Material preparation for potency testing of tetanus toxoid vaccine

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Tetanus is a more remarkable and globally prevalent disease of human and vertebrate animals. The causative agent of tetanus, spastic paralysis, a vaccine preventable disease, caused by the second most poisonous substance known, the tetanus toxin (TetX). Despite an effective vaccine, tetanus is an ongoing problem in some developing countries. This study aimed for toxin production (in minimum quantity without using fermentor) which was then confirmed through Minimum Lethal Dose (animal testing) and used for potency testing and efficacy of tetanus toxoid (Adsorbed) vaccine.

Key words: Endogenous proteases, polypeptide chain, tetanus, trismus, toxin.

INTRODUCTION

Clostridium tetani is an obligate anaerobe, cosmopolitan, readily endospore, and Gram positive bacillus (Farrar et al., 2000; Kenneth, 2004). Mostly present in habitats like soil, dust, and intestinal tracts of various animals, horses, chicken and humans (Allen et al., 2002). This organism is the causative agent of tetanus, spastic paralysis, a vaccine preventable disease (World Health Organization; www.who.int_vaccines_diseases_diseases_Neonatal_Tetanus. html) caused by the second most poisonous substance known, the tetanus toxin (TetX), with a human lethal dose of 1 µg/kg. The WHO definition of adult tetanus requires in any case one of the subsequent signs: trismus (inability to open the mouth) or risus sardonicus (sustained spasm of the facial muscles); or painful muscular contractions. Even if this definition requires a history of injury or wound, tetanus may also occur in patients who are incapable to remember a specific wound or injury (Who Technical Note, 2010). Tetanus is more remarkable and globally prevalent disease of human and vertebrate animals and is not transmitted from person to person (Afridi et al., 2005) and is caused by C. tetani, that produces two exotoxins, hemolysin, comparatively unimportant and other potent neurotoxin, tetanospasmin (Lee et al., 2000) and has been reported for over 24 centuries, and was first described in Egypt (Cook et al., 2001). The toxin is formed initially as a single polypeptide chain, with a molecular weight of approximately 150,000 Da. This toxin is cleaved into two subunits with molecular weights of approximately 107,000 Da and 43,000 Da by the action of endogenous proteases. Tetanospasmin then enters presynaptic neurons and disables neurotransmitter release, most importantly, the inhibitory neurotransmitters gamma-aminobutyric acid (GABA) and glycine. These results in a disinhibition of end-organ neurons, such as motor neurons and those of the autonomic nervous system, resulting in muscular stiffness and spasms that are typical of tetanus (Daniel, 2005) and may persist for several months. Unimmunized
survivors of tetanus become sufferers a second time. 74kb mega-plasmid is responsible for tetanospasmin (Marvaud, 2000). Toxin produced at the end of germination phase, and the main action of the toxin is a blockage of transmitter release from nerve terminals (Collingridge et al., 1982).

**METHODOLOGY**

**Media**

(Culture media) Thioglycolate and Mueller Miller (M.M) used.

**Toxin formation**

To formulate the toxin from pure culture present in thioglycolate tubes, sub-cultured in Mueller Miller media for seed preparation and incubated for 48 hours at 37°C under strict anaerobic condition. Repeated twice same step to obtain pure and healthy seed. Contamination at any step can lead to impure toxin production, so strict aseptic techniques are used for toxin production at each step. After following the incubation period, culture purity was confirmed by microscopy and verified by biochemical and in-vivo testing. Then, seed culture from all the three tubes was inoculated into 250 ml M.M media in conical flask, and incubated at 37°C for 48 h, after the 48 h incubation, the Zinc dust was added to the same flask and continued the incubation process for further 96 hours under strict anaerobic conditions at same temperature. This inoculation step is also very much crucial and important to formulate pure toxin of *Clostridium tetani*. These six days provided culture for incubation, gave opportunity to organism to utilize all the nutrient sources including all proteins, vitamins and amino-acids (provided in the M.M media), and to burst out to release the toxin. After the following six days of incubation, the toxin was filtered out by using the filter pumps and filtration units. Finally, toxin became purified by passing it through 0.2 µ filter unit. Since it was an experimental work, after the completion of experiment, the culture and toxin were decontaminated through moist heat method (autoclaving).

**RESULTS**

**Toxin conformation**

Pure toxin was injected in mice. By keeping them under observation, showed the following results. Figure 1 shows that after 12 h observation, mice have very minor effect of rigidity in tail muscles. After 24 h, mice showed the tetanus symptoms with sever stiffness in hind portion of the body especially limbs, as shown in (Figure 2). Keeping mice under observation till 48 – 72 h, following the strictness in all body muscles, mice become dead (Figure 3). Ultimately, it is confirmed through animal testing that material prepared for potency of tetanus toxoid vaccine was pure.
Future prospects

Benefits of this experiment include:

- Easy production of Tetanus Toxin for Pharmaceutical companies to establish their internal QC and improvement of their tetanus toxoid vaccine production.
- This study shows the cost effective and time saving toxin production.
- Decreases the dependence on fermentor for toxin production in low quantity. This will result in saving the cost for fermentor.

Discussion

According to the study of Daniel (2005), tetanus toxin blocks inhibitory neurotransmitters in the central nervous system which at last results in muscular stiffness and spasms, which favors this study (showing stiffness and spasms).

REFERENCES

www.who.int_vaccines_diseases_diseases_ Neonatal Tetanus. html)