

肾虚衰老与线粒体 DNA 氧化损伤关系的研究

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摘要 目的:观察肾虚衰老与外周血线粒体 DNA 缺失及线粒体呼吸链复合酶活力的相关性。方法:用聚合酶链反应(PCR)和酶动力学技术。结果:老年肾虚组线粒体 DNA 缺失较青年组和老年对照组明显增多,呼吸链复合酶 I、IV 的活力较青年组和老年对照组明显降低。补肾方五子衍宗丸可明显减少线粒体 DNA 缺失,提高呼吸链复合酶 I、IV 的活力。结论:提示线粒体 DNA 的氧化损伤可能是肾虚的重要原因之一。

主题词 肾虚/遗传学 DNA,线粒体/遗传学

中医认为,肾虚与衰老密切相关。本研究从自由基对线粒体 DNA 氧化损伤入手,于 1999 年 2 月~2001 年 5 月观察了肾虚老年人外周血线粒体 DNA 缺失及其呼吸链复合酶的活力,并观察了五子衍宗丸对其的影响,以期在基因水平上探讨肾虚的物质基础。

1 材料和方法

1.1 观察对象及分组:60~80 岁男性 48 例,为经临床心、肺、肝、脾、尿液、血压等检测正常的健康老年人,符合中华医学会老年医学学会规定标准^[1],再结合中医虚证辨证标准,选出无肾虚者 10 例,为老年对照组,平均年龄 71.70 ± 5.87 岁。老年肾虚者 38 例,从随机数字表中查取随机数字,随机数字奇数者为治疗组 19 例,平均年龄 69.16 ± 4.90 岁,服用五子衍宗胶囊;随机数字偶数者为对照组 19 例,平均年龄 69.32 ± 6.67 岁,服用安慰剂,两组年龄差异无显著性。本研究采用单盲法。五子衍宗药粉(按药典配方:枸杞子 400g,菟丝子 400g,覆盆子 200g,五味子 50g,车前子 100g)购自北京同仁堂医药集团,依照药典服用量分装胶囊,每粒胶囊 1g,1 日 3 次,每次 3 粒。淀粉购自北京医药公司,分装胶囊,其外形、用量与治疗组完全一样。疗程 3 个月。另自本院血库选择 20~35 岁的健康献血男性 10 例,为青年组。

1.2 肾虚辨证标准:参照中医虚证辨证参考标准进行^[2]。肾虚证的主要依据:(1)腰膝酸痛(外伤性除外);(2)胫酸膝软或足跟痛;(3)耳鸣或耳聋;(4)发脱或齿摇;(5)健忘;(6)尿后有余沥或失禁;(7)性功能减退。具有上述 3 项者选为观察对象。

1.3 主要试剂及仪器:Tag 酶、dNTP、蛋白酶 K、胰 RNA 酶、SDS、重蒸酚、丙烯酰胺、还原辅酶 I、甘露醇等购自北京华美生物工程公司,细胞色素 C 为 Sigma 原装,其它试剂均为国产分析纯。PE-9600 型 PCR 扩增仪、Pharmacia LKNUltrascan XL 型激光扫描仪、岛津 UV-2500 紫外分光光度计等。

1.4 外周血白细胞线粒体复合酶 I、IV 活力测定:外周血白细胞线粒体提取参照文献^[3]。静脉取血 10ml,肝素抗凝,分离白细胞。在冰浴中超声破碎,低温高速离心,提取线粒体。用酶动力学方法检测,参照伍期专方法进行^[4]。

1.5 白细胞线粒体 mtDNA 缺失的检测:采用聚合酶链反应(PCR)方法。

1.5.1 PCR 引物:由北京赛百盛生物工程公司合成。参照文献^[5]自行设计:L8171(5' -- TGCTCTGAAATCTGTGGAGC -- 3' 8171 -- 8190)和 H8403(5' -- ATTATGGTGGGCCATACGGTAGTA -- 3' 8403 -- 8378)用于扩增野生型 mtDNA 片断,反映正常 mtDNA 含量,以此作为内参照;L8148(5' -- ACCGGGGGTATACTACGGTC -- 3' 8148 -- 8169) H13811(5' -- GCGAGGGCTGTGAGTTT TAG -- 3' 13811 -- 13792)用于扩增缺失型 mtDNA 片断,反映片断缺失型 mtDNA 含量。

1.5.2 白细胞线粒体 DNA 提取及 PCR 反应条件:静脉取血 0.5ml,EDTA 抗凝,采用玻璃奶吸附法提取线粒体 DNA^[6]。PCR 的反应条件为 94℃ 预变性 5 分钟,1 个循环;94℃ 变性 1 分钟,56℃ 退火 1 分钟,72℃ 延伸 1 分钟,30 个循环;72℃ 延伸 10 分钟。

