Tamibarotene/AD project
-Beyond Aβ-

Pleiotropic action of RARα/β agonist as potential therapeutics for AD

Rationale & Preclinical Data

Research Foundation
ITSUU Laboratory
Number of patients suffering from AD is increasing year by year.
Urgent need for novel disease-modifying therapy for AD.
According to PhRMA report 2012, 81 medicines for AD are under development. Many pharmaceutical companies are focusing on amyloid beta-targeting therapies. Recent several failures of Phase III trials of gamma-secretase inhibitors and Abeta antibodies are questioning whether these approaches are truly appropriate for a complex disease like AD.
Retinoic Acid Receptors RARs

Retinoids exert their various biological actions by binding to RAR and regulating transcription of specific RAR target genes.

- Regulation of cell differentiation and proliferation
- Morphogenesis, vertebrate development and growth
- Immunity

What's next then? Brain!

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 immunity in vertebrates.

Retinoids exert their biological actions by binding to their cognate nuclear retinoic acid receptor RAR, which heterodimerizes with another nuclear receptor RXR, and regulating transcription of specific RAR target genes.

There are three subtypes of each of the RARs and RXRs; RAR\(\alpha,\beta,\gamma\) and RXR\(\alpha,\beta,\gamma\).

Now, what's next?

“Adult brain” is next frontier for basic and clinical biology of retinoid.
Retinoids in Clinical Use

Natural Retinoic Acid and mimics

- **ATRA**
  - Pan-RAR (& RXR)
  - acne, APL

- **Acitretin**
  - Pan-RAR
  - psoriasis

- **13-cis-RA**
  - Prodrug
  - Isomerize to ATRA or 9cRA
  - acne

- **9-cis-RA**
  - Pan-RAR&RXR
  - Kaposi’s sarcoma (topical)

Synthetic Retinoids

- **Tamibarotene Am80**
  - RARα,β-specific
  - APL (Japan)

- **Tazarotene**
  - RARβ,γ-specific
  - acne, psoriasis (topical)

- **Adapalene**
  - RARβ,γ-specific
  - acne (topical)

- **Bexarotene LG1069**
  - Pan-RXR
  - cutaneous T-cell lymphoma

This is a list of retinoid in clinical use.

These retinoids are used for cancer (mainly, APL) and dermatological diseases.

It is remarkable that our Am80 is the only RARα/β-specific agonist among them.
Why tamibarotene could be effective for AD?

Beyond Amyloid!!

Pleiotropic action of RARα/β agonist as potential therapeutics for AD

1. Decrease Amyloid β Accumulation
2. Improve Neurotransmission
3. Suppress Neuroinflammation
4. Enhance Neural Outgrowth/Regeneration
5. Maintain BBB and Suppress Angiogenesis

Now, we believe that tamibarotene should be pleiotropically effective for AD. We have accumulated considerable preclinical evidences with help from some collaborators for several years.

Fortunately, many other supporting evidences have been reported from other investigators.

There are 5 key actions of tamibatotene as a therapeutic for AD listed here.
This illustration summarizes pleiotropic action of tamibarotene (Am80).
Synthetic Retinoid Tamibarotene (Am80)

- RARα/β-specific retinoid
- Approved for refractory acute promyelocytic leukemia (APL) in Japan, 2005
- Clinical Trials ongoing in USA, EU and China for APL and other diseases.
- Not cytotoxic. Tolerable for long term use.

In case of APL therapy, high dose of retinoid is necessary to induce differentiation of APL cells whose phenotype is tightly linked to chromosomal translocation involving RARα gene (PML-RARα).

Tamibarotene (Am80) has been approved for therapy of APL in Japan since 2005. Tamibarotene is also effective in patients with ATRA-resistant APL. Tambarotene is not a cytotoxic anticancer agent but a differentiation-inducing agent. In case of APL therapy, high dose of retinoid is necessary to induce differentiation of APL cells whose phenotype is tightly linked to chromosomal translocation involving RARα gene (PML-RARα).

