Tamoxifen affects an autoimmune model, but not a viral model, of multiple sclerosis

Sandesh Rimal, MS
niensandesh@gmail.com

Sandesh Rimal[1], Sundar Khadka[1,2], Fumitaka Sato[1], Seiichi Omura[1], Ijaz Ahmad[1], Ah-Mee Park[1], Cong Thanh Nguyen[1], Reona Shiro[1], Kota Moriguchi[1,3], and Ikuo Tsunoda[1]

[1]Kindai University , Osaka, Japan, [2]Duke University, North Carolina, USA, and [3]Japan Self Defense Forces Hanshin Hospital, Hyogo, Japan

PDF poster

KINDAI UNIVERSITY

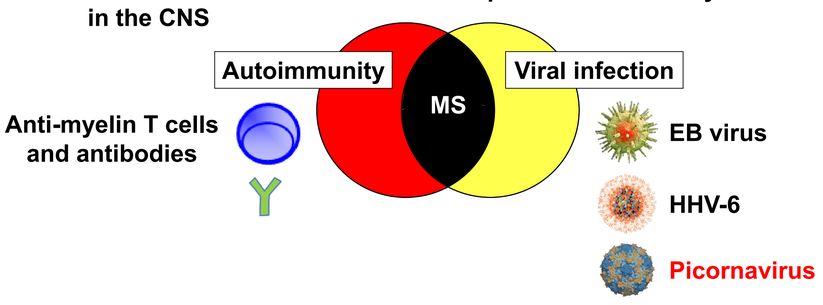
Abstract

[Background] Although the precise cause of multiple sclerosis (MS) is not clear, autoimmune responses and viral infections have been suggested to play crucial roles in MS pathogenesis. Estrogen has been reported to be protective in human MS and its autoimmune model, experimental autoimmune encephalomyelitis (EAE), by modulating T helper (Th)1 / Th17 versus Th2 responses. Tamoxifen (TAM) is an estrogen receptor antagonist used commonly to treat estrogen receptor-positive breast cancer. Epidemiologically, breast cancer incidence was greater in MS patients, highlighting the need to examine the safety of TAM in MS. Since TAM has also been reported to suppress various viral infections and modulates immune responses, TAM may modulate neuroinflammation caused by viral etiology or autoimmunity. Theiler's murine encephalomyelitis virus (TMEV) infection in mice is a viral model of MS, inducing inflammatory demyelination 1-month post infection. Thus, we investigated whether TAM could affect an autoimmune (EAE) and the TMEV models. **[Methods]** SJL/J mice were sensitized subcutaneously with $PLP_{139-151}$ peptide and treated with TAM either before (days –11 to –7; prophylactic group) or after (days 10 to 14; therapeutic group) EAE induction. For TMEV induction, mice were inoculated with TMEV intracerebrally and treated with TAM either before (days –11 to –7; prophylactic group) or after (days 17 to 21; therapeutic group) TMEV infection. Control mice received vehicle. We monitored neurological signs and body weight daily for two-three months. After the observation period, we killed mice and analyzed the central nervous system (CNS) tissues for neuropathology, viral persistence, and T-cell infiltration. We used spleen cells for cytokine and anti-TMEV lymphoproliferative assays and sera for anti-TMEV antibodies. **[Results]** Prophylactic TAM treatment exacerbated EAE; therapeutic treatment suppressed EAE. TAM treatment also increased anti-PLP cellular immunity. In TMEV infection, all mice developed inflammatory demyelination in the CNS. Both prophylactic and therapeutic groups had no effects on neuropathology, viral persistence, or CD3+ T-cell infiltration. The levels of TMEV-specific lymphoproliferative response, cytokines [interferon (IFN)-γ, interleukin (IL)-4, IL-10, and IL-17], and anti-TMEV IgG2c and IgG1 antibodies were also comparable among the control and two treatment TAM groups. **[Conclusions]** TAM treatment had contrasting effects depending upon treatment schedule. On the other hand, TAM treatment did not affect chronic inflammatory demyelination in the Theiler's virus model. Therefore, therapeutic TAM treatment could be safe for use in breast cancer patients with MS.

