ANTIDOTES IN DEPTH (A20)

DIGOXIN-SPECIFIC ANTIBODY FRAGMENTS

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Digoxin-specific antibody fragments (DSFab) are indicated for the management of patients with toxicity related to digoxin and digitoxin, as well as oleander, squill, and toad venom which contain other cardioactive steroids. DSFab have an excellent record of efficacy and safety, and should be administered early in both established and suspected cardioactive steroid poisoning.

HISTORY

The production of antibody fragments to treat patients poisoned with digoxin followed the development of digoxin antibodies for measuring serum digoxin concentrations by radioimmunoassay (RIA).[11] This RIA technique permitted the correlation between serum digoxin concentration and clinical digoxin toxicity.[4,18,24,57]

In 1967, Butler and Chen suggested that purified antidigoxin antibodies with a high affinity and specificity should be developed to treat digoxin toxicity in humans.[11] The digoxin molecule alone, with a molecular weight of 780 daltons, was too small to be immunogenic. But digoxin could function as a hapten when joined to an immunogenic protein carrier such as albumin. These investigators immunized sheep with this conjugate to generate antibodies. [77,79] The immunized sheep subsequently produced a mixture of antibodies that included antialbumin antibodies and antidigoxin antibodies. The antibodies were separated and highly purified to retain the digoxin antibodies, while removing the antibodies to the albumin and all other extraneous proteins. The antibodies that were developed have a high affinity for digoxin, and sufficient cross-reactivity with digitoxin and other cardioactive steroids to be clinically useful for the treatment of all cardioactive steroid poisonings.[13,69,70]

Intact IgG antidigoxin antibodies reversed digoxin toxicity in dogs.[12] Unfortunately, the urinary excretion of digoxin was delayed, and free digoxin was released after antibody degradation occurred. Furthermore, there was significant concern for developing hypersensitivity reactions. To make such antibodies both safe and effective in humans, whole IgG antidigoxin antibodies were cleaved with papain, yielding two antigen-binding fragments (Fab), with a molecular weight of 50,000 daltons each, and one Fc fragment of 50,000 daltons.[12] Affinity chromatography is used to isolate and purify the DSFab following papain digestion. Since the Fc fragment does not bind antigen and increases the potential for hypersensitivity reactions, it was eliminated. When compared with whole IgG antibodies the advantages of DSFab include a larger volume of distribution, more rapid onset of action, smaller risk of adverse immunologic effects, and more rapid elimination.[12,45,48,50] Ultimately, in 1976 Digibind was first used successfully,[78] and in 1986—it became commercially available,[22] Digibind, a relatively pure, very safe, and extremely effective Fab product, was produced. Another commercial product, DigiFab, approved by the US Food and Drug Administration (FDA) in 2001 is also currently available.[23] It is very similar to Digibind except that it is prepared using a digoxin derivative (digoxin-dicarboxymethoxylamine) as the hapten.

PHARMACOLOGY

Immediately following intravenous (IV) administration, DSFab antibody fragments bind intravascular unbound digoxin. Uncomplexed antibodies then diffuse into the interstitial space, binding free digoxin there. A concentration gradient is then established, which facilitates movement into the interstitial or intravascular spaces of both the free intracellular digoxin and digoxin that is dissociated from its binding sites (the external surface of Na^+-K^+-adenosine triphosphatase [ATPase] enzyme) in the heart and in skeletal muscle.[71] The dissociation rate constant of digoxin for Na^+-K^+-ATPase, therefore, affects the time course for binding to DSFab and, consequently, the onset of action.[52,72]

The binding affinities of DSFab for digoxin and digitoxin are about 10^9 to 10^{11} M^{-1} and 10^8 to 10^9 M^{-1}, and are greater than the affinities of digoxin or digitoxin for the Na^+-K^+-ATPase pump receptor.[22]

