

· 基础研究 ·

间歇性缺氧大鼠早期动脉粥样硬化中血管生成性T淋巴细胞的表达变化

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[摘要] 目的:探讨慢性间歇性缺氧(chronic intermittent hypoxia, CIH)暴露下,发生早期动脉粥样硬化血管病变时,大鼠外周血中血管生成性T淋巴细胞(angiogenic T lymphocyte, Tang)水平的变化。方法:将健康成年Sprague Dawley(SD)大鼠随机分为2组:正常饲养组(RA组)和慢性间歇性缺氧组(IH组),其中IH组接受间歇性缺氧暴露,每天8 h,共6周。间歇性缺氧模式设定为:每2 min 1个循环,40 s内向仓内充入氮气使氧气浓度从21%降到8%并稳定20 s,然后充入正常空气使氧气浓度在40 s内恢复到21%并维持20 s。造模完成后,使用流式细胞法检测Tang及内皮祖细胞(endothelial progenitor cell, EPC)水平;ELISA法检测高密度脂蛋白胆固醇(high-density lipoprotein cholesterol, HDL-C)、低密度脂蛋白胆固醇(low-density lipoprotein cholesterol, LDL-C)、总胆固醇(total cholesterol, TC)、甘油三酯(triglycerides, TG)及同型半胱氨酸(homocysteine, Hcy);HE染色观察胸主动脉病理改变并测量动脉内中膜厚度(intima-media thickness, IMT)。结果:流式结果显示,与RA组相比,IH组小鼠Tang水平显著升高[(22.975±1.866)% vs. (15.713±1.746)%, $P < 0.001$],EPC水平差异无统计学意义[(0.040±0.028)% vs. (0.028±0.012)%, $P > 0.05$];HE染色结果显示,IH组小鼠IMT比RA组显著增厚[(121.275±30.896)μm vs. (84.075±7.452)μm, $P < 0.05$];ELISA实验表明,与RA组比,IH组Hcy水平显著升高[(6.2±0.6)μmol/L vs. (5.5±0.6)μmol/L, $P < 0.05$],但HDL-C、LDL-C、TC、TG水平差异无统计学意义。结论:CIH大鼠血管发生了动脉粥样硬化早期病变,同时Tang细胞水平升高,提示Tang细胞水平与早期动脉粥样硬化的发生发展有关。

[关键词] 血管生成性T淋巴细胞;慢性间歇性缺氧;动脉粥样硬化**[中图分类号]** R543.5**[文献标志码]** A**[文章编号]** 1007-4368(2024)01-012-05**doi:** 10.7655/NYDXBNSN230636

Changes in angiogenic T lymphocytes under early atherosclerosis in rats exposed to chronic intermittent hypoxia

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[Abstract] **Objective:** To explore the changes in levels of angiogenic T lymphocyte (Tang) in the peripheral blood of rats exposed to chronic intermittent hypoxia (CIH) during early atherosclerotic vascular changes. **Methods:** Healthy adult Sprague Dawley (SD) rats were randomly divided into two groups: the normal feeding group (RA group) and the chronic intermittent hypoxia group (IH group), with the IH exposed to intermittent hypoxia for 8 h per day for a total of 6 weeks. The intermittent hypoxia pattern was set to repeat every 2 min, with 40 s of nitrogen gas to reduce the oxygen concentration from 21% to 8% and stabilize for 20 s, followed by normal air to restore the oxygen concentration to 21% for 40 s and maintain for 20 s. After modeling, levels of Tang and endothelial progenitor cells (EPC) were measured by flow cytometry. Levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), total cholesterol (TC), and homocysteine (Hcy) were measured by ELISA. pathological changes were observed in HE stained pathological sections of the thoracic aorta. **Results:** Flow cytometry results showed that compared to the RA group, the levels of Tang in rats of the IH group were significantly elevated [(22.975±1.866)% vs. (15.713±1.746)%, $P < 0.001$], and

