

胸腺因子 D 对老年大鼠肝脏过氧化脂质、谷胱甘肽及膜流动性的影响

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Effect of thymic factor D on lipid peroxide, glutathione, and membrane fluidity in liver of aged rats

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ABSTRACT The effects of thymic factor D (TFD) on aging were investigated in ♂ Wistar rats at the age of 24 months. Thymic factor, polypeptides extracted from swine thymus, was given to aged rats in the dose of 2 mg·kg⁻¹ sc every other day for 3 months. It was shown that TFD decreased the content of malondialdehyde (MDA) and lipofuscin in the liver of aged rats, accompanied by an increase of glutathione (GSH). Besides, the declined fluidity of hepatic mitochondrial and microsomal membranes in aged rats was recovered to the level of young adult rats by administration of TFD. It is suggested that TFD may possess beneficial effects on retarding the senile metabolic changes in hepatic cells.

KEY WORDS thymic factor; liver; aging; lipid peroxides; glutathione; membrane fluidity

摘要 本实验旨在研究胸腺因子 D 对老化的影响。胸腺因子 D (TFD) 为猪胸腺提取的分子量小于 14.4 kDa 的复合多肽。给老年 ♂ Wistar 大鼠 sc TFD 2 mg·kg⁻¹, 隔日一次, 共 3 月, 可明显降低肝脏丙二醛 (MDA) 及脂褐素生成, 增高谷胱甘肽 (GSH) 含量, 并使降低的肝线粒体、微粒体膜流动性得以恢复到成年大鼠水平。TFD 可能对延缓衰老有益。

关键词 胸腺因子; 肝; 衰老; 脂质过氧化; 谷胱甘肽; 膜流动性

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胸腺因子 D (thymic factor D, TFD) 为猪胸腺提取的分子量小于 14.4 kDa 的复合多肽, 具免疫调节功能, 主要用于治疗免疫缺陷病、难治性肺结核和急、慢性病毒性肝炎等⁽¹⁾。我们曾报道胸腺的存在对于维持大鼠肝脏的正常抗氧化功能具有重要作用⁽²⁾。目前普遍认为过氧化脂质 (lipid peroxides, LPO) 增多是机体衰老的重要标志。为了研究胸腺提取物对老化的影响, 本文观察了 TFD 对老年大鼠肝脏过氧化脂质、抗氧化物谷胱甘肽 (glutathione, GSH) 及生物膜流动性的作用。

MATERIALS AND METHODS

胸腺因子 D 注射液 (2.5 mg·ml⁻¹), 福州金山药厂产品, 批号 881013。经高效液相色谱法分析, 其组分组成及组分相对百分含量均不同于国内 9 种胸腺素、胸腺肽及美国 thymus factor。它是另一种胸腺激素免疫调节剂。其 uv 吸收光在波长 250 ± 1 nm 处有最大吸收峰, 以此可与其它胸腺激素鉴别。

硫代巴比妥酸, 1, 6-二苯基-1, 3, 5-, 已三烯 (DPH, sigma); 邻苯二醛 (Fluka); N-乙基马来酰亚胺 (上海生物化学研究所东风试剂厂); 其余试剂均为国产 AR。

Wistar ♂ 大鼠, 本院实验动物中心供给, 自由摄食饮水, 18-25℃ 喂养。分组: (1) 成年对照组: 7 月龄, 注射生理盐水 (NS); (2) 老年组: 24 月龄, 注射 NS; (3) 老年 + TFD 组: 24 月龄, 自 21 月龄始 sc TFD 2 mg·kg⁻¹ 隔日一次, 共 3 月。

脂质过氧化产物丙二醛 (MDA) 测定 用硫代巴比妥酸显色法⁽³⁾。

脂褐素含量测定 用荧光法⁽⁴⁾。

谷胱甘肽含量测定 用荧光法⁽⁵⁾。

膜流动性测定 以 DPH 作为荧光探针, 通过测定荧光偏振度 (P) 定量了解肝线粒体、微粒体膜脂分子的运动情况即微粘度 (η)⁽⁶⁾。微粘度越大, 膜流动

性越小,反之亦然.

RESULTS

对老年大鼠肝脏脂质过氧化的影响 结果显示老年大鼠肝脏脂质过氧化产物 MDA 和脂褐素含量比成年对照大鼠明显增多,TFD 对老年鼠的脂质过氧化有显著抑制作用(Tab 1).

