

Virobiome Derived Peptide T: Anti-Inflammatory Peptides for Treating Neuro-Aids and Neurodegenerative Diseases

Abstract

The identification of biologically significant, receptor-targeting epitopes from the “virobiome”, the diverse population of viruses which engage the host immune system, especially those determinants which may control host immunity or be viral entry receptor binding sites, is an important goal for the development of anti-viral drugs, vaccines, and immunomodifying therapies. We [1] and others [2] have observed that numerous viruses, including members of the herpesvirus, poxvirus, and lentivirus families encode peptides that block innate immunity, presumably to help them overcome immune surveillance. A virus, especially a lentivirus, which is well adapted to exist in a nuanced balance within the complete, natural, and physiological host immune system, would be able, over millions of iterations (viral replication cycles), to perfect an escape from immune surveillance by modulating the entire innate immune network. Identifying those innate immune modifying peptide epitopes provides a rational basis for drug development. Contrast this to a typical “pharma” screening approach that a priori seeks to identify a “pure” or specific receptor target, to then be tested in artificial systems, yielding few hits, which typically are of low potency. These types of screens are most suited for detecting either agonist or antagonist activity. However, a more desirable pharmacologic feature, which the virobiome might provide, is partial or mixed agonist/antagonist activity, as this is most suited to provide a balanced modulation of immunity, and avoids substantial suppression of what are certainly useful host immune function(s). Small peptides derived from the HIV envelope protein have been identified that block HIV entry at chemokine receptors, protect neurons, and which antagonize cytokine, chemokine, and TLR/MyD88 inflammatory pathways. This has allowed creation of orally active, potent, peptides to provide treatments in diverse conditions with an underlying inflammatory pathogenesis such as psoriasis, Neuro-AIDS, neuropathies, and Alzheimer’s disease (AD), to name a few examples.

Keywords: Virobiome; Peptide T; Neuro-AIDS; Neurodegeneration; Neuroinflammation; Chemokine antagonist; Oral peptide

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Abbreviations: DAPTA: D-Ala1-Peptide T-Amide; VIP: Vasoactive Intestinal Peptide; PACAP: Pituitary Adenylate Cyclase-Activating Polypeptide; GHRH: Growth Hormone Releasing Hormone; HIV: Human Immunodeficiency Virus; MMSE: Mini-Mental State Examination; FDG: Fluorodeoxyglucose; DBPC: Double-Blind Placeno Controlled; HAART: Highly Active Antiretroviral Therapy

Introduction

We have exploited the HIV virobiome, specifically the envelope protein gp120, to identify highly potent receptor-active peptides that act as functional antagonists of multiple chemokine receptors. This was accomplished by searching the gp120 sequence for small local homologies to known signaling peptides, such as neuropeptides of the VIP/PACAP/GHRH family [1, 3-5].

We identified a discreet octapeptide domain (peptide T site) near the V2 stem of gp120 [1] and related peptides derived from these sequences that preferentially block infection of R5-tropic

HIV isolates [6-8]. Our initial studies used an early passage patient isolate, later shown to have an R5/X4 (dual-tropic) receptor phenotype, although none of the chemokine receptors, nor the significance of chemokine receptor utilization, were known at the time of this work (circa 1985). The predominant antiviral effect for R5 vs X4 HIV isolates explains the early controversy related to lack of antiviral effects with X4 lab adapted isolates [9].

While peptide T was the first receptor targeted anti-viral for HIV (this class came to be called “entry-inhibitors”), as the AIDS epidemic expanded in the mid-1980’s it became evident that the virus, while establishing infection in the CNS, did not replicate in neurons. Nevertheless, profound cognitive and motor deficits were being reported, adding to the great stigma and suffering this disease carried at the time. In our initial reports [1] we noted a cortical distribution of gp120 binding to receptors in primate brain and hypothesized that gp120 killing of susceptible neurons would explain the observed patient cognitive deficits. We proposed that peptide T, by blocking gp120 binding [1], later shown to be at

CCR5 [8], would be efficacious in preventing neuronal loss in HIV infection, now known to occur very early after infection.

