Industry Day Theme : Sustainable Medical Technologies (SMT)

Process for Production of Lethal Toxin Neutralizing Factor (LTNF) for Snakebite Treatment

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CHEMICAL ENGINEERING IITD

Abstract

Current treatments for snake bites include commercially available antivenin which are composed of treated horse serum. Issues with these antivenin include limited availability, unregulated manufacturing process and severe adverse reactions. There is an urgent need for the development of safe and cost effective process to manufacture antivenin.

The present research involves process development and techno-economic analysis for production and purification of lethal toxin neutralizing factor (LTNF) through recombinant technology. Multimeric LTNF was expressed as inclusion bodies (IBs) in *E. Coli*. Expression of LTNF-multimer was confirmed with Tricine PAGE, LC-MS for mass and sequence. LTNF-multimer was successfully produced at 1L scale fermenter with 4 g/L titre. IBs were isolated and solubilized in solubilisation buffer. The product was captured using Cation exchange chromatography with ~98% recovery. The semipurified LTNF-multimer was subjected for cleavage reaction with ~98% conversion. The reaction mixture was further purified by preparative reverse phase chromatography to obtain purified LTNF-monomer. After ultrafiltration steps, the mixture was shown to neutralize rattlesnake venom in mice. Preliminary antivenin activity experiments described here suggest a potential life-saving therapy for snake bite. Further activity testing is undergoing for four major classes of poisonous snake venom.

Introduction

- The estimated number of death due to envenomings are 1,000,000 people around the world and 20,000 in India every year.
- Existing treatments for snake bites include heat treated multivalent antisera produced from horse or sheep.
- Current manufacturing practices for producing the antisera involve envenoming of horses with a specific venom pool to generate antibodies are unregulated, cause side effects and pose challenges for manufacturing.
- There is a dire need for designing a process for manufacturing antivenin that follows the principles of good manufacturing practice (GMP) and can be used for production of safe and cost efficacious antidote for combating envenomation.

Materials and Methods

Production
Fermentation
Homogenization
IB Solubilization

Purification
CEX
Enzymatic React[n]
RP HPLC

Animal Testing
Intravenous peptide injection in mice

Conclusions

- The process for manufacturing the recombinant LTNF has been developed with overall recovery of 78-80%.
- A manufacturing plant capable of producing 10 Kg of LTNF per batch was simulated and techno economic analysis was performed.
- The production cost/gram of LTNF was estimated to be $6 (Rs. 422), significantly lower in cost to that of currently available antivenin in the market.
- Antivenin activity for rattle snake is shown positive. Further, antivenin activity against four major class of venomous snake venom is underway.

Industrial Significance

- Developed process is safe & significantly cheaper than current antivenin in the market.
- GMP compliant process developed in our lab have commercial potential and technology transfer to healthcare companies, governments and WHO.

Technology Readiness Level: Purified peptide animal studies are undergoing presently.

Results

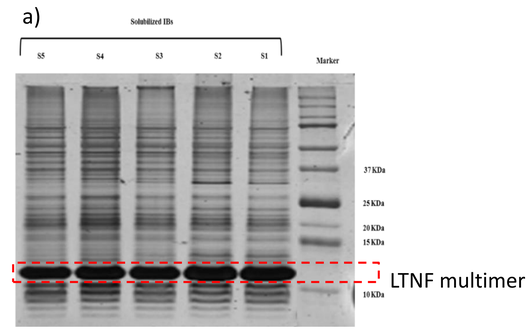

Fig 2. Results showing the process development of LTNF peptide: a) Tricine gel analysis, b) Optimisation of inducer.

