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沙棘油对高脂小鼠诱发阿尔兹海默症的预防作用

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摘要 目的:通过检测沙棘油作用高脂小鼠海马神经元内微管相关蛋白(Tau)及脑源性神经营养因子(BDNF)的表达水平,探讨沙棘油对高脂小鼠诱发阿尔兹海默综合症的预防作用。**方法:**40只KM小鼠,随机取10只为正常对照组;30只以高脂饲料喂养建立高脂模型(HF),按10 mg/kg以生理盐水(阴性对照)、沙棘油(实验)、辛伐他丁(阳性对照)灌胃3 w。取小鼠海马组织进行HE染色、免疫组织化学检测和蛋白印迹分析,检测不同组别小鼠海马神经元内Tau蛋白及BDNF表达的变化。**结果:**高脂模型组与正常组比较,海马神经元结构在光镜下有明显差别;阴性对照组小鼠海马神经细胞数目减少,神经元内有黄色颗粒样沉淀;实验及阳性组海马损伤有改善,斑块状淀粉样蛋白减少;免疫组化及蛋白印迹显示各组间两种蛋白表达水平不同。**结论:**沙棘油对高脂小鼠海马体内Tau蛋白表达有抑制作用,加速淀粉样前体蛋白的代谢,降低了由 β -淀粉样蛋白沉积诱发阿尔兹海默病的风险;而对BDNF表达有促进作用,能防止神经元受损伤死亡、改善神经元的病理状态、促进受损神经元再生。即沙棘油能有效预防高脂人群并发阿尔兹海默综合征。

关键词:沙棘油;微管相关蛋白;脑源性神经营养因子;海马神经元

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Preventive Effect of Seabuckthorn Oil on Alzheimer's Disease Induced by Hyperlipidemia Mice

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ABSTRACT Objective: In order to investigate the prevention effect of seabuckthorn oil on Alzheimer's disease induced by hyperlipidemia mice, the expression level of microtubule-associated protein (Tau) and brain derived neurotrophic factor (BDNF) of seabuckthorn oil-affected hyperlipidemia mice. **Methods:** 40 KM mice were randomly divided into 2 groups with 10 mice for normal control group and 30 mice for high-fatty model group, the high-fatty model group were administered by gavage once a day for 3 weeks with 10 mg/kg NaCl (negative control group), seabuckthorn oil (experimental group) and simvastatin (positive control group), respectively and with 10 mice for each group, the expression level of Tau and BDNF from mice hippocampus tissues were measured by HE, IHC and WB methods. **Results:** There was significant difference of the hippocampal neuron between high-fatty model group and normal control group; the mice hippocampus nerve cells decreased in the negative control group and yellow granular precipitated in the neuron; meanwhile, hippocampal damage was improved and the amyloid protein decreased in the experimental and positive control group, IHC and WB results showed that there was significant difference among groups. **Conclusion:** The above results revealed that seabuckthorn oil could inhibited the expression of Tau of hyperlipidemia mice hippocampus and accelerated metabolism of amyloid precursor protein, so that decrease the risk of β -amyloid precursor protein deposit-induced Alzheimer's disease; while seabuckthorn oil could promote the expression of BDNF, so that prevent injury and death of neurons, improve pathological state of neurons and promote regeneration of injured neurons, indicating seabuckthorn oil could effective prevent hyperlipidemia people concurrent Alzheimer's disease.