Tamibarotene will be effective at lower doses for AD than those used for APL, and tolerable enough for long term use in AD therapy.
Pleiotropic action of RARα/β agonist as potential therapeutics for AD

1. Decrease Amyloid β Accumulation

Transcriptional induction of ADAM10, the physiologically relevant α-secretase. Induction of phagocytic microglia cells.

<Key action 1>
Tamibarotene could decrease cerebral accumulation of amyloid beta. Because, production of amyloid beta could be reduced by induction of alpha-secretase, and cerebral clearance of amyloid beta could be accelerated by induction of phagocytic microglia.
Retinoids decrease Aβ in the brain

• Vitamine A deficiency leads to Aβ accumulation and memory defects
  Jonathan (2004); Misner (2001); Cocco (2002) …

• ATRA administration to APP/PS1 mice reduced Aβ accumulation and tau hyperphosphorylation, and attenuated spatial memory defect
  Ding (2008)

• Retinoid induce ADAM10 (α-secretase)
  Tippmann (2009)
  Jarvis (2010) shown by Am580 in tg2576 mice
  Donmez (2010) shown in APP/PS1 mice

Vitamine A deficiency leads to Abeta accumulation. Jonathan, Maden 2004
EurJNeurosci 20: 896

ATRA administration reduced Abeta and P-tau, and attenuate memory defects in APP/PS1 transgenic mice. Ding (2008); next page.

Synthetic retinoids also reduced Abeta in APP model mice, probably because of ADAM10 induction.

A longevity gene Sirt1 may be involved (Prof. Guarente group, MIT).
ATRA decreased Aβ accumulation, suppressed activation of astrocytes & microglia cells and attenuated spatial memory defect in APP/PS1 mice

Data from Ding’s report in 2008.
ATRA administration reduced cerebral Abeta deposits and attenuated memory defects. Decrease of microglia suggests alleviation of neuroinflammation.
Am80 decreased brain Aβ in APP23 mice

Kawahara (2009)
Biol Pharm Bull
32: 1307

APP23 mice
male, 20 weeks

control (n=13)

Am80 (n=13)

0.5 mg/kg/day, oral, for 14 weeks

Data reported by Dr. Kawahara, one of our collaborator, Kumamoto Univ., in 2009. Oral administration of Am80 significantly reduced insoluble fraction of Abeta42.
Am80 with HX630 improves cognitive defects in APP23 mice

Kawahara (unpublished)

Oral administration of Am80 along with our RXR agonist HX630 attenuated memory defects in APP23 transgenic mice.

*HX630 is a RXR agonist developed by Kagechika & Shudo

Data personally reported by Dr. Kawahara.

Oral administration of Am80 along with our RXR agonist HX630 attenuated memory defects in APP23 transgenic mice.
Amyloid β inhibits retinoic acid synthesis exacerbating Alzheimer disease pathology which can be attenuated by an retinoic acid receptor α agonist

Data reported from Prof. Corcoran’s group of King’s College London, UK. They used Am580, which has been originally developed by Kagechika & Shudo. Am580 reduced Abeta plaques and Tau phosphorylation in tg2576 mice.


Related patent: “Therapeutic Aryl-Amido-Aryl Compounds and Their Use” US Application 2012/0149737

King’s College London, the Wellcome Trust and Advent Venture Partners have announced the formation of a new UK biotechnology company, CoCo Therapeutics Ltd, to progress these Prof. Corcoran’s research into the development of RARa agonists for AD in March 2013.
Both of Am80 and Am580 were originally developed by Kagechika & Shudo in 80’s

- selectivity -

ATRA pan-RAR/RXR agonist
ATRA binds to and activates all subtypes of RAR and also RXR

Am80 RARα > RARβ >> RARγ, RXRs

Am580 same as Am80

The amide bond is, just only, inverted!
Potency and selectivity of Am580 are almost same as those of Am80

Both of Am80 and Am580 were our (Kagechika & Shudo’s) compounds.
2. Improve Neurotransmission

- Improve cholinergic pathway
- Increase dopaminergic transmission
- Effects on LTP/LDP

<Key action 2>
Tamibarotene could improve neurotransmission.

