Drug Metabolites and Immunoassays

Many drugs are converted to metabolites to varying degrees, and these metabolites may or may not be bioactive. The degree of bioactivity is not always clearly known. Blood often contains a mixture of parent drug and metabolites, with the concentrations of the metabolites often not being very predictable. The parent drug occasionally requires biotransformation before it becomes bioactive. The relative amounts of the parent drug and its different metabolites may be influenced by genetic factors, by differences in renal or hepatic function, and, potentially, by other drugs. Such drugs that are often subjected to therapeutic drug monitoring include theophylline, carbamazepine, some tricyclic antidepressants, cyclosporin A, quinidine, procainamide, lidocaine, and digoxin.

Immunoassays are often used in therapeutic drug monitoring. They may be highly analytically specific for the parent drug, and thus avoid assay of metabolites and other drugs; this has sometimes been a stated aim. However, the antibodies used in such assays may still cross-react with some endogenous compounds, as with digoxin assays. Different commercial assays can have quite variable cross-reactivities with metabolites of the parent drug.

In this issue, Miller et al. (1) may be commended for their attempt to find commercial digoxin immunoassays in which the degree of cross-reactivity with metabolites of digoxin parallels their pharmacologic activity. The authors suggest that their findings with the digoxin model may be important because “they demonstrate that the concept of developing antibodies that mimic the ligand response to natural receptors is apparently achievable” and “may represent a marked improvement in TDM over current immunoassays for measuring drugs in serum.” To what extent was their objective achieved?

Digoxin is a compound with a steroid nucleus, a lactone ring at the 17 ring position, and three sugars at the other end at the 3 position. It is biotransformed mainly in the liver, through oxidation catalyzed by the cytochrome P450 system (2) to the one-, two-, and three-deglucylated metabolites called the bis- and monodigitoxosides and 3β-digoxigenin, which is converted to 3α-(epi)-digoxigenin (3, 4). Conjugation then results in the formation of the 3-epi-glucuronide and the 3-epi-sulfate of digoxigenin. Conjugation of the monodigitoxosides also occurs (5). These are more polar end-metabolites and their production appears to be a major route of metabolism that can vary greatly from individual to individual (6, 7). Reduction of the double bond in the lactone ring to dihydro metabolites also occurs with considerable variation and depends, at least in part, on specific intestinal bacteria (8). There are probably other metabolites as well (4, 6).

The bioactivity of the different metabolites varies considerably, with most studies indicating a progressive reduction in activity as each sugar is removed. The greatest loss of bioactivity occurs with reduction of the lactone ring and formation of the dihydro metabolites (8). Information about the bioactivity of these metabolites is limited, as is information even on the amount of the conjugated and unidentified polar metabolites that in some (3, 6), but not all (9), reports have been considered the major urinary components of metabolized digoxin.

Miller et al. (1) compare the cross-reactivity of the mono- and bisdigitoxosides, digoxigenin, and the dihydro metabolites in four commercially available digoxin immunoassays with the bioactivity of the metabolites determined with eight biologic and biochemical assays reported in the literature. They also make a comparison with a previously published digoxin RIA (10).

The eight assays documenting biological activity of digoxin and metabolites included use of whole heart, or samples of heart, from mouse, cat, guinea pig, and human; the methods of assessing activity included toxicity, inotropic, inhibition of Na⁺,K⁺-ATPase, and competitive displacement of ouabain from Na⁺,K⁺-ATPase of human heart tissue determined by a radioreceptor assay (11). Results varied considerably, with some activities for bis- and monodigitoxosides being higher than that of digoxin; but were most lower, with means of 91% and 81% of digoxin activity. All studies showed lower values for digoxigenin (mean 23%) and for dihydroidigoxin (0.7% to <4.2%). The one immunoassay based on use of a monoclonal antibody showed cross-reactivities of 75%, 47%, 0.7%, and 0.05% of digoxin for the bis- and monodigitoxosides, digoxigenin, and dihydroidigoxin, respectively, and the highest correlation (r = 0.94) with results obtained with the radioreceptor activity assay; in this last assay, the relative activities of the metabolites were 52%, 47%, 11%, and <1% of digoxin activity, respectively.

