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Review Article

Forensic relevance of glucuronidation in phase-II-metabolism of alcohols and drugs

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ABSTRACT

Forensic toxicology means detecting toxic or pharmacologically active substances in body fluids and organs and the evaluation and judgement of the respective results. In the legal judgement, not only the taken in active drugs, but also their metabolites are to be included. Regarding metabolism one distinguishes phase-I- and phase-II-metabolism. In the phase-I-metabolism, active substances are converted by oxidation, reduction or hydrolysis, but influencing the polarity of more lipophilic substances often not decisively. The pharmacological activity is often preserved or even increased. In phase-II-metabolism a highly hydrophilic substance – mostly glucuronic acid – is coupled to the active substances or the respective phase-I-metabolites. This reaction step decisively increases hydrophilicity of lipophilic substances, thus enhancing renal elimination and often also abolishing pharmacologically and/or toxicologically effects. Nevertheless the interaction of different drugs and alcohols in glucuronidation and the glucuronides of phase-II-metabolism still do not play a substantial role in the forensic-toxicological analysis and interpretation of results so far. However, in vitro investigations since 1999 in our lab show that such interactions are not unlikely. For valid interpretation of complex cases in the future it may become necessary not only to quantify drugs and the phase-I-metabolites but also the phase-II-metabolites and discuss possible interactions in the metabolism.

1. Introduction

As a branch of the legal medicine, forensic toxicology contributes substantially to the cause of death clarification. Among unnatural deaths, suicides are of rather low forensic interest, whereas poison adductions by other persons are of highest relevance. This applies to cases with deadly poisoning as well as to cases with not deadly poisoning. A distinct differentiation between self adduction and foreign adduction is difficult by toxicological analyses only. However, the intake of a poison in very high doses concedes the case for self adduction. Hence, the intake of a barely lethal dose of a toxic substance often indicates a foreign adduction. But the dose is of course only one of several questions to be answered. Therefore for an appropriate judgement of poisoning cases, all analytic possibilities must be exhausted. This includes the active substances as well as their respective metabolites.

2. Metabolism

Among other duties, the removal of foreign substances from the body is a major function of metabolism. This also counts to forensic-toxicological relevant, psychoactive substances, like alcohols and legal or illegal drugs. Many of these active substances are lipophilic, and for this reason cannot be eliminated renal. Hence, the organism owns enzyme systems, which can convert such active substances in more hydrophilic substances, which can be eliminated a lot easier. One has to differentiate between phase-I- and phase-II-metabolism.

2.1. Phase-I-metabolism

Phase-1-metabolism is characterized by oxidative, reductive or hydrolytic reactions, usually increasing hydrophilicity. Nevertheless, these reactions do not generally enhance the removal of the drug from the body. Take, for example, the demethylation of benzodiazepines. Diazepam has a clearly shorter half-life (24–48 h) than the metabolit nordiazepam (50–80 h) [1]. Same counts to clobazam (10–50 h) and norclobazam (35–133 h). [2] Besides, these metabolites are pharmacologically active. Therefore, from these reactions arises no benefit for the organism. On the contrary, the increased duration of drug effect is to be led back substantially on this phase-I-metabolism. Even more impressive, for example, is tilidine, which constitutes only a non-active prodrug for the active metabilite nordilidine. The oxidation of ethanol to acetaldehyde is likewise no detoxication, in fact acetaldehyde is substantially more toxic than ethanol. With a genetically conditioned, decreased activity of acetaldehyde dehydrogenase, this becomes apparent by typical symptoms of intoxication.

Other oxidative reactions, like the metabolism of diazepam to temazepam and oxazepam, highly increase hydrophilicity, which contrasts to demethylation. However, the substantially shorter

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half-life of temazepam (6–25 h) and oxazepam (6–20 h) [1] is not to be led back substantially on the raised hydrophilicity, but on the possibility of a following phase-II-reaction.

2.2. Phase-II-metabolism

Phase-II-reactions mean conjugation of activated endogenous substrates with alcoholic or phenolic hydroxyl and amino groups of suitable drugs or phase-I-metabolites. Glucuronic acid is being transferred by UDP-glucuronyltransferases from activated glucuronic acid – Uridin-5-diphosphoglucuronic acid (UDPGA). Glucuronic acid is a relatively strong acid, but also very hydrophil, as it contains four alcoholic hydroxyl-groups. These coupling reactions do not only increase hydrophilicity and the removal from the body, the organism has one further benefit from these reactions: the immediate inactivation of pharmacological or toxicological effects, at least in most cases. Known exceptions are only the glucuronides of the opiates morphine and codeine in position 6. Thus, phase-II-metabolism plays a major role in the inactivation of many toxicological relevant substances. These include benzodiazepines, opiates, paracetamol and others. But which parameters are relevant for the coupling activity? The chemical structure is important, but also interactions with competing physiological and not physiological substrates and pharmacogenetics.

