

健脑益智冲剂对多发梗塞性痴呆大鼠模型大脑皮层单胺类神经递质和 P 物质含量的影响

梅建勳¹ 张云岭² 张伯礼¹ 王永炎³

摘要 为探讨一种治疗多发梗塞性痴呆(MID)有较好临床疗效的中药复方制剂——健脑益智冲剂的疗效机理,应用 MID 大鼠模型,采用高压液相—电化学检测技术和免疫组织化学染色方法,观察中药对该模型大脑皮层单胺类神经递质多巴胺(DA)、肾上腺素(E)、去甲肾上腺素(NE)、5-羟色胺(5-HT)和 P 物质含量的影响。结果术前中药组可提高造模后 E 的含量;术后中药组和术后西药组(氢化麦角碱, Dihydroergotamine)可降低造模后 5-HT 的含量;3 个用药组均可提高该模型 P 物质含量,以术前中药组明显。表明该方药可调整 MID 后大脑皮层紊乱的单胺类神经递质和 P 物质的代谢,这可能是它改善 MID 学习记忆的物质基础。

主题词 痴呆,多发性梗塞性/中医药疗法 大脑皮质/代谢

@ 健脑益智冲剂

血管性痴呆(Vascular dementia, VD)/多发梗塞性痴呆(Multi-infarct dementia, MID)和早老性痴呆(Alzheimer's dementia, AD)是老年期痴呆的主要类型,东北亚地区由于脑血管病高发,其 VD/MID 的发病率明显高于 AD。近 10 年来中医药对本病进行了尝试性研究,显示出一定优势,但由于选例标准、观察方法、疗效评定标准存在差异,且缺乏严格随机对照、追踪随访,另外在基础实验研究方面也较为薄弱,因此其研究的可靠性受到影响。近年来我们参照 WHO 正式公布的美国精神病学会血管性痴呆诊断标准(DSM-IV),选取 MID 轻中症 120 例,以目前公认的简短精神状态检查法(MMSE)和 Blessed 行为量表(BBS)对健脑益智冲剂进行疗效评估,总有效率为 76.7%^[1]。基础实验研究表明,本方可明显改善 MID 大鼠模型的学习记忆能力^[2]。在此基础之上,本文进一步研究健脑益智冲剂对该模型大脑皮层单胺类神经递质和 P 物质含量的影响,以期探讨其疗效机制,现报告如下。

1 材料与方法

1.1 动物分组:一级 Wistar 大鼠 47 只,10 月龄,体重 320±40g,雌雄各半,由天津医学实验动物中心提

供。随机分为空白对照组(5 只)、假手术组(5 只)、模型组(7 只)、术前中药组(10 只)、术后中药组(10 只)、术后西药组(10 只)。

1.2 MID 大鼠模型的复制:参照文献^[3]方法,在无菌条件下,同种 Wistar 大鼠左心室内采血 6ml,于 37℃ 温箱内自然干燥,研碎成细粉状,用 200μm 筛孔过筛,应用时取直径小于 20μm 的血栓栓子 1mg 加生理盐水 0.5ml,摇匀成混悬液。

模型制作: Wistar 大鼠以 10%水合氯醛按 35mg/100g 体重腹腔麻醉,颈正中切开皮肤,剥离胸骨舌骨肌、肩胛舌骨肌与胸锁乳突肌,暴露颈总动脉、颈外动脉与颈内动脉,暂时夹闭颈总动脉,于颈外动脉逆行插管至颈内、颈外动脉分界处,注入栓子溶液 0.3ml,推注同时开放颈总动脉,使栓子通过颈内动脉进入颅内至大脑各动脉,造成多发性脑梗塞,然后结扎颈外动脉,缝合皮肤。

1.3 处理方法:空白对照组:正常喂养,并予捉抓,不给任何药物。

假手术组:手术方法同模型制作,只在穿刺时以生理盐水 0.3ml 代替栓子溶液,术后不给任何药物。

模型组:单纯造模,术前、术后均不给任何药物。

术前中药组:健脑益智冲剂(由国家“九五”重点科技攻关课题组提供,主要成分为制何首乌、蜜炙黄芪、全当归、川芎、葛根、远志、石菖蒲),连续灌胃 10 天,1.2g/kg,每日 1 次,然后造模。