Key words: Seabuckthorn oil; Microtubule-associated protein; BDNF; Hippocampal neuron

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前言

沙棘多生长在贫瘠干燥、寒冷的山区。我国是世界上沙棘资源最多的国家。沙棘油通常指沙棘的种子油和果实油,是沙棘的精华,具有很高的医药价值。临床研究表明,沙棘油具有降

低血脂、升高高密度脂蛋白、增强机体免疫力、健脾养胃、健脑益智、抗衰老、抗辐射、抗肿瘤、抗氧化等功能^[1,2]。阿尔兹海默病(Alzheimer's disease, AD)是慢性进行性中枢神经系统变性病导致的痴呆,是痴呆最常见的病因和最常见的老年期痴呆。AD以渐进性记忆障碍、认知功能障碍、人格改变以及语言障碍等神经精神症状为特征。阿尔兹海默病是近年来老年人群发病率较高的疾病,周期短,病情发展较快,危害性大。有研究表明高脂人群Tau表达可见异常,而异常高表达的Tau蛋白能促进淀粉

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样前体蛋白代谢,加剧 β -淀粉样蛋白沉积,诱发阿尔茨海默病^[3-6]。脑源性神经营养因子 (brain derived neurotrophic factor, BDNF),具有防止神经元受损伤死亡、改善神经元的病理状态、促进受损神经元再生及分化等生物效应^[7],本文研究沙棘油对高脂小鼠脑组织中 Tau、BDNF 表达水平的影响,探讨高脂在 AD 发生中的作用,为高脂血症和 AD 的预防与治疗提供实验依据。

1 材料和方法

1.1 主要材料和仪器

野生沙棘果(购自内蒙古自治区);SD 昆明小鼠(大连医科大学 SPF 中心);BDNF 多克隆抗体 (SANTA 公司);Tau 多克隆抗体、免疫组化试剂盒、ECL 发光试剂盒等(武汉博士德生物工程有限公司);超声细胞破碎仪(宁波新芝生物科技股份有限公司);离心机(上海安亭离心机厂);电热恒温干燥箱(上海申光仪器仪表有限公司);旋转蒸发器(上海精密仪器厂);真空离心浓缩仪、酶标仪(Thermo electron corporation, USA)。

1.2 方法

1.2.1 沙棘油的提取 沙棘果籽,粉碎至 40 目,取 10 g 加入 50 mL 正己烷,索氏浸提 5 次,提取液进行旋蒸、浓缩,得沙棘油。

1.2.2 高脂模型建立 取 40 只 18 g~22 g 小鼠,随机分为 4 组,1 组正常饲料,其余 3 组高脂饲料(普通饲料 + 0.3 % 胆固醇 + 5 % 猪油)^[8]饲养 8w,建立高脂模型。

1.2.3 给药及沙棘油对血脂水平的影响 模型组分别按 10

mg/kg 给以生理盐水(阴性对照)、沙棘油(实验组)、辛伐他丁(阳性对照)灌胃 3 w。小鼠禁食 16 h 取血,酶法检测血清 TC 和 TG。

1.2.4 HE 染色 取小鼠海马组织(0℃)置 10%甲醛液 48 h,石蜡包埋,矢状切片(4 μ m),脱蜡、染色、封片。

1.2.5 免疫组织化学 石蜡切片置电热恒温干燥箱中 80℃ 烘烤 3 h。脱蜡,水化。细胞通透、封闭内源性过氧化物酶,抗原修复暴露抗原决定簇。正常山羊免疫血清封闭,室温孵育,滴加按 1:400 稀释的 Tau 多克隆抗体,4℃ 过夜。滴加生物素标记的二抗,室温 10 min。滴加辣根酶标记链霉卵白素工作液,室温 10 min。DAB 显色,苏木素复染,盐酸酒精分化,流水冲洗返蓝 1 min,脱水,二甲苯透明,封片。

1.2.6 蛋白印记分析 取小鼠海马组织,每 20 mg 加入 100 μ L~150 μ L 细胞裂解液(含 PMSF 10 mmol/ml),0℃ 研磨,4℃、12,000 rpm 离心 5 min,取上清,考马斯亮蓝法检测蛋白质含量。各组取 50 μ g 蛋白质常规 SDS-PAGE 电泳,分离胶、浓缩胶浓度分别为 10 % 和 5 %,电压 80 v,半干式电转仪转膜。封闭,BDNF、Tau 抗体及二抗结合,化学发光、显影,结果以 Image J 影像分析系统分析。

