

Purpose of research / research hypothesis

Chronic otitis media with acquired cholesteatoma (COMwAC) is still a major therapeutic problem. During the development of the cholesteatoma, "filling" and proliferation of epithelial keratocytes from the external auditory canal to the middle ear cavity, and the formation of chronic inflammation due to activation of the immune system. As a result, middle and inner ear structures are destroyed, leading to hearing loss, facial palsy, balance disorders, as well as intracranial and intratemporal complications. The reasons for the formation and development of cholesteatoma are not fully explained. Treatment of choice is surgical treatment. In our previous studies we have shown the important role of innate immunity in the development of OMCwAC. Our preliminary studies have demonstrated the presence of the HMGB1 protein and its RAGE receptor, as well as the presence of Toll-like receptors in the cholesteatoma microenvironment.

At present more and more data point to the key role of extracellular vesicles (EVs) in the pathogenesis of chronic inflammatory conditions, because EV acts as carriers of various proteins, including inflammatory mediators, modulating the activities of other cells. Taking into account the results of our preliminary studies, we hypothesize that the release of EV-HMGB1 from the cholesteatoma microenvironment, also spreading systemically through the cardiovascular system, leads to the development of local and general inflammation. Because EV can be absorbed by endocytosis by immune cells such as macrophages, but also by other cells (fibroblasts, vascular endothelium), we want to evaluate the direct effect of HMGB1 (EV-HMGB1) on these cells in studies *in vitro* as well as *ex vivo*. We also assume that the assessment of EV and serum levels of HMGB1 may be a marker of inflammation and correlate with the clinical progression of cholesteatoma or recurrence.

Research Methodology

The concept of this project is based on a systematic approach, with the view that the role of EV-HMGB1 will be evaluated in 2 complementary experimental platforms: 1) *in vitro* matrix model of cholesteatoma on epithelial cell lines; 2) by evaluating the *ex vivo* biological material obtained from patients treated due to cholesteatoma. This will allow you to evaluate the impact of EV-HMGB1, whose role has not yet been investigated in COMwAC. The project is planned to use modern research techniques, functional *in vitro* cellular testing, flow cytometry, immunohistochemistry and molecular biology techniques. An important aspect of the present project is the systemic approach, which is that we will investigate the role of EV-HMGB1 not only using *in vitro* model but also correlate the *ex vivo* results of the presence of HMGB1 in EV with clinical status.

Impact of expected results on the development of science

The implementation of the research tasks specified in our project will make it possible to clarify the principles of a new mechanism important in the development of OMCwAC, chronic inflammation with bone destruction. The result of the project will be to clarify the potential usefulness of EV-HMGB1 as a biomarker. In the future, this may contribute to the development of new therapeutic methods or improvements to existing ones. During the implementation of the project, cooperation between Polish and American institutions will be strengthened. As a whole, this project will contribute to the further dynamic development of Polish science as well as the general knowledge in the field of cholesteatoma biology of the acquired middle ear.