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THE LEVEL OF ENDOTOXIN CONTAMINATION IN BIOPREPARATIONS

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This study is concerned with detection of the bacterial endotoxin as a contamination of various virus and bacterial vaccines. The LAL test (Limulus Amoebocyte Lysate) with S-2423 substrate was applied.

INTRODUCTION

The assay of pyrogens is one of the elements of control and safety of biopreparations. The presence of bacterial endotoxin in the biopreparations may be an indicator of their pyrogenicity.

Our former studies (l, 2, 3) were aimed at checking the applicability of the LAL test (Limulus Amoebocyte Lysate), gel and quantitative with chromogenic substrate (S – 2423), for bacterial endotoxin detection in various biopreparations (vaccines, immunoglobulins, albumins, antibiotics).

The aim of the present study was to test the effect of some compounds included in vaccines (aluminium hydroxide, formaldehyde, merthiolate) on development of color reaction in test between amoebocyte lysate, endotoxin and chromogenic substrate; an attempt was also made to determine the level of bacterial endotoxin in biopreparations.

MATERIALS AND METHODS

Material. Standard endotoxin E. coli ET101 (manufactured by Chromogenix) was used for an evaluation of the accuracy of the LAL test. Al.$(OH)_3$ (Alhydrogel), 1 % formaldehyde (lot: 7/96) and merthiolate (lot: 1I10697) obtained from the Serum and Vaccine Production Plant (Cracow, Poland) were used as components of some vaccines for testing the effect on color reaction in the LAL test. The presence of endotoxin was assayed in the following groups of biopreparations: group I – virus vaccines against hepatitis A and B (12 lots); group II – various virus vaccines (influenza, measels, rubella, mumps, meningitis, combined measles + mumps + rubella) (24 lots); group III – toxoids: 1) monovalent (Te, Di, d), 2) Al$(OH)_3$ adsorbed (14 lots) and nonadsorbed (1 lot), bivalent (Di-Te, Td) Al$(OH)_3$ adsorbed (8 lots) and nonadsorbed (1 lot); 3) combined DTP adsorbed on Al$(OH)_3$ (10 lots); group IV – various types of bacterial vaccines (6 lots): polyvalent pneumococcal , Haemophilus influenzae, DTPer+HBsAg vaccines. A total of 77 preparations were assayed.

Methods. The bacterial endotoxin in the above biopreparations was detected by quantitative LAL test (Coatest Endotoxin, manufactured by Chromogenic) with S-2423 substrate, the procedures used being as in previous studies (1, 2). The principle of method is that endotoxin activates a number of enzymes (LAL reagent), which cause a split of substrate and release of pNA, proportional to the endotoxin concentration. Absorbance was measured photometrically at 405 nm, by Ultraspec Plus spectrophotometer (Pharmacia – LKB). Endotoxin contents were read from a standard curve of endotoxin E. coli ET 101, which was linear in the range 0,15 – 1,2 EU/ml.

RESULTS AND DISCUSSION

As found in a pilot study, standard curve for endotoxin ET 101 shows linear relation between absorbance (A_{405}) of the released p-nitroaniline; the endotoxin concentration is expressed as EU/ml. With 12 – 13 determinations for each of the 4 concentration points at standard curve (0,15; 0,3; 0,6; 1,2 EU/ml), standard deviation (SD) from the arithmetic means ($\bar{x}$) was within the limits of 0,015 – 0,074 and standard error (SE) of 0,024 – 0,05. Calculation by Ch^2 distribution showed differences in readings at the individual concentrations of the standard curve to be statistically insignificant and to have no effect on endotoxin values. The calculated recovery amounted to 98 – 102 %, what confirms precision of the quantitative technique applied.

A study on the effect of formaldehyde, $Al(OH)_3$ or merthiolate which are components of some vaccines, on the development of color reaction in the LAL test showed these chemicals to have no effect on the course of the reaction, or their effect being of little importance.

At the present time, major part of biopreparations have no defined limits of the admissible content of bacterial endotoxin. Some producers only give requarments for their own preparation; e.g. among virus vaccines tested, only 3 had proper requairements (Tab. I).

