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Toxic Substance-induced Hippocampal Neurodegeneration in Rodents as Model of Alzheimer's Dementia

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Abstract

BACKGROUND: Alzheimer's Dementia (AD) cases are increasing with the global elderly population. To study the part of the brain affected by AD, animal models for hippocampal degeneration are still necessary to better understand AD pathogenesis and develop treatment and prevention measures.

AIM: This study was a systematic review of toxic substance-induced animal models of AD using the Morris Water Maze method in determining hippocampal-related memory impairment. Our aim was reviewing the methods of AD induction using toxic substances in laboratory rodents and evaluating the report of the AD biomarkers reported in the models.

METHODS: Data were obtained from articles in the PubMed database, then compiled, categorized, and analyzed. Eighty studies published in the past 5 years were included for analysis.

RESULTS AND DISCUSSION: The most widely used method was intracerebroventricular injection of amyloid-β substances. However, some less technically challenging techniques using oral or intraperitoneal administration of other toxic substances also produce successful models. Instead of hippocampal neurodegeneration, many studies detected biomarkers of the AD pathological process while some reported inflammation, oxidative stress, neurotrophic factors, and changes of cholinergic activity. Female animals were underrepresented despite a high incidence of AD in women.

CONCLUSION: Toxic substances may be used to develop AD animal models characterized with appropriate AD pathological markers. Characterization of methods with the most easy-handling techniques and more studies in female animal models should be encouraged.

Introduction

Alzheimer's dementia (AD) is a problem involving deteriorating cognitive function due to neurodegeneration and a leading cause of disability and dependency in the elderly. It is characterized by memory decline, impaired executive function, and communication problems. The number of cases is currently increasing along with the increasing older population. There were more than 55 million people living with dementia worldwide in 2019, and this is anticipated to triple by 2050 [1]. Although aging is a risk factor for cognitive decline, AD itself is not part of the normal aging process [1], [2]. Until now, no effective dementia therapy is available; hence, prevention efforts are very important to be developed by modifying risk factors to reduce or slow down the pathological process [3]. The study of tissue pathology and molecular biomarkers in the brain of patients with AD is limited due to the invasive examinations required and infrequent autopsies done. Therefore, the use of animal models is still needed to better understand AD pathogenesis, find biomarkers for early diagnosis and develop treatment and prevention modalities.

Rodents are widely used as animal models of neurodegeneration and AD, due to their simplicity in handling and testing compared to larger mammals. Their brain anatomy is analogous to humans. Compared to other brain areas, the hippocampus of animal models of AD shows the most significant changes in the DNA methylation, mRNA (transcriptome), protein (proteome), and metabolite (metabolome) levels. The molecular changes in the hippocampus are correlated with the decrease of cognitive function in animal models, which is in line with the clinical symptoms of AD [4], [5], [6].

At present, various methods are used in inducing AD in animal models, such as using natural aging processes, transgenic animals of AD, various surgery techniques to occlude arteries leading to the brain, and administering a variety of toxic substances. Natural aging processes match the development of AD in humans [6], [7]. However, longer time is needed

to develop the signs and symptoms. Moreover, the increased mortality rate of aging animals complicates the study design. Mostly involving mice as reviewed in several publications [8], [9], transgenic animals of AD are useful in examining certain pathways in disease pathogenesis. However, the technique requires expensive facilities and high-end expertise that are not suitable for many studies especially in drug development research. Requiring only relatively modest facilities, induction of AD using a variety of toxic substances is a widely used method in drug development studies. However, reviews on toxic substance-induced AD models are limited.

Since cognitive dysfunction is the most prevalent symptom in humans, this parameter should occur in good AD animal models. The Morris Water Maze (MWM) test is the main test for examining cognitive impairment in rodent models of hippocampal neurodegeneration [10], [11]. This systematic review aimed to evaluate the use of toxic substances in rat models of cognitive impairment examined by the MWM test. The technical issue of toxic substance delivery as well as the histological and biochemical parameters presented in the reports will be discussed.

