·论 著·

低压低氧诱导大鼠阻塞性睡眠呼吸暂停综合征后咽部肌肉结构和功能的改变

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[摘要] **目的**：观察低压低氧诱导大鼠阻塞性睡眠呼吸暂停综合征(obstructive sleep apnea syndrome，OSAS)后大鼠咽部肌肉结构和功能的变化，探讨咽腔重建以及OSAS形成可能的生物力学机制。**方法**：24只成年SD大鼠，雌雄各半，分为低压低氧诱导雄鼠(A)组、对照雄鼠(B)组、低压低氧诱导雌鼠(C)组、对照雌鼠(D)组，共4组(n=6)。低压低氧诱导组(A、C)大鼠每天6 h，连续22 d进入低压氧仓(压力53.9 kPa，氧浓度10%～11.2%)，对照组(B、D)不接受低压低氧诱导。低压低氧诱导完成后(实验第23天)处死全部大鼠，行咽肌环张开角、咽肌环内径、咽部组织病理检查。**结果**：A、B、C、D组大鼠咽肌环张开角分别为：(55±24)°、(15±8)°、(38±20)°、(35±15)°；内径分别为：(0.4±0.2)、(0.2±0.1)、(0.3±0.1)、(0.3±0.1) cm。A组咽肌环张开角、内径较B、C、D组明显增大(P<0.05)，C、D组间无明显区别。光镜下可见：A组咽肌肌纤维肥大、排列紊乱，横纹模糊不清，上皮下组织充血水肿、炎症细胞浸润；C组可见局部横纹不清；B、D组肌纤维排列整齐，上皮下组织无炎症。电镜下可见：A组肌原纤维结构紊乱、肌丝溶解，部分Z带呈锯齿状排列、有断裂消失，肌质网囊状扩张、空泡样变性，肌细胞核轻度固缩；C组可见局部肌丝溶解。**结论**：22 d间歇性低压低氧诱导可能通过改变咽肌结构和功能，重建咽腔，从而诱导大鼠OSAS形成。

[关键词] 低压低氧；睡眠呼吸暂停，阻塞性；咽肌；生物力学
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Structural and functional changes of pharyngeal muscle in rats with altitude hypoxia-induced obstructive sleep apnea syndrome

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[**ABSTRACT**] **Objective**: To observe the changes of structure and function of pharyngeal muscle in SD rats with altitude hypoxia induced obstructive sleep apnea syndrome (OSAS), in an attempt to investigate the remodeling of pharyngeal space and the possible biomechanical mechanism of OSAS. **Methods**: Twenty-four SD rats(12 male and 12 female) were randomly assigned to 4 groups with 6 rats in each group. Rats in group A and B were male and those in group C and D were female. Rats in group A and C were placed in altitude chamber and treated with altitude hypoxia 6 h per day for 22 days. The pressure in the chamber was 53.9 kPa with an oxygen concentration of 10.0%-11.2%. Rats in group B and D, serving as controls, received no treatment. All rats were sacrificed on the 23rd day. The open-angle and the inside diameter of rats' pharyngeal-ring and the pathology of pharyngeal tissues were examined in all rats. **Results**: The open-angles of rats' pharyngeal-ring of group A, B, C and D were (55±24)°, (15±8)°, (38±20)°, and (35±15)°, respectively; and the inside diameters of the pharyngeal-ring(cm) were 0.4±0.2, 0.2±0.1, 0.3±0.1, and 0.3±0.1, respectively. The open-angle and the inside diameter of rats' pharyngeal-ring in group A were significantly higher (P<0.05) than those of group B, C, and D, with no significant difference found between group C and D. Microscopic findings showed that, compared with group B, the muscle fibers of group A were fatter and were arranged disorderly, with unclear transverse striation, dropsical and congestive subcutaneous tissues infiltrated with inflammatory cells. Local unclear transverse striation of muscle fiber was seen in group C; the muscle fibers in group B and D were orderly arranged and no inflammatory cells were seen subcutaneously. Electron microscope showed disarranged muscle fibers with dissolved myofilament, disturbance and disappearance of the Z line in part of muscle fibers, expanded sarcoplasmic reticulum, and slightly condensed muscle cell nuclei. Local myofilament dissolving was found in group C. **Conclusion**: Twenty-two days' intermittent altitude hypoxia can induce OSAS in SD rats through changing the structure and function of pharyngeal muscle and remodeling the pharyngeal space.

