·癫痫临床与基础研究·

艾地苯醌对癫痫发作致海马损伤神经保护作用及其机制研究

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【摘要】 **目的**　探讨艾地苯醌(IDBN)对癫痫大鼠海马神经元损伤的预防性保护作用及其作用机制。**方法**　共48只Wistar大鼠随机分为正常对照组、IDBN预防组(预防组)、癫痫组和IDBN 25 mg组、50 mg组、100 mg组,观察不同处理组大鼠治疗前后行为学变化,检测海马组织超氧化物歧化酶(SOD)、谷胱甘肽过氧化物酶(GSH-Px)活性和丙二醛(MDA)含量,观察海马组织学形态及神经元线粒体超微结构。**结果**　不同处理组大鼠海马组织SOD、GSH-Px和MDA表达水平差异具有统计学意义(均$P=0.000$)。IDBN不同剂量组以100 mg组SOD和GSH-Px活性最强、MDA含量最低;SOD和GSH-Px活性高于癫痫组($P=0.000,0.000$)、IDBN 25 mg组($P=0.000,0.000$)和50 mg组($P=0.004,0.005$),MDA含量低于癫痫组($P=0.000$)、IDBN 25 mg组($P=0.000$)和50 mg组($P=0.002$)。与正常对照组和预防组相比,癫痫组大鼠海马神经元出现不同程度损伤,经艾地苯醌处理后损伤减轻且随剂量的增加其程度逐渐减轻;癫痫组大鼠海马神经元线粒体结构破坏明显,可见变形、肿胀,部分线粒体呈空泡化;经艾地苯醌处理后损伤减轻,且随剂量的增加其程度逐渐减轻。**结论**　艾地苯醌可通过抑制癫痫大鼠体内氧化应激损伤作用保护海马神经元结构及功能。

【关键词】 苯醌类;　癫痫;　海马;　细胞凋亡;　超氧化物歧化酶;　谷胱甘肽过氧化酶;　丙二醛;　疾病模型,动物

Neuroprotective effect and mechanism of idebenone on hippocampal damage induced by epileptic seizure

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【**Abstract**】 **Objective**　To investigate the protective effect of idebenone (IDBN) on hippocampal neuron injury in epileptic rats and its mechanism. **Methods**　Forty-eight Wistar rats were randomly divided into normal control group, IDBN prevention group (prevention group), epilepsy group, IDBN 25, 50 and 100 mg groups. Behavioral changes of rats in different treatment groups before and after treatment with IDBN were observed. The activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and the content of malondialdehyde (MDA) in hippocampal were detected. The ultrastructural changes of hippocampal neurons and mitochondria were observed. **Results**　The expression levels of SOD, GSH-Px and MDA in different treatment groups were significantly different ($P=0.000$, for all). The activity of SOD and GSH-Px was the highest and the content of MDA was the lowest in IDBN 100 mg group. The activity

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of SOD and GSH-Px in IDBN 100 mg group were higher than those in epilepsy group (P = 0.000, 0.000), IDBN 25 mg group (P = 0.000, 0.000) and 50 mg group (P = 0.004, 0.005), while the content of MDA was lower than that in epilepsy group (P = 0.000), IDBN 25 mg group (P = 0.000) and 50 mg group (P = 0.002). Compared with normal control group and prevention group, hippocampal neurons in epilepsy group showed different degrees of damage, which was relieved after treatment with IDBN and gradually decreased with the increase of dose. In epilepsy group, the mitochondrial structure of hippocampal neurons was damaged obviously, with deformation and swelling, and some mitochondria were vacuolated. After treatment with IDBN, the damage was relieved, and the 100 mg group had the least damage. **Conclusions** IDBN can protect hippocampal neuron structure and function by inhibiting oxidative stress injury in epileptic rats.

【Key words】 Benzoquinones; Epilepsy; Hippocampus; Apoptosis; Superoxide dismutase; Glutathione peroxidase; Malondialdehyde; Disease models, animal

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癫痫是一种常见的神经系统疾病，严重影响患者生活质量，给家庭和社会带来沉重负担[1-2]。氧化应激是癫痫发作的重要病理生理学机制，线粒体作为其关键调控靶点，发生结构异常或功能障碍时可导致癫痫发作[3-5]；线粒体相关遗传性疾病主要表现为反复癫痫发作，长期发作可使线粒体形态结构异常，并加重线粒体功能障碍[4,6-7]。目前临床常用的抗癫痫药物（AEDs）主要有钠通道阻滞药、钙通道阻滞药、兴奋性氨基酸受体阻断药等，但疗效均不尽如人意，因此有必要进一步探究癫痫发病机制，寻找新的治疗靶点。艾地苯醌（IDBN）是一种辅酶Q10类似物，可激活线粒体呼吸活性，改善神经元能量代谢，提高神经元对葡萄糖的利用率，促进ATP生成，同时具有较强的抗氧化及自由基清除能力，主要用于慢性脑血管病或颅脑创伤等引起的神经损伤的治疗[8-10]。艾地苯醌近年开始用于癫痫领域的动物实验与临床研究，目前有关该药在癫痫致神经损伤中作用机制的报道较少，本研究拟通过构建癫痫大鼠模型，观察艾地苯醌对癫痫致海马神经元损伤的预防性保护作用及可能作用机制，以为癫痫治疗提供新的思路及作用靶点。