Benefits in neuroinflammation and neurodegeneration

Initial studies that showed peptide T, and its degradation resistant analog D-ala1-peptide T-amide (DAPTA), was protective to the neurotoxic effects of gp120 in neuronal cultures [10-13], in part by releasing protective chemokines [14]. The neuro-protective effects of DAPTA were also shown in animals treated with gp120, where the loss of synapses and dendritic arbor, along with behavioral delays, were reversed [15]. The neurotoxic effects of gp120 were therefore shown to be a cause of cortical neuronal loss and dendritic pathologies [16,17] in neuro-AIDS. The mechanisms of gp120 neurotoxicity were gradually revealed by us and others to be indirect, and related to activation of microglia by gp120 [18,19], effects which are blocked by DAPTA [20]. Activated microglia and astrocytes are now well appreciated to be mediators in diverse neuropathologies, which peptide T analogs can treat.

Although the anti-viral actions of these peptides was the initial subject of investigation, it gradually became clear that the peptide T family of peptides had useful immunomodulating [14,21-24] and neuronal sparing [14,25] effects beyond HIV that could be exploited therapeutically, especially in neurodegenerative conditions with an inflammatory pathogenesis. DAPTA lowered M1 cytokines IL-1, IL-6, IL-8, IL-23, TNF α , and enhanced M2 cytokines such as IL-4 and IL-10 [26]. Some examples of possible DAPTA treatment benefits include Alzheimer's disease [20,25], neuropathies of diverse origin [27-29], cancer pain [30], excitotoxicity [31] and stroke/cerebral ischemia [32]. Clinical benefits have been shown for neuro-AIDS (below), suppression of growth hormone [5], a cause of developmental delays in pediatric HIV, which DAPTA has restored [33], as well as in non-HIV related skin conditions like psoriasis [34,35].

Clinical trial results in neuro-aids

Peptide T, or more correctly DAPTA, entered human clinical trials for neuro-AIDS endpoints in 1986. Improvements in MRI brain scans and cognitive testing were subsequently reported [36,37]. The cognitive benefits in neuro-AIDS were confirmed in further controlled testing, which showed significant group (active vs. placebo, $p=.003$) and time ($p=.001$) effects [38], absent any toxicities. A three-site DBPC trial in 215 randomized subjects of intra-nasal spray DAPTA (2 mgs, TID) was conducted in the early 1990's. The main endpoint was change in global cognitive score at 6 months on a battery of 23 tests. While no significant difference was found between the DAPTA and the placebo group on the global cognitive score, 2 of 7 domains, working memory ($p.04$) and speed of information processing ($p=.008$), did show improvement in the DAPTA group. A treatment effect was also reported for patients whose CD4 cell numbers were above 200 cells/ μ L at baseline (non-AIDS).

Overall, this cohort was minimally cognitively impaired (MMSE=28). However, among those with greater and clinically significant cognitive deficit >0.5 on the global Z-score, a pre-planned sub-group analysis showed that DAPTA was associated with improved performance while deterioration was more common in the placebo group ($P=.02$) [39]. Although anti-viral

measurements were not a primary endpoint in this trial, those data were reported and showed that DAPTA reduced the viral load (-0.54 log, $p<.03$) [40]. The finding of anti-viral effect in this study is remarkable as the patients were not enrolled based on viral load, there was no dose optimization for anti-viral effect, their R5 or X4 viral phenotypes which would determine sensitivity to an anti-viral effect was unknown, and since the study was done in the pre-HAART era, DAPTA was essentially tested as monotherapy.

An in-hospital phase 2 study of DAPTA at 15 or 1.5mg/day for 4 weeks in nine IV drug users with early AIDS dementia also showed improved neurocognitive performance, at the higher dose compared with the lower dose or the placebo ($P<.05$) [41]. In a further study, benefits on functional brain imaging were reported in a 39-year old man with AIDS Dementia Complex who received 12 weeks of intranasal DAPTA (0.4mg TID, 1.2mgs per day). This study demonstrated that 34 of 35 brain regions having low FDG activity showed remission after therapy [42], consistent with the MRI and cognitive benefits identified in the phase 1 and 2 studies. (op.cit., above).

Orally active peptides

While DAPTA is substantially protected from degradation in plasma it is rapidly digested in the stomach [43]. Furthermore, its clinical use has primarily been by nasal spray, which requires long term storage of a liquid drug product. DAPTA has been shown to lose activity by aggregation upon storage. Aggregation of the drug product was a concern in the DBPC trial [39] as stored product was used for the several years it took to complete the study and gelling of study medication was reported by the patients. This has limited DAPTA further clinical development. To overcome this significant obstacle, we created an analog of DAPTA (RAP-103) that is fully protected to degradation, and retains picomolar potency.

