·基础研究·

别嘌醇与非布司他对家兔快速心房起搏房颤模型心房电重构的影响

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【摘 要】 目的 探讨黄嘌呤氧化酶(XO)抑制剂(别嘌醇与非布司他)对家兔快速心房起搏(RAP)房颤模型心房电重构的影响。**方法** 24只健康雄性新西兰白兔,随机分为4组,假手术组(S组)、快速心房起搏组(P组)、快速心房起搏-别嘌醇干预组(ALL组)及快速心房起搏-非布司他干预组(FP组)。使用酶联免疫吸附测定(ELISA)法检测血清中超敏C反应蛋白(hs-CRP)、生长分化因子-15(GDF-15)、半乳糖凝集素-3的活性及左心房组织匀浆中超氧化物歧化酶(SOD)、XO、黄嘌呤脱氢酶(XDH)的活性。在起搏之前及4周的RAP之后分别进行电生理检查,测定基础起搏周长为200 ms时的心房有效不应期(AERP 200)和房颤诱发率。实时荧光定量聚合酶链式反应及蛋白质印记法检测钙离子电压门控通道亚基α 1 C(Cav1.2)、钾离子电压门控通道亚族D3(Kv4.3)的信使RNA(mRNA)和蛋白表达。采用SPSS 26.0统计软件进行数据分析。根据数据类型分别采用单因素方差分析或χ^2检验进行组间比较。**结果** 与S组相比,P组SOD活性明显降低,XO、XDH、hs-CRP、GDF-15及半乳糖凝集素-3水平显著升高,差异均有统计学意义($P<0.01$)。应用XO抑制剂后,SOD活性回升明显,XO、hs-CRP、GDF-15及半乳糖凝集素-3活性均显著降低,差异均有统计学意义($P<0.05$)。FP组SOD显著高于ALL组,XDH、hs-CRP、GDF-15及半乳糖凝集素-3活性显著低于ALL组,差异均有统计学意义($P<0.05$);但FP组与ALL组XO水平比较,差异无统计学意义($P>0.05$)。此外,FP组XDH水平明显低于P组,差异有统计学意义($P<0.01$),但ALL组XDH水平与P组比较,差异无统计学意义($P>0.05$)。RAP后P组的AERP 200明显低于S组($P<0.01$),然而FP组的AERP 200显著高于P组和ALL组($P<0.05$)。除S组外,P组、ALL组和FP组在RAP后的房颤诱发率均显著高于起搏之前基线状态下的房颤诱发率($P<0.05$);应用XO抑制剂干预后,房颤诱发率有一定程度的降低,在FP组尤为明显。RAP可导致左心房组织中Cav1.2和Kv4.3mRNA及蛋白表达水平下调,但是应用XO抑制剂干预可部分抑制这些改变,在FP组尤为明显。**结论** XO抑制剂(尤其是非布司他)可能通过减轻炎症反应和氧化应激损伤,抑制左心房组织中Cav1.2、Kv4.3表达水平的下调,进而延长AERP,降低房颤诱发率,改善心房电重构。

【关键词】 心房颤动;心房电重构;黄嘌呤氧化酶;非布司他

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Effects of allopurinol and febuxostat on atrial electrical remodeling in a rabbit model of atrial fibrillation induced by rapid atrial pacing

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【Abstract】 Objective The purpose of this study was to investigate the effects of xanthine oxidase (XO) inhibitors (allopurinol and febuxostat) on atrial electrical remodeling in a rabbit model of atrial fibrillation (AF) induced by rapid atrial pacing (RAP). **Methods** Twenty-four healthy male New Zealand white rabbits were randomly divided into four groups: sham-operated group (S group), RAP group (P group), RAP-allopurinol group (ALL group), and RAP-febuxostat group (FP group). Enzyme-linked immunosorbent assay (ELISA) kit was used to detect the activity of high-sensitivity C-reactive protein (hs-CRP), growth differentiation factor-15 (GDF-15) and galectin-3, and the activity of superoxide dismutase (SOD) and the levels of XO and xanthine dehydrogenase (XDH) in left atrial tissues. Before pacing and four weeks after RAP, electrophysiological examinations were performed to evaluate the atrial effective refractory period at 200 ms (AERP 200) and the inducibility of AF. Real-time fluorescence quantitative polymerase chain reaction (PCR) and Western blotting were used to detect the messenger RNA (mRNA) and protein expressions of calcium voltage-gated channel subunit α1C (Cav1.2) and potassium voltage-gated channel subfamily D3 (Kv4.3). SPSS statistics 26.0 was used for data analysis. According to different data type, one-way analysis of variance or χ^2 test was used for data comparison between groups. **Results** Compared with S group, the SOD activity was significantly decreased, and the levels of XO, XDH, hs-CRP, GDF-15 and galectin-3 were increased in

