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脑科学与神经物理

阿尔茨海默病大鼠氢质子磁共振波谱研究

林富春[1],杨期东[2],何建川[3],游箭[3],邵志成[3],马海涵[2],邵阳[1,2,3]
1.南京医科大学第二附属医院神经内科,江苏 南京 210011;2.中南大学湘雅医院神经内科,湖南 长沙 410008;3.川北医学院医学影像四川省重点实验室,四川 南充 637000

【摘 要】**目的:**探讨海马注射β-淀粉样肽($A\beta_{1-42}$)诱发阿尔茨海默病(AD)大鼠模型的病理学变化及氢质子磁共振波谱(^1H MRS)特性。**方法:**健康SD大鼠40只,实验随机分为假伤组(Sham组)、阿尔茨海默病模型(AD组)。采用大鼠海马注射$A\beta_{1-42}$复制AD模型。苏木精-伊红染色法染色观察海马CA_1区神经元形态,免疫化学染色观察Aβ的沉积,采用Morris水迷宫法进行大鼠记忆功能的测定。^1H MRS检测AD鼠脑中的N-乙酰天门冬氨酸(NAA)、胆碱(Cho)、肌酸(Cr)和乳酸(Lac)水平。**结果:**与Sham组比较,AD组游全程时间延长,错误次数增加,差异显著(均$P<0.01$)。与Sham组比较,AD组双侧海马CA_1区神经元数目减少,核固缩增加;且与注射对侧比较,$A\beta_{1-42}$注射侧大鼠海马CA_1区神经元数目显著减少,核固缩显著增加。免疫化学染色Sham组呈阴性;AD组双侧抗$A\beta_{1-42}$呈阳性,且与注射对侧比较,注射侧阳性显著增加。^1H MRS结果Sham组双侧半球无显著性差异。与Sham组比较,AD组双侧^1H MRS海马NAA/Cr明显降低、Cho/Cr增加、Lac/Cr增加;在AD组,与注射对侧半球比较,注射侧半球海马NAA/Cr明显降低($P<0.05$)、Cho/Cr显著增加($P<0.01$)、Lac/Cr显著增加($P<0.01$)。**结论:**$A\beta_{1-42}$能引起大鼠海马CA_1区神经元损伤,记忆功能下降,注射Aβ后4周NAA/Cr已有明显改变,与免疫组化发现$A\beta_{1-42}$表达增强结果一致,提示利用^1H MRS检测NAA/Cr改变,可能有助于AD早期临床诊断。

【关键词】阿尔茨海默病;β-淀粉样肽;氢质子磁共振波谱
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^1H-proton magnetic resonance spectroscopy features of Alzheimer's disease model in rats

LIN Fuchun[1], YANG Qidong[2], HE Jianchuan[3], YOU Jian[3], SHAO Zhicheng[3], MA Haihan[2], SHAO Yang[1,2,3]
1. Department of Neurology, the Second Affiliated Hospital of Nanjing Medical University, Nanjing 210011, China; 2. Department of Neurology, Xiangya Hospital of Central South University, Changsha 410008, China; 3. Sichuan Key Lab of Medical Imaging, North Sichuan Medical College, Nanchong 637000, China

