

基于PI3K/Akt/Caspase-9信号通路研究严氏强心饮对心力衰竭大鼠心肌细胞凋亡的影响

朱思行¹, 严世芸¹, 徐燕², 秦嫣³, 陈丽云^{1*}, 贾美君^{2*}

¹ 上海中医药大学科技人文研究院, 上海 201203;

² 上海中医药大学附属曙光医院, 上海 200021;

³ 上海中医药大学附属市中医院, 上海 200071

* 通信作者: 陈丽云, E-mail: cly304@126.com; 贾美君, E-mail: sunnymj@163.com

收稿日期: 2021-10-20; 接受日期: 2022-01-15

基金项目: 上海市科学技术委员会“科技创新行动计划”启明星培育扬帆专项(22YF1449200); 国家重点研发计划项目(2019YFC1709203); 国家自然科学基金青年基金项目(81603581)

DOI: 10.3724/SP.J.1329.2022.02007

开放科学(资源服务)标识码(OSID):



摘要 目的: 基于PI3K/Akt/Caspase-9信号通路研究严氏强心饮对心力衰竭大鼠心肌细胞凋亡的影响, 探讨严氏强心饮治疗心力衰竭可能的作用机制。**方法:** 选择60只Wistar雄性大鼠, 采用随机数字表法分为正常组、模型组、严氏强心饮低剂量组、严氏强心饮中剂量组、严氏强心饮高剂量组和培哚普利组, 每组10只。除正常组外, 其余5组通过注射阿霉素溶液诱导心肌损伤, 进行心力衰竭大鼠模型造模。严氏强心饮低、中、高剂量组分别给予低、中、高剂量严氏强心饮灌胃, 各组分别含4.325、8.65、17.3 g/(kg·d)生药; 培哚普利组予培哚普利溶液灌胃, 剂量为4 mg/(kg·d)。灌胃剂量以10 mL/(kg·d)计算, 正常组和模型组给予同体积蒸馏水灌胃。末次给药1 d后, 采用超高分辨率小动物超声影像系统对大鼠进行M型超声心动图检测[左室射血分数(LVEF)、左室短轴缩短率(LVFS)、左室舒张末期内径(LVIDd)及左室收缩末期内径(LVIDs)]; 采用ELISA法检测大鼠血清B型钠尿肽(BNP)含量; 采用激光共聚焦显微镜观察心肌细胞凋亡情况并计算心肌细胞凋亡指数; 采用Western blot法检测凋亡相关蛋白Bax、Bcl-2及PI3K/Akt/Caspase-9信号通路蛋白的表达情况。**结果:** ① 心脏超声检测结果: 与正常组比较, 模型组左室前壁运动波形不明显, 接近直线运动, 且LVEF、LVFS明显下降($P<0.05$), LVIDd、LVIDs明显升高($P<0.05$), 左室明显扩大, 收缩能力减弱。与模型组比较, 严氏强心饮低、中、高剂量组左室前壁运动波形的幅度均有不同程度的增长, 且随着给药浓度升高, LVEF、LVFS逐渐升高($P<0.05$), 严氏强心饮中剂量组LVIDd降低($P<0.05$), 严氏强心饮中、高剂量组LVIDs均明显降低, 严氏强心饮中剂量组降低最明显($P<0.05$)。② 血清BNP结果: 与正常组比较, 模型组BNP含量明显升高($P<0.05$)。与模型组比较, 严氏强心饮低、中、高剂量组BNP含量均明显降低, 且随着给药浓度的升高, BNP呈下降趋势($P<0.05$)。③ 心肌组织病理学检测结果: 正常组心肌细胞基本没有凋亡, 几乎检测不到阳性信号; 模型组大鼠心肌细胞凋亡明显增加($P<0.05$); 与模型组比较, 严氏强心饮低、中、高剂量组与培哚普利组细胞凋亡均明显降低($P<0.05$), 随着严氏强心饮给药浓度的升高, 细胞凋亡数呈下降趋势($P<0.05$)。④ PI3K/Akt/Caspase-9信号通路蛋白表达水平和细胞凋亡因子情况: 与模型组比较, 严氏强心饮低、中、高剂量组PI3K和Akt蛋白表达、Bcl-2含量、Bcl-2/Bax比值均明显升高, 且随着给药浓度升高而逐渐升高($P<0.05$); 严氏强心饮低、中、高剂量组Caspase-9和Caspase-3蛋白表达均明显降低, 严氏强心饮中、高剂量组Bax含量明显降低, 且随着给药浓度升高而逐渐降低($P<0.05$)。**结论:** 严氏强心饮能够改善

引用格式: 朱思行, 严世芸, 徐燕, 等. 基于PI3K/Akt/Caspase-9信号通路研究严氏强心饮对心力衰竭大鼠心肌细胞凋亡的影响[J]. 康复学报, 2022, 32(2): 131-139.
ZHU S X, YAN S Y, XU Y, et al. Effect of Yanshiqiangxin decoction on cardiomyocyte apoptosis in heart failure rats based on PI3K/Akt/Caspase-9 signaling pathway [J]. Rehabilitation Medicine, 2022, 32(2): 131-139.
DOI: 10.3724/SP.J.1329.2022.02007

心力衰竭大鼠的心功能,降低血清BNP和心肌细胞凋亡,其作用机制可能与调节PI3K/Akt/Caspase-9信号通路有关。

关键词 心力衰竭;心脏功能;PI3K/Akt/Caspase-9信号通路;细胞凋亡;严氏强心饮

心力衰竭(heart failure, HF)是心室舒张或收缩功能受损,以动脉缺血和/或静脉淤血为主要特征的一组临床综合征,常常是由于心脏结构或功能异常导致的,是大多数心血管疾病的最终归宿^[1],常见于各种心脏疾病的危重期或晚期阶段。近年来, HF的患病率、病死率和再住院率居高不下^[2],严重危害人们的身心健康。细胞凋亡是各种生物体内较常见的细胞死亡方式,心肌细胞数量减少是导致心肌功能障碍的决定性因素^[3]。PI3K/Akt信号通路对细胞生长、合成、维持、凋亡等方面都起着重要的作用,研究表明,通过对PI3K/Akt信号通路的调节,减缓心肌细胞的凋亡进程是控制HF的有效途径^[4-5]。

