

Report of the supervisor on Bc. thesis by Carmen Ziebermayr „DETECTION OF PILOCARPINE IN TICK SALIVA AND ITS EFFECT ON THE HOST IMMUNITY“

The subject of Carmen's thesis was chosen due to the problem with pilocarpine contamination of tick saliva. Pilocarpine, a muscarinic cholinomimetic agent, is used for the induction of tick salivation. The presence of pilocarpine in tick saliva was first reported in 2004 by Ribeiro et al. and since that time immunologists working with tick saliva have to take the effect of contaminating pilocarpine on immunological experiments into consideration. But little is known about the effect of pilocarpine on cells of the immune system.

Therefore Carmen with the kind help of the Laboratory of Analytical Biochemistry determined pilocarpine content in two samples of tick saliva using HPLC and mass spectrometry and tried to determine its effect on the production of cytokines by mouse splenocytes, mainly the dose-response relationship.

Carmen determined the concentration of pilocarpine in tick saliva being between 0.2 – 1.5 mg/ml. It is lower than that reported by Ribeiro for related tick species *Ixodes scapularis* but higher than that determined for *Amblyoma americanum*. She also showed that these concentrations can influence (inhibit) production of two important cytokines by mouse splenocytes stimulated with LPS. It is very important information for us and removal of pilocarpine from tick saliva or at least the system of appropriate controls should be included into our experiments.

As I have mentioned in my report on the Bc. thesis by Barbora Svobodova, Carmen suffered from the lack of knowledge in immunology as well. I am not very familiar with the study programme of biological chemistry, but I believe that students are very busy, having less time for the work on their thesis in comparison with our students of biology for example. There is also no experience with the length and general form of the thesis in the Biological chemistry programme. Therefore Carmen's thesis seems to be too short and not detailed and it really is. Nevertheless, Carmen did good piece of work. She was capable of creative thinking, designing experiments, carrying them out, interpreting the results and discussing them critically, although shortly. In the Discussion she offered several ways how to solve the problem of pilocarpine contamination of tick saliva.

Conclusion: I recommend the Bc. thesis by Carmen Ziebermayr for the defence.

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