Abstract: Objective To investigate the pathological changes and ^1H-proton magnetic resonance spectroscopy (^1H MRS) features of experimental rat injected with $A\beta_{1-42}$ in hippocampus to induce Alzheimer's disease (AD). **Methods** Forty healthy Sprague Dawley (SD) rats were randomly divided into Sham group and AD group. The SD rats in AD group were injected with $A\beta_{1-42}$ in hippocampus to induce AD, while the SD rats in Sham group were injected with normal saline. The morphology of neurons in hippocampus CA_1 area was observed with hematoxylin-eosin (HE) staining; the deposition of Aβ was assessed by immunochemical staining; the memory function was evaluated by Morris water maze. Herein, we used ^1H MRS images to evaluate the levels of NAA, Cho, Cr and Lac in the brains of AD rats. **Results** Compared with those in Sham group, the abilities of learning and memory in AD group were reduced for the rats in AD group extended their time for swimming the whole process and increased the mistake times ($P<0.01$). Under microscopy, AD group displayed obviously decreased number of neurons and higher karyopyknosis rate in the hippocampus CA_1 area as compared with Sham group. Within AD group, the number of neurons in the hippocampus CA_1 area was significantly lower and karyopyknosis rate was higher in the injected lateral as compared with contra-lateral. Immunohistochemical staining was negative for $A\beta_{1-42}$ in Sham group, but positive in AD group. In AD group, the injected lateral showed a higher positive rate of $A\beta_{1-42}$ than the contralateral. ^1H MRS revealed that no significant difference was found in the level of compounds (NAA, Cho, Cr and Lac) between two laterals in Sham group. Compared with Sham group, AD group showed

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【作者简介】林富春,医师,硕士研究生,从事神经疾病等方面研究,E-mail: linfuchun91@hotmail.com
【通信作者】邵阳,博士,教授,主任医师,从事神经疾病发病机制等方面研究,E-mail: shine771@126.com

a decrease in NAA/Cr, and increases in Cho/Cr and Lac/Cr. Within AD group, the comparison demonstrated that the injected lateral had a decrease in NAA/Cr (P<0.05) and increases in Cho/Cr (P<0.01) and Lac/Cr (P<0.01). **Conclusion** $A\beta_{1-42}$ could cause significant injury to neurons in the hippocampus CA_1 area in rats, resulting in reduced memory functions. The NAA/Cr changed significantly at 4 weeks after the injection of $A\beta_{1-42}$, in accord with the changes in the expression of $A\beta_{1-42}$ found in immunohistochemical staining, suggesting that detecting NAA/Cr changes by ^{1}H MRS might be helpful for the early clinical diagnosis of AD.

Keywords: Alzheimer's disease; amyloid-β; ^{1}H-proton magnetic resonance spectroscopy

前 言

阿尔茨海默病(Alzheimer's Disease, AD)是老年期痴呆常见的类型,严重影响老年人的生活质量[1]。至今尚无活体诊断AD特异性的生物学工具,临床疑似AD诊断主要依据神经病学表现、病史、体征,辅以神经心理、智能量表或神经影像学等测评结果,要对患者精准治疗,诊疗水平亟待提高[2-3]。

磁共振质子波谱技术(^{1}H-proton Magnetic Resonance Spectroscopy, ^{1}H MRS)借助研究磁共振现象和化学移位分析化合物代谢的技术。^{1}H MRS可以实现活体无创检测细胞水平代谢,研究代谢物的异常改变[4-7]。^{1}H MRS主要检测3种化合物变化:N-乙酰天门冬氨酸复合物(NAA)、胆碱复合物(Cho)和肌酸-磷酸肌酸复合物(Cr)[8-11]。研究显示细胞外β-淀粉样肽(Aβ)的早期聚集引起轴突损害是导致AD病理机制重要环节之一。本研究通过Aβ注射海马建立AD大鼠模型,观测AD大鼠模型脑区^{1}H MRS变化,探讨AD大鼠脑代谢的变化规律,为临床诊疗提供依据。

1 材料与方法

1.1 实验动物分组

选择经预先训练且已学会游迷宫(标准为逃避潜伏期在30 s以内)健康SD大鼠40只,3~4月龄,雄性,体质量200~250 g,由重庆医科大学实验动物中心提供。实验分为假伤组(Sham组)、AD模型(AD组),每组20只。实验采用$A\beta_{1-42}$注射海马复制AD大鼠老年痴呆模型。