HF属于中医“心水”“心痹”“心悸”等范畴,其病机关键为阳虚不化、水饮内停、痰浊瘀阻等,温阳利水是HF的主要治法之一^[6]。中医药在心脏治疗和康复方面疗效显著^[7],有其独特优势。严氏强心饮是国医大师严世芸教授创制的治疗HF的经验方和基础方,具有补益心肾、温阳利水的作用。前期临床研究证明,严氏强心饮能够改善HF患者的临床症状,提高其生活质量^[8-9]。但其具体作用机制尚不完全清楚。基于此,本研究拟构建阿霉素诱导的HF大鼠模型,观察严氏强心饮对HF大鼠的干预效果,并探讨其作用机制是否与PI3K/Akt信号通路的调控有关,为严氏强心饮的后续临床应用提供新的科学依据。

1 材料与方法

1.1 实验动物

选择SPF级Wistar雄性大鼠60只,体质量约200 g,购自北京维通利华实验动物技术有限公司(实验动物生产许可证号:SCXK2019-0001)。适应性喂养2周后,按体质量由高到低进行编号,采用随机数字表法分为正常组、模型组、严氏强心饮低剂量组、严氏强心饮中剂量组、严氏强心饮高剂量组和培哌普利组,每组10只。

1.2 实验药物

实验药物为严氏强心饮水煎液。严氏强心饮(制附子12 g,茯苓15 g,猪苓15 g,白术12 g,白芍12 g,淫羊藿20 g,补骨脂12 g,鹿角9 g)采用传统方法水

煎并水浴浓缩,分别配制成含生药0.432 5、0.865、1.73 g/mL的提取液。所用中药均购自上海中医药大学附属曙光医院中药房。

1.3 主要试剂与设备

1.3.1 主要试剂 阿霉素(阿拉丁控股集团有限公司, A183027);培哌普利(MedChemExpress公司, HY-B0130);异氟烷(上海玉研科技有限公司, S10010533);BNP ELISA试剂盒(CUSABIO公司, CSB-E07972r);TUNEL试剂盒(Roche公司, 116848 17910);DAPI试剂盒(Sigma公司, D9542);PI3K抗体(Abcam公司, ab191606);Akt抗体(4060T)、Caspase-9抗体(9507s)、Caspase-3抗体(9664T)均购自CST公司;Bax抗体(ab32503)、Bcl-2抗体(ab59348)均购自Abcam公司。

1.3.2 实验设备 超高分辨率小动物超声影像系统(VisualSonics公司, 型号: Vevo2100);激光共聚焦显微镜(Leica公司, 型号: TCS SPE);电泳仪(BIO-RAD公司, 型号: 165-8000)。

1.4 模型制备及干预方法

在避光条件下,用0.9% NaCl溶液将阿霉素粉针配成浓度1 mg/mL的溶液(避光),培哌普利配成浓度0.4 mg/mL的溶液。参照相关文献方法^[10],除正常组外,其余各组大鼠每周按2 mg/(kg·d)的剂量给予尾静脉注射阿霉素溶液进行造模,共注射6周;正常组尾静脉注射等容积0.9% NaCl溶液。同时,严氏强心饮低、中、高剂量组分别给予低、中、高剂量严氏强心饮灌胃^[11],各组含生药分别为4.325、8.65、17.3 g/(kg·d);培哌普利组予培哌普利溶液灌胃,剂量为4 mg/(kg·d)。灌胃剂量以10 mL/(kg·d)计算,正常组和模型组给予同体积蒸馏水灌胃。

1.5 观察指标

1.5.1 大鼠心脏超声检测 末次给药1 d后,将大鼠麻醉及心脏部位脱毛处理后,采用超高分辨率小动物超声影像系统(图像深度调为2 cm,频率为21 MHz)对大鼠进行M型超声心动图检测。主要测量指标包括左室射血分数(left ventricular ejection fraction, LVEF)、左室短轴缩短率(left ventricular fractional shortening, LVFS)、左室舒张末期内径(left ventricular internal dimension diastole, LVIDd)及左室

收缩末期内径(left ventricular internal dimension systole, LVIDs)。

1.5.2 大鼠血清B型钠尿肽含量检测 心脏超声检查完毕后,对大鼠进行腹主动脉采血。血液静置2 h后离心(4℃, 3 000 r/min, 10 min)。利用ELISA试剂盒检测各组大鼠血清B型钠尿肽(B-type natriuretic peptide, BNP)含量。操作步骤均严格按试剂盒说明书进行,分别设标准孔、空白孔、待测样品孔,每孔加入相应的试剂100 μL,封板后置37℃温育2 h后,每孔先后加入100 μL生物素抗体、100 μL HRP-avidin、90 μL TMB,并置37℃孵育,避光显色后加入终止液终止反应,测量各孔的光密度值(optical density, OD)。

1.5.3 心肌组织病理学检测 用异氟烷麻醉大鼠后,在其膈肌处剪一横行切口,充分暴露胸腔,分离心包后迅速摘取大鼠心脏,用4℃克氏液冲洗干净后置于4℃克氏液中,于显微镜下快速分离主动脉和上、下腔静脉以及肺动脉、肺静脉,并用滤纸吸干表面水分。切取一部分左室心肌组织置入脱水盒内,用自动组织脱水机常规梯度脱水、透明、浸蜡后进行人工石蜡包埋,用石蜡切片机制片。将处理好的切片用4%多聚甲醛处理(室温)30 min后,置于二甲苯中脱蜡2次,5 min/次;再置于无水乙醇中浸泡5 min,95%乙醇中浸泡5 min,70%乙醇中浸泡5 min;然后用0.5% Triton X-100处理(室温)5 min后,加入配置好的TUNEL标记液,37℃避光孵育60 min,加入DAPI染色液,孵育5 min;最后用抗荧光淬灭封片液封片,待干后用激光共聚焦显微镜,调至×200倍镜下观察心肌细胞凋亡情况并拍照,采用Image J软件计算心肌细胞凋亡指数。