[**KEY WORDS**] altitude hypoxia; sleep apnea, obstructive; pharyngeal muscles; biomechanics
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睡眠呼吸暂停综合征(sleep apnea syndrome，SAS)是一种严重危害人类生命健康的疾病，近二十多年来逐渐为人们所认识，可分为三型：阻塞性睡眠呼吸暂停(OSA)、中枢性睡眠呼吸暂停(CSA)和混

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合性睡眠呼吸暂停(MSA)。OSAS 是其中最常见的类型,约占全部睡眠疾病的 70%。流行病学调查显示,30～60 岁中年人群中该病的发病率男性达 4%,女性为 2%[1],未经治疗的重症患者 8 年生存率仅为(63±17)%[2]。

目前国外 OSAS 的研究很多,但因研究目的不同,各自模拟特点、实验对象(猪、狗、鼠)也各异[3～5]。以往的研究[5,6]中,大鼠 OSAS 形成采用低氧混合气方法诱导。这些方法采用了自然环境中不存在的低氧混合气体,使大鼠氧饱和度下降。本研究拟采用低压低氧处理法模拟 OSAS 患者自然病程的环境,诱导大鼠 OSAS 形成,探讨 OSAS 对咽部肌肉结构和功能的影响。

1　材料和方法

1.1　*实验动物及仪器*　24 只成年 SD 大鼠,雌雄各半,雄性体质量约(300 ± 20) g ,雌性体质量约(220±20) g。海军医学研究所航空医学研究室提供动物低压氧仓。

1.2　*实验方法*　动物适应性饲养 1 周后开始实验。动物自由进食摄水,温度保持在 24℃,自然光照时间和日夜变化。雄雌大鼠随机分为低压低氧处理雄鼠(A)组、对照雄鼠(B)组,低压低氧处理雌鼠(C)组、对照雌鼠(D)组共 4 组(n=6)。A、C 组大鼠每天置于全透光分格低压氧舱中, 每格放一大鼠(每格每天放置 10 g 钠石灰),以(15～20) m/s 的速率,模拟上升至海拔(5 000±50) m 高度(压力约为 53.9 kPa,氧浓度 10%～11.2%)后停留 6 h,而后按(10～15) m/s的速率,下降至海平面高度,依此法持续 22 d。对照组不进行低压低氧处理,正常饲养。观察大鼠生长情况及各项生理反应。

1.3　*检查项目和方法*

1.3.1　*咽肌环张开角和咽肌环内径*　第 23 天上午,大鼠称质量后以戊巴比妥钠(40 mg/kg)腹腔注射麻醉,从下颌处剪开口腔,迅速暴露软腭、咽,与颈椎及周围组织分离,置于室温下(25℃)krebs 液(NaCl 120.0 mmol/L,KCl 4.7 mmol/L, NaH_2PO_4 1.2 mmol/L, $MgSO_4$ 1.2 mmol/L, $NaHCO_3$ 20.0 mmol/L, Dextrose 10.0 mmol/L,$CaCl_2$ 0.1～6.5 mmol/L)中,迅速切为 3 mm 厚肌环,以眼科手术剪周向修剪并测定咽肌环内径,尔后于咽后壁中央将咽肌环剪开,肌环置于盛有室温 krebs 液玻璃皿中,使咽肌环处于零应力状态,按张开角定义测量计算得到咽肌环的张开角[7]。

1.3.2　*咽肌组织显微结构和超微结构*　处死大鼠后分离咽肌,标本分别置于 2.5%戊二醛磷酸缓冲液留待电镜检查,10%甲醛溶液中留待石蜡包埋切片 H-E 染色。

1.4　*统计学处理*　实验数据以 SARS 统计软件进行 t 检验。

2　结　果

2.1　*咽肌环张开角和咽肌环内径的变化*　经 22 d 低压低氧处理后 A 组较 B、C、D 组咽肌环张开角、内径有显著的增加($P<0.05$), C、D 组间无明显变化。见表 1。

表 1　大鼠咽肌力学特性

Tab 1　Mechanical characteristics of pharyngeal muscle in rats

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

Group	Open-angles (α/°)	Inside diameters (l/cm)
A	55±24	0.4±0.2
B	15±8*	0.2±0.1*
C	38±20*	0.3±0.1*
D	35±15*	0.3±0.1*

