

# 还精煎对老年小鼠胸腺超微结构及性激素受体的作用观察

生化教研室 赵伟康 潘迎宪 万叔援  
上海中医学院基础部 电镜室 徐凤仙 郭振英 严凌鹤

**内容提要** 本文观察了以填补肾精为主的延缓衰老方剂还精煎对老年小鼠胸腺超微结构及胸腺性激素受体等的影响。结果表明还精煎能明显提高老年小鼠胸腺重量；改善胸腺超微结构的老年性变化；使胸腺胞浆蛋白质、核 RNA 和 DNA 含量显著增加；并减少胸腺核雌二醇 ( $E_2$ ) 受体和双氢睾酮 (DHT) 受体数量，从而减弱  $E_2$ 、DHT 对胸腺的致萎缩作用。还精煎的上述作用提示填补肾精法延缓衰老可能与调整下丘脑—垂体—性腺—胸腺轴功能有关。

许多临床和实验研究证明：神经内分泌系统和免疫系统在衰老进程中起着重要的作用<sup>(1)</sup>。近年来，许多学者对在发育及衰老中胸腺和神经内分泌之间的关系进行了大量的研究，认为胸腺—神经内分泌网的衰退在机体衰老中占主导地位<sup>(2)</sup>。其中以下丘脑—垂体—性腺—胸腺轴 (HPG—thymic axis) 的研究较为突出<sup>(3)</sup>。我们根据中医“肾藏精，主生殖”，“精气夺则虚”，和“天癸竭，精少，肾脏衰，形体皆极”等记载，以及填补肾精为主的延缓衰老方剂——还精煎，具有延缓生殖系统和胸腺依赖性免疫功能的衰老作用等<sup>(4,5)</sup>，进一步观察还精煎对老年小鼠胸腺超微结构及胸腺性激素受体的影响，试图从性激素与胸腺及其依赖性免疫功能的关系中，进一步研究还精煎对下丘脑—垂体—性腺—胸腺轴的药理作用，探讨“肾精”与下丘脑—垂体—性腺—胸腺轴的关系。

## 材料与方法

一、实验动物：C<sub>57</sub>BL/6J 雄性小鼠，由上海生物制品研究所提供。动物分成三组。青年组：4 月龄，正常饮水及饲料；老年组：16 月龄，正常饮水及饲料；老年用药组：16 月龄，把还精煎药汁拌于混合饲料中制成药物饼干每晨饲予动物，待食完后再供给一般饲料。药物剂量约为成年人的 20 倍。从 12 月龄开始，喂 2 周停 1 周，连续 4 个月。

二、胸腺重/体重比值测定：胸腺重以毫克湿重为单位，体重以克为单位。两者相除即为比值。

三、胸腺超微结构的电镜观察：动物处死后，迅速取出胸腺。固定于二甲砷酸钠缓冲液配制的 2% 戊二醛溶液内 (pH 7.3)。4℃ 冰箱内固定 2 小时，经缓冲

液充分冲洗后，用 1% 锇酸溶液作后固定，再经各级酒精脱水，Epon812 包埋后，用 LKB 超薄切片机切片，厚度约为 400~500Å，铅铀染色，日立 H-600 电镜观察。

四、胸腺胞浆蛋白、核 RNA 和 DNA 含量测定：蛋白测定采用 Lowry 法；RNA 和 DNA 测定分别采用苔黑酚法和二苯胺法。

五、胸腺胞浆及核  $E_2$  和 DHT 受体测定：胞浆受体采用 DCC 法 (葡聚糖—活性炭吸附法)<sup>(6)</sup>。核受体采用 Anderson 的核交换法<sup>(7)</sup>。

六、统计学处理采用 t 检验法。

七、还精煎药物组成：主要由生熟地、首乌、女贞子、桑椹子、牛膝、川断、锁阳、潼蒺藜、菟丝子等中药组成。方中不含参、芪益气药和鹿角、动物胶类等名贵药材<sup>(8)</sup>。

## 实验结果

一、胸腺重/体重比值的变化：由表 1 可见，还精煎能明显延缓老年小鼠胸腺重量及胸腺重/体重比值的减退。

二、胸腺超微结构的电镜变化：与青年组相比，

表 1 还精煎对老年小鼠胸腺重及胸腺重/体重比值的影响 ( $M \pm SE$ )