材料与方法

一、实验材料

1.实验动物　清洁级健康雄性Wistar大鼠共计48只，体重220～250 g，由山东大学实验动物中心提供[许可证号：SCXK（鲁）20190001]。于室温22 ℃、相对湿度50%、12 h昼-12 h夜循环照明环境中饲养，自由摄食、进水适应性饲养1周进行动物实验。本研究经山东大学动物伦理委员会审核批准（审批号：KYLL-2017-565）。

2.试剂与仪器　（1）主要药品与试剂：艾地苯醌（纯度100%，规格30 mg）购自齐鲁制药有限公司，氯化锂（纯度≥99.98%，规格100 g）及匹罗卡品（纯度≥98%，规格5 g）购自美国Sigma公司，地西泮（规格10 mg/2 ml）购自哈药集团三精制药有限公司，总超氧化物歧化酶（SOD）活性检测试剂盒、谷胱甘肽过氧化物酶（GSH-Px）活性检测试剂盒、丙二醛（MDA）检测试剂盒均购自南京建成生物工程研究所。（2）主要设备与仪器：BH-5生物光学显微镜购自日本Olympus公司，JEOL JEM-2100F透射电子显微镜购自德国Siemens公司，高速冷冻离心机购自美国Thermo Fisher公司，UV1800紫外分光光度计购自美国Alpha Innotech公司。

二、实验方法

1.动物分组　按照随机数字表法将大鼠分为正常对照组、IDBN预防组（预防组）、癫痫组和IDBN治疗组（25 mg组、50 mg组和100 mg组），每组各8只动物。IDBN剂量参照文献[11]方法，IDBN治疗组大鼠灌胃剂量分别为25、50和100 mg/（kg·d），预防组大鼠以IDBN 100 mg/（kg·d）灌胃，正常对照组和癫痫组大鼠则以等体积生理盐水灌胃；各组均连续灌胃3 d。IDBN治疗组和癫痫组大鼠灌胃后3 d于腹腔注射氯化锂（127 mg/kg），20 h后皮下注射氢溴酸东莨菪碱（1 mg/kg）以减轻胆碱能反应，30 min后经腹腔注射匹罗卡品（50 mg/kg）；预防组大鼠灌胃后3 d仅于腹腔注射等体积生理盐水。据Racine分级标准[12]评估癫痫发作等级：0级，无惊厥；Ⅰ级，面部痉挛抽动，包括眨眼、动须、节律性咀嚼等；Ⅱ级，Ⅰ级＋节律性点头；Ⅲ级，Ⅱ级＋前肢痉挛；Ⅳ级，双侧前肢阵挛、抽搐，伴身体立起；Ⅴ级，持续站立、倾倒、失平衡、四肢抽搐。从癫痫发作开始观察大鼠

行为学改变，发作达Ⅳ或Ⅴ级，且持续30 min以上者视为模型构建成功。癫痫组于癫痫发作后1 h腹腔注射地西泮（10 mg/kg）终止发作，建模过程中癫痫组有1只大鼠死亡，及时补充后继续实验，建模成功率达8/8。

2.化学比色法检测氧化应激反应　每组随机选取5只大鼠，断头切取适量海马组织，按质量体积比1∶9加入磷酸盐缓冲液冰浴研磨，制备组织匀浆，4 ℃、3500转/min离心10 min，取上清液。（1）SOD活性检测：参照试剂盒说明书，加入0.05 ml上清液和1.30 ml工作液混匀，37 ℃水浴40 min，显色、室温静置10 min，紫外分光光度计测定550 nm波长处光密度值。（2）GSH-Px活性检测：参照试剂盒说明书，加入0.20 ml上清液和2.10 ml工作液混匀，于37 ℃水浴5 min，显色、室温静置15 min，紫外分光光度计测定412 nm波长处光密度值。（3）MDA含量检测：参照试剂盒说明书，加入0.20 ml上清液和5.20 ml工作液混匀，95 ℃水浴40 min，冷却后于4 ℃、3500转/min离心10 min，取上清液，再以紫外分光光度计测定532 nm波长处光密度值。

3.HE染色观察海马组织学形态变化　每组各3只大鼠断头、切取适量海马组织，以质量分数为4%多聚甲醛固定24 h，制备厚度为2.50 μm石蜡切片；常规HE染色，BH-5生物光学显微镜采集图像，观察各组大鼠海马神经元变化。

4.海马神经元线粒体超微结构观察　取HE染色所用大鼠海马组织CA1和CA3区，制成大小约为1 mm×1 mm×1 mm组织块，置于预冷质量分数为2.5%戊二醛溶液，依次漂洗后以质量分数1%锇酸固定2 h，漂洗、脱水、包埋，制备厚度为0.50～1 μm组织切片；甲苯胺蓝染色、制备厚度为50～70 nm组织切片；柠檬酸铅和醋酸铀双重染色15～30 min，漂洗、质量分数0.2%氢氧化钠分化、漂洗、干燥，于JEOL JEM-2100F透射电子显微镜下观察海马神经元线粒体超微结构。

