University of Fribourg / Faculty of Science and Medicine / Department of Chemistry

Contributions to the Discovery and Synthesis of Biomarkers for the Retrospective Verification of Exposure to Chlorine and Novichoks

Severin Vital Martz

The first part of this thesis is contributed to the so called dual-use chemical chlorine (Cl_2). Cl_2 is a chemical with a wide range of commercial applications that, however, has recently been misused as a chemical weapon in the Syrian Arabic War due to its high toxicity and availability. Unambiguous biomarkers for the retrospective verification of exposure to this toxic chemical are currently still lacking, calling for further research in this field. The first aim of this work was to find and/or synthesize potential chlorinated or oxidized adducts of proteins that may be used for the retrospective verification of exposure to Cl_2.

A systematic screening of the 20 proteinogenic amino acids before and after Cl_2 exposure was carried out to lay a solid empirical foundation. Based on the results of this screening, potential albumin-based Cl_2-biomarkers were synthesized. The practical relevance of these substances was subsequently tested by means of proteomics experiments with human blood plasma. Lastly, a practice-oriented comparative intoxication study with human hair was carried out. Chlorotyrosines were detected in hair up to 10 months after exposure and were furthermore confirmed to be the most relevant markers by means of a computational, untargeted approach. The projects demonstrated how challenging the search for Cl_2 specific biomarkers is. Chlorotyrosines in hair, together with additional markers, such as oxidation products in human blood plasma, might provide convincing evidence of Cl_2 intoxications.

The second part of this dissertation aimed to synthesize nonapeptidyl and tyrosine-based biomarkers of the nerve agent family “the Novichoks”. Compounds of the Novichok family are supposedly the most toxic chemicals every synthesized by humans. They have been recently used as a chemical weapon in Salisbury and Amesbury (UK), as well as in Tomsk (RU). As the Novichoks belong to the nerve agent class of chemical weapons they were recently included in the Chemical Weapons Convention (CWC) in 2020. For organizations like the Organisation for the Prohibition of Chemical Weapons (OPCW) and its designated laboratories, straightforward and time-efficient synthetical strategies to obtain reference standards for the retrospective verification of exposure to the wide variety of these recently listed compounds are therefore essential. Nerve agents are organophosphorus electrophiles that show an exceptionally high toxicity through the inhibition of the enzyme acetylcholinesterase (AChE) that is present in neurons of mammals. They form specific adducts with cholinesterases which can be obtained from enzymatic digests or be synthesized by using solid phase peptide synthesis (SPPS). In this dissertation, the tyrosine-based adduct of the Novichok compound A-230 was exemplarily synthesized to investigate whether previously established synthetical approaches for other nerve agents were also successful for biomarkers of this new subclass. Additionally, an alternative synthetic strategy based on a “direct poisoning approach” was further developed. However, further experiments are needed to make the synthetical approach applicable to the Novichoks, which would greatly facilitate the work of designated laboratories of the OPCW when dealing with these compounds. The work carried out in this dissertation intends to make a small contribution to a world free of chemical weapons.

The Jury:

Prof. Dr. Christian G. Bochet, University of Fribourg, thesis supervisor,
Dr. Christophe Curty, Spiez Laboratory, co-examiner
Prof. Dr. Katharina Fromm, University of Fribourg, co-examiner,
PD Dr. Martin Lochner, Institute of Biochemistry and Molecular Medicine, co-examiner