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Clostridium Perfringens Toxins

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Abstract

Clostridium perfringens is an important pathogen known to cause critical clinical disease. *C. perfringens* type A strains produces two types of toxins which is alpha-toxin and endotoxin. They have different attachment, internalization mechanisms and structures that lead them to diverse functions and several diseases. Alpha-toxin is a protein produces by the bacteria and cause gas gangrene once it invades the cell bilayer membrane's fluidity. However, endotoxin cause Gastrointestinal (GI) Disease by rapid pores creating, invasion of the toxin to multiple receptors in intestine lumen, tight junction disruption. Although there are similarities in general molecular structure for both toxins, they obtain unique differentiae in the active domains. However, alpha-toxin and endotoxin have endogenous phospholipase C (PLC) and C-terminal domain that responsible for the virulence biological activities respectively. Despite that low dose of the endotoxins leads to apoptosis and high dose leads to oncosis, the endotoxin has been used now in clinical purposes such as cancer therapy and vaccination. Additionally, *C. perfringens* is not only foodborne human diseases but also non-foodborne disease since it has been founded in soil, water and animal's gut.

Food poising can be prevented successfully through rapid cooking from 55 to 15°C within 90 minutes and store in refrigerator/freezer temperatures. This paper will illustrate the mode of action of both toxins and how they lead to cause illness.

Keywords: Clostridium perfringens; alpha-toxin; C-domain; central loop domain, CPE, superoxide production

Introduction

Clostridium perfringens is a Gram-positive, encapsulated, spore forming, non-motile, anaerobic bacterium. It inhabits in anaerobic environments like inside the gut of humans or

animals and in the soil. There are 5 types: a, b, c, d, e [4, 6]. Approximately 5% of all the *C. perfringens* strains produce *C. perfringens* enterotoxin (CPE). Most CPE positive strains are *C. perfringens* A strains. They are responsible for mild to moderate food poisoning in humans [1]. *C. perfringens* A strains are also important as they are causative agents of gas gangrene and also cause hemolysis, aggregation of platelets, O_2^- generation, and blood vessel contraction [2, 8].

Types of C. perfringens toxins [5,19]:

Toxinotype	Typing toxins			
	Alpha a	Bet	Epsilon	Iota
A	X			
B	X	X	X	
C	X	X		
D	X		X	
E	X			X

1. Alpha toxin: Alpha toxin belongs to a family of bacterial zinc- metallo phospholipase C enzymes [7]. It is a crucial virulence factor for the organism. A three dimensional analysis of

alpha toxin revealed that the structure is divided into 2 main domains- N-domain consisting of nine tightly packed α helices and C-domain is made of eight –stranded antiparallel β-sandwich motif as shown in Figure 1[2].

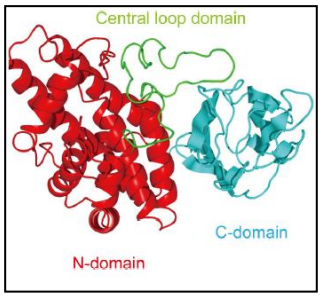

Figure 1: Three domains of *C. perfringens* alpha toxin

C-domain plays a role in binding to membranes [15]. Binding of toxin to liposomes and hydrolysis of phosphatidylcholine (PC) in liposomes is dependent on phase transition temperature (Tm) of PC. The membrane damage activity of alpha toxin is also dependent on membrane fluidity[17]. The central loop domain (55-93 aa) has an important role of interacting with ganglioside GM1a [13]. The binding of alpha toxin to membrane takes place in presence of GM1a/Trk A complex activating PLCγ-1 through Trk A [2,10,14]. Endocytosis of alpha toxin takes place to activate MEK/ERK pathway finally leading to cytotoxicity and cell death (Figure 2 a) [11,12]. In ganglioside deficient cells release of 1,2, diacyl glycerol(DG) and ceramide from the membrane leads to endocytosis(Figure 2 b)[1,18].