Methods

The search was conducted on the PubMed database on December 9, 2020, at 6:42 Western Indonesian Time by entering the keyword combination (((((((dementia) OR (dementia Alzheimer)) OR (Alzheimer)) AND ((degenerati*) OR (neurodegenerati*))) AND (hippocamp*)) AND ((rat) OR (rats))) AND (Morris Water Maze). The inclusion criteria were original reports on hippocampal degeneration and AD using rats as animal models, and MWM as a test for spatial memory examination. The efficacy studies of alternative medicine and drug developments were included when they provided data of untreated models and normal control groups. The exclusion criteria were non-English articles, lack of full text, not using toxic substances as induction technique, have non-significant results in MWM, and studies which used transgenic animal modeling. There was no year limitation in our first search.

The data obtained were compiled in a spreadsheet and categorized based on modeling techniques, characteristics of animal models, tested parameters, and modeling mechanisms in causing hippocampal degeneration and clinical symptoms of AD. The obtained data from the past 5 years (2016–2021) were then analyzed to provide a more detailed description of each model.

Results

The screening of articles

From this search, 255 articles published within 1998 to 2021 were obtained and selected based on the inclusion and exclusion criteria. The filtering process is described in Figure 1. From the screening of titles and abstracts, 61 articles were excluded because they did not meet the criteria, including three articles which were not original articles, three articles written in non-English language, one article which was an incomplete manuscript (only abstract found), and 54 articles which did not use toxic substances. From the remaining 194 full texts, 34 articles were excluded in the rescreening step, that is, ten articles used transgenic animal modeling, two articles aimed to model neurological disease other than AD, three articles using mice, two articles did not test the control group (normal control nor induction control), one article had insignificant results on MWM, and 16 articles used more than one induction. From the 160 articles obtained, 80 articles published in the past 5 years (2016–2021) were included in the study for further analysis.

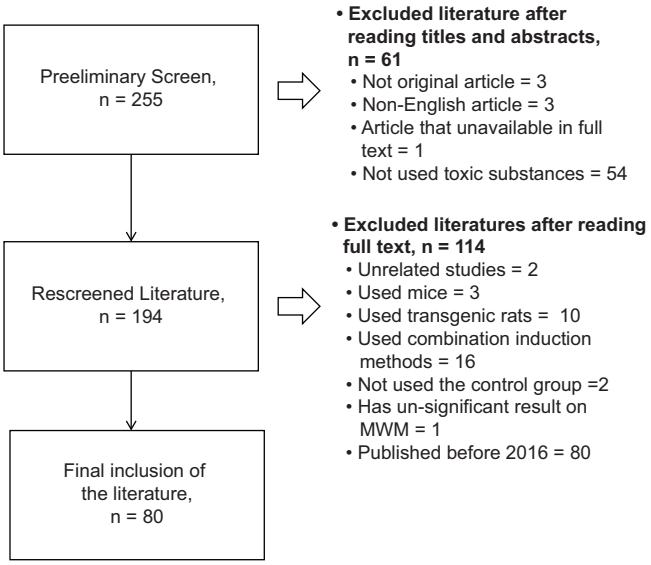

Figure 1: Screening flowchart for systematic review

Increased reports on this subject were apparent since the number of reports published before 2010 periods were only 21 studies, while 59 studies reported between 2011 and 2015, and 80 studies reported between 2016 and early 2021. The most widely used technique in the past 10 years is the injection of amyloid-β (Aβ) and streptozotocin (STZ). However, over the past 10 years, many other techniques have emerged which have not been used in older studies, that is, the use of trimethyltin (TMT), d-galactose, okadaic acid, monosodium glutamate (MSG), lipopolysaccharide (LPS), high-fat high glucose (HFHG) diet, high salt-cholesterol diet (HSCD), virus vectors as carriers of toxic substances, colchicine, cuprizone, letrozole, and scopolamine. All the studies

included in further analysis conducted the MWM test and showed significant results, both in the acquisition test, the probe test, and/or both.

Experimental animals and modeling methods

Most of the reports used male Wistar or Sprague-Dawley (SD) rats; each reported by 43 and 30 studies, respectively. Only five studies used female rats (two Wistar rats and three SD rats) (Table 1). Meanwhile, another two studies used male rats but did not mention the strain. The average age of the rats at the necropsy was 5 months. One study induced neonate rats with MSG [12] and the testing and necropsy were done in adulthood.

