EFFECT OF USING DIFFERENT ANTI-FOAMS ON TOXIN PRODUCTION OF CLOSTRIDIUM PERFRINGENS TYPE A.

Elham F. El-Sergany, Taha, M.M., El-Helw, H.A., Hala El-Sawy
Anaerobic bacterial Vaccine Research Department, VSVRI, Abbassia, Cairo

ABSTRACT

In this study, three types of anti-foaming agent (Polyethylene glycol, simethicone, and charcoal) were used in the process of preparation of C. perfringens type A toxoid. It was found that simethicone and polyethylene glycol when added to toxin producing medium gave a high yield of alpha toxin (α toxin) of C. perfringens type A (120, and 90 Minimum Lethal Dose (MLD) respectively) in comparison to that of medium without anti-foaming agent, which gave alpha toxin with 60 MLD. Medium with added charcoal gave least amount of α toxin (30 MLD). Two formula of C. perfringens type A vaccines were prepared with Simethicone and polyethylene glycol as anti-foams, and the third one was prepared without anti-foam as control one. Testing of these vaccines in rabbits revealed that simethicone as anti-foam in toxin producing medium of C. perfringens type A produced high level of toxin production and reduced the dose of vaccine used

Key words: C. perfringens type A, Anti-foam, Vaccine.

1. INTRODUCTION:

Clostridium perfringens α-toxin, which possesses lethal, hemolytic, dermonecrotic and phospholipase C (PLC) activities, has been reported to be a major pathogenic factor in the development of gas gangrene caused by the microorganism (Titball, 1993; Awad et al., 1995; Sakurai, 1995; Titball, 1997a, b; Bryant et al., 2000a, b). The production of Alpha toxin from C. perfringens type A, requires culturing of cells either in large flasks or in continuously stirred tank fermentors. Foaming can lead to reduced yield of toxins since it bursting bubbles can damage proteins (Holmes et al., 2006) and can also result in a loss of sterility if the foam escapes (Varley et al., 2004). In bioreactors, foaming can lead to over-pressure if a foam-out blocks an exit filter. To prevent the formation of foam, mechanical foam breakers, ultrasound or, most often, the addition of chemical antifoaming agents (or "antifoams") are routinely employed (Varley et al., 2004). In this study, three antifoams (Poly ethylene Glycol, Simethicone, Charcoal) that are widely used in controlling the foaming (Jahic et al., 2003; Charoenrat et al., 2005; Jungo et al., 2007) were chosen to analyze effect of their de-foaming action in toxin production medium and testing its effect on toxin yield and further more in preparation of vaccine.

2. MATERIAL AND METHODS:

2.1. Strains:

C. perfringens type A isolate was previously isolated from rabbits and identified in Anaerobic bacterial vaccine research department, Veterinary Serum and Vaccine Research Institute was used in preparation of α toxin. Robertson’s Cooked meat medium (Oxoid) Smith and Holdman (1968) was used for rehydration and culturing of lyophilized strain of C.
Effect of using different antifoams on toxin production of *C. perfringens* type A.

*perfringens* type A Production Medium (Roberts *et al.*, 1970). It was used for production of *C. Perfringens* exotoxins.

2.2. Antifoams:

1-Polyethylene Glycol 6000 (Titan Biotech Limited, 1013), it was added to toxin production medium at concentration of 1% (w/v) according to Wiebe *et al.*, (2001).

2-Simethicone it was supplied from Pharco pharmaceutical Co., it is derivatives of silicone polymers, it was added to toxin production medium at concentration of 0.05% (v/v) according to Keill (1976).

3-Vegetable charcoal class as organic anti-foam it was supplied from South Egypt Drug Industries Co. (SEDICO), it was added to toxin production medium at concentration of 0.05% (w/v) according to Dworschack *et al.*, (1954).

2.3. Preparation of vaccines:

Briefly *C. perfringens* type A was cultivated in cooked meat broth and incubated anaerobically at 37°C for 24 hrs. and then inoculated into toxin production medium (Roberts *et al.*, 1970) which added to it the different anti-foams and incubated at 37°C for 5 hrs. The minimum lethal dose of produced toxin was determined according to Fu *et al.*, (2004) by injecting mice weighted between (14-16 g) intravenously with 0.1 mL aliquots of tenfold serial dilutions of toxin samples and observing the mice 24 h for toxicity. Two mice were used for each amount of toxin dilutions.

The prepared toxin was inactivated by adding of 37% formalin at 0.5% (v/v) to inoculated medium and left at 37°C for 7 days until complete inactivation of toxin and convert to toxoid. The produced toxoids were Centrifuged and the supernatant were taken where 2% Aluminum Hydroxide Gel (Suprex, Copenhagen, Denmark), it was added to each toxoid at concentration of 20% (v/v). Safety and sterility tests were applied on prepared vaccines according to OIE (2008).

Three types of vaccines were formulated according to addition of antifoams to toxin production medium (Vaccine #1 Polyethylene Glycol); (Vaccine #2 Simethicone); and (Vaccine #3 Control without antifoam).

