

超声波降血脂驱动脉粥样硬化斑的实验病理研究

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Experimental Pathologic Studies of Effects of Ultrasound on Lipidemia Reduction and Plaque Ablation

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Although ultrasonic therapy on the coronary heart disease have been identified as in effect, no studies have evaluated the capability of the ultrasound eliminating atherosclerotic lesions. The purpose of the present study was to elucidate the roles of the ultrasound in the hyperlipidemia reduction and the plaque ablation on the basis of the experimental animal models.

Methods Thirty Chinese white rabbits were divided into 3 groups: model, natural eliminating and ultrasonic therapeutic group, according the age and the body weight. In every group the experimental hyperlipidemia and atherosclerosis were produced by feeding high lipid diets for 110 days. Then model group served as control to assess on the atherosclerotic lesions, other two groups were given to normal diets maintaining 63 days so as to evaluate the role of ultrasonic plaque ablation. In the therapeutic group ultrasound was irritated every day for 15 min in front of the heart area, using pulsed mode, frequency 800 kHz and intensity $1.0 \text{ W} \cdot \text{cm}^{-2}$. At the all experimental process the serum total cholesterol level was measured

once every twenty days. At the end of experiment samples from coronary arteries and aortae were taken using different sections. Light sections were stained with hematoxylin-eosin and weigert and examined by light microscopy lesions with point lattice test system. Ultrathin sections were only to qualitative investigation.

Results In the experimental hundred-tenth days the serum total cholesterol level of the three groups was distinctly higher than that of the beginning of experiment. By the end of experiment the serum total cholesterol level; the incidence of coronary arterial sections with lesion, and the volume density of atherosclerotic lesions of coronary arteries and aortae in the therapeutic groups were $26.01 \pm 8.15 \text{ mmol} \cdot \text{L}^{-1}$, 7.86%, 36.59% $\pm$ 6.98%, 7.95% $\pm$ 3.16%, respectively. These results were remarkably lower than those of the natural eliminating groups ($P = 0.05 \sim 0.01$).

Conclusions Our results suggest that the ultrasound has the significant effects to decrease concentrations of the serum total cholesterol and to eliminate the atherosclerotic plaques. This study was provided the theoretical base to treat coronary heart diseases with the ultrasound.

KEY WORDS Ultrasonic therapy; Hyperlipidemia; Atherosclerosis

摘要 为探讨超声波对冠心病奏效的机理,在人工高脂血症及动脉粥样硬化家兔模型的常规饲养63天中,对超声波治疗及自然消退两组做了比较观察。结果表明,实验终期血清总胆固醇水平、冠状动脉截面粥样硬化病变率、冠状动脉及主动脉粥样硬化病变体积密度等指标,超声波治疗组均较自然消退组分别减少43.7%、44.4%、30.3%及48.4%,统计学差异非常显著

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($P < 0.05 \sim 0.01$)。这显示,超声波具有降低血脂和剔除粥样硬化病变的明显作用,为治疗冠心病提供了理论基础。

关键词 超声波治疗;高脂血症;动脉粥样硬化

1929年Harvey等首先将超声波用于心脏直接照射以来,一些学者先后进行了心脏疾病的超声波治疗研究,结果皆因方法不当引起休克等严重副作用而将心脏定为“超声禁区”。1971年日本山中^[1]总结了以往超声波照射心脏失败的经验,改进了治疗方法,采用低频低强度超声波照射,获得了实验性心肌梗塞治疗的成功。此后,Koeppen^[2]将超声波用于人患心脏疾病的治疗,于心前区多次照射未发现对心脏及微循环功能有任何不良影响。Weiss^[3]对心肌梗塞患者心前区进行多次照射,获得疗效,提出也适用于老年患者。1975年后,我国学者开始将超声波用于冠心病的治疗^[4],作者等迄今已累积不少这方面的临床资料,证明超声波对冠心病具有明显疗效^[5]。本文目的是通过动物实验探讨超声波对冠心病疗效的机理,以为心、脑、肢体动脉粥样硬化(atherosclerosis, As)的治疗提供理论基础。

1 材料和方法

1.1 造型与治疗

大耳白兔30只,雌雄各半,体重2.5~3.0 kg,分模型组(model group, MG)、自然消退组(natural eliminating group, NEG)及超声波治疗组(ultrasound therapeutic group, UTG),每组10只。各组喂以高脂混合饲料(蛋黄粉5 g、猪脂1.5 g、基础饲料43.5 g)110天以造成高脂血症(hyperlipidemia, HL)及As。第111天,MG按下述方法处置,用以评定As发生程度,其余两组改喂常规饲料,持续63天。此间UTG,应用VD10205型或74-Ⅱ型超声波治疗仪,以脉冲式、频率800 kHz、声强 $1.0 \text{ W} \cdot \text{cm}^{-2}$ 于心前区(剪毛,涂液体石蜡)移动声头照射15 min,每天1次。