Table 1: Number of articles published on 2016–2021 using chemical substance and route of administration

Toxic substances	Number of article	Route of Administration			
		ICV	IP	Oral	Sub-cutaneous
Amyloid β**	35 [13], [14], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33], [34]. [35], [36], [37], [38], [39], [40], [41], [42], [43], [44], [45], [46], [47]	35	0	0	0
Streptozotocin**	16 [48], [49], [50], [51], [52], [53], [54], [55], [56], [57], [58], [59], [60], [61], [62], [63]	16	0	0	0
Aluminum chloride	4 [64], [65], [66], [67]	0	1	3	0
192IgG-saporin	4 [68], [69], [70], [71]	4	0	0	0
d-galactose	3 [72], [73], [74]	0	1	0	2
Scopolamine	2 [75], [76]	0	2	0	0
Okadaic acid	2 [77], [78]	2	0	0	0
Ibotenic acid	2 [79], [80]	2	0	0	0
Lipopolysaccharide	2 [81], [82]	0	2	0	0
Trimethyltin	2 [83], [84]	0	2	0	0
High-fat-high glucose diet	2 [85], [86]	0	0	2	0
Monosodium glutamate	1 [12]	0	0	0	1
Virus vector-APP	1 [87]	1	0	0	0
Colchicine	1 [88]	1	0	0	0
Cuprizone	1 [89]	0	0	1	0
High salt and cholesterol diet**	1 [90]	0	0	1	0
Letrozole**	1 [91]	0	0	1	0
Total number of articles	80	61	8	8	3

**Used female rat: Aβ 2 articles (33,45); STZ 1 article (61); HSCD (90); Letrozol (91)

In the 2016–2021 periods, there was a rapid development in the number and type of induction techniques in research using AD modeling. In the 80 studies reported within the past 5 years, we found 17 modeling techniques Table 1.

The length of interval between induction and MWM test with all methods varied with the different types of induction. The interval was 7 weeks on average, while the fastest was 30 min after induction (of scopolamine injection) [75], and the longest was 32 weeks (HFHG diet) [85]. The most widely used administration technique for introducing toxic substances was through the intracerebroventricular (ICV) route (Table 1). Most toxic substance administrations through ICV route used a single dose, except for STZ administration that needed multiple doses. Four studies of ICV administration used multiple doses of Aβ. The most widely used administration technique for introducing toxic substances was through the (ICV) route (Table 1). Most toxic substance administrations through ICV route used a single dose, except for STZ administration that needed multiple doses. Four studies of ICV administration used multiple doses of Aβ. The ICV route of delivery resulted in the shortest interval time such as reported in ICV colchicine induction (1 week), ICV STZ induction (1–3 weeks), okadaic acid (2 week), 192 Ig-Saporin (2–3 weeks), and ibotenic acid (2–5 weeks). Although we found one article reported a short 1-week interval, other reports of ICV Aβ induction reported longer intervals of 8 weeks between induction and the MWM tests. By ICV administration, the toxic substance directly accumulates in the central nervous system without any problems in crossing the brain-blood barrier. Therefore, the effect is likely to be faster than systemic administration [92].

Toxic substances administration through intraperitoneal (IP) injection and oral route were also commonly used. LPS, scopolamine, TMT, aluminum chloride, and d-galactose were toxic substances administered through the IP route, while aluminum chloride, cuprizone, and letrozole were toxic substances administered orally. Almost all of the toxic substances given through IP injection and oral routes were done in multiple doses and in a relatively longer time, except TMT that was given at single dose orally 20 days before the MWM test. Among the parenteral routes, subcutaneous administration requires a longer absorption time [93], [94] and a relatively longer interval between induction and behavioral test (12 weeks for MSG, 6–8 weeks for d-galactose, and 6–12 weeks for aluminum). Subcutaneous injection was reported by three studies; two of them used d-galactose; and the other one gave MSG injection to their animal models. Oral induction, such as HFHG [85] and HSCD [90], was seen to have the longest interval, which was about 15–32 weeks.