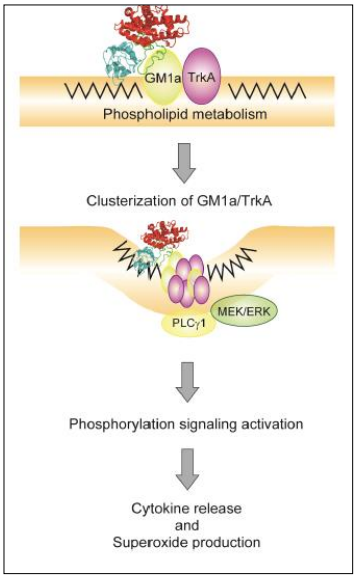

Figure 2a:alpha toxin lead to superoxide production

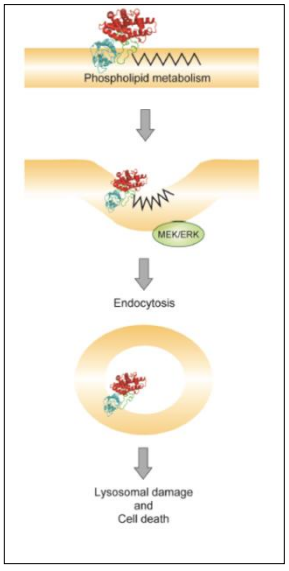

Figure 2b: alpha toxin lead to cell death

Moreover, Alpha toxin can cause septic shock and hypoxia. There is degeneration of tissue and muscles causing buildup of gas. Leucocytes cannot reach infection site due to clogging of blood

vessels [3]. Alpha toxin has phospholipase C and sphingomyelinase enzymes that are responsible for phosphatidylcholine (PLC) and sphingomyelin (SM) hydrolysis[16]. PLC and SM are components of eukaryotic membranes and after their hydrolysis DG and ceramide are generated respectively. The toxin induces carboxyfluorescein (CF) leakage and phospharylcholine (PC) release. This promotes cell lysis and finally death (Figure 3).

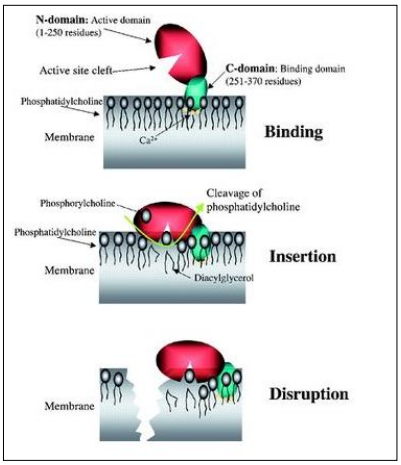

Figure 3: Alpha toxin attachment and insertion into host cell [3].

Alpha toxin induces production of intercellular mediators –intercellular adhesion molecule 1, IL-8, TNF-α, platelet activating factor, leukocyte adhesion molecule. Due to this there is increased vascular permeability and edema [3].

2. *Clostridium perfringens* enterotoxin (CPE): CPE is responsible for diarrhea and type A food poisoning in humans. Inactivation of *cpe* gene in the human food poisoning strain leads to generation of an virulent strain. Figure 4 shows the histologic damage induced by CPE positive strain SM101(food poisoning strain) . The eosin and hematoxylin stained tissue sections of rabbit ileal loop are shown in the figure below. Concentrated vegetative culture lysates or Duncan-

Strong (DS) sporulation culture lysates of SM101 or MRS101 (*cpe* null mutant of SM101) were compared. No damage in SM101 vegetative lysate and MRS101 mutant while complete villus damage, necrosis and epithelial desquamation was observed with wild type DS SM101 and complementing strain (Figure 4)[1,19].

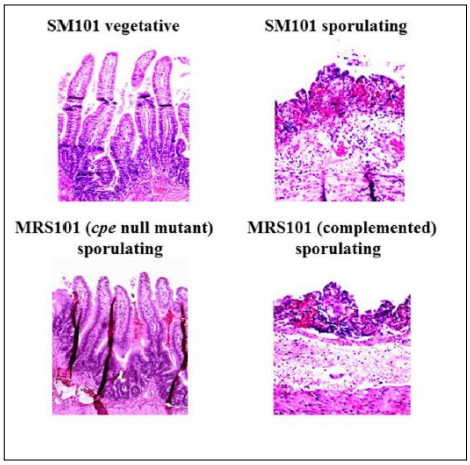

Figure 4: Tissue sections of rabbit ileal loop

The Structure of CPE monomer: There are two domains of CPE monomer consist of C-terminal CPE binding domain (yellow-red) as shown in Figure 5 and N-terminal oligomerization or membrane insertion domain. N terminal domain is important for cytotoxicity and pore formation.