1.2 制备模型的建立

大鼠用10%的水合氯醛(35 mg/kg)腹腔注射麻醉后,将鼠头固定于立体定位仪上,手术切开头皮,充分暴露前囟视野,参考《大鼠脑立体定位图谱》行注射目标区域定位:在大鼠左侧海马区(以Bregma点为0点,AP-3.0 mm, ML-2.0 mm, DV-2.9 mm),立体定位仪上将微量注射器缓慢进针到定位区域,待大鼠平稳后缓慢注入已处理呈凝聚态的$A\beta_{1-42}$ 5 μL,留针5 min,使$A\beta_{1-42}$充分弥散。再用微量进样器注射5 μL生理盐水,留针15 min后,缓慢撤针,缝合切口。Sham组注入等容积生理盐水,注射时间及留针时间同上。注射完毕后,手术部位涂碘伏,缝合头皮,术后7 d腹腔内注射给予青霉素,每只4万U/d。分笼饲养。

1.3 行为学检测

大鼠记忆功能的测定采用Morris水迷宫法。Morris水迷宫(Morris Water Maze)购自中国医学科学院,形状是不锈钢的圆桶形水池,直径130 cm,高50 cm。池壁4个入水点将水池等分为4个象限,任选其中1个象限,正中放置1个直径11 cm、高29 cm的平台。选用定位航行试验:时间4 d,上、下午各2次,从4个入水点将大鼠面向池壁分别放入水中,记录其逃避潜伏期(在3 min内爬上平台的时间)。在术后2、4周重新进行水迷宫实验,计算成绩。

1.4 大鼠海马的活体^{1}H MRS检查

GE Signa1.5T超导型磁共振成像仪测定AD模型大鼠海马的^{1}H MRS变化。采用MRS扫描仪系统预设软件完成基线校准、信号平均、代谢物识别,自动计算各代谢物波峰曲线下面积。处理工作站自动生成以Cr为参照的各种代谢物信号强度的比值。

1.5 组织灌注和固定

在建立模型后不同观测时间点,以10%水合氯醛麻醉大鼠,经升主动脉先后以0.9%生理盐水100 mL冲洗3次、随后用0.1M PBS(pH, 7.4)4%多聚甲醛灌注固定。解剖获取大脑,于4 ℃ 4%多聚甲醛中后固定2 h,储存于4 ℃ 30% 0.1 mol/L PBS蔗糖溶液中,直至组织下沉。

1.6 病理学观察

采用苏木精-伊红染色法(Hematoxylin-eosin staining, HE),简之,将标本切片、充分水洗(过夜)后,行脱水、透明、石蜡包埋、切片和染色,封片,显微镜下观察。

1.7 免疫荧光组化步骤

$A\beta_{1-42}$兔多克隆抗体(Abcam公司,英国)、免疫组织化学染色试剂盒购于博士德生物工程公司(武

汉)。按照染色试剂盒说明操作,滴加一抗工作液(1:50),3,3'-二氨基联苯胺(DAB)显色,苏木素复染,封片。显微镜下观察拍照。结果判定:背景无颜色,胞浆内着棕黄色颗粒为阳性细胞。

1.8 统计学处理

实验结果均采用均数±标准差表示。两样本均数的比较采用独立样本的t检验;所有数据均由SPSS15.0软件计算完成。P<0.05代表差异具有统计学意义。

2 结 果

2.1 大鼠行为学变化

术前各组大鼠学习记忆能力基本一致。Sham组各时间点大鼠学习记忆能力无明显差异。随时间的推移,与Sham组比较,AD模型组的游全程时间逐渐延长,注射$A\beta_{1-42}$后2、4周错误次数显著增加(P<0.01)(表1)。

表1 老年性痴呆模型大鼠水迷宫学习成绩变化(n=20, $\bar{x}\pm s$, s)

Tab.1 Score of Morris water maze for experimental rats injected with $A\beta_{1-42}$ in hippocampus(n=20, *Mean±SD*, s)