The most widely used typical marker of AD was the presence of amyloid plaque or Aβ (26 articles), followed by neurofibrillary tangle (NFT) or p-tau (11 articles), glycogen synthase kinase 3 beta (GSK3β) (four articles), beta secretase (βACE) (four articles), amyloid precursor protein (APP) (three articles), and presenilin-1 (PS-1) (one article) Table 2. Examination of typical markers of AD was performed through *in situ* and biochemical techniques. *In situ* studies of amyloid plaque markers used mostly immunohistochemical staining techniques and rarely using special staining of Congo Red. Biochemical and qualitative level measurements used enzyme-linked immunosorbent assay, polymerase chain reaction, and Western blotting to determine levels of the typical marker of AD in the level of protein and mRNA.

In addition to examining the typical markers of AD, these studies also examined the markers of the AD pathological process, such as markers of inflammation, oxidative stress, neurotrophic factors, the change of cholinergic activity, and markers of neurodegeneration

(apoptosis, neuronal and hippocampal tissue damage, change of neuronal morphology, decrease of neurogenesis, or neuronal death through both quantitative and qualitative methods). Seventeen studies examined inflammation-related markers, including interleukin-1 (IL1), IL6, IL10, tumor necrosis factor-alpha, nuclear transcription factor-kappa B, peroxisome proliferator-activated receptor γ, XB1, and inducible nitric oxide synthase. Twenty-six studies examined markers of oxidative stress, such as malondialdehyde, reactive oxygen species, and the level of antioxidants such as total antioxidant capacity, total thiol groups, thiol, superoxide SOD, catalase, glutathione peroxidase, and glutathione. Ten studies examined markers of neurotrophic factors, such as brain-derived neurotrophic factor and vascular endothelial growth factor, and signaling components such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha, cAMP response element-binding protein, sirtuin, protein kinase B (p-AKT), and extracellular signal-regulated kinase. Nineteen studies examined altered cholinergic enzymes, such as activities of choline acetyltransferase and acetylcholinesterase.

Most of the studies (52 studies) examined markers of degenerative neurons such as synaptic damage, pyknotic nuclei, cytoplasmic swelling, neuron shrinkage, vacuolization, lower number of neurons, and decreased volume of the hippocampus. Molecular markers including apoptosis markers: B-cell associated X-protein, B-cell lymphoma protein 2, Caspase-3, terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL), and fluoro jade; neuronal and glial responses: Microtubule associated protein 2, neuronal nuclei, glial fibrillary acidic protein, ionized calcium-binding adaptor molecule 1; and the neurogenesis marker: Bromodeoxyuridine was also frequently reported Tables 2 and 3.

Discussion

The use of chemical substances to induce animal models has been shown to be successful in causing memory impairment [95] as shown with the result of the MWM test. This test examines spatial memory impairment and has been correlated with hippocampal neurodegeneration in rat models [10], [11], [68]. Wistar and SD rats are widely used with equal proportions in AD modeling. Both strains of rats are widely available and handled easily with comparable MWM test results.

The pathological process of AD is related to the deposition of amyloid plaque and tau-protein hyperphosphorylation that causes NFT. The formation of Aβ deposition comes from the cleavage of APP by βACE through the amyloidogenic pathway and subsequently produces C-terminal fragment β (CTFβ). The CTFβ fragment is cleaved by secretase-γ which contains the protein PS-1 to produce Aβ peptide. The βACE has been shown to be elevated in AD patients, as APP and PS-1 mutations are also responsible for increased Aβ deposition in patients. The Aβ deposition will cause synaptic disturbance and then lead to excessive tau-protein hyperphosphorylation. Tau-protein hyperphosphorylation leads to pathological intracellular tau protein accumulation to form NFT and subsequently leads to neurodegeneration. Tau phosphorylation is facilitated by tau kinases, including GSK3β and cyclin-dependent kinase 5 (CDK5). Amyloid-deposition also causes the formation of hydrogen peroxide which triggers lipid peroxidation and finally produces an aldehyde compound that is toxic to nerve cells, namely, 4-hydroxynonenal (4-HNE). The formation of 4-HNE also leads to the formation and aggregation of NFT. The Aβ and NFT are neurotoxic and cause oxidative stress and inflammation, hence leading to neuronal death [3], [96], [97], [98].

