博士論文（要約）

Development of Poly(amino acid)-based

Immunomodulating Polymers for Antitumor Therapy

(ポリアミノ酸ブロックを有した免疫改善型高分子

による抗腫瘍療法の開発)

フアン　ジョージ　ロ

George Lo Huang

Abstract

A significant number of clinical diseases are caused by dysregulation of the immune response. In cancer, immunosuppression is a key feature, and the approaches directed to overcome the immunosuppressive pathways are making a great strides in the treatment of cancer. The immune response is strongly influenced by the enzymatic catabolism of tryptophan and arginine in local environments. As such, amino acid homologs of tryptophan and arginine have been demonstrated to inhibit such enzymatic activity, leading to profound changes in immune response. Nevertheless, the in vivo application of such inhibitors has been limited by their rapid clearance and poor accumulation in the diseased tissues. Since the enhanced permeability and retention (EPR) of large macromolecules is a hallmark of both inflammatory autoimmune diseases and cancer, here we are developing polymeric prodrugs aimed at taking advantage of the EPR effect for increasing the drug levels in target tissues to effectively modulate the immune responses. To this end, we have synthesized poly(amino acid)s from small molecule amino acids homologues capable of inhibiting the immunomodulating enzymes indoleamine 2,3-dioxygenase-1 (IDO) and Arginase 1 (ARG1). Thus, the N-Phenyl Carbamate (NPC) of the IDO inhibitor methyl-L-tryptophan (MLT) was polymerized by ring opening polymerization to produce homo-poly(methyl-L-tryptophan) (p(MLT)), as well as poly(ethylene glycol)-poly(methyl-L-tryptophan) (PEG-p(MLT)). These polymers released MLT after enzymatic cleavage, inhibiting IDO in vitro. In addition, polymeric versions of the Arg1 inhibitor Nω-hydroxy-Arginine (NOHA) were prepared by modifying homo-poly(L-ornithine) (p(Orn)) and PEG-p(Orn), which are being evaluated in vitro.

Summary

Amino acid depletion of arginine and tryptophan in cancer is a well-studied phenomenon that has been shown to lead to resistance to immunotherapy through T cell inhibitory pathways[1,2,3]. In particular, ARG1 and IDO have been shown to be the main culprit in amino acid depletion of arginine and tryptophan, respectively[4,5]. Depletion of tryptophan has been shown to lead to T cell anergy and apoptosis, while kynurenine, a metabolic byproduct of tryptophan synthesized from IDO, stimulates regulatory T cell proliferation, leading to an immunologically suppressed microenvironment. Like tryptophan, depletion of arginine also leads to T cell anergy. However, arginine is also a substrate used by macrophages to regulate immune response and wound healing. Arginine can be metabolized by tumor associated macrophages by ARG1 into ornithine, which is converted to polyamines which support cell proliferation. Thus, there is clinical interest in developing ARG1 and IDO inhibitors for enhancing immunotherapies, such as immune checkpoint inhibition, which rely on T cell activity within the tumor microenvironment[6].

Inhibitors of both IDO and ARG1 are often homologs of their substrate amino acids. These amino acids demonstrate relatively weak inhibitory constants (MLT IC_{50} = 20 μM[1], NOHA IC_{50} = 3.6 μM[8]), and require good pharmacokinetics in order to accumulate in tumors in a meaningful concentration. For example, 1-Methyl-D-Tryptophan (MDT), the

D enantiomer of MLT, which is entering phase III clinical trials for melanoma, presents poor tumor uptake and rapid renal clearance. Thus, even though 2 g of IDO inhibitor are taken orally, its C_{max} is only 12 μM, which is lower that its 35 μM IC_{50}, suggesting limited pharmacological efficacy[1,9].

A possible method to overcome the poor pharmacokinetics of amino acid-based inhibitors is to formulate tumor-targeted drug delivery systems (DDS) for enhancing the drug levels in tumors. However, because the effective dose of these amino acid-based inhibitors is so high, the development of traditional DDS, such as liposomes or polymer-drug conjugates, for tumor targeting is unrealistic, as such strategies would increase the amount of non-therapeutic excipient material in each dose. Herein, we hypothesized that developing polymeric forms of the amino acid inhibitors MDT, MLT, and NOHA (**Figure 2**) could be a viable approach for enhancing the pharmacokinetics and the drug levels in tumors toward superior efficacy.

Polymeric NOHA was synthesized by pendant chain modification of PEG-p(Ornithine) through two synthesis reactions followed by deprotection. This synthesis strategy demonstrates the relative ease of synthesizing modified arginines, and allows for the potential to synthesize a variety of various polymers that may be hydrolyzed into inhibitors or substrates of either inducible nitric oxide synthase (iNOS) or arginase 1 (ARG1). Although no biological activity of PEG-P(NOHA) has been demonstrated as of this time, the successful synthesis of PEG-P(NOHA) suggests great potential in developing immune modifying polymers based on monomers of modified arginine.

