EXPERT REVIEWS

Retinoic acid as a therapeutic option in Alzheimer's disease: a focus on cholinergic restoration

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Retinoic acid is a potent cell differentiating factor, which through its nuclear receptors affects a vast range of promoter sites in brain neuronal and glial cells in every step of embryonic and postnatal life. Its capacities, facilitating maturation of neurotransmitter phenotype in different groups of neurons, pave the way for its application as a potential therapeutic agent in neurodegenerative diseases including Alzheimer's disease. Retinoic acid was found to exert particularly strong enhancing effects on acetylcholine transmitter functions in brain cholinergic neurons, loss of which is tightly linked to the development of cognitive and memory deficits in course of different cholinergic encephalopathies. Here, we review cholinotrophic properties of retinoic acid and its derivatives, which may justify their application in the management of Alzheimer's disease and the related neurodegenerative conditions.

Keywords: acetyl-CoA • Alzheimer's disease • cholinergic neurons • energy metabolism • neuroprotection • neurotoxicity • retinoic acid

Alzheimer's disease & cholinergic deficits

There are several neurodegenerative diseases, in which dementia is a key clinical symptom. The neurodegenerative diseases include different forms of Alzheimer's disease (AD) [1], thiamine deficiency [2], aluminum overload [3], Parkinson's and Lewy body dementias [4] and hypoxia/anoxia/hypoglycemia-induced encephalopathies [5,6]. There are progressive pathologies, which depending on specific regional localization in the brain, present different combinations of vast range of short- and long-term memory, learning and cognitive deficits. In their final stages, they lead to disappearance of self-maintenance capacity and interactive contacts with surrounding environment. Each of these pathologies may also be characterized by specific pathomorphological and pathobiochemical alterations [1]. In the AD, the degree of the impairment of different cognitive functions, assessed by Mini Mental State Examination scales, was found to correlate with deficit markers of cholinergic neurotransmission, particularly located within septo-hippocampal and basalo-cortical pathways, as well as with amyloid-β (Aβ) deposition [1,7]. They included the decreases in levels and activities of choline acetyltransferase (ChAT), vesicular acetylcholine transporter (VAChT), high-affinity choline transporter as well as in cholinergic neurons density, acetylcholine (ACh) levels and its quantum release rates [1,8–11]. Interestingly, in elderly subjects, in stage of mild cognitive impairment, frontal cortex and hippocampus display increased or unchanged activities of ChAT despite evidence of significant amyloid pathology [7,12]. These data suggest the existence of compensatory reactions in cholinergic neurons under neurotoxic conditions early in the progression of AD [7,12]. Similar deficiencies in the markers of cholinergic metabolism were also reported for autopsy brains affected by other above-mentioned pathologies [4,11,13]. On the other hand, other transmitter systems seem to be less affected by these conditions [9]. Therefore, the whole group of the diseases is classified as the cholinergic encephalopathies [13].

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The deficiency of cholinergic neurotransmission in the encephalopathic brains is usually caused by variable combinations of neuronal death and functional defects in those surviving such conditions. Several pathogenic signals are identified as possible factors contributing to the neuronal injury yielding onset and progress of AD. They include: focal or general hypoxia/anoxic episodes, frequent in elderly people [5], hypoglycemia, diabetes [14], thiamine pyrophosphate deficits [2], excitotoxic stimulation, nitric oxide excess [15], zinc redistribution [16] and aluminum and iron overload [3]. Each of them may aim single or multiple targets at various cellular-intercellular and molecular levels. One has to be stressed that fractional contribution of individual neurotoxic signals to the overall picture of the disease is not entirely clear [13,17].

Disturbances in the cholinergic neurotransmission in cellular and animal models of AD have been reported to involve all steps of transmitter metabolism including the uptake, the reuptake and/or the synthesis of choline and acetyl-CoA precursors of ACh moiety, its vesicular accumulation and quantal release [13,16,18,19].

AD & failure of energy metabolism

Under resting conditions, the brain consumes about 20% of the whole body glucose and the oxygen supply [6,13]. Glucose is almost exclusive energy precursor in the brain, which through glycolytic pathway in cytoplasm is converted to pyruvate. The latter, after transporting into mitochondria, is metabolized by pyruvate dehydrogenase complex (PDHC) to acetyl-CoA and oxidized in tricarboxylic acid cycle (TCA) cycle, yielding ATP. Neurons constitute 10% of brain cells, but produce and utilize 70% of its whole energy pool. Therefore, in neuronal compartments rates of metabolic fluxes through glycolytic and high-energy pathways are at least 20-times faster than in glial compartments [13]. Over 70% of energy produced in neurons is utilized for restoration of their membrane potentials, after depolarization takes place with a frequency of 10–50 Hz. Accordingly, neuronal activities of PDHC, ketoglutarate dehydrogenase complex (KDHC) and the most of TCA enzymes are several times higher than those in glial cells [13,20]. Thus, transmitter functions make neurons more susceptible to conditions limiting glucose or oxygen supply or other conditions inhibiting energy production.