3. Pharmacogenetics

The individual genetic background can be apparently rather different. Meanwhile is known that Glucuronidation is catalyzed by different isoenzymes. These are divided into two families – UGT1 and UGT2 – and this again in three subfamilies – UGT1A, UGT2A and UGT2B. Today we know more than 26 different human isoenzymes. Twelve of these isoenzymes are commercially available yet. Some of them are highly specific for certain substrates, but others are more unspecific and therefore able to glucuronidate synthetic substances as well as their metabolites [3,4] (Table 1).

Today we assume an interindividual polymorphism of gene expression that influences the ability to eliminate drugs, alcohols and other substances. For example we know that 5–10% of the population show an only low activity in UGT1A1 and show, therefore, raised concentrations of bilirubine in the blood, because glucuronidation is essential for the removal of bilirubine. This is known as "Gilberts Syndrome". Paracetamol is glucuronidated in particular by UGT1A6. But in 1992, de Morais et al. [5] could show that patients with Gilbert's syndrome eliminate substantially less paracetamolglucuronide than healthy persons. Obviously there is a correlation between the activities of UGT1A1 and 1A6.

The glucuronidation of congener alcohols is highly individual as the working group of Bonte found out [6]. In several drinking trials, some participants showed reproducible high, others rather low rates of glucuronidation.

After heroin consumption, we have observed in some cases an unexpectedly low rate of glucuronidation, which cannot be explained regarding the time of consumption. Whether enzyme activity is decreased or competition reactions with other active substances are the cause of the observed low glucuronidation, deserves further study.

Table 1
Specifity of several UDP-glucuronyl-transferases for different substrates [3,4].

Families	
UGT1	UGT2
Subfamilies	
UGT1A	UGT2A, UGT2B
UGT-specificity	
1A1 Bilirubine...	2B3 Aliphatic alcohols...
1A4 Amitriptyline...	
1A6 Paracetamol...	2B7 Morphine, codeine...
1 A9 Propofol...	

4. Drug interactions

Beside the genetic differences, interactions of different active substances may appear. Unfortunately, in vivo studies are not practicable for ethical reasons, for example, oxazepam intake with or without consumption of considerable amounts of alcoholic beverages. Here, we are limited to the knowledge that we gain from acute intoxications. Therefore, in vitro studies are more suitable than in vivo studies.

From the forensic point of view, Iwersen and Schmoldt carried out such studies first with rat liver microsomes in 1998. Aliphatic alcohols are metabolized by UGT2B [4].

5. Enzymatic investigations in vitro

Enzyme kinetics describes how quickly enzyme-catalyzed reactions run off. The turnover of a substrate is depending upon the substrate concentration, provided that temperature, pH, enzyme and cosubstrate concentration are constant. However, under the respective conditions there is a maximum velocity (V_{max}) which cannot be increased by rise of the substrate concentration, because all available enzymes are completely bound in a complex with the substrate. When just half of the enzymes are bound to substrate, the velocity of the enzymatic turnover is also just half of the maximum velocity (V_{max}). The concentration of the substrate at this point is called "Michaelis Menton constant" (K_M). K_M describes the affinity between the enzyme and the respective substrate. With high K_M, the affinity is low, with a low K_M you have a high affinity between enzyme and substrate. With a twice reciprocal axis configuration in the "Lineweaver Burk" diagram, K_M and V_{max} arises from the axis intersections $1/K_M$ or 1/V max. Generally, enzymatic reactions can be restrained by inhibitors, which themselves can also be a substrate. In competitive inhibition, substrate and inhibitor exclude themselves mutually from binding to the enzyme. The inhibitor can be edged out by raised substrate concentrations, however, from the enzyme, so that V_{max} does not change in contrast to K_M. By contrast, in noncompetitive inhibition, the inhibitor does not bind to the active site of the enzyme. However, the tertiary structure of the enzyme is changed through the binding, which leads to a decrease of enzyme activity. This leaves K_M unchanged, whereas V_{max} is decreased. In a mixed or combinated inhibition, V_{max} is not influenced, however, K_M is increased or decreased.

In 1999, we started such in vitro studies in our lab [7–10]. We use commercially available, human liver microsomes, in each case as a pool of at least 20 corpses. The following parameters were determined:

- Affinity of substrate and enzyme (K_m).
- Maximum velocity V_{max}.
- In inhibition trials: also the constant of inhibition (K_i) and the type of inhibition (competitive inhibition, mixed inhibition, like Fig. 1, noncompetitive inhibition).

In the following, I will present some of the results of our working group in Cologne.

The Table 2 shows that, with increasing chain length, the enzyme's affinity to the substrate increases. From ethanol to *n*-pentanol, the affinity increases by around factor 26, from 366 to 14 mM. Moreover, the turnover speed rises by the factor 35. Branched mol-