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**PHD THESIS**  
**NEW PERSPECTIVES IN THE THERAPY OF  
INFECTIOUS AND TUMOR ENT-RELATED  
DISEASES**

**- R E S U M E -**

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## RESUME

Otitis media, purulent otitis, cholesteatomatous otitis represent an extremely frequent pathology in ENT practice, especially the pediatric one that I deal with in particular; a pathology with great challenges from a therapeutic point of view.

Through the research carried out, we try to identify therapeutic schemes that contribute to obtaining improved results in the treatment of abobe mentioned pathologies.

In the studies of the present work, several objectives were proposed that aimed at complementary research directions in order to make a contribution in the field of common and particular conditions in the ORL sphere that are based on bacterial infections and/or benign and malignant tumor proliferations. The present paper is structured, according to the drafting rules, into two main parts: (a) the general part - which is allocated to literature studies and which presents the latest scientific information in the field and (b) the special part which reproduces the results of the experimental studies conducted during years of doctorate, together with the tailoring of the applied materials and methods, discussions based on the data presented in the literature and particular conclusions.

Preclinical evaluations of some compounds associated with the therapy and prognosis of bacterial and tumoral conditions in the ENT sphere are of real importance today. Due to intrinsic and extrinsic factors, a series of changes are signaled that are visible both in the daily life of patients and in the evolution of various microorganisms. Biofilm plays a crucial role in the prognosis and progression of various bacterial infections that are often associated with chronic conditions and cause long-term problems for patients. The stability and resistance mechanisms of biofilm bacteria present unique and evolving

challenges for both patients and clinicians. It has been shown that biofilm infections can alter inflammatory immune responses, alter immune cell metabolism, affecting the development and activation of immune responses. Innovative therapeutic agents in infections are crucial to counteract the severe complications of biofilm-associated infections.

**The general part** is structured in three chapters, in which the emphasis was placed on the following: (a) current aspects related to common conditions in the field of ENT, detailing information on otitis (risk factors in the occurrence of recurrent infections, prevention and treatment methods), (b) the role of biofilm in ENT conditions (introduction, biofilm and malignancies, along with biofilm therapeutic approach) and (c) particular ENT conditions (introduction, complications and intratemporal sequelae).

**The experimental part** had three main research directions, in close correlation with each other. The first direction of research is related to the *in vitro* evaluations of some compounds associated with the therapy and prognosis of bacterial and tumoral conditions in the ENT sphere. Epigallocatechin gallate-loaded polyurethane structures were evaluated for biological activity using pharyngeal and squamous cell carcinoma cells. Compounds from natural sources are an alternative effectively used in the study of antibacterial and antitumor drugs. Between the early 1980s and the end of 2019, more than 160 antibacterial drugs were approved. Of these, only about 22% are exclusively synthetic. Antibacterial drugs of natural origin approved at the beginning of 1981 are: netilmycin sulfate (1981), micromomicin sulfate (1982), myokamycin (1985), mupirocin (1985), carumonam (1988), fosfomicin trometamol (1988), isepamycin (1988), teicoplanin (1988), RV-11 (1989), daptomycin (2003), fidaxomicin (2011). Biological products have been approved in number four and vaccines more than 30, especially as prophylactic agents for young children. Approximately 48% of the total number of drugs approved in the specified period are part of the categories of natural compounds or derived from natural compounds, highlighting their importance