Striking, the common feature of cholinergic encephalopathies, is inhibition of glucose uptake and energy metabolism in affected brains [6,21–23]. Reductions of neuronal PDHC activity and acetyl-CoA synthesis are also considered as characteristic features of AD [13,21,22]. Acetyl-CoA serves as a key substrate for energy production and *N*-acetyl-L-aspartic acid (NAA) synthesis in mitochondria of majority of neurons and for ACh synthesis exclusively in cytoplasmic compartment of cholinergic neurons [13,20,22]. The consumption of acetyl-CoA for ACh synthesis may make the neurodegeneration susceptibility of cholinergic neurons greater than that of non-cholinergic cells [13,24].

Suppressions of aconitase, isocitrate dehydrogenase and KDHC, as well as respiratory chain complexes activities were also reported for cellular and animal models of AD and thiamine encephalopathy [2,6,13,20]. Studies on cell cultures revealed that losses in enzyme activities appear almost instantly after their exposure to multiple primary pathogenic signals known to be involved in early pathogenesis of AD or vascular encephalopathies [13,16]. For instance, a short time (1–10 min) exposure of cholinergic neurons or brain nerve terminals to pathophysiologically relevant concentrations of Zn^{2+}, peroxynitrite radicals or aluminum was found to evoke irreversible inhibition of aconitase and transient one of PDHC or KDHC [16,24,25]. These alterations affected negatively further steps of ACh metabolism: vesicular accumulation, storage and quantal release [13,18]. Compatible with these findings are loses of cholinergic neurons and decreased activities and levels of ChAT, and VAChT mRNA and proteins, detected in autopsied human AD brains [7,10,11,19,21].

These findings remain in accordance with MRI/PET studies of AD patients displaying deficits in ^{18}F-deoxyglucose uptake in pathology-affected brain regions [23,26,27]. Molecular scanning also revealed deficits of NAA in brains of those patients [22]. NAA is produced exclusively in neuronal mitochondria, from acetyl-CoA and L-aspartate, by *N*-acetyl-aspartate transferase [28]. Therefore, NNA level is recognized as an indirect indicator of acetyl-CoA content and energy/ATP production through TCA in neuronal mitochondria [22,28].

Aβ & cholinergic cytotoxins in AD

Excessive accumulation of Aβ peptides, in form of its oligomeric and polymeric deposits in extracellular compartments of the brain, and intraneuronal deposits of neurofibrillary tangles are a hallmark of AD, useful for its clinical diagnosis [23,26,27]. In addition, early monomeric/oligomeric $A\beta_{1\text{-}42}$ forms, accumulating in the intracellular cytoplasmic synthesizing compartment, are thought to be the most neurotoxic forms of the peptide. Their excessive accumulation may result from extensive amyloidogenic proteolysis of amyloid precursor protein (APP) by β-secretases including β-amyloid cleaving enzyme [1]. Primary accumulation of Aβ takes place in brains carrying relatively rare mutations in *APP* gene (<1% of all cases) yielding early onset cases of AD. Also mutations of *PS1* and *PS2* genes coding presenilin peptides may cause appearance of γ-secretase complex with increased affinity to C-terminal fragment of APP [1]. On the other hand, in most frequent cases of sporadic AD, Aβ excess seems to be due to the secondary increase of amyloidogenic APP proteolysis inside the neurons previously impaired by cytotoxic signals [1–3,5]. Also, people who carry susceptibility genes such as *apoE4* were found to display several-fold greater prevalence of late-onset AD, with multiple mechanisms being involved [29–31]. That does not exclude possibility for secondary neurotoxicity accumulating Aβ, which may combine with different primary cytotoxic signals, thereby aggravating neurodegenerative processes [3,13,32].

Cholinergic neuronal cells in culture display preferential susceptibility to Aβ and vast range of other neurodegenerative signals [13,20]. It might result from the fact that unlike neurons of another transmitter systems, and glial cells, cholinergic neurons utilize acetyl-CoA not only for energy/ATP and NAA production, but also for ACh neurotransmitter synthesis. Studies on cholinergic neuronal cell cultures and isolated brain nerve terminals revealed that the majority of neurodegenerative signals evokes inhibition of PDHC activity, which is the only source of acetyl-CoA in the brain under non-ketotic conditions [6,13,20,24]. Therefore, the decrease of acetyl-CoA synthesis in cholinergic neurons in such conditions results in much faster depletion of this energy precursor than in other types of brain cells [13,20]. In addition, cytotoxicity-evoked extensive depolarization, hampers high-affinity extracellular choline uptake and activates phospholipase-D-dependent autophagy of plasma membrane phospholipids in cholinergic neurons to provide choline for ACh synthesis [33].