PHARMACOKINETICS

The pharmacokinetics of Digibind versus DigiFab (previously named DigiTAb) were compared in human volunteers, in a study financed by Protherics.[88] Each subject received 1 mg of digoxin intravenously as a 5-minute bolus, followed 2 hours later by a 30-minute IV infusion of 76 mg (an equimolar neutralizing dose) of either Digibind or DigiFab. Free and total digoxin (free plus DSFab bound) were assayed using an ultrafiltration method over 48 hours. At 30 minutes after infusion of either DSFab, the serum free digoxin concentration was below the level of detection of the assay and remained so for several hours. A few patients in both groups had free digoxin concentrations rebound to peak concentrations of 0.5 ng/mL at approximately 18 hours and the area under the plasma drug concentration versus time curve (AUC) for 2 to 48 hours, for free digoxin, was similar for both treatment groups. The elimination half-life of total digoxin averaged 18 hours for DigiFab and 21 hours for Digibind, while the distribution half-life was 1 hour for each. The volumes of distribution were 0.3 L/kg for DigiFab versus 0.4 L/kg for Digibind.[22,23] The systemic clearance of DigiFab was higher than Digibind, accounting for the shorter elimination half-life of DigiFab (15 hours versus 23 hours).[88] Urine sampling over the first 24 hours demonstrated mostly free digoxin and very little free DSFab for both groups. The authors postulate that during renal excretion, the DSFab digoxin complex is metabolized in the kidney by the proximal tubular cells, releasing free digoxin and unmeasured DSFab metabolites.[88]

Similar findings were first described by Smith and associates in 1976, following the first clinical use of Digibind in a patient who gave a history of ingesting 90 (0.25 mg) digoxin tablets.[78] Total serum digoxin concentration, which was 17.6 ng/mL before Digibind were given, rose to 226 ng/mL 1 hour after the start of the Digibind infusion and

remained there for 11 hours, before falling off the next 44 hours, with a half-life of 20 hours.[78] Fab concentrations peaked at the end of the infusion and then apparently exhibited a biphasic or triphasic decline, probably reflecting distribution into different compartments, as well as excretion and catabolism. Free serum digoxin concentrations were undetectable for the first 9 hours, then rose to a peak of 2 ng/mL at 16 hours, and fell to 1.5 ng/mL at both 36 hours and 56 hours at which time sampling stopped. An analysis of renal elimination based on an incomplete collection suggested that digoxin was excreted only in the bound form during the first 6 hours, but by 30 hours after Fab administration all digoxin in the urine was free digoxin.

In order to better match availability of DSFab to liberated digoxin, one study compared a loading dose of DSFab followed by an infusion to the total DSFab dose infused over a short amount of time.[68] The former strategy increased the ratio of digoxin bound to uncomplexed DSFab in the plasma from 50% to 70%.[68] The authors hypothesized that a too rapid infusion regimen would result in the elimination of DSFab before the fragments could optimally bind the digoxin redistributing from tissue sites.[68]

Digoxin takes several hours to distribute from the blood to the tissue compartment. As expected, a rodent model demonstrated that DSFab were more effective when administered prior to complete distribution of digoxin.[65] Once distribution is complete, increasing the dose of DSFab improved efficacy, as measured by comparing the AUC of digoxin to that of the Fab-digoxin complex.[65]

Pharmacokinetic studies in patients with renal failure demonstrate that the half-life of DSFab is prolonged 10-fold, with no change in the apparent volume of distribution (Vd).[82] In this situation serum DSFab concentrations remain detectable for 2 to 3 weeks. Total serum digoxin concentrations generally follow DSFab. Case reports demonstrate that free digoxin concentrations reappear up to 10 days following administration of DSFab to patients with severe renal dysfunction, as compared with 12 to 24 hours in patients with normal renal function.[16,25,28,43,54,55,74,76,82–84,89] In one series of patients with end-stage renal disease, the maximum average concentration of free digoxin was 1.30 ± 0.7 ng/mL and occurred at 127 ± 40 hours.[84] The mechanism for this rebound is unclear. Following the peak, there is a slow decline that parallels the elimination of DSFab.

