鼠 Ach 诱导的胸主动脉舒张率比较差异无统计学意义($P>0.05$)。AS + VB_6 组小鼠 Ach 诱导的胸主动脉舒张率显著低于 AS 组($P<0.05$);AS + LiCl 组、AS + VB_6 + LiCl 组与 AS 组小鼠 Ach 诱导的胸主动脉舒张率比较差异无统计学意义($P>0.05$)。AS + VB_6 + LiCl 组小鼠 Ach 诱导的胸主动脉舒张率显著高于 AS + VB_6 组($P<0.05$)。6 组小鼠硝普钠诱导的胸主动脉舒张率比较差异均无统计学意义($P>0.05$)。AS 组小鼠胸主动脉中 NHE1 蛋白表达量百分比显著高于对照组($P<0.05$);VB_6 组与对照组小鼠胸主动脉中 NHE1 蛋白表达量百分比比较差异无统计学意义($P>0.05$)。AS + VB_6 组小鼠胸主动脉中 NHE1 蛋白表达量百分比显著低于 AS 组($P<0.05$);AS + LiCl 组、AS + VB_6 + LiCl 组与 AS 组小鼠胸主动脉中 NHE1 蛋白表达量百分比比较差异无统计学意义($P>0.05$)。AS + VB_6 + LiCl 组小鼠胸主动脉中 NHE1 蛋白表达量百分比显著高于 AS + VB_6 组($P<0.05$)。**结论** VB_6 可通过抑制 NHE1 蛋白的表达来改善 AS 小鼠的血管内皮损伤。

关键词: 维生素 B_6;钠氢交换蛋白 1;内皮损伤;动脉粥样硬化;氧化应激
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Effect of vitamin B_6 on vascular endothelial injury of atherosclerosis mice and its mechanism