2.4. Vaccination:

Fifteen rabbits were assigned into three groups, each group (n=5). Groups (1, 2, 3) vaccinated with (vaccine #1, #2, #3 respectively). Each rabbit in each group vaccinated subcutaneously with two doses of 2 ml, 21 day apart containing 60 MLD. Blood samples obtained from ear vein of rabbits after 14 days from second dose, sera were separated and stored at -20°C until determination of antitoxin titers in them.

Antitoxin Titration:

2.5. Antiserum:

Diagnostic antiserum for α toxin of *C. perfringens* type A, was obtained from Welcome, Diagnostics Dart ford, England. It was used as control positive among the used toxin in toxin neutralization test and ELISA for evaluation of produced vaccine.

a. Toxin Neutralization Test:

It was done according to British Veterinary Pharmacopoeia (2010), briefly L+ dose of α toxin of *C. perfringens* type A firstly determined, then equal amount from serial diluted tested sera and L+ dose of α toxin were mix then incubated at 37 °C/1hour. From each mixed dilution, 2 mice were injected with 0.2 ml intravenously, and then observed for 24 hours.

b. Enzyme Linked Immunosorbent Assay (ELISA):

Indirect ELISA was used to detect the antibodies against toxin of *C. perfringens* type A, according to Harlow and Lane (1988). The antibody titers were determined by a least squares-weighted modification of the parallel line model according to Grabowska *et al.*, (2002).
Table 1: Effect of using different anti foam agents in toxin production medium of *C. Perfringens* type A

<table>
<thead>
<tr>
<th>Anti-Foaming Agent</th>
<th>Minimum Lethal Dose (MLD)/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethylene Glycol 6000</td>
<td>90</td>
</tr>
<tr>
<td>Simethicone</td>
<td>120</td>
</tr>
<tr>
<td>Charcoal</td>
<td>30</td>
</tr>
<tr>
<td>Control group</td>
<td>60</td>
</tr>
</tbody>
</table>

MLD: Minimum Lethal Dose

Table 2: Vaccines composition for 2ml dose for rabbits

<table>
<thead>
<tr>
<th>Types of Vaccines</th>
<th>MLD/ml</th>
<th>Volume of toxoid/ml</th>
<th>Volume of Gel/ml</th>
<th>Volume of sterile distilled water/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine #1 (Polyethylene glycol)</td>
<td>90</td>
<td>0.66</td>
<td>0.4</td>
<td>0.93</td>
</tr>
<tr>
<td>Vaccine #2 (Simethicone antifoam)</td>
<td>120</td>
<td>0.5</td>
<td>0.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Vaccine #3 Control group</td>
<td>60</td>
<td>1</td>
<td>0.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

MLD: Minimum Lethal Dose

Table 3: Antibody titers measured by toxin neutralization test in sera of rabbits vaccinated with different types of vaccines

<table>
<thead>
<tr>
<th>Types of vaccines</th>
<th>Antibody titers expressed as IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine #1 (containing Polyethylene glycol)</td>
<td>5</td>
</tr>
<tr>
<td>Vaccine #2 (Simethicone antifoam)</td>
<td>5</td>
</tr>
<tr>
<td>Vaccine #3 Control group</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 4: antibody titers measured by ELISA in sera of rabbits vaccinated with different types of vaccines

<table>
<thead>
<tr>
<th>Types of vaccines</th>
<th>Antibody titers expressed as ELISA Units/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine #1 (containing Polyethylene glycol)</td>
<td>5.9</td>
</tr>
<tr>
<td>Vaccine #2 (Simethicone antifoam)</td>
<td>5.7</td>
</tr>
<tr>
<td>Vaccine #3 Control group</td>
<td>5.5</td>
</tr>
</tbody>
</table>
3. RESULTS

There was double increase in Minimum lethal dose of α toxin of *C. perfringens* type A (120 MLD/ml) when simethicone was used as anti-foam in toxin production medium in comparison with control medium that gave (60 MLD/ml), followed by Polyethylene glycol gave higher MLD (90 MLD/ml) than control one. While charcoal gave minimum MLD (30 MLD/ml) also lower than control group, (table 1). Preparation of vaccines against *C. perfringens* type A using different anti-foam agents in medium with fixed dose of 60 MLD/ml, accordingly using 0.66ml; 0.5ml, and 1.0ml from *C. perfringens* type A toxoid with polyethylene glycol 6000; simethicone, and control group respectively. As showed in table (2). Tables (3 & 4) illustrated antibody titers measured by toxin neutralization test and ELISA in sera of vaccinated rabbits with *C. perfringens* type A toxoid. Antibody titers were 5 IU/ml in all 3 groups of vaccinated rabbits measured by toxin neutralization test, and antibody titers were 5.9; 5.7, and 5.5 IU/ml in groups (1, 2, and 3) respectively measured by ELISA.