The main strategy for synthesizing methyl tryptophan polymers of controlled length and dispersity was through the use of ring opening polymerization. As ring opening polymerization requires the synthesis of reactive amino acid intermediates, homopolymers of MLT and MDT were first synthesized through simple condensation reactions to determine their biological activity before proceeding to the synthesis of reactive monomers for ring opening polymerization. After synthesis of P(MLT) and P(MDT), P(MLT) was determined to be the superior prodrug, as it was hydrolysable by chymotrypsin and able to release monomeric MLT, whereas P(MDT) was not.

The most common way of synthesizing polymers of amino acids is through ring opening polymerization of N-carboxyanhydrides (NCA). Thus, we began to prepare for polymerization of MLT by synthesizing MLT-NCA by the triphosgene method. However, after multiple failed attempts to synthesize MLT-NCA, alternative methods to synthesize MLT polymers of controlled length and dispersity were pursued. To this end, we have succeeded in synthesizing an N-Phenyl Carbamate (NPC) of MLT, which is capable of generating an in situ NCA after being heated in solution. By using MLT-NPC, we were able to synthesize block copolymers of PEG-P(MLT) that can self-assemble into a micelle, function as a drug delivery system, and simultaneously release the immune modulatory small molecule MLT through enzymatic hydrolysis.

After synthesis of PEG-P(MLT), the biological characteristics of the polymer were studied in vitro. PEG-P(MLT) polymers were labelled with Alexa Fluor 647-NHS ester and

then self assembled into micelles. Alexa Fluor labelled PEG-P(MLT) micelles were demonstrated to be uptaken by THP-1 human monocytes, and that these polymers were demonstrated to be enzymatically released by chymotrypsin. Next, both the micelles and the digest were demonstrated to successfully inhibit IDO in THP-1 human monocytes. Finally, polymers were assessed for cytotoxicity and immunogenicity. Cytotoxicity against a range of cells demonstrated no cytotoxicity, and no immunogenicity was displayed against THP-1 and RAW 264.7 murine macrophages, indicating that PEG-P(MLT) are well tolerated.

New random block copolymers of PEG-P(Glu(Otbu)-MLT) and PEG-P(Lys(Cbz)-MLT) have been synthesized in order to facilitate potential drug loading of PEG-P(MLT) polymers. The pendant group of glutamic acid and lysine are capable of direct drug conjugation by amide or ester bond, and can also be used for polyion complex of polynucleotide drugs. The next step in our work is to load the PEG-P(MLT) micelles with drugs that may synergize with the immunomodulatory effect of MLT in order to demonstrate the potential of our drugs. As micelles are best used to load small molecule drugs, we are currently evaluating the loading ability of small molecule immune modifiers, as well as cytotoxic antitumor drugs that are known to induce immunogenic cell death.

Conclusion

Our novel polymers based on amino acid-inhibitors are designed such that the copolymer is made from the drug itself, reducing the excipient material to just hydrophilic PEG in the case of the block copolymers, which is an FDA approved polymer for drug and protein conjugation. We believe this is the first attempt to synthesize a polymeric form of a drug through NCA polymerization. This approach is valuable to the field of bioengineering by providing a novel approach to designing polymeric micelles by using the drugs as the building block for the nanoparticle. The enzymatic activation of p(MLT) is provides us with a strong rationale to further develop p(MLT) based polymers for drug delivery and IDO inhibition. This has been confirmed in vitro by the ability of the digested MLT to inhibit KYN production in macrophages by HPLC, and further in vivo validation is underway. We believe that these novel immunomodulating polymers may have strong therapeutic applications in oncology and may one day improve outcomes for patients suffering from cancer.

1. Hou, D. Y. *et al.* Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses. *Cancer Res.* **67**, 792–801 (2007).
2. Geiger, R. *et al.* L-Arginine Modulates T Cell Metabolism and Enhances Survival and Anti-tumor Activity Article L-Arginine Modulates T Cell Metabolism and Enhances Survival and Anti-tumor Activity. *Cell* **167**, 829-836.e13 (2016).
3. Gallina, G. *et al.* Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8 + T cells. **116**, 2777–2790 (2006).
4. Rath, M., Müller, I., Kropf, P., Closs, E. I. & Munder, M. Metabolism via arginase or nitric oxide synthase : two competing arginine pathways in macrophages. **5**, 1–10 (2014).
5. Miyajima, M. *et al.* Metabolic shift induced by systemic activation of T cells in PD-1-deficient mice perturbs brain monoamines and emotional behavior. **18**, (2017).

6. Buchbinder, E. I. & Desai, A. CTLA-4 and PD-1 Pathways. **39**, 98–106 (2016).

7. Haile, L. A., Gamrekelashvili, J., Manns, M. P., Korangy, F. & Greten, T. F. CD49d Is a New Marker for Distinct Myeloid-Derived Suppressor Cell Subpopulations in Mice. *J. Immunol.* **185**, 203–210 (2010).

8. Pudlo, M. Arginase Inhibitors : A Rational Approach Over One Century. 475–513 (2017). doi:10.1002/med

9. Soliman, H. H. *et al.* A phase I study of indoximod in patients with advanced malignancies. *Oncotarget* **7**, 22928–22938 (2016).