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阿尔茨海默病实验动物模型评述

李少创[1,2],韩诚[1,2*],秦亚莉[1,2],赵雅飞[1,2],魏文一[1,2],杨杰[1,2],帅月圆[1,2],郭栋[1]

(1. 山西中医药大学, 山西 晋中 030619; 2. 中医脑病山西省重点实验室,山西 晋中 030619)

【摘要】 阿尔茨海默病(Alzheimer's disease,AD)是一种病因未明且与年龄相关的不可逆性神经退行性疾病临床表现以认知和记忆功能丧失为主。目前,对于该病的发病机制及药物治疗效用的探索已成为现代脑科学研究的热点之一,但是其复杂的发生机制和病理学变化对实验动物模型的选择提出了重大挑战。本文就常用实验动物种类的特点、多种动物模型的甄选和模型构建方法进行了详细的评述。AD 常用动物模型可分为自然动物模型、物理干预模型、化学干预模型、基因干预动物模型,以及其他类型。本文对这些模型构建方法、病理变化情况和适用实验类型进行总结和评述,希望能为研究者选用和建立实验动物模型提供参考。

【关键词】 阿尔茨海默病;动物模型;构建方法;评价
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A review of experimental animal models of Alzheimer's disease

LI Shaochuang[1,2], HAN Cheng[1,2*], QIN Yali[1,2], ZHAO Yafei[1,2], WEI Wenyi[1,2], YANG Jie[1,2], SHUAI Yueyuan[1,2], GUO Dong[1]

(1. Shanxi University of Traditional Chinese Medicine, Jinzhong 030619, China. 2. Shanxi Key Laboratory of Chinese Medicine Encephalopathy, Jinzhong 030619)
Corresponding author: HAN Cheng. E-mail: hc@sxtcm.edu.cn

【Abstract】 Alzheimer's disease (AD) is an irreversible and age-associated neurodegenerative disease with unclear etiology, and is characterized by a gradual loss of cognitive and memory functions. At present, investigation into the pathogenesis of AD and the efficacy of drug treatment has become one of the hotspots in the field of brain science. It is challenging to select proper experimental animal models of AD because of its complicated etiological mechanisms and pathological changes. This article gives a detailed review of the characteristics of multiple laboratory animals and related AD models, as well as the method of model construction. In general, AD animal models can be divided into natural, physical intervention, chemical intervention, genetic intervention, and other animal models. This paper has summarized and commented on the method of models' construction, the changes of pathology and applicable types of experiments, hoping to provide reference for researchers to select and establish experimental animal models.

【Keywords】 Alzheimer's disease; animal models; construction method; evaluation
Conflicts of Interest: The authors declare no conflict of interest.

阿尔茨海默病(Alzheimer's disease,AD)是一种病因尚不明确的认知和记忆功能逐渐丧失的不可逆转的神经变性类疾病,多发于老年人群。据统计全球约有 5000 万 AD 患者[1],中国的患病情况亦

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[作者简介]李少创(1993—),男,硕士研究生,研究方向:证候规律研究。Email:2945758772@qq.com
[通信作者]韩诚(1987—),男,博士,讲师,硕士生导师,研究方向:中医藏象理论研究。Email:hc@sxtcm.edu.cn

不容乐观。据推测,随着中国老年人口的快速增加,未来 20 年中国的痴呆患者将大幅增加[2],这会极大的增加中国家庭和医疗系统以及社会的负担[3]。

AD 患者可表现出进行性记忆减退,执行功能受损,日常活动困难,思维和行为方式改变,以及语言功能损害等临床症状[4]。AD 的特征性病理表现为由神经元外蛋白质片段——β 淀粉样蛋白(β-amyloid,Aβ)的异常聚集,神经元内 Tau 蛋白的异常积累,神经胶质增生和神经元丢失导致的大脑萎缩[5],同时伴有脑血管淀粉样变性,炎症和重大突触改变等[6-8]。面对这一种破坏性较强的退行性神经疾病,其复杂的病因和病理变化,以及严重的社会危害性,对临床医生和科研工作者提出了重大挑战,而实验动物模型的正确选择则是解决这一难题的前提条件。本文通过总结当前各类 AD 实验动物模型的建立方法,针对其优势、适用条件和不足进行了详细评述,以期为研究者选用和建立实验动物模型提供可靠的参考依据。

1 实验对象的选择

人类和动物在生理上有极大的相似性[9],因此可以通过在动物身上模拟构建各类疾病模型,探索疾病的生物学机制,验证相关治疗方法的有效性。目前,用于制备 AD 模型的动物包括黑腹果蝇[10]、秀丽隐杆线虫[11]、斑马鱼[12]、小鼠、大鼠、犬[13]、恒河猴[14-15]和黑猩猩[16]等。实验动物在种属和生理条件上不尽相同,因此研究者可以根据所要研究的疾病机制和需要模拟的神经病理变化来选择合适的实验动物(见表 1)。

表 1 AD 实验动物的选择

Table 1 Selection of AD experimental animals

动物 Animal	所属 Belonging	生理特点 Physiological characteristics	优势 Advantages	不足 Disadvantages	适用范围 Scope of application	参考文献 References
黑腹果蝇 Drosophila melanogaster	昆虫纲双翅目果蝇科 *Insecta* *Diptera* *Drosophilidae*	低等生物,遗传物质较少 Lower organisms with less genetic material	遗传易操作 Genetically manageable	脑结构与人差距较大 There is a big gap between brain structure and humans	Tau 蛋白异常研究及转基因 Tau protein abnormality research and genetic modification	[10]
秀丽隐杆线虫 Caenorhabditis elegans	线虫纲小杆线虫目小杆科 *Chromadorea* *Rhabditida* *Rhabditidae*	体积小,寿命短 Small size, short life	体积小,寿命短,易于繁殖和实验操作 Small size, short life span, easy for reproduction and experimental operation	脑结构与人差距较大 There is a big gap between brain structure and humans	AD 发展中各种事件的顺序 Sequence of various events in AD development	[11]
斑马鱼 Zebrafish	辐鳍鱼纲鲤形目鲤科 *Actinopterygii* *Cypriniformes* *Cyprinidae*	体型小,具有与人相同 γ-分泌酶复合物 Small size, with the same γ-secretase complex as human	体型小,易于大规模饲养和基因操作,具有与人相同的直系 PSEN1 基因和 PSEN2 基因 Small size, easy for large-scale breeding and genetic manipulation, with the same direct line PSEN1 gene and PSEN2 gene as human	脑结构与人差距较大,缺乏明显的高级认知行为 There is a big gap between brain structure and human, lack of obvious high-level cognitive behavior	早老素 1 基因和早老素 2 基因转基因动物 Transgenic animals with presenilin 1 gene and presenilin 2 gene	[12]
小鼠 Mice	哺乳纲啮齿目鼠科 *Mammalian* *Rodentia* *Muridae*	与人具有高度直系同源,具有哺乳动物生理学上的相似性,并且体型小 It has a high degree of orthology with humans, has similarity in mammalian physiology, and is small in size	脑结构与人相近,饲养和维护成本低 The brain structure is similar to that of humans, and the cost of feeding and maintenance is low	培育转基因品系周期长,时间成本高 Cultivating genetically modified strains has a long cycle and high time cost	最常用的转基因动物,用来评估认知功能障碍 The most commonly used genetically modified animals to assess cognitive dysfunction	–

