Peptide Derived From Rabies Glycoprotein as Potential Therapeutics Against Clinical Rabies: A Graduate Proposal

Nirajan Bhattarai, Graduate Student1; Marvin K. Schulte, PhD2; Sabina Yerasingh, Graduate Student3
1Department of Biomedical and Pharmaceutical Sciences, Idaho State University

Aims

The goal of our research is to use small peptides derived from RGP to study the molecular basis of the RGP/nAChR interaction. This information can be used as the basis for rational drug design of peptides and peptidomimetics that may provide treatment for clinical rabies by displacing virus from the receptors and restoring function.

Specific Aims

1) To evaluate the selectivity of rabies glycoprotein for neuronal nAChRs.
2) To determine key residues present in nAChR that are critical to binding and selectivity of RGP.
3) To generate drug design model and optimize peptide ligands by reducing flexibility and maximizing the strength of interactions.

Introduction

The fatality rate associated with rabies is 100% with fifty-five thousand annual reports of human death (Fooks et al., 2017). Although rabies can be successfully combated with preventive measures, including pre- and post prophylaxis (PrEP and PEP) treatment (Van de Burgwal et al., 2017), it still causes a death every 10-20 minutes with a child mortality rate (<15 yrs) of 40-50 %, in developing countries in Africa and Asia. Once observable clinical symptoms arise, the disease is nearly 100% fatal (Fooks et al., 2017). There are currently no available treatement options for the clinical rabies stage, thus palliative care is the only option left for victims.

The development of therapeutics for clinical rabies has been impeded by a lack of knowledge of the molecular mechanism of rabies in the CNS. Nevertheless, it had been documented that the short ectodomain of rabies glycoprotein (RGP) is homologous to three-fingered snake toxins such as bungarotoxin. RGP, binds to the orthosteric binding site of nicotinic acetylcholine receptors (nAChR), antagonizing function and altering host behavior (Hueffer et al., 2017). Specifics of this interaction are unknown and the role of nicotinic receptor inhibition in CNS dysfunction resulting from rabies infection has not been determined.

Methods and Materials

Approach

1. Synthesis of peptide
2. Binding assay using surface plasmon resonance
3. Functional assay using two electrode voltage clamp technique
4. Protein-peptide docking analysis and modelling

Experimental Design

Figure 1. Surface plasmon Resonance

Figure 2. Two electrode voltage Clamp

Preliminary Study

1. Work produced by a collaborator of Schulte lab.
2. 5 group of 12 animal used.
3. 2 groups are uninfected or untreated respectively.
4. Enhancement of survival on 2mg and 4mg dFBr treatment
5. No survival on 6mg dFBr treatment. 

Note: dFBr is positive allosteric modulator (PAM) of alphabeta2 nAChR

Figure 3: pET peptide expression system

Figure 4: Functional effect of peptide derived from rabies glycoprotein of varied pathogenicity

1. Work produced by Hueffer K et al from Schulte lab
2. 3 peptides vary with single mutation at 183 or 196 position
3. a = alanine at 183 (derived from pathogenic strain)
4. b = proline at 183 (derived from pathogenic strain)
5. c = aspartate at 196 (derived from non pathogenic strain)

Note: Peptide derived from non-pathogenetic strain with aspartate at 196 position is PAM to alphabeta2 nAChR as dFBr

References


Innovation

Pathogenic rabies glycoprotein tweaking to therapeutics

- Elucidate mechanism of rabies pathogenicity
- Basis of rational drug design (peptide and peptidomimetics)

Peptide based solution to rabies glycoprotein pathogenicity

- Reduce drug-drug interaction
- Reduce metabolite side effects; product is amino acid

Significance

Why this research?

- No treatment modalities for clinical rabies
- Unclear molecular Mechanism of CNS rabies pathogenicity

Impact:

- Molecular mechanism for Rabies Pathogenicity
- A model for rational drug design against clinical rabies
- Identification of specific target
- Peptide based modulation of nAChR

Conclusion

This project is designed to create groundwork for design and discovery of peptide based therapeutic against clinical rabies. This can be achieved by elucidating the molecular mechanism and selectivity of rabies glycoprotein in CNS. Further, key residues involved in viral interaction will be identified to generate a drug design model for new peptides and peptidomimetics against clinical rabies. Flexibility will be constrained by an effective approach to achieve higher affinity of peptide candidates.

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Contact Information

Nirajan Bhattarai
Department of Biomedical and Pharmaceutical Science
Idaho State University
Email: bhattarai@isu.edu
Phone: +12083173452