Potential limitations of the conclusions regarding the preferred immunoassay include: the limited cross-reactivity (0.7%) of digoxigenin with digoxin (ideally, it probably should have been ≥10%); the virtually absent (0.05%) cross-reactivity of dihydroidigoxin with digoxin [reports of ≤10% of the bioactivity of digoxin being attributable to dihydroidigoxin are not uncommon (12, 13)]; selection of an in vitro test for comparing bioactivities; and failure to include any of the polar compounds including conjugates in the comparisons.

The importance of conjugated and other polar metabolites is difficult to assess with the available data and with the wide variation in the amounts of these metabolites in different individuals. If one considers unchanged digoxin and all digoxin metabolites, the proportion of polar metab-
ATPase

Although with even the ventricular include metabolites quoted (1) includes the 61–109% cross-reactivity between β-digoxigenin glucuronide and digoxin found in five of the same digoxin RIAs of the early 1980s. Although limited studies (7, 14, 15) indicate that the inotropic bioactivity of the polar metabolites is slight, it is possible that these metabolites mediate augmentation of arrhythmias through the central nervous system (15).

The problems in digoxin immunoassays caused by the cross-reactivities of digoxin metabolites (and possibly in other immunoassays in which there are active metabolites) may be small compared with the problems caused by factors that influence the relation between digoxin dose and variations in the concentration of digoxin in serum. These factors (16) include physical exercise and, sometimes, acute renal failure (17), which seem to alter the apparent distribution of digoxin because of reduced binding; thyroid function; lack of a standard time for conducting the assay after dosing with digoxin; cross-reactivities with mainly endogenous digitalis-like factors; substances that can alter absorption, elimination, and biotransformation, such as some drugs; and, especially, renal function. These factors, and the few convincing data indicating that use of the serum digoxin assay can save lives or provide cost benefit, make some physicians consider clinical, electrophysiologic, and other laboratory tests to be of more value than assays of serum digoxin, so that they tend to use intuitive or fixed dosing without conducting the assay.

Of more recent concern (18) is the way in which digitalis glycosides function through differential binding to and inactivation of multiple distinct Na⁺,K⁺-ATPase isoforms that are differentially expressed and regulated throughout the cardiovascular system. These include the α-1 isoform, which predominates in the ventricular myocardium, and the α-2 and α-3 isoforms, which may localize in conducting system structures. Their differential presence or expression, perhaps as a result of genetic or endocrine factors, could dissociate the contractile and conduction functions of digoxin, and even predispose an individual to digitalis toxicity (18).

Improved mortality has not been clearly shown in patients treated with digoxin, and the use of oral digoxin for symptomatic congestive heart failure has, for many physicians, become a second-line approach to therapy, with vasodilators, especially inhibitors of angiotensin-converting enzyme, being used primarily; as in atrial fibrillation, digoxin is used mainly for patients with systolic dysfunction (19). Nevertheless, several large prospective trials support the benefit of digoxin, alone or in combination with other treatments, in congestive heart failure, and currently this drug remains the only reasonably safe and effective oral, long-term, inotropic agent (19).

Computer surveillance (16) may be used to monitor some, but not all, of the factors that influence serum digoxin concentration and to aid in the interpretation of the data so obtained. In spite of some individuals’ negative opinions and the known limitations of the assay, I suggest that the serum digoxin assay is also important, e.g., to assess other factors such as patient compliance, clinically ineffective dosage, and evidence of toxicity. Therefore, every step taken to improve the digoxin immunoassay is important, and the attempt of Miller et al. (1) to provide a “bioactive average” is a valuable step in improving our understanding of the relation between the cross-reactivity of metabolites and their bioactivity; indeed, we may hope that their work sets an example and stimulates others to improve the immunoasays of other drugs with active metabolites.

References


M. H. Gault

Faculty of Medicine
Memorial University of Newfoundland
St. John’s, Newfoundland
Canada, A1B 3V6
Fax 709-737-6995