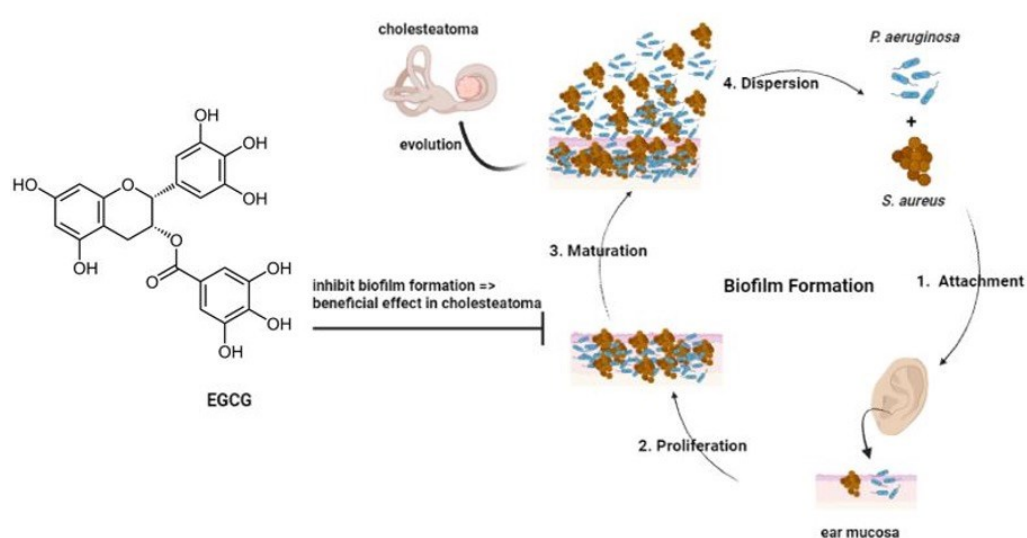


and role in the therapeutic approaches of various old and new pathologies. Polyurethane carriers, based on nano and microparticles, have a very good capacity to encapsulate large amounts of active substances. The advantages of these carriers are multiple, among them a low cost of raw materials, the possibility of changing the degradation period of the macromolecular chains by balancing the ratio between the components used in the aqueous phase and the possibility of changing the size of the particles by using different amounts of chain extension. In the framework of the present study, a drug release system was obtained from polyurethane used for the transmembrane transfer of epigallocatechin gallate (compound of natural origin). It has been proven that the system consists of almost homogeneous particles with a high encapsulation efficiency, with a diameter around 200 nm, with an average tendency to form agglomerations, very stable during heating processes. These characteristics make it a biomedical system compatible with the biological environment according to the specifications of the specific standards. The data obtained from the evaluations of the behavior of the cells taken in the study (pharyngeal tumor cells and squamous cell carcinoma) indicated that the transporter system loaded with epigallocatechin gallate exerts a cytotoxic activity that deserves to be studied in more detail and *in vivo* studies.

The second research direction, the second objective, aimed at the analysis of active biomaterials as therapeutic solutions in the treatment of ENT infections, focusing on cholesteatoma. A cholesteatoma may involve the middle ear, mastoid, or both, invade the labyrinth, or even extend beyond the temporal bone. A cholesteatoma can be a cyst-like structure with no signs of infection. A cholesteatoma that is present in association with chronic inflammation of the middle ear (mastoid) would be defined as a cholesteatoma with chronic suppurative otitis media. Occasionally children with advanced disease secondary to cholesteatoma may present with mastoid pain, fever, and irritability, mimicking acute mastoiditis, and rarely this may lead to the formation of a mastoid abscess. In these cases, a systemic treatment in the

form of intravenous antibiotics is necessary. The ability of pathogens to form biofilms facilitates their survival in adverse conditions, allowing them to proliferate and colonize host tissues as well as inert surfaces such as implants, producing negative human health reactions and resistance to antimicrobial drugs. More controversial is the situation where biofilms are polymicrobial. A common example of coinfection is that between *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which can worsen the disease and prevent the choice of antibiotic therapy, making the person's recovery slower. Biofilm formation can be considered as one of the main causes by which bacteria develop resistance to several drugs. The unreasonable use of antibiotics has led to the development of multi-resistant microorganisms. Natural products derived from plants but also microorganisms and marine species represent an invaluable source of anti-biofilm agents. Compounds isolated from plants as well as extracts have been shown to have important antimicrobial and anti-biofilm effects. The anti-biofilm properties of natural products refer to inhibition of polymer matrix formation, reduction of production of virulence factors and suppression of cell adhesion, thus blocking communication between bacterial cells and biofilm development. In the present study, the effect of epigallocatechin in combination with ciprofloxacin was analyzed to see the possible synergistic effect against bacterial species with a defining role in the appearance of biofilm related to cholesteatoma (bacteria, biofilm and cholesteatoma - perspectives of innovative therapeutic approaches) and at the same time the role of finished materials was analyzed in the cholesteatoma approach. There are approved single-dose products for treatment (combination of ciprofloxacin and a poloxamer) and research is ongoing in various directions. Plants and natural compounds extracted from plants have a significant antibacterial role and represent an alternative to antibiotic resistance. Several studies have been conducted to evaluate whether green tea and its main phytochemical, epigallocatechin-3-gallate, have antimicrobial properties. The data obtained showed that both aqueous and alcoholic extracts

and ECGC were effective against *S. aureus* and *P. aeruginosa*. Due to the massive acceleration of the development of bacterial resistance globally and the lack of new antimicrobial substances, new strategies to eradicate infectious diseases are needed. An alternative would be to combine antibiotics with each other or antibiotics with natural compounds to extract potential synergistic effects, knowing that plants are recognized for their antimicrobial effects.