续表 1

动物 Animal	所属 Belonging	生理特点 Physiological characteristics	优势 Advantages	不足 Disadvantages	适用范围 Scope of application	参考文献 References
大鼠 Rat	哺乳纲啮齿目鼠科 *Mammalian Rodentia Muridae*	具有哺乳动物生理学上的相似性,并且体型较小 Similar to mammalian physiology and small in size	相比小鼠,脑容量更大,脑结构与人更为相近,饲养和维护成本低 Compared with mice, the brain capacity is larger, the brain structure is more similar to that of humans, and the cost of feeding and maintenance is low	培育转基因品系周期长,时间成本高 Cultivating genetically modified strains has a long cycle and high time cost	用作转基因动物,用来评估认知功能障碍 Used as a genetically modified animal to assess cognitive dysfunction	–
犬 Dog	哺乳纲食肉目犬科 *Mammals Carnivora Canidae*	可以患有与人相似的年龄相关性认知功能障碍 May suffer from age-related cognitive dysfunction similar to humans	可以自然地再现 AD 的关键方面,包括 Aβ 病理学,神经元变性以及学习和记忆障碍 Can naturally reproduce key aspects of AD, including Aβ pathology, neuronal degeneration, and learning and memory impairment	不会出现神经斑块和神经缠结 No nerve plaques and tangles	可作为自然动物模型,并可进行 Aβ 靶向药物的测定 It can be used as a natural animal model and can be used for the determination of Aβ targeted drugs	[13]
恒河猴 Rhesus Monkey	哺乳纲灵长目猴科 *Mammals Primates Cercopithecidae*	大脑在功能网络的整体架构和组织功能网络上具有很大的相似性,并且系统发育上更接近人类,视网膜具有其他哺乳动物不具有的特征(黄斑,中央凹)。药物动力学与人更为接近。进化具有高度保守性 The brain has great similarities in the overall structure of the functional network and the organizational functional network, and is closer to humans in system development, and the retina has features (macular, fovea) that other mammals do not have. Pharmacokinetics are closer to humans. Evolution is highly conservative	大脑结构比较接近人类,可以接受训练并执行感知和认知任务 The brain structure is closer to human beings and can be trained to perform perceptual and cognitive tasks	价格高,可用性受限,维护要求高,以及道德考虑 High price, limited availability, high maintenance requirements, and ethical considerations	人类临床和电生理研究 Human clinical and electrophysiological research	[14-15]
黑猩猩 Chimpanzee	哺乳纲灵长目人科 *Mammals Primates Hominidae*	大脑在功能网络的整体架构和组织功能网络上具有很大的相似性,并且系统发育上更接近人类,视网膜具有其他哺乳动物不具有的特征(黄斑,中央凹)。药物动力学与人更为接近 The brain has great similarities in the overall structure of the functional network and the organizational functional network, and is closer to humans in system development, and the retina has features (macular, fovea) that other mammals do not have. Pharmacokinetics are closer to humans	大脑结构最为接近人类,可以接受训练并执行感知和认知任务 The brain structure is closest to human beings and can be trained to perform perceptual and cognitive tasks	价格高,可用性受限,维护要求高,以及道德考虑 High price, limited availability, high maintenance requirements, and ethical considerations	人类临床和电生理研究 Human clinical and electrophysiological research	[16]

2 实验动物模型的构建

由于阿尔茨海默病发病机制尚不明确,学者围绕 AD 患者临床表现出的衰老特征和该疾病所特有的病理特征来进行合理推测并提出了众多发病假说,AD 模型的构建思路即是以各种假说为依据的。目前尚未真正模拟出与 AD 患者所有病理特征完全相符的模型,一方面,阿尔茨海默病的病理特征呈现出多样性特点;另一方面,动物和人类在生理上相似度虽然很高,但二者之间仍存在着一些差异。此外,动物模型设计的充分性、合理性会极大程度的影响受试药物效应的可靠性,进而影响将科学研究成果应用于临床。

因此,选择合适的实验动物模型是解决这一难题的前提条件。目前,可用的 AD 动物模型包括:自然动物模型、人工干预动物模型、遗传动物模型、其他动物模型。

2.1 自然动物模型

年龄是 AD 发病的重要危险因素之一[17]。自然动物模型以高龄老化作为 AD 的发病基础,可自发形成。一方面符合衰老或老化生理特征,另一方面无需人为干预,减少了人为因素参与所产生的误差,主要包括自然衰老模型和快速衰老模型。

2.1.1 自然衰老模型

衰老过程在动物(如啮齿类动物和犬科动物,以及非人灵长类动物)和人类所表现的体征(如认知功能下降、记忆能力衰退)比较相似[18]。该模型不需要干预因素,可自发形成,模型动物的老化症状与 AD 患者相似。但此类动物不会自发地形成 AD 典型的病理特征(如淀粉样蛋白沉积),可以用于开展生理性老化与 AD 之间关系的相关研究[19-20]。常用的衰老动物为小鼠和大鼠,小鼠衰老期在 12 ~ 24 月龄,大鼠衰老期在 21 ~ 32 月龄[21-22]。但是,由于造模周期较为漫长,受试动物很可能会在衰老过程中变生其他疾病或死亡,导致动物个体之间差异性增大或组内动物数量减少,最终导致实验失败。

2.1.2 快速衰老模型

此模型最早由日本京都大学 Takeda 等[23]培育出来,并将其命名为快速老化小鼠(senescence-accelerated mouse, SAM),包括快速衰老系 P 系(SAMP)和正常衰老系 R 系(SAMR)。P 系小鼠的衰老进程明显加快,可作为研究衰老相关机制实验的受试动物。R 系保留了动物的正常衰老特性,可作为 P 系实验动物的对照样本。SAMP 系小鼠可表现出 AD 特有的 Aβ 聚集、tau 蛋白异常过度磷酸化、神经元丢失等病理改变[24]。SAMP 系当中的 SAMP8 小鼠由于老化迅速和 AD 病理特征性改变发生早的特点,受到很多研究者的青睐,是较为理想的动物模型。

2.2 人工干预模型

2.2.1 物理干预模型

以 AD 疾病发生病理假说为基础,采用各种物理干预手段对实验动物进行干预,来制备相应动物模型。具体建模方法见表 2。

(1)胆碱能损伤模型

胆碱能损伤假说认为,AD 痴呆的严重程度与胆碱能神经元丧失的程度呈正相关性[25-26]。并且认知功能的正常与否依赖于足够的胆碱能神经传递[27],乙酰胆碱作为重要的神经递质之一,其在脑内神经元含量的减少,会引起认知和记忆能力的下降。一方面,在 AD 患者大脑中存在着广泛的神经元和突触缺陷,其中基底前脑神经元的退化在很大程度上影响了神经递质传递的有效性,从而导致 AD 认知能力的下降[28]。另一方面,乙酰胆碱脂酶活性增强,加速乙酰胆碱的分解,并且胆碱乙酰转移酶活性降低导致乙酰胆碱合成减少[29],更加剧了乙酰胆碱的匮乏程度,导致神经元之间的传递障碍,最终发展为 AD[30]。

本方法通过手术损伤海马伞的方法,造成动物胆碱能系统损坏、动物空间定向困难和记忆缺陷,进而制备胆碱能损伤模型[31-32]。

(2)颈总动脉结扎模型

研究表明,老年人脑血流量减少、血流减慢,脑部神经元长期处于慢性缺血缺氧状态,随时间延长,出现认知记忆障碍等 AD 病理特征表现[33]。

通过结扎受试动物颈总动脉来建立 AD 模型,包括单侧颈总动脉永久性结扎法[34]、双侧颈总动脉永久性结扎法[35-37],另有一侧颈总动脉闭塞一侧颈总动脉的狭窄法、双侧颈总动脉狭窄法、不对称双侧颈动脉狭窄法[38]。此模型通过结扎颈总动脉使脑组织处于缺血状态,随后出现空间学习记忆障碍。

