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Quantitative measurement of endotoxin, haemolysis and water extractable protein from four brands of surgical latex gloves

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Abstract

In this study, an effort was made to evaluate the quantity of water extractable protein, endotoxin level and percentage haemolysis from four different brands of commercially available surgical latex gloves. The estimation of protein, endotoxin content and percentage haemolysis was carried out as per International standard. The result of the study indicated that the protein, endotoxin and percentage haemolysis were under acceptable limit. Hence, it can be concluded that the protein content of all the four brands of surgical latex gloves can be considered as pyrogen free and non hemolytic, hence safe.

Keywords: Blood compatibility, endotoxin, hypersensitivity, immunogenic, latex gloves, water extactable protein

Introduction

Surgical gloves offer barrier protection for both health care workers and patients against contact with blood, body fluids and microorganisms. Therefore surgical gloves must be safe and effective for the intended use. Allergic potential of latex products with reference to latex protein has recently become a parameter of importance in the manufacturing of safer latex products. Exposure to latex medical products caused multitude of allergic reactions ranging from non-immunogenic irritation (Type1V) to life threatening (Type 1) hypersensitivity reactions. Water soluble protein is believed to be one among the allergens, which is heat stable and is present on the surface of the latex gloves. Cornstarch powder in the gloves may bind the latex protein in the surgical gloves, which allows the antigen to reach both the wearer's skin (more easily when the hand becomes moist during surgery) and patient's skin (Beezhold and Beck, 1992). Gloves with high extractable protein contents are generally associated with high allergen contents (Swanson *et al.*, 1994), while those with low extractable proteins tend to have low allergen contents. There are three major types of latex related immunological reactions or latex allergy they are i) irritant dermatitis, ii) delayed hypersensitivity (Type IV allergy) and iii) immediate reaction (Type I allergy) (Beezhold and Beck, 1992)

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Endotoxin is an inflammatory agent and will be able to produce irritation on skin, induce respiratory problems, fever and shock. It is an adjuvant for both delayed hypersensitivity and IgE production and has been shown to magnify antigen specific mediator release (Beutler, 1989 and Nordan, 1986). Specific cellular and humoral receptors exist for endotoxins that enhance their ability to induce pro-inflammatory cytokines and chemokines such as TNFá, IL-6, and IL-8 (Tobias *et al.,* 1989 and Nordan, 1986). In addition, endotoxin acts synergistically with allergen resulting in a magnification of specific allergen induced mediator release and it was suggested that many of the clinical problems associated with natural latex products involve similar clinical sequelae and one of the cause for such clinical symptoms may be with presence of endotoxin (Norm , 1994).

Blood represents one of the most complex biochemical systems in living organisms, and its various components play an integral role in several life functions, such as transport of oxygen, destruction of invading pathogens and repair of damaged tissue. Because of the range and criticality of functions, any source of toxicity to blood cells can cause significant harm. Hemolysis is the breakdown of red blood cells, which can be by mechanical damage or by material mediated leachants, and it directly impairs the ability of the circulatory system to carry oxygen to body tissues. In the present study, an attempt was made to evaluate the water

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extractable protein, endotoxin level and haemolytic properties of four different brands of commercially available surgical gloves.

Materials and Methods

Commercially available four different brands of surgical gloves (H1A, H2B, H3C, and H4D), Di-sodium hydrogen- ortho- phosphate, sodium-di-hydrogen -ortho- phosphate, Sodium deoxycholate, trichloroacetic acid, phosphotungstic acid, sodium carbonate, ACD (Acid Citrate Dextrose), sodium carbonate, sodium tartarate, sodium hydroxide, from (sd -fine chemicals, India). Folin reagent, chicken egg albumin (concentrations from 2 to 200μg/ml from Sigma Aldrich), rabbit blood, human blood, Limulus Amebocyte Lysate (LAL) reader, LAL water, LAL reagent (Charles River Germany) were procured for this study.

Estimation of water soluble protein from surgical latex gloves using the modified Lowry method

Extraction of water soluble protein content of four different brands of commercially available surgical latex gloves was carried out as per modified Lowry method (ASTM D 5712-99, ASTM Standards, 1989). The various step involved in this process are:

Standard preparation

Extraction of gloves in phosphate buffer

A sample glove (from different brands of commercially available surgical gloves) was weighed and surface area (11.5 dm^2) was measured and extracted in phosphate buffer solution (pH 7.4$\pm$ 0.2) as per modified Lowry method. The extraction was carried out at 25 $\pm$ 5°C for 120 $\pm$ 5 minutes. At the end of extraction period the extract was collected into a propylene centrifuge tube and centrifuged at 500g for 15 min to remove particulate matter. The supernatant solution was again filtered through a 0.45 μm filter at room temperature into a polypropylene tube.

Colour development

Add 0.3 ml Folin reagent to all the above 30 tubes, mixed thoroughly and allowed to stand for 30 minutes at room temperature. Absorbance of the solution was measured at 700nm in a spectrophotometer (Shimadzu UV 1601). The absorbance of the extract of gloves in the absence of copper sulphate was subtracted from the absorbance of the extract of gloves in presence of copper sulphate to correct the interferences.