术后中药组:造模后,健脑益智冲剂连续灌胃 10 天,1.2g/kg,每日 1 次。

术后西药组:造模后,喜得镇(Hydergine,氢化麦角碱, Dihydroergotamine,由天津华津制药厂与瑞士山德士制药公司合资生产)连续灌胃 10 天,0.2mg/kg,每日 1 次。

1.4 单胺类神经递质检测:使用日本岛津(Shimadzu)公司 LC-6A 高效液相色谱仪,LC-6A 型高压泵,CTC-6A 柱温箱,L-ECD-6A 电化学检测器,LC-6B 控制系统,C-R4A 数据处理机,美国 UHQ 超纯水器,瑞典 LKB 公司 2021 型活动冷室,日本 SANYO 台式高速离心机。色谱条件:日本岛津公司 ODS-C18 色谱柱(4.5×150mm),流速为 1.0ml/

基金项目:“九五”国家重点科技攻关课题(96-906-09-03)

1 天津中医学院中医工程研究所,天津市南开区玉泉路 88 号(300193)

2 北京中医药大学

3 中国中医研究院

收稿日期:1999-09-20

分,柱温为 35℃,L-ECD-6A 设定电位+0.7V,检测范围为 2nA(全刻度)。

造模后第 8 天,大鼠快速断头取出全脑,在冰盘上立即分离大脑皮层,置-80℃冰箱中备用,检测时取样品超声粉碎,3000r/分钟离心 10 分钟,4℃,上清液移入一预冷的 1.5ml 的 Eppendorf 管中,备用提取去甲肾上腺素(NE)、肾上腺素(E)、多巴胺(DA)、5-羟色胺(5-HT)的含量。标准品 NE、E、DA、5-HT 均是美国 Sigma 公司产品。将标准品与样品做相同处理,按下列公式进行定量测定。用于 NE、E、DA 测定的计算公式:含量=补正系数×面积×内部标准量×100/内部标准峰面积×试样量。用于 5-HT 测定的计算公式:含量=补正系数×面积×100/试剂量。补正系数由分析标准试样求得。

1.5 P 物质免疫组织化学染色:造模后第 8 天,取注入栓子一侧大脑皮层组织,10%甲醛固定,石蜡包埋,切片,SPTM 试剂盒由美国 ZYMED 公司提供,实验方法及步骤按试剂盒操作,于光学显微镜下观察结果。

1.6 数据处理:应用成对样品 *t* 检验及方差分析判断差异程度及来源,数据均以 $\bar{x} \pm s$ 表示。

2 结果

2.1 健脑益智冲剂对 MID 大鼠模型大脑皮层单胺类神经递质的影响:实验结果(见附表)显示,较空白对照组,MID 大鼠模型大脑皮层的 E 和 5-HT 含量出现明显异常,E 的含量降低($P < 0.01$),而 5-HT 的含量增高($P < 0.05$)。在 3 个用药组中,术前中药组可明显提高造模后 E 的含量($P < 0.01$),术后中药组和术后西药组可明显降低造模后 5-HT 的含量(P 均 < 0.01)。

附表 各组大脑皮层单胺类神经递质含量比较(ng/mg, $\bar{x} \pm s$)

组别	例数	DA	E	NE	5-HT
空白对照组	5	0.38±0.24	0.50±0.15**	2.12±0.38	1.69±0.11*
假手术组	5	0.48±0.28	0.40±0.10**	1.73±0.37	1.73±0.49
模型组	7	0.32±0.23	0.23±0.09	2.22±0.76	2.18±0.56
术前中药组	10	0.38±0.15	0.47±0.16**	2.20±0.26	2.02±0.34
术后中药组	10	0.13±0.06	0.22±0.16	1.39±0.26	1.27±0.34**
术后西药组	10	0.23±0.13	0.33±0.17	1.29±0.25	1.31±0.20**