心肌细胞凋亡指数=凋亡心肌细胞数/心肌总细胞数×100%

1.5.4 PI3K/Akt/Caspase-9信号通路蛋白表达水平及凋亡因子检测 将待测的大鼠左室心肌组织放置于培养皿中,加入1×RIPA 100 μL的裂解液,待充分溶解后提取蛋白,并用BCA法检测蛋白浓度。取15 μg总蛋白进行凝胶电泳(电压分别为80、120 V),再通过半干转将蛋白质转移至PVDF膜,转膜时间为90 min;用含5%脱脂奶粉的PBST封闭2 h后,孵于1:1 000稀释好的一抗,4℃过夜。洗膜后加入以

1:2 000稀释的二抗,室温孵育2 h后,将膜放入发光成像仪中,滴加发光试剂曝光。以β-actin为内参,检测PI3K、Akt、Caspase-9、Caspase-3、Bcl-2和Bax的蛋白表达。

1.6 统计学方法

采用SPSS 26.0软件进行统计学分析。计量资料符合正态分布,数据采用($\bar{x} \pm s$)表示,组间比较采用单因素方差分析,两两比较方差齐采用LSD-*t*检验,方差不齐用校正*t*检验。 $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 6组大鼠存活情况

在造模及给药过程中,共有7只接受阿霉素注射的大鼠死亡,其中模型组、严氏强心饮低剂量组、严氏强心饮中剂量组、严氏强心饮高剂量组和培哚普利组分别死亡2、1、1、1、2只。最终纳入实验研究大鼠正常组、模型组、严氏强心饮低剂量组、严氏强心饮中剂量组、严氏强心饮高剂量组和培哚普利组分别为10、8、9、9、9、8只。

2.2 6组心脏超声检测结果比较

M模式下大鼠心脏超声结果显示,与正常组比较,模型组左室前壁运动波形不明显,接近直线运动,且LVEF、LVFS明显下降($P < 0.05$),LVIDd、LVIDs明显升高($P < 0.05$),左室明显扩大,收缩能力减弱,提示HF模型复制成功。给予不同剂量严氏强心饮干预后,严氏强心饮低、中、高剂量组左室前壁运动波形的幅度较模型组均有不同程度的增长,且随着给药浓度升高,LVEF、LVFS逐渐升高($P < 0.05$),LVIDd仅在严氏强心饮中剂量组降低($P < 0.05$)。与模型组比较,LVIDs在严氏强心饮中、高剂量组均明显降低($P < 0.05$),严氏强心饮中剂量组降低最明显($P < 0.05$)。与严氏强心饮中剂量组比较,严氏强心饮高剂量组LVIDd、LVIDs差异均无统计学意义($P > 0.05$)。见图1、表1。

2.3 6组血清BNP含量比较

与正常组比较,模型组BNP含量明显升高($P < 0.05$)。与模型组比较,严氏强心饮低、中、高剂量组BNP含量均明显降低,且随着给药浓度的升高,BNP含量呈下降趋势($P < 0.05$)。见表2。

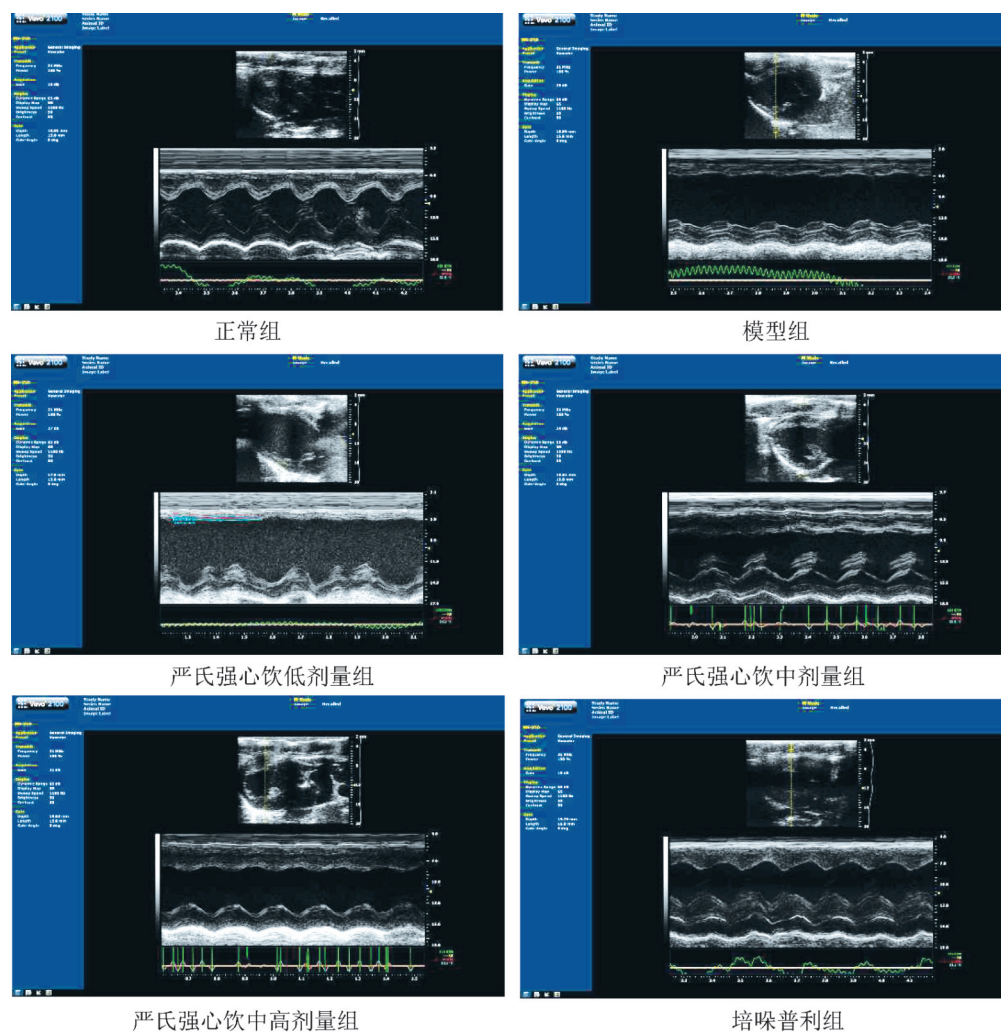


图1 6组大鼠心脏超声检测图

Figure 1 Cardiac ultrasound patterns in six groups

表1 6组心脏超声检测指标比较($\bar{x} \pm s$)