The typical AD markers such as amyloid plaques and NFT are important parameters to be examined in an AD animal model, yet they were only reported by a limited number of studies. Other typical markers, such as protein PS-1, APP, GSK3β, and βACE, were reported less frequently. Instead, most of the studies examined neurodegeneration markers and markers of processes leading to neurodegeneration such as oxidative stress, inflammation, and neuronal damage. These markers are reported possibly due

Table 2. Number of articles reporting each type of parameter for each toxic-substance-induced animal model

Toxic substances	Typical marker of dementia	Stress oxidative marker	Inflammation marker	Neurotrophic factor	Cholinergic activity	Neuro-degeneration marker
Amyloid β (Aβ)	14	9	3	4	3	25
Streptozotocin (STZ)	6	5	3	1	5	10
192Ig-saporin	0	0	0	1	3	2
Alumunium chloride	3	2	2	1	2	4
d-galactose	1	2	2	1	0	2
High fat high glucose (HFHG) diet	2	1	1	0	0	1
Ibotenic acid	1	2	0	0	2	1
Lipopolysaccharide (LPS)	0	0	1	0	0	1
Okadaic acid	2	1	2	0	0	2
Scopolamine	0	2	0	2	1	0
Trimethyltin (TMT)	0	0	0	0	1	1
Colchicine	0	1	0	0	0	1
Cuprizone	1	0	0	0	1	0
Monosodium glutamate (MSG)	1	0	1	0	0	0
High salt & cholesterol diet (HSCD)	0	1	1	0	0	0
Letrozole	1	0	1	0	1	1
Virus vector-APP	1	0	0	0	0	1
Total number of articles	33	26	17	10	19	52

Table 3. Markers detected in hippocampus of AD animal model

Typical marker of dementia	Stress oxidative markers	Inflammation markers	Neurotrophic factor	Markers of Cholinergic activity	Neuro-degeneration marker
Amyloid plaque	MDA (malondialdehyde)	NF-κB	BDNF (brain derived neurotrophic factor)	AchE (Acetylcholine esterase)	Casp-3 (caspase-3)
p-Tau (phosphorylated tau)	ROS (reactive oxygen species)	TNF-α	PGC1-α	ChAT (choline acetyltransferase)	BAX
APP (amyloid precursor protein)	NO (nitric oxide)	IL-1 (interleukin 1)	SIRT (sirtuin)		BCL-2
Presenilin-1 (PS1)	Nitrit	IL-6 (interleukin 6)	CREB		p-AKT
GSK3β (glycogen synthase kinase 3 beta)	FOXO1 (forkhead box protein O1)	IL-10 (interleukin 10)	VEGF (vascular endothelial growth factor)		ERK
βACE (Beta secretase)	PCO (plasma protein carbonyl)	PPAR-γ			GFAP (glial fibrillary acidic protein)
	TTG (total thiol group)	XB-1			TUNEL
	H_2O_2 (hydrogen peroxide)	iNOS			JNK
	4-HNE (4-hydroxynonenal)				Fluoro Jade
	GSH (glutathione)				IBA-1
	GPx (glutathione peroxidase)				Neu-N
	SOD (superoxide dismutase)				BrdU
	CAT (catalase)				MAP-2
	TAC (total antioxidant capacity)				Change of neuron morphology:
					Decrease number of neuron using stereology technique
					Change in density of neuron
					Neuron impairment (pyknotic nuclei, vacuolization)
					Synaptic impairment

to the clinical similarity between neurodegenerative processes and AD, especially if the disease includes hippocampal degeneration [99], [100].

Two toxic substances were most frequently used in the studies, that is, Aβ and STZ. ICV administration of Aβ injection is the most widely reported induction technique especially after 2010. The injection of the substance in the form of protein aimed directly into the ventricle of the brain was mostly done by a single dose injection. Only four studies performed the injection in divided doses. These techniques consistently reported amyloid plaque formation in the hippocampus [19]. Increased levels of Aβ and the formation of amyloid plaques stimulate further processes related to AD pathology such as formation of NFT, oxidative stress, inflammation, decrease of neurotrophic factor, change in cholinergic activity, and death of neurons [3], [96], [98]. Because of its consistency in producing AD characteristics, this model of direct delivery to the target organ is increasingly popular despite the technical challenges [19].