Low levels of ACh are present in extracellular compartments inside and outside the brain despite of the presence of cholinesterases [34–37]. It implies that extracellular ACh may originate both from cholinergic and non-cholinergic compartments. Recently, relatively high activities and levels of non-cholinergic cell-derived ChAT protein have been detected in cerebrospinal fluid of neurological patients [36]. Based on the foregoing it has been claimed that certain fraction of ACh pool may be generated by ChAT in distant non-cholinergic extracellular compartments of the brain and exert anti-inflammatory effects on lymphocytes and microglial cells [36,37]. This could constitute a potential target for anti-inflammatory effects of cholinesterase inhibitors used in AD therapy [36,38]. On the contrary, other reports provide either no evidence for ChAT or negate its involvement in extracellular ACh synthesis in the brain [39,40]. Nevertheless, irrespective of the origin, extracellular ACh may modify frequency of γ-oscillations and immune functions in the brain [36,40].

Neuroprotective compounds in AD

A number of compounds have been tested on animals and cellular models as potential therapeutics to treat AD using different animal and cellular models of the disease. So far, only some reversible or pseudo-irreversible acetylcholinesterase/butyrylcholinesterase inhibitors/muscarinic M_2 receptor antagonists/M1 receptor agonists and GABA antagonists were found to be modestly effective in AD patients in the early phase of mild cognitive impairments [38,41]. The acetylcholinesterase inhibitors exert their procognitive effects by increasing ACh levels within cholinergic synapses, due to the inhibition of its breakdown in the synaptic cleft. On the other hand, GABA and NMDA glutamate receptor antagonists overcome inhibitory signals from GABA-ergic terminals, and mitigate excitotoxic stimulation by excess of glutamate, respectively directed to postsynaptic neurons of cholinergic, and other transmitter systems [38].

Also number of compounds that may support neuronal survival improving acetyl-CoA and oxidative/energy metabolism by another mechanisms were tested [13,14]. They include thiamine/thiamine pyrophosphate, lipoic acid, L-carnitine and neurotrophins as well [38,42–44]. All of them were found to improve energy/acetyl-CoA metabolism in cultured cholinergic neuronal cells, through direct protection of several enzymes such as PDHC, aconitase, KDHC and enzymes of oxidative chain [13,20]. So far, none of these compounds has proved to be fully an effective anti-AD agent in clinic [19,38]. On the other hand, hundreds of plant-derived polyphenols have been tested as scavengers of free oxygen, nitroso or fatty acid radicals. Their effects are rather non-specific, resulting from attenuation of detrimental effects of free radicals on key enzymes of energy and neurotransmitter metabolism [43,44].

There are various cytokine-type peptides such as NGF, brain-derived neurotrophic factor or bone morphogenic protein-9, which were found to exert neurotrophic and neuroprotective effects in different cellular and animal whole brain models of neurodegeneration [45–47]. Studies on cultured cholinergic cells revealed that neuroprotective effects are mediated by number of specific surface membrane receptors such as TrkA, TrkB or NT3/4, followed by retrograde axonal propagation of the neurotrophic signal [45–47]. On the contrary, non-specific neurotrophin p75 receptors, when overexpressed or activated by high concentrations of neurotrophins, exerted cholinosuppressive effects in all-*trans* retinoic acid (RA)-differentiated cholinergic cells, and aggravated cytotoxic effects of Aβ or nitric oxide excess [18,48].

All-trans RA for brain neurodegeneration

RA and 9-*cis*-RA are the key biological active derivatives of oxidative metabolism of vitamin A (retinol) catalyzed by two specific alcohol and aldehyde dehydrogenases, respectively [49]. The liver is the main site of RA synthesis, but the brain also has synthetic capacity of this compound [49]. Thus, brain RA may originate both from endogenous and exogenous synthesis. RA binds to nuclear RA receptors α, β or γ (RAR) [49–51]. They may form different kinds of heterodimer complexes with retinoid X receptors α, β or γ (RXR) that are activated by binding with 9-*cis* RA [50,51]. Each of heterodimers may interact with various sets of retinoid response elements modulating expression of different arrays of genes [50,51]. That explains appearance of vast range of RA-evoked regulatory and adaptive reactions in brain cells including increased expression of different neuronal transmitter phenotypes, maturation/differentiation of stem cells, anti-inflammatory reactions, direct and indirect neuroprotective effects, regulation of various peptidases, such as those involved in Aβ formation [50,52]