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Abstract: **Objective** To investigate the effect of vitamin B_6 (VB_6) on vascular endothelial injury of atherosclerosis (AS) mice and its mechanism. **Methods** Thirty-six $ApoE^{-/-}$ mice were randomly divided into control group, AS group, VB_6 group, AS + LiCl group, AS + VB_6 group and AS + VB_6 + LiCl group, with 6 mice in each group. The mice in the AS group, AS + LiCl group, AS + VB_6 group and AS + VB_6 + LiCl group were fed with high-fat diet for 12 weeks to establish the AS model; the mice in the control group and VB_6 group were given regular diet and normal drinking water for 12 weeks. After 12 weeks, the mice in the control group were given conventional diet and the same volume of physiological saline as the VB_6 group daily by gavage; the mice in the VB_6 group were given routine diet and VB_6 (50 mg · kg^{-1}) by gavage daily; the mice in the AS + LiCl group were given high-fat diet continuously and LiCl (1 mg · kg^{-1}) by gavage daily; the mice in the AS + VB_6 group were given high-fat diet continuously and VB_6 (50 mg · kg^{-1}) by gavage daily; the mice in the AS + VB_6 + LiCl group were given high-fat diet continuously and VB_6 (50 mg · kg^{-1}), LiCl (1 mg · kg^{-1}) by gavage daily; all mice were intervened for 4 weeks. After intervention, the serum nitric oxide (NO), malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity of mice in each group were measured by enzyme linked immunosorbent assay. Hematoxylin-eosin staining was used to observe the morphology of thoracic aortic tissue of mice in each group and the percentage of AS plaque area to total vascular area was calculated. The vasodilatation rate of thoracic aorta was detected by isolated vascular ring experiment. The expression of sodium/hydrogen exchanger 1 (NHE1) protein in thoracic aorta was detected by immunohistochemistry. **Results** Compared with the control group, the NO level and SOD activity in the serum of mice in the AS group decreased, while the MDA level increased ($P<0.05$); there was no significant difference in the NO, MDA levels and SOD activity in the serum of mice between the VB_6 group and the control group ($P>0.05$). Compared with the AS group, the serum NO level and SOD activity of mice in the AS + VB_6 group increased, while the MDA level decreased ($P<0.05$); there was no significant difference in serum NO, MDA levels and SOD activity of mice between the AS + LiCl group, AS + VB_6 + LiCl group and AS group ($P>0.05$). Compared with the AS + VB_6 group, the serum NO level and SOD activity of mice in the AS + VB_6 + LiCl group decreased, while the MDA level increased ($P<0.05$). The percentage of AS plaque area to total vascular area of mice in the AS group was significantly higher than that in the control group ($P<0.05$); there was no significant difference in the percentage of AS plaque area to total vascular area of mice among the VB_6 group and the control group ($P<0.05$). The percentage of AS plaque area to total vascular area of mice in the AS + VB_6 group was significantly lower than that in the AS group ($P<0.05$); there was no significant difference in the percentage of AS plaque area to total vascular area of mice between the AS + LiCl group, AS + VB_6 + LiCl group and AS group ($P<0.05$). The percentage of AS plaque area to total vascular area of mice in the AS + VB_6 + LiCl group was significantly higher than that in the AS + VB_6 group ($P<0.05$). In the control group, the vascular endothelium of mice was smooth with orderly arrangement of cells; in the AS group, AS + LiCl group and AS + VB_6 + LiCl group, the tissue structure of vascular of mice was disordered and the vascular endothelium was rough; in the VB_6 group and AS + VB_6 group, the vascular wall structure of mice was normal, the vascular endothelium was smooth, and the cells were arranged orderly. The vasodilatation rate of thoracic aorta of mice induced by acetylcholine (Ach) in the AS group was significantly lower than that in the control group ($P<0.05$); there was no significant difference in the vasodilatation rate of thoracic aorta of mice induced by Ach between the VB_6 group and the control group ($P>0.05$). The vasodilatation rate of thoracic aorta of mice induced by Ach in the AS + VB_6 group was significantly lower than that in the AS group ($P<0.05$); there was no significant difference in the vasodilatation rate of thoracic aorta of mice induced by Ach between AS + LiCl group, AS + VB_6 + LiCl group and AS group ($P>0.05$). The vasodilatation rate of thoracic aorta of mice induced by Ach in the AS + VB_6 + LiCl group was significantly higher than that in the AS + VB_6 group ($P<0.05$). There was no significant difference in the vasodilatation rate of thoracic aorta of mice induced by sodium nitroprusside among the six groups ($P>0.05$). The percentage of NHE1 expression in the thoracic aorta of mice in the AS group was significantly higher than that in the control group ($P<0.05$); there was no significant difference in the percentage of NHE1 expression in the thoracic aorta of mice between the VB_6 group and the control group ($P>0.05$). The percentage of NHE1 expression in the thoracic aorta of mice in the AS + VB_6 group was significantly lower than that in the AS group ($P<0.05$); there was no significant difference in the percentage of NHE1 expression in the thoracic aorta of mice among the AS + LiCl group, AS + VB_6 + LiCl group and the AS group ($P>0.05$). The percentage of NHE1 expression in the thoracic aorta of mice in the AS + VB_6 + LiCl group was significantly higher than that in the AS + VB_6 group ($P<0.05$). **Conclusion** VB_6 can improve vascular endothelial injury in AS mice via inhibiting the expression of NHE1 protein.

Key words: vitamin B_6; sodium/hydrogen exchanger 1; endothelial injury; atherosclerosis; oxidative stress

动脉粥样硬化(atherosclerosis,AS)是一种由于动脉壁周围脂质堆积而导致动脉壁增厚硬化、血管腔狭窄的病理状态,是多种心脑血管疾病的主要病因。动脉内皮细胞损伤是 AS 的一种初始病理现象[1-4]。

钠氢交换蛋白 1(sodium/hydrogen exchanger 1, NHE1)是一类存在于细胞膜表面的离子转运蛋白,通过将细胞内 H^+ 与细胞外 Na^+ 按照 1∶1 的比例进行交换来维持细胞内酸碱平衡[5-6]。有研究表明,巨噬细胞中 NHE1 的活化会促进细胞凋亡,进而导致 AS 或其他心血管疾病的发生[7]。维生素 B_6(vitamin B_6, VB_6)可通过转化为其活化形式磷酸吡哆醛来减轻血管内皮细胞的形态改变[8-9]。但 NHE1 在 VB_6 改善 AS 血管内皮损伤中的作用尚不清楚。基于此,本研究通过建立 AS 模型来探讨 NHE1 在 VB_6 改善载脂蛋白 E 基因敲除(apolipoprotein E gene knockout, $ApoE^{-/-}$)小鼠血管内皮细胞功能中的作用及机制。