1.2 血清总胆固醇测定

实验全程中,每隔20天左右,由各组兔耳动脉采血4 ml,于日本制生物化学分析仪用三酸比色法测定血清总胆固醇(total cholesterol, TC)水平。

1.3 冠状动脉制样及病变定量

实验终期,三组家兔以4%戊巴比妥钠5~6 ml注入耳缘静脉麻醉,开胸取出心脏及主动脉。心脏用2%多聚甲醛-2.5%戊二醛的 $0.1 \text{ mol} \cdot \text{L}^{-1}$ 磷酸缓冲液(pH 7.4)经左右冠状动脉口灌注固定10 min,再于该液中浸泡固定48 h。然后从每个心脏的肺动脉瓣环下开始,每隔3 mm横贯左右心室切取6片组织,连续编号,同向切面向上做石蜡包埋。每个包埋块间隔30 μm 切两张切片,分别做苏木素伊红及弹力纤维染色。光镜观察,计数冠状动脉大支(管径300 μm 以上)、中支(管径50~300 μm)及小支(管径50 μm 以下)截面数及病发数;用方格点测试系统计数落在各级冠脉截面粥样病变及非病变区点数,按 $\text{VDi} = \text{Pi}/\text{Pt}$ 公式计算粥样硬化病变体积密度(volume density, VD)^[6]。

1.4 主动脉制样及病变定量

主动脉剪成12 cm长,内膜向内疏松剥离,按常规做石蜡包埋。每个包埋块以30 μm 间隔切4张切片,做苏木素伊红及弹力纤维染色各两张,按上述方法求出主动脉全长切面病灶的VD。

1.5 超薄切片制备及定性观察

从部分病例的冠状动脉及主动脉病灶取材,按电镜样品制备常规于LKB4801A型超薄切片机制作半薄切片,次甲基蓝一天青Ⅱ及碱性复红染色,光镜观察定位。然后制取超薄切片,常规电子染色,H600A型电镜下定性观察和拍照。

1.6 统计学处理

治疗前后对比用 t 检验,组间比较用 t 及 χ^2 检验。

2 结果

2.1 血清总胆固醇水平

实验过程中三组血清TC水平见Table 1。可见造型至第110天,三组TC水平较实验开始时增高4.9~5.1倍。常规饲养第63天,每组TC水平均明显下降($P < 0.001$),但UTG降低的幅度较NEG大(-88.0%对-78.6%),两组均数差异非常显著($P < 0.01$)。

2.2 动脉粥样硬化病变

2.2.1 冠状动脉 主要病变是内皮细胞变性、肿胀,细胞间及与基膜间连接处分离,有的胞浆含为数不等的脂滴,胞体突入管腔或脱落。内皮下层胶原纤维和弹力纤维增生,内弹力膜断裂,少量脂质沉着,有平滑肌细胞浸入和泡沫细胞形成(Figure 1,2)。中膜平滑肌细胞变形、分离、排列紊乱,有灶状增生,与内膜的平滑肌

Table 1. The role of ultrasound in reducing the serum total cholesterol level ($\bar{x} \pm s$, mmol $\cdot$ L⁻¹)

Groups	n	beginning of experiment	model produced for 110 days	treated for 63 days
model	10	1.10 $\pm$ 0.21	5.62 $\pm$ 1.16	
natural eliminating	10	1.11 $\pm$ 0.81	5.55 $\pm$ 1.29	1.19 $\pm$ 0.43
ultrasound therapeutic	10	1.14 $\pm$ 0.27	5.58 $\pm$ 1.35	0.67 $\pm$ 0.21*

* compared with natural eliminating, $P < 0.01$.

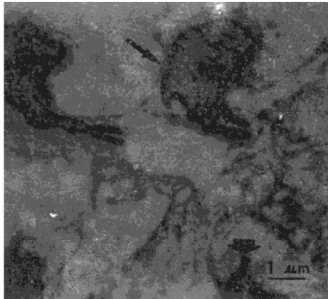


Figure 1. Electron micrograph of the tunica intima of coronary artery in model group showing the swollen endothelial cells (small arrow) and the proliferative smooth muscle cells (rough arrow) in the tunica intima (8 000 $\times$).

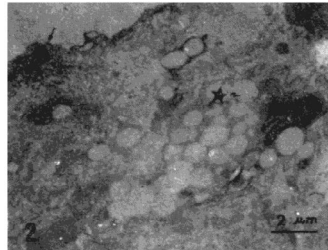


Figure 2. Electron micrograph of the tunica intima of coronary artery in natural eliminating group shows the foam cells (★) in the tunica intima and lipides in the endothelial cells (arrow, 5 000 $\times$).