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P group, and the differences were statistically significant ($P<0.01$). After using XO inhibitor, the activity of SOD recovered significantly, and the activities of XO, hs-CRP, GDF-15 and galectin-3 were significantly decreased, and the differences were statistically significant ($P<0.05$). The activity of SOD in FP group was significantly higher than in ALL group, whereas the activities of XDH, hs-CRP, GDF-15 and galectin-3 in FP group were obviously lower compared with ALL group ($P<0.05$). However, there was no significant difference in the level of XO between FP group and ALL group ($P>0.05$). Additionally, the activity of XDH in FP group was significantly lower than in P group, and the differences were statistically significant ($P<0.01$). Nevertheless, there was no significant difference in the level of XDH between ALL group and P group ($P>0.05$). After RAP, AERP 200 was significantly lower in P group than S group ($P<0.01$), and the AERP 200 of FP group was significantly higher than that of P group and ALL group ($P<0.05$). Except for S group, AF inducibility of P group, ALL group and FP group was significantly higher than the baseline before pacing ($P<0.05$). After the intervention of XO inhibitors, the AF inducibility was reduced to a certain extent, especially in FP group. RAP could reduce down-regulation of Cav1.2 and Kv4.3 mRNA and protein expression levels in left atrial tissue, and the intervention of XO inhibitors could partially inhibit these changes, which was particularly evident in FP group. **Conclusion** XO inhibitors (especially febuxostat) could inhibit the down-regulation of Cav1.2 and Kv4.3 expression levels in left atrial by reducing inflammation and oxidative stress damage, thereby prolonging AERP, reducing the inducibility of AF, and mitigating atrial electrical remodeling.

【Key words】 atrial fibrillation; atrial electrical remodeling; xanthine oxidase; febuxostat

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心房颤动(简称房颤)是临床实践中最常见的持续性心律失常。鉴于目前治疗方案如抗心律失常药物、抗凝药物、电复律、导管消融、外科消融、左心耳封堵及其他疗法的局限性[1],并针对房颤发生发展过程中的病理生理学机制,寻求一种能有效延缓房颤进展及导管消融术后房颤复发的新型房颤上游治疗药物尤为重要。近年来,越来越多的证据表明,导致房颤发生和维持的主要机制是心房电重构和结构重构[2],其中,心房电重构在房颤的发生和维持中起着重要的作用。炎症和氧化应激参与房颤发生发展的过程[3],黄嘌呤氧化酶(xanthine oxidase, XO)是心房组织中活性氧(reactive oxygen species, ROS)的主要来源之一,XO 在多种心血管疾病的氧化还原信号转导中发挥着关键作用[4]。别嘌醇及非布司他均为 XO 抑制剂,目前关于 XO 抑制剂参与心房电重构的研究尚少。本研究通过建立家兔快速心房起搏慢性房颤模型,评价 XO 抑制剂对家兔快速心房起搏房颤模型中心房电重构的影响,探讨其产生心脏保护效应的潜在可能机制。

