Tamibarotene: A Candidate Retinoid Drug for Alzheimer’s Disease

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Tamibarotene (Am80), a synthetic retinoid approved in Japan for treatment of acute promyelocytic leukemia (APL), is a retinoic acid receptor (RAR) agonist with high specificity for RARα and RARγ over RARβ. Temporarily and spatially specific expression of RARs suggests their pivotal roles in the adult brain. Am80 is considered to be a promising candidate drug for treatment of Alzheimer’s disease (AD) because of its transcriptional controls of multiple target genes involved in etiology and pathology of AD. In APP23 AD model mice, administration of Am80 decreased the deposition of insoluble amyloid-β(42). In senescence-accelerated mice (SAMP8), Am80 ameliorated the decrease of cortical acetylcholine, as well as reducing anxiety in behavioral tests and improving the sleep deficit. Am80 also affected a significant improvement of memory in the rat scopolamine-induced memory deficit model. Like other retinoids, Am80 also has an immunomodulatory effect and reduces secretion of proinflammatory cytokines and chemokines by astrocytes and microglia surrounding amyloid-β plaques. In a rat experimental autoimmune encephalomyelitis model, Am80 reduced inflammatory cytokines and showed significant efficacy. Retinoids also promote differentiation of neural stem cells, and Am80 improved the recovery of spinal cord-injured rats. Am80 may also improve vascular factors involved in onset and/or progression of AD. Am80 has been in clinical use for treatment of APL in Japan since 2005, and has been reported to have fewer side effects than other retinoids. We have recently started a clinical study to evaluate the efficacy and safety of Am80 for the treatment of Alzheimer’s disease.

Key words retinoid; tamibarotene; Alzheimer’s disease; amyloid beta; acetylcholine; memory

INTRODUCTION

Alzheimer’s disease (AD) mainly affects elderly individuals, and is becoming an increasing socioeconomic problem as average life expectancy increases, particularly in developed countries. Drugs currently used to treat AD are neurotransmission modifiers, which are expected only to ameliorate the symptoms. Anti-amyloid therapies designed to reduce amyloid-β (Aβ) production or to promote Aβ clearance, including active or passive immunotherapies, have been proposed, and tau protein and mitochondrial dysfunction are also potential targets for therapy,1–2) though the latter approaches are at a very early stage. To assist the clinical evaluation of candidate drugs, it remains important to identify and validate appropriate biomarkers.

The etiology of AD is thought to be complex and the cause of early onset also remains unclear, though genetic factors definitely participate in the case of early-onset familial AD. Environmental factors and infection may also play a role. A variety of factors may also contribute to progression of the disease. Therefore, multi-target therapies may prove to be more effective than single-target therapies. Multi-drug therapy, with each drug having a different target, would be one possible approach. Another possibility would be to use a single drug that acts on plural targets related to Aβ, inflammation, neurotransmission, etc., involving different pathways or physiological categories.3–5) Retinoids may be good candidates for the latter approach, because they directly regulate a number of key genes and proteins in diverse pathways, and their principal biological targets include neurotransmission, Aβ, inflammation, neurogenesis, the blood–brain barrier, and others.4,5)

