Mass spectrometry for research and clinical implementation of TDM & CT

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Scope of the lecture:
The scope is a review of current applications of various hyphenated low and high-resolution mass spectrometry techniques (e.g. GC, LC, CE, MALDI, or paper spray coupled to quadrupole, ion trap, time-of-flight, or Orbitrap analyzers) in the field of TDM and clinical/forensic toxicology in both, research and practice.

Learning objectives:
1. Overview of current MS techniques
2. Typical applications in TDM and CT/FT
3. Discussion of advantages and limitations of these methods
4. Perspectives of MS in research and practice

Extended abstract:
In clinical pharmacology (CP) and in clinical toxicology (CT), the desirable or undesirable effects of drugs or poisons are assessed by the blood (plasma/serum) concentrations of the acting compounds/metabolites and/or corresponding biomarkers in correlation to pharmacological, pharmacokinetic, and clinical data. In contrast to therapeutic drug monitoring (TDM), in many CT and forensic toxicology (FT) cases, the applied drugs or poisons are unknown, so that a targeted or comprehensive screening has to be performed before quantification in blood.

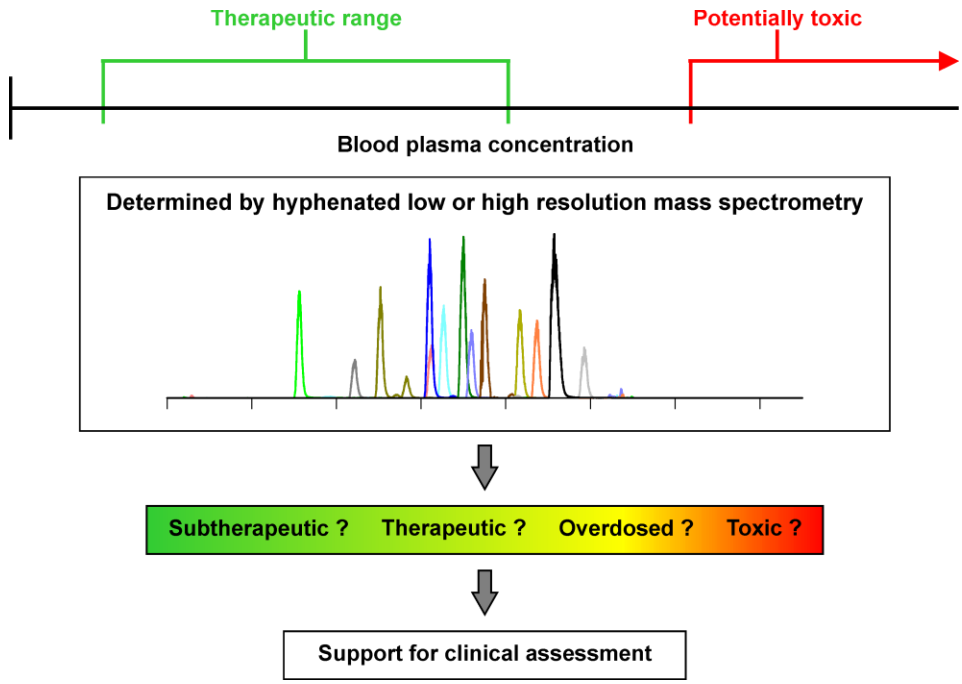

Figure 1. Concentrations of drugs or poisons in blood support clinical assessment of effects.

Over the years, various bioanalytical techniques have been applied for determination of blood levels. Today, besides immunoassays (IA), various hyphenated low and high-resolution (HR) mass spectrometry (MS) techniques are used such as gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis (CE), inductively coupled plasma (ICP), matrix-assisted laser desorption/ionization (MALDI), or paper spray ionization (PSI) coupled to quadrupole (Q), ion trap (IT), time-of-flight (TOF), or Orbitrap (OT) analyzers.

