Monitoring of Unbound Digoxin in Patients Treated with Anti-Digoxin Antigen-binding Fragments: A Model for the Future?

Digoxin is one of the few therapeutic drugs for which antidotal therapy is available (1). Administration of Fab fragments has successfully been used to reverse the effects of life-threatening digoxin overdoses for over 20 years. Digoxin-specific Fab fragments are produced by cleaving sheep digoxin-specific IgG with papain to form two Fab fragments (50 000 Da each) and one 50 000-Da Fc fragment (2). As determined by equilibrium dialysis with one such preparation of Fab fragments, the intrinsic affinity constant for binding of digoxin was $10^{10}$ L/mol (3). Depending on the experimental conditions used (e.g., K+ concentration and type of membrane isolated), the affinity constant for binding of digoxin to its receptor (the sodium pump) has been reported to be in the range $10^{-9}$-$10^{-8}$ L/mol (4, 5). The rationale for using Fab fragments to reduce toxic effects of digoxin is their greater affinity for digoxin when compared with the sodium pump. As a consequence, the extracellular unbound digoxin concentration is lowered, and further equilibration of receptor-bound digoxin with the fluid in the extracellular space leads to the eventual release of digoxin from its receptor sites. The affinity of digoxin for binding to Fab fragments is 10-fold lower than that of digoxin; however, it is high enough to allow clinical utility of Fab fragments in cases of digoxin intoxication as well (3).

The dose of Fab fragments should be approximately equimolar to the total body digoxin load, which is determined according to the serum digoxin concentration and/or patient’s medical history (6). Digibind® (Glaxo Wellcome) is the most common brand of anti-digoxin Fab fragments used in the United States and worldwide. Boehringer-Mannheim also produces anti-digoxin Fab fragments intended for use as an antidote. Anti-digoxin Fab fragments have been suggested and used as antidotes in intoxications caused by other digitalsis-like molecules such as oleandrin (7–9), bufadienolide-containing aphanoradisiac (10), digitoxin (11), and foxglove extract (12); they have also been successfully used to reverse hypertension believed linked to increased digoxin-like immunoreactive factors in blood (13). In 90% of digitalsis-induced intoxicated patients, a median initial favorable response time of 19 min was achieved after administration of Digibind (14). Hyperkalemia associated with digitalsis poisoning is also reversed after administration of Fab fragments (2).

Fab fragments have a volume of distribution of 0.4 L/kg, an elimination half-life of 16–20 h, and a systemic clearance of 0.324 mL·min⁻¹·kg⁻¹ (15). Both renal and nonrenal (i.e., metabolism and/or removal by the reticuloendothelial system) routes are responsible for the elimination of Fab-digoxin complexes with approximately 65% of both Fab as well as Fab-digoxin complexes eliminated by the kidneys (15). Such complexes are not removed by hemodialysis or even by continuous arteriovenous hemofiltration (16). In patients with reduced or no renal function, Fab fragments can remain in plasma for 2–3 weeks after administration because of decreased renal elimination.

After administration of Fab fragments, there is approximately a 10- to 30-fold increase in total digoxin concentration in the serum, whereas the unbound fraction of the drug decreases rapidly (17). Because the unbound fraction of digoxin is the pharmacologically active form, its accurate and reliable measurement in serum collected at various times after administration of Fab fragments may be clinically important (18). The rationale here is that rapid removal of all bioactive digoxin from a patient in need of the drug is not clinically efficacious, and an ability to titrate the unbound digoxin would seem optimal. Although no definitive evidence has yet been presented, the concept seems rational. Furthermore, the pharmacokinetics of digoxin after administration of Fab fragments becomes dependent on the disposition of Fab (19). Monitoring of unbound digoxin concentrations after administration of Fab fragments may be appropriate to establish or eliminate toxicity in renal failure patients, to assess the need for more Fab to be administered, and to establish the need for reintroduction of digoxin (15). However, specimens collected from patients treated with digoxin Fab fragments usually give grossly erroneous and misleading values for digoxin concentrations by most immunoassays (20). Assay antibody and Fab fragments have similar affinities for digoxin and the tracer. Therefore, the most likely mechanism of interference of Fab fragments in digoxin assays is by binding of the assay tracer components. In fact, over the last few years, over a dozen publications have addressed the issue of discrepant digoxin values caused by the presence of this antidote in blood. One study in particular noted discrepancies between several immunoassays for digoxin as long as 14 days after a patient had been treated with Fab (21). Generally, immunoassays involving wash steps are less prone to interference by Fab fragments because the unbound Fab fragments are removed before the addition of tracer during the wash step (17).