1. RETINOIDS AND THEIR BIOLOGICAL EFFECTS

Retinoids are analogues of all-trans-retinoic acid (ATRA), an active metabolite of vitamin A (retinol), and are specific modulators of cell proliferation, differentiation, morphogenesis and immunology in vertebrates, as has been extensively reviewed.5–10) The term retinoid is often used loosely, e.g., to include (1) structurally vitamin A-related compounds, including vitamin A and its biological precursor carotenoids,11) (2) all-trans-retinoic acid (a metabolite of vitamin A that activates retinoic acid receptor (RAR)α, β, and γ) and its synthetic analogs that bind to RARs with high affinity in an agonistic (similar biological activities to ATRA) or antagonistic manner; this definition is close to the original definition of Sporn,12) (3) compounds that activate retinoid X receptors (RXRα, β, and γ), which are nuclear receptors structurally and functionally...
related to RARs and activated by 9-cis-RA (9cRA), but distinct from them.12,13 (4) compounds that modify the activities of ATRA by influencing metabolism, biosynthesis or other pathways acting on co-factors or transporters, without binding to RARs or RXRs. We prefer to use the term retinoid in a strict sense, that is, confined to compounds in category (2). It should be noted that the activity of vitamin A is essentially due to RA generated by metabolism in vivo, except for the participation in vision via rhodopsin. Normally, endogenous ATRA is maintained at low nanomolar levels in serum and tissues under strict metabolic control, although much larger (low micromolar) amounts of retinol are found in serum and tissues, while retinyl esters are stored in liver at high micromolar levels.14 9cRA, which activates RARs as ATRA, can be hardly detected in serum and tissues.15 Therefore, its physiological role in vivo is still a subject of controversy. It is important to recognize that vitamin A itself is not active at all.

The endogenous active retinoid, ATRA, equally binds to and activates RARα, β, and γ. It is important to note that ATRA also could bind to and activate RXRs, β, and γ, as does 9cRA16 (Shudo, unpublished). Many synthetic analogs with a variety of structures have been prepared, and often show different receptor selectivity and pharmacological effects from ATRA, including different adverse effects. Some act as antagonists which inhibit the activity of ATRA.17,18

It is now clear that retinoids play a key role in the differentiation and proliferation of various stem cells and progenitor cells, including nerve cells.19 Retinoids initially attracted attention in the field of skin science, because they markedly suppress skin keratinization (an effect that stimulates skin rejuvenation). In 1978, Strickland and Madhvi discovered that retinoic acid stimulates differentiation of embryonic stem cells into various types of cells.20 At approximately the same time, it was reported that retinoids stimulate final differentiation of the acute promyelocytic leukemia (APL) cell line HL60, in which differentiation is blocked midway.21

2. ATRA AND TAMIBAROTENE FOR APL THERAPY

Nearly 50000 papers on retinoids have been indexed in PubMed, most of them concerning ATRA. Clinically, ATRA is extremely effective in the treatment of APL; most patients achieve complete remission.22 Several clinically used synthetic retinoids have advantages over ATRA in terms of receptor selectivity, potency, stability or bioavailability.17,18 Among them, tamibarotene (Am80) is effective in APL patients who experience relapse after ATRA treatment and acquire RA resistance.23-25 Am80 is a receptor subtype-selective retinoid (RARα= RAR/β>RARγ) and is inactive towards RXRs, β, and γ26; this selectivity is practically advantageous from the viewpoints of low adverse effects and high target selectivity. For example, ATRA is highly irritant to skin, in which it activates RARγ27,28 and it also activates RXRs, which in turn activate various other nuclear receptors.

3. PREREQUISITES OF DRUGS FOR TREATMENT OF AD

Candidate drugs for use in the treatment of AD should in principle have the following effects: (1) suppression of subacute accumulation of Aβ, a component of senile plaque of Alzheimer’s patients, which is produced from amyloid precursor protein (APP) by β- and γ-secretases, in the brain of gene-modified animal models, (2) suppression of brain inflammation in animal models generated by intracerebral injection of lipopolysaccharide (LPS) or other agents, (3) stimulation of functional, emotional and histological regeneration in animal models of cholinergic or glutaminergic nerve destruction, etc., as reflected by elevation of acetylcholine (Ach) level, choline acetyltransferase (Chat) activity, etc., (4) improvement of cognition and learning in animal models of impairment, (5) stimulation of nerve regeneration and neurite growth, and (6) tolerability during prolonged use.

During screening of drugs for the treatment of AD, requirements (1), (2) and (3) are evaluated first. If pharmacologically acceptable compounds that satisfy these requirements are discovered, the next requirement (4) is a behavioral pharmacological study. However, the results of such a study depend upon the experimental conditions, choice of animals, method of evaluation and so on, and are not always reliable or reproducible. More importantly, effects observed in experimental animals cannot necessarily be extrapolated to humans. Nevertheless such studies have an important role.