Because,
retinoids induce vesicular ACh transporter (VACHT), which is a responsible gene for ACh storage, and increase cerebral Ach level,
and dopamine receptor D2R gene is a RAR-target gene.
RARs are also suggested to be involved in hippocampal LTP (long term potentiation) and/or LTD (long term depression).
In this scenario, tamibarotene can also work as symptomatic treatment.
RAR signaling is involved in spatial learning and memory.

Morris Water Maze

RARβ/ RXRγ KO
RARβ KO
RXRγ KO
Wild

RARβ/RXRγ knockout mice show deficits in spatial learning and memory.

Data from Chiang’s report in 1998 (Ron Evans’s group).
RARβ/RXRg knockout mice show deficits in spatial learning and memory.
Why RARβ? It is still mystery.
Related reports.
Misner (2001) PNAS 98: 11714-9 <PMID 11553775>
Nomoto (2012) Mol Brain <PMID 22316320>
Am80 rescues Scopolamine-induced Memory deficit

**SAMP8 mice**

**model of age-related defects in learning/memory**

- Senescence-accelerated mouse (SAM) lines were developed for studies of age-related disorders in Japan.
- SAMP8 line is useful for studies of age-related deterioration in learning and memory abilities.
- SAMP8 shows an emotional disorder characterized by reduced anxiety-like behaviors, sleep defect, circadian rhythm disorders, etc.

Aging is the most prominent risk factor for AD.  
*SAMP8 mouse is a good model for age-related memory defects.*  
*The senescence-accelerated prone (SAMP) mouse model of accelerated aging was established through phenotypic selection (Takeda, 1999). SAMP8 (P8), a substrain of SAMP, is known to exhibit an age-related deterioration in learning and memory abilities compared with normal aging mice (SAMR1; R1) (Flood and Morley, 1998). In addition, P8 is known to show an age-related increase of amyloid beta protein (Aβ), amyloid precursor protein (APP) and relative gene expression (Kumar et al., 2000a, Morley et al., 2000, Nomura and Okuma, 1999). These findings suggest that P8 is an appropriate rodent model for age-related cognitive deficits such as those observed in Alzheimer’s disease (AD) (Butterfield and Poon, 2005, Morley, 2002).*
Am80 Improves reduced anxiety-like behaviors of SAMP8 mice

Representative movement tracks in open field tests are shown. The distance moved by the SAMR1 group (accelerated aging) increased compared to the SAMR1 group (normal aging). Am80 normalized this abnormal movement of SAMP8 mice.

The movement distance and rearing actions in SAMP1 mice increased compared to SAMRI1 mice.

SAMP8 mice shows anti-anxiety behavior and low exploratory behavior.

Am80 normalized these abnormalities of SAMP8 mice.
Effects of Am80 treatment on the light/dark box test endpoints of latency of the first exit (A), time spent in the dark box (B), and the number of transitions (C).
AD patients often have problems with sleeping. These are data reported by Dr. Kitaoka, one of our collaborators, Univ. Tokushima, in 2010.

• Am80 improved the decrease in REM sleep in SAMP8 mice
• Am80 improved decreased cortical ACh in SAMP8 mice.
• In SAMP8 mice, hippocampal expression of RARa and TTR decreased.
• Am80 restored reduced cerebral ACh contents in SAMP8 mice, probably increasing VACHT expression.