Another substance that is used to induce hippocampal degeneration in animal models of dementia is STZ [48], [57], which is a toxic compound that is widely employed in inducing pancreatic beta cell death in animal models of diabetes [58]. It has been revealed that the PI3K/AKT/GSK-3β pathway of the insulin signaling cascade is downregulated upon administration of ICV STZ and downregulation of this pathway is responsible for the emergence of insulin resistance [55]. The ICV pathway is the STZ entry route of choice in AD modeling. In AD modeling, administration of STZ through the ICV pathway causes increased levels of Aβ, formation of NFT, induces oxidative stress, neuroinflammation, decreases of neurotrophic factors, changes in cholinergic activity, apoptosis, and other neurodegenerative changes through insulin signaling impairment leading to cognitive and memory deterioration as found in AD [50], [55]. Several studies used the IP route, which was relatively easier than the ICV route. However, this method has not been reported in the past 5 years. Although insulin resistance is correlated with GSK3β activity, tau hyperphosphorylation and amyloid formation [55], previous STZ-induced animal models using the IP route had not reported biomarkers of AD.

Insulin signaling pathway is implicated in AD model induced by HFHG diet through increased CDK5 transcriptional activity that causes hyperphosphorylation of various substrates such as neurofilament, APP and p-tau [85]. Other than STZ, aluminum chloride, MSG, HFHG, and dan letrozole were other toxic substances that induce the appearance of typical AD markers through impaired insulin signaling [12], [65], [85], [91]. Normally, insulin binds to the insulin receptor (IR) and further leads to the activation of PI3K/AKT and inactivation of GSK-3β. Insulin resistance is characterized by abnormal GSK-3β activity, responsible for hyperphosphorylation of tau protein, a significant contributor to AD pathogenesis. Other mechanisms in AD pathogenesis may involve reducing the activity of insulin degrading enzymes (IDE) that are responsible for the degradation of insulin as well as Aβ. In a mouse model, IDE gene knockout creates the tendency of excessive APP generated Aβ accumulation in neuronal cells [55].

Biomarkers of AD such as tau hyperphosphorylation and Aβ formation have been

reported in AD models induced by other toxic substances. Okadaic acid induced AD biomarkers through inhibition of serine/threonine phosphatase 1 (PP1) and 2A (PP2) [78]. Ibotenic acid impaired cholinergic neurons in the nucleus basalis of Meynert, a similar sign found in AD. Although the mechanism was not clearly stated, one article reported that APP and amyloid were expressed on ibotenic acid induction [80]. Cuprizone was also one of the toxic substances reported to induce amyloid plaques although the exact mechanism was not elucidated. Cuprizone is able to induce neuronal demyelination and oxidative stress in the brain, causing AD clinical symptoms [67]. One study also reported that d-galactose induction lead to the presence of Aβ and βACE. D-galactose itself has previously been used to induce oxidative stress and brain impairment [74]. TMT administration has been reported to induce memory impairment, typical markers of AD, hippocampal degeneration, neuroinflammation, and decreased neurotrophic factors [68], [100], [101], [102]. In addition, a transcriptomic high-throughput analytical study of TMT revealed differential expression of AD-associated genes such as PS-1 and p-tau [101].

In the HFHG and HSCD induced AD models, the dietary cholesterol cannot pass the blood-brain barrier directly, but it is thought to influence central nervous system homeostasis by increased transport of its circulatory breakdown product, an endogenous selective estrogen receptor namely 27-hydroxycholesterol, into the brain. Most studies investigating the role of cholesterol in increasing the risk of AD has focused on how cholesterol affects APP processing and Aβ protein clearance. The cholesterol-fed animal model of AD shows a multitude of pathological findings similar to those seen in AD patients including Aβ deposits, NFT, and significant increase of markers associated with neurodegeneration in the hippocampus as well as cognitive deficits [103].