For TDM, LC coupled to tandem MS (LC-MS/MS) is the gold standard and has removed, e.g. in case of immunosuppressants, the immunoassays in many institutions because they are affected e.g. by cross reactivity, interferences with other drugs, metabolites, or endogenous factors.[1-3] However, pitfalls have to be considered also when using LC-MS.[4] If biomarkers fit better e.g. with the prediction of transplant rejection, such biomarkers can be determined by MS techniques as well.[3,5] Underlying metabolomics and proteomics studies also need MS approaches desireably with HR apparatus.[3,6] In case of very low-dosed drugs, modern very sensitive LC-MS systems are needed such as hybrids of Q with IT, TOF or OT analyzers. Such high sensitivity allows also miniaturization of TDM e.g. using dried blood spots for easier sampling and storage.[7]

In CT and FT, similar developments could be observed for quantification of drugs (of abuse), poisons, and/or their metabolites in blood.[8,9] However, in emergency toxicology where in contrast to TDM and FT, not series of determinations have to be performed but often single analyses for a variety of compounds, the analytical strategy can be different. The procedures must be available around the clock, suitable for all toxicologists on duty, robust, reliable, and fast. Examples have been described using GC-MS or LC-MS/MS and fast one-point calibration.[10,11] In comparison with full calibration TDM methods, they showed acceptable results for blood level assessment to support the clinical diagnosis and therapy. In addition, GC-MS is still established for determination of alcohols and other solvent including toxic glycols.[12] However, for rather polar compounds such as cardiovascular drugs or very low-dosed drugs such as fentanyl derivatives or synthetic cannabinoids of the group of so-called new psychoactive substances (NPS), LC-MS/MS particularly with TOF or OT analyzers is mandatory for blood quantification.[8]

In CT and FT, IA prescreening for a limited number of drugs of abuse developed for workplace drug testing in the USA is still common if the prevalence of positives is low and a huge number of samples have to be screened. As most NPS cannot be detected by IA and in case of high prevalence, the positives must be confirmed,[13] hyphenated MS is more and more replacing the IAs for drug testing. However, targeted MS drug screening also covers only a limited number of known compounds, although modern multi-analyte LC-MS/MS approaches with selected reaction monitoring allowed including several hundred analytes.[14] The identification power of course depends on the selectivity and number of monitored transitions.[9]

For comprehensive drug screening, mostly performed in urine, GC-MS has still its place in CT and FT thanks to well-established reference libraries with over 10,000 selective electron impact spectra and sophisticated search algorithms.[15,16] However, for the reasons mentioned before, LC-MS^n screening with a corresponding reference library was established as suitable complement to GC-MS.[17] In the meantime, LC-HR-MS/MS with TOF or OT analyzers was also applied successfully for targeted and comprehensive screening providing various advantages such as very high identification power together with comparable easy method development, high flexibility, robustness, sensitivity, and selectivity.[8] The first HR-MS reference library with focus on metabolites for urine screening was developed and successfully used for urine screening.[18] Detection of metabolites increases selectivity, allows confirmation of the body passage, and finally, minimizes the risk of false negative LC-MS results possibly caused by ion suppression of the target analyte. Even, the risk of false

positive results can be reduced by consideration of metabolic patterns.

The knowledge of such patterns is the prerequisite for developing metabolite-based screening approaches. As in drug discovery and development, such studies are also important for toxicological risk assessment if the metabolites show pharmacologic and/or toxic effects. The flood of NPS, which are sold and consumed without any preclinical testing, stimulated metabolism studies in animals or human liver preparations such as primary hepatocytes, cell cultures, S9 fraction, microsomes, or cytosol because controlled human studies are not allowed.[8,19] In this research field, HR-MS dominates because of its unique identification power and sensitivity.[20] It is also mostly involved in studies on drug binding at proteins or drug transporters.[21]

Finally, the high sensitivity of modern apparatus opens new fields of research and practice, e.g. for miniaturizing by dried urine spot[22] or paper spray urine screening (Michely/Meyer/Maurer, poster presentation No. 71, IATDMCT meeting 2017, Kyoto) as well as e.g. for detection of protein adducts of chemical warfare agents.[23] MALDI coupled MS can be used for microanalysis of drugs e.g. in hair or tissue samples.[24,25]

As stated elsewhere,[8] HR-MS devices seem to come very close to the jack-of-all-trade for applications in human toxicology, especially in CT and FT, but also TDM. They provide very high identification power together with comparably easy development of qualitative and quantitative methods. Qualitative methods are characterized by high flexibility, robustness, sensitivity, and selectivity. Thus, HR-MS will gain even more attraction when cheaper equipment and more user-friendly software packages will be provided.

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