5.统计分析方法　采用SPSS 24.0统计软件进行数据处理与分析。正态性检验采用Shapiro-Wilk检验，呈正态分布的计量资料以均数±标准差（$\bar{x}\pm s$）表示，采用单因素方差分析，两两比较行LSD-t检验。以$P\leqslant 0.05$为差异具有统计学意义。

结　果

不同处理组大鼠SOD、GSH-Px和MDA表达水平差异具有统计学意义（均$P=0.000$，表1）。IDBN不同剂量组与其他各组比较，以100 mg组SOD活性最强，分别高于正常对照组（$P=0.000$）、预防组（$P=0.000$）、癫痫组（$P=0.000$），以及IDBN 25 mg组（$P=0.000$）和50 mg组（$P=0.004$）；虽然IDBN 25 mg组和50 mg组GSH-Px活性低于正常对照组（$P=0.000$，0.000）和预防组（$P=0.000$，0.000），但50 mg组高于癫痫组（$P=0.000$），而IDBN 100 mg组同时高于癫痫组（$P=0.000$）、25 mg组（$P=0.000$）和50 mg组（$P=0.005$）；IDBN 25 mg组和50 mg组MDA含量高于正常对照组（$P=0.000$，0.000）和预防组（$P=0.000$，0.000），而IDBN 100 mg组MDA含量最低，分别低于癫痫组（$P=0.000$）、25 mg组（$P=0.000$）以及50 mg组（$P=0.002$，表2）。

HE染色显示，正常对照组和预防组大鼠海马神经元形态和结构完整清晰，胞核着色均匀、核仁明显；癫痫组大鼠海马神经元不同程度损伤，神经元稀疏、排列无序，可见肿胀、变形、坏死的神经元，胞核固缩、碎裂；与癫痫组相比，IDBN不同剂量组大鼠海马神经元损伤呈剂量依赖性逐渐减轻，25 mg组可见神经元脱失、肿胀，部分神经元空泡化，少数神经元胞核固缩；50 mg组可见神经元排列较规整，脱失、肿胀程度减轻，少量神经元胞质存在空晕；100 mg组大部分神经元形态及结构正常，排列较规整，仅有个别神经元脱失（图1，2）。

超微结构观察显示，正常对照组和预防组大鼠海马神经元线粒体嵴排列紧密，形态及结构正常；癫痫组大鼠海马神经元线粒体变形、肿胀，结构破坏，部分线粒体呈空泡化，大片线粒体嵴缺失；与癫痫组相比，IDBN不同剂量组大鼠海马神经元线粒体损伤呈剂量依赖性减轻，25 mg组和50 mg组线粒体变形、肿胀、嵴脱失，100 mg组线粒体结构基本正常，偶见少量线粒体嵴脱失（图3）。

讨　论

癫痫系多种病因使大脑神经元高度同步化异常放电所致，目前全球患病率为1%～2%[2]。截至2021年，我国有近1000万例癫痫患者，患病率为4.5/1000～7.0/1000[13]，各年龄段均可发病，以青少年高发；临床表现主要包括感觉、运动、意识、精神、行为、自主神经功能障碍，并可伴认知功能、情感、睡眠障碍等，是多因素、多机制介导的复杂作用结果，发病机制尚未完全阐明[14-15]。目前认为癫痫发

表1 不同处理组大鼠海马组织SOD、GSH-Px活性及MDA含量比较($\bar{x} \pm s$)

Table 1. Comparison of the activity of SOD and GSH-Px and content of MDA in the hippocampus of rats in different treatment groups ($\bar{x} \pm s$)

组别	例数	SOD(U/mgprot)	GSH-Px(U/mgprot)	MDA(nmol/mgprot)
正常对照组(1)	5	247.87±16.84	122.72±11.17	2.12±0.22
预防组(2)	5	244.47±15.10	121.92±12.08	2.15±0.13
癫痫组(3)	5	179.92±12.43	65.58± 6.89	4.36±0.32
IDBN 25 mg组(4)	5	204.77±15.21	70.49±11.72	3.89±0.34
IDBN 50 mg组(5)	5	263.32±11.36	96.81± 7.09	3.48±0.75
IDBN 100 mg组(6)	5	292.05±15.23	115.84± 7.71	2.34±0.83
*F*值		38.999	33.988	18.697
*P*值		0.000	0.000	0.000

SOD,superoxide dismutase,超氧化物歧化酶;GSH-Px,glutathione peroxidase,谷胱甘肽过氧化物酶;MDA,malondialdehyde,丙二醛;IDBN,idebenone,艾地苯醌

表2 不同处理组大鼠海马组织SOD、GSH-Px活性及MDA含量的两两比较

Table 2. Pairwise comparison of the activity of SOD and GSH-Px and content of MDA in the hippocampus of rats in different treatment groups