细胞相移行,有的转变成泡沫细胞。

冠状动脉壁病变多为灶型,少数为环周型,致使管腔狭窄。按管腔面积百分比估计,大都减少25%左右,为Ⅰ级病变;减少26%以上(即Ⅱ级以上病变)者,约占5%。

2.2.2 主动脉 基本病变与冠状动脉相同。光镜下,病变主要是内膜增厚,厚度为100~150 μ m,含大量泡沫细胞及增殖的平滑肌细胞,内弹力膜有破坏(Figure 3,4)。增厚的内膜有时发生小溃疡,血小板沉着。内膜侧中膜平滑肌细胞有灶状增殖。

粥样硬化病灶,以升主动脉最多,主动脉弓次之,胸主动脉最少。

2.3 动脉病变的发生率及体积密度

动脉粥样硬化病变的发生率及病变体积密

度见 Table 2 和 Table 3。可见冠状动脉受累断面发生率,以及冠状动脉及主动脉病灶的 VD, UTG 均较 NEG 明显减少($P < 0.05 \sim 0.01$)。

3 讨论

各组动脉的病变,主要是内皮细胞变性、分离和脱落,泡沫细胞形成与堆积,平滑肌细胞增殖与变异,以致内膜灶状或片状增厚,受累冠状动脉狭窄主要为Ⅰ级。因此本模型与一些学者复制的动脉粥样硬化早期模型^[7]相一致,便于对超声波疗效的观察。

本研究证明,冠状动脉断面病发率及其病变的 VD,以及主动脉全长切面病灶的 VD,皆以 UTG 最小,表明超声波有驱除 As 病灶的明显作用。超声波驱除 As 病变作用的机理迄今未见文献报道,可能与其降血脂及清除病灶异常大分子作用有关。本实验证明,超声波有降 TC 的明显作用,而且随着 TC 水平的降低,As

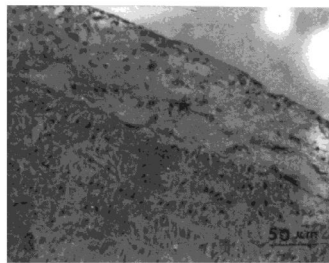


Figure 3. Light micrograph of the aortic wall in model group shows the thickness of the tunica intima (★) increased obviously (200×).

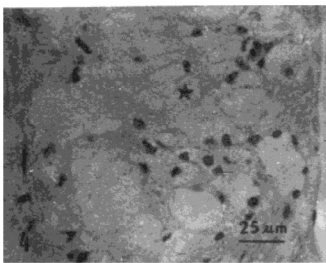


Figure 4. Light micrograph of the aortic wall in ultrasound therapeutic group has the foam cells(★) in the tunica intima thickened obviously(400×).

Table 2. The incidence of the transverse sections with atherosclerotic lesions in the 4 516 various transverse sections of coronary arteries.

Groups	n	the number and the percentage of (%)			mount
		large branches	medial branches	small branches	
model	8	4/153 (2.61)	85/533 (15.95)	153/931 (16.43)	242/1 617 (14.97)
natural eliminating	9	7/148 (4.73)	92/560 (16.43)	138/948 (14.56)	237/1 656 (14.31)*
ultrasound therapeutic	8	0/116 (0.00)	39/424 (9.20)	60/703 (8.53)	99/1 243 (7.96)**
amount	25	11/417 (2.64)	216/1 517(14.24)	351/2 582(13.59)	578/4 516 (12.80)

* P>0.05, compared with model group; ** P<0.01, compared with natural elimination group.

Table 3. The volume density (VD) of the atherosclerotic lesions in arteric walls ($\bar{x}\pm s\%$).

Groups	n	coronary arteries	aortae
model	6	49.15±6.92	21.77±5.26
natural eliminating	7	52.53±13.23*	15.42±4.95*
ultrasound therapeutic	8	36.59±6.98***	7.95±3.16**

* P>0.05, compared with model group; ** P<0.05, *** P<0.01, compared with natural eliminating groups

病变的 VD 也明显减少,说明超声波降 TC 有使 As 病变消退的明显作用。超声波降血脂作用,是否与其增强血脂氧化和排泄以及其反射波抑制肠道吸收和肝脏合成胆固醇有关,有待深入研究。

自然消退组的 TC,虽自然下降至实验前的水平,但 As 病变与模型组比较却无明显改善,提示 As 消退尚须另一机制同时参与。一般

认为,超声波可增强细胞原浆微流、细胞容积运动及细胞膜渗透性等,并能激发组织细胞的粒子的高频振荡。这种生物理化效应有可能促使粥样病灶中脂质等异常大分子移动、分离和释放。这对于清除 As 病灶可能具有重要作用。

本研究提示,超声波对冠心病治疗的有效性,可能是通过其降血脂、消斑块或伴溶栓^[8]等途径而改善心肌缺血的结果。因此,超声波用于

脑或肢体 As 的治疗也应当是有效的。

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