1 材料与方法

1.1 实验试剂及仪器

注射用青霉素钠(80 万 U/支,华北制药股份有限公司,国药准字 H13020431);别嘌醇片(100 mg/片,上海信谊万象药业股份有限公司,国药准字 H31020334);非布司他片(40 mg/片,江苏万邦生化医药集团有限责任公司,国药准字 H20130058);钙离子电压门控通道亚基 α 1 C(calcium voltage-gated channel subunit α1C,Cav1.2)一抗、钾离子电压门控通道亚族 D3(potassium voltage gated channel subfamily D3,Kv4.3)一抗(Abcam);甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase,GAPDH)一抗(索莱宝);酶联免疫吸附测定(enzyme-linked immunosorbent assay,ELISA)试剂盒(易科拜德科技有限公司);锦江多导电生理仪(四川锦江电子有限公司);心脏电生理刺激仪(苏州市东方电子仪器厂);房颤模型起搏器(乐普上海医疗器械有限公司)。

1.2 实验动物及分组

24 只健康新西兰白兔,体质量 3.0~3.5 kg,雄性,清洁级,由中国人民解放军总医院实验动物中心提供(合格证号:1103331911000262),随机分为 4 组,每组 6 只,分别为假手术组(S 组),快速心房起搏组(P 组),快速心房起搏-别嘌醇干预组[ALL 组,50 mg/(kg·d),i.g.]及快速心房起搏-非布司他干预组[FP 组,10 mg/(kg·d),i.g.]。

1.3 动物模型的建立

家兔通过耳缘静脉注射 3% 的戊巴比妥钠(30 mg/kg)进行麻醉。将房颤模型起搏器环形电极缝合固定于左心耳游离壁起搏最佳的部位,手术完成后,给予家兔每日肌肉注射青霉素钠 80 万 U,2 次/d,共注射 7 d,同时家兔正常喂养,使其恢复 1 周。

ALL 组的家兔在起搏器植入术后按照 50 mg/(kg·d)的剂量灌胃别嘌醇,FP 组的家兔按照 10 mg/(kg·d)的剂量灌胃非布司他,共灌胃 5 周。S 组和 P 组的家兔接受等量的口服安慰剂。P 组、ALL 组及 FP 组的家兔在术后恢复 1 周后,打开起搏开关,按照 AOO 模式,输出电压 2.7 V,脉宽 0.37 ms,以 600 次/min 的频率进行快速心房起搏 4 周。S 组的家兔接受起搏器植入手术,术后不打开起搏器的起搏开关。

1.4 电生理研究

在家兔快速心房起搏之前及4周的快速心房起搏之后分别进行电生理检查。以2 ms脉宽,2倍于起搏阈值的电压,在200 ms的基础周期长度上测量心房有效不应期(atrial effective refractory period at 200 ms, AERP 200)。8个基础刺激(S1)之后紧跟着一个过早的额外刺激(S2)。S1-S2间隔以5 ms的步长减少,直到S2不能夺获心房产生心房反应,然后增加10 ms,S1-S2间隔以2 ms的步长减少,直到提前出现的S2不能夺获心房。未能夺获心房产生心房反应的最长S1-S2被定义为AERP 200。采用三次重复试验所测出的AERP 200的平均值进行数据分析。应用心脏电生理刺激仪进行房颤诱发率的测量,以3倍于起搏阈值的电压,60 ms的周长,1 000次/min的高频快速刺激,每次持续10 s,每只家兔重复10次,记录房颤诱发率。房颤定义为持续时间超过1 000 ms的快速、不规则的心房节律伴不规则的房室传导比例。房颤诱发率=各组动物成功诱发出房颤的次数/各组动物进行高频刺激的总次数×100%。

1.5 样本的制备

家兔安乐死后经颈静脉穿刺取血,血液离心取上清液。将左心房组织切成约100 mg小块,放入液氮中冻存,以用于后续检测。

1.6 ELISA检测

按照试剂盒操作说明书,检测左心房组织中氧化应激指标:超氧化物歧化酶(superoxide dismutase, SOD)、XO及黄嘌呤脱氢酶(xanthine dehydrogenase, XDH)的活性;血清中炎症相关指标:超敏C反应蛋白(high-sensitivity C-reactive protein, hs-CRP)、生长分化因子-15(growth differentiation factor-15, GDF-15);纤维化相关指标:半乳糖凝集素-3的活性。