Background

Multiple sclerosis (MS)

- Immune-mediated disease characterized by inflammatory demyelination in the central nervous system (CNS)
- Effector: T helper (Th) 1 / Th17 cells and antibodies
- Higher incidence of breast cancer in multiple sclerosis patients
- Immuno-modulation suppresses disease activity
  - Disease-modifying drugs are effective
  - Pregnancy and estrogen improve MS
- Viral etiology of MS
  - Epstein–Barr (EB) virus and human herpesvirus 6 (HHV-6)
  - Animal model: Theiler's virus model
- Virus-induced demyelination
  - Direct viral infection of oligodendrocytes, myelin-forming cells, causes demyelination
  - Uncontrolled anti-viral immune responses cause demyelination in the CNS



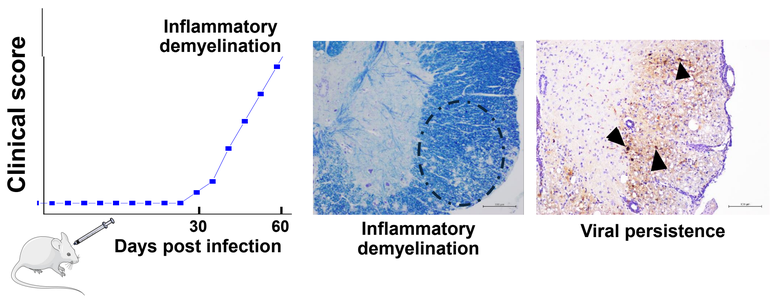
Experimental autoimmune encephalomyelitis (EAE)

- Most widely used as an autoimmune model of MS
- Induced by sensitization with myelin proteins (myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP)) emulsified in complete Freund's adjuvant
  - $CD4^+$ T-cell-mediated immune response against the CNS
- EAE animals develop ascending paralysis with inflammation, demyelination, and axonal degeneration similar to MS.

Myelin antigens → T-cell activation → CNS infiltration → Inflammation & cytokines → Demyelination → Paralysis

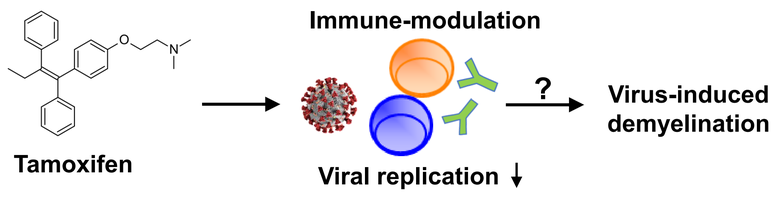
Theiler's virus

- Non-enveloped, positive-sense, single-stranded RNA virus belonging to the family *Picornaviridae*
- Induces inflammatory demyelination 1-month post infection with persistent viral infection in the CNS
- Previously, we detected increases in estrogen-related genes: *Esrrb, Esrrg, Greb1,* and *Esr1* (Omura et al., 2019).



Tamoxifen

- Estrogen receptor antagonist, use for treatment of estrogen receptor-positive breast cancer
- Used for the Cre/loxP system to control gene expression or gene knockout in a time- and tissue-specific manner
- Modulates immune responses
- Suppress various viral infections (e.g. SARS-CoV-2, HIV, Zika virus)



Aim

- Is tamoxifen safe for MS patients with breast cancer?

"To assess the effects of tamoxifen on two MS models"

Methods

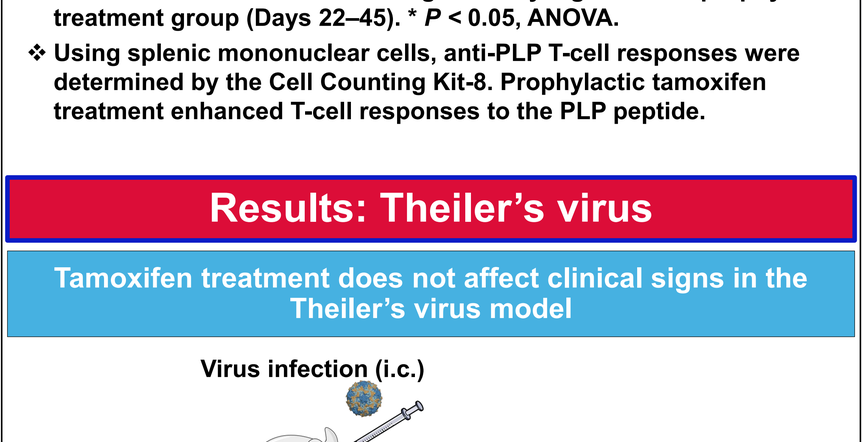
- Two MS models: EAE and Theiler's virus infection
  - SJL/J mice were sensitized subcutaneously (s.c.) with the myelin proteolipid protein $(PLP)_{139-151}$ peptide for EAE induction.
  - SJL/J mice were inoculated intracerebrally (i.c.) with 2×10^5 PFUs of Theiler's virus.
  - We treated mice intraperitoneally (i.p.) with tamoxifen for five consecutive days before (prophylactic) or after (therapeutic) induction of MS models.
  - We monitored clinical signs for 2–4 months.