4. DISCUSSION

Foam is the dispersion of a gas in a continuous liquid phase, and thus foam dispersions possess bulk densities closer to that of a gas rather than a liquid (Vardar-Sukan, 1991). A general definition of foam, applicable to bioreactors, determines foam to occur when gas holdup in a gas-liquid dispersion is greater than 90% (Schubert et al., 1993). Other authors have quantified the gas content of foam to be in the range of 60-90% (Van’t Riet and Tramper, 1991). Anti-foam are defined as strongly surface active substances, which replace foam forming components and lower surface tension of liquids. Anti-foams are dispersed by stirring and foam is destroyed by bubble coalescence, which decrease the available surface area for gas liquid mass transfer (de Haut, 2001). Anti-foams typically are added to medium or broth before foaming occurs, it was used to knock down foam after it has formed (Ghildyal et al., 1988). As showed in table (1) there was double increase in minimum lethal dose of α toxin of *C. perfringens* type A (120 MLD/ml) when simethicone was used as anti-foam in toxin production medium in comparison with control medium that gave (60 MLD/ml), followed by Polyethylene glycol gave higher MLD (90 MLD/ml) than control one. Preferred anti-foaming agents are Dimethicone and simethicone. Simethicone is described in the USP dictionary of USAN and International Drug Names, US Pharmacopeia as a mixture of poly (dimethylsiloxane) and silicone dioxide, the calculated average of dimethylsiloxane units is 200 to 350, and also non-irritating and biocompatible, so that is meant non-damaging to tissue (Carter et al., 2001). From the above results shown in table (1) that using of charcoal as antifoam had negative effect on toxin production. This may be due to adsorption of protein content in medium resulting in precipitation of protein and also insolubility in medium. Therefore preparation of vaccine from it must be excluded. Preparation of vaccines from toxin production media containing antifoams (Polyethylene glycol and Simethicone) and control medium devoid of antifoam shown that in table (2) so using fixed dose of 60 MLD/ml for each vaccine. Results illustrated in tables (2 & 3& 4) revealed that using of specific dose of 60 MLD/ dose gave antibody titers of 5 IU/ml and this titer is over the permissible limits (4 IU/ml) according to United States Department of Agriculture (USDA) USDA (2002), which surpass requirements to receive a conditional license pass standardized test by the development of a serum antibody concentration of at least 4 international antitoxin units per ml in at least 80 % of vaccinated animals that were seronegative prior to vaccination. So that using *C. perfringens* type A toxoid for vaccination
of rabbits and poultry was allowed, and also from the results that using of half the dose of control group when using Simethicone as antifoam in toxin producing medium gave also 5 IU/ml, which meet requirements of vaccine potency. Therefore, reduce the cost of vaccine into the half. There was great correlation between results obtained from toxin neutralization test and ELISA for measuring antibody titers and this come with accordance with Grabowska et al., (2002) who found that the calculation of ELISA titer by weighted parallel line model is preferable for the evaluation of antibody level for ELISA data, particularly for samples with medium and low antibody levels. They suggested using of ELISA as replacement test for toxin neutralization test as a precise, accurate and not require the use of laboratory animals, which is a global trend for reducing using lab animals.

It could be concluded from the above results that, using of simethicone as anti-foam in toxin producing medium of \textit{C. perfringens} type A is recommended as it produce high level of toxin production and reduced the dose of vaccine used.

5. REFERENCES


Effect of using different antifoams on toxin production of C. perfringens type A.


Smith, L.D., and Holdman, L.V. (1968): The pathogenic anaerobic bacteria. Charles Thomas Publisher, USA.


USDA (2002): Center for Veterinary Biologics Notice NO. 02-25, Conditional Licenses for Products Containing Clostridium perfringens Type A.


تأثر استخدام مضادات الرغوة على إنتاج السم المفرز من الكلوسترديم بيرفينجينز نوع أ.

إلهام فضل السراجاني – مدحت محمد طه – حامد عادل الحلو - هالة الصاوي أحمد
معهد بحوث الأصل واللقاحات البيطرية - العباسية - القاهرة

المتخصص العربي

في هذه الدراسة تم استخدام ثلاثة مواد من مضادات الرغوة (بولي إيثيلين جليكول، ساميثيكون، فحم النباتي) في عملية تحضير توكسيد الألفا لعترة الكلوسترديم بيرفينجينز نوع (أ). وجد عند إضافة ساميثيكون وبولي إيثيلين جليكول إلى الوسط الغذائي المخصص لإنتاج السم أنها أعطت ناتج عالي من سم الألفا لعترة الكلوسترديم بيرفينجينز نوع (أ) بالمقارنة بالوسط الغذائي المستخدم بدون مضادات الرغوة. ولكن عند استخدام المحمض النباتي أعطت أقل ناتج من سم الألفا. تم تحضير ثلاثة لقاحات لتوكسيد الألفا لعترة الكلوسترديم بيرفينجينز نوع (أ) الأول باستخدام بولي إيثيلين جليكول والثاني باستخدام ساميثيكون أما الثالث بدون مضاد رغوة (كمجموعة ضابطة) وباختيار اللقاحات الثلاثة في الأربات وجد أنها أعطت خمسة وحدات دولية/مللي في كل اللقاحات على الرغم من استخدام نصف الجرعة بالنسبة لللقاح المحضر باستخدام ساميثيكون.

(مجلة بنها للعلوم البيطرية: عدد 27(1):1-7، سبتمبر 2014)