1　材料与方法

1.1　实验动物

$ApoE^{-/-}$ 小鼠 36 只,6 ~ 8 周龄,购自北京 Huafukang 动物实验中心(雌雄各占 50%)。小鼠饲养于新乡医学院无特定病原体级动物房,温度(23 ± 1) ℃,湿度 40% ~ 60%,12 h 光暗循环,小鼠可自由获取食物和水。

1.2　主要药物、试剂与仪器

NHE1 抑制剂 LiCl、VB_6、乙酰胆碱(acetylcholine, Ach)、硝普钠(sodium nitroprusside, SNP)购自美国 Sigma Chemical Co 公司;NHE1 一抗购自美国 Santa Cruz 公司,超氧化物歧化酶(superoxide dismutase, SOD)、丙二醛(malondialdehyde, MDA)、一氧化氮(nitric oxide, NO)测定试剂盒购自南京建成生物工程研究所;K-3 型酶标仪购自美国 Thermo 公司,SMZ-800N 体式显微镜购自日本 Nikon 公司,组织包埋机、RM-2125-RTS 超声振动切片机购自德国 Leica 公司。

1.3　实验方法

1.3.1　动物分组、模型建立及各组干预措施

36 只小鼠适应性喂养 1 周后,随机分为对照组、AS 组、VB_6 组、AS + LiCl 组、AS + VB_6 组和 AS + VB_6 + LiCl 组,每组 6 只。AS 组、AS + LiCl 组、AS + VB_6 组和 AS + VB_6 + LiCl 组小鼠给予高脂饮食(质量分数 0.15% 胆固醇和 21.00% 脂肪)12 周建立 AS 模型;对照组和 VB_6 组小鼠常规饮食、正常饮水 12 周。12 周后,对照组小鼠常规饮食,每日给予与 VB_6 组等体积的生理盐水灌胃;VB_6 组小鼠常规饮食,每日灌胃给予 VB_6(50 mg · kg^{-1});AS + LiCl 组小鼠继续给予高脂饮食,每日灌胃给予 LiCl(1 mg · kg^{-1});AS + VB_6 组小鼠继续给予高脂饮食,每日灌胃给予 VB_6(50 mg · kg^{-1});AS + VB_6 + LiCl 组小鼠继续给予高脂饮食,每日灌胃给予 VB_6(50 mg · kg^{-1})和 LiCl(1 mg · kg^{-1});6 组小鼠均干预 4 周。

1.3.2　血清中 MDA、NO 水平和 SOD 活性检测

给药结束后,小鼠禁食禁水 12 h,腹腔注射戊巴比妥钠(30 mg · kg^{-1})麻醉大鼠。摘除眼球,采血 1 mL 于抗凝管中,低温离心机 4 000 r · min^{-1} 离心 10 min,取血清,应用酶联免疫吸附试验试剂盒测定血清中 NO、MDA 水平和 SOD 活性,其中血清中 NO 水平检测采用微板法,血清中 MDA 水平检测采用硫代巴比妥酸法,血清中 SOD 活性检测采用水溶性四氮唑-8 法,严格按照试剂盒说明书进行操作。

1.3.3　胸主动脉组织形态学观察

眼球取血后切开小鼠胸腔,分离胸主动脉。取部分胸主动脉组织于 40 g · L^{-1} 多聚甲醛溶液中固定 16 h,体积分数 70% 乙醇浸泡 2 min,油红 O 染色 30 min,体积分数 70% 乙醇漂洗组织至发白,体式显微镜拍照观察染色结果。另取胸主动脉组织进行脱水、透明、浸蜡和包埋等制备成石蜡切片,苏木精-伊红(hematoxylin and eosin, HE)染色后,使用连接 QImaging Retiga CCD 相机的显微镜观察胸主动脉组织形态学并拍照。采用 Image J 软件的 IHC Profiler[10] 插件处理图片,并计算 AS 斑块面积占血管总面积的百分比(每组血管斑块所占面积/血管总面积 × 100%)。

