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Chapter 29
Post-modern Medicolegal and Forensic Toxicology

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Abstract The ongoing progress in analytical and life sciences is opening up new perspectives in post-modern medico-legal and forensic toxicology. In times of personalized medicine, the interpretation of analytical results in clinical and forensic cases should also be based on genetic aspects, pharmacogenomics in particular. Pharmacologic and toxic effects of drugs or poisons may be influenced by the genotype and phenotype of an individual, but also by the isoenzymes involved in their metabolism and membrane transport. Further individual factors such as body mass, age, sex, kidney and liver function, and drug-drug (food-drug) interactions may have an impact. Detailed knowledge of all these factors is a prerequisite for evidence-based case interpretation. In this chapter, the current knowledge of possible risks in variations of the effects of relevant therapeutic drugs, herbal drugs, and drugs of abuse will be presented. A critical discussion of the impact on the interpretation of analytical results in clinical and forensic toxicology will follow.

29.1 Introduction

In times of ante-modern forensic toxicology (FT), drug concentrations were determined, for example, by spectrophotometry after thin-layer chromatographic separation and elution of the scraped spots following internal approaches. In modern FT, the blood concentrations are determined using the latest high-end mass spectrometry equipment in high resolution [1, 2] following all international

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guidelines for method validation and internal and external quality control [3] in an accredited laboratory. However, this analytically determined "true blood level" was/is often interpreted toxicologically by simply comparing it with a published reference list, e.g. by Schulz et al. [4]. Such lists are mainly based on data coming from controlled clinical trials of pharmaceutical companies or single case reports. Of course, this procedure is too simple for evidence-based case interpretation in times of P5 medicine and justice. While the pharmacokinetic variability within such trials is up to ten times, the variability among the real population may range up to 100 times [5]. Case reports are often well documented only for the medical or for the analytical part [6].

In (post-)modern toxicology, various effects influencing the individual drug/poison response have to be considered, such as age, gender, body-mass index, drug-drug or drug-food interactions, lifestyle and nutrition, the microbiome, and particularly the genome and epigenome [7, 8]. All effects may, for example, increase or decrease the bioavailability and plasma elimination half-life of a drug. Pharmacogenomics (PGx) plays a major role in the relationship of P5 medicine and justice [9]. In contrast to pharmacogenetics describing only the genetic variations, PGx describes multifactorial variations of drug responses caused by variable gene expression (polymorphisms), influence of drugs on genes (epigenetics), and time-dependent changes of gene expression [10]. These genetic and post-translationally acquired variations then describe the phenotype.

PGx is nowadays well established in P5 medicine, such as in oncology, transplantation, and psychiatry with the goal to find the right dose of the right drug for the right indication for the right patient at the right time [7]. Sim and Ingelman-Sundberg [11] discussed important pharmacogenomic biomarkers influencing treatment response and/or incidence for adverse drug reactions. However, the prerequisite is that the drugs are characterized during drug development concerning the influence of polymorphically expressed target proteins (receptors, ion channels, or enzymes) and proteins involved in drug transport or metabolism.

As the new psychoactive substances (NPS) are neither tested for preclinical nor clinical pharmacology and toxicology before distribution and consumption, such data have to be elucidated by, for example, academic institutions. The author's group, for example, has studied metabolism intensively, including the kinetics of the involved (iso)enzymes [12–20], the cytochrome P450 (CYP) inhibition potential of NPS [21, 22], and the possible interaction with drug transporters [23]. In pharmacokinetics, various drug transporters are involved in absorption, distribution, and elimination [24, 25]. For elucidating possible impact on drug response, PGx variations or interactions have to be tested for. Meyer et al. [23] described that the investigated NPS were no substrates of the major efflux transporter P-glycoprotein (P-gp), but some were potent inhibitors. Thus, PGx variations or

interactions will have no impact on the effect of these NPS, but they can produce interaction with substrates of P-gp. For example, loperamide and domperidone, both effluxed by P-gp at the blood-brain barrier, are discussed to act centrally by coadministration with P-gp inhibitors such as verapamil or quinidine.

For personalized case interpretation, Wong et al. [9] discussed various advantages and disadvantages of PGx as an adjunct biomarker in personalized justice. It is advantageous that the DNA is stable in postmortem settings and may provide a personalized approach for assessing the relation of drug response with the postmortem drug concentrations. However, there are only limited postmortem reference data available in contrast to clinical medicine. As already discussed, the legal interpretation is challenging as many posttranslational modifications have to be considered as well as interactions with inhibitors or inducers of drug metabolizing or transporting proteins.

In the following, published examples for the impact of PGx and/or interactions on real case interpretation will be discussed.