A proof-of-concept study in rats showed that oral administration of RAP-103 (0.05-1mg/kg) for 7 days fully prevents mechanical allodynia and inhibits the development of thermal hyperalgesia after partial ligation of the sciatic nerve in rats [27]. In this study, we further showed that DAPTA and RAP-103 blocked both CCR2 as well as the closely related chemokine receptor CCR5. Moreover, RAP-103 could reduce spinal microglial activation and monocyte infiltration, and inhibit the inflammatory cytokine responses evoked by peripheral nerve injury, the cause of neuropathic pain.

Our findings suggest that targeting CCR2/CCR5 should provide greater efficacy than targeting CCR2 or CCR5 alone, and that the dual CCR2/CCR5 functional antagonist RAP-103 has the potential for clinical use in neuropathic and other pain conditions. Because this analog shares multiple DAPTA mechanisms to reduce microglial activation, shift the cytokine balance, protect neurons and spare dendritic arbor, it is a prime candidate for further clinical development in the multiple neuroinflammatory conditions already discussed, for which benefits in pre-clinical animal testing models have been shown, as noted in the many citations of this review.

Conclusion

Virobiome derived peptides have led to creation of an entirely new and novel class of innate-immune modulating peptides with proven human benefits in neuro-AIDS that may be useful in

many other neuroinflammatory diseases with few, if any, effective treatments. Of particular interest are findings that DAPTA prevented neuronal losses and protected synapses in neuro-AIDS and aging rodent models, and blocked microglial and astrocyte activation in brain, results highly suggestive of a beneficial effect in Alzheimer's disease and other neurodegenerative conditions. Benefits in animal models of AD, as well as ischemic stroke and neuropathic pain result in part from DAPTA's action to shift the cytokine profile from an M1 (inflammatory) to an M2 (repair) response, actions which have particular utility in brain injuries and neurodegeneration from diverse causes.

Virobiome-derived peptides like DAPTA, have been shown in multiple human studies to be safe and efficacious by blunting innate immune cytokines and chemokines. Next-generation orally active peptides have shown proof-of-concept benefits in neuropathic pain models, via effects at clinically validated receptor targets. Virobiome-derived oral peptides can therefore provide significant treatment and patient benefits over current antibody-based pharmaceuticals which seek to reduce inflammatory cytokines including IL-1, IL-6, IL-8, IL-23, and TNF α . Their small size allows rapid tissue distribution and entry into the brain, and peptides in general have an excellent safety profile, compared to many small molecule therapeutics.

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Conflict of Interest

The author is an inventor of the subject peptides.