1.3.4　离体血管环实验检测胸主动脉对 Ach 或 SNP 的舒张反应

将胸主动脉切成环状(长 3 ~ 4 mm),悬挂并固定在灌注 pH 值 7.4 Krebs 缓冲液的器官室中。缓冲液通入体积分数 95% O_2 和 5% CO_2,加入去氧肾上腺素(1 μmol · L^{-1})诱发胸主动脉的收缩反应。收缩前,给胸主动脉环施加 2.0 ~ 6.0 g 张力 90 min。在此期间,每 15 min 更换 1 次 Krebs 缓冲液,共 3 次;将胸主动脉环置于 60 mmol · L^{-1} KCl 溶液中 30 min,然后用 1 μmol · L^{-1} 去氧肾上腺素收缩血管环,待收缩反应达坪值后,再依浓度梯度加入 Ach(0.003、0.030、0.300、3.000 μmol · L^{-1})或 SNP(0.003、0.030、0.300 μmol · L^{-1}),观察血管环张力的变化,以药物诱发血管舒张幅度与最大收缩幅度的比值作为血管舒张率[11]。

1.3.5　免疫组织化学法检测胸主动脉中 NHE1 蛋白的表达

将胸主动脉石蜡切片脱蜡,用 0.1 mol · L^{-1} 柠檬酸钠缓冲液(pH = 6.0)进行热修复(92 ~ 98 ℃,30 min),体积分数 3% 过氧化氢室温孵育 5 ~ 10 min,体积分数 3% 牛血清白蛋白封闭 20 min,加 NHE1 一抗(滴度为 1∶100),4 ℃过夜孵育。第 2 天用磷酸缓冲盐溶液洗 3 次,每次 5 min,与相应二

抗在37 ℃温箱中孵育1 h，3,3′-二氨基联苯胺显色，苏木精复染，脱水，透明，封片。使用连接QImaging Retiga CCD相机的显微镜观察切片中NHE1蛋白的表达并拍照（棕色部分为NHE1蛋白表达）。采用Image J软件的IHC Profiler[11]插件处理图片，计算NHE1蛋白表达量百分比，NHE1蛋白表达量百分比=NHE1蛋白表达阳性细胞的平均灰度值（染色强度）/阳性面积（染色面积）×100%。

1.4 统计学处理

应用SPSS 22.0软件进行统计学分析。计量资料以均数±标准差（$\bar{x} \pm s$）表示，多组间比较采用单因素方差分析，组间两两比较采用Tukey′s t检验，$P<0.05$为差异有统计学意义。

2 结果

2.1 6组小鼠血清中NO、MDA水平和SOD活性比较

与对照组相比，AS组小鼠血清中NO水平和SOD活性显著下降，MDA水平显著升高，差异有统计学意义（$P<0.05$）；VB_6组与对照组小鼠血清中NO、MDA水平和SOD活性比较差异无统计学意义（$P>0.05$）。与AS组相比，AS+VB_6组小鼠血清中NO水平和SOD活性显著上升，MDA水平显著下降，差异有统计学意义（$P<0.05$）；AS+LiCl组、AS+VB_6+LiCl组与AS组小鼠血清中NO、MDA水平和SOD活性比较差异无统计学意义（$P>0.05$）。与AS+VB_6组相比，AS+VB_6+LiCl组小鼠血清中NO水平和SOD活性显著下降，MDA水平显著升高，差异有统计学意义（$P<0.05$）。结果见表1。