(3)化学干预模型

化学干预模型主要以 AD 疾病发生病理假说为理论基础,通过向动物脑部特定区域以及身体各部

表 2　人工干预 AD 动物模型分类表

Table 2　Classification table of artificial intervention AD animal models

模型 Models	动物 Animal	化学物质/物理方法 Chemical substances/physical methods	操作方法 Method of operation	损伤部位 Injury site	优点 Advantages	缺点 Disadvantages	参考文献 References
胆碱能损伤模型 Cholinergic injury model	Wistar 大鼠/SD 大鼠 Wistar rat/SD rat	物理损伤 Physical damage	–	海马伞 Hippocampal fimbria	模拟胆碱系统损害,空间定向和记忆障碍 Simulate cholinergic system damage, spatial orientation and memory impairment	不出现 Aβ 和 tau 病理 No Aβ and tau pathology	[31-32]
颈总动脉结扎模型 Common carotid artery ligation model	SD 大鼠/Wistar 大鼠/C57BL/6J 小鼠 SD rat/Wistar rat/C57BL/6J mice	物理结扎 Physical ligation	–	颈动脉 Carotid artery	脑慢性缺血,认知障碍 Chronic cerebral ischemia, cognitive impairment	不出现 Aβ 和 tau 病理 No Aβ and tau pathology	[34-37]
Aβ 注射模型 Aβ infusion model	BALB/c 小鼠 BALB/c mice	$A\beta_{1-42}$	410 pmol, 3 μL, 脑定位注射, 注射时长 1 min, 留针 3 min 410 pmol, 3 μL, brain localization injection, injection time 1 min, needle retention 3 min	侧脑室(Bregma 点后 0.5 mm, 中线旁开 1.0 mm, 深度 2.5 mm) Lateral ventricle (0.5 mm behind Bregma point, 1.0 mm lateral to midline, 2.5 mm in depth)	–	–	[39]
	SD 大鼠 SD rats	$A\beta_{1-40}$	1 g/L, 1 μL, 脑定位注射, 注射时长 5 min, 留针 5 min 1 g/L, 1 μL, brain localization injection, injection time 5 min, needle retention 5 min	海马齿状回背侧(前囟后 3.3 mm, 右侧旁开 2.0 mm, 硬脑膜下 3.0 mm, 门齿钩平面低于耳间线平面 2.4 mm) The dorsal side of the hippocampal dentate gyrus (3.3 mm posterior to the bregma, 2.0 mm lateral to the right, 3.0 mm below the dura mater, and the incisor hook plane is 2.4 mm below the interaural line)	Aβ 沉积, 炎症反应, 学习记忆障碍 Aβ deposition, inflammation, learning and memory impairment	不符合 AD 渐进性发病的特点 Aβ 聚集在注射局部 Does not meet the characteristics of the progressive onset of AD, Aβ accumulates at the injection site	[40]
	SD 大鼠 SD rats	$A\beta_{25-35}$	10 μg/μL, 脑定位注射, 左右各 1 μL, 5 min 注射完, 留针 5 min 10 μg/μL, brain localization injection, 1 μL each on the left and right, after 5 min injection, keep the needle for 5 min	双侧海马 CA1 区(前囟为零点, 穿刺点位于前囟后 3.5 mm, 中线右侧旁开 2 mm, 以微量注射器自脑表面垂直进针 3 mm) CA1 area of the hippocampus on both sides (the bregma is the zero point, the puncture point is 3.5 mm behind the bregma, 2 mm on the right side of the midline, and the needle is vertically inserted 3 mm from the brain surface with a micro syringe)	–	–	[41]
鹅膏蕈氨酸注射模型 Ibotenic acid infusion model	SD 大鼠 SD rats	IBO	5 μg/μL, 1 μL	Meynert 基底核(前囟后 1.0 mm, 中线旁开 3.0 mm, 深 7.3 mm。) Meynert basal nucleus (1.0 mm behind bregma, 3.0 mm next to midline, 7.3 mm deep.)	Aβ 沉积和 tau 蛋白增加, 以及记忆障碍 Aβ deposition and tau protein increase, and memory impairment	不出现神经纤维缠结 No neurofibrillary tangles	[44]
链脲菌素注射模型 Streptozotocin infusion model	大鼠 Long Evans rats	STZ	40 mg/kg, 注射时长 3 min 40 mg/kg, injection time 3 min	双侧脑内(前囟后 1.0 mm, 中线右侧旁开 1.0 mm, 颅骨下 2.5 mm) In both sides of the brain (1.0 mm behind the bregma, 1.0 mm lateral to the right side of the midline, 2.5 mm below the skull)	Aβ 沉积, tau 蛋白过度磷酸化, 胆碱能缺失, 氧化应激 Aβ deposition, tau protein hyperphosphorylation, cholinergic loss, oxidative stress	不出现神经纤维缠结和老年斑 No neurofibrillary tangles and age spots	[48]
D-半乳糖注射模型 D-galactose infusion model	小鼠 Swiss albino mice	D-gal	150 mg/kg, 每天 1 次, 连续注射 42 d 150 mg/kg, once a day, continuous injection for 42 d	皮下注射/腹腔注射 Subcutaneous injection/Intraperitoneal injection	组织的氧化应激和炎症, 认知和胆碱系统障碍, tau 蛋白过度磷酸化 Tissue oxidative stress and inflammation, cognitive and cholinergic system disorders, tau protein hyperphosphorylation	不出现 Aβ 和神经纤维缠结以及老年斑 No Aβ, neurofibrillary tangles and age spots	[49-50]

续表 2

模型 Models	动物 Animal	化学物质/物理方法 Chemical substances/physical methods	操作方法 Method of operation	损伤部位 Injury site	优点 Advantages	缺点 Disadvantages	参考文献 References
三氯化铝注射模型 Aluminum trichloride infusion model	Wistar 大鼠 Wistar rats	三氯化铝 Aluminum trichloride	100 mg/kg,连续注射 60 d 100 mg/kg, continuous injection for 60 d	腹腔注射 Intraperitoneal injection	Aβ 聚集,神经元变性,学习和记忆障碍 Aβ aggregation, neuronal degeneration, learning and memory impairment	造模时间长,中枢胆碱能未降低,NFTs 不同于 AD 患者 Modeling time is long, central cholinergic is not reduced, NFTs are different from AD patients	[54]
冈田酸注射模型 Okadaic acid infusion model	SD 大鼠 SD rats	OKA	40 ng/μL,5 μL,注射时长 5 min,留针 5 min 40 ng/μL, 5 μL, injection time 5 min, needle retention 5 min	侧脑室(前囟后 0.8 mm,中线旁开 1.5 mm,垂直进针 3.6 mm) Lateral ventricle (0.8 mm posterior to the bregma, 1.5 mm lateral to the midline, 3.6 mm vertical needle insertion)	表现出 Tau 蛋白过度磷酸化和 Aβ 病理表现 Shows Tau protein hyperphosphorylation and Aβ pathological manifestations	不出现神经纤维缠结 No neurofibrillary tangles	[57]
东莨菪碱注射模型 Scopolamine infusion model	Wistar 大鼠 Wistar rats	SCOP	0.2 mL/150 g,连续注射 14 d 0.2 mL/150 g, continuous injection for 14 d	腹腔 Abdominal cavity	出现空间和记忆障碍 Space and memory impairment	不出现 Aβ 典型的病理特征,神经元纤维缠结 No typical pathological features of Aβ, neurofibrillary tangles	[58]

位(皮下、腹腔等)注射不同化学物质来模拟构建 AD 疾病模型。具体建模方法见表 2。

2.2.2 Aβ 注射模型

β-淀粉样蛋白沉积所形成的老年斑(senile plaques,SP)是 AD 病理特征主要表现之一。Aβ 注射模型通过向脑内各区域注射不同长度 Aβ 多肽片段($Aβ_{1-42}$[39]、$Aβ_{1-40}$[40]、$Aβ_{25-35}$[41])来达到急性损伤,进而模拟构建 AD 模型[42-43]。

此模型需要 35 ~ 42 d 的时间,影响因素单一,且属于急性损伤,与 AD 慢性起病的特点不符。此外,Aβ 容易停留聚集在注射部位,而不是像 AD 患者脑内的弥散状态。

2.2.3 鹅膏蕈氨酸注射模型

AD 病理特征之一表现为明显的神经元缺失,引发认知和记忆的严重障碍。将鹅膏蕈氨酸(Ibotenic acid, IBO)注入与学习记忆强相关的 Meynert 基底核,模型动物表现为大脑内神经元缺失、Aβ 蛋白沉积和 Tau 蛋白水平增加以及胆碱能系统损坏[44]。另有 Aβ 结合 IBO 共同使用来模拟 AD 模型[45]。

2.2.4 链脲菌素注射模型

向动物侧脑室注射链脲菌素(streptozotocin, STZ)来破坏脑部能量代谢,使动物出现相应的 Aβ 沉积、tau 蛋白高度磷酸化、胆碱能功能异常、氧化应激等[46]。

STZ 模型与 AD 许多病理特点相符,但此模型在注射 STZ 后要等待 3 个月后来观察 AD 病理特征[47],且动物死亡率较高[48]。

2.2.5 D-半乳糖注射模型

D-半乳糖(D-galactose, D-gal)具有还原性,会引发动物组织的氧化应激和炎症,进而导致神经元的衰老。

通过皮下注射 D-gal 来建立亚急性衰老模型,导致认知和胆碱能功能异常[49-50]。此模型造模相对简便,但大脑内不出现 Aβ 沉积等病理表现。

2.2.6 三氯化铝注射模型

金属元素在 AD 的发病中占有重要的作用[51]。此模型通过向小鼠腹腔注射三氯化铝来建立空间学习记忆损伤模型[52]。脑组织中高浓度的铝使 Aβ 聚集加快,神经元变性坏死,空间学习和记忆能力遭到损害[53]。此模型通过腹腔注射三氯化铝造成动物脑组织中 Aβ 增多以及神经元的变性,进而模拟 AD 病理特征[54]。

另有口服三氯化铝来构建 AD 疾病模型[55]。

2.2.7　冈田酸注射模型

冈田酸(okadaic acid, OKA)是蛋白磷酸酶选择抑制剂,在向受试动物脑内不同位置注射后会诱导 Tau 蛋白过度磷酸化[56]。

模型动物在 OKA 注射后可以诱导产生记忆损伤,tau 磷酸化的增加以及特殊脑区中的 β-淀粉样蛋白的形成[57]。

2.2.8　东莨菪碱注射模型

东莨菪碱(scopolamine, SCOP)为胆碱能拮抗剂,腹腔注射后可出现胆碱功能障碍和氧化应激[58]。此模型可以造成动物学习记忆障碍,但缺乏 tau 蛋白过度磷酸化和 Aβ 沉积等 AD 典型的病理改变。

3　转基因动物模型

转基因动物模型以遗传学说为其基础,将人或动物的疾病相关基因作为目的基因进行修饰,再拼接一个特定的启动子后植入动物(最常用为小鼠,少数为大鼠、果蝇、斑马鱼)受精卵内。再将受精卵导入假孕动物体内使其稳定遗传以复制 AD 特定病理特征。