Table 1 Comparison of cardiac ultrasound in six groups ($\bar{x} \pm s$)

组别	n	LVEF/%	LVFS/%	LVIDd/mm	LVIDs/mm
正常组	10	84.28±1.76	58.97±5.81	184.13±47.07	48.84±28.45
模型组	8	30.07±0.78 ¹⁾	16.65±2.98 ¹⁾	380.82±48.27 ¹⁾	255.95±49.84 ¹⁾
严氏强心饮低剂量组	9	35.19±1.16 ²⁾	18.6±1.88	406.59±101.79	253.73±50.56
严氏强心饮中剂量组	9	40.99±1.2 ²⁾³⁾	33.57±11.21 ²⁾³⁾	248.11±28.48 ²⁾³⁾	100.61±36.34 ²⁾³⁾
严氏强心饮高剂量组	9	48.83±2.37 ²⁾³⁾⁴⁾	30.41±6.75 ²⁾³⁾⁴⁾	329.58±17.86	145.98±29.97 ²⁾
培哚普利组	8	68.75±1.37 ²⁾	39.7±1.02 ²⁾	278.07±37.07 ²⁾	87.25±14.14 ²⁾

注:与正常组比较,1) $P<0.05$;与模型组比较,2) $P<0.05$;与严氏强心饮低剂量组比较,3) $P<0.05$;与严氏强心饮中剂量组比较,4) $P<0.05$ 。

Note: Compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$; compared with the low dose Yanshiqiangxin decoction group, 3) $P<0.05$; compared with the medium dose Yanshiqiangxin decoction group, 4) $P<0.05$.

表2 6组大鼠血清BNP含量比较($\bar{x} \pm s$) pg/mL

Table 2 Comparison of serum BNP content in six groups ($\bar{x} \pm s$) pg/mL		
组别	<i>n</i>	BNP
正常组	10	172.36 $\pm$ 9.01
模型组	8	348.02 $\pm$ 11.99 ¹⁾
严氏强心饮低剂量组	9	313.55 $\pm$ 9.60 ²⁾
严氏强心饮中剂量组	9	272.55 $\pm$ 9.65 ²⁾³⁾
严氏强心饮高剂量组	9	207.06 $\pm$ 10.49 ²⁾³⁾⁴⁾
培哚普利组	8	183.08 $\pm$ 8.67 ²⁾

注:与正常组比较,1) $P<0.05$;与模型组比较,2) $P<0.05$;与严氏强心饮低剂量组比较,3) $P<0.05$;与严氏强心饮中剂量组比较,4) $P<0.05$ 。

Note: Compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$; compared with the low dose Yanshiqiangxin decoction group, 3) $P<0.05$; compared with the medium dose Yanshiqiangxin decoction group, 4) $P<0.05$.

2.4 6组心肌组织病理学检测结果比较

心肌组织细胞由DAPI+TUNEL双染色后,两者图像经软件融合后得到merge图片,其中阳性凋亡细胞核呈绿色荧光。图像中的心肌细胞凋亡免疫荧光结果显示:正常组细胞基本没有凋亡,几乎

检测不到阳性信号;经阿霉素造模后,模型组大鼠心肌细胞凋亡较正常组明显增加($P<0.05$);与模型组比较,培哚普利组细胞凋亡数最少($P<0.05$),随着严氏强心饮浓度的升高,其细胞凋亡数量呈下降趋势($P<0.05$)。见表3、图2。

表3 6组心肌细胞凋亡情况比较($\bar{x} \pm s$) %

Table 3 Comparison of cardiomyocyte apoptosis in six groups ($\bar{x} \pm s$) %		
组别	<i>n</i>	心肌细胞凋亡指数
正常组	10	0.01 $\pm$ 0.01
模型组	8	58.93 $\pm$ 3.46 ¹⁾
严氏强心饮低剂量组	9	52.13 $\pm$ 1.36
严氏强心饮中剂量组	9	35.87 $\pm$ 2.48 ²⁾³⁾
严氏强心饮高剂量组	9	15.40 $\pm$ 2.16 ²⁾³⁾⁴⁾
培哚普利组	8	9.53 $\pm$ 0.70 ²⁾

注:与正常组比较,1) $P<0.05$;与模型组比较,2) $P<0.05$;与严氏强心饮低剂量组比较,3) $P<0.05$;与严氏强心饮中剂量组比较,4) $P<0.05$ 。

Note: Compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$; compared with the low dose Yanshiqiangxin decoction group, 3) $P<0.05$; compared with the medium dose Yanshiqiangxin decoction group, 4) $P<0.05$.