与模型组比较,* $P < 0.05$,** $P < 0.01$

2.2 健脑益智冲剂对 MID 大鼠模型大脑皮层 P 物质含量的影响:模型组大脑皮层组织切片有浅棕黄色物质分布,着色程度极浅,表明 P 物质含量减少。术前中药组可见有深棕黄色物质广泛分布,其着色程度较明显,近似空白对照组,并优于其他实验治疗组。术后中药、术后西药组表现相似,可见各脑区组织切片有棕

黄色物质分布,阳性反应程度强于模型组。

3 讨论

脑梗塞缺血直接影响单胺类神经递质的合成释放再摄取降解全过程,是造成其代谢异常的主要原因。各脑区神经元对缺血敏感性不同,单胺类神经递质的代谢异常可有时相差异,而单胺能神经元在脑区中分布又不同,因此不同脑区对缺血应答的递质变化亦有差异,这似可解释本实验中所观察到的大脑皮层组织中各种单胺类递质变化的差异性。

近年来的研究证实,单胺类神经递质可以直接或间接参与大脑功能活动,主要表现在:(1)儿茶酚胺能易化条件性学习行为的获得,促进短时记忆的储存而成为长时记忆;(2)儿茶酚胺通过改变觉醒及运动而影响学习行为的执行;(3)肾上腺能系统是一种特殊的学习加强系统, α -肾上腺能系统在平时受胆碱能系统抑制性的调节,而学习可激活 α -肾上腺能系统,使胆碱能的抑制解除而使学习行为易化。P 物质是具有生物活性的多肽,作为神经调质与传统神经递质(如单胺类神经递质等)共存,既相互协同又相互抑制,共同起到复杂的神经调节作用,不仅可影响神经元的兴奋和抑制过程,而且参与学习和记忆行为等高级神经功能调节,当中枢胺类神经元受到病理因素如梗塞缺血损害时,便可造成其含量的异常变化,进而影响脑的功能活动。从本实验结果看,针对 MID 大鼠模型大脑皮层神经递质和神经调质的异常变化,中药具有明显的拮抗作用,推论这可能不仅是健脑益智冲剂改善 MID 大鼠模型学习记忆能力的物质基础,同时也是其临床治疗的疗效机制之一。

MID 在病机上存在着因虚致实、由实转虚、虚实错杂的变化,提示应依据疾病发展各个时期和轻重程度的差异进行论治。MID 早期属本虚标实,本虚乃肾虚精亏、脑髓失养,标实乃风、痰、瘀夹杂,治疗当补肾益气、祛痰活血。早期治疗不仅可以改善智能障碍症状,还可以减轻甚或消除神经系统症状和自觉症状,以延缓病程进展,促使病势逆转。随病程自然进展,多见以虚为主或虚中夹实,严重者气血阴阳俱虚,治疗十分困难。

参考文献

- 张云岭,梅建勋,张伯礼,等.健脑益智冲剂治疗多发梗塞性痴呆轻中度的临床观察.北京中医药大学学报,1998,21(5):42-44.
- 梅建勋,张云岭,张伯礼.多发梗塞性痴呆(MID)动物模型的改进与应用.中风与神经疾病杂志,1999,16(2):100-102.
- Kaneko D, Nakamura N, Ogawa T. Cerebral infarction in rats using homologous blood emboli: development of a new experimental model. Stroke, 1985, 16(1): 76-84.

ABSTRACTS FROM ORIGINAL ARTICLES

Clinical Observation on Treatment of 240 Cases of Chronic Hepatitis of Syndromeless Differentiation Type with Self-Made Wen Gan Tablet

Wang Huameng

In order to probe into change of syndrome types and the necessity of treatment, 346 cases of chronic hepatitis of syndromeless differentiation type were randomly divided into two groups. 240 cases in the treatment group were treated with Wen Gan Tablet and 106 cases in the control group were treated with starch or did not receive any treatment. Within 6 months, it was investigated whether the patient in syndromeless differentiation type changed and the changed syndrome types. Results showed that the syndrome type was changed in 44 cases of the treatment group, and 62 cases in the control group, accounting for 18.33% and 58.49% respectively, with significant difference ($P < 0.01$). It is indicated that intensive treatment can be used for chronic hepatitis of syndromeless differentiation type to reduce attack of chronic hepatitis.