表1 6组小鼠血清中NO、MDA水平及SOD活性比较

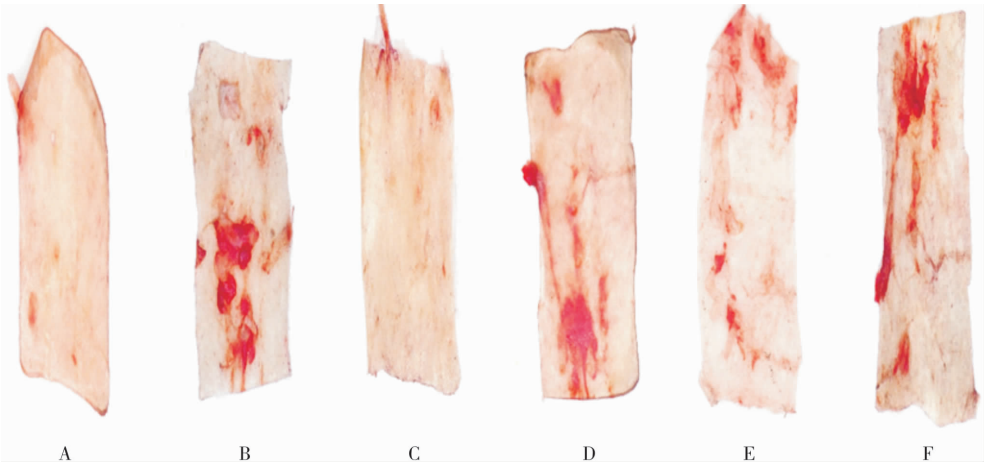
Tab.1 Comparison of serum NO, MDA levels and SOD activity of mice among the six groups （$\bar{x} \pm s$）

组别	n	MDA/(μmol·L^{-1})	NO/(μmol·L^{-1})	SOD/(kU·L^{-1})
对照组	6	3.24±0.44	38.71±2.43	148.90±6.48
AS组	6	13.44±0.98^a	10.06±0.76^a	41.53±3.83^a
VB_6组	6	4.15±0.38	39.76±2.62	145.20±5.50
AS+LiCl组	6	13.79±0.79	10.92±0.80	37.05±2.31
AS+VB_6组	6	8.15±0.72^b	22.76±1.32^b	111.70±3.36^b
AS+VB_6+LiCl组	6	14.39±0.68^c	10.59±0.60^c	42.89±2.48^c

注：与对照组比较^a$P<0.05$；与AS组比较^b$P<0.05$；与AS+VB_6组比较^c$P<0.05$。

2.2 6组小鼠AS斑块面积占血管总面积的百分比比较

6组小鼠AS斑块情况见图1。对照组、AS组、VB_6组、AS+LiCl组、AS+VB_6组和AS+VB_6+LiCl组小鼠AS斑块面积占血管总面积的百分比分别为（1.70±0.60）%、（37.55±4.25）%、（1.55±0.39）%、（37.71±5.32）%、（15.41±5.32）%、（35.45±4.63）%。AS组小鼠AS斑块面积占血管总面积的百分比显著高于对照组，差异有统计学意义（$P<0.05$）；VB_6组与对照组小鼠AS斑块面积占血管总面积的百分比比较差异无统计学意义（$P<0.05$）。AS+VB_6组小鼠斑块面积占血管总面积的百分比显著低于AS组，差异有统计学意义（$P<0.05$）；AS+LiCl组、AS+VB_6+LiCl组与AS组小鼠AS斑块面积占血管总面积的百分比比较差异无统计学意义（$P<0.05$）。AS+VB_6+LiCl组小鼠AS斑块面积占血管总面积的百分比显著高于AS+VB_6组，差异有统计学意义（$P<0.05$）。



