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Vaccine

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Effect of different detoxification procedures on the residual pertussis toxin activities in vaccines

Chun-Ting Yuen [a], Catpagavalli Asokanathan [b], Sarah Cook [b], Naomi Lin [b], Dorothy Xing [b]

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Abstract

Pertussis toxin (PTx) is a major virulence factor produced by Bordetella pertussis and its detoxified form is one of the major protective antigens in vaccines against whooping cough. Ideally, PTx in the vaccine should be completely detoxified while still preserving immunogenicity. However, this may not always be the case. Due to multilevel reaction mechanisms of chemical detoxification that act on different molecular sites and with different production processes, it is difficult to define a molecular characteristic of a pertussis toxoid. PTx has two functional distinctive domains: the ADP-ribosyltransferase enzymatic subunit S1 (A-protomer) and the host cell binding carbohydrate-binding subunits S2–5 (B-oligomer); and in this study, we investigated the effect of different detoxification processes on these two functional activities of the residual PTx in toxoids and vaccines currently marketed worldwide using a recently developed *in vitro* biochemical assay system. The patho-physiological activities in these samples were also estimated using the *in vivo* official histamine sensitisation tests. Different types of vaccines, detoxified by formaldehyde, glutaraldehyde or by both, have different residual functional and individual baseline activities. Of the vaccines tested, PT toxoid detoxified by formaldehyde had the lowest residual PTx ADP-ribosyltransferase activity. The carbohydrate binding results detected by anti-PTx polyclonal (pAb) and anti-PTx subunits monoclonal antibodies (mAb) showed specific binding profiles for toxoids and vaccines produced from different detoxification methods. In addition, we also demonstrated that using pAb or mAb S2/3 as detection antibodies would give a better differential difference between these vaccine lots than using mAbs S1 or S4. In summary, we showed for the first time that by measuring the activities of the two functional domains of PTx, we could characterise pertussis toxoids prepared from different chemical detoxification methods and this study also highlights the potential use of this *in vitro* biochemical assay system for in-process control.

Introduction

Pertussis toxin (PTx) is a major virulence factor produced by *Bordetella pertussis* and is one of the major protective antigens in vaccines against whooping cough. Ideally, PTx in the vaccine should be completely detoxified while still preserving immunogenicity. However, this may not always be the case and a safety test for residual PTx activities present in these vaccines are required by authorities before they are released to the public. PTx has two functional distinctive domains: the enzymatic subunit S1 (A-protomer), which is an ADP-ribosyltransferase and the subunits S2–5 (B-oligomer), which is a carbohydrate binding molecule that facilitates host cell binding and entry of PTx into the cells [1]. The current detoxification processes include chemical treatments of the toxin with formaldehyde and/or glutaraldehyde, hydrogen peroxide and genetic detoxification [2]. Formaldehyde reacts with side chains of lysine, arginine, cysteine, histidine and N-terminal amino acids and formaldehyde-glycine Schiff-base adduct could also react with the primary N-terminal amino group, arginine, tyrosine as well as asparagine, glutamine and tryptophan [3]. Glutaraldehyde is a bifunctional protein cross-linking reagent and reacts similarly to formaldehyde to form more complex high molecular weight protein species [4], [5], [6], [7]. In PTx toxoid prepared by oxidation of hydrogen peroxide, nearly all methionine and cysteine were oxidised and tryptophan, histidine, tyrosine and asparagine were also modified [8]. Genetic detoxification of PTx could be achieved by substitution of two amino acids (9K/129G) at the enzymatic active site in subunit S1 and the resultant recombinant inactive PTx molecule could then be stabilised by low dose of formaldehyde [9].