	动物数 (只)	胸腺重 (mg)	胸腺重 (mg)/ 体重 (g)
青年组	5	63.20 $\pm$ 2.06	2.43 $\pm$ 0.17
老年组	8	37.58 $\pm$ 0.89 $\Delta$	1.24 $\pm$ 0.02 $\Delta$
老年用药组	10	48.80 $\pm$ 0.61* $\Delta$	1.70 $\pm$ 0.05* $\Delta$

$\Delta$  与青年组相比  $P < 0.01$ ；\* 与老年组相比  $P < 0.001$

老年组胸腺的淋巴细胞与上皮性网状细胞均发生明显变化(图1、2)。



图1 老年组胸腺。上皮性网状细胞与淋巴细胞内线粒体肿胀变性，内质网池扩张。×12000

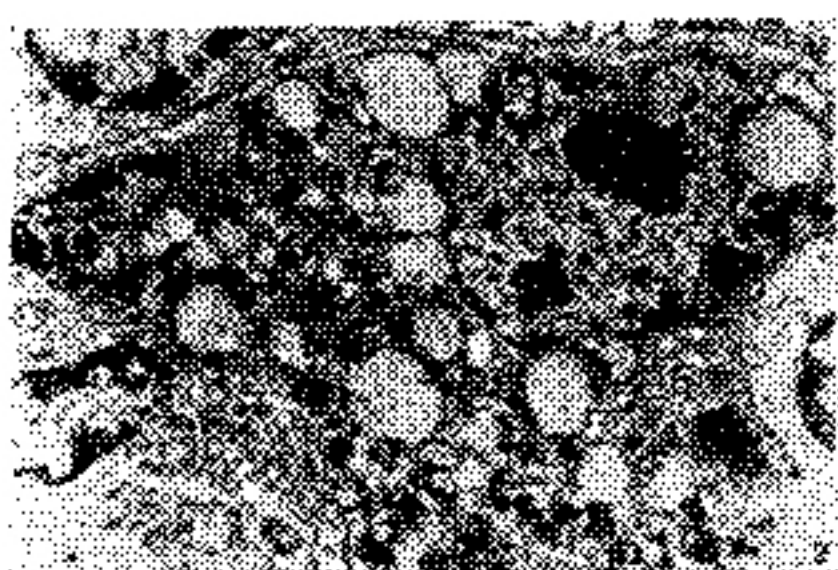


图2 老年组胸腺。巨噬细胞线粒体肿胀变性，脂褐质增多。×12000

淋巴细胞的细胞质内线粒体肿胀变性，嵴消失，游离核糖体含量减少。上皮性网状细胞的细胞核内染色质浓缩，线粒体显著变性肿胀，嵴断裂或消失，内质网池扩张。吞噬细胞内脂褐质与溶酶体增加，内质网显著扩张，线粒体变性肿胀。动物饲以还精煎后胸腺组织的超微结构接近正常(图3、4)。上皮性网状细胞结构清晰，线粒体无肿胀变性，粗面内质网与滑面内质网分布正常，有游离核糖体及张力微粒，细胞核内可见核仁，常染色质均匀分布于核质内，异染色



图3 老年用药组胸腺。上皮性网状细胞与淋巴细胞结构清晰。×20000



图4 老年用药组胸腺。细胞超微结构清晰。×20000

质分布于核周围。淋巴细胞超微结构接近正常，线粒体与内质网无变性现象。

三、胸腺胞浆蛋白质和核RNA、DNA含量的变化：还精煎能显著提高胸腺胞浆蛋白质含量及核DNA、RNA含量见(表2)。

四、胸腺胞浆和核E<sub>2</sub>受体数量的变化：见表3。

表2 还精煎对老年小鼠胸腺胞浆蛋白质、核DNA和RNA含量的影响 (M±SE)

	动物数	胞浆蛋白质	核DNA	核RNA
		mg/100mg 组织		
青 年 组	5	1.61±0.08	1.90±0.11	1.48±0.07
老 年 组	7	1.39±0.08	1.43±0.05△	0.90±0.02△
老年用药组	7	2.43±0.05*△	2.26±0.11*	1.23±0.02*△

△与青年组相比P<0.01；\*与老年组相比P<0.001

表3 还精煎对老年小鼠胸腺胞浆和核E<sub>2</sub>受体数量的影响 (M±SE)

	动物数	胞浆E <sub>2</sub> 受体	核E <sub>2</sub> 受体
	(只)	(fmole/mg蛋白)	(fmole/mgDNA)
青 年 组	5	12.31±4.08	4.27±0.71
老 年 组	7	7.27±0.72	7.79±0.75*
老年用药组	7	11.65±2.64	3.26±0.40**

