Methods of Endotoxin Removal from Biological Preparations: a Review

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ABSTRACT

PURPOSE: Endotoxins, also called lipopolysaccharides (LPS), are major contaminants found in commercially available proteins or biologically active substances, which often complicate study of the biological effects of the main ingredient. The presence of small amounts of endotoxin in recombinant protein preparations can cause side effects in host organism such as endotoxin shock, tissue injury, and even death. Due to these reactions, it is essential to remove endotoxins from drugs, injectables, and other biological and pharmaceutical products. An overview of this subject is provided by this article. METHODS: An extensive review of literature with regard to methods for removal of endotoxin from biotechnological preparations was carried out. RESULTS: A short history of endotoxin is presented first. This is followed by a review of chemical and physical properties of endotoxin and its pathophysiological effects when the body is exposed to LPS excessively or systemically. The techniques of endotoxin determination and interaction of endotoxin with proteins is also presented, taking into consideration the established techniques as well as the state of the art technology in this field. A review of techniques of endotoxin removal from biotechnological preparations is described, emphasizing how endotoxin removal can be carried out in an economical way based on a number of processes discussed in the literature (e.g., adsorption, two-phase partitioning, ultrafiltration and chromatography). Different methods are mentioned with relatively high protein recoveries; however, special attention is given to two-phase aqueous micellar systems, which are valuable tools for endotoxin removal from pharmaceutical proteins on a small scale because they provide a mild environment for biological materials. CONCLUSIONS: Efficient and cost-effective removal of endotoxins from pharmaceutical and biotechnology preparations is challenging. Despite development of novel methods, such as the two-phase aqueous micellar systems, in recent years, more research is needed in this field.

INTRODUCTION

Endotoxins are lipopolysaccharides (LPS) derived from cell membrane of Gram-negative bacteria and are responsible for its organization and stability. In pharmaceutical industries it is possible to find endotoxins during production processes or in the final product. Although endotoxins are linked within the bacterial cell wall, they are continuously liberated into the environment. The release does not happen only with cell death but also during growth and division. Since bacteria can grow in nutrient poor media, such as water, saline, and buffers, endotoxins are found almost everywhere. A single *Escherichia coli* contains about 2 million LPS molecules per cell. Endotoxin elicits a wide variety of pathophysiological effects. In conditions where the body is exposed to LPS excessively or systemically (as when small concentrations of LPS enter the blood stream), a systemic inflammatory reaction can occur, leading to multiple pathophysiological effects, such as endotoxin shock, tissue injury, and death (1-3). However, endotoxin does not act directly against cell or organs but through activation of the immune system, especially through monocytes and macrophages, with the release of a range of pro-inflammatory mediators, such as tumor necrosis factor (TNF), interleukin (IL)-6 and IL-1. Pyrogenic reactions and shock are induced in mammals upon intravenous injection of endotoxin at low concentrations (1 ng/mL) (4).

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