In group I – virus vaccines for hepatitis A (3 lots), endotoxin was detected in an amount of 0,0; 0,08; 7,75 EU/ml, with the corresponding limit of 30 EU/ml. In 8 lots of hepatitis B vaccine (Engerix B) endotoxin was not detected; only in one vaccine (H-B-Vax recomb) it was present at a level of 1,47 EU/ml. In group II – various virus vaccine, higher differentiation in the endotoxin contens was observed in the individual vaccines, as can be seen in Table I.

Bacterial endotoxin was not detected in the inlluenza vaccines (Vaxigrip, Fluarix) and in Trimovax vaccine (measles, mumps, rubella), as well as in four out of the five measles vaccines (Rouvax). Endotoxin amounts detected in all other preparations ranged from 0,06 to 76,56 EU/ml, but they did not exceed the defined limits. However, in the individual lots of influenza vaccine (Influvac) high dispersion of the results for endotoxin concentrations was observed (5,52 – 76,56 EU/ml), though the values also were within normal limits.

The results for endotoxin detected in toxoid preparations and combined vaccines (group III) and in other types of bacterial vaccines (group IV) are presented in Table II.

In group II in monovalent toxoid preparations adsorbed on $Al(OH)_3$, high dispersion in endotoxin contens detected was observed, from 1,36 to 64,26 EU/ml. The dispersion was much higher in case of bivalent preparations, from 0,0 to 605,54 EU/ml.

Table I. *The Content of endotoxin estimated by LAL test (Coatest Endotoxin – Chromogenix) in various types of virus vaccines.*
Zawartość endotoksyny oznaczonej za pomocą testu LAL (Coatest Endotoxin – Chromogenix) w szczepionkach wirusowych.

Group	Preparation	Manufacturer	Number of samples	The level of endotoxin EU/ml	The limit of endotoxin EU/ml
I	**Hepatitis A vaccine:**				
	Havrix™	SKB	2	0,0–0,08	≤ 2
	Avaxim	P-M	1	7,75	not specified
	Hepatitis B vaccine:				
	Engerix™ – B	SKB	8	0	≤ 30
	H-B-VAX recomb.	MSD	1	1,47	≤ 10
II	**Influenza vaccine:**				
	Vaxigrip	P-M	2	0	< 100
	Fluarix	SKB	1	0	≤ 200
	Influvac	SD	4	5,52–76,56	≤ 100
	Measle vaccine:				
	Rouvax	P-M	5/1	0/2,88	not specified
	Rubella vaccine:				
	Rudivax	P-M	3	0,0–2,4–3,5	not specified
	Mumps vaccine:				
	Mumps Vax	MSD	2	0,15–2,25	not specified
	Measles, Mumps, Rubella Vaccine:				
	Trimovax	P-M	2	0,0–0,06	not specified
	M-M-R. II™	MSD	1	7,03	
	Virus Meningoencephalitis vaccine:				
	FSME-IMMUN	Immuno AG	2	0,11–0,13	not specified
	Encepur	Be	1	0,12	< 2
Total			36		

Legends
Manufacturers: SKB → SmithKline–Beecham; PM → Pasteur Merieux; MSD → Merck-Sharp-Dohme; SD → Solvay-Duphar, Be → Behring

Table II. The content of endotoxin estimated by LAL test (Coatest Endotoxin – Chromogenix) in various types of bacterial vaccines.
Zawartość endotoksyny oznaczonej za pomocą testu LAL (Coatest Endotoxin – Chromogenix) w szczepionkach bakteryjnych.

