Structural and immunochemical studies of polysaccharides from some pathogenic actinomycetal strains

Abstract

Actinomycosis is a chronic infection caused by microorganisms of the genus *Actinomyces*, with *Actinomyces israelii* being the most common etiologic agent. Other species that have been reported as human pathogens include *Actinomyces naeslundii, A. odontolyticus, A. gerencseriae, A. meyeri* and *A. viscosus*. Recent research in the area of microbiologic identification techniques, especially 16S ribosomal RNA sequencing, have identified new *Actinomyces* species, which are also being reported as human pathogens. *Actinomyces* are part of the normal flora of the oral, gastrointestinal and genital tract. They normally act as commensal organisms and become opportunistic and can cause actinomycosis, which is an inflammatory and chronic suppurative granulomatous infection that forms abscesses and fistulas. Resultant infections involve the oral/cervicofacial, intra abdominal and genitourinary regions. Pulmonary infections are believed to occur due to aspiration of the organisms from the oral cavity. Actinomyces and actinomyces-like bacteria are undervalued in terms of epidemiological surveillance, due to the difficulty in identification and diagnosis, methods are lacking of their detection because of the high relationship of pathogens to probiotic strains. Besides, they are intracellular pathogens and in medicine are still a serious problem. So far, the focus has been on such molecules as fatty acids, phospholipids, glycolipids, with less specificity. In this work there were isolated and purified from 4 strains pathogenic *Actinomycetes* a set of new surface polysaccharides, *Actinomycess israelii, A. naeslundii, A. odontolyticus, Tsukamurella pulmonis*. Results from MALDI-TOF Biotyper method on studied strains indicate that MALDI is a reliable and fast method for *Actinomycetes* identification, thus could be used for identification of clinical isolates. The colony extraction procedure is preferred technique over direct liquid medium procedure. Then, bioimaging experiments have been performed on whole cells. The detection approach using energy-filtered back-scattered electrons allowed to demonstrate the slime formation, extended from the bacteria to the substratum, visible as zones around the bacteria. Results from scanning ion-electron microscopy revealed that the rod-shaped bacteria at the periphery of colony are surrounded with slime, whereas planktonic culture has cells without visible slime structure of biofilm. Further study has been performed on purified polysaccharides. Sugar analysis revealed the diferent complex compositions of polysaccharides from *Actinomyces israelii* and *A. odontolyticus*, while this for *Actinomyces naeslundii* indicated for glucane type and this

from *Tsukamurella pulmonis* an arabinomannan type, proved by methylation analysis for *T. pulmonis* and *A. israelii* and by NMR for all studied polysaccharides. The structure has been determined for *T. pulmonis* polysaccharide, which is an arabinomannan composed of branched tetrasaccharide repeating units where →)-αAra*f*(1→5)-αAra*f*(→ main chain is substituted by αMan*p*(1→2)αMan*p*(1→2)- disaccharide, with additional low amount of linear →6)-α-D-Manp-(1→ mannan. Then there were produced monoclonal antibodies against polysaccharides of tested strains, in order to potential detection their presence in human serum. Monoclonal antibodies obtained against polysaccharides express reactivity with homologous antigens and cross reactivity between *A. odontolyticus* and *A. naeslundi*. Monoclonal antibody against PS of *A. odontolyticus* are cross reacting with bacterial lipopolysaccharides, what might have the practical significance for anti *A. odontolyticus* antibodies for endotoxins detection. It has been shown a serological reactivity of these polysaccharides, which is crutial for the development of specific diagnostics and preventive vaccines. The immunogenicity of polysaccharides was specified, ELISA diagnostic test conditions using polysaccharides as specific antigens might be elaborated. The results have important diagnostic potential, as they establish pathways in research of individual antigens to test. It has been shown that polysaccharides could be useful markers for the diagnosis of actinomycoses and tubeculose-like disesase. Polyclonal rabbit sera have been also obtained, a set of polysaccharides, which is considered as a part of the immunodiagnostic test. The discovery of immunogenic polysaccharides among pathogenic bacteria of the genera *Actinomyces* and *Tsukamurella*, is an innovative approach in immunochemistry of these pathogens and in the research of biomarkers. Together they consist a wide panel of markers, a library of antigens of diagnostic values for direct use in the matrices. Structural studies of polysaccharides with NMR spectroscopy has shown a unique construction of polysaccharide of *Tsukamurella pulmonis*. The greatest hope is associated with polysaccharides, which are excellent substrates to specific diagnostic tests and vaccines, will be included as markers for immunodiagnostics of actinomycetal infections in the ELISA. In clinical practice will be introduced a determination of markers in clinical material for the determination of the presence of actinomycetes, for diagnostics of tuberculose-like diseases in patients of pulmonologic department, neurological and pulmonary branches, monitoring of actinomycetal therapy and actinonomyces in patients on the wards of dermatological, prevention of actinomycetal infections in people prepared for transplantation. Clinical, histochemical and ultrastructural studies of *Tsukamurella pulmonis* infection revealed that this strain is capable to break defense barriers and to spread to remote organs on its own. Immunohistochemical

methods allowed to identify with monoclonal antibodies against *A. israelii* and *T. pulmonis* the infection of mice with *T. pulmonis* strain in the liver. Together with immuno-histochemical data, these data demonstrate that *T. pulmonis* is capable of breaching defense barriers as single infecting agent and of spreading to remote organs even in the absence of other co-infecting pathogens.