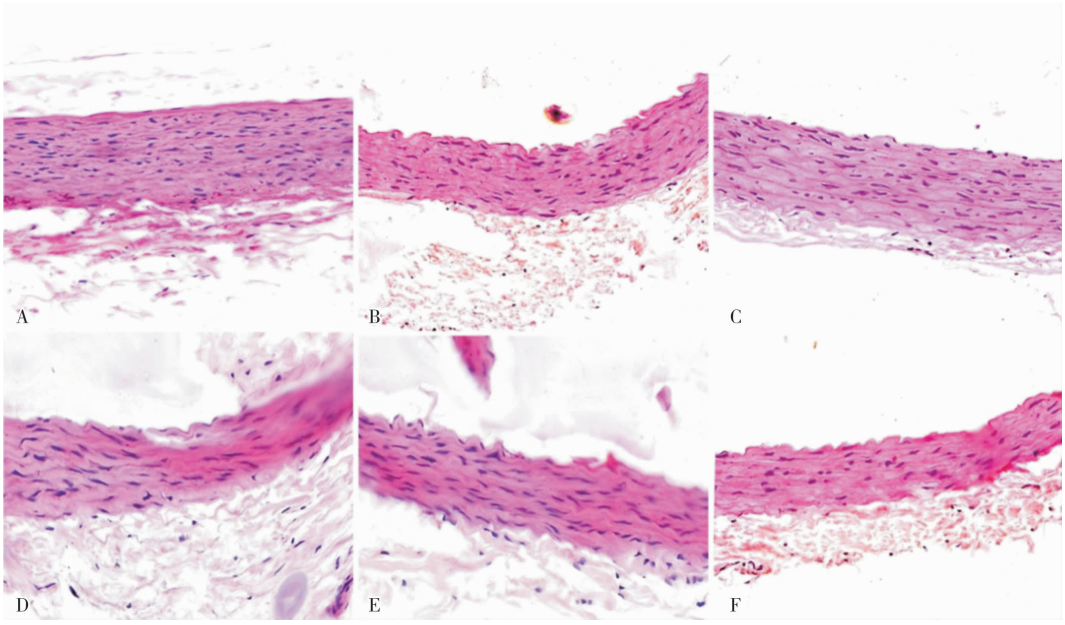
A：对照组；B：AS组；C：VB_6组；D：AS+LiCl组；E：AS+VB_6组；F：AS+VB_6+LiCL组。

图1 6组小鼠AS斑块（油红O染色，×20）

Fig.1 AS plaque of mice in the six groups (oil red O staining, ×20)

2.3 VB_6对6组小鼠胸主动脉组织形态的影响

对照组小鼠血管内皮光滑平整，排列整齐有序；AS组、AS+LiCl组和AS+VB_6+LiCl组小鼠的血管组织结构紊乱，血管内皮粗糙；VB_6组和AS+VB_6组小鼠的血管壁结构正常，血管内皮光滑，细胞排列有序；见图2。



A:对照组;B:AS 组;C:VB_6 组;D:AS + LiCl 组;E:AS + VB_6 组;F:AS + VB_6 + LiCl 组。

图 2　6 组小鼠胸主动脉组织形态学改变(HE 染色,×200)

Fig. 2　Pathological changes of the thoracic aorta of mice in the six groups(HE staining, ×200)

2.4　6 组小鼠胸主动脉对 Ach、SNP 的舒张反应比较

AS 组小鼠 Ach 诱导的胸主动脉舒张率显著低于对照组,差异有统计学意义($P<0.05$);VB_6 组与对照组小鼠 Ach 诱导的胸主动脉舒张率比较差异无统计学意义($P>0.05$)。AS + VB_6 组小鼠 Ach 诱导的胸主动脉舒张率显著高于 AS 组,差异有统计学意义($P<0.05$);AS + LiCl 组、AS + VB_6 + LiCl 组与 AS 组小鼠 Ach 诱导的胸主动脉舒张率比较差异无统计学意义($P>0.05$)。AS + VB_6 + LiCl 组小鼠 Ach 诱导的胸主动脉舒张率显著高于 AS + VB_6 组,差异有统计学意义($P<0.05$)。各组小鼠 SNP 诱导的胸主动脉舒张率比较差异均无统计学意义($P>0.05$)。结果见表 2。