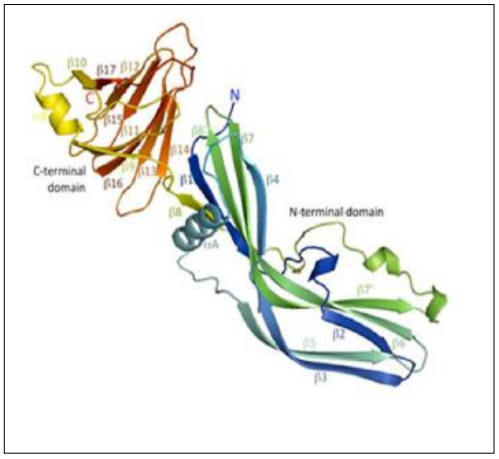

Figure 5: The Structure of CPE monomer

The CPE is produced only during sporulation. *C. perfringens* undergoes asymmetrical cell division during adverse conditions giving rise to dormant spores. The spores can tolerate harsh cooking environments like heat, acidity etc. The survived spores germinate in the contaminated food and grow in large numbers. They are consumed along with contaminated food and after passing through acidic conditions in stomach the vegetative cells again speculate in intestine releasing CPE and cause food poisoning.

Mechanism of action of CPE: CPE first binds to claudin receptors depicted as green boxes that are present on apical surface of host cells and there is formation of a small . Due to formation of several small complexes CPE oligomerization and a prepore formation on the plasma membrane takes place. Active pore CH-1 is formed due to beta barrel. Low dose CPE causes some calcium ion influx leading to cytoplasmic calpain activation and caspase 3 mediated apoptosis. High dose of CPE causes large Ca^{2+} influx and high cytoplasmic calpain activation inducing cell death via oncosis [1]. Figure 6 shows the mechanism action of CPE. This leads to formation of second large complex CH-2 that promotes internalization of occludin and claudin into the cytoplasm.

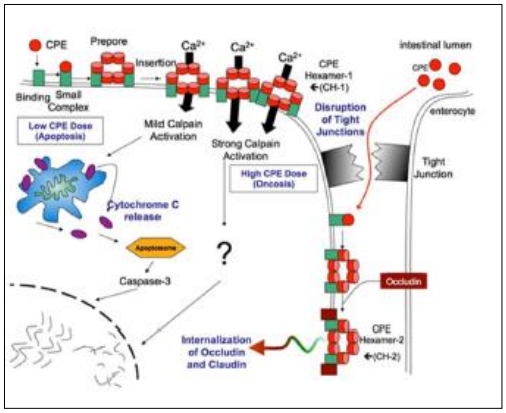

Figure 6: Mechanism of action of CPE

For prevention of CPE the food should be thoroughly cooked at high temperatures. Rapid cooling (within 90 minutes of preparation) of cooked food for storage purpose should be followed. Precooked foods should be reheated at >70^{o}C.

Conclusion

Clostridium perfringens A produce alpha toxin and enterotoxin. Alpha toxin is responsible for fatal disease like gas gangrene and enterotoxin is responsible for food poisoning. Both have unique structures with one binding domain and other catalytic domain. In my opinion, this toxin can be used in blocking the receptors and apoptosis cells especially for cancer disease who already known by its fast disruption. Moreover, I assume that genomic mutation could be applied to the toxin to make positive changes such as cpe gene mutation that have no negative impact on the intestine cell membrane as the experiment explained previously. In addition, changes in toxin gene expression by mutate the gene coding may lead to evolutionary discovery in medical and therapy filed. The toxins are important virulence factors of *C. perfringens*. Furthermore, the alpha-toxin also attacks immune system of the host leading to complete necrosis of tissue and muscle and produces superoxide molecules leading to death. Lastly, Food poisoning can be avoided using properly cooked and stored food and then prevent the whole diseases types correspond to the toxin.

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