研究表明,多个基因都与 AD 的发生有着密切的关系,主要有淀粉样前体蛋白 APP、微管相关蛋白 MAPT、早老素 PSEN1、载脂蛋白 APOE 和髓系细胞-2 上表达的触发受体 TREM2,以及淀粉样蛋白原的剪切酶 BACE1(β-分泌酶 1)[59-61]。由于 AD 两个确定的病理标志是 Aβ 和 tau,这些模型多专注于淀粉样蛋白,仅有少部分关注于 tau。其中 APP 和 PSEN1 基因与 Aβ 的生成有关,APOE 基因参与 Aβ 沉积形成过程。Tau 基因突变可引发 tau 蛋白表达异常,进而出现 tau 蛋白异常磷酸化及神经缠结等 AD 病理特征。AD 转基因模型主要包括 APP 转基因模型、Tau 蛋白转基因模型、PSEN1 转基因模型、APOE 转基因模型、双转基因模型以及多转基因模型等[62]。具体建模方法见表 3。

尽管许多转基因模型在评估新型疗法潜力方面的实用性上仍存在较大争议,但了解可用的不同模型并对其功能进行详细了解,可以帮助研究者选择最佳模型,以探索疾病机制或评估候选药物。转基因动物模型依然是目前 AD 模型中的主要模型,已为人类了解疾病的部分机制做出了巨大贡献,并且在以后的其他实验中可能会开创出疾病新的治疗方法。

3.1　APP 转基因模型

Aβ 沉积是 AD 病理改变的重要特征之一,APP 作为 Aβ 的上游前体蛋白,影响着 Aβ 的产生。过量表达 Aβ 蛋白可以形成 SP,影响着 AD 病理过程的发展。

此模型通过转染或敲除 APP 基因,促进动物脑内 Aβ 产生,进而表现出 Aβ 沉积的病理特征。但模型动物大脑区域中不会出现神经纤维缠结和明显的神经元丢失。

3.2　Tau 蛋白转基因模型

Tau 蛋白是一种细胞内被用作支架的微管相关蛋白,可以促进微管的聚集。其主要存在于轴突中,但体细胞和树突中也存在 Tau 蛋白。在病理条件下,Tau 过度翻译表达以及异常修饰,尤其是过度磷酸化修饰,会影响其与微管结合的亲和力,进而导致 Tau 聚集性增强和清除率下降。

此类模型仿效了 AD 患者 Tau 蛋白异常修饰而被广泛应用,但不能用于模拟 AD 其他病理过程。

3.3　PS 转基因模型

位于 14 号染色体上的 PS-1 基因和位于 1 号染色体上 PS-2 基因共同控制着 APP 剪切酶之一的 γ-分泌酶复合物的形成。PS 基因突变时会影响到 γ-分泌酶复合物结构的稳定性,进而影响到下游 APP 的剪切过程。

PS1 中的突变是家族性 AD 的最常见原因,但它们不会在转基因小鼠模型中形成淀粉样蛋白斑块。因此,PS 突变常与 APP 或其他突变结合使用,来加强转基因后的协同效应。

3.4　双转基因模型

双转基因模型是在单转基因模型的基础上结合另外一种单转基因模型构建而成的。APP 小鼠大脑皮层可形成老年斑,但几乎观察不到神经纤维缠结,为解决问题,人类的 Tau 和 PS 基因被引入到 APP 小鼠当中,建立出 APP/Tau 双转基因小鼠。

APP/PS 双转基因小鼠通过加速表达 Aβ,实现更快速且稳定地形成 Aβ 增多、聚集的病理特征表现。但这类模型与 APP 模型一样,无法形成 Tau 病理。而 APP/Tau 双转基因小鼠可同时出现 Aβ 斑块沉积和神经元纤维缠结。

3.5　多转基因模型

AD 病理存在多种基因缺陷,单转基因模型或双转基因模型难以完全契合 AD 的全部病理特征。因此,研究者创建了多转基因模型来全面模拟 AD 的病理特征。

表 3 常用 AD 转基因动物模型分类表

Table 3 Classification of commonly used AD transgenic animal models

模型 Models	基因 Gene	名称 Name	突变/启动子 Mutation/promoter	动物品系 Animal strains	优点 Advantages	缺点 Disadvantages	参考文献 References
APP 转基因模型 APP transgenic model	APP	PDAPP	hAPP (Ind), human PDGF-β promoter	C57BL/66 × DBA2	6 ~ 8 月龄时形成 Aβ 斑块,胶质增生,脑血管淀粉样变性,3 个月时出现空间记忆障碍 Formation of Aβ plaques at 6 ~ 8 months of age, gliosis, cerebrovascular amyloidosis, and spatial memory impairment at 3 months	无典型 tau 病理改变 No typical tau pathological changes	[63-64]
		Tg2576	hAPP (Swe), isoform 695, hamster PrP promoter	C57BL/6	9 ~ 11 月龄时出现 Aβ 斑块,胶质增生,突触丢失,脑血管淀粉样变性,6 月龄时出现空间和学习记忆障碍 Aβ plaques, glial hyperplasia, loss of synapses, and cerebrovascular amyloidosis appear at 9 ~ 11 months of age, and space, learning and memory disorders appear at 6 months of age	无典型 tau 病理改变 No typical tau pathological changes	[65-66]
		APP23	hAPP (Swe), isoform 751, mouse Thy1 promoter	C57BL/6	6 ~ 8 月龄时形成 Aβ 斑块,过度磷酸化的 tau 蛋白,胶质增生,神经元丢失,脑血管淀粉样变性,3 月龄时出现认知和空间记忆障碍 Formation of Aβ plaques at 6 ~ 8 months of age, hyperphosphorylated tau protein, glial hyperplasia, neuronal loss, cerebrovascular amyloidosis, cognitive and spatial memory impairment at 3 months of age	不出现神经纤维缠结 No neurofibrillary tangles	[67]
		J20	hAPP (Swe, Ind), human PDGF-β promoter	C57BL/6	6 ~ 8 月龄时形成 Aβ 斑块,胶质增生,突触和神经元丢失,脑血管淀粉样变性,2 月龄时出现识别记忆障碍和 3 个月时出现空间记忆障碍 Formation of Aβ plaques at 6 ~ 8 months of age, glial hyperplasia, loss of synapses and neurons, cerebrovascular amyloidosis, recognition and memory impairment at 2 months of age and spatial memory impairment at 3 months	无典型 tau 病理改变 No typical tau pathological changes	[68]
		TgCRND8	hAPP (Swe, Ind), hamster PrP promoter	C3H/He-C57BL/6	3 ~ 5 月龄时形成 Aβ 斑块,胶质增生,突触和神经元丢失,脑血管淀粉样变性,6 月龄时出现进行性工作和空间记忆障碍 Formation of Aβ plaques at 3 ~ 5 months of age, glial hyperplasia, loss of synapses and neurons, cerebrovascular amyloidosis, and progressive work and spatial memory impairment at 6 months of age	无典型 tau 病理改变 No typical tau pathological changes	[69]
		APPNL-G-F knock-in	hAPP (Swe, Ibe, Arc), endogenous APP promoter	C57BL/6	2 月时形成 Aβ 斑块,胶质增生,突触丢失,6 月龄时出现空间记忆障碍 Formation of Aβ plaques at 2 months, glial hyperplasia, loss of synapses, and spatial memory impairment at 6 months of age	无典型 tau 病理改变 No typical tau pathological changes	[70]
		TgSweDI	hAPP (Swe, Dutch, Iowa), mouse Thy1 promoter	C57BL/6	3 月龄时形成 Aβ 斑块,胶质增生,脑血管淀粉样变性,6 月时出现学习记忆障碍 Formation of Aβ plaques at 3 months of age, gliosis, cerebrovascular amyloidosis, and learning and memory impairment at 6 months	无典型 tau 病理改变 No typical tau pathological changes	[71]
		APP693△	hAPP (Osaka), mouse PrP promoter	B6C3F1, back-crossed to C57BL/6	8 月龄时形成 Aβ 斑块,tau 蛋白过度磷酸化,胶质增生,神经元丢失,8 月时出现空间记忆障碍 Formation of Aβ plaques at 8 months of age, hyperphosphorylation of tau protein, glial hyperplasia, neuron loss, and spatial memory impairment at 8 months	不出现老年斑和神经纤维缠结 No senile plaques and neurofibrillary tangles	[72]