All-trans RA & AD

Patients suffering from AD are at risk of multiple nutritional deficits including vitamins and other trace elements due to mental disability and other age-related conditions [16]. Vitamin A status as one of 17 essential nutrients was subjected to

meta-analysis of data from 80 reports, which met inclusion criteria [44]. Among nine reports testing vitamin A status, four showed a decrease and five showed no change of its level in plasma of AD patients [44]. One has to stress that detected levels of vitamin A in the brain tissue, being in the range of 1 μmol/l, are 10^3-times higher than those of derived RA species [51]. On the other hand, experimental works studying retinoid properties employ 1 μmol/l or higher concentrations of RA or other retinoids [42,52–55]. Therefore, they may not reflect the real functional status of these bioactive compounds in the extracellular and the intracellular brain compartments *in vivo* [42,49,53,52].

Recent studies revealed that RA acting in various regions of the brain including hippocampus and hypothalamus is one of the factors regulating circadian and diurnal rhythms including sleep cycles [49]. However, the isolated activation of RAR was unable to correct sleep abnormalities in RA-deficient animals [49]. Thus, it may be the case that RA regulates circadian clock indirectly through interaction with neurons of the cholinergic and the dopaminergic neurotransmitter systems [49]. Given the significance of hippocampal region in memory formation, RA deficiency may contribute to worsening cognitive deficits in AD [44,51].

RA was proven to support survival of cultured brain neuronal and glial cells, and maturation (differentiation) of their native forms, as well as progenitors and stem cells [13,48,53,56]. This property is linked to anti-proliferative effects of retinol derivatives against vast range of neuroblastoma and other classes of neoplastic cell lines [57]. There are also numerous data on potential effective application of RA agonists in decreasing Aβ load and its oligomeric forms in AD [50,58–61]. However, one should note that effective anti-oligomerization concentrations of RA used in above experimental models were 10^3- to 10^4-times higher than those present in the brain [51,62]. They also put less attention to specific interactions between RA and injured cholinergic neurons responsible for cognitive and memory deficits in course of AD [7,11,59]. Therefore, the remaining part of the article will focus on the putative RA effects on brain cholinergic neurons.

Specific cholinotrophic effects of RA

RA & SN56 cholinergic neuroblastoma cells

RA was found to enhance the expression of cholinergic phenotype both in cultured primary and clonal cholinergic cell lines, as well as in the brain *in situ* [20,63,64]. Increased levels of ChAT, ACh, VAChT and rates of quantal ACh release were observed in cultured hybridoma SN56 cells originating from mouse septum upon their exposition to supraphysiological concentrations of RA (Table 1) [20,63,64]. Those effects were mediated by RA binding to different RAR αβγ-RXR αβγ nuclear receptor heterodimer complexes, activating or suppressing broad range of multiple nuclear promoter sites including cholinergic locus [49,53,61,63,65]. It also increased insulin degrading enzyme mRNA/protein, contributing to Aβ clearance [61]. Thereby, RA could alleviate suppression of PDHC, and other enzymes of acetyl-CoA and ACh metabolism by attenuating Aβ load (Table 1) [7,61]. Moreover, RA displayed synergistic effects with other cholinotropic/differentiating peptides like NGF, brain-derived neurotrophic factor and with intracellular pathways rising cAMP/PKA/CREB signaling [13,61,63,65]. That resulted in an additive enhancement of cholinergic phenotype, and morphologic maturation in septal cholinergic clonal neuronal cells [13,64]. It indicates the divergence of these two signaling pathways on the level of separate promoter sites responsive to RAR/RXR and cAMP/CREB within the cholinergic locus (Table 1) [13,61,63,65]. Such properties paved the way for hypotheses, making RA candidate therapeutic agent, which could consolidate structural and functional capacities in those neurons that survive under degenerating conditions [52,60,65]. However, these positive cholinotrophic effects of RA were evident only under basal conditions [64]. It has been shown that cholinergic cells of septal origin, when subjected to any differentiating signal, listed above, became more susceptible to several neurodegenerative signals including nitrosyl radicals, Aβ, zinc excess or thiamine deficits [13,18]. Therefore, particular attention should be focused on improving acetyl-CoA and energy metabolism in neuronal mitochondria, which are inhibited in AD [21]. RA exerted no activating effects on PDHC or aconitase and KDHC activities in basal conditions [18,64]. On the other hand, neurotoxins caused much stronger inhibition of those enzyme in RA, cAMP or NGF-differentiated SN56 cells than in non-differentiated ones [13,18]. These findings suggest that efficient application of either RA or its agonists, as cholinoprotective/tropic agents, in AD should be accompanied by preceding and/or parallel treatment alleviating pathogenic signals suppressing energy metabolism. Such hypothesis is supported by *in vivo* studies on G93A-amyotrophic lateral sclerosis model mice [66]. They revealed that long-term supplementation of those animals with RA decreased cholinergic markers in spinal cord and shortened their lifespan [66].