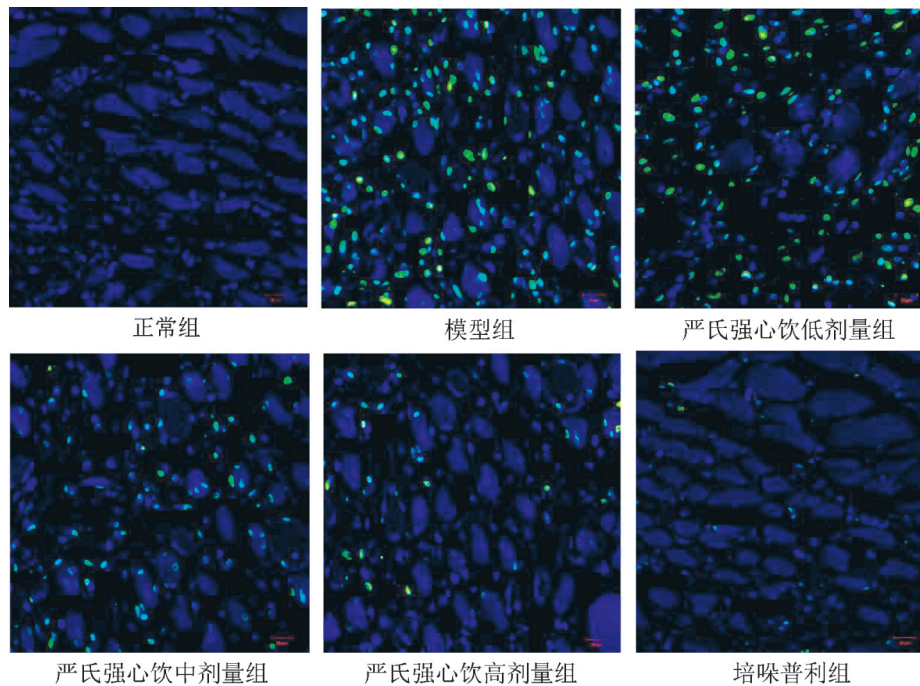


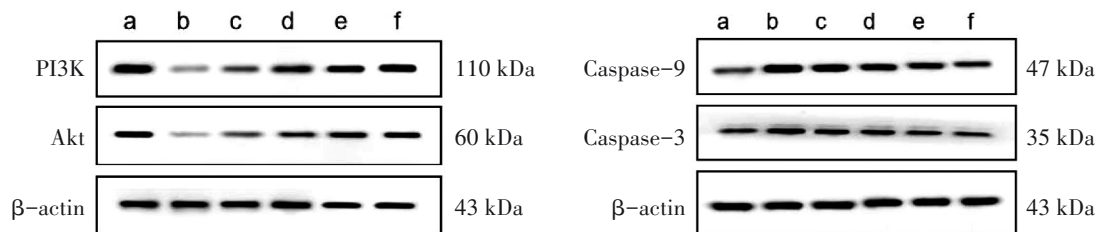
图2 6组心肌组织病理学检测图(×200)

Figure 2 Myocardial histopathology figure in six groups (×200)

2.5 6组 PI3K/Akt/Caspase-9 信号通路蛋白表达水平比较

与正常组相比,模型组 PI3K、Akt 蛋白表达降低, Caspase-9、Caspase-3 蛋白表达升高。与模型组比较,严氏强心饮低、中、高剂量组 PI3K、Akt 蛋白表达

均明显升高($P<0.05$),且随着给药浓度升高而逐渐升高($P<0.05$),严氏强心饮低、中、高剂量组 Caspase-9、Caspase-3 蛋白表达均明显降低($P<0.05$),且随着给药浓度升高而逐渐降低($P<0.05$)。见图 3、表 4。



注:a~f分别代表正常组、模型组、严氏强心饮低剂量组、严氏强心饮中剂量组、严氏强心饮高剂量组、培哚普利组。

Note: a~f represented the normal group, the model group, the low dose Yanshiqiangxin decoction group, the medium dose Yanshiqiangxin decoction group, the high dose Yanshiqiangxin decoction group and the positive drug control group, respectively.

图3 6组 PI3K/Akt/Caspase-9 信号通路蛋白表达

Figure 3 Expression of PI3K, Akt, Caspase-9 signaling pathway protein in six groups

表4 6组 PI3K、Akt、Caspase-9 信号通路蛋白表达比较($\bar{x}\pm s$)

Table 4 Comparison of expression of PI3K/Akt/Caspase-9 signaling pathway protein in six groups ($\bar{x}\pm s$)

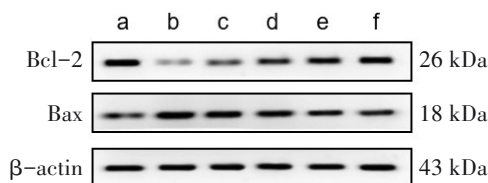
组别	n	PI3K	Akt	Caspase-9	Caspase-3
正常组	10	1.50±0.25	1.49±0.41	0.42±0.05	0.41±0.05
模型组	8	0.39±0.03 ¹⁾	0.74±0.4 ¹⁾	1.00±0.09 ¹⁾	1.01±0.23 ¹⁾
严氏强心饮低剂量组	9	0.74±0.11 ²⁾	0.95±0.38 ²⁾	0.89±0.14 ²⁾	0.87±0.24 ²⁾
严氏强心饮中剂量组	9	1.03±0.19 ²⁾³⁾	1.09±0.36 ²⁾³⁾	0.77±0.11 ²⁾³⁾	0.73±0.16 ²⁾³⁾
严氏强心饮高剂量组	9	1.15±0.08 ²⁾³⁾⁴⁾	1.27±0.37 ²⁾³⁾⁴⁾	0.65±0.07 ²⁾³⁾⁴⁾	0.56±0.12 ²⁾³⁾⁴⁾
培哚普利组	8	1.26±0.22 ²⁾	1.34±0.39 ²⁾	0.51±0.08 ²⁾	0.46±0.07 ²⁾

注:与正常组比较,1) $P<0.05$;与模型组比较,2) $P<0.05$;与严氏强心饮低剂量组比较,3) $P<0.05$;与严氏强心饮中剂量组比较,4) $P<0.05$ 。

Note: Compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$; compared with the low dose Yanshiqiangxin decoction group, 3) $P<0.05$; compared with the medium dose Yanshiqiangxin decoction group, 4) $P<0.05$.