续表 3

模型 Models	基因 Gene	名称 Name	突变/启动子 Mutation/promoter	动物品系 Animal strains	优点 Advantages	缺点 Disadvantages	参考文献 References
Tau 转基因模型 Tau transgenic model	Tau	APP21	hAPP (Swe, Ind), the ubiquitin-C promoter	SD rat	9 月龄时出现弥漫性斑块和血管周围淀粉样蛋白沉积。脑淀粉样血管病,19 月龄时出现长时程增强效应缺陷和学习障碍 Diffuse plaques and perivascular amyloid deposits appeared at 9 months of age. Cerebral amyloid angiopathy, with defects in long-term potentiation and learning disabilities at 19 months of age	无典型 tau 病理改变 No typical tau pathological changes	[73]
		McGill-R-Thy1-APP	hAPP (Swe, Ind), mouse Thy1. 2 promoter	Wistar rat	1 周时海马和皮层中的神经元出现 Aβ。6 月龄时出现细胞外 Aβ 斑块和小胶质细胞密度增加,18 月龄时出现神经元丢失,20 月龄时胆碱能神经支配减少,3 月时出现恐惧反应缺陷,4 ~ 6 月龄时出现空间和工作记忆缺陷以及视觉辨别能力严重缺陷 Aβ appeared in neurons in the hippocampus and cortex at 1 week. The density of extracellular Aβ plaques and microglia increased at 6 months of age, neuron loss at 18 months of age, cholinergic innervation decreased at 20 months of age, fear response defects appeared at March, 4 ~ 6 months Spatial and working memory deficits and severe deficits in visual discrimination at age	无典型 tau 病理改变 No typical tau pathological changes	[74-77]
		hTau	hMAPT (all 6 isoforms, non-mutant), human tau promoter	C57BL/6	9 月龄时出现 tau 病理,神经元丢失,12 月龄时出现认知和空间记忆障碍 Tau pathology and neuron loss at 9 months of age, cognitive and spatial memory impairment at 12 months of age	不出现 Aβ 斑块 No Aβ plaques	[78]
		rTg4510	hMAPT (h0N4R P301 L), human CaMKIIα promoter	mixed: 129S6 (activator) × FVB (responder)	8 月龄时出现神经纤维缠结,突触和神经元丢失,3 ~ 5 月龄出现空间记忆障碍 Neurofibrillary tangles appear at 8 months of age, synapses and neurons are lost, and spatial memory disorders appear at 3 ~ 5 months of age	不出现 Aβ 斑块 No Aβ plaques	[79-80]
		TauP301S Line19 or PS19	hMAPT (h1N4R P301S), mouse PrP promoter,	(C57BL/6 × C3H) F1	8 月龄时出现神经纤维缠结,胶质增生,7 月龄时出现空间和学习记忆障碍 Neurofibrillary tangles and glial hyperplasia appear at 8 months of age, and space, learning and memory disorders appear at 7 months of age	不出现 Aβ 斑块 No Aβ plaques	[81]
PS 转基因模型 PS transgenic model	PS	PS1M146VKI	PS1M146VK	129/C57BL/6	神经元丢失 Neuron loss	不出现 Aβ 沉积和 Aβ 斑块,也不会出现神经纤维缠结 No Aβ deposits, Aβ plaques, and no neurofibrillary tangles	[82]
		PS1P117 L	P117 L	B6D2	可以形成 Aβ 斑块 Can form Aβ plaques	不出现 tau 病理表现 No pathological manifestations of tau	[83]
		PS1v97 L-Tg	V97 L	C57BL/6	表现出神经纤维缠结和神经元丢失 Exhibits neurofibrillary tangles and neuron loss	不出现 Aβ 沉积和 Aβ 斑块 No Aβ deposits and Aβ plaques	[84]
APOE 转基因模型 APOE transgenic model	APOE	APOE2 Knock-in	APOE2 inserted with expression regulated by endogenous regulatory elements and the mouse APOE gene inactivated	C57BL/6	Aβ 增多 Increased Aβ	无典型 tau 病理改变 No typical tau pathological changes	[85]

续表 3

模型 Models	基因 Gene	名称 Name	突变/启动子 Mutation/promoter	动物品系 Animal strains	优点 Advantages	缺点 Disadvantages	参考文献 References
双转基因模型 Double transgenic model	APP+PS	APPswe/PSEN1dE9 or APP/PS1	m/hAPP (Swe), hPSEN1 (m/hAPP (Swe), hPSEN1 (△E9), mouse PrP promoters	(C57BL/6 × C3H)F2	6 ~ 9 月龄时形成 Aβ 斑块,胶质增生,突触和神经元丢失,脑血管淀粉样变性,6 月龄时出现空间和记忆障碍 Formation of Aβ plaques at 6 ~ 9 months of age, glial hyperplasia, loss of synapses and neurons, cerebrovascular amyloidosis, and space and memory impairment at 6 months of age	无典型 tau 病理改变 No typical tau pathological changes	[86]
		APPPS1-21 or APPPS1	hAPP (Swe), hPSEN1 (L166P), mouse Thy1 promoter	C57BL/6J	6 ~ 8 月龄形成斑块,胶质增生,突触和神经元丢失,脑血管淀粉样变性,7 月龄时出现空间和学习记忆障碍 Plaque formation at 6 ~ 8 months of age, glial hyperplasia, loss of synapses and neurons, cerebrovascular amyloidosis, and space and learning and memory impairment at 7 months of age	无典型 tau 病理改变 No typical tau pathological changes	[87]
		5×FAD	hAPP (Swe, Fl, Lon), hPSEN1 (M146 L, L286 V), mouse Thy1 promoter	C57BL/6×SJL	2 月龄时形成 Aβ 斑块,胶质增生,突触和神经元丢失,脑血管淀粉样变性,3 月时出现进行性空间记忆障碍,6 月龄出现学习记忆障碍 Formation of Aβ plaques at 2 months of age, glial hyperplasia, loss of synapses and neurons, cerebrovascular amyloidosis, progressive spatial memory impairment at 3 months, learning and memory impairment at 6 months of age	无典型 tau 病理改变 No typical tau pathological changes	[88]
		PSAPP	APPK670 N/M671 L PS1M146 L, PrP promoters	Tg2576	Aβ 沉积和斑块 Aβ deposits and plaques	无典型 tau 病理改变 No typical tau pathological changes	[89]
		APP+PS1	hAPP (Swe Ind), PSEN1 L166P, mouse PrP promoters	Inbred Fischer 344 rats	Aβ 斑块,脑血管淀粉样变性,12 ~ 14 月龄时出现记忆损伤 Aβ plaques, cerebrovascular amyloidosis, memory impairment at 12 ~ 14 months of age	无典型 tau 病理改变 No typical tau pathological changes	[90]
		TgF344-AD	hAPP (Swe), PSEN1: deltaE9, mouse PrP promoters	Fischer 344 rats	6 ~ 26 月龄海马和皮质中出现 Aβ 斑块,6 月龄时蓝斑处出现 tau 过度磷酸化,6 ~ 24 月龄出现空间和记忆缺陷 Aβ plaques appear in the hippocampus and cortex at 6 ~ 26 months old, tau hyperphosphorylation appears at the locus coeruleus at 6 months old, and space and memory deficits appear at 6 ~ 24 months old	–	[91]
	APP+tau	TAPP	tauP301 L/APPsw, mouse PrP promoters	Tg2576×JNPL3	3 月龄时出现 tau 病理特征,6 月龄时表现出 Aβ 形成的斑块 Tau pathological features appear at the age of 3 months, and plaques formed by Aβ appear at the age of 6 months	–	[92]
多基因转基因模型 Multigene transgenic model	APP+PS +tau	3×Tg	hAPP (Swe), hPSEN1 (M146 V), hMAPT (h0N4RP301 L), mouse Thy1.2 promoter (APP, MAPT), endogenous PSEN1 promoter	C7BL/6, 129X1/SvJ, 129S1/Sv	6 月龄时形成 Aβ 斑块,12 月龄时出现 tau 病理表现,胶质增生,4 月龄时出现进行性学习记忆障碍,6 月龄出现空间记忆障碍 Formation of Aβ plaques at 6 months of age, tau pathological manifestations and glial hyperplasia at 12 months of age, progressive learning and memory impairment at 4 months of age, and spatial memory impairment at 6 months of age	–	[93]

多转基因模型是根据需要将三个或三个以上易感基因结合在一起来建立基因模型。此类模型可以用于测试 AD 特定的病理分子机制或研究候选药物对特定病理的作用和疗效,也可用于比较两类转基因模型之间的差异。3xTg 模型是典型的多转基因模型,同时具有 APP、PS1、Tau 三个基因突变。

该模型可表现出 Aβ 沉积形成的斑块和 tau 病理改变,以及神经纤维缠结。

4　其他动物模型

除了自然动物模型、人工干预动物模型、遗传动物模型以外,研究者还基于近年出现的新的发病假说创建了相关动物模型。主要有自身免疫模型、人源肠道菌群动物模型。

4.1　自身免疫模型

AD 的一个重要病理特征为 Aβ 蛋白在大脑和脑血管系统的沉积[94],AD 神经变性严重程度与脑内 Aβ 抗体的水平密切相关[95]。动物实验证明主被动免疫疗法均能减少转基因小鼠的体内的 Aβ 含量并可挽救记忆缺陷[96]。于是研究者不再局限于研究淀粉样斑块沉积,而将研究视角聚焦于自身免疫层面。虽然免疫疗法也像动物实验一样可以降低患者体内的 Aβ 含量,但免疫疗法临床试验均未能改善患者的记忆力。