1.7 实时荧光定量聚合酶链式反应

提取总RNA,进行逆转录成互补DNA,对各样本进行实时荧光定量聚合酶链式反应(quantitative real time polymerase chain reaction, qRT-PCR)检测。引物系列见表1。以GAPDH作为内参对照,S组心房肌组织为样本对照,根据所得的循环阈值(cycle threshold, Ct)值,计算$2^{-\triangle\triangle Ct}$值,进而比较Cav1.2和Kv4.3的信使RNA(message RNA, mRNA)水平在各组之间的差异。

1.8 蛋白质印记法

使用蛋白裂解液提取左心房组织总蛋白。应用二喹啉甲酸法测定蛋白浓度,通过十二烷基硫酸钠聚丙烯酰胺凝胶电泳检测相关蛋白的表达水平。一抗稀释倍数如下:Cav1.2(1∶500)、Kv4.3(1∶500)、GAPDH(1∶2500)。Image Pro-Plus软件用于检测与分析目的条带的净光密度,之后比较两组目的条带的净光密度的比值,内参为GAPDH。

表1 目的基因PCR引物序列

Table 1 Sequences of primers used for quantitative real-time PCR

Gene	Primer sequence
Cav1.2 (F)	5′-GCTGTGTCTGCTGCCTCTGAAG-3′
Cav1.2(R)	5′-TCGGTGTCGCCTTGGTTGTTG-3′
Kv4.3 (F)	5′- ATCATTGGCGACTGCTGCTACG-3′
Kv4.3 (R)	5′-CGGCATGGACTCCTGGTTGTTC-3′
GAPDH (F)	5′-TCTACCTTCGATGCTGGGGC-3′
GAPDH (R)	5′-TCTCGTCCTCCTCTGGTGCT-3′

Cav1.2: calcium voltage-gated channel subunit α1C; Kv4.3: potassium voltage-gated channel subfamily D3; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; F: forward; R: reverse.

1.9 统计学处理

采用SPSS 26.0统计软件进行数据分析。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,组内两两比较采用Tukey检验。计数资料以例数(百分率)表示,组间比较采用χ^2检验。使用GraphPad Prism 7.0制作统计图。$P<0.05$为差异有统计学意义。

2 结 果

2.1 别嘌醇与非布司他对氧化应激指标、炎症相关指标和纤维化相关指标的影响

与S组相比,P组SOD活性明显降低,XO、XDH、hs-CRP、GDF-15及半乳糖凝集素-3水平显著升高,差异均有统计学意义($P<0.01$)。应用XO抑制剂后,SOD活性回升明显,XO、hs-CRP、GDF-15及半乳糖凝集素-3活性均降低显著,差异均有统计学意义($P<0.05$)。FP组SOD显著高于ALL组,XDH、hs-CRP、GDF-15及半乳糖凝集素-3活性显著低于ALL组,差异均有统计学意义($P<0.05$);但FP组与ALL组XO水平比较,差异无统计学意义($P>0.05$)。此外,FP组XDH水平明显低于P组,差异有统计学意义($P<0.01$),但ALL组XDH水平与P组比较,差异无统计学意义($P>0.05$;表2)。

2.2 别嘌醇与非布司他对AERP 200及房颤诱发率的影响

电生理检查过程中家兔的心电图和心腔内电图见图1。正常状态下家兔的肢体导联心电图和心腔内电图记录的心房电信号见图1A及图1B。以600次/min进行快速心房起搏时记录的肢体导联心电图见图1C。从图1D及图1E中可以看出,进行心腔内电生理检查,S1-S2程序性刺激,测量不能夺获心房产生心房反应的最长S1-S2间期,即为AERP 200(图中为65 ms)。图1F为典型的房颤诱发过程的流程图:快速心房起搏(1000次/min)维持10 s,导致P波消失,出现不规则的RR间期,成功诱发心房颤动。

表2 别嘌呤醇与非布司他对SOD，XO，XDH，hs-CRP，GDF-15及半乳糖凝集素-3水平的影响

Table 2 Effects of allopurinol and febuxostat on levels of SOD, XO, XDH, hs-CRP, GDF-15 and galectin-3