29.2 Evidence-Based Case Interpretation

29.2.1 Missing Drug Effect Caused by PGx Variations and/or Interactions

29.2.1.1 Tramadol in Personalized Pain Management

Tramadol is an enantioselectively metabolized by the polymorphically expressed CYP 2D6 to the more potent opioid receptor agonist *O*-demethyl tramadol. In the context of personalized therapy in pain management, Stamer et al. [26] could show that a patient with a CYP 2D6 poor metabolizer genotype formed the lowest blood concentrations of the active metabolite, while the ultra-rapid metabolizers formed the highest. They could confirm the clinical response correspondingly. In a further study, they gave a CYP 2D6 inhibitor to the ultra-rapid metabolizers and, as expected, they could be transferred to functional poor metabolizers with low blood concentration of the acting metabolite. Again, the blood levels correlated with the clinical outcome. This example demonstrates that the case interpretation would not be correct when the blood levels of the parent drug would have been correlated with published data.

29.2.1.2 Missing Pain Treatment Under Oxycodone

Lee et al. [27] described the case of a patient under pain treatment with oxycodone, but without analgesic effects. The anesthetist wanted to monitor his adherence by

urine drug testing for oxycodone. The test, performed with an assay focused on the parent drug only, was negative. After intake under supervision and still no response, they thought of possible PGx variations or interactions. Oxycodone is also a prodrug bioactivated by CYP 2D6. The patient was genotyped as CYP 2D6 poor metabolizer explaining the missing analgesic effects, but what was the reason for the negative urine test? They found that the patient was under treatment of the tuberculostatic rifampicin for years. Thus, the patient showed a significant induction of CYP 3A4, the enzyme responsible for the major metabolizing step leading to the pharmacologically inactive *N*-dealkyl metabolite. This example shows the above-mentioned complexity of evidence-based case interpretation. Incomplete drug testing with missing targets may lead to misinterpretation of the adherence test, drug-drug interaction as well as genetic variations in forming the acting metabolite, making a simple case interpretation impossible.

29.2.2 Poisoning Caused by PGx Variations and/or Interactions

29.2.2.1 Narcotic Syndrome After Codeine Administration

Gasche et al. [28] described a narcotic syndrome of a patient under therapeutic doses of codeine. Genotyping revealed that the patient was a CYP 2D6 ultra-rapid metabolizer forming a higher rate of the acting *O*-demethyl metabolite morphine. He was additionally under co-administration of the potent CYP 3A4 inhibitors clarithromycin and voriconazole. Thus, the main metabolizing step, namely the formation of the inactive *N*-demethyl metabolite, was blocked, resulting in even more morphine production. Finally, the patient suffered from an acute renal failure resulting in a limited elimination of the morphine-6-glucuronide, which can pass the blood-brain barrier and act as potent opioid. Again, this case shows that all aspects of PGx, interactions, and the body functions have to be considered when interpreting analytical results.

29.2.2.2 Fatal Poisoning After Breast Feeding of the Mother Under Codeine Treatment

Madadi et al. described a fatal morphine poisoning of a newborn after breast feeding of the mother under codeine treatment. The mother was a CYP2D6 ultra-rapid metabolizer forming high concentrations of morphine. This example

shows that PGx and interactions must also be considered in such cases when the mother may be accused of having killed the newborn by application of morphine.

29.3 Conclusions

In post-modern toxicology, it is a must—at least in any unclear cases—to consider pharmacogenomic variations and interactions in interpretation of analytical results. However, it is essential that the above-mentioned limitations are considered of genotyping detecting only the genetic risks. Antemortem, phenotyping [29] is preferred to detect genetic and acquired posttranscriptional variations. The future will show whether in postmortem cases, direct determination of the modified proteins (metabolizing enzymes, transporters etc.) can be determined by high-resolution mass spectrometry. On the other hand, over-interpretation of all above-mentioned aspects by untrained experts must be excluded.

References

1. Meyer MR, Helfer AG, Maurer HH (2014) Current position of high-resolution mass spectrometry for drug quantification in clinical & forensic toxicology. Bioanalysis 6:2275–2284
2. Maurer HH, Meyer MR (2016) High-resolution mass spectrometry in toxicology—current status and future perspectives. Arch Toxicol 90(9):2161–2172
3. Wille SMR, Peters FT, Di Fazio V, Samyn N (2011) Practical aspects concerning validation and quality control for forensic and clinical bioanalytical quantitative methods. Accred Qual Assur 16:279–292
4. Schulz M, Iwersen-Bergmann S, Andresen H, Schmoldt A (2012) Therapeutic and toxic blood concentrations of nearly 1000 drugs and other xenobiotics. Crit Care 16:R136
5. Bengtsson F (2004) Therapeutic drug monitoring of psychotropic drugs. Ther Drug Monit 26:145–151
6. Maurer HH (2007) Demands on scientific studies in clinical toxicology. Forensic Sci Int 165:194–198
7. Schwab M, Schaeffeler E (2012) Pharmacogenomics: a key component of personalized therapy. Genome Med 4:93
8. Meyer UA, Zanger UM, Schwab M (2013) Omics and drug response. Annu Rev Pharmacol Toxicol 53:475–502
9. Wong SH, Happy C, Blinka D et al (2010) From personalized medicine to personalized justice: the promises of translational pharmacogenomics in the justice system. Pharmacogenomics 11:731–737