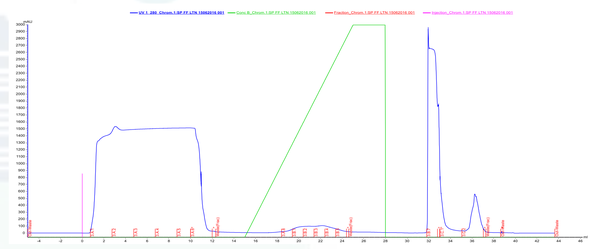

Fig 3. Preparative chromatogram of capture step

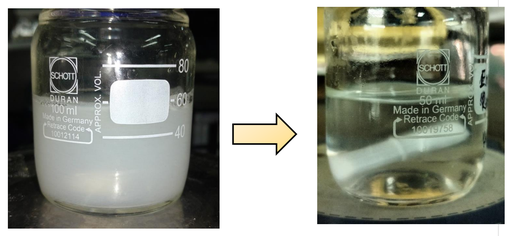

Fig 4. Cleavage through enzymatic reaction at 0 hr & 2 hr

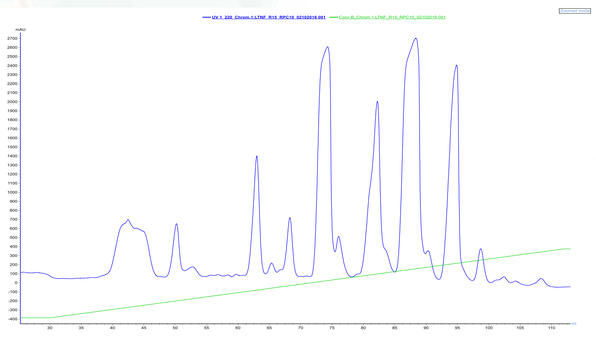

Fig 5. Preparative chromatogram of RP HPLC

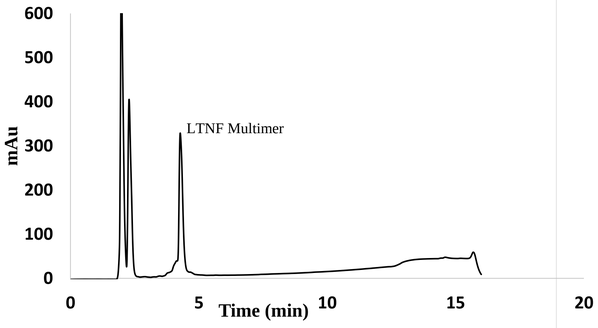

Fig 6. HPLC chromatogram of Solubilized IBs

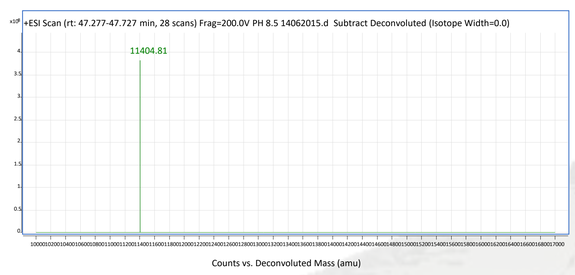

Fig 7. LC-MS mass confirmation of LTNF multimer peak

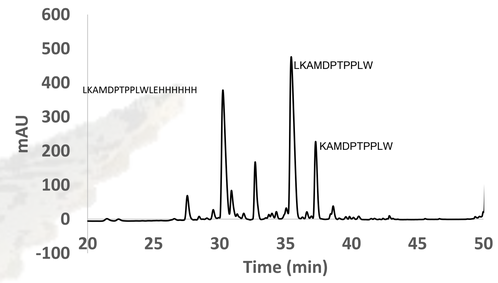

Fig 8. HPLC chromatogram of cleavage reaction labelled sequences were confirmed by LC-MS

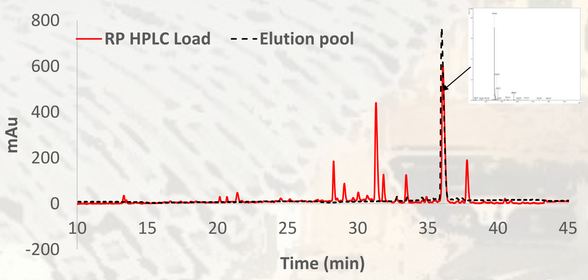

Fig 9. HPLC chromatogram of Load and elution fraction of polishing step and LTNF monomer confirmation through MALDI-TOF

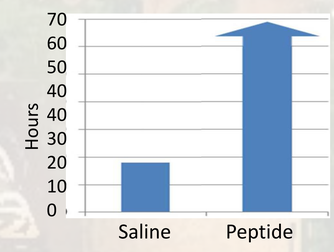

Fig 6. Survival time post injection of venom in mice incubated with saline or peptide

References

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Acknowledgement

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