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

Item	S group	P group	ALL group	FP group
SOD(U/L)	186.16±10.13	131.49±9.07**	149.52±5.40**##	168.33±6.91**##△△
XO(U/L)	31.83±2.90	46.00±5.28**	40.43±2.38**#	37.23±2.22*##
XDH(U/L)	17.66±3.20	31.08±2.18**	27.38±2.25**	22.70±2.88*##△
hs-CRP(μg/ml)	4.38±0.93	8.72±0.87**	7.25±0.41**##	6.34±0.29**##△
GDF-15(ng/L)	45.29±4.06	80.91±4.50**	69.93±5.52**##	59.30±5.02**##△△
Galectin-3(ng/L)	4 464.94±193.54	8 758.40±429.72**	7 140.70±262.58**##	6 050.48±516.72**##△△

SOD: superoxide dismutase; XO: xanthine oxidase; XDH: xanthine dehydrogenase; hs-CRP: high-sensitivity C-reactive protein; GDF-15: growth differentiationfactor-15; RAP: rapid atrial pacing. S group: sham-operated group; P group: RAP group; ALL group: RAP with 50 mg/(kg·d) allopurinol group; FP group: RAP with 10 mg/(kg·d) febuxostat group. Compared with S group, *$P<0.05$, **$P<0.01$; compared with P group, #$P<0.05$, ##$P<0.01$; compared with ALL group, △$P<0.05$, △△$P<0.01$.

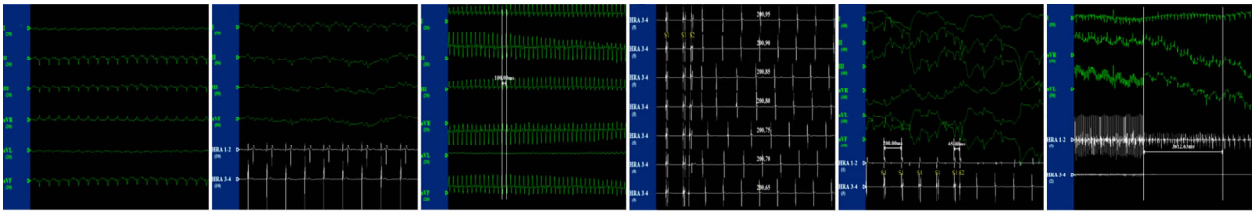


图1 电生理检查过程中家兔心电图

Figure 1 ECG recordings of rabbits during electrophysiological studies

A: ECG of limb leads under normal state; B: ECG of limb leads and potential of left atrium under normal state; C: ECG of limb leads during rapid atrial pacing at 600 beats per minute; D: intracardiac electrophysiological examination and S1-S2 programmed stimulation; E: AERP measurement; F: representative atrial fibrillation episode induced by high-frequency atrial burst pacing at 1000 beats per minute. ECG: electrocardiogram; AERP: atrial effective refractory period. S2 (stimulate interval=65 ms) was unable to induce a propagated atrial response.

在快速心房起搏之前的基线状态下，各组家兔的AERP 200之间的比较无显著差异($P>0.05$；图2A)。快速心房起搏4周后各组家兔心房不应期的情况见图2B。由图2B中可以看出，快速心房起搏4周后，P组的AERP 200明显低于S组[(73.53±5.06)和(95.25±5.01) ms]，差异有统计学意义($P<0.01$)。然而，应用XO抑制剂处理后，FP组的AERP 200显著高于P组，并且FP组的AERP 200显著高于ALL组，差异均有统计学意义($P<0.01$或$P<0.05$)。

基线状态及快速心房起搏4周后各组房颤诱发率情况见图2C。由图2C中可以看出，假手术对家兔的房颤诱发率无显著影响，差异无统计学意义($P>0.05$)。除S组以外，P组、ALL组和FP组快速心房起搏4周后的房颤诱发率均显著高于起搏之前基线状态下的房颤诱发率(16.7%和10.0%，$P>0.05$；58.3%和8.3%，46.7%和11.8%，35.0%和15.0%，$P<0.05$)。然而，与P组相比，快速心房起搏4周后的房颤诱发率在应用XO抑制剂干预的组中有一定程度的降低，FP组尤为明显。结果显示，快速心房起搏可导致AERP 200明显缩短，房颤的诱发率明显增加，应用XO抑制剂干预可部分抑制这些改变，并且在非布司他干预组尤为明显。