EFFICACY

A large study evaluating adults and children with acute and chronic digoxin toxicity established the efficacy of Digibind.[1] Of the 150 patients treated, 148 were evaluated for cardiovascular manifestations of toxicity prior to treatment: 79 patients (55%) had high-grade atrioventricular (AV) block, 68 (46%) had refractory ventricular tachycardia, 49 (33%) had ventricular fibrillation, and 56 (37%) had hyperkalemia. Ninety percent of patients had a response to DSFab within minutes to several hours of Digibind administration. Complete resolution of all signs and symptoms of digoxin toxicity occurred in 80% of cases. Partial response was observed in 10% of patients, and of the 15 patients who did not respond, 14 were moribund or later found to not be digoxin toxic. The spectacular success of DSFab for patients with digoxin toxicity is demonstrated by the fact that of the 56 patients who had cardiac arrest caused by digoxin, 54% survived hospitalization, as compared with 100% mortality before the availability of these antibody fragments for treatment.[1,5] Newborns, infants, and children have all been successfully treated with Digibind.[5,29,41,73]

ADVERSE EFFECTS AND SAFETY

DSFab are generally safe and effective. Reported adverse effects include hypokalemia as a consequence of reactivation of the Na^+-K^+-ATPase; withdrawal of the inotropic or atrioventricular nodal blocking effects of digoxin, leading to congestive heart failure or a rapid ventricular rate in patients with atrial fibrillation; and, rarely, allergic reactions.[22,23] In the multicenter study of 150 patients treated with Digibind, the only acute adverse clinical manifestations were hypokalemia in six patients (4%), worsening of congestive heart failure in four patients (3%), and transient apnea in a several hours old neonate.[1] There were no other reactions reported in any of the patients in this series. In a postmarketing surveillance study of Digibind that included 451 patients, two patients with a prior history of allergy to antibiotics reportedly developed rashes.[59] One of these patients developed a total body rash, facial swelling, and a flush during the infusion. The other experienced a pruritic rash. Two other reported adverse reactions were thrombocytopenia and rigors and were probably unrelated to the use of Digibind.[59] One patient received Digibind on three separate occasions over the course of 1 year for multiple suicide attempts, with no adverse effects.[8]

During the clinical trials with DigiFab, one patient developed pulmonary edema, bilateral pleural effusions, and renal failure, most likely caused by the loss of the inotropic and chronotropic digoxin effects.[23] Phlebitis and postural hypotension were related to the infusion of DigiFab in two healthy volunteers.[23]

Both products warn that patients with allergies to papain, chymopapain, or other papaya extracts may be at risk for an allergic reaction because trace amounts of these residues may remain in the DSFab.[23,50] Manufacturers of DigiFab state that because some literature suggests a resemblance between both dust mite allergens and some latex allergens with the antigenic structures of papain, patients may exhibit cross-allergenicity amongst all three. Patients with an allergy to sheep protein or those who have previously received ovine antibodies or ovine Fab may also be at risk for allergic reactions, although this is not reported.