Neuro-regeneration with small molecules is not yet widely accepted as a potential clinical strategy, and alternatives such as cell transplantation and gene therapy are often considered more promising. However, neuro-regeneration with retinoids appears to be feasible.19 The central nervous system contains stem cells whose differentiation undoubtedly involves retinoids. In addition, safety is an absolute requirement, and in this respect, the use of small-molecular drugs may have some advantages.

4. THERAPEUTIC POTENTIAL OF TAMIBAROTENE FOR AD

So far, no compound satisfying all of the aforementioned requirements has been found. Indeed, most drug discovery efforts have been focused on a single target. However, Am80 is intrinsically a multi-target drug and it meets at least some of the above criteria. As it is already in clinical use, there is
a considerable body of safety data, though for the new indication of AD it will be necessary to carry out additional animal studies. First, however, we will review the pharmacological data obtained during the last decade, in order to illustrate the rationale for clinical treatment of AD with Am80.

4.1. Amyloid β Ethemendy et al. reported alleviation of age-related deficit in CA1 long-term potentiation efficacy, and improvement of age-related relational memory deficit by ATRA in vivo. On the other hand, disruption of the retinoid signaling pathway caused deposition of Aβ in the adult rat brain. Accumulation of Aβ in the brain is considered a hallmark of AD, though it may not be a gold standard marker. Even though, reducing Aβ production or aggregation is generally considered to be an important requirement for therapy.

In APP23 transgenic mice with Swedish mutation, prolonged administration of Am80 (0.5 mg/kg/d from 20 to 34 weeks of age) tended to reduce the insoluble Aβ/(40) level in the brain and significantly decreased the level of insoluble Aβ(42), though the soluble Aβ level did not differ from that in control mice. This decrease of insoluble Aβ(42) may be an outcome of retinoid-induced potentiation of either α-secretase transription or phagocytosis by alternatively activated (= phagocytic) microglia (vide infra). Although Am80 had no significant effect on the learning deficit of APP23 mice in the Morris water maze (MWM) test, coadministration of Am80 with a specific RXR agonist HX630, which strongly enhances the biological effects of RARs in the presence of RXR agonist, resulted in a highly significant improvement in the MWM test concomitantly with a decrease of Aβ production (Kawahara, unpublished).

Decreased intracellular and extracellular Aβ production was reported by Jarvis et al. in Tg2576 mice given synthetic retinoid Am580 (1 mg/kg intraperitoneally (i.p.) every 3 d, or 3.6 mg/kg/d fed daily from 3 to 7 months of age), an isomer of Am80, as a selective RARα agonist (this compound also activates RARβ, but not RAR2 or RARs, like Am80). Am580 had similar effects to Am80 on Aβ/(40) and Aβ/(42). Further, i.p. administration of Am580 to Tg2576 mice for 3 to 7 months dramatically increased sAPPα, a product of α-secretase processing, in cortices. Am580 did not influence the production of β- and γ-secretases in cell cultures. Similarly, treatment with ATRA (20 mg/kg, 3 times a week, starting at 5 months age, for 8 weeks) in APP-PS1 double-transgenic mice, which exhibit faster Aβ accumulation and disease progression than Tg2576 mice, caused a marked decrease of Aβ aggregation and improvement of three-dimensional learning and memorization in the MWM test. Decreased activation of non-phagocytic inflammatory microglia and reactive astrocytes was also observed, together with a reduction of tau phosphorylation.

Retinoids, including Am80, increase gene transcription of ADAM10 (a member of the disintegrin and metalloprotease, ADAM, family) and increase the activity of α-secretase, which cleaves a different site of APP protein, precluding Aβ generation and leading to release of the soluble neuroprotective protein sAPPα. Increase or activation of ADAM10 may be effective for reducing toxic Aβ without affecting γ-secretase activity; this is important because γ-secretase participates in Notch signaling, which is essential for the maintenance of mammalian neural stem cells. Administration of Am80 to 13-month-old SAMP8 mice (vide infra) improved the mRNA expression and protein production of ADAM10, which are decreased significantly in hippocampus of these mice.