*TTR inhibits Abeta aggregation by its association to Abeta in monomeric form. It is also possible that TTR facilitates Abeta degradation directly or indirectly, transports of Abeta from CNS into serum. TTR may also inhibit Abeta production by inhibition of gamma secretase cleavage (reviewed by Li, PMID:22112803).
Am80 recovers ADAM10 in aged SAMP8 mice


These are additional data reported by Dr. Kitaoka, in 2013.
Oral administration of Am80 significantly improved reduced hippocampal expression of ADAM10 in aged SAMP8 mice.
**Pleiotropic action of RARα/β agonist as potential therapeutics for AD**

3. Suppress Neuroinflammation

Suppress inflammatory microglia

Regulation of Th17/Treg balance

*<Key action 3>*

**Tamibarotene could suppress neuroinflammation in AD patients.**

*Neuroinflammation is now recognized as a prominent feature in Alzheimer's pathology and a potential target for therapy.*

*One of its hallmarks is activation of microglia cells.*

**Tamibarotene suppresses inflammatory microglia and reduces various inflammatory cytokines.**

**Tamibarotene could regulate balance between inflammatory Th17 cells and regulatory T cells.**
Immunoregulatory actions of retinoids

Involvement of vitamin A (retinol) in immunity have been recognized since its discovery early in 20th century.

Even though, its detailed cellular and molecular mechanism begins to be elucidated in the past several years.

The effects of retinoids on T cell development are especially attracting great attentions.

In classical Th1/Th2 paradigm, retinoid seems to prefer Th2-bias.

In novel Th17/Treg paradigm, retinoid prefers Treg-bias, i.e. immune tolerance rather than autoimmunity.

With regard to microglia cells, retinoid suppresses inflammatory M1 cells and induces phagocytic microglia M2 cells.
**Am80 suppress neuroinflammation in EAE mice**

3 mg/kg AM80, orally every other day.  
spinal cords on Day 15


Data from Klemann’s report in 2009  
(Our collaborator Prof. Yamamura’s group, National Center of Neurology and Psychiatry, Japan).

Am80 was orally administered into EAE mice, a model for multiple sclerosis.  
Neuroinflammation in EAE mice was suppressed by Am80.  
Expression of brain Th17 and IL23R was reduced.  
In spinal cord, amount of IL-6 was significantly decreased by Am80.
Inhibition of IL-12/IL-23 signaling reduces Alzheimer’s disease–like pathology and cognitive decline

Johannes vom Berg¹, Stefan Prokop²,³, Kelly R Miller⁴, Juliane Obst³, Roland E Källin³, Ileana Lopategui-Cabezas⁵,⁶, Anja Wegner⁷, Florian Mair¹, Carola G Schipke⁵,⁶, Oliver Peters⁷, York Winter⁴, Burkhard Becher¹,² & Frank L Heppner⁵,⁷

Recently, vom Berg reported that genetic deletion of IL-12 and/or IL-23 subunits reduces Abeta plaques load in APPPS1 mice. This suggested that involvement of inflammatory microglia cells in AD-like pathology in APP mice.
Am80 suppresses microglia activation and neural damage induced by intracerebral hemorrhage
collagen-induced ICH mice


Data from another our collaborator Matsushita’s report in 2011 & 2012
(Prof. Katsuki’s group, Univ. Kumamoto, Japan).
Am80 was orally administered into murine intracerebral hemorrhage model induced by collagenase.
Daily oral administration of Am80 (5 mg/kg) starting from 1 day before or from up to 6 hours after intrastriatal injection of collagenase Prominent expression of RARα was observed in activated microglia.
The number of activated microglia in the perihematoma region was lower in Am80-treated mice.
Am80 treatment also reduced areas affected by hemorrhage-associated oxidative stress and attenuated heme oxygenase-1 expression in activated microglia.
Am80 10 mg/kg p.o. for 5 days before LPS injection into the midbrain substantia nigra of male C57BL/6 mice.

**Increased BDNF expression by Am80 should be involved.**

Another data of Dr. Katsuki in 2009

Am80 protected dopaminergic neurons in substantia nigra from LPS-injection (in vivo).

Am80 protected dopaminergic neurons in rat midbrain slice culture from injury mediated by lipopolysaccharide-activated microglia (ex vivo).

Neuronal expression of RARα and RARβ in midbrain slice culture.

