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INCQS

ASSESSMENT OF PYROGENIC CONTAMINATION WITH LIPOTEICHOIC ACID (LTA) IN THE MONOCYTE ACTIVATION TEST (MAT) AND RABBIT PYROGEN TEST (RPT)

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INTRODUCTION

All injectable products for human use subject to health surveillance must be pyrogen free. The contamination of these products by pyrogens can be dangerous and is considered a serious public health problem for causing febrile reactions, pyrogenic shock, organ failure and even death.

LTA is a non-endotoxin pyrogen of great importance in the pathogenesis of sepsis. RPT is able to detect all types of pyrogens but involves a great number of animals to be used. The Bacterial Endotoxin Test (BET) cannot fully replace RPT since it only detects endotoxins. MAT is sensitive to all types of pyrogens and is based on the same biological mechanism responsible for the fever reaction in humans.

ICCVAM has recommended its utilization for other pyrogens than endotoxin since its equivalence to RPT can be demonstrated[1].

OBJECTIVE

This work aims to evaluate the ability of MAT to detect LTA contamination in sodium chloride by the establishment and comparison of the dose-response curve of LTA in rabbits and the concentration-response curve of LTA by the MAT.

METHODOLOGY

LPS from *E. coli* serotype O55:B5 (Sigma, reference No. L2880) and LTA from *S. aureus* (Sigma, reference No. L2515) as non-endotoxin stimulation was used. The solutions were diluted in pyrogen-free solution of 0,9% Sodium Chloride (Sanobiol lot: 13,050,857).

a) the establishment of the dose-response curve of LTA in rabbits

Adult New Zealand rabbits were injected with different LTA doses (100; 1,000; 10,000; 50,000; 75,000; 100,000; 125,000; 250,000; 500,000 ng of LTA/Kg, 1mL/Kg of body weight) into the marginal ear vein. After the injection, rectal temperatures of the animals were recorded with a PyroMon® system (ELLAB, Denmark) over a 3 hours period. Fever was considered as an individual variation of temperature ≥ 0.5 ºC. The result was expressed by the mean of individual temperature rise[2,3].

b) establishment of the concentration-response curve of LTA by MAT

For MAT a pool of human whole blood cryopreserved[4,5] was and the proinflammatory cytokine IL-1β release was measured by ELISA (R&D Systems). The same points of the curve tests in rabbits were tested in MAT assay.

c) Endotoxin Equivalent

The pyrogenic contamination of the preparations was estimated quantitatively tested by constructing a concentration-response curve of LPS standard (0.125, 0.25, 0.5, 1.0, 5.0 EU/mL) versus the value of Optical Density (OD) of the release of IL-1β by ELISA of different concentrations of LTA. Thus the results of LTA were expressed in equivalent endotoxin unit per ml (EEU / ml)[6,1].

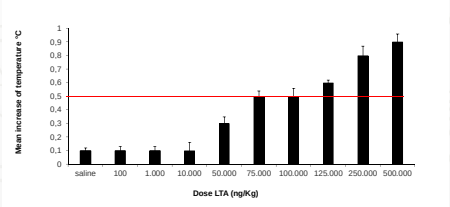

Figure 1: The dose-response curve *S. aureus* lipoteichoic acid in rabbits. The results are expressed by a higher concentration by temperature increase/animals with standard error. The animals received doses of 100; 1,000 and 10,000 (n = 3); 50,000; 75,000; 100,000; 125,000; 250,000 and 500,000 ng/kg and the control group (n = 8) in the marginal ear vein. The red line represents the cut-off temperature of 0.5 ° C, which corresponds fever.

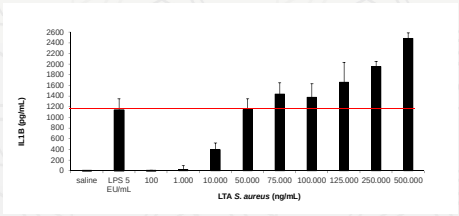

Figure 2: Response to the release of IL-1β to the stimulus of different concentrations of LTA from *S. aureus* by ELISA in cryopreserved blood. Blood pool 4-5 the cryopreserved donor was used. Mean of four independent experiments in duplicate and standard error. The red line represents the cut at the concentration of 5 EU/mL of LPS from *E. coli* O55: B5, which corresponds to the threshold dose fever in humans and rabbits.

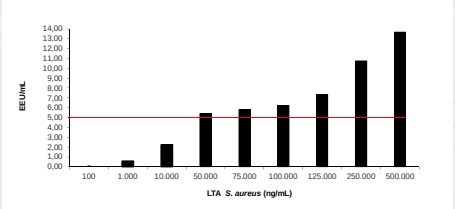

Figure 3: Equivalence of different concentrations of LTA from *S. aureus* to Endotoxin Units (EEU/mL) based on the concentration-response curve of LPS versus the value of Optical Density in the LTA release of IL-1β ELISA. The red line represents the cut at the concentration of 5 EU/mL of LPS from *E. coli* O55: B5.

RESULTS

Rabbit fever response was observed from 75.000 ng of LTA/Kg (Figure 1) and in MAT the limit detection was established in 50.000 ng/mL of LTA (Figure 2), or 5.41 UEE/mL (Figure 3). MAT showed to be more sensitive than RPT.

CONCLUSIONS

Results suggest that MAT was efficient in detecting LTA and may contribute to the acceptance of this test by the Brazilian regulatory agencies and the may be a good assay for the replacement of animals in RPT.

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