2.5　6 组小鼠胸主动脉中 NHE1 蛋白表达量百分比比较

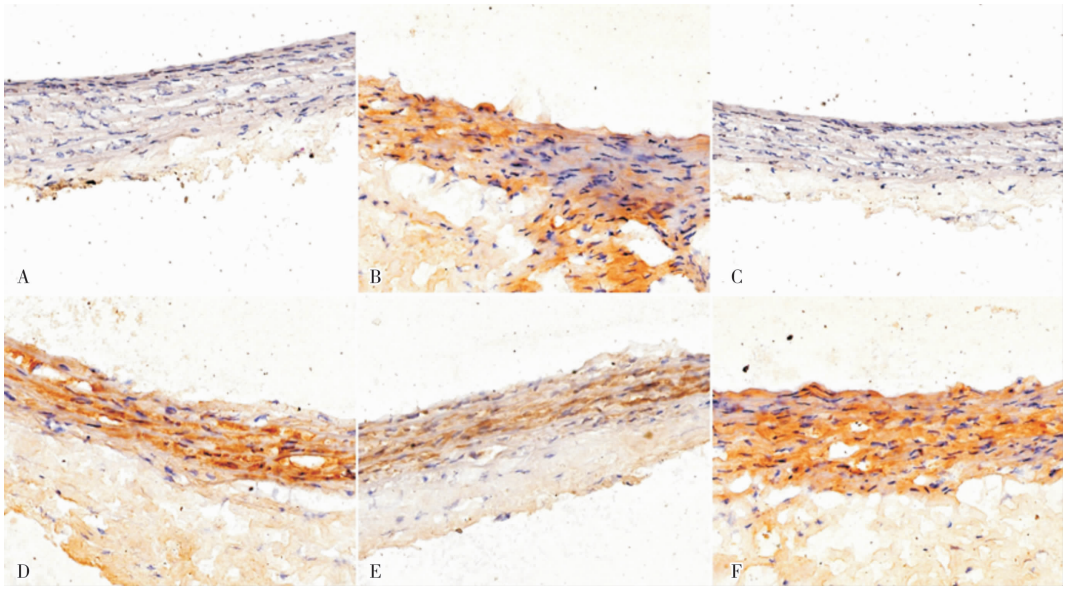
6 组小鼠胸主动脉中 NHE1 蛋白表达结果见图 3。对照组、AS 组、VB_6 组、AS + LiCl 组、AS + VB_6 组和 AS + VB_6 + LiCl 组小鼠胸主动脉中 NHE1 蛋白表达量百分比分别为(9.011 ± 2.244)%、(85.870 ± 2.706)%、(9.133 ± 1.824)%、(69.180 ± 4.701)%、(30.330 ± 3.312)%、(85.280 ± 2.284)%。AS 组小鼠胸主动脉中 NHE1 蛋白表达量百分比显著高于对照组,差异有统计学意义($P<0.05$);VB_6 组与对照组小鼠胸主动脉中 NHE1 蛋白表达量百分比比较差异无统计学意义($P>0.05$)。AS + VB_6 组小鼠胸主动脉中 NHE1 蛋白表达量百分比显著低于 AS 组,差异有统计学意义($P<0.05$);AS + LiCl 组、AS + VB_6 + LiCl 组与 AS 组小鼠胸主动脉中 NHE1 蛋白表达量百分比比较差异无统计学意义($P>0.05$)。AS + VB_6 + LiCl 组小鼠胸主动脉中 NHE1 蛋白表达量百分比显著高于 AS + VB_6 组,差异有统计学意义($P<0.05$)。

表 2　6 组小鼠胸主动脉舒张率比较

Tab. 2　Comparison of the vasodilatation rate of thoracic aorta of mice among the six groups ($\bar{x} \pm s$)

组别	n	血管舒张率/%	
		Ach	SNP
对照组	6	91.00 ± 5.83	93.00 ± 8.27
AS 组	6	43.17 ± 7.49[a]	93.00 ± 3.52
VB_6 组	6	89.33 ± 4.59	95.17 ± 2.04
AS + LiCl 组	6	46.00 ± 8.74	94.67 ± 4.18
AS + VB_6 组	6	70.33 ± 5.99[b]	95.83 ± 2.48
AS + VB_6 + LiCl 组	6	60.20 ± 5.46[c]	95.17 ± 2.53