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there was no statistical difference in EPC levels $[(0.040 \pm 0.028)\% \text{ vs. } (0.028 \pm 0.012)\%, P > 0.05]$; H E staining results showed that the IMT of rats thoracic aorta in the IH group was significantly increased compared to the RA group $[(121.275 \pm 30.896) \mu\text{m} \text{ vs. } (84.075 \pm 7.452) \mu\text{m}, P < 0.05]$; ELISA results showed that the Hcy level in the IH group was significantly higher compared to the RA group $[(6.2 \pm 0.6) \mu\text{mol/L} \text{ vs. } (5.5 \pm 0.6) \mu\text{mol/L}, P < 0.05]$, but there were no statistical differences in HDL-C, LDL-C, TC, and TG levels. **Conclusion:** Early atherosclerosis occurs in the blood vessels of CIH rats and the levels of Tang elevated, suggesting that the level of Tang is related to the occurrence and development of early atherosclerosis.

[Key words] angiogenic T lymphocyte; chronic intermittent hypoxia; atherosclerosis

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阻塞性睡眠呼吸暂停低通气综合征(obstructive sleep apnea hypopnea syndrome, OSAHS)是动脉粥样硬化性疾病的独立危险因素,在动脉粥样硬化性心脏病患者中的发生率高达40%~80%^[1]。慢性间歇性缺氧(chronic intermittent hypoxia, CIH)是 OSAHS 重要的病理生理学特征^[2],引起血液循环中氧合水平出现周期性变化,引发脂质代谢紊乱、氧化应激和免疫炎症改变,并通过其相互作用,最终导致动脉粥样硬化的发生。

动脉粥样硬化的起始改变是内皮损伤和功能障碍,随后受损组织分泌一系列促血管生成因子趋化成血管细胞至损伤部位修复内皮损伤以及形成新生血管^[3]。血管生成性T淋巴细胞(angiogenic T lymphocyte, Tang)是T淋巴细胞亚群之一,也是一类成血管细胞,其主要细胞表面标志物包括CD3、CD31、CXCR4,通常被标记为CD3⁺CD31⁺T淋巴细胞。研究表明,Tang可通过分泌促血管生成因子白细胞介素-8(interleukin-8, IL-8)、基质金属蛋白酶-9(matrix metalloproteinases-9, MMP-9)和粒细胞集落刺激因子(granulocyte-colony stimulating factor, G-CSF)促进内皮祖细胞(endothelial progenitor cell, EPC)增殖、分化,参与损伤血管内皮修复或新生血管形成^[4]。Weil等^[5]研究发现,心脑血管疾病患者外周血中Tang水平降低。迄今尚未有CIH暴露下Tang对早期动脉粥样硬化发生发展影响的相关性研究。

本研究拟通过建立CIH大鼠动脉内皮损伤模型,观察CIH暴露下大鼠血管病变及外周血Tang表达水平的变化,为进一步寻求OSAHS相关性动脉粥样硬化性疾病的干预及治疗策略提供线索。

1 材料和方法

1.1 材料

16只成年雄性健康Sprague Dawley(SD)大鼠

(体重210~230 g)购买于常州卡文斯实验动物有限公司。实验于南京医科大学附属无锡人民医院实验动物中心进行。实验过程严格按照《实验动物的饲养和使用指南(第八版)》要求执行。此实验获得南京医科大学附属无锡人民医院动物保护伦理委员会的批准(实验伦理号:220214501)。

FITC荧光Anti-CD3抗体、FITC荧光Anti-CD31抗体、FITC荧光Anti-CD34抗体(Santa Cruz公司,美国),CD133抗体(Abcam公司,美国),FITC标记山羊抗兔IgG(Biosharp公司,北京),高密度脂蛋白胆固醇(high-density lipoprotein cholesterol, HDL-C)、低密度脂蛋白胆固醇(low-density lipoprotein cholesterol, LDL-C)、甘油三酯(triglycerides, TG)、总胆固醇(total cholesterol, TC)、同型半胱氨酸(homocysteine, Hcy)酶联免疫试剂盒(上海酶联生物)。

