**Molecular mechanism of the toxicity of the antifungal antibiotic amphotericin B**

Owing to a dramatic increase in systemic mycoses, in particular associated with the HIV pandemic and organ transplantation, effective treatment of fungal infections is an important and topical issue. Amphotericin B (AmB) belongs to a group of life saving antibiotics, used as a gold standard to treat deep-seated mycotic infections. AmB is a pharmaceutical known for more than half a century and is in use, due to its high effectiveness, despite of the severe side effects, in particular nephrotoxicity and hepatotoxicity, which can be even lethal to patients. Despite decades of intensive research on the mode of action of AmB, exact molecular mechanisms responsible for the pharmaceutical activity of the drug and for the toxic side effects are not fully understood. Within the present research proposal, the experiments are designed, aimed to bring precise knowledge on molecular mechanisms responsible for self-organization of molecules of AmB in the environments important from the physiological standpoint: water, lipid membranes and proteins. The process of spontaneous organization of this antibiotic is not only interesting but also very important owing to the fact that both the therapeutic activity and the toxic side effects of the drug are directly linked to this process. The results of our previous studies show that molecules of AmB tend to form two types of dimers (pairs stabilized by intermolecular forces) that are able to associate into tetramers (two dimers can form a tetrameric structure). The AmB tetramer has very distinct and important biologically property, its structure enables incorporation into lipid membranes in the form of a transmembrane channel, which can potentially affect physiological ion transport and lead to cell death. In our opinion, such a mechanism can be a key factor responsible for toxic side effects of the antibiotic. Most of our experiments will be carried out with application of molecular spectroscopy methods combined with microscopic imaging. FLIM (Fluorescence Lifetime Imaging Microscopy) is one of such techniques. In this advanced microscopic technique, imaging is based upon analysis of fluorescence of molecules of interest (in our case AmB) and in particular on one of the physical characteristics which is the fluorescence lifetime. Typically, molecules excited by ultrafast laser pulses emit fluorescence after time periods in the order of nanoseconds (one billionth part of a second) and lifetime characteristics are extremely sensitive to environment and molecular organization of fluorescence emitting particles. This particular property will be applied in our study of molecular organization of AmB. The technique is extremely sensitive, so it allows to detect single molecules. We will use a single molecule approach to analyze formation of AmB-protein complexes as potential carriers of the antibiotic to biomembranes. The fact that we can engineer number of AmB molecules attached to a single protein opens possibility to delivery monomeric antibiotic to biomembranes, avoiding risk of tetramer-presence-related toxic side effects. At the same time, AmB can act as an antimycotic pharmaceutical via interaction with membrane sterols, in particular with ergosterol present in fungi. This is generally accepted mode of action of the drug. We hope very much that expected results of this research project will be important for the activity of numerous research centers, aimed to design effective antimycotic pharmacological formula of amphotericin B with minimized toxicity to patients.