4.2　人源肠道菌群动物模型

随着这几年对肠道菌群的研究加深,越来越多的研究表明肠道菌群与 AD 的发生发展有着密不可分的联系[97]。因此,有研究人员将 AD 患者粪便的菌群提取液经口灌胃小鼠来制备人源肠道菌群动物模型[98]。该模型可以探索肠道微生物群对 AD 发病机制的远程贡献背后的特定信号介质或机制。

5　问题与展望

阿尔茨海默病已成为危害老年人的全球性疾病,受到了世界认知科学、神经科学、心理学等领域学者的广泛关注。目前常用的 AD 实验动物模型众多,表现出的疾病症状及病理改变各不相同,各种动物模型都有各自的长处与不足,因此需要根据疾病发生原理和实验目的不同选择相应的模型。

但是,即便 AD 的相关研究成果层出不断,为何至今仍然鲜有有效的治疗方法和手段问世。笔者认为,AD 有效治疗方法开发的主要障碍是缺乏适当的模型,而构建恰当的疾病模型需要明确疾病发生的机制。AD 发病机制尚不明确,未能形成定论或达成统一认识,模拟构建出完全符合疾病发病机制特点的模型似乎是遥不可及的。此外,AD 是一种复杂的慢性神经退行性疾病,并且可能是人类所特有的,当前单一病因途径或单一致病因素的观点限制了 AD 在动物身上的模拟,以及动物模型的发展。就此,我们一方面需要在 3R 原则的指导下尝试开发新的实验动物种类;另一方面则可以从整合医学理念出发,从整体和宏观角度综合判别疾病发生机制和多靶点治疗途径,以拟定更加完善且符合疾病本质的研究方案和治疗手段。

参　考　文　献(References)

[1] Alzheimer's Disease International, Patterson C. World Alzheimer Report 2018[R/OL]. [2018-09-21]. https://www.alzint.org/resource/world-alzheimer-report-2018/#: ~: text = The% 20World% 20Alzheimer% 20Report% 202018% 20highlights% 20an%20urgent,some%20of%20the%20complex%20questions% 20surrounding%20dementia%20research.

[2] Jia L, Quan M, Fu Y, et al. Dementia in China: epidemiology, clinical management, and research advances [J]. Lancet Neurol, 2020, 19(1): 81-92.

[3] Xu J, Wang J, Wimo A, et al. The economic burden of dementia in China, 1990-2030: implications for health policy [J]. Bull World Health Organ, 2017, 95(1): 18-26.

[4] Tarawneh R, Holtzman DM. The clinical problem of symptomatic alzheimer disease and mild cognitive impairment [J]. Cold Spring Harb Perspect Med, 2012, 2(5): a006148.

[5] Iqbal K, Grundke-Iqbal I. Neurofibrillary pathology leads to synaptic loss and not the other way around in Alzheimer disease [J]. J Alzheimers Dis, 2002, 4(3): 235-238.

[6] Katsumoto A, Takeuchi H, Takahashi K, et al. Microglia in Alzheimer's disease: risk factors and inflammation [J]. Front Neurol, 2018, 9: 978.

[7] Dansokho C, Heneka MT. Neuroinflammatory responses in Alzheimer's disease [J]. J Neural Transm, 2018, 125(5): 771-779.

[8] Tönnies E, Trushina E. Oxidative stress, synaptic dysfunction, and Alzheimer's disease [J]. J Alzheimers Dis, 2017, 57(4): 1105-1121.

[9] Denes AS, Jékely G, Steinmetz PR, et al. Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in bilateria [J]. Cell, 2007, 129(2): 277-288.

[10] Sivanantharajah L, Mudher A, Shepherd D. An evaluation of Drosophila as a model system for studying tauopathies such as Alzheimer's disease [J]. J Neurosci Methods, 2019, 319: 77-88.

[11] Luo Y. Alzheimer's disease, the nematode Caenorhabditis elegans, and ginkgo biloba leaf extract [J]. Life Sci, 2006, 78(18): 2066-2072.

[12] Newman M, Ebrahimie E, Lardelli M. Using the zebrafish model for Alzheimer's disease research [J]. Front Genet, 2014, 5: 189.

[13] Sarasa M, Pesini P. Natural non-trasgenic animal models for research in Alzheimer's disease [J]. Curr Alzheimer Res, 2009, 6(2): 171-178.

[14] Toledano A, Álvarez MI, López-Rodríguez AB, et al. ¿Existe la enfermedad de Alzheimer en todos los primates? Patología Alzheimer en primates no humanos y sus implicaciones fisiopatológicas (II) [J]. Neurologia, 2014, 29(1): 42-55.

[15] Heuer E, Rosen RF, Cintron A, et al. Nonhuman primate models of Alzheimer-like cerebral proteopathy [J]. Curr Pharm Des, 2012, 18(8): 1159-1169.

[16] Edler MK, Munger EL, Meindl RS, et al. Neuron loss associated with age but not Alzheimer's disease pathology in the chimpanzee brain [J]. Philos Trans R Soc Lond B Biol Sci, 2020, 375(1811): 20190619.

[17] Hyman BT, Phelps CH, Beach TG, et al. National institute on aging-Alzheimer's association guidelines for the neuropathologic assessment of Alzheimer's disease [J]. Alzheimers Dement, 2012, 8(1): 1-13.

[18] Liberman MC. Noise-induced and age-related hearing loss: new perspectives and potential therapies [J]. F1000Res, 2017, 6: 927.

[19] Van Dam D, De Deyn PP. Drug discovery in dementia: the role of rodent models [J]. Nat Rev Drug Discov, 2006, 5(11): 956-970.

[20] Van Dam D, De Deyn PP. Animal models in the drug discovery pipeline for Alzheimer's disease [J]. Br J Pharmacol, 2011, 164(4): 1285-1300.

[21] 王喆, 李瑞生. 衰老动物模型的研究进展 [J]. 中国比较医学杂志, 2013, 23(3): 67-70.
Wang J, Li RS. Research progress of aging animal model [J]. Chin J Comp Med, 2013, 23(3): 67-70.

[22] 向婷婷, 张长城, 刘朝奇, 等. 竹节参总皂苷干预自然衰老大鼠肝脏炎症的实验研究 [J]. 中国药理学通报, 2017, 33(6): 848-853.
Xiang TT, Zhang CC, Liu CQ, et al. Effects of total saponins of Panax japonicus on liver inflammation natural aging rats [J]. Chin Pharmacol Bull, 2017, 33(6): 848-853.

[23] Takeda T, Hosokawa M, Takeshita S, et al. A new murine model of accelerated senescence [J]. Mech Ageing Dev, 1981, 17(2): 183-194.

[24] Takeda T. Senescence-accelerated mouse (SAM) with special references to neurodegeneration models, SAMP8 and SAMP10 mice [J]. Neurochem Res, 2009, 34(4): 639-659.

[25] Francis PT, Palmer AM, Sims NR, et al. Neurochemical studies of early-onset Alzheimer's disease [J]. N Engl J Med, 1985, 313(1): 7-11.

[26] Perry EK, Tomlinson BE, Blessed G, et al. Neuropathological and biochemical observations on the noradrenergic system in Alzheimer's disease [J]. J Neurol Sci, 1981, 51(2): 279-287.

[27] Gerretsen P, Pollock BG. Drugs with anticholinergic properties: a current perspective on use and safety [J]. Expert Opin Drug Saf, 2011, 10(5): 751-765.

[28] Bartus RT, Dean RL 3rd, Beer B, et al. The cholinergic hypothesis of geriatric memory dysfunction [J]. Science, 1982, 217(4558): 408-414.

[29] 董云芳, 冯慧利, 陈芳, 等. 金思维对东莨菪碱致记忆障碍小鼠脑胆碱能系统的影响 [J]. 中国实验动物学报, 2018, 26(1): 107-113.
Dong YF, Feng HL, Chen F, et al. Effect of herbal formula GAPT on cholinergic system in mice with scopolamine-induced memory impairment [J]. Acta Lab Anim Sci Sin, 2018, 26(1): 107-113.

[30] Lombardo S, Maskos U. Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment [J]. Neuropharmacology, 2015, 96: 255-262.

[31] Hefti F, Dravid A, Hartikka J. Chronic intraventricular injections of nerve growth factor elevate hippocampal choline acetyltransferase activity in adult rats with partial septo-hippocampal lesions [J]. Brain Res, 1984, 293(2): 305-311.

[32] 龙大宏, 姚志彬, 何蕴韶, 等. 神经生长因子对老年鼠穹窿海马伞损伤后齿状回胆碱能突触重构的作用 [J]. 神经解剖学杂志, 1997, 13(1): 51-54.
Long DH, Yao ZB, He YS, et al. Effect of nerve growth factor on cholinergic synapse remodeling in dentate gyrus after fimbria fornix injury in aged rats [J]. Chin J Neuroanat, 1997, 13(1): 51-54.