组间两两比	SOD		GSH-Px		MDA	
	*t*值	*P*值	*t*值	*P*值	*t*值	*P*值
(1):(2)	0.371	0.714	0.132	0.896	0.075	0.940
(1):(3)	7.420	0.000	9.400	0.000	6.997	0.000
(1):(4)	4.706	0.000	8.102	0.000	5.536	0.000
(1):(5)	1.688	0.104	4.263	0.000	4.248	0.000
(1):(6)	4.824	0.000	1.132	0.269	0.693	0.496
(2):(3)	7.048	0.000	9.269	0.000	6.922	0.000
(2):(4)	4.334	0.000	7.978	0.000	5.461	0.000
(2):(5)	2.059	0.050	4.131	0.000	4.172	0.000
(2):(6)	5.196	0.000	1.000	0.328	0.618	0.544
(3):(4)	2.714	0.012	0.761	0.454	1.461	0.158
(3):(5)	9.107	0.000	5.137	0.000	2.749	0.011
(3):(6)	12.244	0.000	8.269	0.000	6.304	0.000
(4):(5)	6.393	0.000	4.083	0.000	1.288	0.210
(4):(6)	9.530	0.000	7.035	0.000	4.846	0.000
(5):(6)	3.137	0.004	3.131	0.005	3.558	0.002

SOD,superoxide dismutase,超氧化物歧化酶;GSH-Px,glutathione peroxidase,谷胱甘肽过氧化物酶;MDA,malondialdehyde,丙二醛

作与神经元凋亡、氧化应激、离子通道异常及免疫反应等因素相关。

钾离子、钠离子、钙离子等与癫痫发作密切相关,在通过离子通道后可使神经元膜电位发生改变,引起神经元兴奋或抑制,若离子通道发生功能障碍,可导致神经元发生兴奋性或抑制性异常改变,诱导癫痫发作[16];而免疫反应则可通过促进突触重塑、损伤血-脑屏障、降低癫痫发作阈值等引起癫痫发作。研究显示,癫痫患者血清T淋巴细胞亚群存在明显异常[17],且在癫痫患者和癫痫模型动物中均发现血清白细胞介素-1β(IL-1β)、肿瘤坏死因子-α(TNF-α)和环氧合酶-2(COX-2)等炎性因子水平升高,提示神经炎症可使神经元兴奋性增高、癫痫发作阈值降低[18]。活性氧(ROS)是机体正常氧化呼吸的代谢产物,氧化产物增多或抗氧化剂减少均可使机体内的氧化-抗氧化过程失衡,引起氧化应激[19-20]。线粒体是机体内源性活性氧的主要来源,其内膜上附着的线粒体DNA(mtDNA)无组织蛋白保护,而催化线粒体DNA复制的DNA聚合酶γ不具有校对功能,故对活性氧所致损伤异常敏感[21]。癫痫发作可产生大量活性氧,引起线粒体功能障碍;线粒体氧化损伤和功能障碍则可导致神经元能量代谢异常,增加癫痫易感性,进一步加重癫痫所致的神经损伤[7,22]。Goldenthal等[23]对40例线粒体病(MD)患儿进行随访观察,其中15例(37.50%)临床表现为癫痫发作,以反复发作者线粒体功能障碍尤为严重;因此有学者认为颞叶癫痫(TLE)与线粒体功能障碍有关[24]。艾地苯醌为线粒体靶向治疗药物,可以透过血-脑屏障,具有较强的抗氧化及抗自由基作用[10],而癫痫发作可使神经元处于高能量代谢状态,进而产生过量的氧自由基,引起线粒体损伤;本研究癫痫组大鼠海马神经元线粒体变形、肿胀,部分呈空泡化,经艾地苯醌处理后其线粒体损伤程度明显减轻,提示该药可能通过改善线粒体功能而达到神经保护作用。

SOD和GSH-Px是机体主要的抗氧化酶,SOD可对氧自由基进行特异性清除,催化超氧阴离子自由基歧化生成氧气和过氧化氢,其活性降低可导致机体清除活性氧能力下降[25-26];GSH-Px具有清除细胞内活性氧、阻断过氧化连锁反应的作用,在维护细胞结构及功能完整性方面具有重要作用[27],而

