

证的研究

心虚证大鼠心肌荧光组织化学及超微结构的实验研究

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摘要 目的:观察心虚证对心肌细胞微细结构及超微结构的影响。方法:选用健康大鼠随机分为造模组和对照组,于造模 48h、72h 分别取心肌组织作冰冻切片、超薄切片,采用荧光组织化学及电镜技术,观察心肌细胞内糖原和 DNA 的分布、含量及超微结构的变化。结果:心虚证造模组大鼠的心肌细胞在 48h 后过碘酸-Schiff 反应(荧光 PAS 反应)明显减弱,72h 后 DNA 荧光反应阳性物明显低于对照组;电镜观察心肌细胞内线粒体肿胀、变形,嵴断裂,数量减少,基质密度降低,肌原纤维有断裂,糖原减少。经立体学定量分析,线粒体密度与对照组相比差异有显著性($P < 0.01$)。结论:心虚证可导致心肌细胞的损伤,影响心肌代谢及细胞结构。本研究为心虚证的病因病理学提供一定的实验依据,可指导临床治疗。

主题词 心虚/病理学 心肌/超微结构 @ 荧光组织化学

中医学认为,心主血脉,主神志。脉为血之府,全身的血均在脉中运行,且有赖于心脏的正常搏动而输送到全身,发挥濡养作用。心主神志即心藏神,神指人体生命活动的外在表现,指人的精神、意识、思维活动。张介宾在《类经》中指出:“情致之伤,虽五脏各有所属,然求其所由,则无不从心而发”^[1]。血是神志活动的物质基础,推动血液运行的动力是心气,只有心气充沛,才能保证机体脏腑器官生理功能的进行。因此,心主神明和心主血脉的功能是密切相关的,二者相互影响,相互协调。目前,对心虚证的临床研究较深入,基础研究较少,采用荧光组织化学及电镜技术,研究心虚证大鼠心肌细胞内糖原和 DNA 的分布、含量及超微结构变化少见报道。本实验研究试图探讨心虚证与心脏功能障碍及结构损伤之间的关系,为中医独特的病因学提供科学依据。

1 材料和方法

1.1 动物分组:实验动物选取 Wistar 系雄性成年大鼠 40 只,体重 200~250g,由山东大学实验动物中心提供。大鼠在安静、温暖、避强光的环境中饲养 1 周后,随机分为实验造模组和对照组,室温 23~25℃。

1.2 动物模型的建立:在自然光照条件下,于实验室内采用小站台水环境技术剥夺大鼠快速眼动相(REM)睡眠方法建立心虚证动物模型^[2],按 Mendelson^[3]的工作条件,将实验组大鼠放在高于水面 1.5cm 的小站台上,站台直径为 4.5cm,同时于实验室中放置一个定时钟,30 分钟响铃 1 次。对照组大鼠放在高于水面 1.5cm 的大站台上,站台直径为 13.5cm。实验期间,两组大鼠均可自由饮水和摄食。

1.3 操作方法:于造模的 48h、72h,分别取实验组及对照组大鼠各 10 只,快速断头处死;在冰盒上迅速取心肌组织(组织块宜小)。

1.3.1 荧光组织化学方法:组织块经 Carnoy 液固定后,用 AO 恒冷箱切片机进行冰冻切片(厚度 30μm),分两套收集切片。过碘酸-Schiff 反应(荧光 PAS 反应)方法如下,切片经 1%高碘酸水溶液,入吖啶黄 Schiff 试剂染色,在 1%盐酸乙醇中分化,梯度乙醇脱水,二甲苯透明,DPX 封片,待观察。荧光 Feulgen 染色时可切片入 1N 盐酸水解,蒸馏水洗后入吖啶黄 Schiff 试剂染色,再经蒸馏水洗后,入 1%盐酸乙醇中分化,脱水、透明、封片步骤同上,待观察。以上切片在 POLYVAR 万能显微镜下观察,激发滤片 BG12,屏障滤片 515~530。

1.3.2 电镜标本采集及样本处理:于造模的 72h 在大鼠心脏取 1mm³ 心肌组织,置入 4%戊二醛固定液中,4℃条件下固定 8h,用 pH7.4 的 PBS 冲洗 3 次,再用 1%四氧化锇后固定 4h,用 PBS 充分漂洗后,梯度乙醇脱水。用 812 树脂包埋,LEKB-V 型超薄切片机切片,醋酸双氧铀和枸橼酸铅染色。用 JEOL-1200EX 透射电镜观察(加速电压 80kV,放大倍率在 1.0 万~3.0 万倍之间)并摄影。

1.4 指标测定

1.4.1 荧光组织化学反应:(1)荧光阳性物定性观测。(2)荧光阳性物半定量观测:观察阳性荧光物的密度和强度。将阳性荧光物分为强阳性(+++),阳性(++),弱阳性(+)。