[33] 高风超, 陈翔, 田新英. 血管性痴呆危险因素及发病机制的研究进展 [J]. 医学综述, 2014, 20(6): 1068-1071.
Gao FC, Chen X, Tian XY, et al. Study progress of the risk factors and pathogenesis of Vascular Dementia [J]. Med Recapitulate, 2014, 20(6): 1068-1071.

[34] 张振强, 贾亚泉, 王自闯, 等. 化痰通络汤预先灌胃的合并高脂血症脑缺血大鼠神经系统功能、脑组织病理变化观察 [J]. 山东医药, 2018, 58(43): 47-50.
Zhang ZQ, Jia YQ, Wang ZC, et al. Observation of nervous system function and pathological changes of brain tissues in rats with hyperlipidemia and cerebral ischemia after pretreatment of Huatan Tongluo decoction [J]. Shandong Med J, 2018, 58(43): 47-50.

[35] 胡久略, 商健, 侯紫君, 等. 温肾醒脑方对血管性痴呆大鼠认知功能的影响及机制研究 [J]. 中华中医药学刊, 2019, 37(10): 2450-2455.
Hu JL, Shang J, Hou ZJ, et al. Effect of Wenshen Xingnao decoction on cognitive function in Vascular Dementia rats and its mechanism [J]. Chin Arch Tradit Chin Med, 2019, 37(10): 2450-2455.

[36] 孙成成, 刘剑刚, 刘美霞, 等. 双侧颈总动脉结扎致大鼠脑血流低灌注不同时程的神经功能障碍和病理学变化 [J]. 实验动物与比较医学, 2020, 40(6): 470-476.
Sun CC, Liu JG, Liu MX, et al. Neural dysfunction and pathological changes in different time courses of cerebral blood flow hypoperfusion induced by bilateral common carotid artery ligation in rats [J]. Lab Anim Comp Med, 2020, 40(6): 470-476.

[37] Washida K, Hattori Y, Ihara M. Animal models of chronic cerebral hypoperfusion: from mouse to primate [J]. Int J Mol

Sci, 2019, 20(24): 6176.

[38] 侯外方, 张茂森, 张琳, 等. 血管性认知障碍动物模型的研究进展 [J]. 中国实验动物学报, 2021, 29(4): 542-552.
Hou WF, Zhang MS, Zhang L, et al. Research progress using animal models of vascular cognitive impairment [J]. Acta Lab Anim Sci Sin, 2021, 29(4): 542-552.

[39] 刘鑫, 余盈, 何蔚. 欧前胡素对 $A\beta_{1-42}$致阿尔茨海默病模型小鼠的神经保护作用 [J]. 赣南医学院学报, 2020, 40(5): 451-455.
Liu X, Yu Y, He W. Neuroprotective effects of imperatorin in a mouse model of Alzheimer's disease induced by $A\beta_{1-42}$ [J]. J Gannan Med Univ, 2020, 40(5): 451-455.

[40] 易健, 刘柏炎, 蔡光先. 超微六味地黄汤对老年痴呆大鼠认知功能和脑组织碱性成纤维生长因子表达的影响 [J]. 中国实验方剂学杂志, 2011, 17(21): 139-142.
Yi J, Liu BY, Cai GX. Study on effect of Ultra-powder Liuwei Dihuang decoction on recognize and bFGF in Alzheime's disease rats bFGF [J]. Chin J Exp Tradit Med Formulae, 2011, 17(21): 139-142.

[41] 赵娣, 李爽, 徐婧. 蒲公英总黄酮改善 $A\beta_{25-35}$诱导的阿尔茨海默病大鼠记忆功能障碍的观察 [J]. 中国药物评价, 2019, 36(1): 41-45.
Zhao D, Li S, Xu J. The protection effects of total flavonoids from dandelion on memorizing ability in AD rats induced by $A\beta_{25-35}$ [J]. Chin J Drug Eval, 2019, 36(1): 41-45.

[42] Facchinetti R, Bronzuoli MR, Scuderi C. An animal model of Alzheimer disease based on the intrahippocampal injection of amyloid β-peptide (1-42) [J]. Methods Mol Biol, 2018, 1727: 343-352.

[43] 杨晶莹, 肖移生, 姜劼琳, 等. 黄精丸对阿尔茨海默病大鼠大脑的抗氧化作用及 $A\beta_{1-42}$, APP 蛋白表达的影响 [J]. 中国实验方剂学杂志, 2020, 26(6): 32-38.
Yang JY, Xiao YS, Jiang JL, et al. Effect of Huangjingwan on antioxidant functions and $A\beta_{1-42}$ and APP protein expressions in brain of Alzheimer's disease rats [J]. Chin J Exp Tradit Med Formulae, 2020, 26(6): 32-38.

[44] 陈惠芳, 莫友胜, 杨从, 等. 槲皮素对鹅膏蕈氨酸致痴呆大鼠模型海马神经元的保护作用及机制 [J]. 中药新药与临床药理, 2018, 29(5): 552-556.
Chen HF, Mo YS, Yang C, et al. Protective effect and mechanism of quercetin on hippocampal neurons in IBO-injured rats [J]. Tradit Chin Drug Res Clin Pharmacol, 2018, 29(5): 552-556.

[45] Shabani S, Sarkaki A, Ali Mard S, et al. Central and peripheral administrations of levothyroxine improved memory performance and amplified brain electrical activity in the rat model of Alzheimer's disease [J]. Neuropeptides, 2016, 59: 111-116.

[46] Zhou M, Chen S, Peng P, et al. Dulaglutide ameliorates STZ induced AD-like impairment of learning and memory ability by modulating hyperphosphorylation of tau and NFs through GSK3β [J]. Biochem Biophys Res Commun, 2019, 511(1): 154-160.

[47] Melita SP, Jelena OB, Martina KB, et al. Cerebral amyloid angiopathy in streptozotocin rat model of sporadic Alzheimer's disease: a long-term follow up study [J]. J Neural Transm (Vienna), 2011, 118(5): 765-772.

[48] Nataniel LC, Enrique JR, Stephanie JS, et al. Intracerebral streptozotocin model of type 3 diabetes: Relevance to sporadic Alzheimer's disease [J]. J Alzheimers Dis, 2006, 9(1): 13-33.

[49] Wang C, Cai Z, Wang W, et al. Piperine regulates glycogen synthase kinase-3β-related signaling and attenuates cognitive decline in D-galactose-induced aging mouse model [J]. J Nutr Biochem, 2020, 75: 108261.

[50] 孙荣花, 李爽, 邵莹, 等. D-半乳糖致小鼠亚急性衰老模型方法优化 [J]. 中药药理与临床, 2015, 31(4): 293-297.
Sun RH, Li S, Shao Y, et al. Method optimization of D-galactose induced mice subacute aging model [J]. Pharmacol Clin Chin Mater Med, 2015, 31(4): 293-297.

[51] Wang P, Wang ZY. Metal ions influx is a double edged sword for the pathogenesis of Alzheimer's disease [J]. Ageing Res Rev, 2017, 35: 265-290.

[52] 李莉, 李晓秀, 郭鹏骥, 等. 衰老模型大鼠空间记忆的改变及抑郁样行为的研究 [J]. 中国医科大学学报, 2017, 46(9): 816-820.
Li L, Li XX, Guo PJ, et al. Changes in spatial memory and depressive-like behavior in an aging rat model [J]. J Chin Med Univ, 2017, 46(9): 816-820.

[53] 张志慧, 贾振华, 康健生, 等. Aβ 损伤人脑微血管内皮细胞培养液对正常神经元凋亡的影响及通心络胶囊的干预作用 [J]. 中国实验方剂学杂志, 2017, 23(17): 166-171.
Zhang ZH, Jia ZH, Kang JS, et al. Effect of Aβ injury-based human brain microvascular endothelial cell culture solution on apoptosis of normal neurons and intervention effect of Tongxinluo capsule [J]. Chin J Exp Tradit Med Formulae, 2017, 23(17): 166-171.

[54] Mustafa HN. Neuro-amelioration of cinnamaldehyde in aluminum-induced Alzheimer's disease rat model [J]. J histotechnol, 2020, 43(1): 11-20.

[55] Ahmad Rather M, Justin Thenmozhi A, Manivasagam T, et al. Neuroprotective role of Asiatic acid in aluminium chloride induced rat model of Alzheimer's disease [J]. Front Biosci (Schol Ed), 2018, 10: 262-275

[56] Kaushal A, Wani WY, Bal A, et al. Okadaic acid and hypoxia induced dementia model of Alzheimer's type in rats [J]. Neurotox Res, 2019, 35(3): 621-634.

[57] Song XY, Hu JF, Chu SF, et al. Ginsenoside Rg1 attenuates okadaic acid induced spatial memory impairment by the GSK3β/tau signaling pathway and the Aβ formation prevention in rats [J]. Eur J Pharmacol, 2013, 710(1-3): 29-38.

