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Lipid tucaresol as an adjuvant for methamphetamine vaccine development

K. C. Collins^a, J. E. Schlosburg^{a,b}, J. W. Lockner^a, P. T. Bremer^a, B. A. Ellis^a, and K. D. Janda^{a,*}

^aDepartments of Chemistry and Immunology, The Skaggs Institute for Chemical Biology, Worm Institute of Research and Medicine (WIRM), The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States

^bCommittee on Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, California 92037, United States

Abstract

The immunopotentiator tucaresol was modified for incorporation into liposomes, where it was found to be a superior adjuvant to MPLA for vaccination against methamphetamine.

Methamphetamine abuse has been an increasing global problem over the last few decades,¹ and was estimated to cost over \$23 billion in 2005 in the US alone.² Methamphetamine is highly addictive, flooding the brain with multiple neurotransmitters, including dopamine, serotonin and norepinephrine, causing a euphoric high.³ Repeated exposure depletes neurotransmitter levels and damages the corresponding transporter systems,⁴ resulting in severe withdrawal on attempted abstinence, and consequently a gravely high relapse rate.⁵ Furthermore, the complex neurochemistry behind the drug's psychoactive effects makes development of treatments difficult; there are no approved medications for methamphetamine addiction.^{6,7} The only current treatments are behavioural therapies⁸, which require a significant support network that addicts frequently lack, and which have demonstrated only limited improvement in long-term abstinence rates.⁹ One attractive therapeutic approach in development is active immunisation against methamphetamine; a successful vaccine would aid abstinence by sequestering the drug in the event of a relapse, minimising its pharmacological effects.

Small molecules like methamphetamine are invisible to the immune system, requiring conjugation to a T cell epitope to make them immunogenic; the peptidic nature of this carrier results in presentation of the MHC class II-antigen complex to the immune system, initiating antibody isotype switching from IgM to IgG, effecting a specific and long-lasting immune response. Vaccines against nicotine and cocaine have reached clinical trials, but candidates against methamphetamine are still in early stages of development.¹⁰ One area of

vaccine optimisation is the choice of adjuvant, which is used to enhance the local immune response by increasing (local) inflammation, stimulating antigen presenting cells and acting as a depot.¹¹ Aluminium hydroxide (alum) has been the historically dominant adjuvant, but alternatives are being pursued in an effort to improve safety, increase the strength of the immune response and to access alternative immune response profiles.¹² Liposomes have been explored as vaccine delivery systems since 1974¹³ and are currently at the forefront of vaccine research due to their ability to safely deliver both antigen and adjuvant in a versatile and readily-optimisable manner at relatively low cost.

Monophosphoryl lipid A (MPLA) is the only non-alum adjuvant approved for use (in conjunction with alum) in both the US and Europe^{14, 15} A detoxified derivative of bacterial lipopolysaccharide (LPS), MPLA is believed to enhance the immune system *via* a combination of mechanisms including agonism of toll-like receptor 4 (TLR4), which invokes a signal cascade that results in the production of proinflammatory cytokines¹⁶ and antigen-specific effector CD4+ and memory CD8+ T cells.¹⁷ Synthetic MPLA (also termed phosphorylated hexaacyl disaccharide, PHAD or glycopyranoside lipid A, GLA) has also more recently been investigated as an alternative to the multi-component, potentially heterogeneous, bacteria-derived MPLA.¹⁸ Direct comparison has shown enhanced results using the synthetic version,¹⁹ which being homogeneous, allows for precise control over the vaccine components. MPLA has been incorporated into liposomes (L(MPLA)) where it has shown stronger immunostimulation than alum, and synthetic L(MPLA) has been applied to drugs of abuse vaccines, where it has successfully elicited high antibody titres against heroin.²⁰

Another class of molecular adjuvant under investigation in clinical trials is the Quillaja saponins, which effect immunostimulation by providing T cells with direct costimulatory signals; the aldehyde moieties they contain are believed to mimic carbonyl groups on the surface of antigen-presenting cells (APCs), forming Schiff bases with free lysine residues on the surface of T cells.²¹ MPLA and saponins are complex small molecules and herein we propose that a readily synthetically accessible adjuvant, tucaresol, may be a suitable substitute. Tucaresol is an orally bioavailable aldehyde-containing immunopotentiator, whose application in vaccines has been primarily limited to systemic use to enhance DNA-based vaccines,²² but when used as a local adjuvant it has demonstrated enhanced Th cell priming compared to both alum and saponin Quil A.²³ Tucaresol has been shown to elicit both cellular and humoral responses,²² with characteristics of both Th 1 and Th 2-type immunity;²⁴ we have proposed that this mixed response is ideal for drugs of abuse vaccines.²⁵ Despite tucaresol being identified as an immunopotentiatory agent two decades ago, its use in vaccines has not flourished. Although tucaresol stimulates T cells, it has not been directly associated with the antigen as part of the vaccine design, and is usually administered orally or in a separate injection. We envisaged that incorporation of tucaresol into liposomes would enhance immunopotentiality by recruiting the liposomes to the T cells, promoting uptake of the liposomes and thus the associated antigen. Modification of tucaresol *via* lipidation would generate an amphiphilic molecule capable of being incorporated into the lipid bilayer of liposomes. We proposed that esterification of the acid

would be a facile method to achieve this and here provide the first reported example of a lipid analogue of tucaresol (lipid tucaresol, **LT1**, Figure 1).