1.5.3 PCR 产物鉴定及定量:PCR 产物经特异性酶切位点 XbaI 限制性内切酶酶切(识别序列为 T▼CTAGA)来证明。PCR 扩增产物经 8% 聚丙烯酰胺凝胶电泳,硝酸银染色,观察结果。用激光光密度扫描仪进行扫描,取得积分吸光度,计算缺失型 mtDNA 与野生型 mtDNA 含量的百分比。

1.6 统计学方法:采用 SPSS 8.0 统计软件的方差分析、配对 *t* 检验和 *t* 检验。

2 结果

2.1 PCR 产物鉴定:PCR 产物经 XbaI 限制性内切酶酶切(识别序列为 T▼CTAGA),证明人白细胞约有

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表 1 各组外周血白细胞缺失 mtDNA/内参 mtDNA 及其线粒体复合酶 I、IV 活力比较($\bar{x} \pm s$)

组别	例数	缺失 mtDNA/内参 mtDNA (%)	复合酶 I	复合酶 IV
			nmol · mgPr ⁻¹ · min ⁻¹	
青年组	10	0.40 ± 0.26	81.16 ± 15.54	47.93 ± 9.95
老年对照组	10	2.19 ± 0.82 [*]	54.27 ± 12.19 [*]	33.85 ± 8.16 [*]
老年肾虚组	38	7.31 ± 4.75 [△]	38.61 ± 8.77 ^{*△}	23.18 ± 6.42 ^{*△}

与青年组比较, * $P < 0.01$; 与老年对照组比较, $\Delta P < 0.01$

表 2 治疗前后两组外周血白细胞缺失 mtDNA/内参 mtDNA 及其线粒体复合酶 I、IV 活力比较($\bar{x} \pm s$)

组别		缺失 mtDNA/内参 mtDNA (%)	复合酶 I	复合酶 IV
			nmol · mgPr ⁻¹ · min ⁻¹	
治疗组	治疗前	7.64 ± 3.42	37.53 ± 9.96	22.83 ± 6.70
	治疗后	4.17 ± 3.07 ^{**}	46.75 ± 10.76 ^{**}	28.76 ± 8.30 [*]
	差值	3.48 ± 2.53 [#]	9.21 ± 9.18 [#]	5.93 ± 11.10
对照组	治疗前	6.99 ± 5.88	39.76 ± 7.43	23.55 ± 6.28
	治疗后	6.11 ± 4.08	41.27 ± 7.88	24.38 ± 8.63
	差值	0.87 ± 2.13	1.51 ± 4.19	0.82 ± 4.07

与治疗前比较, * $P < 0.05$, ** $P < 0.01$; 与对照组差值比较, # $P < 0.01$

5.0kb 的缺失。

2.2 老年肾虚与外周血白细胞线粒体 DNA 缺失、线粒体复合酶 I、IV 活力的关系:见表 1,结果表明,老年肾虚组和老年对照组外周血白细胞 mtDNA 缺失率明显高于青年组,其线粒体复合酶 I、IV 活力较青年组明显降低($P < 0.01$);老年肾虚组外周血白细胞 mtDNA 缺失率及线粒体复合酶 I、IV 活力又较老年对照组明显增高和降低($P < 0.01$)。

2.3 补肾方五子衍宗丸对老年肾虚外周血白细胞线粒体 DNA 缺失及其线粒体复合酶 I、IV 活力的影响,见表 2。治疗前两组白细胞线粒体 DNA 缺失及其线粒体复合酶 I、IV 活力无明显差异($P > 0.05$)。治疗后治疗组白细胞缺失 mtDNA/内参 mtDNA 较治疗前明显降低($P < 0.01$),白细胞线粒体复合酶 I、IV 活力均较治疗前明显升高($P < 0.05$ 或 $P < 0.01$)。对照组与治疗前相比,各项指标的变化差异无显著性($P > 0.05$)。白细胞 mtDNA 缺失及线粒体复合酶 I 治疗前与治疗后的差值比较,治疗组与对照组差异均有显著性($P < 0.01$)。

3 讨论

本研究结果发现无论是无肾虚老年人还是肾虚老年人其外周血白细胞 mtDNA 缺失率明显高于青年人,线粒体复合酶 I、IV 活力明显低于青年人。说明 mtDNA 的氧化损伤在正常的青、老年人均可出现,这种损伤随年龄增加,由于 mtDNA 损伤比例较小,正常 mtDNA 代偿了损伤 mtDNA 所至的不良影响,临床无任何表现。当 mtDNA 损伤累计(累计效应)到一定程度,ATP 的生成低于所需能量最低域值时(阈值效应),导致细胞能量的缺陷,临床上则出现了一系列衰

老的变化。这可能是本研究发现的肾虚老年人外周血白细胞 mtDNA 缺失率及线粒体复合酶 I、IV 活力较无肾虚老年人明显增高和降低的原因。因此 mtDNA 损伤可能是导致肾虚衰老的重要原因之一。