2.3 XO抑制剂对心房离子通道基因的影响

本研究检测了左心房组织中Cav1.2和Kv4.3的mRNA及蛋白表达水平(图3)。与S组相比，快速心房起搏导致各组Cav1.2 mRNA及蛋白表达水平显著降低，且差异有统计学意义($P<0.05$)。然而，与P组相比，XO抑制剂的处理显著增加了Cav1.2 mRNA及蛋白表达水平，尤其是在FP组中，并且FP组的Cav1.2 mRNA及蛋白表达水平显著高于ALL组，差异有统计学意义($P<0.01$)。各组Kv4.3 mRNA及蛋白表达水平的变化趋势与Cav1.2的变化趋势相同。本研究结果显示，快速心房起搏影响了左心房组织中离子通道基因的活性，导致Cav1.2和Kv4.3mRNA水平及蛋白表达水平下调，但是这些改变可以被XO抑制剂(尤其是非布司他)的干预所逆转。

3 讨 论

本研究对家兔进行4周的快速左心房起搏，成功建立了家兔慢性房颤模型并诱发出了氧化应激损伤、炎症反应和心房电重构。本研究证实XO抑制剂(尤其是非布司他)可能通过减轻炎症反应和氧化应激损伤，上调左心房组织中Cav1.2、Kv4.3表达水平，进而延长AERP，降低房颤诱发率，改善心房电重构。

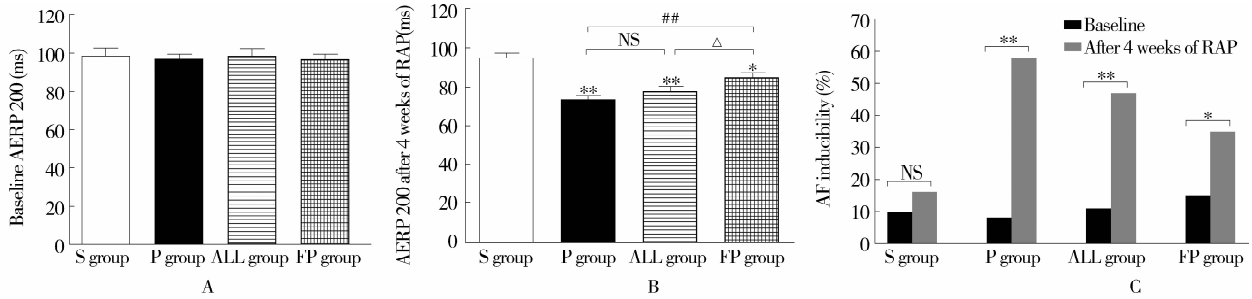


图 2 别嘌呤醇与非布司他对 AERP 200 及房颤诱发率的影响

Figure 2 Effects of allopurinol and febuxostat on AERP 200 and inducibility of AF ($n=6$)

A: baseline AERP 200 in four groups; B: AERP 200 after 4 weeks of RAP; C: effects of allopurinol and febuxostat on inducibility of AF. NS: not significant; XO: xanthine oxidase; AERP: atrial effective refractory period; RAP: rapid atrial pacing; AF: atrial fibrillation. S group: sham-operated group; P group: RAP group; ALL group: RAP with 50 mg/(kg·d) allopurinol group; FP group: RAP with 10 mg/(kg·d) febuxostat group. Compared with S group, $^{*}P<0.05$, $^{**}P<0.01$; compared with P group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; compared with ALL group, $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$.