1.2 方法

1.2.1 实验分组及CIH大鼠模型制备

16只SD大鼠标号称重后随机分为2组:正常饲养组(RA组)和间歇性缺氧组(IH组),每组8只。饲养条件为:每4只大鼠饲养在1个鼠笼中,大鼠可自由取食足够的灭菌饲料及干净饮用水,室温保持在20~24℃,室内保持相对安静,噪音小于50 dB,室内照明12 h调整1次,保持通风换气。因自然界中大鼠习性为昼伏夜出,本实验在大鼠白天睡眠时将IH组大鼠放入CIH仓(无锡市怀信生物公司)进行间歇性缺氧实验8 h,其余时间则放回正常动物房内,与RA组大鼠在相同环境下饲养。每日上午8点将IH组大鼠置于CIH仓中,在电脑控制仪上将循环模式设置为:每2 min 1个循环,40 s内氧气浓度降到8%之后稳定20 s,然后氧气浓度在40 s内恢复到21%之后维持20 s。将CIH仓内氧气浓度维持在8%时,经大鼠耳朵测量其血氧饱和度,能够降低至80%以下,可以模拟重度OSAHS患者夜间

呼吸暂停时的缺氧情况,且2 min循环1次的模式,达到了睡眠呼吸暂停低通气指数 ≥ 30 次的重度OSAHS诊断标准。IH组大鼠每日循环8 h后取出,置于正常空气环境下饲养,连续间歇性缺氧6周,共42 d。

1.2.2 大鼠胸主动脉HE染色

使用3%戊巴比妥钠(30 mg/kg)对大鼠进行腹腔注射麻醉,进行心脏穿刺取血,每只大鼠取约5 mL全血,置于4 °C冰箱内待下一步检测;开胸将大鼠胸主动脉剥离,截取胸主动脉,分离好的组织用预冷的PBS将表面冲洗干净,固定、脱水、透明、渗透、包埋、切片、脱蜡、复水、染色后用中性树脂封固。在光镜下观察大鼠胸主动脉的病理改变,并测量平均动脉内中膜厚度(intima-media thickness, IMT)。

1.2.3 HDL-C、LDL-C、TG、TC、Hcy水平检测

取大鼠全血3 000 r/min离心15 min,分离血清后,通过ELISA法检测HDL-C、LDL-C、TG及Hcy。

1.2.4 流式细胞术检测

取大鼠全血裂红后进行流式双标检测Tang及EPC占单个核细胞(mononuclear cell, MNC)比例,检测指标分别为 $CD3^+CD31^+$ 和 $CD34^+CD133^+$ 。调整细胞悬液浓度至 1×10^6 个/mL,取样本1 mL分别加入1 μ g未稀释CD3、CD31抗体置于4 °C孵育30 min,加入1 mL PBS上机检测Tang水平;另外,再取 1×10^6 个/mL的细胞样本1 mL加入1 μ g CD34抗体,4 °C

孵育30 min,然后加入1 μ g未稀释CD133抗体4 °C孵育1 h,因本实验所用CD133抗体非荧光抗体,故再加入1 μ g CD133相对应的二抗(FITC标记山羊抗兔IgG)4 °C孵育30 min,随后加入1 mL PBS上机检测。

1.3 统计学方法

本研究中计数资料用均数 \pm 标准差($\bar{x} \pm s$)表示,使用R语言统计分析与可视化软件(3.6.3版本),R包:stats(4.2.1版本)、ggplot2(3.3.3版本)进行数据统计与作图分析,使用Shapiro-Wilk检验进行正态性检验,独立样本 t 检验进行组间差异比较, $P < 0.05$ 为差异具有统计学意义。