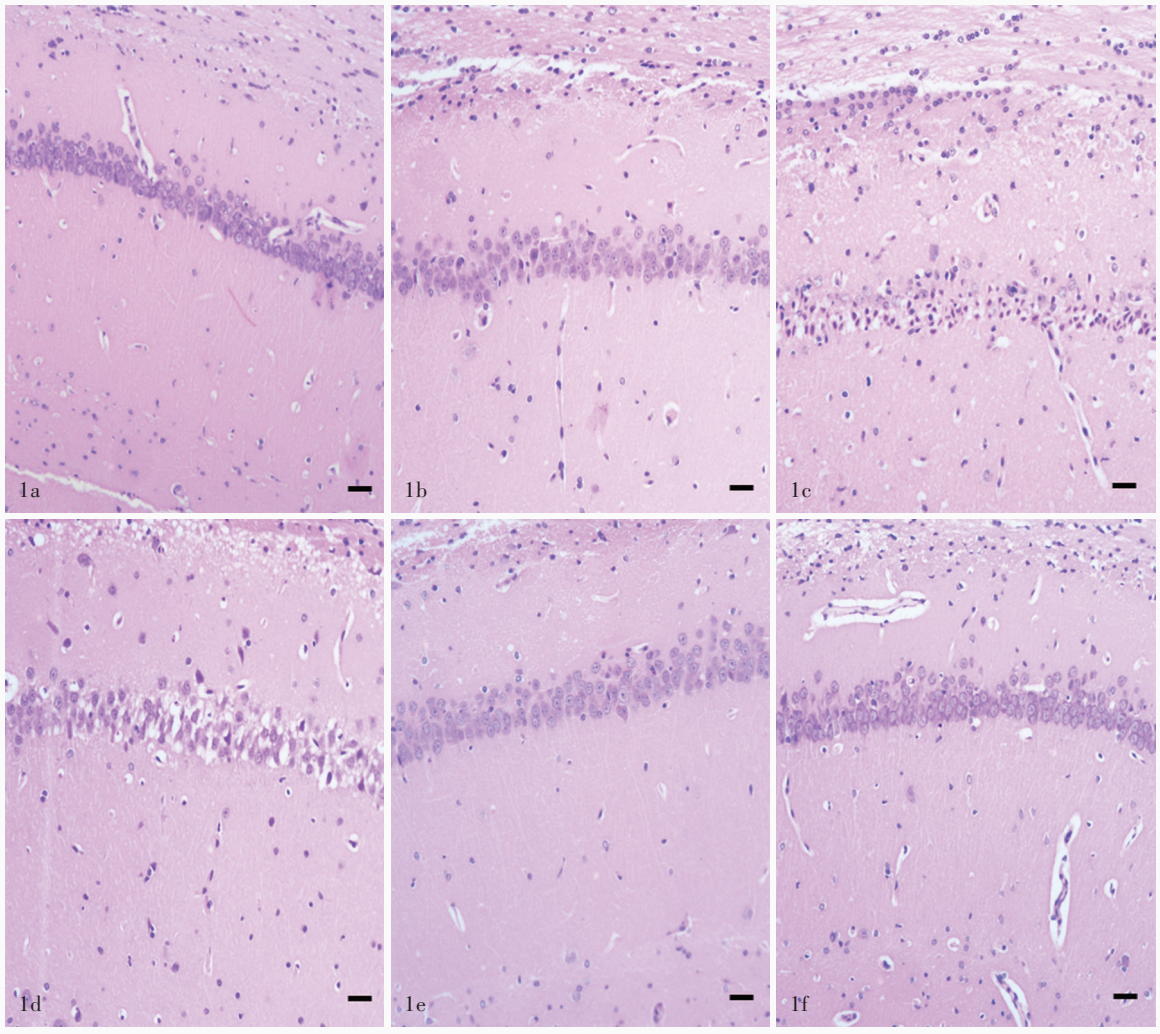

图1　光学显微镜观察海马组织CA1区所见　HE染色　中倍放大　1a　正常对照组神经元形态和结构完整清晰，胞核着色均匀、核仁明显　1b　预防组神经元结构完整　1c　癫痫组神经元肿胀、变形、坏死　1d　IDBN 25 mg组神经元脱失、肿胀，部分神经元空泡化，少数胞核固缩　1e　IDBN 50 mg组神经元排列有序，神经元肿胀、脱失减轻，少量神经元胞质中存在空晕　1f　IDBN 100 mg组神经元形态及结构基本正常，个别神经元脱失

Figure 1　Light microscopy of hippocampal CA1 region　HE staining　Median power magnified　In the normal control group, the morphology and structure of neurons were intact and clear, the nuclei were uniformly stained, and the nucleoli were obvious (Panel 1a). In prevention group, hippocampal neurons were structurally intact (Panel 1b). In epilepsy group, neurons were swollen, deformed and necrotic (Panel 1c). In the IDBN 25 mg group, neurons were lost and swollen, some neurons were vacuolated, and a few nuclei appeared pyknosis (Panel 1d). In the IDBN 50 mg group, neurons were arranged orderly, swelling and loss of neurons were reduced, and a few neurons had halo in their cytoplasm (Panel 1e). In the IDBN 100 mg group, neuron morphology and structure were basically normal, and some neurons lost (Panel 1f).

SOD、GSH-Px活性降低可使机体出现过氧化反应，进而导致组织细胞损伤。MDA是一种细胞脂质过氧化的分解产物，其含量可反映机体自由基累积及氧化应激损伤情况[28]。因此SOD、GSH-Px和MDA常被视为体内氧化应激的重要指标。有研究表明，中枢神经系统在各种诱因引起的氧化应激情况下均可能出现SOD和GSH-Px活性降低，以及MDA含量升高[8,29-31]。动物实验显示，过表达SOD的转基因小鼠可对抗癫痫发作所致的神经损伤，而缺乏SOD的小鼠出现癫痫发作易感性和神经元退化增加情况[32]，且癫痫大鼠GSH-Px活性降低尚可引起脂质过氧化和蛋白质氧化增强[33]。一项纳入29项临床研究计636例癫痫患者和665名健康对照者的Meta分析显示，癫痫患者血清SOD和GSH-Px活性均明显低于健康对照者[34]，而MDA含量高于健康对照者[35]。有研究显示，抗癫痫药物可使癫痫患者血清MDA水平降低[36]。本研究结果显示，与正常对照组相比，癫痫组大鼠海马神经元SOD和GSH-Px活性