注:与对照组比较[a] $P<0.05$;与 AS 组比较[b] $P<0.05$;与 AS + VB_6 组比较[c] $P<0.05$。



A:对照组;B:AS组;C:VB_6组;D:AS+LiCl组;E:AS+VB_6组;F:AS+VB_6+LiCl组。

图3 6组小鼠胸主动脉中NHE1蛋白表达(免疫组织化学染色,×200)

Fig. 3 Expression of NHE1 protein in thoracic aorta of mice in the six groups(immunohistochemistry staining, ×200)

3 讨论

AS是一种与脂质代谢障碍有关的全身性疾病,主要病变为动脉内膜中脂质沉积、内膜纤维化及粥样斑块形成等。NHE1通过使细胞内H^+和细胞外Na^+交换来维持细胞内pH值与Na^+浓度的动态平衡。NHE1活化可导致细胞内pH下降、Na^+浓度增加,引起细胞内Ca^{2+}超载进而导致钙蛋白酶活化[12-13],这一过程被认为是导致糖尿病微小血管并发症发生的关键因素[5]。有研究表明,血管钙蛋白酶活性的增加可导致内皮功能异常[14]。VB_6参与糖类和脂肪的代谢,在人体中起着极其重要的作用[15]。WANG等[16]研究显示,补充B族维生素可以提高大鼠血脂代谢酶的活性,从而调节血清中脂质水平,对AS有一定的预防作用。

本研究结果发现,与对照组相比,AS组小鼠的AS斑块明显,血管内皮损伤严重,Ach介导的血管内皮依赖性舒张反应显著降低,血清中NO水平和SOD活性显著下降,MDA水平显著升高;给予VB_6治疗后,以上各项指标均得到明显改善。上述结果说明,$ApoE^{-/-}$ AS小鼠补充VB_6可以显著缩小胸主动脉斑块面积,维持血管内皮细胞功能,保持细胞形态完整、排列有序。为了进一步探讨VB_6防治AS的机制,本研究检测了胸主动脉中NHE1的表达,结果显示,补充VB_6后可显著改善AS小鼠血管内皮功能障碍,降低胸主动脉中NHE1的表达水平。值得注意的是,本研究观察到LiCl可逆转VB_6对AS小鼠的改善作用。上述结果说明,VB_6可通过抑制NHE1的表达来改善AS小鼠的氧化应激,改善内皮舒张功能和血管内皮的损伤。

VB_6通过抑制NHE1而改善AS的具体机制有待进一步的实验来揭示。通过查阅大量相关文献推测,作为内皮细胞功能的重要调节因子,多种信号通路相关因子可能在这一过程中发挥作用,如人蛋白激酶Cβ、环前列腺素合酶、细胞外调节蛋白激酶1/2、磷酸化丝氨酸/苏氨酸激酶、蛋白激酶B、活化磷酸腺苷蛋白激酶、血管紧张素Ⅱ、内皮素、微RNA(microRNA,miRNA)等[17-22]。其中,miRNA由小的非编码RNA组成,与信使RNA的特定3′-非翻译区域结合,抑制信使RNA翻译或促进信使RNA降解。有研究通过RNA深度测序,在人脐静脉内皮细胞中鉴定出400多个miRNA,这些miRNA对于维持内皮细胞的稳态是必不可少的。同时,一些miRNA,如miR-130、miR-133a和miR-199a/b,在静态内皮细胞中不存在,但在血管平滑肌细胞和心肌细胞中表达较多[23];病理生理条件下,它们在内皮细胞中表达异常[24]。他汀类药物或其他药物可抑制这些miRNA,使糖尿病、高脂血症等患者的血管内皮功能正常化。因此,推断VB_6可能通过特异性miRNA逆转NHE1的表达来改善动脉粥样硬化,具体机制有待进一步研究来探讨。

4 结论

VB_6可通过抑制NHE1表达来改善$ApoE^{-/-}$ AS小鼠血管内皮功能障碍。由于血管内皮功能障碍是包括AS、糖尿病和高血压在内的多种心血管疾

病进展的一个普遍起始点[25-26],因此,这一发现可能在新药开发中具有重要的应用价值。而本研究为 AS 的防治提供了理论依据,并为进一步的研究奠定了基础。

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