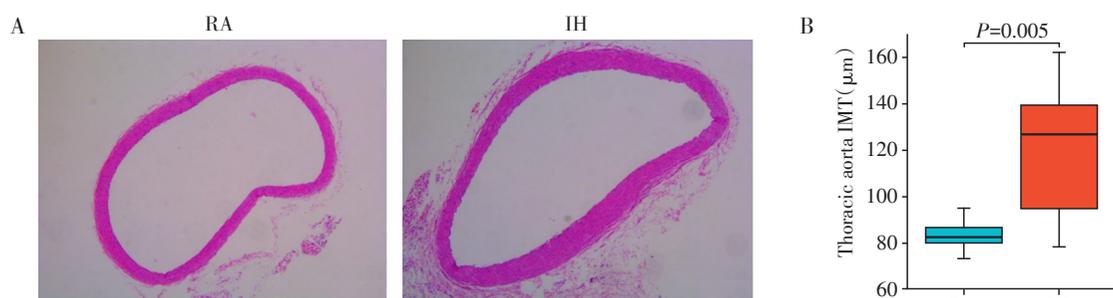
2 结果

2.1 CIH大鼠胸主动脉内中膜增厚

经过6周的间歇性缺氧暴露后,取大鼠胸主动脉切片做HE染色,IH组病理切片中未见动脉斑块及脂质空泡(图1A),胸主动脉内中膜厚度IH组[(121.275 \pm 30.896) μ m]对比RA组[(84.075 \pm 7.452) μ m]显著增厚($P < 0.01$,图1B)。

2.2 CIH大鼠Hcy、TC、TG、HDL-C、LDL-C水平改变

使用ELISA方法测定大鼠血清中Hcy、TC、TG、HDL-C、LDL-C水平。IH组比RA组Hcy水平显著升高($P < 0.05$,图2A)。TG、HDL-C、LDL-C、TC水平,IH组对比RA组差异均无统计学意义($P > 0.05$,图2B~E)。



A: HE staining for aorta in the two groups($\times 50$). B: Comparison of thoracic aortic intima-media thickness in the two groups($n=8$).

图1 间歇性缺氧暴露可导致大鼠胸主动脉内中膜增厚

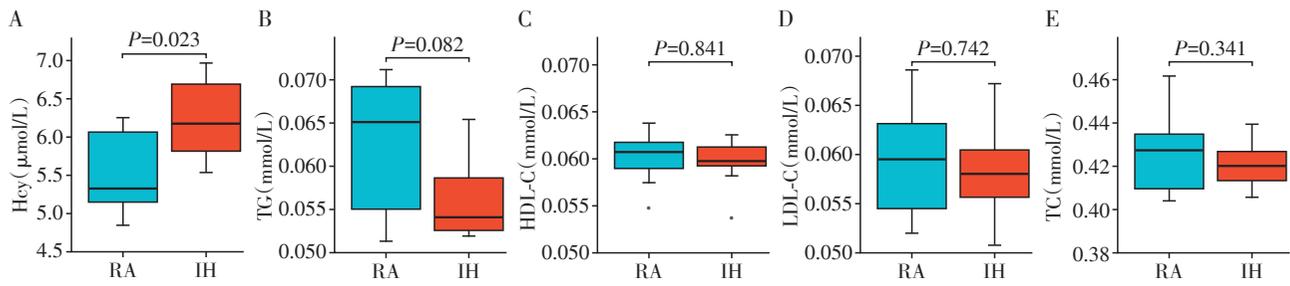
Figure 1 Intermittent hypoxia exposure caused a thickening of thoracic aorta IMT in rats

2.3 CIH大鼠Tang水平显著升高

与RA组相比,IH组Tang/MNC比值增高[(22.975 \pm 1.866)% vs. (15.713 \pm 1.746)%],差异具有统计学意义($P < 0.001$,图3A、B);IH组EPC/MNC的比值增高[(0.040 \pm 0.028)% vs. (0.028 \pm 0.012)%],但差异无统计学意义($P > 0.05$,图3C、D)。