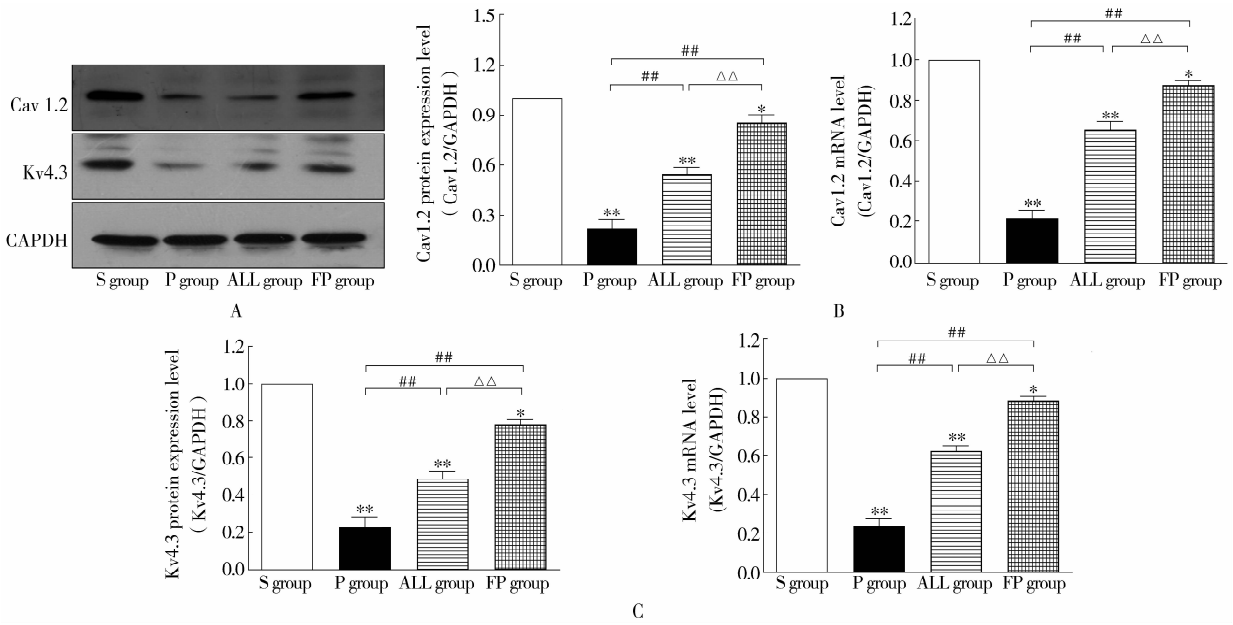


图 3 别嘌呤醇与非布司他对左心房组织中 Cav1.2 和 Kv4.3 表达的影响

Figure 3 Effects of allopurinol and febuxostat on expression of Cav1.2 and Kv4.3 in left atrium ($n=6$)

A: Western blot analysis of protein expression levels of Cav1.2 and Kv4.3 in samples from P group, S group , ALL group and FP group; B: quantitative analysis of mRNA and protein expression levels of Cav1.2; C: quantitative analysis of mRNA and protein expression levels of Kv4.3. RAP: rapid atrial pacing; Cav1.2: calcium voltage-gated channel subunit α1C; Kv4.3: potassium voltage gated channel subfamily D3; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. S group: sham-operated group; P group: RAP group; ALL group: RAP with 50 mg/(kg·d) allopurinol group; FP group: RAP with 10 mg/(kg·d) febuxostat group. Compared with S group, $^{*}P<0.05$, $^{**}P<0.01$; compared with P group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; compared with ALL group, $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$.

炎症和氧化应激可促进心房重构[4,5],房颤时炎症因子和氧化应激标志物的改变均证实了这一点。hs-CRP 是最常见的炎症标志物,同时也是当前存在房颤,未来有可能发展为房颤以及房颤射频消融术后或电复律后房颤复发的强有力的预测因子[6,7]。GDF-15 是一种新型的心血管系统炎症标志物,血清中 GDF-15 水平的高低与房颤事件的发生成正相关[8]。本研究结果显示,与 S 组相比,P 组家兔血清中 hs-CRP 和 GDF-15 水平明显升高,表明 4 周的快速心房起搏诱导了严重的炎症反应;然而,使用 XO 抑制剂干预后,血清中 hs-CRP 和 GDF-15 水平显著降低,且在 FP 组中最为明显。结果表明,XO 抑制剂可以抑制快速心房起搏所诱导的炎症反应。SOD、XO 及 XDH 是反映氧化应激的重要指标[5],本研究结果显示,4 周的快速心房起搏导致家兔左心房组织匀浆中 SOD 的活性降低,XO 和 XDH 的水平升高。应用 XO 抑制剂处理后,SOD 活性回升明显,而 XO 和 XDH 活性降低显著,说明 XO 抑制剂可抑制快速心房起搏所诱导的氧化应激反应。半乳糖凝集素-3 水平与左房纤维化程度独立相关,半乳糖凝集素-3 水平升高,预示着心房重构过程的开始[9]。本研究结果显示,P 组半乳糖凝集素-3 的水平与 S 组相比显著升高,应用 XO 抑制剂处理后,半乳糖凝集素-3 的水平与 P 组相比有一定程度的降低,在 FP 组中尤为明显。结果表明,XO 抑制剂(尤其是非布司他)可能通过减轻心房组织的炎症反