Tab 1. Effect of thymic factor D (TFD) on malondialdehyde (MDA), lipofuscin, glutathione (GSH), and oxidized glutathione (GSSG) content in the liver of δ aged Wistar rats. Control: 7-month-old. Aged: 24-month-old. TFD: 2 mg·kg⁻¹ sc every other day for 3 months. $\bar{x} \pm s$. **P*>0.05, ***P*<0.05, ****P*<0.01 vs aged groups.

	Control <i>n</i> =6	Aged <i>n</i> =6	Aged+TFD <i>n</i> =5
MDA, nmol/mg protein			
Homogenate	285±25***	340±33	298±25**
Microsomal	453±67***	616±32	507±47***
Mitochondria	422±41***	528±30	378±61***
Lipofuscin, U/g liver			
	90±7***	133±28	98±9**
GSH, mg/g liver			
	1.32±0.06***	1.05±0.08	1.25±0.10***
GSSG, mg/g liver			
	0.71±0.03*	0.73±0.05	0.74±0.04*
GSH : GSSG ratio			
	1.85±0.13***	1.44±0.11	1.69±0.06***

对老年大鼠肝脏谷胱甘肽含量的影响 老年大鼠 GSH 减少,氧化型谷胱甘肽(GSSG)无明显变化,GSH/GSSG 比值降低. TFD 可使老年大鼠降低的 GSH 含量增高,提高其 GSH/GSSG 比值(Tab 1).

对老年大鼠肝线粒体、微粒体膜流动性的作用 老年大鼠的肝线粒体、微粒体膜微粘度增高,表示其膜流动性下降. TFD 可使老年大鼠的膜流动性增高,接近成年对照鼠水平(Tab 2).

Tab 2. Effect of TFD on membrane fluidity of liver microsome and mitochondria in δ aged rats. $\bar{x} \pm s$. ****P*<0.01 vs aged group.

Group	<i>n</i>	Fluorescence polarization <i>P</i>	Microviscosity η (poise)
Microsome			
Control	6	0.188±0.018***	1.39±0.22***
Aged	6	0.217±0.013	1.80±0.20
Aged+TFD	5	0.183±0.018***	1.33±0.10***
Mitochondria			
Control	6	0.173±0.018***	1.20±0.19***
Aged	6	0.203±0.015	1.58±0.17
Aged+TFD	5	0.187±0.017***	1.37±0.19***

DISCUSSION

过氧化脂质增多是机体衰老的重要标志,老年人和老龄动物体内过氧化脂质及其与蛋白质的结合物脂褐素显著增多⁽⁷⁾. 本实验观察到 TFD 能够降低老年大鼠肝脏脂质过氧化产物丙二醛及脂褐素含量,提示该药对延缓衰老可能有益.

TFD 抑制脂质过氧化,可能与其提高 GSH 含量有关. GSH 在细胞内具有维持膜稳定性,降解由代谢所产生的过氧化物及自由基的作用. 老年动物组织内 GSH 降低使得细胞易受过氧化物及自由基等的损伤⁽⁶⁾,而 TFD 能够增高老年大鼠肝脏 GSH 含量,使其抗氧化能力增强,从而抑制脂质过氧化的反应.

本实验还观察到 TFD 可纠正老年大鼠降低的肝线粒体、微粒体膜流动性. 这对于维持生物膜的正常生理功能具有重要意义. 生物膜正常功能需要膜脂质处于适当的流动状态. 膜流动性改变,将影响膜上酶的活性、膜的转运功能、膜受体的功能及其表达等. TFD 提高老年大鼠肝脏生物膜流动性,可能与其增强细胞抗氧化能力,抑制膜脂质过氧化有关. 我们以往的研究曾发现大鼠肝微粒体在体外经 Fe²⁺-半胱氨酸诱发 O₂和 OH 自由基增多之后,丙二

醛含量明显增高,同时膜的流动性急剧下降⁽⁹⁾。脂质过氧化影响膜流动性的机理可能是由于丙二醛进入膜脂水相,与膜蛋白及膜脂上的NH₂交联形成Schiff's碱而使膜的刚性增加⁽¹⁰⁾,交联作用还使膜胆固醇从脂质双层内侧向外侧移动受阻,使膜胆固醇增加,膜流动性下降⁽¹¹⁾。因此,TFD之所以能使老年大鼠已降低的膜流动性得以恢复,可能主要是由于它增强老年大鼠抗氧化功能的作用所致。

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