2 结果

2.1 沙棘油对高脂小鼠血脂水平的影响

与正常组比较,高脂模型各组小鼠的血脂水平均升高;与模型组比较,沙棘油组、阳性对照组血脂水平降低($P < 0.05$ 有显著差异)见表 1。

表 1 灌胃 3W 不同组别小鼠血脂水平($\bar{x} \pm s, n=8$)

Table 1 Blood lipid of mice after 3W by intragastric administration($\bar{x} \pm s, n=8$)

	Normal	Negative control	Seabuckthorn oil-treated	Positive control
TC (mmol/L)	0.53 $\pm$ 0.20	1.02 $\pm$ 0.30	0.71 $\pm$ 0.3	0.58 $\pm$ 0.3
TG (mmol/L)	2.5 $\pm$ 0.49	5.23 $\pm$ 0.62	3.20 $\pm$ 0.65	2.8 $\pm$ 0.48

2.2 HE 染色

正常组海马神经元染色清晰,排列紧密,形态完整,细胞核大而圆,着色较浅,位于细胞中央;阴性组海马神经细胞数目减

少,排列松散,核小而固缩;淀粉样蛋白增多。实验组与阳性对照组海马损伤有较明显改善,斑块状淀粉样蛋白减少,细胞排列趋于整齐(图 1)。

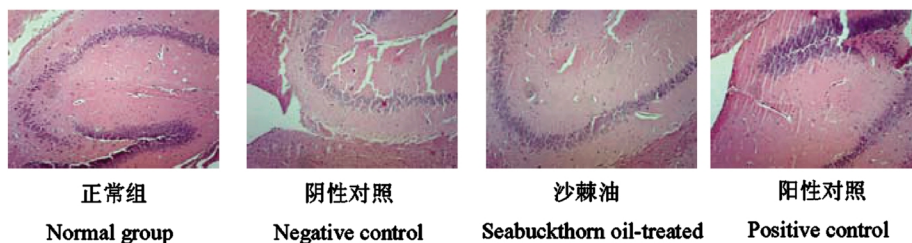


图 1 不同组别海马组织 HE 染色光镜观察

Fig.1 HE staining and light microscope observation method of hippocampal tissue

2.3 免疫组织化学

阴性对照(高脂模型)组染色比正常组深,阳性对照(辛伐他丁)染色明显变浅,沙棘油组次之,表明沙棘油对目的蛋白 Tau 的表达有一定抑制作用。

2.4 蛋白印记分析

BDNF 在模型组的表达低于正常对照组,沙棘油作用后表达有所升高;模型组 Tau 蛋白表达水平明显高于其他各组,且沙棘油和阳性对照组的表达量均低于正常组(见图 3、图 4)。

