CHAPTER 4

DISCUSSION

Pseudomonas aeruginosa, particularly, is a frequently encountered gram-negative organism in hospital acquired infection.[1] Inspite of low virulence in healthy individuals, it may cause serious and lethal infections in debilitated or immunosuppressed hosts such as cancer, burn, urinary, lower respiratory tracts and cystic fibrosis patients, etc. P. aeruginosa usually produces a variety of extracellular products that may contribute to its pathogenicity including hemolysin, proteases, enterotoxin, exotoxin and endotoxin. Endotoxin, which was originally designated as lipopolysaccharide endotoxin[1,34] was a potentially important virulent factor[1,38,41] which in the experiment induced hemorrhagic and death of mice. Numerous methods of production, concentration and purification of this endotoxin were developed. Most of them based on modification of combined techniques such as precipitation ion exchange chromatography, gel filtration, and electrophoresis.[32,34]

With this study, we provided a simple method in preparation of partially purified endotoxin by using 60 % final concentration of ammonium sulfate for precipitation and concentration, and employing column chromatography with Sephadex G-200 for purification. Applying of spectrophotometry for the detection of endotoxin during the purification was more convenient an accurate than the animal model procedure.

The preparation showed four peaks (Figure 2). There was only one main peak was toxic to the experimental animal (table 3). Immunodiffusion test confirmed the components contained in this crude endotoxin preparation by revealing a heavy precipitin lines (Figure 6) but no precipitin lines for fraction endotoxin. From these result it was proved that the crude endotocin was an antigen which was common in the cells of the immunotype of P. aeruginosa.[41] The mouse LD_{50} of endotoxin was 130 mcg.[52,53]

The role of endotoxin in the pathogenesis of mice, our microscopic observation were resembled with those made by liu[8] and Markley et al[58] who report the liver necrosis, edematous and hemorrhagic lung and necrotic and hemorrhagic kidney in mice after giving endotoxin intraperitoneally. Our microscopic observations were the same as Nipaporn[30] who report about the exotoxin. Our histopathalogic and immunofluorescent studies revealed the main site of tissue injury and endotoxin localization was spleen but minor effects were also observed in kidney and liver. It would be emphasized that the mice in our study were subjected to a single large dose of crude endotoxin. On the other hand, concerning human infections, the host would be exposed to the continuous release of small amount of many toxic substances, each of which induces different biochemical and pharmacological effects. Since the endotoxin of P. aeruginosa was toxic to the experimental animals, it was hardly believed that it did not play a role in pathagenesis. The work presented here may well pertray the clinical effect of the endotoxin.

The obtained immune sera had a highly specific antitoxin, as judged by double immunodiffusion reaction perform with concentrated P. aeruginosa crude endotoxin, and had neutralizing activity against crude endotoxin in mice as report in this study. Freshly prepared of double gel diffusion, the precipitin lines were appeared both fractioned endotoxin and crude endotoxin. For prolong storage of fractioned endotoxin the protein might be denatured, since the precipitin line was not appeared in figure 6 .

Chiyoji et al reported that mice immunized with original endotoxin protein were protected from infection due to P. aeruginosa.(41)

Pollack et al who briefly reported that exotoxin and endotoxin were present early in the serum of most patient with P. aeruginosa septicemia increase with survival, and were protective. This suggested that antibodies passively provided or actively engendere engendered to both exotoxin and endotoxin may have therapeutic or prophylactic potential.(59)

Several type of whole cell and subcellular Pseudomonas vaccines have been tested in experimental and clinical situation to control such infection with varying degrees of success.(27,60,61,62,63)

These findings suggested that the feasibility of producing a toxoid in studying of immunoprophylaxis and/or treatment of Pseudomonas diseases may be valuable for future investigation.