\*与青年组相比P<0.05；\*\*与老年组相比P<0.01

(1)老年组胸腺胞浆E<sub>2</sub>受体数量比青年组低，老年用药组胸腺胞浆E<sub>2</sub>受体数量趋于增加。(2)老年组胸腺核E<sub>2</sub>受体数量比青年组明显增高。而还精煎可明显降低老年小鼠核E<sub>2</sub>受体数量，使之恢复到接近青年组水平。

五、胸腺胞浆和核DHT受体数量的变化：由表4可见：(1)老年组胞浆DHT受体较青年组有减少趋势，老年用药组胞浆DHT受体进一步减少，与老年组



相比无差异。(2)老年组核 DHT 受体明显高于青年组。还精煎能使核 DHT 受体显著下降达到青年组水平。

表 4 还精煎对老年小鼠胸腺胞浆和核 DHT 受体数量的影响 (M±SE)

	动物数 (只)	胞浆 DHT 受体 (fmole/mg 蛋白)	核 DHT 受体 (fmole/mg DNA)
青年组	5	16.52±2.73	3.36±0.88
老年组	7	6.56±3.48	6.44±0.62△
老年用药组	7	2.89±0.35△	2.56±0.40*△

△与青年组相比  $P < 0.01$ ; \* 与老年组相比  $P < 0.05$

## 讨 论

一、人或动物到性成熟期胸腺即开始退化,随年龄增长而逐渐萎缩。本实验结果表明:与青年组相比,老年组小鼠胸腺重/体重比值明显下降。胸腺组织超微结构变化明显,尤以上皮性网状细胞和淋巴细胞更为突出,与文献报道基本一致<sup>(9)</sup>。老年小鼠胸腺蛋白质、RNA 和 DNA 含量下降的生化测定结果与上皮性网状细胞核染色质浓缩,淋巴细胞内游离核糖体含量减少,以及两者的线粒体肿胀变性,嵴消失等电镜观察结果又相符合。而还精煎能延缓老年小鼠胸腺的萎缩,提高胸腺重/体重比值;明显延缓老年小鼠胸腺组织超微结构的老化,使之接近于青年小鼠;并显著提高老年小鼠胸腺蛋白质、RNA 和 DNA 含量水平。

二、胸腺具有内分泌功能。胸腺上皮性网状细胞能分泌多种胸腺激素,它们能促进 T 淋巴细胞的成熟,是胸腺依赖性免疫的重要因素之一。电镜观察结果表明,还精煎似能通过延缓胸腺上皮性网状细胞的衰老,而保持机体的正常免疫功能。

三、性激素对胸腺具有明显的抑制和致萎缩作用<sup>(10,11)</sup>。性激素核受体的增加,可增强性激素对胸腺的抑制和致萎缩作用,而还精煎则可通过降低核  $E_2$  和 DHT 受体数量对胸腺起保护作用。

四、衰老时胸腺胞浆  $E_2$  和 DHT 受体数量明显减少而核  $E_2$  和 DHT 受体数量则显著增加,反映衰老时  $E_2$  和 DHT 受体由胞浆转移到核的活化能力明显提高,核膜通透性可能发生改变,从而增强了  $E_2$  和 DHT 对胸腺的致萎缩作用。而还精煎能使胸腺核  $E_2$  受体数量减少,并相应使胞浆  $E_2$  受体增加,其作用原理可能改善

了上述功能。至于胸腺 DHT 受体,还精煎不仅能使其核受体数量减少,而且也使其胞浆受体趋于减少,这可能一方面改善了上述功能,同时又抑制了 DHT 受体的合成,有待进一步研究。

五、中医学在“养生、延年”方面有着丰富的经验,并有独特的理论,是中医优势所在。根据中医理论,衰老的调控属于“肾中精气”的功能范畴,人体的生长、发育、壮盛、衰老与“肾中精气”的盛衰有关。以填补肾精为主的还精煎能调整老年动物胸腺  $E_2$  和 DHT 受体数量,延缓胸腺的萎缩和胸腺超微结构的老年性改变,保持正常的免疫功能。联系肾阳虚患者下丘脑—垂体—性腺轴有不同水平、不同程度的功能紊乱,而补肾药物有纠正作用,提示填补肾精法延缓衰老及中医“肾中精气”可能与调整下丘脑—垂体—性腺—胸腺轴功能有关,值得进一步探讨。