中医认为“肾”为先天之本,主生长发育衰老的过程。五子衍宗丸为补肾益精的代表方,方中枸杞子、菟丝子补肾,覆盆子滋精,五味子生津,车前子利肾固精。主要用于肾气不足、阳痿早衰、精寒无子、小便余沥等症。研究证明,该方有明显改善老年肾虚衰症状、降低血中过氧化脂质含量、提高 SOD 活力等延缓衰老的作用^[7]。本研究结果表明,该方可明显减少肾虚老年人外周血白细胞线粒体 DNA 缺失,提高肾虚老年人线粒体复合酶 I、IV 活力。说明补肾可以减少 mtDNA 氧化损伤,证明了肾虚与 mtDNA 氧化损伤的相关性,并在基因水平提供了补肾延缓衰老的作用机理。

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ABSTRACTS FROM ORIGINAL ARTICLES

Clinical observation on treatment of 34 cases of primary hypertension of type of stagnation of phlegm with Fang Ji Huang Qi Capsules combined with western drug

Xiao Yan, Wen Wangxiu, Cheng Kanglin, et al.

Objective: To investigate effects of Fang Ji Huang Qi Capsules on the level of blood uric acid and blood pressure in the patient of primary hypertension of type of stagnation of phlegm.

Methods: 61 patients were divided into the treatment group (34 cases) treated with Fang Ji Huang Qi Capsules plus Norvasc and the control group (27 cases) treated with Norvasc. They were treated for 6 weeks and blood uric acid level, dynamic blood pressure and TCM clinical symptoms were observed. **Results:** The blood uric acid level decreased significantly, the valley to peak ratio of dynamic blood pressure increased and cumulative score of TCM clinical symptoms decreased significantly in the treatment group as compared with the control group ($P < 0.05$). **Conclusion:** Fang Ji Huang Qi Capsules can effectively improve blood uric acid level, decrease blood pressure and improve clinical symptoms for the patient of primary hypertension of the type of stagnation of phlegm.

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Key words: Hypertension/TCM therapy, @Fang Ji Huang Qi Capsules

(Original article on page 271)

Clinical study on modified Wei Er Fang for treatment of 30 cases of gastric precancerous lesion

Chen Yuanling, Pan Yibin

For observation on clinical therapeutic effect of modified Wei Er Fang on gastric precancerous lesion (GPL), 90 cases of GPL were divided into 3 groups, the treatment group treated with modified Wei Er Fang, the control group I treated with Wei Fu Chuan and the control group II with Bismuth subcitrate, 30 cases each group. Results indicated that the total effective rate for clinical symptoms was 96.67% in the treatment group which was significant different from 60.00% in the control group I ($P < 0.05$). There was no significant difference among the 3 groups in pathological therapeutic effect of gastroscopy ($P > 0.05$), but the therapeutic effect on heterotypic proliferation in the treatment group was superior to those in the two control groups. It is showed that modified Wei Er Fang has a better action of reversing gastric precancerous lesion.

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Key words: Gastric tumor/TCM therapy, precancerous state, / TCM therapy. @Wei Er Fang

(Original article on page 275)

Clinical study on changes of blood LPO, SOD, GSH — Px and $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ in red blood cell membrane in the patient of blood — heat syndrome

Fang Bangjiang, Zhou Shuang, Chen Ruquan

In order to study on pathological essence of blood — heat syndrome and changes of anti — oxidation system of the organism with blood — heat syndrome, blood LPO content, SOD and GSH — Px activities and activity of $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ in red blood cell membrane in 30 cases of blood — heat syndrome were observed and the normal group were used as control. Results indicated that LPO content increased very significantly ($P < 0.01$), SOD and GSH — Px and $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activities decreased significantly ($P < 0.05$) in the patient of blood — heat syndrome. It is suggested that defense function of the antioxidation system decreases and lipid peroxidation strengthens in blood — heat syndrome.

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Key words: Blood heat/zymology

(Original article on page 287)

Research on the relation between kidney deficiency senility and oxidized injury of mitochondrial DNA

Wang Xuemei, Fu Hong, Liu Gengxin

The relation between mitochondrial DNA (mtDNA) deletions, respiratory chain complex enzymes and kidney deficiency syndrome in the senility was studied by using polymerase chain reaction (PCR) and enzyme kinetic technique. Results indicated that the mtDNA deletion increased significantly and the activities of mitochondrial respiratory chain complex enzyme I, IV in the senile kidney deficiency group decreased significantly as compared with the youth group and the senile control group. Wu Zi Yan Zong Pill, a prescription for tonifying the kidney, could reduce the mtDNA deletion and raise the activities of mitochondrial respiratory chain complex enzyme I, IV in the senile kidney deficiency group. It is suggested that the oxidized injury of mtDNA can be regarded as one of the important reasons in kidney deficiency syndrome.

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Key words: Kidney — deficiency syndrome/genetics, mitochondrial DNA/genetics

(Original article on page 294)