RA & SH-SY5Y neuroblastoma cells

Extensive data on RA effects on neuronal differentiation and susceptibility to neurotoxic inputs were collected for human neuron-like SH-SY5Y cell line. Those cells were found to co-express dopaminergic, glutamatergic, acetylcholinergic and adenosine neurotransmitter systems [53]. RA caused morphologic and biochemical differentiation of SH-SY5Y toward morphologically mature neuronal cells with markedly increased cholinergic and noradrenergic/dopaminergic phenotypes and elevations of mRNAs for ChAT (six-times) and tyrosine hydroxylase (three-times) [67]. On the other hand, dopamine excess or miRNA-432 exposure caused differentiation with more significant expression of dopaminergic phenotype [67]. Also transcriptional profile of RA-treated SH-SY5Y cells suggests the upregulation of dopamine-linked and the suppression of ACh-linked genes [53]. It must be stressed that the expression of the majority of transmitter-linked genes was directly dependent on RA treatment. It suggests that post-translational mechanisms may be involved in RA-induced differentiation of these cells [53].

Table 1. Retinoic acid interactions with cholinergic neurons in different experimental models of Alzheimer's disease.

Experimental model	Neurotoxin's applications (mmol/l)	Parameter – relative alteration (±% difference from non-differentiated controls)				Ref.
SN56 cholinergic cells Non-differentiated		Non-viable	PDHC	ChAT	Acetyl-CoA	
	+SNP 1.0 10 min	+15	–8	+1	0	
	+$A\beta_{25-35}$ 0.001 24 h	+11	–7	–10	–9	
	+Zn 0.1 30 min	+22	–31	–25	–23	
Differentiated RA 0.001 48 h dbcAMP 1.0 48 h RA 0.001 + dbcAMP 1.0 48 h	+Amprolium 2.0 48 h	+5	–27	0	–37	[13,16,20,42,64]
	None	0	+2	+145	–23	
	None	0	–28	+110	+15	
	None	+2	–22	+305	–24	
	+Zn 0.1 30 min	+42	–52	+236	–67	
	+SNP 1.0 10 min	+27	–56	+135	–60	
	+SNP 1.0 +LA 0.005 48 h	+7	–25	+283	–17	
	+$A\beta_{25-35}$ 0.001 24 h	+31	–38	+46	–57	
	+$A\beta_{25-35}$ 0.001+LA 24 h	+4	–29	+247	–26	
	+ Amprolium 2.0 48 h	+13	–56	+300	–46	
SN56 cholinergic cells Differentiated RA 0.001 48 h	None	ChAT mRNA	VAChT mRNA		ACh level	[63]
		+225	+100		+311	
SH-SY5Y cells	None	ChAT mRNA	miRNA432		Neurite length	[67]
Differentiated RA 0.01 7 d		+507	+462		+525	
SH-SY5Y cells, ADAM10 construct transfected RA 0.002 48 h	None LY294002	ADAM10 +170 –15				[54]
SH-SY5Y cells	Exposition 48 h	Propidium iodide(+)		MTT reduction		[69]
Non-differentiated	Rotenone 0.01	+2.4				
	Lactacystin 2.5 µg/ml			–80.0		
Differentiated RA 0.01 7 d	Rotenone 0.01	+28.7				
	Lactacystin 2.5 µg/ml			0		
BE(2)M17 glycinergic neuroblastoma		SNAP25	Glycine release		Ca channel activity	[56]
Differentiated RA 0.01	None	+120	+50		+200	
RA 0.01 treated human embryonic stem cells 7 d +BMP9		ChAT mRNA	Ap75 mRNA		AChE mRNA	[73]
	None	+1100	+3900		+220	
	Lhx8 siRNA nucleofected	+1100	+40		0	

Alterations are given as percent difference from respective non-differentiated control values of 100%, or absolute fractional content for non-viable cells. Escape latency alterations are given as seconds.
ChAT: Choline acetyltransferase; IDE: Insulin degrading enzyme; PDHC: Pyruvate dehydrogenase complex; RAR/RXR: Retinoic acids receptors; SNP: Sodium nitroprusside – nitric oxide donor.

Table 1. Retinoic acid interactions with cholinergic neurons in different experimental models of Alzheimer's disease (cont.).