Am80 increased tissue level of brain-derived neurotrophic factor (BDNF) mRNA.
**Pleiotropic action of RARα/β agonist as potential therapeutics for AD**

4. Enhance Neural Outgrowth/Regeneration

Neural outgrowth
Neural stem cells in adult brain
Neurotrophic and neuroprotective effects

<Key action 4>

Tamibarotene could enhance neural outgrowth and adult neurogenesis.

Tamibarotene has neurotrophic and neuroprotective effects, probably via BDNF/TrkB pathway.
ATRA can be involved in adult neurogenesis.

Vitamin A deficiency causes reduced hippocampal neurogenesis, which is reversed by ATRA.
They suggested that RARβ is involved in neural outgrowth and neurogenesis in spinal cord injury (SCI) model.
Am80 improves spinal cord injury-Induced hindlimb motor dysfunction in rats

This is data of our collaborator Prof. Takenaga.
In rat SCI model, Am80 improved hindlimb motor function.
Spinal expression of TrkB (receptor for BDNF) increased by Am80.
Pleiotropic action of RARα/β agonist as potential therapeutics for AD

5. Maintain BBB and Suppress Angiogenesis

<Key action 5>

Tamibarotene could enhance neural outgrowth and suppress vascular remodeling and angiogenesis.

Neurovascular dysfunction could have a significant role in the pathogenesis of AD.

Normal function of blood-brain barrier (BBB) may be important for the clearance of cerebral Abeta.

The accumulation of Abeta on cerebral blood vessels, known as cerebral amyloid angiopathy (CAA), is a feature of aging and AD.

Vascular disorders may be important features in chronic neurodegeneration in AD.

And, many data suggest an association between cardiovascular diseases, such as atherosclerosis, and AD.

Am80 and Am580 normalize the excessive vascular permeability of blood–retinal barrier (BRB) by modulating expression of tight junction proteins in capillary endothelium (Nishikiori, 2007). Architecture of BRB is similar as that of BBB.

Suppressive action of Am80 on Th17 can be also beneficial for maintenance of BBB function., because BBB endothelial cells express IL-17 receptor on their surface and IL-17 disrupts BBB tight junctions.

Extensive cerebral angiogenesis in Tg2576 mouse and AD patients is reported. Am80 is anti-angiogenic in vitro and in vivo.

Suppressive effect of Am80 on KLF5, which is involved in vascular remodeling, in vascular smooth muscle cells may also be beneficial (Fujii 2005, Circ Res). Am80 reduces atherosclerosis in apolipoprotein E-knockout mice (Takeda 2004, Circ J).
Pleiotropic action of tamibarotene as potential therapeutics for AD

From Bench to Bedside

Now, “from bench to bedside”.
**Tamibarotene/AD Study Design**

*Phase Ila (placebo-controlled double blind)*
4 mg, once daily, 24 weeks  
Total Enrollment: 50 (tamibarotene:placebo 2:1, donepezil add-on)  
Locations: Osaka City University Hospital and others, Japan  
Period: From May 2010 to December 2012 (extended)  
ClinicalTrial.gov NCT01120002 Japic CTI-101115

**Endpoints**
- ADAS-JCog [0,12, 24w]
- MMSE, ADCS-ADL, CIBIC-Plus [0,12,24w]

**Inclusion Criteria**
- mild to moderate AD <MMSE 10 – 26>  
- Age from 55 to 80 (for women, menopause ≥ 2 years)  
- Treated with a stable dose of donepezil and willing to continue the same during the trial period

**Exclusion Criteria**
- Any cause of dementia not due to Alzheimer’s disease  
- Triglyceride > 400 mg/dL

*Design of Tamibarotene/AD study in progress*
Research Foundation ITSUU Laboratory

- established in 1915
- Address: 2-28-10 Tamagawa, Setagaya-ku, Tokyo 158-0094, Japan
- President and CEO: Koichi Shudo, Ph.D.
- Research Activities: mainly in Chemistry, but also in Biology & Pharmacology
- No. of employees: 7

Founder
Dr. H. Kondo

Thank you very much for your interests