INDICATIONS FOR DSFab

DSFab are indicated for life-threatening, or potentially life-threatening, toxicity from any cardioactive steroid.[22] Patients with known or suggestive cardioactive steroid exposure with progressive bradydysrhythmias, including symptomatic sinus bradycardia, or second- or third-degree heart block unresponsive to atropine, and patients with severe ventricular dysrhythmias, such as ventricular tachycardia or ventricular fibrillation, should also be treated with DSFab. Ventricular tachycardia with a fascicular block is likely to be a digoxin-toxic rhythm.[26,49] Any patient with a potassium concentration exceeding 5 mEq/L in the setting of an acute or chronic overdose that is attributable to a cardioactive steroid in the presence of other manifestations of digoxin toxicity should also be treated.[6] Acute ingestions greater than 4 mg in a healthy child (or more than 0.1 mg/kg), or 10 mg in a healthy adult, may require DSFab, with the threshold lower in patients with significant medical illness. Serum digoxin concentrations are not representative of myocardial concentrations until tissue distribution takes place. Following ingestion, a time delay of 4 to 6 hours is usually required for digoxin to achieve an equilibrium distribution from the serum to the myocardium. Serum concentrations of ≥10 ng/mL soon after an acute ingestion may predict the need for treatment with DSFab. Because the elderly appear to be at greatest risk of lethality, the threshold for treating patients older than 60 years of age should be lower.[7] Before the advent of DSFab, mortality in patients older than 60 years of age was 58%, as compared to 8% in patients younger than 40 years of age, and 34% in patients between 40 and 50 years of age.[7] A rapid progression of clinical signs and symptoms, such as cardiac and gastrointestinal toxicity and an elevated or rising potassium concentration, in the presence of an acute overdose, suggests a potentially life-threatening exposure and the need for DSFab.

Cardioactive steroid toxicity causes intracellular myocardial hypercalcemia, and the administration of exogenous calcium may further

exacerbate conduction abnormalities and potentially result in cardiac arrest, unresponsive to further resuscitation. Thus, in a patient with an unknown exposure who is clinically ill with characteristics suggestive of poisoning by a cardioactive steroid, a calcium channel blocker, or a β-adrenergic antagonist, DSFab should be administered early in the management, and always prior to calcium use. If digoxin or another cardioactive steroid is involved, the toxic effects can be reversed, obviating the need to administer calcium and avoiding the danger of giving calcium to a cardioactive-steroid-toxic patient. Also, as it may be difficult to distinguish clinically between digoxin poisoning and intrinsic cardiac disease, the administration of DSFab can help establish the diagnosis.

A recent computer-based simulation model compared the treatment of non–life-threatening digoxin toxicity with standard therapy. The authors concluded that treatment with DSFab could decrease length of hospitalization by 1.5 days.[21]

ONSET OF RESPONSE

In the multicenter study of 150 patients, the mean time to initial response from the completion of the Digibind infusion (accomplished over 15 minutes to 2 hours) was 19 minutes (range, 0–60 minutes), and the time to complete response was 88 minutes (range, 30–360 minutes).[17] Time to response was not affected by age, concurrent cardiac disease, or presence of chronic or acute ingestion.[1]

DOSING

The dose of DSFab depends on the total body load (TBL) of digoxin. Adults and children receiving digoxin therapeutically who develop chronic digoxin toxicity require small doses of DSFab because their total body burden of digoxin is smaller when toxicity develops. Children with acute overdoses require DSFab doses based on the amount of digoxin ingested, in a manner similar to adults with acute ingestions.

Estimates of digoxin TBL can be made in three ways: (1) estimate the quantity of digoxin acutely ingested and assume 80% bioavailability (milligrams ingested × 0.8 equals TBL); (2) obtain a serum digoxin concentration (SDC) and, using a pharmacokinetic formula, incorporate the apparent Vd of digoxin and the patient's body weight (in kilograms); or (3) use an empiric dose based on the average requirements for an acute or chronic overdose in an adult or child.

Each of these methods of estimating the dose of DSFab has limitations. History of ingestion is often unreliable, and empiric doses based on averages may overestimate or underestimate Fab requirements. Using the pharmacokinetic formula assumes a steady-state Vd of 5 L/kg. This is not accurate in the acute setting. In addition, the 5 L/kg Vd is a population average that varies both with each individual and in certain diseases, such as the decreases that occur in patients with renal disease (thought to be due to displaced tissue binding) and hypothyroidism.[87]

Sample calculations for each of these methods are shown in Tables A20–1, A20–2, and A20–3. Each vial of DSFab contains 38 mg (Digibind) or 40 mg (DigiFab) of purified DSFab that will bind

