Editorial

Drug Testing and Analysis

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Analysis of drugs for the therapy of anticholinesterase poisoning

Anticholinesterase poisoning is caused by inhibition of the enzyme acetylcholinesterase (AChE). Consequently the cleavage of acetylcholine (ACh) in the synaptic clefts is suppressed, thus hindering from deactivation of the neurotransmitter at the muscarinic and nicotinic receptors. Thereby a permanent overstimulation of effector cells is induced that affects a cholinergic crisis. Clinical signs and symptoms after poison incorporation include miosis (constriction of pupils), enhanced secretion of body fluids (e.g. sweat, saliva, tears) and fasciculation of muscles. In fatal cases, central and peripheral respiratory paralysis will cause death. Inhibition can occur by proteins such as fasciculins found in snake venom or small molecules, which can act as reversible inhibitors such as carbamates (e.g. pyridostigmine) or quasi-irreversible inhibitors. The most dangerous subgroup among the irreversible inhibitors is formed by organophosphorus compounds (OPCs) including nerve agents and a wide range of pesticides.

Even though existing stockpiles of chemical weapons including OPC nerve agents declared by state parties to the *Chemical Weapon Convention* (CWC) await destruction under the control of the *Organisation for the Prohibition of Chemical Weapons* (OPCW), these deadly poisons are still of critical concern not only for the military but also for the civilian population. The use of fatal chemical substances like OPCs has to be taken into consideration in scenarios of future terroristic attacks. The disposal of the nerve agent sarin in the Tokyo subway in 1995 has shown that non-state actors are capable of carrying out a chemical attack. In addition, several hundreds of thousands of people die from accidental or suicidal intake of OPC pesticides each year worldwide, especially in the Asia-Pacific region. Therefore, there is a permanent need for effective clinical countermeasures that will help to save the lives of poisoned patients and enable rapid and sufficient recovery.

First responders require effective antidotes and application systems that are deployable on-site, allowing the patient to be transferred to a nearby intensive care unit (ICU) of a hospital for further treatment. When hospitalized, therapies and regimens with state-of-the-art drugs have to be provided assuring best reconstitution of health in a reasonable short time.

Conservative strategies comprise the (1) symptomatic therapy, that antagonizes the ACh effect on muscarinic receptors by administration of atropine or scopolamine; (2) the causal therapy, that reactivates inhibited AChE by a chemical reaction performed with oximes (e.g. pralidoxime, obidoxime, HI 6); and (3) the anticonvulsive therapy with benzodiazepines, that prevents or stops central nervous seizures. More recent therapeutic concepts intend to apply (1) stoichiometric bioscavengers (e.g. recombinant butyrylcholinesterase, BChE), that bind OPCs to destroy their toxic properties; or (2) catalytic bioscavengers (e.g. paraoxonase 1) that catalyze the hydrolysis of OPCs to nontoxic products; and (3) synthetic molecules of non-biological origin that either bind or catalytically detoxify OPCs (e.g. cyclodextrins). Complementary to post-exposure medication, prophylactic therapeutics might be administered in specific cases where successive exposure to poison cannot be excluded entirely, especially in military scenarios. Current prophylactics (carbamates, e.g. pyridostigmine, physostigmine) inhibit AChE reversibly thereby protecting a sufficient portion of AChE from irreversible phosphylation by OPC agents and conserving essential residual enzyme activity. New approaches also consider the use of bioscavengers with sufficient plasma half-life.

This special issue focuses on analytical methods and aspects that are to be considered for the measurement of the relevant antidotes and related drugs in pharmaceutical preparations and biological fluids for accompanying therapeutic drug monitoring, pharmacokinetic studies, efficacy testing, stability studies, and forensic analysis.

Analyses of atropine by gas and liquid chromatography (GC and LC) for drug monitoring in man[1] and enantioselective pharmacokinetic study in swine[2] are addressed. A radioligand binding assay is described as a useful tool to identify compounds with affinity to muscarinic receptor subtype 5 that may be helpful to define molecular structures and pharmacophores of new potential antidotes for the treatment of OPC poisoning.[3] With respect to oxime antidotes, novel findings on stability of HI 6 in injection solutions obtained from, for example, chromatographic, NMR-spectroscopic and enzymatic assays are presented.[4,5] Furthermore, an LC-UV method for pralidoxime quantification in urine samples[6] as well as a comprehensive review article[7] are included. The OPC hydrolyzing potency of the enzymes DFPase[8] and organophosphorus hydrolases [9] are addressed in additional papers indicating their potential as novel catalytic bioscavengers. Approaches based on the isolation and mass spectrometric analysis of protein adducts that allow detection and verification of exposure and poisoning with carbamates and OPCs are reviewed.[10] The valuable properties of classic Ellman-based assays to explore inhibition and reactivation kinetics of cholinesterases are also outlined in a concise review.[11] The growing need for liquid chromatography-electrospray

ionization-tandem mass spectrometry (LC-ESI-MS/MS) data processing for compound identification in general is addressed by a research paper presenting the current status of spectral libraries and search algorithms.[12] In addition, perspective papers point out current technical and methodological trends and progress in mass spectrometry instrumentation[13] as well as use of attenuated-total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)[14] and stable isotope labelling for synthesis of internal standards for mass spectrometry, applications in nuclear magnetic resonance (NMR) spectroscopy and for studying drug biotransformation.[15]

Regarding this diversity of presented techniques and applications, we hope that this special issue will be of interest not only

to toxicologists, pharmacologists, pharmacists, and medicinal chemists but to researchers with an interest in analytical chemistry in general. We hope the papers provide novel ideas, insights, and valuable overviews.

This issue would not have been possible without the great help and support of the chief editor of *Drug Testing and Analysis*, Professor Dr Mario Thevis and of Paul Trevorrow, Wiley-Blackwell. We wish to express our sincere gratitude and warm thanks to both of them for giving us the opportunity to realize the special issue on *Analysis of Drugs for the Therapy of Anticholinesterase Poisoning*. We also would like to thank all authors and referees from highly recognized and respected institutions and authorities worldwide whose expertise, efforts, and engagement allowed us to arrange this compilation of current research papers and exceptional reviews and perspectives focused on analytical procedures and techniques for the measurement and characterization of the most important current antidotes and promising new therapeutics administered for the treatment of anticholinesterase poisoning. We would like to wish you an interesting and rewarding read.

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