Experimental model	Neurotoxin's applications (mmol/l)	Parameter – relative alteration (±% difference from non-differentiated controls)						Ref.
Primary neuronal culture embryonal mice brain		Non-viable	$A\beta_{1-42}$		ADAM10		APPα	[72]
None	+Aβ 0.01 (viability only) for Aβ, ADAM10, APPα –0.0001, for viability 0.001 agonist concen trations were used	+35	N.A.		N.A.		N.A.	
+RA 0.001		–	–		+365		–	
+RARα agonist AM580		+10	–85		+550		+1350	
+RARβ agonist CD2019		+32	+93		–65		0	
+RARγ agonist CD437		+55			–35		+150	
Primary cultured neurons from brains NSE-apoE3, NSE-apoE4 transgenic mice	None	I	II	III	IV	V	Hsp70	[84]
	Mitochondrial respiratory complexes levels in apoE4 vs apoE3 neurons	–60	–37	–66	–53	–50	–13	
AβPP23 8.5 m old mice Oral applications 18 days	Aβ accumulation in the brain	Escape latencyΔs/ 4 days		$A\beta_{1-42}$		IDEmRNA		[61]
None		–19		–		–		
+RARαβ agonist Am80	5.0 mg/kg/d	–19		–12		+25		
+RXR n.s. agonist HX630	0.5 mg/kg	–18		–18		–100		
Am80 + HX630	5.0 + 0.5 mg/kg/d	–27		–52		–165		
Percutaneous human brain cortex autopsy samples	Clinical stage	ChAT		Pittsburgh compound B		$A\beta_{1-42}$		[7]
	Mild cognitive impairment	–12		+82		+88		
	Alzheimer's disease	–31		+216		+314		

Alterations are given as percent difference from respective non-differentiated control values of 100%, or absolute fractional content for non-viable cells. Escape latency alterations are given as seconds.
ChAT: Choline acetyltransferase; IDE: Insulin degrading enzyme; PDHC: Pyruvate dehydrogenase complex; RAR/RXR: Retinoic acids receptors; SNP: Sodium nitroprusside – nitric oxide donor.

This potential for multidirectional differentiation makes the SH-SY5Y cells useful cellular model both for AD and Parkinson's disease. Strongest evidence for RA link with AD therapy results from its ability reducing Aβ burden in those neurons [52,55]. Recent findings demonstrate that RA-treated SH-SY5Y cells start expressing $p42^{IP4}$ (centaurin α1 protein), which yields several-fold elevation of metalloendopeptidase nardilysin activity, thereby promoting non-amyloidogenic α-secretase pathway of APP degradation (Table 1) [55]. However, in brain neurons, disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) with α-secretase activity are principal enzymes for non-amyloidogenic proteolytic processing of the APP [1,52]. The promoter in ADAM10 gene inserted into SH-SY5Y cells was also activated over twofold upon exposition to RA [54]. This activation was abolished by LY294002, a PI3K inhibitor, indicating significance for cooperation of neurorophin/PI3K/AKT and RA in neuroprotective mechanisms (Table 1) [54,68]. These data remain in accordance with hypothesis that high cholinergic activity prevents Aβ accumulation [19,37]. There are also indications that RA directly inhibits γ-secretase preventing proteolysis of APP-C99 peptide, direct precursor of Aβ1-42 [52]. RA-cholinergically/dopaminergically differentiated SH-SY5Y cells appeared to be more susceptible than the non-differentiated ones to complex I inhibitor-rotenone induced energy deficits [69]. On the other hand, the differentiated cells displayed greater resistance than the non-differentiated ones to detrimental effects of proteasome inhibitor lactacystin (Table 1) [69]. These data indicate that neuroprotective potential of RA may be limited to highly specific cytotoxic conditions and neuronal phenotypes. This finding remains in accordance with similar data on cAMP/RA differentiated cholinergic SN56 cells demonstrating their high susceptibility to inhibitors of PDHC and TCA cycle enzymes [13,20]. Differentiation may also alter positive cholinotropic properties of NGF to neurosuppressive ones due to overexpression of p75 receptors [18,70]. These findings reveal possible side effects of RA therapy in AD, resulting in a parallel increase in cholinergic phenotype and cytotoxic vulnerability in brain cholinergic neurons. However, the interpretation of these data must consider the fact that experimentally used RA concentrations are 10^3- to 10^4-times higher than those found in the brain [49,71].

Cholinotropic effects of RA in primary neuronal cultures & whole brain

Studies on mice primary cortical neuron cultures demonstrated that RARα agonists but not RARβ or γ agonists were capable of increasing their ADAM10 expression, thereby preventing loss of viability upon exposition to high concentrations of $A\beta_{(1-42)}$ (Table 1) [72]. RA was found to be necessary to reinforce effects of neurotrophic factors, such as BMP9, or NGF to differentiate human embryonic stem cells, toward basal forebrain cholinergic neuron phenotype containing high levels of ChAT and p75 receptors [73]. This process was blocked by specific blocker of cholinergic differentiation home box gene family – Lhx8 siRNA (Table 1) [73,74].