2.6 6组心肌组织凋亡因子比较

与正常组比较,模型组 Bcl-2 含量、Bcl-2/Bax 比值均明显降低,而 Bax 含量明显升高($P<0.05$)。与模型组比较,严氏强心饮低、中、高剂量组 Bcl-2 含量明显升高($P<0.05$),随着严氏强心饮浓度的升高而逐渐增多($P<0.05$);严氏强心饮中、高剂量组 Bax 含量随着严氏强心饮浓度的增加而逐渐下降($P<0.05$),而 Bcl-2/Bax 比值随着给药浓度升高而逐渐提高($P<0.05$)。见图 4、表 5。



注:a~f分别代表正常组、模型组、严氏强心饮低剂量组、严氏强心饮中剂量组、严氏强心饮高剂量组、培哚普利组。

Note: a~f represented the normal group, the model group, the low dose Yanshiqiangxin decoction group, the medium dose Yanshiqiangxin decoction group, the high dose Yanshiqiangxin decoction group and the positive drug control group, respectively.

图4 6组心肌组织凋亡因子检测结果

Figure 4 Myocardial tissue apoptosis factor assay results in six groups

3 讨论

3.1 严氏强心饮可改善 HF 大鼠心脏功能,减少心肌细胞凋亡

经胸超声心动图是评估心脏结构和功能的首选方法,LVEF可反映左心室收缩功能^[11-12]。本研究

表5 6组心肌组织凋亡因子比较($\bar{x} \pm s$)Table 5 Comparison of myocardial tissue apoptosis factor in six groups ($\bar{x} \pm s$)

组别	n	Bcl-2	Bax	Bcl-2/Bax
正常组	10	1.02±0.13	0.77±0.11	0.23±0.02
模型组	8	0.27±0.06 ¹⁾	1.58±0.04 ¹⁾	0.03±0.01 ¹⁾
严氏强心饮低剂量组	9	0.45±0.11 ²⁾	1.50±0.10	0.05±0.02
严氏强心饮中剂量组	9	0.66±0.11 ²⁾³⁾	1.31±0.04 ²⁾³⁾	0.09±0.02 ²⁾³⁾
严氏强心饮高剂量组	9	0.86±0.10 ²⁾³⁾⁴⁾	1.10±0.04 ²⁾³⁾⁴⁾	0.16±0.05 ²⁾³⁾⁴⁾
培哚普利组	8	0.89±0.13 ²⁾	1.02±0.04 ²⁾	0.15±0.03 ²⁾

注:与正常组比较,1) $P < 0.05$;与模型组比较,2) $P < 0.05$;与严氏强心饮低剂量组比较,3) $P < 0.05$;与严氏强心饮中剂量组比较,4) $P < 0.05$ 。

Note: Compared with the normal group, 1) $P < 0.05$; compared with the model group, 2) $P < 0.05$; compared with the low dose Yanshiqiangxin decoction group, 3) $P < 0.05$; compared with the medium dose Yanshiqiangxin decoction group, 4) $P < 0.05$.

结果显示,严氏强心饮低、中、高剂量组LVEF、LVFS逐渐升高,且严氏强心饮高剂量组改善效果更明显;严氏强心饮中剂量组LVIDd降低,与模型组比较,严氏强心饮中、高剂量组LVIDs均明显降低,这提示严氏强心饮可改善HF大鼠心脏功能。BNP主要由心室肌合成,当心脏负荷增大或心室扩大时,心肌细胞受牵拉而合成并释放BNP入血增加,血浆BNP含量升高,并与心功能损伤的严重程度呈显著正相关,故血清BNP的检测被推荐用于HF筛查、诊断和鉴别诊断、病情严重程度及预后评估^[13-14]。本研究结果显示,HF大鼠血清BNP含量、心肌细胞凋亡数量随着严氏强心饮浓度的升高而逐渐降低,这提示严氏强心饮能够有效抑制HF大鼠心肌细胞的凋亡,且呈一定剂量依赖性,提高HF大鼠的心脏功能。

3.2 严氏强心饮改善HF大鼠心脏功能可能与PI3K/Akt/Caspase-9信号通路调节有关

本研究结果显示,与模型组比较,随着严氏强心饮给药浓度升高PI3K、Akt、Bcl-2蛋白表达以及Bcl-2/Bax比值均逐渐升高,而Caspase-9、Caspase-3、Bax蛋白表达均逐渐降低。这提示严氏强心饮改善HF大鼠心脏功能可能与调节PI3K/Akt/Caspase-9信号通路有关。PI3K/Akt信号通路的激活,可以通过直接或间接的形式抑制细胞凋亡,从而减缓心力衰竭的发展进程^[15]。Caspase家族分为包括Caspase-9在内的凋亡启动因子和包括Caspase-3在内的凋亡执行因子两类^[16],两者都是Akt下游的靶蛋白,是各类细胞凋亡的最好途径,其能够破坏细胞骨架,裂解DNA,导致细胞凋亡^[17]。因此,对Caspase-9和Caspase-3蛋白表达的调节,是有效抑制心肌细胞凋亡的关键。严氏强心饮可以激活PI3K/

Akt信号通路,使Akt活化后,降低Caspase-9表达,抑制Caspase级联反应,使下游的Caspase-3不被激活,实现调控细胞凋亡的启动和执行程序,抑制细胞凋亡,从而减缓心力衰竭的发展进程。抗凋亡蛋白Bcl-2和促凋亡蛋白Bax之间的平衡是调控细胞凋亡的关键^[18],严氏强心饮可以激活PI3K,促使其下游的Akt发生磷酸化而被激活参与信号传导,从而促进Bcl-2的释放并抑制Bax的表达,增加HF大鼠心肌细胞抗凋亡能力,减少心肌细胞的凋亡数量。

4 小 结

严氏强心饮能够有效降低HF大鼠血清BNP水平,减少心肌细胞凋亡数量,改善HF大鼠心脏功能,并能上调PI3K、Akt、Bcl-2蛋白表达,下调Caspase-9、Caspase-3、Bax蛋白表达,提示其保护心肌细胞凋亡的作用机制可能与调节PI3K/Akt/Caspase-9信号通路有关。