Recently, Cramer et al. reported that an RXR-selective agonist (so-called “rexinoid”) bexarotene (LG1069, Targettin), which is approved for treatment of cutaneous T cell lymphoma, enhance apolipoprotein E (ApoE)-dependent Aβ clearance from the brain and improve behavioral defects in APP/PS1 mice. Probably, bexarotene also binds to RXR in RAR-RXR heterodimer and enhances RXR signaling as HX630 does.

4.2. Suppression of Inflammation Neuroinflammation is a prominent feature in Alzheimer’s pathology and a potential target for therapy. Anti-inflammatory therapies, particularly nonsteroidal anti-inflammatory drugs, have received considerable experimental and clinical attention.

LPS-interferon (IFN)γ-induced inflammation of central nervous system cells was powerfully suppressed by Am80. The loss of neuronal viability in organotypic midbrain slice cultures was rescued by submicromolar concentrations of Am80. The neuroprotective effect of Am80 on midbrain neuron cells was also observed in an LPS-induced inflammation model in vivo. In that study, gene expression and protein synthesis of brain-derived neurotrophic factor (BDNF) were markedly enhanced, suggesting that the neuroprotective activity of Am80 was mediated at least in part by BDNF. In an intracerebral hemorrhage model in mice, Am80 significantly attenuated neuronal damage in the striatum, and this effect was associated with suppression of activation of microglia/macrophages in the perihematoma region. A similar suppression of inflammatory cell death caused by oxidative stress, nitroxide or other stresses seems likely to occur in other brain areas. Jarvis et al. reported that Am580 significantly suppressed cell death of cultured cortical neurons exposed to 10 μM Aβ. RA also showed evident suppression of inflammation around sites of aggregation.

Experimental autoimmune encephalomyelitis (EAE) is a model that develops inflammation and demyelination in the central nervous system, resembling multiple sclerosis in humans. DA rats immunized with complete Freund’s adjuvant (CFA) supplemented with myelin basic protein developed severe EAE, which reached its peak 12 to 14 d after immunization. Am80 (1 and 3 mg/kg/d) administered orally for 12 d after immunization significantly diminished the clinical symptoms and the infiltration of inflammatory cells in a dose-dependent manner. The mRNA levels of interleukin-6 (IL-6), IFN-γ and tumor necrosis factor-α (TNF-α) in spinal cord were found to parallel the clinical symptoms of the disease in the Am80-treated rats. The expression of IL-6 mRNA was more rapidly and substantially reduced than that of the other two cytokine mRNAs. These findings suggest that the inhibition of EAE is, at least in part, related to the inhibition of IL-6 production.

A similar experiment in B6 mice examined in more detail the immunological effects of Am80 treatment in the EAE model: Am80 (3 mg/kg/d per os (p.o.)) significantly improved the clinical score of the EAE mice. CD4+ effector T cells were classically categorized into two subsets: T helper type 1 (Th1) and type 2 (Th2) cells, and more recent research has delineated a further subset, Th17 cells, which are activated CD4+ T cells characterized by the production of the cytokine IL-17 and involved in chronic inflammation. Am80 inhibited the in vitro Th17 differentiation of splenocytes, and instead
up-regulated Foxp3 expression, a key transcription factor in regulatory T cells. Am80 administration suppressed both the differentiation and the inflammatory function of Th17 cells in vivo.48) These mice have been viewed as a model of multiple sclerosis, but the inflammatory lesions seen in this model (e.g., expression of IL-6, IFNγ and MCP-1) resemble those observed in neurodegenerative diseases, including AD.