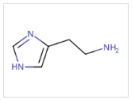
Due to multilevel reaction mechanisms of chemical detoxification, characterisation of sites of modification of the resultant PTx toxoid (PTd) is often difficult. Varying degrees of alternation either an enhancement or a decrease in the PTx antibody binding or neutralising epitope of the PTd had been reported [2]. Presence of heterogeneous high molecular weight species had also been shown in PTds detoxified with increasing formaldehyde concentration [10]. In a recent study of chemically detoxified PTx using a patented procedure [11] with formaldehyde, glutaraldehyde and both, Oh et al. [12] found that different aldehydes could affect differently on the PTx functional domains. They showed that formaldehyde inactivated the enzymatic subunit S1 better than glutaraldehyde while the opposite was true for the carbohydrate binding subunits S2–5. The authors could also demonstrate that under their detoxification conditions, the stability or reversion of the toxoids were different between the chemical reagents.

In one of our earlier studies on carbohydrate binding activity of PTx and PTd, we demonstrated that a glutaraldehyde-detoxified PTd lost almost all of its ability to bind trisialylated tri-antennary *N*-glycans but the binding capacity to neutral tri-antennary *N*-glycans was less affected [13]. The degrees of carbohydrate binding activities had also been shown to relate to local reactogenicity due to changes to vaccine detoxification and production methods in Japan [14]. This clearly illustrates that different production methods of PTd would result in vaccines with different biochemical and biological residual PTx activities. Here we reported our further investigation into the functional changes of PTx in different PTds and pertussis vaccines, of which some are currently marketed worldwide, using the *in vitro* biochemical and *in vivo* biological assay methods

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Reference pertussis toxin

B. pertussis toxin (PTx, NIBSC Code: 90/518) was prepared in-house at National institute for Biological Standards and Control (NIBSC). This preparation has a nominal protein concentration of 20μg/ampoule and an assigned unitage of 2,100 IU/ampoule calibrated against the 1st IS, JNIH-5 by histamine sensitisation test (HIST) [15]. …

Vaccines and toxoids

Acellular pertussis vaccines used in this study was all licensed, commercially available products (Table 1). The pertussis antigens PTd, filamentous haemagglutinin …

ADP-ribosyltransferase activities in PTd containing vaccines

ADP-ribosyltransferase activities were measured in 49 (V1), 6 (V2) and 8 (V3) batches of G-, F- and (G+F)-detoxified vaccines respectively (Table 1; Fig. 1). Of the 3 types of vaccines, F-detoxified vaccines (V2) gave the lowest mean enzyme activity at 16±6mEU/μg toxoid. The G-detoxified vaccines (V1) gave the highest mean enzyme activity at 104±40mEU/μg toxoid and the (G+F)-detoxified vaccines (V3) gave an intermediate mean enzyme activity at 55±16 mEU/μg toxoid. All these vaccines were noted …

Discussion

Mechanism of inactivation of bacterial toxins by chemical treatments of cross-linking agents such as formaldehyde and glutaraldehyde is often complex, difficult to control and could result in a wide array of molecules with varying degrees of different cross-linkages and aggregation [2], [19]. The exact sites and extents of modification are therefore difficult to characterise. Pertussis toxin molecule has two biochemically active functional domains: the carbohydrate binding B-oligomer (subunits …

Authors' contribution

Chun-Ting Yuen, Catpagavalli Asokanathan and Dorothy Xing contributed to the concept, design, analyses, interpretation of the study and drafting of this manuscript. Sarah Cook and Naomi Lin performed most of the *in vitro* biochemical assays. Catpagavalli Asokanathan and Dorothy Xing performed all the *in vivo* HIST. …

Acknowledgement

We are grateful for the support provided by the Biological Services Division at NIBSC. …

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Quantitative determination of bioactive proteins in diphtheria tetanus acellular pertussis (DTaP) vaccine by liquid chromatography tandem mass spectrometry

2019, Journal of Pharmaceutical and Biomedical Analysis

> *Citation Excerpt :*
> ...Detoxification is necessary for almost all bioactive proteins of DTaP vaccine. Mechanism of inactivation of these proteins is complicated and difficult to control [36,37]. In this study, the LC–MS/MS method developed was used to determine non-detoxified and detoxified proteins (Table 7)....

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