3 讨论

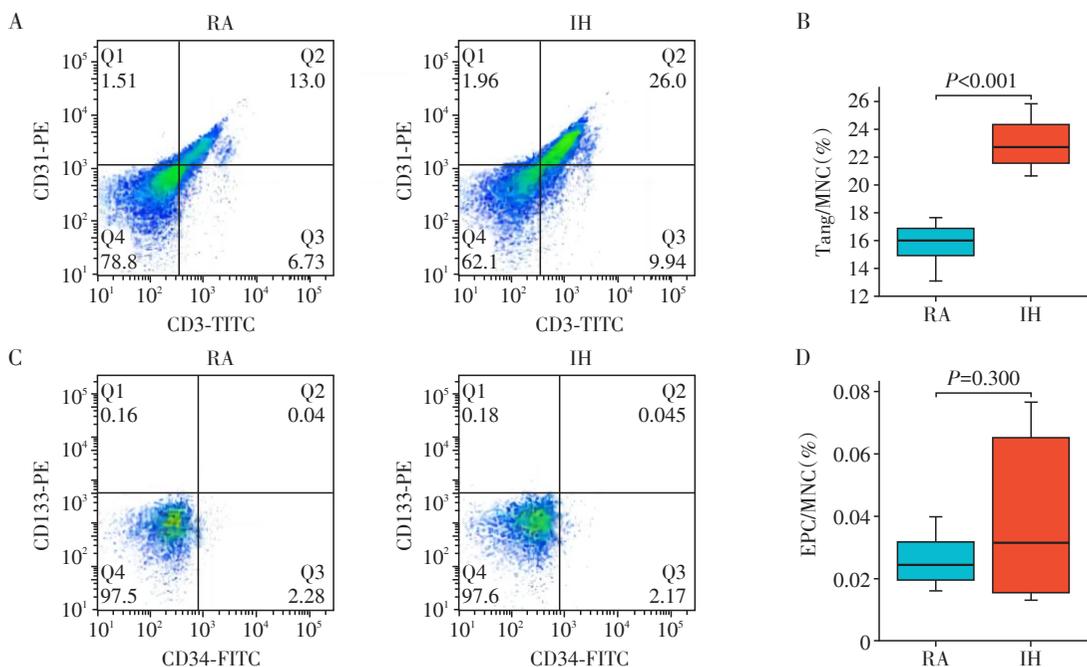
作为动脉粥样硬化的独立危险因素,长期CIH暴露会导致动脉粥样硬化斑块的产生^[6]。动脉粥样硬化病变通常是从动脉内膜开始,经过脂质和复合糖类沉积、内皮损伤及血小板聚集、血栓形成,进而



A: Comparison of Hcy expression levels in the two groups. B: Comparison of TG expression levels in the two groups. C: Comparison of HDL-C expression levels in the two groups. D: Comparison of LDL-C expression levels in the two groups. E: Comparison of TC expression levels in the two groups ($n=8$).

图2 RA组与IH组大鼠Hcy、TC、TG、HDL-C、LDL-C的水平

Figure 2 Levels of Hcy, TC, TG, HDL-C, and LDL-C in rats in the RA and IH groups



A, B: The proportion of Tang in MNC in each group was detected by flow cytometry (A) and bar graph (B). C, D: The proportion of EPC in MNC in each group was detected by flow cytometry (C) and bar graph (D) ($n=8$).

图3 RA组与IH组大鼠Tang及EPC水平

Figure 3 Levels of Tang and EPC in rats from the RA and IH groups

成纤维细胞增生及钙质沉着,并伴有动脉内中膜的重构和钙化,导致动脉壁增厚变硬、血管腔狭窄^[7]。Cuspidi等^[8]研究发现,动脉粥样硬化斑块和管腔狭窄反映的是动脉粥样硬化过程的更晚期阶段,而在动脉粥样硬化早期阶段,血管内皮损伤后,血管发生适应性重构导致的IMT增厚应是更重要标志。有研究证实,中重度OSAHS患者颈动脉内中膜厚度与缺氧程度成正比,CIH能够导致Hcy显著升高,造成血管内皮损伤,并导致血脂代谢异常^[9]。而本研究在进行动脉内皮损伤大鼠造模过程中发现,在IH组大鼠中并未观察到血清HDL-C、LDL-C、TG、TC显著

变化,但发现IH组相比RA组,血清Hcy水平显著升高且胸主动脉IMT显著增厚,而IH组大鼠胸主动脉病理切片血管壁完整、未见动脉斑块及脂质空泡,证实CIH暴露6周的大鼠动脉为应对血管内皮损伤发生了内膜适应性重构,处于动脉粥样硬化的早期阶段。

循环EPC水平是血管内皮损伤与修复平衡的衡量标准之一,在具有各种心血管疾病危险因素如高血压、肥胖、血脂异常、糖尿病等疾病的患者中会降低^[3]。本研究检测了造模后大鼠循环Tang及EPC水平,发现IH组大鼠Tang水平显著升高,IH组大鼠