* $P<0.05$ *vs* group A

2.2　*咽肌组织显微结构和超微结构*　光镜下:A 组肌纤维型可辨,但纤维肥大,排列紊乱,横纹模糊不清,上皮下组织充血水肿、炎症细胞浸润(图 1A);B 组肌纤维排列整齐,横纹清晰,鳞状上皮排列规整(图 1B);C、D 组结构类似,肌型清晰、排列整齐,鳞状上皮下组织无明显炎症,但 C 组可见灶性横纹不清。电镜下:A 组咽肌肌原纤维结构紊乱,有肌丝溶解,部分 Z 带呈锯齿状排列,有断裂消失,肌质管囊状扩张,大小不一、髓鞘样变性、肌细胞核轻度固缩(图 2A);B 组咽肌肌纤维结构规整,明带及暗带清楚,H 带内 M 线清晰,明带内 Z 线明显,排列规则(图 2B); C、D 组肌纤维结构整齐,明暗带清楚,结构同 B 组类似,但 C 组局部可见肌丝溶解,较 A 组为轻。B、C、D 组未见明显核固缩。

3　讨　论

OSAS 这一疾病,由于发病率高,在我国正逐步引起人们的重视,由于人体研究的局限性,动物模型对该病的临床和基础研究有重要意义。本研究拟采用低压低氧处理诱导大鼠 OSAS 形成,进一步探讨 OSAS 对咽部肌肉结构和功能的影响。

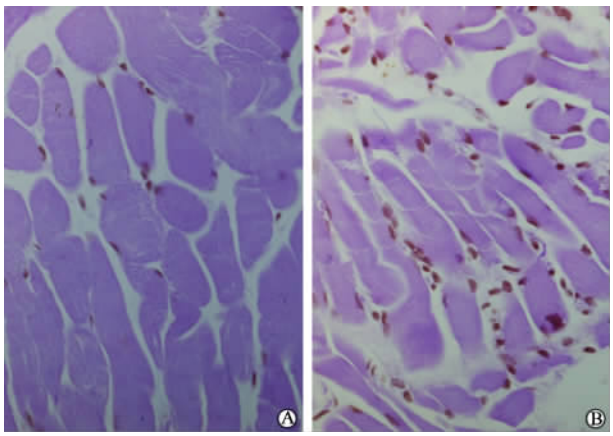

图 1　低压低氧处理 A 组(A)、对照 B 组(B)大鼠咽部肌肉光镜下结构

Fig 1　Pharyngeal muscle structure of altitude hypoxia group A(A) and control group B(B) under microscope(H-E, ×100)

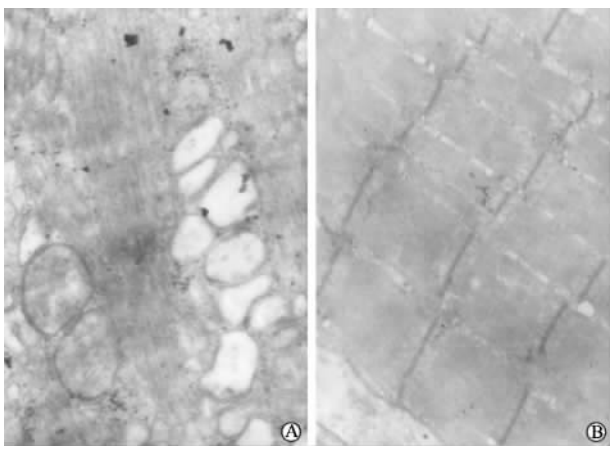

图 2　低压低氧处理 A 组(A)、对照 B 组(B)咽部肌肉电镜下结构

Fig 2　Pharyngeal muscle structure in altitude hypoxia group A(A) and control group B(B) under electron microscope(×6 000)

国外采用低氧诱导动物 OSAS 形成的报道已有很多，但均采用低氧混合气方法。McGuire 等[5]以 N_2、CO_2、O_2 混合气供大鼠呼吸，氧分压 6%～8%、二氧化碳分压 10%～14%。Fletcher 等[6]将大鼠置于盒内，以管道输入纯 N_2 12 s，使盒内的氧浓度降至 2%～3%并持续 3～6 s，之后输入压缩空气，使盒内氧气浓度逐渐恢复到 20.9%。这些方法均采用了自然环境中不存在的低氧混合气体，使大鼠 SpO_2 下降。航空生理学认为，低压低氧的生理学作用主要是由低氧引起的。本研究中低压氧仓内气体组成比例与空气没有区别，消除了气体组分改变可能造成的不良影响。因而较前述实验中使用的低氧混合气法，低压低氧法提供的气体，更类似于 OSAS 患者自然病程中的环境，减少了 N_2、CO_2 浓度升高可能对实验结果的影响。