1.4.2 电镜技术:(1)电镜定性观测:观察心肌细胞线粒体、糖原、肌原纤维等的分布、含量及毛细血管的超

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附表 两组心肌线粒体密度比较($\bar{x} \pm s$)

组别	造模时间	标本数	线粒体占核周胞质比例(%)
对照组	72小时	10	23.48±1.60
造模组	72小时	10	16.34±2.36*

与对照组比较, * $P < 0.01$

微结构。(2)电镜定量观测:用形态学计量法经立体学定量分析,观测线粒体密度。

2 结果

2.1 荧光组织化学反应

2.1.1 荧光PAS反应:对照组大鼠的心肌细胞胞质内,分布有大量的糖原,呈金黄色荧光颗粒,近肌膜处尤为明显,金黄色荧光颗粒聚集排列成金黄色线样结构,荧光阳性物呈强阳性(+++),其他部分呈绿色荧光。实验组大鼠心肌细胞胞质内的金黄色荧光颗粒明显减少,荧光强度减弱,荧光阳性物呈阳性或弱阳性(++~+);尤其在造模72h荧光阳性物呈弱阳性(+),分布在肌膜附近,其他部分呈绿色荧光。

2.1.2 荧光Feulgen染色:对照组大鼠的心肌细胞内,有体积较大的亮金黄色荧光物(为心肌细胞核内的DNA),位于心肌细胞的中央,荧光阳性物呈强阳性(+++),周围部分呈暗绿色荧光。实验组大鼠心肌细胞内的亮金黄色荧光物,大小不等,荧光强度减弱,荧光阳性物呈阳性(++);造模72h大鼠心肌细胞内的荧光阳性物呈弱阳性(+),数量明显减少,荧光强度明显减弱,周围部分呈暗绿色荧光。

2.2 电镜技术

2.2.1 电镜定性观测:对照组心肌细胞形态结构完整,肌质较丰富,内含各种细胞器及丰富的线粒体和糖原,肌原纤维完整。线粒体体积较大,为圆形或卵圆形,呈串状排列在肌丝区之间,线粒体嵴结构清晰,线粒体周围有糖原分布,闰盘处可见细胞连接。毛细血管内皮细胞结构完整,形态正常,内皮含大量吞饮小泡。造模组心肌细胞轮廓较模糊,肌质内细胞器结构不完整。线粒体大小悬殊,排列不规律,且出现肿胀和畸形,线粒体嵴变形、扭曲,甚至断裂、消失,呈空泡状,基质密度减少,糖原分布稀疏,肌原纤维有断裂。细胞核局部有凹陷,染色质聚集呈块状或溶解,核仁松散或消失。闰盘局部细胞连接有损伤。毛细血管内皮的吞饮小泡数量减少。

2.2.2 电镜定量观测:附表示,心肌线粒体密度造模组与对照组比较,差异有显著性($P < 0.01$)。

3 讨论

该造模法剥夺大鼠REM睡眠,再加上间断的定时铃声刺激,均可使深度睡眠或梦中的大鼠突然坠

入水中,由梦中惊醒,该情况反复发生,造成不寐,模拟了中医“惊”和“劳”的病因。反复的刺激造成生化之源不足,心失所养,心不主血,神不内守,致大鼠出现心悸、失眠、易惊、体倦无力、心神不宁等。根据孙福立等^[2]心血管功能指标的测定,大鼠出现心率加快、血压降低,与中医所称的心悸、细数和细弱的脉象相吻合。

正常情况下,心肌细胞周围分布有大量的毛细血管,心肌细胞内线粒体丰富,以保证氧和能量的供给及氧化代谢的正常进行,使心肌有规律地进行舒缩活动,维持其射血功能,濡养机体。本实验结果表明,实验造模组大鼠心肌细胞的荧光PAS反应及荧光Feulgen染色的DNA反应均较对照组弱,反应产生的荧光阳性物强度降低,且随造模时间的延长反应强度明显降低。心肌超微结构显示,线粒体数量及形态结构变化较大,糖原减少,细胞器及细胞核均有不同程度的损伤;肌纤维间的毛细血管内皮受损,吞饮小泡减少。这说明造模组大鼠因不良刺激、过于疲劳,致机体耗氧量增加,使心肌相对缺氧。在缺氧情况下,心肌的磷酸肌酸及ATP被大量消耗,有氧化障碍,糖酵解加强,糖原分解加速,故糖原明显减少甚至消失,致使荧光PAS反应减弱,能量代谢障碍,同时还影响到DNA的合成和代谢,致使染色质内的DNA被酶解成片段,导致荧光DNA反应减弱。由于糖酵解释放的能量有限,能量供给相对不足,引起心肌收缩力下降,血压下降,心肌缺血缺氧及代谢障碍,导致心肌超微结构的改变,出现电镜下观察到的线粒体的改变、肌原纤维的改变、毛细血管的改变等结构变化及线粒体数量减少。线粒体被称为细胞内的“能量工厂”,其主要功能是进行氧化磷酸化,合成ATP,为细胞生命活动提供直接能量。同时,线粒体亦是细胞内最易受损伤的一个敏感的细胞器,它可显示细胞受损伤的程度。本实验结果从心肌细胞的功能及结构变化解释、印证了心虚证的临床表现,反映了心虚证与心脏功能障碍及结构损伤之间的关系,为临床诊断治疗心虚证提供实验依据^[5]。