References

- Pert CB, Hill JM, Ruff MR, Berman RM, Robey WG, et al. (1986) Octapeptides deduced from the neuropeptide receptor-like pattern of antigen T4 in brain potently inhibit human immunodeficiency virus receptor binding and T-cell infectivity. *Proc Natl Acad Sci USA* 83(23): 9254-9258.
- Alcami A (2003) Viral mimicry of cytokines, chemokines and their receptors. *Nat Rev Immunol* 3(1): 36-50.
- Ruff MR, Martin BM, Ginns EI, Farrar WL, Pert CB (1987) CD4 receptor binding peptides that block HIV infectivity cause human monocyte chemotaxis. Relationship to vasoactive intestinal polypeptide. *FEBS Lett* 211(1): 17-22.
- Sacerdote P, Ruff MR, Pert CB (1987) Vasoactive intestinal peptide 1-12: a ligand for the CD4 (T4)/human immunodeficiency virus receptor. *J Neurosci Res* 18(1): 102-107.
- Mulrone SE, McDonnell KJ, Pert CB, Ruff MR, Resch Z, et al. (1998) HIV gp120 inhibits the somatotrophic axis: a possible GH-releasing hormone receptor mechanism for the pathogenesis of AIDS wasting. *Proc Natl Acad Sci USA* 95(4): 1927-1932.
- Redwine LS, Pert CB, Rone JD, Nixon R, Vance M, et al. (1999) Peptide T blocks GP120/CCR5 chemokine receptor-mediated chemotaxis. *Clin Immunol* 93(2): 124-131.
- Ruff MR, Melendez-Guerrero LM, Yang QE, Ho WZ, Mikovits JW, et al. (2001) Peptide T inhibits HIV-1 infection mediated by the chemokine receptor-5 (CCR5). *Antiviral Res* 52(1): 63-75.
- Polianova MT, Ruscetti FW, Pert CB, Ruff MR (2005) Chemokine receptor-5 (CCR5) is a receptor for the HIV entry inhibitor peptide T (DAPTA). *Antiviral Res* 67(2): 83-92.
- Sodroski J, Kowalski M, Dorfman T, Basiripour L, Rosen C, et al. (1987) HIV envelope-CD4 interaction not inhibited by synthetic octapeptides. *Lancet* 1(8457): 1428-1429.
- Brenneman DE, Westbrook GL, Fitzgerald SP, Ennist DL, Elkins KL, et al. (1988) Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature* 335(619): 639-642.
- Brenneman DE, Buzy JM, Ruff MR, Pert CB (1988) Peptide T sequences prevent neuronal cell death produced by the envelope protein (gp120) of the human immunodeficiency virus. *Drug Devel Res* 15(4): 361-369.
- Pollicita M, Ruff MR, Pert CB, Polianova MT, Schols D, et al. (2007) Profound anti-HIV-1 activity of DAPTA in monocytes/macrophages and inhibition of CCR5-mediated apoptosis in neuronal cells. *Antivir Chem Chemother* 18(5): 285-295.
- Bachis A, Biggio F, Major EO, Mocchetti I (2009) M- and T-tropic HIVs promote apoptosis in rat neurons. *J Neuroimmune Pharmacol* 4(1): 150-160.
- Brenneman DE, Hauser J, Spong CY, Phillips TM, Pert CB, et al. (1999) VIP and D-ala-peptide T-amide release chemokines which prevent HIV-1 GP120-induced neuronal death. *Brain Res* 838(1-2): 27-36.
- Hill JM, Mervis RF, Avidor R, Moody TW, Brenneman DE (1993) HIV envelope protein-induced neuronal damage and retardation of behavioral development in rat neonates. *Brain Res* 603(2): 222-233.
- Wiley CA, Masliah E, Morey M, Lemere C, DeTeresa R, et al. (1991) Neocortical damage during HIV infection. *Ann Neurol* 29(6): 651-657.
- Masliah E, N. Ge, M. Morey, R. De Teresa, R. D. Terry, and C. A. Wiley. 1992. Cortical dendritic pathology in human immunodeficiency virus encephalitis. *Lab Invest* 66(3): 285-291.
- Lipton SA (1992) Requirement for macrophages in neuronal injury induced by HIV envelope protein gp120. *Neuroreport* 3(10): 913-915.
- Gendelman HE, Lipton SA, Tardieu M, Bukrinsky MI, Nottet HS (1994) The neuropathogenesis of HIV-1 infection. *J Leukoc Biol* 56(3): 389-398.
- Rosi S, Pert CB, Ruff MR, McGann-Gramling K, Wenk GL (2005) Chemokine receptor 5 antagonist D-Ala-peptide T-amide reduces microglia and astrocyte activation within the hippocampus in a neuroinflammatory rat model of Alzheimer's disease. *Neuroscience* 134(2): 671-676.
- Raychaudhuri SK, Raychaudhuri SP, Farber EMP (1998) Anti-chemotactic activities of peptide-T: a possible mechanism of actions for its therapeutic effects on psoriasis. *Int J Immunopharmacol* 20(11): 661-667.
- Raychaudhuri SP, Farber EM, Raychaudhuri SK (1999) Immunomodulatory effects of peptide T on Th 1/Th 2 cytokines. *Int J Immunopharmacol* 21(9): 609-615.
- Tufano MA (2002) Immunomodulatory effects of peptide T on human keratinocyte cells. *Br J Dermatol* 147(11): 663-669.