Sica 等[8]采用间歇性呼吸低氧混合气法诱导大鼠 OSAS 形成，使大鼠产生了睡眠呼吸暂停，发生了类似 OSAS 的病理生理变化。本实验中观察到大鼠在入仓开始减压后，即出现呼吸加快、变深、腹式呼吸明显，即呼吸幅度加大、吸气压力升高，这些改变，同 OSAS 患者吸气负压增大的特点是相似的；实验中同时也观察到大鼠在仓内有耳郭、足爪色泽变浅、口唇轻度紫绀等现象，表明大鼠的血氧饱和度降低，提示我们对大鼠的低压低氧处理可模拟 OSAS 患者的低氧状态。

上气道是 OSAS 患者呼吸暂停发生的关键部位[9]。Fung[10]指出，血管零应力状态可用张开角来描述，他同时指出活组织的生长取决于应力。柳兆荣等[7]通过研究大鼠的气管张开角以探讨气管在不同力学状态下的重建。在本研究中，我们通过咽肌环张开角的变化来研究咽部的肌肉功能状态。根据生物力学原理，在一定的压力作用下，咽肌将产生适应性生长，使其结构与所处的力学环境相适应，不同部分咽肌的非均匀性生长促使其结构发生非均匀性分布，进而导致咽肌在零应力状态下张开角的变化。实验中，我们对大鼠的咽肌环张开角和内径测量后发现，低压低氧处理雄鼠组较对照雄鼠组、低压低氧处理雌鼠组张开角和内径明显变大($P<0.05$)。说明低压低氧处理雄鼠在 22 d 的低压低氧处理时血氧饱和度低，导致吸气负压持续增大，咽部肌肉发生适应性生长，维持咽腔固有形态能力下降，咽肌功能受低压低氧处理的影响而下降。喻筱红等[11]研究发现，OSAS 患者中咽张大肌肌纤维萎缩、减少，肌肉功能紊乱，电镜下可见肌原纤维结构紊乱、Z 带锯齿状。O'Halloran 等[12]研究发现，睡眠呼吸暂停能显著减少 OSAS 模型大鼠的胸骨舌骨肌的活动性和肌电活动，并引起膈肌的功能减退。本实验发现，低压低氧处理雄鼠咽部肌肉结构、功能发生了类似变化。提示大鼠经低压低氧处理后，发生了类似于 OSAS 患者的病理变化。大鼠的咽肌组织结构改变造成维持咽腔开开放能力的下降。模型大鼠咽部肌肉的结构、功能的改变，同 OSAS 患者咽肌维持咽腔开放能力下降的特点是类似的。

Young 等[1]发现，OSAS 患者中男性发病率是女性的两倍。Mohsenin[13]研究发现，女性气道结构优于男性，与男性症状相同的女性患者 OSA 较男性轻。在我们的研究中，相同时间低压氧处理后，低压

低氧处理雄鼠较其他各组咽肌力学特性、病理均有明显改变,对照雄鼠的咽部肌肉功能指标较对照雌鼠小,雌性动物在相同低氧处理后,OSAS 发病率较雄性低,结果与 OSAS 疾病的性别分布特点相类似。低压低氧处理雌鼠较对照雌鼠无明显改变,由于本实验样本数较少,对这一现象尚不能做出肯定性结论。

SD 大鼠是生活在正常大气环境的健康动物,低压低氧处理后的 SD 大鼠,咽肌环张开角、咽肌环内径、病理较对照组均发生了改变,这些咽部结构和功能的改变类似于 OSAS 患者,提示经低压低氧处理后的大鼠,可用于 OSAS 咽部肌肉功能的进一步研究。

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Augmented humoral and cellular immune responses of a hepatitis B DNA vaccine encoding HBsAg by protein boosting

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[**ABSTRACT**] Several reports have indicated that combinatorial regimens with DNA and protein vaccines can elicit both strong immune responses, to circumvent the limits of each vaccine. Surprisingly little was known on HBV vaccine. Here, we investigated the immunization effects of several regimens in BALB/c mice. The level of total antibody and isotypes of IgG were determined by ELISA. Cellular immune responses were assayed by measuring the production of cytokines and CTL activity after restimulation for 7 days *in vitro* with tumor cells CT26/S stably expressing HBsAg. The efficacy of immunoprotection against the challenge of transplanted CT26/S was also examined. The regimen involving twice priming pVAX(S) encoding HBsAg and once recombinant HBsAg protein (rHBsAg) boosting, induced strong and homogenous antibody responses. This regimen induced significant stronger responses of interleukin-12 and gamma interferon (IFN-gamma) in splenocytes, and elicited stronger $CD8^+$ CTL responses and greater immunopretectional efficacy than those elicited by immunization with rHBsAg or pVAX(S) alone. Our regimen may thus provide a strategy for developing an improved immunization against HBV and many other pathogens.

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