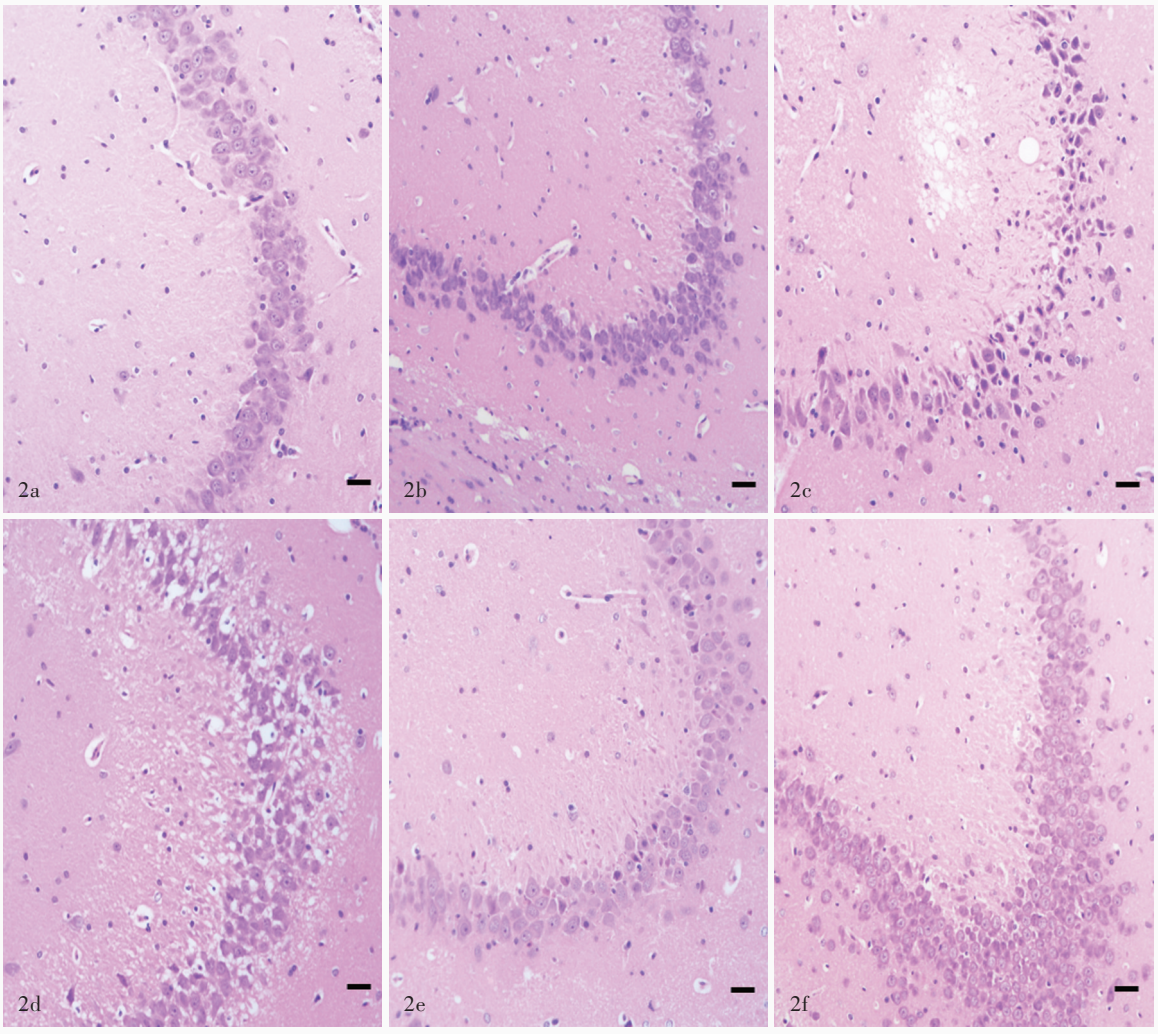

图2　光学显微镜观察海马组织CA3区所见　HE染色　中倍放大　2a　正常对照组神经元形态和结构完整清晰，胞核着色均匀、核仁明显　2b　预防组神经元形态和结构完整清晰　2c　癫痫组神经元肿胀、变形，胞核固缩、碎裂　2d　IDBN 25 mg组神经元脱失、肿胀，部分神经元空泡化，少数胞核固缩　2e　IDBN 50 mg组神经元排列有序，神经元肿胀、脱失减轻，少量神经元胞质中存在空晕　2f　IDBN 100 mg组神经元形态及结构基本正常，个别神经元脱失

Figure 2　Light microscopy of hippocampal CA3 region　HE staining　Median power magnified　In normal control group, the morphology and structure of neurons were intact and clear, the nuclei were evenly colored, and the nucleoli were obvious (Panel 2a). In prevention group, the morphology and structure of neurons were complete and clear (Panel 2b). In epilepsy group, neurons were swollen and deformed, and nuclei were pyknosis and fragmentation (Panel 2c). In IDBN 25 mg group, neurons were lost and swollen, some neurons were vacuolated, and a few nuclei were pyknosis (Panel 2d). In IDBN 50 mg group, neurons were arranged orderly, swelling and loss of neurons were reduced, and a few neurons had halo in their cytoplasm (Panel 2e). In IDBN 100 mg group, the morphology and structure of neurons were basically normal, and some neurons lost (Panel 2f).