In some digoxin immunoassays that require acid precipitation of proteins, pretreatment of sample with acid releases the Fab-sequestered digoxin; subsequently, total digoxin present in the serum including the bound and the unbound fractions is measured. This usually gives very large measured values. There appears to be no clinical value in measuring total serum digoxin concentrations in patients treated with the antidote.

One method for measuring the unbound digoxin is the use of ultrafiltration before the immunoassay. Although this procedure is cumbersome, it has been used successfully (18, 22). In the study reported in this issue, Ocal and Green (23) have used the Stratus and AxSYM digoxin immunoassays to analyze three samples from a patient treated with Digibind. In addition, they also analyzed several samples to which Digibind was added at different
amounts to a sample containing digoxin. These authors concluded that there was good correlation between these assays in measuring unbound digoxin in the presence of Digibind. We believe that ultrafiltration of a portion of these samples followed by measurement of the unbound digoxin by an immunoassay (previously shown to be free of matrix-dependent error) might have been used to monitor the accuracy of these immunoassays in measuring unbound digoxin in the presence of Fab fragments. The problem of bias caused by matrix in the immunoassays used for measuring the concentration of digoxin in the ultrafiltrate has been previously recognized (24). Ujhelyi et al. (17) have also reported a mean prediction error of 0.62 μg/L (0.54–0.70 μg/L) for the Baxter Dade Stratus digoxin immunoassay as a result of bias between ultrafiltrate and serum matrices. Because the Abbott TDx fluorescence polarization immunoassay digoxin assay measures the sample after protein precipitation, the measured matrix is protein-free. Therefore, we agree with the recommendation by Ujhelyi et al. of using that assay to monitor the unbound digoxin concentration in the ultrafiltrates as reference in their study. However, just because the Stratus and the AxSYM results for measuring unbound digoxin in Fab-treated patients correlate, one cannot conclude that the results are accurate as well. Therefore, as recommended in the recently established Guidelines for Therapeutic Drug Monitoring developed by the National Academy of Clinical Biochemistry (24), the authors might have prepared a pool of digoxin-free serum ultrafiltrate. Then varying concentrations of digoxin could have been added to the ultrafiltrate and the serum followed by analysis for digoxin. If results for the serum and the ultrafiltrate were statistically equivalent, the assay would have been better documented as suitable to monitor digoxin in the ultrafiltrates of the correlation samples.

Serum samples to which various concentrations of digoxin and Fab fragments have been added may not accurately represent samples collected from antidote-treated patients. Ocal and Green (23) have used three samples from a Digibind-treated patient in addition to several samples to which Fab was added by titrating their stock digoxin sample. We commend them for using actual patient samples for the correlation studies; however, use of an appropriate reference to monitor accuracy of their results would have buttressed their conclusions.

Despite several reports in the literature, including the current paper by Ocal and Green (23) on the methodology for measuring unbound digoxin in Fab-treated patients, the issue of therapeutic range has not been adequately addressed. It is not clear if the fraction of digoxin bound to albumin (≈20% of that in serum) is also measured as “free” by the immunoassays attempting to directly measure the pharmacologically active fraction of digoxin in the serum. Until this question is addressed, we believe that the current therapeutic ranges for digoxin should be used with caution in patients treated with the antidote; this point should be considered before redigitalizing Fab-treated digoxin-overdosed patients.

An important question is whether the concept of treating poisoned patients with Fab raised against other drugs or poisons can be more generally applied beyond digitalis intoxication. Thorough discussion of this topic is beyond the scope of this editorial. Nevertheless, the following fundamental questions need to be addressed as part of assessing if the unbound concentration of digoxin or any other drug can be clinically useful after treatment of an overdose with a binding agent. First, should such monitoring be performed in all patients treated with Fab fragments or should it be reserved for selected patients such as those with renal failure? Second, what reference interval should be used for the unbound digoxin in Fab-treated patients? Third, what controls should be used (i.e., would regular controls containing only digoxin suffice in monitoring the performance of the assay in presence of Fab)? Fourth, how often and how long after administration of Fab fragments should unbound digoxin concentrations be monitored? Fifth, because Fab fragments are also used to treat other cardiac glycoside poisonings, such as from ingestion of oleander plants, can monitoring serum in terms of digoxin equivalents be useful in assessing the antidotal therapy? We believe that both the analytical and clinical issues related to monitoring unbound digoxin in the presence of Fab fragments be done with these questions in mind and thank Ocal and Green for bringing this important issue once again to the forefront of laboratory medicine.

References


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