10. Kalow W (2006) Pharmacogenetics and pharmacogenomics: origin, status, and the hope for personalized medicine. Pharmacogenomics J 6:162–165
11. Sim SC, Ingelman-Sundberg M (2011) Pharmacogenomic biomarkers: new tools in current and future drug therapy. Trends Pharmacol Sci 32:72–81
12. Maurer HH (2010) Chemistry, Pharmacology, and Metabolism of Emerging Drugs of Abuse. Ther Drug Monit 32:544–549
13. Meyer MR, Maurer HH (2012) Current status of hyphenated mass spectrometry in studies of the metabolism of drugs of abuse, including doping agents. Anal Bioanal Chem 402:195–208
14. Meyer MR, Richter LHR, Maurer HH (2014) Methylenedioxy designer drugs: mass spectrometric characterization of their glutathione conjugates by means of liquid chromatography-high-resolution mass spectrometry/mass spectrometry and studies on their glutathionyl transferase inhibition potency. Anal Chim Acta 822:37–50
15. Meyer MR, Robert A, Maurer HH (2014) Toxicokinetics of novel psychoactive substances: Characterization of N-acetyltransferase (NAT) isoenzymes involved in the phase II metabolism of 2C designer drugs. Toxicol Lett 227:124–128
16. Meyer MR, Schütz A, Maurer HH (2014) Contribution of human esterases to the metabolism of selected drugs of abuse. Toxicol Lett 232:159–166
17. Welter-Luedeke J, Maurer HH (2015) New psychoactive substances: chemistry, pharmacology, metabolism, and detectability of amphetamine derivatives with modified ring systems. Ther Drug Monit 38:4–11
18. Schaefer N, Wojtyniak JG, Kettner M et al (2016) Pharmacokinetics of (synthetic) cannabinoids in pigs and their relevance for clinical and forensic toxicology. Toxicol Lett 253:7–16
19. Richter LHR, Kaminski YR, Noor F, Meyer MR, Maurer HH (2016) Metabolic fate of desomorphine elucidated using rat urine, pooled human liver preparations, and human hepatocyte cultures as well as its detectability using standard urine screening approaches. Anal Bioanal Chem 408:6283−6294
20. Wagmann L, Meyer MR, Maurer HH (2016) What is the contribution of human FMO3 in the N-oxygenation of selected therapeutic drugs and drugs of abuse? Toxicol Lett 258:70
21. Dinger J, Meyer MR, Maurer HH (2016) In vitro cytochrome P450 inhibition potential of methylenedioxy-derived designer drugs studied with a two cocktail approach. Arch Toxicol 90:305–318
22. Dinger J, Woods C, Brandt SD, Meyer MR, Maurer HH (2016) Cytochrome P450 inhibition potential of new psychoactive substances of the tryptamine class. Toxicol Lett 241:82–94
23. Meyer MR, Wagmann L, Schneider-Daum N, Loretz B, de Souza-Carvalho C, Lehr CM, Maurer HH (2015) P-glycoprotein interactions of novel psychoactive substances - stimulation of ATP consumption and transport across Caco-2 monolayers. Biochem Pharmacol 94:220–226
24. Kitamura S, Maeda K, Sugiyama Y (2008) Recent progresses in the experimental methods and evaluation strategies of transporter functions for the prediction of the pharmacokinetics in humans. Naunyn Schmiedebergs Arch Pharmacol 377:617–628
25. Wilson AGE (ed) (2016) New Horizons in Predictive Drug Metabolism and Pharmacokinetics. Royal Society of Chemistry, London
26. Stamer UM, Zhang L, Stuber F (2010) Personalized therapy in pain management: where do we stand? Pharmacogenomics 11:843–864
27. Lee HK, Lewis LD, Tsongalis GJ, McMullin M, Schur BC, Wong SH, Yeo KT (2006) Negative urine opioid screening caused by rifampin-mediated induction of oxycodone hepatic metabolism. Clin Chim Acta 367:196–200

28. Gasche Y, Daali Y, Fathi M, Chiappe A, Cottini S, Dayer P, Desmeules J (2004) Codeine intoxication associated with ultrarapid CYP2D6 metabolism. N Engl J Med 351:2827–2831
29. Ghassabian S, Chetty M, Tattam BN et al (2009) A high-throughput assay using liquid chromatography-tandem mass spectrometry for simultaneous in vivo phenotyping of 5 major cytochrome p450 enzymes in patients. Ther Drug Monit 31:239–246