降低、MDA含量升高，且IDBN不同剂量组神经元损伤程度呈剂量依赖性减轻，其中IDBN 100 mg组海马神经元线粒体结构基本正常，说明癫痫发作可以引起自由基堆积及氧化应激损伤，而艾地苯醌预处理可在一定程度上改善这种损伤；此外，预防组大鼠未出现海马神经元线粒体损伤，且SOD、GSH-Px活性和MDA含量与正常对照组无明显差异，提示艾地苯醌100 mg对癫痫致线粒体损伤的预保护作用更佳，结合既往研究结果，考虑艾地苯醌可能通过作用于氧化应激的调控过程改善癫痫发作引起的海马神经元线粒体损伤。本研究艾地苯醌剂量参考文献[11]的给药方法，未来需纳入更多剂量组别并进行相应的毒理学试验，以明确最佳预防性保护剂量。

综上所述，本研究通过构建氯化锂-匹罗卡品癫痫大鼠模型，发现癫痫大鼠海马组织出现明显的神

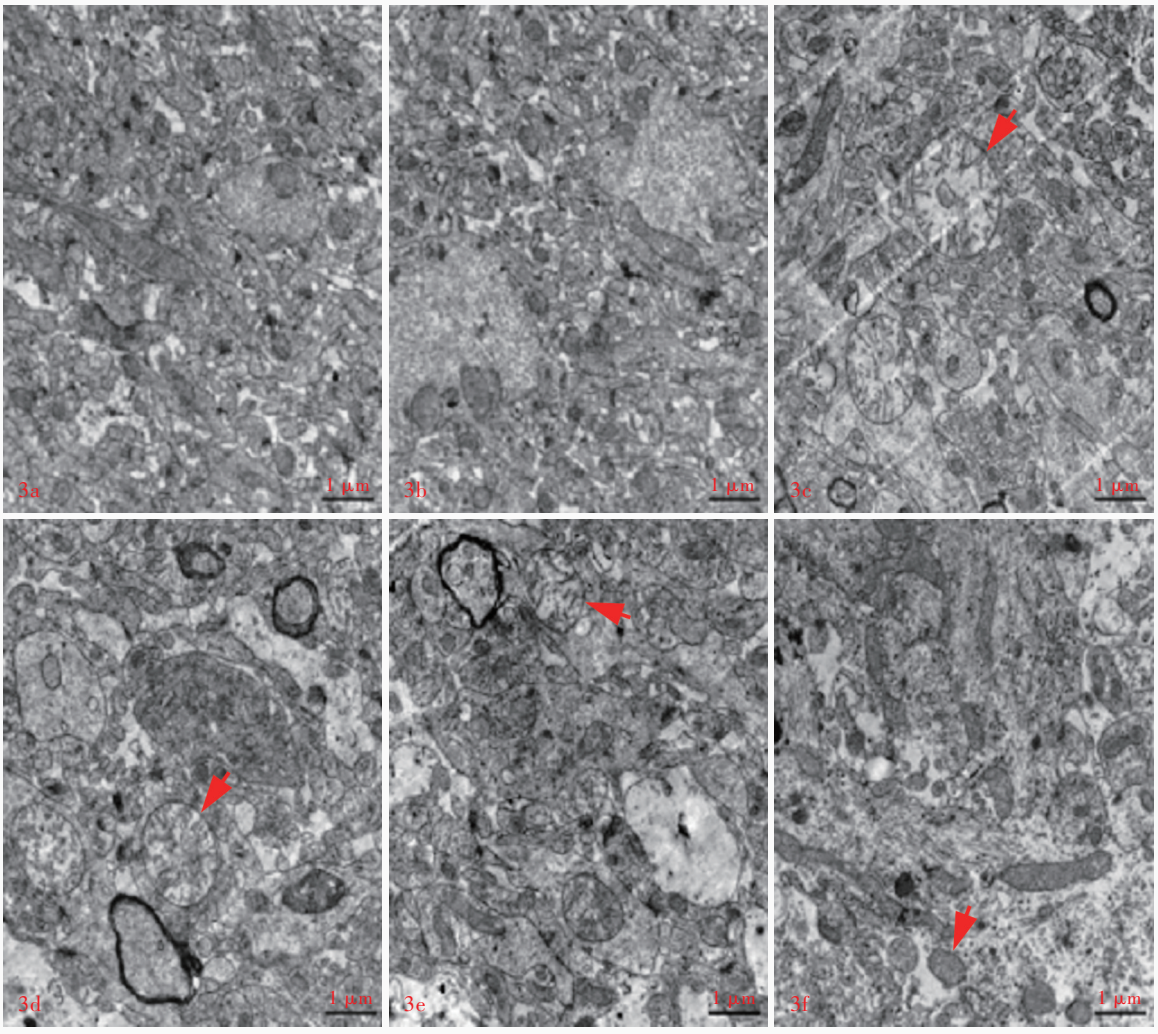

图3　透射电子显微镜观察海马神经元线粒体超微结构　柠檬酸铅与醋酸铀双重染色　3a　正常对照组神经元线粒体嵴排列紧密、形态及结构正常　3b　预防组神经元线粒体形态和结构正常　3c　癫痫组神经元线粒体变形、肿胀，结构破坏，部分线粒体呈空泡化（箭头所示）　3d　IDBN 25 mg组神经元线粒体肿胀明显，变形和嵴脱失，但较癫痫组轻微（箭头所示）　3e　IDBN 50 mg组神经元线粒体肿胀明显减少，结构仍完整，嵴缺失不明显（箭头所示）　3f　IDBN 100 mg组神经元线粒体结构基本正常（箭头所示）

Figure 3　Transmission electron microscopy of mitochondrial ultrastructure in hippocampal neurons　Double staining with lead citrate and uranium acetate　In control group, the mitochondrial cristae of normal neurons were closely arranged, and their morphology and structure were normal (Panel 3a). In prevention group, the morphology and structure of neuron mitochondria were normal (Panel 3b). In epilepsy group, the mitochondria of neurons were deformed, swollen, structurally destroyed, and some mitochondria were vacuolated (arrow indicates, Panel 3c). In IDBN 25 mg group, the swelling, deformation and crista loss of neurons mitochondria were obvious, but the degree was lighter than that of epilepsy group (arrow indicates, Panel 3d). In IDBN 50 mg group, the swelling of neuron mitochondria