The loss of the cholinergic phenotype is a key feature of AD being associated tightly with deficits of memory and spatial learning [11]. The majority of *in vivo* animal studies investigating RA-neuroprotection remain compatible with observations gained from cell culture studies. Aβ accumulating in brains of Tg2576 AD-model mice inhibited endogenous RA synthesis, which was attenuated by RAR antagonist [75]. This was accompanied by elevations of neprilysin and insulin degrading enzyme activities and Aβ clearance from the brain [75]. Moreover, some RA derivatives such as acitretin cross blood–brain barrier and accumulate two- to fivefold, over extracellular compartment levels, allowing their pharmacological activity [76]. Therefore, RA alone or combined with peptide-neurotrophins, could help to induce formation or maintain population of brain cholinergic neurons both during its physiologic maturation and under neurodegenerating conditions [73]. RA is one of the compounds necessary for differentiation of human fibroblast-derived induced pluripotent stem cells toward cholinergic neuron phenotype [77]. Such RA-modified cholinergic cells, when transplanted into brains of APP transgenic mice, alleviate their spatial memory dysfunctions [77]. It seems that only activation of RARα may be efficient in controlling amyloid overload. RARα-specific agonists (AM580, BMS 194753) applied either intraperitoneally or orally to Tg2576 AD mice were demonstrated to cross blood–brain barrier reaching over 100-times higher levels in brain regions over those in plasma [72]. Therefore, they reached effective concentrations yielding several-fold increases in ADAM10 expression and totally attenuating Aβ burden in Tg2567 brains (Table 1) [70].

RA & non-cholinergic neuronal cells

RA neurotrophic effects were also observed in human neuroblastoma M17 cells, in which it brought about maturation toward functional glycinergic phenotype expressing voltage-gated calcium channels, quantal release of glycine and nicotinic α7 surface receptors facilitating their responsiveness to cholinergic signaling (Table 1) [56].

RA was proven to possess strong anti-proliferative activity causing differentiation of stem and non-differentiation of neuroblastoma cells toward mature dopaminergic phenotypes [78]. It also protected primary cultures of nigrostriatal dopaminergic neurons against methamphetamine-induced neurotoxicity [78]. Administration of RA to rats early after 6-hydroxy-dopamine or methamphetamine application that evoked Parkinson's disease model, reduced their rotational behavior and increased depolarization-evoked dopamine release from lesioned striatum [79]. RA effects on dopamine phenotype may differ depending on the type of neuronal cell. Thus, in brain-derived, neuronal cultured clonal cells, neuro-2A, RA caused morphologic maturation along with increased expression of choline kinase involved in plasma membrane formation [80,81]. However, it had no effect on their dopaminergic phenotype. Moreover, RA suppressed cAMP-induced conversion of neuro-2A cells to dopaminergic phenotype decreasing cAMP-elevated tyrosine hydroxylase and dopamine levels [82]. It indicates that RA, when used for treatment of AD might exert, under specific conditions, dopamine-suppressive effects in some groups of brain neurons.

Lipoic acid-non-specific RXR ligand

Structural-affinity studies for number of RXR agonists revealed that structurally RA-unrelated β-lipoic and sulfanilic acids displayed much higher potent agonistic binding affinities than RA or its derivative-agonist Targretin [83]. This indicates that β-lipoic (LA) and sulfanilic acids may be potential lead compounds for development of RXR-interacting drug against AD [83]. However, there are no specific data whether those compounds exert RA agonistic or antagonistic effects. Most probable beneficial effects may result from the fact that LA is cofactor of PDHC and KDHC reactions [42]. It possesses capacity reversing direct and indirect inhibitory effects of Zn and peroxynitrite radicals on PDHC/KDHC, in cultured cholinergic cells, through protection of active centers of their E2 and E3 subunits [25,42]. Preincubation of SN56 differentiated neurons or their homogenates with LA protected iron-sulfur clusters present in active centers of aconitase and respiratory chain complexes [42]. Such conditions are thought to be an early neurotoxic event in the process of the neurodegeneration [13,20]. These properties of LA allowed maintaining viability, and high expression of cholinergic phenotype in SN56 cholinergic septum-derived neuronal cells subjected to neurotoxic signals [42]. On the contrary, delayed incubation with LA failed to restore activities of aconitase and other enzymes of brain energy metabolism [24]. It might be the reason for lack of significant improvement in cognitive status of AD patients after chronic treatment with LA [38]. Also outcomes of putative LA interactions with RXR remain to be tested [83].