[58] Haider S, Tabassum S, Perveen T. Scopolamine-induced greater alterations in neurochemical profile and increased oxidative stress demonstrated a better model of dementia: A comparative study [J]. Brain Res Bull, 2016, 127: 234-247.

[59] 唐伟, 张营丽, 王威, 等. 阿尔茨海默病发病机制的研究进

展[J]. 医学与哲学, 2014, 35(1): 49-51.
Tang W, Zhang YL, Wang W, et al. Progress of research on pathogenesis of Alzheimer's disease [J]. Med Phil, 2014, 35(1): 49-51.

[60] 张雅梦. 新发 PSEN1 M139I 基因突变在早发型家族性 Alzheimer 病中的病理功能研究[D]. 青岛: 青岛大学; 2020.
Zhang YM. A novel presenilin 1 M139I mutation and its pathological function in a Chinese family with early-onset Alzheimer's disease [D]. Qingdao: Qingdao University; 2020.

[61] Guerreiro R, Wojtas A, Bras J, et al. TREM2 variants in Alzheimer's disease [J]. N Engl J Med, 2013, 368(2): 117-127.

[62] Blackmore T, Meftah S, Murray TK, et al. Tracking progressive pathological and functional decline in the rTg4510 mouse model of tauopathy [J]. Alzheimers Res Ther, 2017, 9(1): 77.

[63] Rockenstein EM, McConlogue L, Tan H, et al. Levels and alternative splicing of Amyloid β Protein Precursor (APP) transcripts in brains of APP transgenic mice and humans with Alzheimer's disease [J]. J Biol Chem, 1995, 270(47): 28257-28267.

[64] Games D, Adams D, Alessandrini R, et al. Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein [J]. Nature, 1995, 373(6514): 523-527.

[65] Hsiao K, Chapman P, Nilsen S, et al. Correlative memory deficits, abeta elevation, and amyloid plaques in transgenic mice [J]. Science, 1996, 274(5284): 99-102.

[66] Holcomb L, Gordon MN, McGowan E, et al. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes [J]. Nat Med, 1998, 4(1): 97-100.

[67] Sturchler-Pierrat C, Abramowski D, Duke M, et al. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology [J]. Proc Natl Acad Sci U S A, 1997, 94(24): 13287-13292.

[68] Mucke L, Masliah E, Yu GQ, et al. High-level neuronal expression of β1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation [J]. J Neurosci, 2000, 20(11): 4050-4058.

[69] Chishti MA, Yang DS, Janus C, et al. Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695 [J]. J Biol Chem, 2001, 276(24): 21562-21570.

[70] Saito T, Matsuba Y, Mihira N, et al. Single App knock-in mouse models of Alzheimer's disease [J]. Nat Neurosci, 2014, 17(5): 661-663.

[71] Davis J, Xu F, Deane R, et al. Early-onset and robust cerebral microvascular accumulation of amyloid β-protein in transgenic mice expressing low levels of a vasculotropic Dutch/Iowa mutant form of amyloid beta-protein precursor [J]. J Biol Chem, 2004, 279(19): 20296-20306.

[72] Tomiyama T, Nagata T, Shimada H, et al. A new amyloid beta variant favoring oligomerization in Alzheimer's-type dementia [J]. Ann Neurol, 2008, 63(3): 377-387.

[73] Agca C, Fritz JJ, Walker LC, et al. Development of transgenic rats producing human β-amyloid precursor protein as a model for Alzheimer's disease: transgene and endogenous APP genes are regulated tissue-specifically [J]. BMC Neurosci, 2008, 9: 28.

[74] Leon WC, Canneva F, Partridge V, et al. A novel transgenic rat model with a full Alzheimer's-like amyloid pathology displays pre-plaque intracellular amyloid-beta-associated cognitive impairment [J]. J Alzheimers Dis, 2010, 20(1): 113-126.

[75] Galeano P, Martino Adami PV, Do Carmo S, et al. Longitudinal analysis of the behavioral phenotype in a novel transgenic rat model of early stages of Alzheimer's disease [J]. Front Behav Neurosci, 2014, 8: 321.

[76] Wilson EN, Abela AR, Do Carmo S, et al. Intraneuronal amyloid beta accumulation disrupts hippocampal CRTC1-dependent gene expression and cognitive function in a rat model of alzheimer disease [J]. Cereb Cortex, 2017, 27(2): 1501-1511.

[77] Petrasek T, Vojtechova I, Lobellova V, et al. The McGill transgenic rat model of Alzheimer's disease displays cognitive and motor impairments, changes in anxiety and social behavior, and altered circadian activity [J]. Front Aging Neurosci, 2018, 10: 250.

[78] Andorfer C, Kress Y, Espinoza M, et al. Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms [J]. J Neurochem, 2003, 86(3): 582-590.

[79] Santacruz K, Lewis J, Spires T, et al. Tau suppression in a neurodegenerative mouse model improves memory function [J]. Science, 2005, 309(5733): 476-481.

[80] Ramsden M, Kotilinek L, Forster C, et al. Age-dependent neurofibrillary tangle formation, neuron loss, and memory impairment in a mouse model of human tauopathy (P301L) [J]. J Neurosci, 2005, 25(46): 10637-10647.

[81] Yoshiyama Y, Higuchi M, Zhang B, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model [J]. Neuron, 2007, 53(3): 337-351.

[82] Guo Q, Fu W, Sopher BL, et al. Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice [J]. Nat Med, 1999, 5(1): 101-106.

[83] Zufferey V, Vallet PG, Moeri M, et al. Maladaptive exploratory behavior and neuropathology of the PS-1 P117L Alzheimer transgenic mice [J]. Brain Res Bull, 2013, 94: 17-22.

[84] Wang Y, Cheng Z, Qin W, et al. Val97Leu mutant presenilin-1 induces tau hyperphosphorylation and spatial memory deficit in mice and the underlying mechanisms [J]. J Neurochem, 2012, 121(1): 135-145.

[85] Mann KM, Thorngate FE, Katoh-Fukui Y, et al. Independent effects of APOE on cholesterol metabolism and brain Abeta levels in an Alzheimer disease mouse model [J]. Hum Mol Genet, 2004, 13(17): 1959-1968.

[86] Jankowsky JL, Fadale DJ, Anderson J, et al. Mutant presenilins specifically elevate the levels of the 42 residue β-amyloid peptide

in vivo: evidence for augmentation of a 42-specific γ secretase [J]. Hum Mol Genet, 2004, 13(2): 159-170.

[87] Radde R, Bolmont T, Kaeser SA, et al. Aβ42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology [J]. EMBO Rep, 2006, 7(9): 940-946.

[88] Oakley H, Cole SL, Logan S, et al. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation [J]. J Neurosci, 2006, 26(40): 10129-10140.

[89] Holcomb L, Gordon MN, McGowan E, et al. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes [J]. Nat Med, 1998, 4(1): 97-100.

[90] Agca C, Klakotskaia D, Schachtman TR, et al. Presenilin 1 transgene addition to amyloid precursor protein overexpressing transgenic rats increases amyloid beta 42 levels and results in loss of memory retention [J]. BMC Neurosci, 2016, 17(1): 46.

[91] Cohen RM, Rezai-Zadeh K, Weitz TM, et al. A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric Aβ, and frank neuronal loss [J]. J Neurosci, 2013, 33(15): 6245-6256.

[92] Lewis J, Dickson DW, Lin WL, et al. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP [J]. Science, 2001, 293(5534): 1487-1491.

[93] Oddo S, Caccamo A, Shepherd JD, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction [J]. Neuron, 2003, 39(3): 409-421.

[94] Mehta PD. Amyloid beta protein as a marker or risk factor of Alzheimer's disease [J]. Curr Alzheimer Res, 2007, 4(4): 359-363.

[95] McLean CA, Cherny RA, Fraser FW, et al. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease [J]. Ann neurol, 1999, 46(6): 860-866.

[96] Bachmeier CJ, Beaulieu-Abdelahad D, Mullan MJ, et al. Epitope-dependent effects of Beta-amyloid antibodies on Beta-amyloid clearance in an *in vitro* model of the blood-brain barrier [J]. Microcirculation, 2011, 18(5): 373-379.

[97] Sochocka M, Donskow-Łysoniewska K, Diniz BS, et al. The gut microbiome alterations and inflammation-driven pathogenesis of Alzheimer's disease-a critical review [J]. Mol Neurobiol, 2019, 56(3): 1841-1851.

[98] 朱华, 刘小海, 李卓, 等. 建立阿尔茨海默症人源肠道菌群动物模型 [J]. 中国实验动物学报, 2021, 29(1): 55-62.
Zhu H, Liu XH, Li Z, et al. Establishment of a human flora-associated mouse modelcorrelated with Alzheimer's disease [J]. Acta Lab Anim Sci Sin, 2021, 29(1): 55-62.

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