#### Inhibitory effect of epigallocatechin-3-gallate (EGCG) against biofilm formation and cholesteatoma development

Our study revealed the antibacterial effect of epigallocatechin-3-gallate (EGCG) at concentrations between 25-100  $\mu\text{g/mL}$  compared to a fluoroquinolone, ciprofloxacin, against bacterial strains *S. aureus* and *P. aeruginosa*. The association of ECGC with ciprofloxacin led to a stronger effect, especially against *S. aureus*, which punctuated the synergistic effect of the two molecules. In the specialized literature, the antibacterial effect of ECGC in association with other antibiotics was also studied and the synergism was also observed, highlighting the fact that an antibiotic from the class of cephalosporins, such as cefepime, associated with epicatechin 3-gallate of natural origin, induced a synergistic eradication effect against the resistant

isolate of *P. aeruginosa*. Thus, combining the antibiotic with ECGC allowed the use of lower concentrations of ECGC and cefepime than when each substance was administered alone. The minimum inhibitory concentration for the natural compound was reduced in the presence of the antibiotic at different concentrations (0.5-4 µg/mL). The association of natural compounds, in this case ECGC, with antibiotics potentiates the antibacterial effect due to the resulting synergy; this combination can be an effective option for fighting infections and even ear infections and cholesteatoma, a pathology that can seriously affect the population. Cholesteatoma is a process of keratinization of the cleft or mastoid of the middle ear. The causes of the development of cholesteatoma are not fully known, but several factors are responsible for the formation of this medical condition. Among these factors we find microbial infection leading to chronic inflammation, collection of cellular debris and increased viscosity of secretions, Eustachian tube dysfunction, invasion of immune system cells, and epithelial hyperplasia. This pathology is generally manifested by pain and the presence of a foul-smelling fluid in the infected ear and up to hearing loss. Histologically, this noncancerous lesion contains keratin debris covered by keratinized squamous epithelium. A prototype device was made, and design parameters were determined based on measurements taken by medical personnel during mastoidectomy operations. The requirements of biomaterials used for middle ear reconstruction, such as biocompatibility, surface energy, resistance to degradation and infection, mechanical stiffness, weight, bone deposition, functional design, ease of tailoring and positioning, imaging artifact, were all taken into consideration. consideration and were evaluated accordingly.

The last research direction focused on highlighting the importance of prompt diagnosis of grade 1 chondrosarcoma at an early stage, based on clinical features, radiological aspects, and histopathological descriptions, considering the fact that this histological grade has a favorable evolution. Chondrosarcoma (CHS) is a malignant soft tissue tumor with cartilaginous

differentiation that accounts for one tenth of all malignant proliferations developed from bone tissues. There are two main subtypes of chondrosarcoma, conventional and unconventional. Conventional CHS accounts for over 85% of all cases and is subdivided into central, peripheral, and periosteal subgroups. Unconventional CHS includes three types: mesenchymal, clear cell, and dedifferentiated. CHS are predominantly present in patients aged between 40 and 60 years. Symptoms of CHS developed in a pre-existing bone are non-specific and include local swelling and pain for a long time. Dyspnea, wheezing, cough, hemoptysis, hoarseness are present in tracheal localization. CHS usually presents as a solitary lesion. In general, CHS are firm tumors with a lobulated appearance, with a smooth, glassy, gray-cut surface. Microscopic examination reveals slightly pleomorphic chondrocytes embedded in a cartilaginous matrix. Sometimes myxoid changes, calcification or necrosis may occur. Typically, atypical chondrocytes have dark, hyperchromatic nuclei. Most important in the treatment of CHS and its prognostic factors is the grading system pronounced on histological criteria. Characteristics useful for grading are cellularity, pleomorphism, multinucleation, and mitoses. The most common are grade 1 tumors (low-grade sarcoma), with a favorable prognosis and slowly progressive growth, although the rate of local recurrence is relatively high. In general, immunohistochemical reactions (IHC) play a limited role in the diagnosis of CHS, which is based on morphological, clinical, and radiological features. The only consistent IHC finding is S100 protein positivity. CHS are resistant to chemotherapy and radiotherapy. Large tumor resection with free margins increases 5-year survival rates to over 75%. The treatment of choice for tracheal and laryngeal CHS is conservative surgery, function-preserving surgery, including laser therapy.

The present study reported two cases of conventional chondrosarcomas, which were diagnosed in the Department of Thoracic Surgery and the Department of Otorhinolaryngology of the Municipal

Emergency Hospital, Timișoara, Romania, between February and June 2021. The malignant cases were peripheral chondrosarcomas, one of the scapulae and the other had an extremely rare tracheal location with microscopic features of conventional low-grade tumors (grade 1).

**The future studies** that should be carried out need to refer to detailing the mechanisms of action of alternative types of treatment, based on innovative formulations, with a beneficial role both in addressing microbial biofilms (whose resistance to current treatments is notorious) but also in combating the malignant processes for which the curative action involves the application of treatments with significant adverse effects. At the same time, awareness of the importance of specific and rapid diagnosis in the case of malignant pathologies in the ENT sphere must be emphasized by realizing specific health programs.