Another key variable in drugs of abuse vaccine design is the choice of hapten. Our best candidate for vaccination against methamphetamine is MH6-SH (Figure 2).^{26, 27} This hapten requires thiol conjugation to the appropriately-activated carrier protein since it contains a secondary amine that would be incompatible with conjugation using activated carboxylic acids. We have found acid hapten conjugations to give more robust, efficient conjugation than their thiol counterparts, with the added benefit that the hapten precursors are stable upon storage. We therefore designed MH6t-CO₂H (**2**, Figure 2) as a new hapten, proposing that switching from a secondary to a tertiary amine should not have a significant impact on antibody production; elsewhere it has been shown that anti-methamphetamine antibodies can still be generated despite the larger perturbation of derivatising this amine to a tertiary amide.²⁸

Having chosen our target adjuvant and hapten, lipid tucaresol **LT1** was therefore synthesised in two steps from 4-(bromomethyl)-benzoic acid *via* esterification with 1-hexadecanol, followed by etherification with 2,6-dihydroxybenzaldehyde (Scheme 1a). MH6t-CO₂H, **2** was synthesised from methamphetamine *via* alkylation of the amine followed by saponification of the ester (Scheme 1b).

MH6t-CO₂H, **2** was then conjugated to two carrier proteins, diphtheria toxoid (DT) and BSA, using standard acid activation. The DT conjugate, MH6t(CO₂H)-DT (25 μ L, 1 mg/mL, hapten density of 13.3), was mixed with liposomes containing either MPLA or **LT1** (25 μ L) with the following composition: DMPC (90 mM), cholesterol (75 mM), DMPG (10 mM) and either MPLA (0.454 mM, 20 μ g/injection) or tucaresol (0.454 mM, 5.6 μ g/injection). The resulting vaccines, MH6t(CO₂H)-DT+[L(MPLA)] and MH6t(CO₂H)-DT+[L(**LT1**)] were used to immunise groups of six Swiss Webster mice (6–8 weeks old) *via* subcutaneous injection on days 0, 14, and 35. Serum was collected *via* tail bleed on days 21 and 42, and *via* cardiac puncture on day 63, and production of anti-methamphetamine antibodies was evaluated by ELISA using MH6t(CO₂H)-BSA coated plates (hapten density of 30.3). Antibody affinities and concentrations were determined by RIA (Figure 3).

Both liposomal vaccines showed a similar immune response, with tucaresol appearing to be the superior adjuvant in this case; L(MPLA) and L(**LT1**) generate similar antibody concentrations (19 and 14 μ g/mL, respectively), but the specificity of the L(**LT1**)-induced antibodies is higher, with a K_d value of 134 nM (compared to 233 nM induced by [L(MPLA)]). This value is also on par with that obtained in a previous study using secondary amine hapten MH6(SH)-KLH with SAS® under a similar vaccination schedule (130 nM at 42 days).²⁶ This suggests that the presence of the extra methyl group in the MH6t hapten does not substantially impact antibody recognition of methamphetamine. However, the concentration of the antibodies elicited by MH6t(CO₂H)-DT+[L(**LT1**)] (14 μ g/mL at 63 days) is lower than that of MH6(SH)-KLH+SAS® (108 μ g/mL at 42 days). The antibody concentration dropped throughout the study of MH6(SH)-KLH+SAS®, and given that our bleed was performed at a later time point, this may account for some of the discrepancy. Analysis of the ELISA data also shows a drop in antibody titre throughout this

study rather than the expected increase after multiple boosts; it is likely that we used a suboptimal vaccination schedule as it can be seen that liposomal vaccination often requires larger spacing between boosts to promote the optimal response. In any case while there is scope for optimisation of the protocol, the direct comparison of MPLA and **LT1**, which was the primary goal of this study, clearly demonstrates the latter's efficacy as a synthetic adjuvant.

Additional scope for improvement of our [L(**LT1**)] vaccine should be achievable through optimisation of the vaccine composition. The concentrations of both MPLA and **LT1** in this study were chosen based on previous work with MPLA.^{19, 20} Tucaresol's effect on the immune response is known to be concentration-dependent, with increasing immunopotential at lower doses, leading to immunosuppression at higher doses.²⁹ It is highly unlikely that the dose of **LT1** used was optimal, and further investigation could result in significant improvement in the immune response. Finally, the location and length of the alkyl chain used for lipidation of tucaresol could also be optimised.

In summary, we have synthesised the first lipid analogue of tucaresol, **LT1**, and shown that it is capable of eliciting an immune response against methamphetamine, demonstrating superior liposomal adjuvancy to MPLA and having great potential for future development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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tucaresol **lipid tucaresol, LT1**

Figure 1.
Structures of tucaresol and lipid tucaresol, **LT1**

MH6-SH **MH6t-CO₂H, 2**

Figure 2.
Structures of MH6-SH and MH6t-CO₂H, **2**

Figure 3. Anti-methamphetamine antibody titres, affinities and concentrations from MH6t(CO₂H)-DT +[L(MPLA)] and MH6t(CO₂H)-DT+[L(LT1)] vaccinated mice (n=6). a. Midpoint titres as determined by ELISA using MH6t-BSA as the coating antigen; b. Dissociation constants (K_ds) as determined by competitive RIA using pooled sera; c. Antibody concentrations as determined by competitive RIA using pooled sera. Data was obtained in duplicate; errors represent SEM; individual data points represent individual mouse titres.

Scheme 1.
Synthesis of lipid tucaresol, **LT1** and MH6t-CO₂H, **2**