应和氧化应激损伤,进而抑制心房重构的进展。

心房电重构的主要特点是离子通道功能障碍,表现为心房肌的动作电位平台期缩短、AERP 的缩短、不应期离散度增加、频率适应性下降和延迟后除极等[10]。Cav1.2 在动作电位的形成及触发心肌细胞兴奋-收缩耦联过程中发挥着重要作用[11]。当动作电位达到 2 相平台期膜电位时,去极化使细胞膜上的 Cav1.2 激活后选择性允许 Ca^{2+} 内流,触发肌浆网钙释放通道释放 Ca^{2+},使细胞内 Ca^{2+} 升高,引起心肌细胞收缩[12]。过快的心房率所诱导的细胞内钙超载可通过短期和长期适应性来代偿,以减少心房动作电位持续时间为代价下调 Cav1.2 的基因表达,引起 L 型钙通道数量减少、Ca^{2+} 进入心房肌细胞减少、L 型钙通道电流降低,进而又使 AERP 缩短,房颤的频率适应性下降,最终导致房颤的易感性增加[12,13]。Kv4.3 在心肌细胞动作电位复极 1 期通道开放生成瞬时外向钾电流(transient outward potassium current,I_{to}),I_{to} 是复极过程中重要的离子流,影响动作电位的形态与时程[14]。Bosch 等[15]对比窦性心律与房颤患者心房肌细胞的 L 型钙通道电流及瞬时外向钾电流的变化,发现与窦性心律的患者相比,房颤患者心房肌细胞 L 型钙通道电流及 I_{to} 明显降低。本研究也部分验证了这一机制,电生理检查结果显示,4 周的快速心房起搏导致 AERP 200 明显缩短,房颤的诱发率明显增加,同时也影响了左心房组织中离子通道基因的活性,这与快速心房起搏之后 Cav1.2 和 Kv4.3 mRNA 及蛋白表达水平的下调相一致。总之,心房电重构改变了心房肌细胞跨膜离子通道基因的表达,进而产生有利于触发房颤的底物并促进房颤的发生和维持,但是这些改变可以被 XO 抑制剂(尤其是非布司他)的干预所逆转。

XO 在多种心血管疾病的氧化还原信号转导中,尤其是房颤的氧化还原信号转导中发挥着关键的作用[16]。Sakabe 等[17]研究证实别嘌呤醇能够抑制犬快速心房起搏所致左室功能障碍动物模型中房颤的发生,同时抑制心房电重构与结构重构。非布司他是一种新型的非嘌呤选择性 XO 抑制剂,与别嘌呤醇相比,非布司他可以选择性抑制黄嘌呤氧化酶而对其他嘌呤和嘧啶代谢酶不产生交叉抑制,且具有更强的 XO 抑制作用,副作用更少[18]。研究证实,非布司他可以通过抑制 XO 活性、直接清除 ROS 而发挥抗氧化作用,进而减弱自由基诱导的氧化应激[16]。本研究首次将 XO 特异性抑制剂非布司他用于房颤病理生理机制的研究,并证实了非布司他可能是一种能够减轻心房重构用于房颤上游治疗的潜在新型治疗药物。

本研究的局限性主要在于研究中使用的 XO 抑制剂的剂量是根据痛风患者高尿酸血症的临床研究选择的,需要进一步的动物实验和临床试验来确定非布司他及别嘌醇治疗房颤的最佳剂量和给药频率,并且 XO 抑制剂改善心房电重构的分子生物学机制有待进一步更深入性研究来证实。

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