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ABSTRACTS FROM ORIGINAL ARTICLES

Clinical observation on 102 cases of alcoholic fatty liver treated with Juge Yigan Decoction

Jia Jingpeng, Jiang Sen

Objective: To explore the ideal method to treat alcoholic fatty liver. **Methods:** 102 cases of alcoholic fatty liver were treated with Juge Yigan Decoction as treatment group, and the effect was compared with control group. **Results:** Effect of treatment group was superior to that of control group. Clinically cured rate, effective rate and ineffective rate in treatment group were 74.5%, 21.6% and 3.9% respectively, superior to 31.6%, 56.1% and 12.3% in control group. There was significant difference between two groups ($P < 0.01$). **Conclusion:** Juge Yigan Decoction is an ideal choice for treatment of alcoholic fatty liver.

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Key Words: Fatty liver, alcoholic/TCM therapy

(Original article on page 750)

Clinical observation on 123 cases of rheumatoid arthritis treated with Sitengyin

Xu Feng, Dai Xiaoming, Zhang Guanghua, et al.

Objective: To observe the effect of Sitengyin on rheumatoid arthritis (RA). **Methods:** 123 cases of RA were treated with Sitengyin, and 67 cases were given Methotrexate and Nimesulide as control. Main symptoms, signs and results of related laboratory tests were compared between two groups before and after treatment. **Results:** Markedly effective rate and total effective rate of treatment group were 72.36% and 95.94% respectively, and those of control group were 65.67% and 86.57%. There was significant difference in efficacy between the two groups ($P < 0.05$). Compared with control group, improvement of main symptoms, signs and results of laboratory tests of treatment group was more remarkable with less side-effect, indicating that efficacy of treatment group was superior to that of control group. **Conclusion:** Sitengyin is definitely effective in treatment of RA, with less side-effect. It is of great value of application and development.

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Key Words: Arthritis, rheumatoid/TCM therapy

(Original article on page 760)

Experimental study on fluorescence histochemistry and ultra-microstructure changes of cardiac muscle of model rats with heart deficiency syndrome

Liu Liqing, Zhou Shengnian

Objective: To observe the effect of heart deficiency syndrome on microstructure and ultra-microstructure of cardiac muscle cells.

Methods: Healthy rats were randomly divided into model group and control group. For model group, cardiac muscle was taken respectively after 48 hours and 72 hours to make frozen slices and ultra-filmy slices. Fluorescence and electric microscope were used to observe changes of distribution, content and ultra-microstructure of heparin and DNA within cardiac muscle cells. **Results:** After 48 hours, fluorescence PAS reaction of cardiac muscle cells of model group reduced significantly, and after 72 hours, electropositive matters in DAN fluorescence reaction was much less than that of control group. Based on tridimensional and quantificational analysis, there was significant difference in chondriosome density between two groups ($P < 0.01$). **Conclusion:** Syndrome of heart deficiency could result in injury of cardiac muscle cells and affect metabolism of cardiac muscle and structure of cardiac muscle cells.

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Key Words: Heart deficiency syndrome/pathology, cardiac muscle/ultra-microstructure

(Original article on page 775)

Effect of Xianqi Decoction on bone mass density, IL-6 and plasma acid phosphatase in experimental osteoporosis rats

Zhang Lijun, Zhang Yan, Xu Qian, et al.

Objective: To investigate the effect of Xianqi Decoction on experimental osteoporosis of rats and its mechanism. **Methods:** 72 female SD rats aged 7 months were randomly divided into two groups, i. e. 60 for operation group, 12 for sham-operation group. Bilateral ovariectomy was performed on operation group. 2 months later, postmenopausal osteoporosis model rats were successfully established. Rats in operation group were randomly divided into model group, western medicine group, Xianqi Decoction low dose group, middle dose group and high dose group. Total six groups were given corresponding medicine by intragastric perfusion. 60 days later, rats were sacrificed after taking blood sample. Serum IL-6, plasma acid phosphatase (ACP) and bone mass density of vertebra and femur were determined. **Results:** Compared with model group, IL-6 and ACP level in three Xianqi Decoction groups was significantly reduced ($P < 0.05$ or $P < 0.01$), and there was no significant difference among Xianqi Decoction groups and western medicine group. Bone mass density in all drug-administered groups was increased ($P < 0.05$).

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Key Words: Osteoporosis/TCM therapy, Bone mass density/drug effect

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