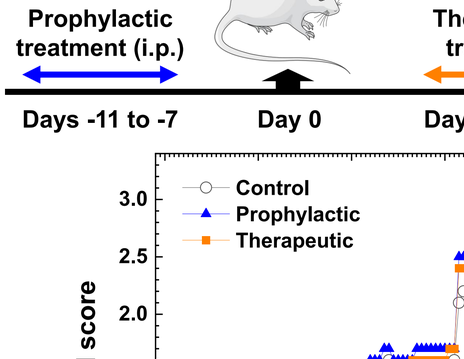
- Clinical score
  - Clinical scores of EAE were evaluated as: 0, no signs; 1, tail paralysis; 2, mild hind limb paresis; 3, moderate hind limb paralysis; and 4, complete hind limb paraplegia.
  - Clinical scores of the Theiler's virus model were evaluated by impairment of righting reflexes.

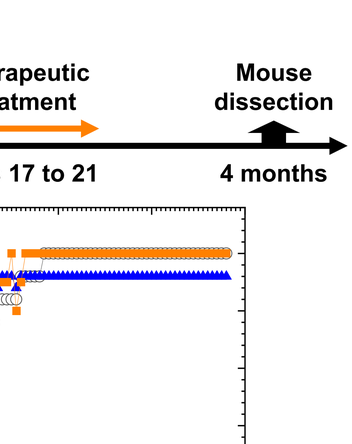
- Sample collection and analysis
  - Spleen: lymphoproliferative assay, cytokine ELISAs
  - CNS tissue: demyelination (Luxol fast blue stain) and viral antigens / $CD3^+$ T-cells (Immunohistochemistry)
  - Serum: anti-viral antibody ELISAs

Results: EAE

Tamoxifen treatment has contrasting effects in PLP-EAE depending on the timing



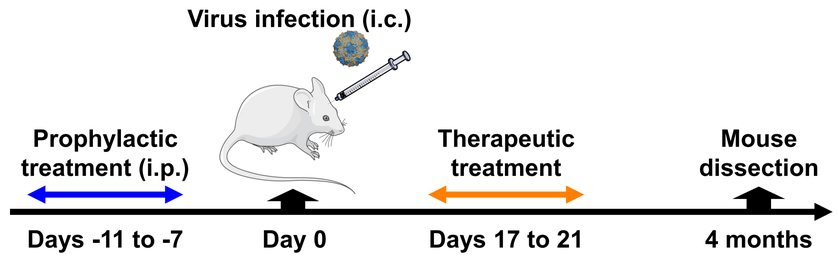




- Clinical signs were evaluated by EAE scoring system. Although prophylactic tamoxifen treatment exacerbated EAE, therapeutic tamoxifen treatment suppressed EAE. * $P < 0.05$, Kruskal-Wallis test.
- The area under the curve was significantly higher in the prophylactic treatment group (Days 22–45). * $P < 0.05$, ANOVA.
- Using splenic mononuclear cells, anti-PLP T-cell responses were determined by the Cell Counting Kit-8. Prophylactic tamoxifen treatment enhanced T-cell responses to the PLP peptide.

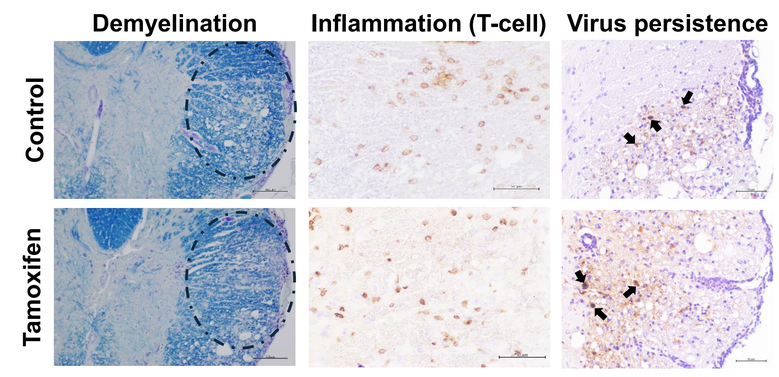
Results: Theiler's virus

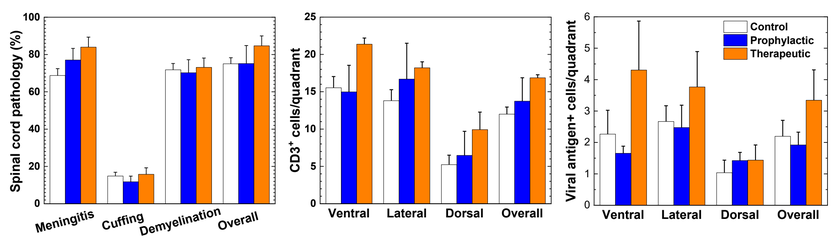
Tamoxifen treatment does not affect clinical signs in the Theiler's virus model



- Clinical signs were evaluated by impaired righting reflexes. Both prophylactic and therapeutic treatment did not affect clinical signs in the Theiler's virus model.