Group	Preparation	Manufacturer	Number of samples	The level of endotoxin EU/ml	The limit of endotoxin EU/ml
III	**Toxoids adsorbed on $Al(OH)_3$:**				
	d	Biomed-Cracow	2	14,28–19,04	
	Te	Biomed-Cracow	6	1,36–64,26	
	Te	Biomed-Warsaw	6	8,50–33,32	not specified
	Di-Te*	Biomed-Cracow	5	223,24–605,54	
	DT	Biomed-Cracow	1	20,06	
	Td	Biomed-Cracow	2	0,0–19,38	
	Polyvalent vaccines:				
	DTP	Biomed-Cracow	10	1382,4–1607,0	
	Toxoids non adsorbed:				
	Di CB 3/96	Biomed-Cracow	1	122,4	
	Te KT 5/96	Biomed-Cracow	1	136,8	
	DT*	Biomed-Cracow	1	57,6	
IV	**Polyvalent pneumococcal vaccine:**				
	Pneumo-23	P-M	1	0,32	< 200
	Haemophilus influenzae type B conjugate vaccine:				
	Act-HIB	P-M	3	29,25–75,57	not specified
	Haemophilus influenzae type B vaccine:				
	Hiberix	SKB	1	0,528	< 30
	Vibrio cholerae vaccine	Biomed–Warsaw	1	1656,00	
	Polyvalent vaccines DTPer + HBsAg vaccine:				
	Tritanrix	SKB	1	1506,00	not specified
Total			41		

Legends * two lots made of Di CB 3/96 and Te KT 5/96 components
Manufacturers: PM → Pasteur Merieux; SKB → SmithKline–Beecham

The combined DTP vaccines contained 2 – 2,5 times more endotoxin (1382,4 – 1607,0 EU/ml) than the highest endotoxin levels found in Di-Te. Such a result was presumably due to the pertussis component (Per), as in the V. cholerae vaccine (group IV) it was due to the presence of V. cholerae endotoxin.

In case of nonadsorbed monovalent toxoid Di and Te the amounts of endotoxin detected was 122,4 and 136,8 EU/ml, respectively.

In DT preparation, also nonadsorbed, prepared from the same components, 2,1 – 2,3 times less endotoxin (57,6 EU/ml) was detected than in the single components.

However, combination of the components into bivalent Di – Te preparation and adsorption on $Al(OH)_3$ gel was followed by an increase in the level of endotoxin to 277,2 EU/ml and 419,0 EU/ml, while $Al(OH)_3$ gel itself gave negative reaction with amoebocyte lysate and S-2423. It can be suggested, therefore, that toxoid adsorption on $Al(OH)_3$ may have an effect on the results of reaction in the LAL test.

At the present time, pharmacopeal requirments that would define the degree of endotoxin contamination of toxoids have not yet been specified. In further works, concerning determination of these requirements for endotoxin contens in bacterial vaccines, it would be purposefull to include studies on the selection of suitable type of LAL test (different producers, various lots of preparations, tests differing insensibility)for determining endotoxin in complex preparations.

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Summary

Bacterial endotoxins as contamination of biopreparations have been estimated by chromogenic LAL test. Study on some compounds (aluminium hydroxide, formaldehyde and mertiolate) being components of vaccines showed no effect on the result of LAL test. The level of endotoxins in virus vaccines with the limits defined in producers certificate was adequate, the level of endotoxin was also low in virus vaccines of undefined requirements. The concentration of endotoxin in bacterial vaccines was differentiated. Considering the results of our experiments, as well as the fact, that the requirements for endotoxin contamination of bacterial vaccines are not available it seems necessary to establish the limits for these group of biopreparations.

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POZIOM ZANIECZYSZCZENIA ENDOTOKSYNĄ BIOPREPARATÓW

Streszczenie

Oceniono poziom zanieczyszczenia endotoksyną bakteryjną biopreparatów stosując test chromogeniczy LAL. Badanie niektórych wyodrębnionych składników szczepionki (wodorotlenek glinu, formaldehyd, mertiolat) wykazało, że nie mają one wpływu na wynik w teście LAL. Poziom endotoksyn w szczepionkach wirusowych mieścił się w granicach limitu deklarowanego przez producenta. W szczepionkach wirusowych nie posiadających jeszcze takich wymogów zawartość wykrytej endotoksyny była również niska. Stężenie endotoksyny wykrytej w szczepionkach bakteryjnych było zróżnicowane. Uwzględniając wyniki naszych badań oraz

brak wymagań dla dopuszczalnego poziomu zanieczyszczeń w szczepionkach bakteryjnych wydaje się niezbędne opracowanie norm dla tych grup biopreparatów.

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