Author's address: Society of Traditional Chinese Medicine, Anhui Lao Zi Research Association, Anhui Province 233600

Key words: hepatitis, virus, human/TCM therapy, @Wen Gan Tablet

(Original article on page 28)

Comparative Study on Ultramicrostructures and Functions of the Thyroid in Rats of Excess-Heat Type and Deficiency-Heat Type

Xu Zhiwei, Chen Qun, Liu Yamei, et al

Serum contents of triiodothyronine (T_3), tetraiodothyronine (T_4), reverse triiodothyronine (rT_3) and thyroid-stimulating hormone (TSH) and changes of structures of the thyroid gland in the rat of excess-heat syndrome and deficiency-heat syndrome were observed with radioimmunoassay and electron microscopic technique, respectively. The rats of excess-heat syndrome and deficiency-heat syndrome were established with administration of Chinese herbs. Results indicated that serum T_3 and T_4 levels increased and the rT_3 level tended to decrease in the deficiency-heat group, and thyroid follicle epithelial cell had more significant changes as compared with that of the excess-heat group. It is suggested that changes of hormones and ultra-microstructures of the thyroid can be served as an important basis for heat syndrome of deficiency type and excess type.

Authors' address: College of Basic Medicine, Guangzhou University of TCM, Guangzhou 510405

Key words: heat of excess type/pathology, heat of deficiency type/pathology, thyroid gland, ultramicrostructure

(Original article on page 43)

Preliminary Study on TCM Crasiology in 10 Strains of Male Rats

Chen Xiaoye, Zhou Yongsheng, Zou Shijie, et al

Ten strains of male rats are commonly used for experimental study at present in Beijing. The rats' outward appearance, stool, picture of the tongue, body temperature, and wet weight of adrenal gland, thymus gland and spleen were observed, and the function of the hypothalamus-pituitary-thyroid axis was determined. Results showed that the constitution of Wistar grade I, II, III, and SD grade III were basically normal. SD grade I had the constitution of mild deficiency-cold, SD grade II had constitution of excess-heat, CD (SD) IGS grade III (small or big body weight) had constitution of mild deficiency. SHR grade I had constitution of mild deficiency-cold, SHR/sp grade I had obvious deficiency-cold constitution; There was a certain difference in TCM constitution among different strains, same strain with different microbic grades, pure line and inbred line, and different ages in rats. It is indicated that the study on TCM crasiology in experimental animals are not only necessary, and also feasibility.

Authors' address: Institute of Basic Medicine, China Academy of Traditional Chinese Medicine, Beijing 100700

Key words: Crasiology/TCM

(Original article on page 45)

Effects of Jian Nao Yi Zhi Instant Granules on Contents of Monoamine Neurotransmitters and Substance P of Cerebral Cortex in Rats of Multi-Infarct Dementia

Mei Jianxun, Zhang Yunling, Zhang Boli, et al

In order to explore the mechanism of Jian Nao Yi Zhi Instant Granules for treatment of Multi-infarct dementia (MID), the contents of dopamine (DA), adrenaline, noradrenaline, 5-hydroxytryptamine (5-HT) and substance P in cerebral cortex were observed in the rat of MID with electrochemical detection of high pressure liquid chromatography and immunohistochemical method. Results indicated that the content of adrenaline in the rat of MID increased in the pre-operation Chinese herb group; 5-HT level decreased in the post-operation Chinese herb group and the post-operation western medicine (dihydroergotamine) group. Contents of substance P elevated in the three groups, with more increase in the pre-operation Chinese herb group. It is concluded that the prescription can regulate abnormal metabolism of monoamine neurotransmitters and substance P in the cerebral cortex after MID. This is possibly material base for improving memory.

Authors' address: Tianjin College of TCM, 300193

Key words: dementia, multi-infarct/TCM therapy cerebral cortex/metabolism @Jian Nao Yi Zhi Instant Granules

(Original article on page 48)