Group	Before injection	Time after injection	
		2 weeks	4 weeks
Sham	20.36±4.14	21.54±4.26	23.75±5.48
AD	38.03±6.12**	45.62±9.34**	25.12±6.40

AD: Alzheimer's disease; **: Compared with Sham group, P<0.01

2.2 ^{1}H MRS改变

Sham组双侧半球^{1}H MRS结果无显著性差异。与Sham组比较,AD模型组,双侧^{1}H MRS海马NAA/Cr明显降低,提示海马注射$A\beta_{1-42}$可能引起海马局部神经元凋亡或变性;Cho/Cr增加提示海马注射$A\beta_{1-42}$可能引起海马局部有胶质增生、细胞增殖现象;Lac/Cr增加提示海马注射$A\beta_{1-42}$可能引起海马局部氧化性损伤。在AD模型组,与注射对侧半球比较,注射侧半球海马,NAA/Cr明显降低(P<0.05)、Cho/Cr显著增加(P<0.01)、Lac/Cr显著增加(P<0.01)(表2)。

表2 老年性痴呆模型大鼠^{1}H MRS变化(n=20, $\bar{x}\pm s$)

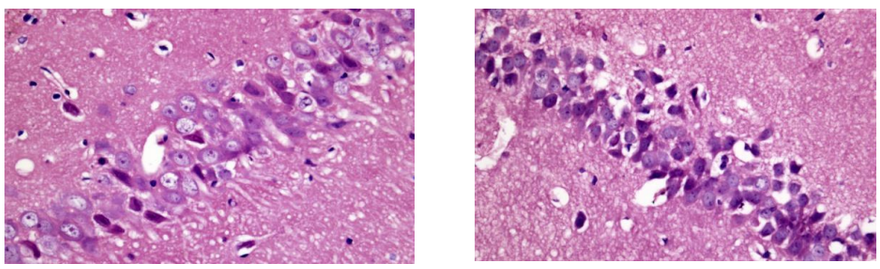
Tab.2 Changes of ^{1}H MRS in experimental rats injected with $A\beta_{1-42}$ in hippocampus (n=20, *Mean±SD*)

Group		NAA/Cr	Cho/Cr	Lac/Cr
Sham	Contralateral	1.27±0.21	1.68±0.34	1.80±0.46
	Injection side	1.21±0.23	1.57±0.26	2.07±0.37
AD	Contralateral	1.02±0.22	2.45±0.52	2.31±0.34
	Injection side	0.72±0.15*	3.32±0.47**	3.71±0.71*

^{1}H MRS: ^{1}H-proton magnetic resonance spectroscopy; *, **: compared with contralateral in AD group, P<0.05, P<0.01

2.3 大鼠脑海马病理学改变

Sham组光镜下可见正常大鼠脑组织神经细胞形态规则,胞浆丰富,嗜碱性,核圆形,核仁清晰可见,双侧半球无显著性差异。AD模型组鼠海马可见大量空泡样结构,神经元数目减少,核萎缩;与对侧半球比较,$A\beta_{1-42}$注射侧半球空泡样结构明显增多,核萎缩数量增加,见图1。



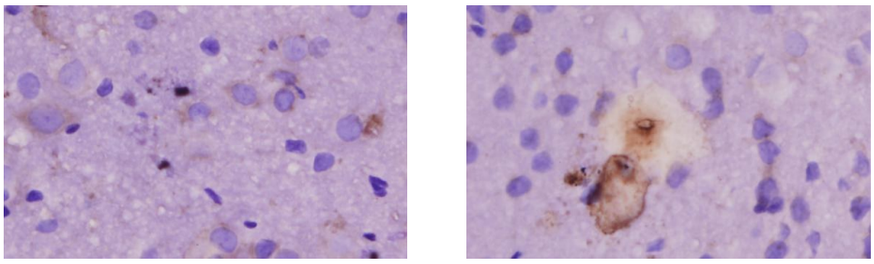
a: Contralateral hemisphere　　**b: Injected hemisphere**