4.3. Neural Growth  In addition to the prevention of neural cell death and amelioration of inflammation, retinoids have a regenerative effect on neural cells.49) Goncalves et al.49) reported that differentiation of adult forebrain neural progenitor cells (NPC) into neurons was induced by RA through sequential RARβ and RARα signaling in vivo; RARγ seemed not to be involved. The proliferated embryonic forebrain NPC expressed glial fibrillary acidic protein and were predominantly uni/bipolar, two characteristics of neuronal progenitor cells. RARα agonist stimulated the differentiation of NPC into cholinergic neurons. Neuroblastoma cells NH-12 were differentiated to neuronal cells by Am80 more efficiently than by RA.50) Am80 combined with BDNF promoted extensive neurite outgrowth and increased expression of neurotrophic tyrosine kinase receptor B (TrkB) gene, which encodes the cognate receptor for BDNF, in cultured SH-SY5Y neuroblastoma cells, as did RA. Thymidine incorporation was dramatically suppressed, but there was little effect on cell viability. Thus, the effect of Am80 on spinal cord injury (SCI) in rats as a neural differentiation model was investigated. Treatment with Am80 (orally or subcutaneously) significantly promoted recovery from SCI-induced motor dysfunction. On day 28 after injury, the lesion cavity size was markedly reduced. Interestingly, expression of TrkB was over 3-fold higher after Am80 treatment than in SCI controls. Many TrkB-positive cells, as well as BDNF-positive ones, were observed around the injured site. These findings suggest that Am80 has potential for treating neurodegenerative disorders.51)

4.4. Improvement of Behaviors and Neurotransmission Spontaneous models may have advantages over gene-modified models to explore the etiopathogenesis of aging-related disorders. Seneescence-accelerated mouse strains (SAM), a group of related inbred strains consisting of senescence-prone inbred strains (SAMP) and senescence-resistant inbred strains (SAMR), have been developed by selective inbreeding of AKR/J strain mice donated by the Jackson Laboratory in 1968. The characteristic feature of SAMP is accelerated senescence, whereas SAMR shows normal aging and can be used as a reference. The prone 8 (SAMP8) mice show age-related behavioral deterioration, including deficits in learning and memory and emotional disorders such as reduced anxiety-like behavior.52)

SAMP8 mice exhibit age-related deterioration in sleep-wake architecture compared with SAMR1 mice. Treatment of 9- to 10-month-old SAMP8 mice with Am80 (2 mg/kg fed daily) improved their sleep deficit as evaluated by electroencephalogram (EEG) and electromyogram analyses. One or four weeks of Am80 administration improved the decrease in rapid eye movement (REM) sleep characteristic of SAMP8 mice. Real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis demonstrated an impairment in the hippocampal retinoid cascade genes RARα and transthyretin (TTR) in SAMP8 in comparison to SAMR1 mice. Am80 treatment induced an increase in mRNA expression levels of vesicular acetylcholine transporter (VACtT) in the brainstem and TTR in the hippocampus. Furthermore, the decreased cortical acetylcholine (ACh) content in SAMP8 was improved by Am80 administration. Decreased non-REM sleep and delta oscillation were also observed in SAMP8 mice.53)

It was reported that expression of the RARβ gene seems to determine the contribution of delta oscillation to the sleep EEG patterns in mice.54) Four-week LES40 (RAR antagonist) administration induced a significant attenuation of wakefulness and delta oscillation magnitude in non-rapid eye movement sleep.55) There was also a significant decrease in the expression of dopamine D1 receptor (DIDR) in the striatum and tyrosine hydroxylase in the midbrain of the treated mice. Therefore, the improvement of sleep disorder by Am80 may be induced by activation of the dopaminergic pathway as well as the cholinergic pathway.

Prolonged administration of Am80 to SAMP8 mice significantly suppressed the symptoms of low anxiety, resulting in improvement of the moving latency of the animals in a light-dark box and in open field behavior experiments to the level seen in the control mice (SAMR1). The distance moved, the number of movements from one compartment to another and the number of standing-up motions were all improved.56) Am80 may have a beneficial effect on ambulatory tendency, a symptom seen in patients with AD.