24. Phipps DJ, MacFadden DK (1996) Inhibition of tumour necrosis factor-alpha explains inhibition of HIV replication by peptide T. *AIDS* 10(8): 919-920.
25. Succi DJ, Pert CB, Ruff MR, Arendash GW (1996) Peptide T prevents NBM lesion-induced cortical atrophy in aged rats. *Peptides* 17(5): 831-837.
26. Ruff MR, Polianova M, Yang QE, Leoung GS, Ruscetti FW, et al. (2003) Update on D-ala-peptide T-amide (DAPTA): a viral entry inhibitor that blocks CCR5 chemokine receptors. *Curr HIV Res* 1(1): 51-67.
27. Padi SS, Shi XQ, Zhao YQ, Ruff MR, Baichoo N, et al. (2012) Attenuation of rodent neuropathic pain by an orally active peptide, RAP-103, which potently blocks CCR2- and CCR5-mediated monocyte chemotaxis and inflammation. *Pain* 153(1): 95-106.
28. Saika F, Kiguchi N, Kobayashi Y, Fukazawa Y, Kishioka S (2012) CC-chemokine ligand 4/macrophage inflammatory protein-1beta participates in the induction of neuropathic pain after peripheral nerve injury. *Eur J Pain* 16(9): 1271-1280.
29. Lee YK, Choi DY, Jung YY, Yun YW, Lee BJ, et al. (2013) Decreased pain responses of C-C chemokine receptor 5 knockout mice to chemical or inflammatory stimuli. *Neuropharmacology* 67: 57-65.
30. Hang LH, Li SN, Dan X, Shu WW, Luo H, et al. (2016) Involvement of Spinal CCR5/PKCγ Signaling Pathway in the Maintenance of Cancer-Induced Bone Pain. *Neurochem Res* 42(2): 563-571.
31. Di Prisco S, Summa M, Chellakudam V, Rossi PI, Pittaluga A (2012) RANTES-mediated control of excitatory amino acid release in mouse spinal cord. *J Neurochem* 121(3): 428-437.
32. Li L, Zhi D, Shen Y, Liu K, Li H, et al. (2016) Effects of CC-chemokine receptor 5 on ROCK2 and P-MLC2 expression after focal cerebral ischaemia-reperfusion injury in rats. *Brain Inj* 30(4): 468-473.
33. Barbey Morel C, McDonnell K, Pert C, Adams M, Farrand D, et al. (2002) A Peptide T Bolus Normalizes Growth Hormone Secretion Pattern in Two Children with AIDS. *Peptides* 23(12): 2279-2281.
34. Delfino M, Fabbrocini G, Brunetti B, Procaccini EM, Santoianni P (1992) Peptide T in the treatment of severe psoriasis. *Acta Derm Venereol* 72(1): 68-69.
35. Farber EM, Cohen EN, Trozak DJ, Wilkinson DI (1991) Peptide T improves psoriasis when infused into lesions in nanogram amounts. *J Am Acad Dermatol* 25(4): 658-664.
36. Wetterberg L, Alexius B, Saaf J, Sonnerborg A, Britton S, et al. (1987) Peptide T in treatment of AIDS. *Lancet* 1(8525): 159.
37. Bridge TP, Heseltine PN, Parker ES, Eaton E, Ingraham LJ, et al. (1989) Improvement in AIDS patients on peptide T. *Lancet* 2(8656): 226-227.
38. Bridge TP, Heseltine PN, Parker ES, Eaton EM, Ingraham LJ, et al. (1991) Results of extended peptide T administration in AIDS and ARC patients. *Psychopharmacol Bull* 27(3): 237-245.
39. Heseltine PN, Goodkin K, Atkinson JH, Vitiello B, Rochon J, et al. (1998) Randomized double-blind placebo-controlled trial of peptide T for HIV-associated cognitive impairment. *Arch Neurol* 55(1): 41-51.
40. Goodkin K, Vitiello B, Lyman WD, Asthana D, Atkinson JH, et al. (2006) Cerebrospinal and peripheral human immunodeficiency virus type 1 load in a multisite, randomized, double-blind, placebo-controlled trial of D-Ala1-peptide T-amide for HIV-1-associated cognitive-motor impairment. *J Neurovirol* 12(3): 178-189.
41. Kosten TR, Rosen MI, McMahon, Bridge TP, O'Malley SS, et al. (1997) Treatment of early AIDS dementia in intravenous drug users: high versus low dose peptide T. *Am J Drug Alcohol Abuse* 23(4): 543-553.
42. Villemagne VL, Phillips RL, Liu X, Gilson SF, Dannals RF, et al. (1996) Peptide T and glucose metabolism in AIDS dementia complex. *J Nucl Med* 37(7): 1177-1180.
43. Kahns AH, Bundgaard H (1991) Facile a-chymotrypsin-catalyzed degradation of the HIV inhibitor [D-Ala1]-Peptide T amide. *Int J Pharmaceutics* 77(1): 65-70.