Not all studies on AD animal model using toxic substances examined NFT and Aβ deposits but instead used biomarkers of neurodegenerative processes, such as markers of neuronal damage, oxidative stress, and inflammation. A study using HSCD reported that it is associated with neuroinflammation marked by activated NF-kB signaling pathway [90]. Inhibiting NF-κB pathways itself could interrupt neuroinflammation and generation of Aβ [90]. Neuronal death due to oxidative stress and inflammatory processes in the hippocampus results in a lower number of hippocampal pyramidal neurons. While hippocampal neurodegeneration is often reported in toxic substance induced AD models, this hallmark of AD neuropathology is an aspect often lacking in transgenic models [103]. Many studies provided data on the density of pyramidal neurons in the hippocampal area from histological sections [24], [28], [29], [50], [51], [58], [61], [62], [87]. Such data are prone to bias from reference traps [104]. Unbiased stereological techniques of total number of pyramidal neurons in the hippocampus [32], [83] may provide more reliable data on reduced number of neurons upon neuronal death.

The length of interval between induction and MWM test varies with different types of induction. Most studies used mostly young adults of 2–5 months old rats. Although aging is a risk factor for cognitive decline, AD itself is not part of the normal process of aging [2], [3]. Therefore, using younger animals induced by toxic substances may reduce the length of time needed to obtain the desired signs and symptoms of AD [7]. In general, oral administration of toxic substances takes more time to produce desirable signs and symptoms compared to parenteral administration. In the gastrointestinal tract, toxic substances may interact with many digestive enzymes, microbiomes and food components that may neutralize the toxin. Furthermore, the detoxification process in hepatocytes may weaken the effect of the toxin. On the other hand, substance metabolism may produce a derivative substance with more potent toxicity [93].

The interval from the start of induction to the MWM test is the longest in animals undergoing diet modification, such as HSFD and HCSD. Obviously, the longer time and special diet for about 15–32 weeks in producing this model require more resources. Nevertheless, diet modification has an advantage in mimicking the slow pathological changes in human metabolism leading to neurodegeneration [93]. ICV route is the most widely use method and produces a relatively fast model with high reproducibility. However, it is a technically demanding method that also needs special equipment. Scopolamine and TMT are toxic substances typically given intraperitoneally to generate neurodegeneration in AD modeling. Scopolamine is injected repeatedly in multiple dosages, while TMT is injected in a single dose. Scopolamine has a rapid effect, but the duration of its effect is short. Therefore, scopolamine must be given repeatedly, every 30 min before the behavioral examination or termination [75]. TMT can be considered as one of the promising alternative AD induction substances due to several factors, for example, easy administration (IP, single dose) and relatively shorter duration from induction into development of AD characteristics.

Studies using female rats were more limited in number, possibly due to its more complicated nature. Using female rats, researchers must consider the influence of hormones such as estrogen in memory function. Therefore, the study is less appealing for many researchers with limited resources. Studies on sex differences in AD rat models are limited to five studies, that is, two articles using Aβ [33], [45], one study using STZ [61], one study used HSCD [90], and one study used letrozole [91]. Those limited studies reported that female rats are comparable to male rats on MWM, AD markers, and neurodegenerative markers upon toxic

induction. Nevertheless, further study on female animal models should be encouraged, because the number of women who suffer from AD is actually more than men with AD [33] and sexual differences in animal models of other brain-related diseases have been reported [105]. Differential hormonal secretion and sex chromosome gene expression may induce different pathogenesis, markers, and therapeutic efficacy in women [106].

Conclusion and Future Direction

At least 17 modeling techniques in rats were developed to support AD research and the most widely used technique was injection of Aβ toxic substances. The memory impairment in the rat models was examined with MWM. The presence of both senile plaques and NFT in brain tissue is other characteristics of AD in humans and should be considered to be examined in the brains of AD animal models. The reduced number of neurons in the hippocampus can provide evidence of neuronal degeneration and should be counted with an unbiased method. Additional parameters that were widely examined in studies using AD modeling were the biomarkers of AD pathological processes, such as markers of inflammation, oxidative stress, neurotrophic factors, the change of cholinergic activity, and markers of neurodegeneration. It is still necessary to develop techniques and selection of toxic substances with optimal results and easy-handling techniques. Future study of AD using female rats needs to be encouraged considering the higher number of women who suffer from Alzheimer's disease compared to men.

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