**Prodrug Metabolites: Implications for Therapeutic Drug Monitoring**

Prodrugs are analogs of active drugs that have been developed to improve the bioavailability and/or tolerability of the latter. Fosphenytoin (1) and mycophenolate mofetil (MMF) (2) are two examples of prodrugs where monitoring of the active drug, the anticonvulsant phenytoin and the immunosuppressant mycophenolic acid (MPA), respectively, is used to guide therapy. The successful application of therapeutic drug monitoring to prodrugs requires a reliable analytical methodology, which in general should be specific for the active drug and should provide timely results so that the clinician can, if necessary, institute appropriate dosage adjustments. Although HPLC-based methodology is the most specific procedure for drug measurements, immunoassays are more rapid; they may, however, be subject to interference through cross-reactivity with structurally related compounds (e.g., prodrugs and drug metabolites). This problem often is aggravated in patients with liver dysfunction or renal insufficiency because of extensive accumulation of drug metabolites.

The use of prodrugs has the following implications for therapeutic drug monitoring:

- Prodrugs themselves may cross-react in immunoassays, thereby confounding pharmacokinetic data and clinical interpretation.
- Prodrugs may be metabolized independently of the active drug, and the resulting metabolites may cross-react in immunoassays for the active drug.
- From a pharmacodynamic perspective, the contributions of prodrug, active drug, and drug metabolites to the overall pharmacological activity need to be known.

In this issue of *Clinical Chemistry*, Annesley et al. (3) report on the isolation and characterization of a novel oxymethylglucuronide metabolite of fosphenytoin, a prodrug of phenytoin. This metabolite has been identified in the plasma of uremic patients receiving fosphenytoin. Phenytoin is a primary anticonvulsant drug that is used for the prophylaxis of generalized tonic-clonic and partial seizures. Because of its narrow therapeutic index, its saturable elimination kinetics, and its concentration-dependent side effects, monitoring of drug plasma concentrations is recommended as a guide to dosage individualization. Another aspect to be considered when monitoring phenytoin is the fact that it is highly protein bound (>90%). Conditions associated with hypoalbuminemia, altered protein binding (e.g., uremia), or the presence of other exogenous substances that may displace phenytoin from its protein binding sites can cause an increase in the free fraction of phenytoin. Because only unbound drug is pharmacologically active, it is useful to measure the free concentration when any of the above conditions are present.

Fosphenytoin is a phosphate ester prodrug of phenytoin with an improved solubility and better tolerance after intramuscular or intravenous administration. It is rapidly hydrolyzed in vivo to phenytoin with a half-life of 5–15 min. The prodrug is not pharmacologically active, but it has been shown to cross-react in various immunoassays for phenytoin. It is therefore recommended that phenytoin concentrations not be monitored using potentially nonspecific immunoassay methods for at least 2 h after intravenous or 4 h after intramuscular fosphenytoin administration (4). Elimination of phenytoin occurs primarily by biotransformation to several inactive hydroxylated metabolites. Some of these metabolites, notably 5-(p-hydroxyphenyl)-5-phenylhydantoin (HPPH), are further metabolized by conjugation with glucuronic acid. Accumulation of the metabolites, particularly the glucuronide conjugate of HPPH (HPPH-G) occurs in patients with renal failure. Some of the earlier immunoassays for phenytoin were found to give erroneously high apparent phenytoin results in patients with renal insufficiency because of cross-reactivity with HPPH-G. The bias was even more substantial for the free phenytoin concentration because HPPH-G is less strongly bound to plasma proteins than phenytoin. Most newer immunoassays for phenytoin, however, do not display any significant cross-reactivity with HPPH-G (5,6).

Against this background, Roberts et al. (6) investigated the accuracy of various immunoassays for monitoring of phenytoin concentrations in patients with renal insufficiency treated with fosphenytoin. With one exception, all immunoassays tested gave falsely increased phenytoin concentrations that were up to 20 times higher than the HPLC results. The sensitive HPLC method used in that study detected no fosphenytoin in any sample, thus eliminating the prodrug as the cause for the discrepancy. The authors reasoned that the bias must be attributable to a hitherto unknown metabolite or adduct of fosphenytoin that can accumulate in renal failure. Further study culminated in the present report (3), which presents definitive evidence for the structure of this novel immunoreactive metabolite derived from fosphenytoin. It is, to our knowledge, the first example of a direct glucuronidation of the reactive site on a prodrug.

A question that still needs to be answered is whether the metabolite possesses pharmacological activity. Roberts et al. (6) reported that four of seven study subjects appeared to have had their dosages of fosphenytoin reduced in response to falsely increased free phenytoin concentrations. Without knowledge of the pharmacological activity of the oxymethylglucuronide metabolite, it is difficult at present to say whether such dosage changes are really justified. A definite answer will require the synthesis of sufficient material to carry out the necessary studies.

Usually, biotransformation of a drug yields metabolites with diminished or no pharmacological activity. There are, however, exceptions to this rule. Morphine-6-glucuronide but not morphine 3-glucuronide is an analgesically active morphine metabolite (7). Recently, an acyl-
glucuronide conjugate of MPA was isolated, characterized, and subsequently found to possess pharmacological activity (8, 9). In contrast, the major metabolite of MPA, the phenolic glucuronide, is pharmacologically inactive.

There are some interesting and informative parallels between the identification of the MPA acylglucuronide and the discovery of the oxymethylglucuronide metabolite of fosphenytoin described by Annesley et al. (3). MPA is administered in the form of the prodrug MMF, a morpholinoethyl ester of MPA. MMF is rapidly hydrolyzed by tissue and plasma esterases to the active drug MPA and is not usually detectable in plasma after oral administration. In analogy to fosphenytoin, MMF shows a strong cross-reactivity in the immunoassay for the active drug MPA and is detectable in plasma during and immediately after intravenous infusion of MMF. As in the studies performed by Annesley and colleagues (3, 4, 6), the first identification of the presence of a novel metabolite came from an observation of a marked bias between an immunoassay and an HPLC procedure specific for the parent drug (8). However, in contrast to the novel phenytoin metabolite, the acylglucuronide of MPA is a metabolite of the active drug. In an outcome study, HPLC and the immunoassay were found to be comparable with respect to the assessment of the risk for acute rejection in pediatric patients (10). For the immunoassay, however, higher cutoffs applied for predose MPA concentration and for areas under the curve of concentration vs time. In this case, the bias caused by the pronounced cross-reaction of the acylglucuronide in the immunoassay apparently did not compromise the predictive power of the test.

It has been postulated that test procedures that detect the parent drug and its active metabolites proportional to their biological activities will be superior to immunoassays in which the response does not mimic bioactivity. Miller et al. (11) compared the results of four commercially available digoxin immunoassays with the biological activity of digoxin and its metabolites determined using a human heart receptor assay. Digoxin undergoes metabolism to various deglycated, reduced, and polar metabolites with a wide spectrum of biological activities. Only one of the immunoassays was found to give digoxin results that closely correlated with the response of the receptor-based assay.

These studies highlight the importance of following up unexpected discrepancies between analytical methods. As discussed above, the additional cross-reactivity of drug metabolites in immunoassays can either produce falsely increased drug concentrations or, in certain cases, might better reflect total pharmacological activity. The study of Annesley et al. (3) should alert workers in this field to the possibility of alternative metabolic pathways involving prodrugs with potential analytical and pharmacodynamic consequences.

References


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