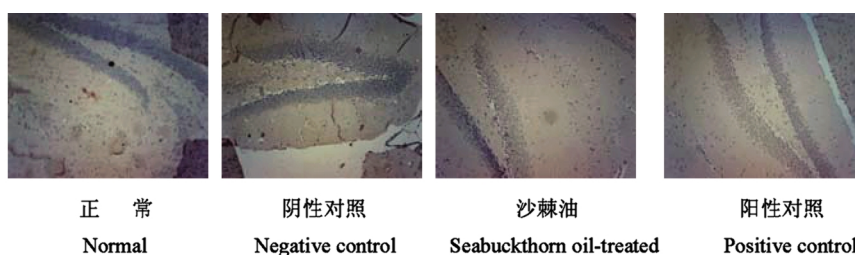


图2 免疫组织化学法检测不同组别间 Tau 的表达水平

Fig.2 The expression of Tau by immunohistochemical method



图3 Western blotting 检测 BDNF 与 Tau 表达水平

Fig.3 The expressions of BDNF and Tau by Western blotting

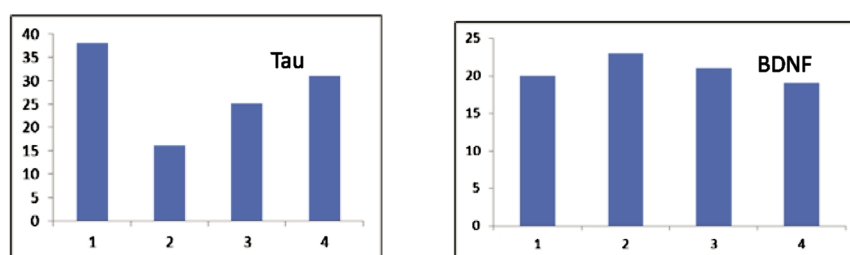


图4 不同组别 Tau、BDNF 占内参的灰度值百分比

Fig.4 The gray percentage of BDNF and Tau

1. 阴性组 2. 阳性组 3. 沙棘油组 4. 正常组

1. Negative control group 2. Positive control group 3. Seabuckthorn oil-treated group 4. Normal group

3 讨论

随着生活质量的改善,高脂血症的发病率呈逐年上升趋势。它是中老年人衰老的病理基础,是心、脑血管疾病的危险因素之一^[9]。近期研究发现高脂血症人群的AD患病率高于正常人群^[6],可能与异常表达的过度磷酸化的Tau蛋白有关,因其丧失维持微管稳定的作用,影响脑组织神经系统功能,促进 β -淀粉样蛋白沉积,诱发AD^[10]。随着人口的老龄化,AD患病人数急剧增加,在85岁以上人群中发病率高达50%^[11],严重影响人类的健康。本研究结果显示,高脂组小鼠Tau蛋白的表达量均高于正常组,且高脂模型组小鼠的Tau蛋白含量高于沙棘油组和阳性对照组,提示高脂血症可能诱发AD的发生。因此,控制高血脂,在降低AD的发病率、提高人类生存质量方面有重要作用。

BDNF (brain derived neurotrophic factor, 脑源性神经生长因子),是由Barde等首先分离和纯化,在中枢神经系统发育过程中,对神经元的存活、分化、生长发育起重要作用;在成熟中枢及周围神经系统的神经元维持生存、生理功能及其损伤后再生修复方面发挥着重要作用;对巩固学习和记忆起重要作用^[12-14];BDNF水平的改变与AD的发病也有一定的关联^[15]。本研究的western blotting结果可见,高脂模型小鼠海马组织中,沙棘油组的BDNF表达水平低于阳性对照组,但两组均高于阴性对照组,即沙棘油可能促进高脂小鼠海马组织BDNF的表达。

本研究HE染色显示不同组别小鼠海马体CA2有差异,高脂模型组神经细胞数目减少,应用阳性药物和沙棘油后的海马组织损伤有所改善,淀粉样蛋白含量减少;免疫组织化学法染色,正常组与高脂模型组的光密度值存在差异,阳性对照及沙棘油组的染色弱于阴性对照组,说明淀粉样蛋白颗粒的形成减少;Western-Blotting的结果中,阴性对照组小鼠海马组织的Tau蛋白表达高于正常组,经沙棘油灌胃,其表达水平有所降低,提示沙棘油可能对Tau蛋白的表达有一定的抑制作用;记忆蛋白BDNF在高脂模型组小鼠海马组织的表达结果显示,服用沙棘油可使其表达水平升高,有益于营养神经细胞,预防AD的发生;但模型组BDNF的表达均高于正常组,可能是由于样本量少,或者观察时间的限制等因素引起。本研究结果提示沙棘油对AD形成的Tau蛋白及BDNF表达有一定的影响,为AD的预防与治疗提供了相关实验依据,但作用剂量关系及机理尚需在样本量、检测技术、检测指标等方面进行更深入的研究。

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