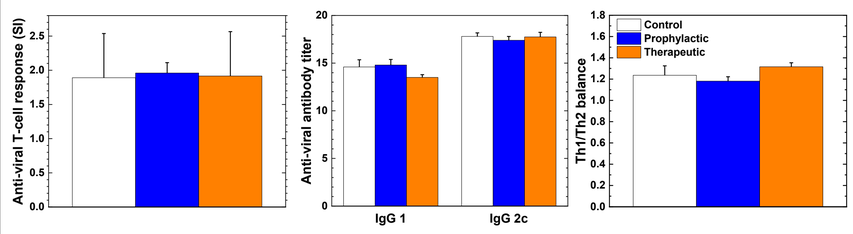
Tamoxifen treatment does not affect demyelination, inflammation, or viral persistence





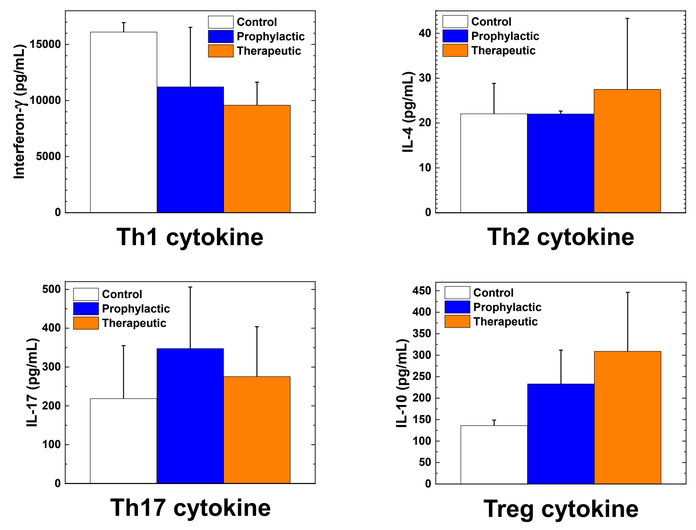
- Using Luxol fast blue stain and immunohistochemistry against $CD3^+$ T cells and Theiler's virus antigen, we found no statistical differences in neuropathology among the three groups.

Tamoxifen treatment does not alter anti-viral T-cell or IgG isotype responses



- Using splenic mononuclear cells, anti-viral T-cell responses were determined by the Cell Counting Kit-8. Tamoxifen treatment did not alter T-cell responses to Theiler's virus.
- Anti-viral antibody titers were quantified by ELISAs. The Th1/Th2 balance was examined as a ratio of IgG2c to IgG1 titers. Tamoxifen treatment did not affect anti-viral IgG1 or IgG2c responses in serum.

Tamoxifen treatment does not change Th cytokine productions significantly



- Splenic mononuclear cells were stimulated with a mitogen, concanavalin A. Four Th-related cytokines [interferon (IFN)-γ, interleukin (IL)-4, IL-10, and IL-17] in the culture supernatants were quantified by ELISAs. Tamoxifen treatment increased IL-17 and IL-10 productions; it decreased IFN-γ levels, although there were no statistical differences.

Conclusions

- Tamoxifen treatment had contrasting effects in EAE depending on the timing of administration. Prophylactic treatment exacerbated EAE; therapeutic treatment suppressed EAE.
- Tamoxifen treatment enhanced anti-PLP cellular immunity in the EAE model.
- Tamoxifen treatment did not affect the Theiler's virus model clinically and histologically.
- Tamoxifen treatment did not alter anti-viral cellular immunity, anti-viral humoral immunity, or cytokine productions in the Theiler's virus model.
- Therapeutic tamoxifen could be safe for use in breast cancer patients with MS.

References

1. Ahmad I. and Rimal S. et al., Gut microbiota in a viral model of multiple sclerosis: modulation and pitfalls by oral antibiotic treatment. 2025, *Cells*. 9;14(12):871.
2. Ahmad I. and Rimal S. et al., The safety of Alcaligenes lipid A in an immune-mediated disease model associated with IgA, Th17 cells, and gut microbiota. 2025, *Int J Mol Sci*. (in press).
3. Sato F. et al., Animal models of multiple sclerosis. 2018, *Neuroinflammation*. pp 37-72, Elsevier inc., Amsterdam.
4. Omura S. et al., Bioinformatics analyses determined the distinct CNS and peripheral surrogate biomarker candidates between two mouse models for progressive multiple sclerosis. 2019, *Front Immunol*. 19;10:516.

Acknowledgements

- The authors have no financial relationships to disclose
- Monbukagakusho (MEXT) Scholarship (S.R.), Ministry of Education, Culture, Sports, Science and Technology, Japan