TABLE A20–1. Sample Calculation Based on History of Acute Digoxin Ingestion

Adult	Child
Weight: 70 kg	Weight: 10 kg
Ingestion: 50 (0.25-mg) digoxin tablets	Ingestion: 50 (0.25-mg) digoxin tablets
Calculation:	Calculation: Same as for adult. Child will require 20 vials.
0.25 mg × 50 = 12.5 mg ingested dose	
12.5 mg × 0.80 (assume 80% bioavailability) = 10 mg (absorbed dose)	
$\frac{10\ \text{mg}}{0.5\ \text{mg/vial}} = 20\ \text{vials}$	

TABLE A20–2. Sample Calculations Based on the Serum Digoxin Concentration (SDC)

Adult	Child	Quick Estimation (for Adults and Children)
Weight: 70 kg	Weight: 10 kg	$\text{No. of vials} = \frac{\text{SDC (ng/mL)} \times \text{Patient Wt (kg)}}{100}$ (Roundup)
SDC = 10 ng/mL	Serum digoxin concentration: 10 ng/mL	
Volume of distribution = 5 L/kg	Volume of distribution: 5 L/kg	
Calculation[a]:	Calculation[a]:	
$\text{No. of vials} = \frac{\text{Total body load (mg)}}{0.5\ \text{mg/vial}}$	$\text{No. of vials} = \frac{10\ \text{ng/mL} \times 5\ \text{L/kg} \times 10\ \text{kg}}{1000 \times 0.5\ \text{mg/vial}}$ (Roundup)	
$= \frac{\text{SDC} \times V_d \times \text{Patient Wt (Kg)}}{1000 \times 0.5\ \text{mg/vial}}$	No. of vials = 1	
$\text{No. of vials} = \frac{10\ \text{ng/mL} \times 5\ \text{L/kg} \times 70\ \text{kg}}{1000 \times 0.5\ \text{mg/vial}}$ (Roundup)		
No. of vials = 7		

[a]1000 is a conversion factor to change ng/mL to mg/L.

TABLE A20–3. Empiric Dosing Recommendations

Acute Ingestion	Chronic Toxicity
Adult: 10–20 vials Child[a]: 10–20 vials	Adult: 3–6 vials Child[b]: 1–2 vials

[a]Monitor for volume overload in very small children.

[b]The prescribing information contains a table for infants and children, with corresponding serum concentrations.

approximately 0.5 mg of digoxin or digitoxin. If the quantity of ingestion cannot be reliably estimated, it is safest to use the largest calculated estimate. The clinician should always be prepared to increase the dose, should symptom resolution be incomplete.

ADMINISTRATION

Digibind should be administered intravenously over 30 minutes via a 0.22-µm membrane filter.[22] The 38-mg vial (which binds 0.5 mg digoxin) must be reconstituted with 4 mL of sterile water for IV injection, furnishing an isoosmotic solution containing 9.5 mg/mL of DSFab. This preparation can be further diluted with sterile isotonic saline for injection (for small infants, addition of 34 mL to the 4 mL [for 38 mL total] achieves 1 mg/mL). After Digibind is reconstituted, it should be used immediately, or if refrigerated, it should be used within 4 hours.[22] Although slow IV infusion over 30 minutes is preferable, Digibind may be given by IV bolus to a critically ill patient.

Each 40-mg vial of DigiFab (which binds 0.5 mg digoxin) should be reconstituted with 4 mL of sterile water for IV injection and gently mixed to provide a solution containing 10 mg/mL of DSFab.[23] The reconstituted product should be used promptly or, if refrigerated, it should be used within 4 hours. This preparation can be further diluted with sterile isotonic saline for injection. DigiFab should be administered slowly as an IV infusion over at least 30 minutes unless the patient is critically ill, in which case the DigiFab can be given by IV bolus. If a rate-related infusion reaction occurs, the infusion should be stopped and restarted at a slower rate. For infants and small children, the manufacturer recommends diluting the 40-mg vial with 4 mL of sterile water for IV injection and administering the dose undiluted using a tuberculin syringe. For very small doses, this preparation can be further diluted with an additional 36 mL of sterile isotonic saline for injection (for a total of 40 mL) to achieve a 1 mg/mL concentration.