图1 注射$A\beta_{1-42}$诱导老年性痴呆大鼠模型病理学变化(HE染色,×400)

Fig.1 Pathological changes in experimental rats injected with $A\beta_{1-42}$ in hippocampus to induce AD (HE staining, ×400)

2.4 大鼠脑海马$A\beta_{1-42}$表达

Sham组抗体为$A\beta_{1-42}$表达阴性(-)。AD模型组,$A\beta_{1-42}$注射后处理4周后,免疫组化结果显示双侧脑组织$A\beta_{1-42}$阳性表达,提示该区域有$A\beta_{1-42}$(图2);与未注射侧比较(+),注射侧脑组织$A\beta_{1-42}$阳性表达明显增强(+++)(P<0.01)。



a: Contralateral hemisphere　　b: Injected hemisphere

图2 注射$A\beta_{1-42}$诱导老年性痴呆大鼠模型脑抗$A\beta_{1-42}$免疫组化变化（抗体为$A\beta_{1-42}$抗体，DAB染色，×400）

Fig.2 Changes of $A\beta_{1-42}$ expressing in experimental rats injected with $A\beta_{1-42}$ in hippocampus to induce AD (Immunohistochemical staining with antibody of $A\beta_{1-42}$, DAB staining, ×400)

3 讨 论

3.1 AD模型方法的评价

Aβ可能是AD形成和发展的关键因素之一。目前较多科学家认同AD病理发生早期Aβ纤维在大脑皮质的堆积导致AD其他的病理症状[1]。本实验经脑立体定位仪向海马区注射凝聚态$A\beta_{1-42}$，与Sham组比较，海马内注射$A\beta_{1-42}$的模型组大鼠在造模2、4周后行为学检查结果显示学会躲避电击所需次数明显增多，提示其学习记忆能力下降。本实验在β_{1-42}注射后处理4周后，脑组织切片，免疫组化染色，结果显示注射侧脑组织$A\beta_{1-42}$阳性表达，提示该区域有$A\beta_{1-42}$；与未注射侧比较，注射侧脑组织$A\beta_{1-42}$阳性表达明显增强。

3.2 ^{1}H MRS对AD诊断价值

MRS是利用核磁共振现象核化学位移进行化合物分析的影像学技术[4-7]。不同化合物由于化学位移的差别，测定其在MR波谱上共振峰的区别，共振核的数目可以通过测定共振峰的面积，反映目标化合物的浓度，可进行定量分析[8-10]。

NAA主要存在于神经元及其轴突内，可以反映轴突致密性、代谢活跃程度，NAA水平的降低反映神经元减少、轴突损伤；Cho分布在细胞膜上，与细胞膜的合成和代谢有关，Cho的升高反映神经细胞膜损伤；Cr是能量代谢物质，特定个体Cr含量在不同代谢条件较稳定。^{1}H MRS检测大脑这几种物质在大脑内的含量的代谢，可以间接反映大脑细胞病理生理特性[13-16]。

本实验显示，与Sham组比较，AD模型组，双侧^{1}H MRS海马NAA/Cr明显降低，提示海马注射$A\beta_{1-42}$可能引起海马局部神经元凋亡或变性；Cho/Cr增加提示海马注射$A\beta_{1-42}$可能引起海马局部有胶质增生、细胞增殖现象；Lac/Cr增加提示海马注射$A\beta_{1-42}$可能引起海马局部氧化性损伤。在AD模型组，与注射对侧半球比较，注射侧半球海马，NAA/Cr明显降低（$P<0.05$）、Cho/Cr显著增加（$P<0.01$）、Lac/Cr显著增加（$P<0.01$）。

综上所述，注射Aβ后4周NAA/Cr已有明显改变，与免疫组化发现$A\beta_{1-42}$表达增强结果一致，提示利用^{1}H MRS检测NAA/Cr改变，可能有助于AD早期临床诊断。

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