RA–apoE interactions

People who carry two copies of *APOE4* gene are at several times greater, although race and gender-independent, risk of late-onset sporadic AD, than subjects carrying *APOE3* or *APOE2* genes [29,30]. Analysis of vast number of aging human populations [30] as well as transgenic animals experimental data unequivocally indicates that apoE4 may increase levels of soluble Aβ in the brain, providing a potential mechanism for AD risk [31]. One of the possible explanations for this phenomenon

came from studies on primary cultures of neurons isolated from brains of transgenic NSE-apoE3 and NSE-apoE4 mice [84]. The mitochondria of apoE4 brain neurons displayed much lower levels of respiratory chain complexes than those from apoE3 phenotype [84]. Thus, neurotoxic properties of apoE4 might reflect lower lipidation levels compared with apoE3, which could reduce stability of apoE4-Aβ complexes, in turn increasing levels of free-toxic Aβ [31]. All natural isomers of RA facilitated apoE production in concentration-dependent pattern [85]. Therefore, depending on apoE phenotype and its lipidation, RA could exert either detrimental or beneficial indirect effects on neurons, respectively [31,85]. Moreover, those differential effects appeared to be neuron specific as mitochondria from GFAP-apoE3 and GFAP-apoE4 astrocytes displayed no differences in levels of their respiratory protein complexes [84].

In conclusion, RA should be considered as one of several, yet not fully documented compounds, for complementary therapeutic strategies in AD. Broad range of interactions of RA derivatives with amyloid-generating systems and their significant positive cholinotrophic properties provide rationale for further searches of efficient application of this class of compounds in AD management [86]. Simultaneous combination of RA with treatments correcting energy metabolism and free radicals level in AD brains seems to be obligatory condition for positive outcome of such putative RA-linked neuroprotective strategies [13,21]. Multiple adverse effects, including teratogenicity should also be taken into account [86].

Expert commentary

There are multiple pathogenic signals that may contribute to onset and progress of AD. These include early pathogenic signals like transient episodes of brain hypoxia, hyper/hypoglycemia, yielding glutamatergic exicitotoxiciy, generation of vast range free radicals, aberrant redistribution of trace metals and many others. They evoke instant functional impairment of neuronal activities, which if not corrected may lead to neuronal death. Vitamin A derivative – RA is known to play a key role in ontogenesis of the brain, contributing to regulation of cell divisions and their differentiation toward mature specific neuronal and glial phenotypes. Particularly, strong effects of RA were observed in the development of cholinergic and dopaminergic transmitter systems. Cholinotropic and anti-amyloidogenic properties of RA described here may provide rationale for its use as complementary compound in multi-directional therapeutic approach in this pathology. Such treatment could help in functional recovery of cholinergic neurons that escaped annihilation in course of AD alleviating Aβ overload and enhancing expression of their neurotransmitter phenotype. However, putative positive effects of RA may be expected in early stages of the AD, in which at least fraction of cholinergic neurons retained its plasticity. One should also remember that increased ACh metabolism is linked with greater demand for energy production, which is depressed in encephalopathic brains. Therefore, eventual clinical use of RA in AD therapy should be linked with alleviation of energy metabolism-suppressing signals.

Five-year view

RA and its agonist provide an attractive option in the management of different neurodegenerative conditions including AD. However, safe and efficient usage of RA will depend on elimination of its potential teratogenic properties and necessary combination with pathogenic signals-eliminating and energy synthesis improving approaches. Also hybrid derivatives of RA with different neuroprotective compounds might enhance their therapeutic potency and attenuate their adverse effects.

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Key issues

- Alzheimer's disease (AD) is the most common prevalent cause of dementia in aging populations. The loss of cholinergic innervation of several cortical areas is responsible for vast range of cognitive and memory deficits in this pathology.
- Retinoic acid and its agonists may offer an alternative approach to AD management increasing expression of cholinergic and other transmitter phenotypes in respective groups of neurons yielding improvement of their neurotransmitter functions.
- Multidirectional interactions with amyloid metabolism on genomic and proteomic levels of amyloid precursor protein, disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), β-amyloid cleaving enzyme and APOE indicate that retinoic acid could be used for causative, Aβ-suppressing treatment of AD.
- Retinoic acid has no positive effects on energy metabolism, impaired by neurodegenerative conditions. Therefore, its efficient use as cholinotrophic/neurotrophic agent would require simultaneous therapy alleviating acetyl-CoA/energy deficits. That would prevent secondary neuronal injuries resulting from neurotransmission-evoked increases in energy demands.

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