Quantitative measurement of Endotoxin level using Limulus Amebocyte Lysate test (LAL test)

Commercially available four different brands of surgical latex gloves were used for the study. The latex gloves were cut into small pieces of 0.5 cm^2. 0.4 g were placed in depyrogenated borosilicate test tubes with 2 ml pyrogen-free water and extracted at 37°C for 1h. The extract was centrifuged at 3000 g for 20 minutes and the supernatant was collected. Fifty-micro liter supernatant was diluted in pyrogen-free water (10 fold) and assayed immediately (Williams, 1997). Endotoxin was measured using the kinetic turbidimetric quantitative LAL test. The extract of the gloves were serially diluted in 10-fold increments with pyrogen free water. To a non-pyrogenic micro titer plate, add 100 ìl each of above dilutions and mixed with 100 μl of freshly prepared Limulus Amebocyte Lysate. These micro titer plates were read under Limulus Amebocyte Lysate reader (Williams *et al.,* 1991).

Determination of percentage hemolysis

Hemolysis test (Mohanan *et al.,* 1991) was carried out using rabbit and human blood (Table 1). 0.4 ml of ACD preserved rabbit blood was added to 25 ml of phosphate buffer saline solution (PBS) containing (1g of gloves sample). Similarly 0.4ml of ACD preserved human blood was added to 25 ml of phosphate buffer saline solution containing 1g of glove sample. For each set separate positive (100% hemolysis induced by replacing the PBS with 25 ml of 0.1% sodium carbonate solution) and negative (0% hemolysis, no gloves sample added) controls were also set up.

Each set of experiments were repeated three times with the same procedure. All the test tubes containing test samples and controls were placed at 35$\pm$1°C, for 60 minutes in an incubator. At the end of the incubation period the tubes were centrifuged at 3000 rpm for five minutes (this procedure was repeated in all the other gloves brand). The percentage hemolysis was calculated by measuring the optical density of the supernatant solution at 545 nm under spectrophotometer as per the following formula:

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Table 1: Experimental Design For Determination of Percentage Haemolysis

No.	Brand code	Weight of test sample (gm)	Volume of PBS (ml)	Volume of blood (ml)
		Rabbit blood		
1	H1A	1.0	25.0	0.4
2	H2B	1.0	25.0	0.4
3	H3C	1.0	25.0	0.4
4	H4D	1.0	25.0	0.4
5	Negative control	—	25.0	0.4
6	Positive control	—	0.1% sodium carbonate (25.0)	0.4
		Human blood		
1	H1A	1.0	25.0	0.4
2	H2B	1.0	25.0	0.4
3	H3C	1.0	25.0	0.4
4	H4D	1.0	25.0	0.4
5	Negative control	—	25.0	0.4
6	Positive control	—	0.1% sodium carbonate (25.0)	0.4

$$\text{Percentage hemolysis} = \frac{\text{O.D. of the test sample} - \text{OD of negative control} \times 100}{\text{O.D. of positive control}}$$

Results and Discussion

Estimation of water soluble protein content from surgical latex gloves using the modified Lowry method

The amount of water extractable proteins from different brands of commercially available surgical latex gloves were H1A is 9.025 μg/ml, H2B is 11.59 μg/ml, H3C is 16.3 μg/ml and H4D is 12.3 μg/ml (Table 2). The experiment was repeated three times. The result of the study indicated that H2B and H4D have comparatively more protein content than H1A and H3C. The protein content of the four different brands of surgical gloves were under acceptable levelcomments provide accepotable levels with ref of pharmacoiea), when compared to reported values.

Quantitative measurement of endotoxin level using LAL test

LAL test is the standard *in vitro* procedure usually employed to evaluate the endotoxin limit of a device/material. The result (Table 3) indicated that the quantity of endotoxin present in the four different brands of commercially available surgical latex gloves (H1A, H2B, H3C, and H4D) passed the LAL test. The amount of endotoxin present in the H1A brand is 0.05 EU/ml, H2B brand is 0.05 EU/ml, H3C brand is 0.9 EU/ml and H4D is 0.5 EU/ml. This suggests that the all the four brands of gloves have endotoxin within the acceptable level

Determination of percentage hemolysis

The results of the hemolysis study (Table 4) indicated that the glove sample H1A induced an average hemolysis of 0.41%, where as H2B, H3C and H4D samples induced an average hemolysis of 3.0%, 3.5% and 2.0% respectively, when treated with rabbit blood. Similarly, when different brands of surgical gloves were treated with human blood, the hemolysis induced by the samples H1A was 2.38%, H2B was 3.0%, H3C was 1.5% and H4D was 2.0%. Since all the values are below 5% they can be considered non-hemolytic as per ASTM standard (ASTM, 2000). This suggests that the percentage hemolysis induced by all the four brands of commercially available surgical latex gloves were non haemolytic. The hemocompatibility of medical devices are usually confirmed before the

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Table 2: Quantitative measurement of water extractable proteins in different brands of surgical latex gloves.

No	Sample Name	Quantity of protein(μg/ml)
1	H1A	09.03
2	H2B	11.59
3	H3C	16.30
4	H4D	12.30

Table 3: Quantity of endotoxin present in four different brands of surgical latex gloves

No	Sample Name	Amount of endotoxin (EU/ml)
1	H1A	0.05 EU/ml
2	H2B	0.05 EU/ml
3	H3C	0.96 EU/ml
4	H4D	0.5 EU/ml

Table 4: Percentage haemolysis induced by four different brands of surgical gloves

No		Percentage Haemolysis (%)	
		Rabbit blood	Human blood
1	H1A	0.41	2.38
2	H2B	3.0	3.0
3	H3C	3.5	1.5
4	H4D	2.0	2.0

medical devices are selected for human application. The hemocompatibility of the gloves is important because it usually come in contact with the blood during surgical or other procedures (Sagnella and Mai, 2005). From the study it is concluded that the four brands of commercially available surgical latex gloves have an acceptable level of water extractable protein and all brands are considered as pyrogen free and non haemolytic.

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