Removal of Endotoxin from Antibody Preparations for Clinical Use

Assessment of Polymyxin-Sepharose CNBr Affinity Chromatography


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ABSTRACT

Despite attempts to maintain asepsis, good manufacturing practices, and the use of terminal sterilization by millipore filtration, the nuclear practitioner is always worried about the possibility of endotoxin contamination. Methods, such as ion-exchange chromatography, have been tried for removing endotoxins during the preparation of radiolabeled antibodies, and so on. As suggested by Stevenson (1990), we evaluated the Issekutz technique (1) of endotoxin removal by affinity chromatography using a polymyxin cyanogen bromide (CNBr) Sepharose column. The endotoxin content of millipore filtrates of heat killed/sonicated suspensions of Pseudomonas pyocyaneus, E. coli were measured using a Sigma (St. Louis, MO) Endotoxin Assay Kit before and after filtration through such columns and compared with the results obtained using gel exclusion and ion-exchange columns of the same length and diameter. Reduction of endotoxin content to undetectable levels by the polymyxin column was observed. The use of such columns for terminal endotoxin removal analogous to terminal sterilization is advocated especially when developing a radiopharmaceutical such as radiolabeled antibodies for in house use.

Index Entries: Endotoxin; antibody preparations; polymyxin-Sepharose CNBr.

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INTRODUCTION

Endotoxin contamination is a recurrent problem in the preparation of solutions for parenteral use in humans. Endotoxins can activate monocyte-macrophage cells to produce a number of biologically active factors, including interleukin-1 (2), interleukin-6 (3), tumor necrosis factor (4), prostaglandins (5), and leukotrienes (6). Some of these factors may have deleterious physiological effects culminating in fever, endotoxic shock, and acute-phase reactions. The sensitivity of mammals to endotoxins is extraordinary since as little as (10 eu) can elicit fever, shock, and even death in susceptible individuals (7).

Issekutz (1) pointed out that gram-negative endotoxins have potent biological effects in humans and in many animal species when administered systemically. In addition, under in vitro conditions, nanogram quantities of endotoxin can influence the behavior of certain cells and even of molecular events in cell-free systems (8,9). These substances are shed from the cell walls of viable or nonviable gram-negative bacteria. These bacteria are very hardy and grow in water with minimal nutrient requirements; therefore endotoxin is a potential contaminant of physiological fluids and aqueous solutions, or the surfaces in contact with such solutions.

Issekutz (1) noted that the cyclolipophilic peptide antibiotic, polymyxin B sulfate, is known to neutralize the biological activity of this group of molecules (10-13), presumably because of its binding with high affinity to the toxic lipid-A moiety of endotoxins (14). He suggested the use of this agent for removal of gram-negative endotoxin from solution by affinity chromatography.

Stevenson (1990) stated in a personal communication that the Issekutz method was promising, and, following his advice, the efficacy of Sepharose 48-Polymyxin column for removal of endotoxins was investigated.

EXPERIMENTAL METHODS

Preparation of Sepharose CNBr-Polymyxin Column

All solutions are made up in pyrogen-free distilled water in sterile plasticware. To make a 5 mL column:

1. Use 1.6 g of CNBr-activated Sepharose 48 (Pharmacia, Uppsala, Sweden) suspended in about 50 mL of 1 mM HCl.
2. Suspend 15 min at room temperature.
3. Wash through glass sinter with 1 mM HCl.
4. Resuspend in 5 mL of 0.1M NaHCO₃, 0.5M NaCl.