参考文献

- [1] 中华医学会心血管病学分会心力衰竭学组,中国医师协会心力衰竭专业委员会,中华心血管病杂志编辑委员会. 中国心力衰竭诊断和治疗指南2018[J]. 中华心血管病杂志, 2018, 46(10):760-789.
Heart Failure Group of Chinese Society of Cardiology of Chinese Medical Association, Chinese Heart Failure Association of Chinese Medical Doctor Association, Editorial Board of Chinese Journal of Cardiology. Chinese guidelines for the diagnosis and treatment of heart failure 2018 [J]. Chin J Cardiol, 2018, 46(10):760-789.
- [2] 周京敏,崔晓通,葛均波. 中国心力衰竭的流行病学概况[J]. 中华心血管病杂志, 2015, 43(12):1018-1021.
ZHOU J M, CUI X T, GE J B. Epidemiological profile of heart failure in China [J]. Chin J Cardiol, 2015, 43(12):1018-1021.

- [3] TERINGOVA E, TOUSEK P. Apoptosis in ischemic heart disease [J]. *J Transl Med*, 2017, 15(1):87.
- [4] 杨明,董竹琴,罗颖,等. 基于PI3K/Akt/mTOR信号通路研究南葶苈子水提液对心力衰竭模型大鼠心室重构的影响[J]. *浙江中西医结合杂志*, 2019, 29(6):442-445,526.
YANG M, DONG Z Q, LUO Y, et al. Effect of aqueous extract from seeds of *descurainia sophia* on ventricular remodeling in rats with heart failure based on PI3K/Akt/mTOR signaling pathway [J]. *Zhejiang J Integr Tradit Chin West Med*, 2019, 29(6): 442-445, 526.
- [5] 杨嘉豪,崔瑞琴,周波,等. 温阳消饮方对慢性心力衰竭大鼠PI3K-Akt信号通路表达及相关凋亡蛋白的影响[J]. *中华中医药杂志*, 2020, 35(1):145-149.
YANG J H, CUI R Q, ZHOU B, et al. Effects of Wenyang Xiaoyin formula on the expression of PI3K-Akt signal pathway and related apoptotic proteins in chronic heart failure rats [J]. *China J Tradit Chin Med Pharm*, 2020, 35(1): 145-149.
- [6] 刘碧绒,李金根,李蒙,等. 心衰中水饮证候要素辨证分析[J]. *中国中医基础医学杂志*, 2021, 27(9):1370-1372,1425.
LIU B R, LI J G, LI M, et al. Syndrome differentiation and treatment with retained fluid syndrome elements in heart failure [J]. *J Basic Chin Med*, 2021, 27(9): 1370-1372, 1425.
- [7] WANG Y, WANG Q, LI C, et al. A review of Chinese herbal medicine for the treatment of chronic heart failure [J]. *Curr Pharm Des*, 2017, 23(34):5115-5124.
- [8] 郭美珠,唐丽萍,黄国毅,等. 强心饮治疗左室射血分数正常的慢性心力衰竭临床研究[J]. *中西医结合心脑血管病杂志*, 2015, 13(11):1252-1254.
GUO M Z, TANG L P, HUANG G Y, et al. Clinical observation on Qiangxin decoction for treatment of chronic heart failure with normal left ventricular ejection fraction [J]. *Chin J Integr Med Cardio/cerebrovascular Dis*, 2015, 13(11): 1252-1254.
- [9] 张鞠华,杨波,严世芸,等. 强心饮治疗阳虚水泛型慢性心力衰竭疗效观察[J]. *现代中西医结合杂志*, 2019, 28(23):2541-2543,2552.
ZHANG J H, YANG B, YAN S Y, et al. Therapeutic effect of Qiangxin decoction on chronic heart failure of type of edema due to Yang insufficiency [J]. *Mod J Integr Tradit Chin West Med*, 2019, 28(23):2541-2543, 2552.
- [10] 严骅,高俊杰,蒋梅先,等. 强心饮对阿霉素诱导大鼠慢性心肌损伤的保护作用[J]. *中国中医基础医学杂志*, 2010, 16(7):557-559.
YAN H, GAO J J, JIANG M X, et al. Protective effect of Qiang Xin Yin on chronic myocardium injury induced by adriacin in rats [J]. *Chin J Basic Med Tradit Chin Med*, 2010, 16(7): 557-559.
- [11] GARBI M, MCDONAGH T, COSYNS B, et al. Appropriateness criteria for cardiovascular imaging use in heart failure: report of literature review [J]. *Eur Heart J Cardiovasc Imaging*, 2015, 16(2):147-153.
- [12] LANG R M, BADANO L P, MOR-AVI V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American society of echocardiography and the European association of cardiovascular imaging [J]. *J Am Soc Echocardiogr*, 2015, 28(1):1-39. e14.
- [13] BOOTH R A, HILL S A, DON-WAUCHOPE A, et al. Performance of BNP and NT-proBNP for diagnosis of heart failure in primary care patients: a systematic review [J]. *Heart Fail Rev*, 2014, 19(4):439-451.
- [14] ROBERTS E, LUDMAN A J, DWORZYNSKI K, et al. The diagnostic accuracy of the natriuretic peptides in heart failure: systematic review and diagnostic meta-analysis in the acute care setting [J]. *BMJ*, 2015, 350:h910.
- [15] 尹红伟,王慈,王帅,等. 心力衰竭发生发展的自噬机制及中医药干预研究进展[J]. *天津中医药大学学报*, 2021, 40(6):685-690.
YIN H W, WANG C, WANG S, et al. Progress of autophagy mechanism and traditional Chinese medicine intervention in heart failure [J]. *J Tianjin Univ Tradit Chin Med*, 2021, 40(6): 685-690.
- [16] 毛德文,陈月桥,王丽,等. Caspase-8及Caspase-3与细胞凋亡[J]. *辽宁中医药大学学报*, 2008, 10(10):148-150.
MAO D W, CHEN Y Q, WANG L, et al. Relationship of Caspase-8 and Caspase-3 to apoptosis [J]. *J Liaoning Univ Tradit Chin Med*, 2008, 10(10): 148-150.
- [17] CROWLEY L C, WATERHOUSE N J. Detecting cleaved Caspase-3 in apoptotic cells by flow cytometry [J]. *Cold Spring Harb Protoc*, 2016, 2016(11):1064-1072.
- [18] 张媛媛,蒋昕钰,李西海,等. 基于PI3K/Akt信号通路探讨电针对膝关节关节炎模型大鼠软骨细胞凋亡的影响[J]. *康复学报*, 2019, 29(4):30-36.
ZHANG Y Y, JIANG X Y, LI X H, et al. Effect of electroacupuncture on chondrocyte apoptosis in knee osteoarthritis rat based on PI3K/Akt signaling pathway [J]. *Rehabil Med*, 2019, 29(4): 30-36.