EPC虽表达升高,但与RA组大鼠EPC水平比较无统计学差异。有文献报道,Tang可促进EPC增殖,维持循环中EPC的水平^[4]。由此推测,在大鼠动脉粥样硬化早期阶段,可能由于IH组大鼠Tang水平显著升高,保证了循环中EPC的水平,进而有效维持了血管损伤与修复的平衡。

此外,Tang表面表达CXCR4受体,CXCR4是稳态趋化因子基质细胞衍生因子-1 α (stromal cell derived factor-1,SDF-1 α)的特异性受体,Tang向外周血中的趋化反应受到SDF-1 α 的调节。研究表明,SDF-1 α 的上游调控因子是缺氧诱导因子-1(hypoxia inducible factor-1,HIF-1),在缺氧条件下HIF-1进入细胞核与靶基因的缺氧反应元件结合,上调SDF-1 α 的表达并释放入血^[10]。因此推测CIH暴露后,缺氧诱导HIF-1/SDF-1 α /CXCR4信号轴激活,细胞内HIF-1水平升高,上调SDF-1 α 表达并释放入外周血中,趋化更多的Tang进入循环,这可能是本研究观察到Tang水平升高的原因,是值得进一步研究的方向。

本研究尚存在不足,未能探讨Tang细胞水平改变对动脉粥样硬化的具体作用及其机制。

综上所述,本研究发现,在间歇性缺氧暴露下,大鼠循环Tang水平升高可能参与血管早期发生适应性重构改变过程,与早期动脉粥样硬化有关。本研究为OSAHS相关性动脉粥样硬化性疾病的干预及治疗策略提供线索。

[参考文献]

- [1] CHEN L W,ZOU S J,WANG J H. Association of obstructive sleep apnea syndrome (OSA/OSAHS) with coronary atherosclerosis risk: systematic review and meta-analysis [J]. *Comput Math Methods Med*, 2022, 2022: 8905736
- [2] PRABHAKAR N R, PENG Y J, NANDURI J. Adaptive

cardiorespiratory changes to chronic continuous and intermittent hypoxia [M]// *Respiratory Neurobiology-Physiology and Clinical Disorders, Part I*. Amsterdam: Elsevier, 2022: 103-123

- [3] ALTABAS V, BILOŠ L S K. The role of endothelial progenitor cells in atherosclerosis and impact of anti-lipemic treatments on endothelial repair [J]. *Int J Mol Sci*, 2022, 23(5): 2663
- [4] HUR J, YANG H M, YOON C H, et al. Identification of a novel role of T cells in postnatal vasculogenesis: characterization of endothelial progenitor cell colonies [J]. *Circulation*, 2007, 116(15): 1671-1682
- [5] WEIL B R, KUSHNER E J, DIEHL K J, et al. CD31+ T cells, endothelial function and cardiovascular risk [J]. *Heart Lung Circ*, 2011, 20(10): 659-662
- [6] REDLINE S, AZARBARZIN A, PEKER Y. Obstructive sleep apnoea heterogeneity and cardiovascular disease [J]. *Nat Rev Cardiol*, 2023, 20(8): 560-573
- [7] CHEN J, LIN S, ZENG Y M. An update on obstructive sleep apnea for atherosclerosis: mechanism, diagnosis, and treatment [J]. *Front Cardiovasc Med*, 2021, 8: 647071
- [8] CUSPIDI C, TADIC M, GHERBESI E, et al. Targeting subclinical organ damage in obstructive sleep apnea: a narrative review [J]. *J Hum Hypertens*, 2021, 35(1): 26-36
- [9] KIM J, MOHLER E R, KEENAN B T, et al. Carotid artery wall thickness in obese and nonobese adults with obstructive sleep apnea before and following positive airway pressure treatment [J]. *Sleep*, 2017, 40(9): zsx126
- [10] ZHANG Y, ZHANG Y Y, PAN Z W, et al. GDF11 promotes wound healing in diabetic mice via stimulating HIF-1 α -VEGF/SDF-1 α -mediated endothelial progenitor cell mobilization and neovascularization [J]. *Acta Pharmacol Sin*, 2023, 44(5): 999-1013

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