Scopolamine is used in cognitive drug research testing, because it impairs power of attention, continuity of attention, quality of working memory, quality of episodic secondary memory, and speed of memory in a dose- and time-dependent manner, and reversal of scopolamine-induced cognitive impairment is a viable model for identifying proognitive compounds. Donepezil as well as non-cholinergic compounds are shown to be effective in this test.57) The learning disorder caused by scopolamine was improved by concomitant treatment with Am80 in a one-trial, step-through, light-dark passive avoidance paradigm.58)

4.5. Vascular Events Vascular dysfunction is also involved in AD pathology. Cerebral hypoperfusion and impaired Aβ clearance across the blood–brain barrier (BBB) may contribute to the onset and progression of AD. The BBB is comprised of specialized capillary endothelial cells firmly linked via intercellular tight junctions and surrounding pericytes and glial cells. The BBB plays a critical role in Aβ clearance from the brain. The accumulation of Aβ on cerebral blood vessels, known as cerebral amyloid angiopathy (CAA), is a feature of aging and AD.59)

Am58060) and Am80 (Nishikiori and Osanai, unpublished) enhanced expression of glial cell line-derived neurotrophic factor (GDNF), but decreased expression of vascular endothelial growth factor (VEGF)/vascular permeability factor in glial cells of the blood–retinal barrier (BRB) in diabetic patients. These actions normalize the excessive vascular permeability of BRB by modulating expression of tight junction proteins in capillary endothelium. Suppressive action of Am80 on Th17 lymphocytes can be also beneficial for maintenance of BBB function. BBB endothelial cells express IL-17 receptor on their surface and IL-17 disrupts BBB tight junctions.61)

RA and Am80 exert potent antiangiogenic activities in the chicken chorioallantoic membrane (CAM) assay and modulate vascular remodeling.62) Am80 has potent effects on vascular reconstruction after physical and drug injury via inhibition
of transcription factor Kruppel-like factor 5 (KLF5). Microvessels derived from AD patients express or release a myriad of factors implicated in vascular activation and angiogenesis. Extensive cerebral angiogenesis in Tg2576 mouse and AD patients is reported. Sunitinib, a multi-target tyrosine kinase inhibitor (TKI) that predominantly targets VEGF, was reported to improve behaviors and to reduce production of various inflammatory cytokines in Tg2576 mice, and Am80 may act through similar mechanisms.

5. SAFETY ISSUES

Am80 has already been approved for use in the treatment of acute promyelocytic leukemia (APL) in Japan since 2005. A serious adverse reaction in the treatment of APL is differentiation syndrome, formerly known as retinoic acid syndrome, but this adverse reaction to retinoids has been definitively concluded to be associated with the underlying condition of APL. This reaction was not seen among 400 patients during prolonged (over two years) use of Am80 after remission of APL, or in clinical studies of patients with Crohn’s disease and multiple myeloma (unpublished results). In the post-marketing surveillance of adverse reactions, common adverse reactions were found to be reversible TG elevation, bone pain, rash, etc. Overall, there were fewer adverse reactions to Am80 than to RA, which frequently induces skin inflammation. Am80, like other retinoids, is absolutely contraindicated in pregnant women. The clearance time of Am80 from the body is short and Am80 is not accumulated in tissues.

6. CLINICAL STUDY

On the basis of these pharmacological and safety data, a clinical trial (ClinicalTrials.gov. NCT 01120002; JAPICCTI-10115(ja)) of Am80 for Alzheimer’s disease is in progress. The trial is an interventional, randomized, placebo-controlled study, in which patients receive two tamibarotene 2 mg tablets or two placebo tablets once daily. Primary outcome measure is the change in Alzheimer’s disease assessment scale (ADAS-JCog). The current trial location is Osaka City University.
CONCLUSION

Am80, tamibarotene, is a potent multi-target drug that acts on multiple pathways considered to be involved in the etiology and pathophysiology of Alzheimer’s disease, as summarized in Fig. 2. It is therefore considered to be a promising agent for treatment of Alzheimer’s disease, and a clinical trial is under way.

REFERENCES