Effect of Yanshiqiangxin Decoction on Cardiomyocyte Apoptosis in Heart Failure Rats Based on PI3K/Akt/Caspase-9 Signaling Pathway

ZHU Sixing¹, YAN Shiyun¹, XU Yan², QIN Yan³, CHEN Liyun^{1*}, JIA Meijun^{3*}

¹ Institute of Science, Technology and Humanities, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China;

² Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 200021, China;

³ Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200071, China

*Correspondence: CHEN Liyun, E-mail: cly304@126.com; JIA Meijun, E-mail: sunnymj@163.com

ABSTRACT Objective: To detect the effect of Yanshiqiangxin decoction on apoptosis of cardiomyocytes in rats with heart failure based on PI3K/Akt/Caspase-9 signalling pathway and to explore the possible mechanism of Yanshiqiangxin decoction in the treatment of heart failure. **Methods:** A total of 60 Wistar male rats were randomly divided into the normal group, the model group, the low dose Yanshiqiangxin decoction group, the medium dose Yanshiqiangxin decoction group, the high dose Yanshiqiangxin decoction group and the Perindopril group according to the random number table method, with 10 cases in each group. Except for the normal group, the remaining five groups were modelled by injecting adriamycin solution to induce myocardial injury and to establish the rat model of heart failure. In the low, medium and high dose Yanshiqiangxin decoction groups, 4.325, 8.65 and 17.3 g/(kg·d) of raw drug were administered by gavage respectively; the Perindopril group was given perindopril solution by gavage at the dose of 4 mg/(kg·d). The gavage dose was calculated as 10 mL/(kg·d), the normal group and the model group were given the same volume of distilled water. One day after the last gavage, M-mode echocardiography [left ventricular ejection fraction (LVEF), left ventricular short-axis shortening (LVFS), left ventricular internal dimension diastole (LVIDd) and left ventricular internal dimension systole (LVIDs)] was performed through an ultra-high resolution small animal ultrasound imaging system; B-type natriuretic peptide (BNP) was measured by ELISA method; the apoptosis of cardiomyocytes was observed through laser confocal microscopy and the apoptosis index was calculated; the expression of apoptosis-related proteins Bax, Bcl-2 and PI3K/Akt/Caspase-9 signalling pathway proteins were detected by Western blot method. **Results:** ① Cardiac ultrasound test results: compared with the normal group, the left ventricular anterior wall motion waveform of the model group was not obvious, close to linear motion, and LVEF and LVFS decreased significantly ($P<0.05$), LVIDd and LVIDs increased significantly ($P<0.05$), the left ventricle was significantly enlarged and the contractility was reduced. Compared with the model group, the amplitude of left ventricular anterior wall motion waveform increased at different degrees in the low, medium and high dose Yanshiqiangxin decoction group, LVEF and LVFS gradually increased with the increase of administration concentration ($P<0.05$), while LVIDd decreased only in the medium dose Yanshiqiangxin decoction group ($P<0.05$), LVIDs of the medium dose Yanshiqiangxin decoction group and the high dose Yanshiqiangxin decoction group were significantly reduced, the medium dose Yanshiqiangxin decoction group decreased the most significantly ($P<0.05$). ② Serum BNP results: compared with the normal group, BNP of the model group was significantly increased ($P<0.05$). Compared with the model group, BNP of the low, medium and high dose Yanshiqiangxin decoction group was significantly decreased, BNP gradually decreased with the increase of administration concentration ($P<0.05$). ③ Myocardial histopathology results: Cardiomyocytes had no apoptosis and almost no positive signal was detected in the normal group; compared with the model group, the number of cardiomyocyte apoptosis of the low, medium, high dose Yanshiqiangxin decoction group and the perindopril group was significantly decreased, the number of cardiomyocyte apoptosis gradually decreased with the increase of administration concentration ($P<0.05$). ④ PI3K/Akt/Caspase-9 signaling pathway protein expression levels and apoptotic factors: compared with the model group, PI3K and Akt protein expression, Bcl-2 content and Bcl-2/Bax ratio of the low, medium and high dose Yanshiqiangxin decoction group were significantly increased, PI3K and Akt protein expression, Bcl-2 content and Bcl-2/Bax ratio gradually increased with the increase of administration concentration ($P<0.05$); Caspase-9 and Caspase-3 protein expression of the low, medium and high dose Yanshiqiangxin decoction group were significantly decreased, Bax content of the medium and high dose Yanshiqiangxin decoction group were significantly decreased, and gradually increased with the increase of administration concentration ($P<0.05$). **Conclusions:** Yanshiqiangxin decoction can improve cardiac function, reduce the content of BNP in serum and cardiomyocyte apoptosis, the mechanism may be related to the regulation of the PI3K/Akt/Caspase-9 signalling pathway.

KEY WORDS heart failure; cardiac function; PI3K/Akt/Caspase-9 signaling pathway; cell apoptosis; Yanshiqiangxin decoction

DOI:10.3724/SP.J.1329.2022.02007