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considered as mainspring of the central aging clock located in hypothalamus. In an *in vitro* experiment about 57% inhibition of mouse brain MAO-B activity was shown by the preparation of Shi Jinmo's recipe. The result was consistent with that of *in vivo* experiment, suggesting a direct inhibitory effect of the medicines on brain MAO-B activity. A weak inhibition (about 20%) on liver MAO-B activity by the preparation was also observed. This met the requirement of a monoamine oxidase inhibitor to be used clinically, since an appropriate level of MAO activity in liver was required for the degradation of certain kinds of monoamines in order to avoid their harmful effect. Feeding the preparation showed no influence on the average body weight of animals, indicating that the medicines had no adverse effects on animal growth. In the life span test using female fruit flies, the preparation was fed to the flies for 3 days. The number of survivors was counted up every day and the life span of each fruit fly was recorded. The results showed that the average life span of *Drosophila melanogaster* was conspicuously increased by this medical treatment (21.63%).

The results were evidences on the anti-aging effect of Shi Jinmo's recipe in terms of the biology of aging.  
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### Effect of "Essence-Restoring Decoction" on Thymic Ultrastructure and Sex Hormone ( $E_2$ and DHT) Receptors in Senile Mice

Zhao Weikang (赵伟康), Pan Yingxian (潘迎宪), Xu Fengxian\* (徐凤仙), et al  
Biochemistry Dept. and \*Electronmicroscopic Dept., Shanghai College of TCM, Shanghai

The clinical effect of "Essence-Restoring Decoction", a kidney essence tonifying recipe on retarding aging, has been reported. This paper presents the pharmacological effects of Essence-Restoring Decoction on thymic ultrastructure and thymic sex hormone ( $E_2$  and DHT) receptors in C<sub>57</sub>BL/6J senile mice. The results showed that "Essence-Restoring Decoction" was able to increase the thymic weight, or the thymic weight (mg) per body weight (g) ( $P < 0.001$ ), and markedly enhance the concentration of thymic cytosol protein as well as nuclear RNA and DNA of the senile mice ( $P < 0.001$ ). The senile change of ultrastructure of thymus was studied under electronmicroscope, the thymus of senile mice had an obvious atrophy. In cytoplasm of thymic lymphocytes, there were swelling degeneration of mitochondria, disappearance of its cristae, and reduction of the ribosome, while in epithelial reticular cell, besides the above change in mitochondria, the nuclear chromatin was concentrated. However, the senile change of the thymus of "Essence-Restoring Decoction"-treated aged mice was retarded. The ultrastructure of its thymus became nearly normal. The amount of thymic cytosol and nuclear  $E_2$  and DHT receptors were estimated respectively. It was found that the Essence-Restoring Decoction was able to reduce the amount of thymic nuclear  $E_2$  receptor ( $P < 0.01$ ) and DHT receptor ( $P < 0.05$ ), thus the effect of the inhibition and atrophy of  $E_2$  and DHT to thymus was reduced. The mechanism of the decrease of nuclear  $E_2$  and DHT receptors may be due to the obstruction on the translocation of the sex hormone receptors from cytosol to nucleus or the change of the permeability of nuclear membrane. It was suggested that the retardation of aging by the method of tonifying kidney essence may be related to the regulating of hypothalamus-pituitary-gonadal thymic axis.

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### Study on Anti-Infection Effect of Chinese Ointment for External Application —The Fc Receptors on Peritoneal Macrophages in Mice

Li Xiulan (李秀兰), et al  
Tianjin Institute of Orthopedics, Tianjin

The Fc receptors are the most characterized markers on the macrophage surfaces. This paper reports the using of EA-rosette formation test to investigate the Fc receptors for the evaluation of anti-infection effect of the Chinese drugs for external application. It was found that EA-rosette forming rate of Sheng Ji ointment (生肌膏) and C. parvum-activated peritoneal macrophages in mice were higher than that of control ( $P < 0.001$ ). The rates of Jin Huang (金黄膏), Yu Hong (玉红膏) and Huang Lian ointments (黄连膏) were also higher than control ( $P < 0.001$ ). The result indicated that these Chinese traditional ointments were good immunostimulants. Therefore Fc receptors were activated by these ointments and the vitality of macrophages was enhanced, due to the mechanisms of its anti-infection effect.

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