was obviously reduced, the structure was still intact, and the crista loss was not obvious (arrow indicates, Panel 3e). In IDBN 100 mg group, the mitochondrial structure of neurons was basically normal (arrow indicates, Panel 3f).

经元坏死、凋亡及缺失，艾地苯醌预处理对癫痫大鼠具有一定的神经保护作用，可为临床合理用药提供理论依据。

利益冲突　无

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2.关键词选取　论著类稿件均应标引3～5个关键词。关键词请尽量选取美国国立医学图书馆编辑的最新版*Index Medicus*中医学主题词表（*MeSH*）内所列的词。若最新版*MeSH*中尚无相应的词，可根据树状结构表选取最直接的上位主题词，必要时可采用习用的自由词排列于最后。关键词中的缩写词应按*MeSH*还原为全称。

3.基金项目著录　论文所涉及的课题如为国家或部、省级等基金项目或属攻关项目，应脚注于文题页左下方，并在圆括号内注明其项目编号，如：“基金项目：××××（项目编号：××××）”。 基金项目名称应按照国家有关部门规定的正式名称书写，多项基金项目应以“；”隔开后逐条列出。并附基金项目证明复印件。由厂商赞助的课题应在资金来源处注明。

4.摘要撰写　论著类稿件须附中、英文摘要。摘要的内容必须包括研究背景（Background）或目的（Objective）、方法（Methods）、结果（Results）及结论（Conclusions）共四部分。一般采用第三人称撰写，不用“本文”、“作者”等主语，不列图、表，不引用文献，不加评论和解释。摘要应客观、如实地反映文章原文，不得添加原文中所没有的内容。中文摘要以不超过800字为宜，英文摘要应与中文摘要相对应。英文摘要中应提供正式对外交流的英文单位名称。其他各类稿件均应附简要的中英文摘要，摘要内容要客观全面地反映文章的中心内容，中英文摘要内容要一致。

《中国现代神经疾病杂志》编辑部关于稿件统计分析方法的要求

《中国现代神经疾病杂志》编辑部对来稿中的统计分析方法一律要求明确研究设计方法，以及详细描述资料性质和结果，具体要求如下：

1.研究设计方法　要求交代研究设计的名称和主要方法。如调查设计应写明是前瞻性、回顾性还是横断面调查研究；实验设计应写明具体设计类型，如自身配对设计、成组设计、交叉设计、析因设计或正交叉设计等；临床试验设计应写明属于第几期临床试验，采用何种盲法措施等。应围绕“重复、随机、对照、均衡”四项基本原则进行概要说明，尤其要说明如何控制重要的非试验因素的干扰和影响。

2.资料及结果的表达与描述　采用均数±标准差（$\bar{x} \pm s$）表示近似服从正态分布的定量资料，采用中位数和四分位数间距[$M(P_{25}, P_{75})$]表示呈偏态分布的定量资料；采用相对数构成比（%）或率（%）表示计数资料，用相对数构成比时分母不能小于20。应写明所用统计分析方法的具体名称、统计量具体值，应尽可能给出确切的P值；当涉及总体参数时，在给出显著性检验结果的同时，给出95%CI。