AVAILABILITY

DSFab are available as Digibind or DigiFab. Vials contain 38 mg or 40 mg of purified lyophilized digoxin-immune ovine immunoglobulin fragments, respectively, with each vial binding 0.5 mg digoxin.

MEASUREMENT OF SERUM DIGOXIN CONCENTRATION AFTER DSFab ADMINISTRATION

Many laboratories are not equipped to determine free serum digoxin concentrations and it is important to remember that after DSFab are administered, total serum digoxin concentrations are no longer clinically useful, because they represent free plus bound digoxin.[2,22,33,38,46,80] The type of test for total digoxin concentrations used can either result in falsely high or falsely low serum concentrations, depending on which phase (solid or supernatant) is sampled.[37,51] If the correct dose of DSFab is administered, the free serum digoxin concentrations should be near zero. Free digoxin concentrations begin to reappear 5 to 24 hours or longer after Fab administration, depending on the antibody dose, infusion technique, and the patient's renal function. Newer commercial methods, using ultrafiltration or immunoassays, make free digoxin concentration measurements easier to perform and, therefore, more clinically useful, but they remain associated with errors in the underestimation or overestimation of the free digoxin level.[32,39,56,63,81,85] Free digoxin concentrations are particularly useful in patients with severe renal dysfunction. Independent of the availability of these data, the patient's cardiac status must be carefully monitored for signs of recurrent toxicity.

Other pitfalls in the measurement and utility of serum digoxin concentrations include endogenous and exogenous factors. Endogenous digoxinlike immunoreactive substances (EDLISs) have been described in infants, in women in the third trimester of pregnancy, and in patients with renal and hepatic failure.[31,34,36,40,42,52,86,87] When EDLISs are free or weakly bound, as in these circumstances, they are measurable by the typical RIA and can account for factitiously high reported serum digoxin concentrations in the absence of digoxin treatment. The role of EDLISs in the body has not been fully elucidated, but they have an effect on both the Na^+-K^+-ATPase pump and the cardioactive steroid receptor site.[35] EDLISs are implicated as a causative factor in hypertension and renal disease.[53] Exogenous factors relate primarily to measurement techniques and interpretation.[44] Digoxin metabolites have varying degrees of cardioactivity.[47] Some metabolites cross-react and are measured by RIA, while others are not. The in vivo production of these metabolites varies in patients, and may depend on intestinal metabolism by gut flora as well as renal and liver clearance.

ROLE OF DSFab WITH OTHER CARDIOACTIVE STEROIDS

DSFab were designed to have high-affinity binding for digoxin and digitoxin. There are structural similarities, however, among all cardioactive steroids. In fact, RIA-determined digoxin concentrations have been reported in patients following poisoning with many nondigoxin cardioactive steroids,[28,49,58,64] suggesting that there is also cross-reactivity between DSFab and other cardioactive steroids. Thus, DSFab may have variable efficacy for all natural cardioactive steroid poisonings, including those unique cardioactive steroids in oleander, yellow oleander, squill, and toad venom.[3,9,10,15,27,30,66] In vitro studies also suggest the binding affinity of Digibind for cardioactive steroids.[19,20,60] One recent GlaxoSmithKline-funded in vitro study demonstrated that although both Digibind and DigiFab bound digoxin equally well, a small difference in the amount of ouabain binding to Fab subpopulations was identified with twice as much bound to Digibind as DigiFab.[61]

The successful reversal, by Digibind, of cardiotoxicity resulting from ingestion of *Nerium oleander* and *Thevetia peruviana* are reported.[14,75] One adult and one child each responded to five vials (200 mg) of Fab, but larger doses may be required in other cardioactive steroid poisonings because of the lower affinity binding of Digibind for these toxins. DigiFab is expected to have similar affinity binding toward cardioactive steroids. Both products are polyclonal, contributing to their broad spectrum of affinity for nondigoxin cardioactive steroids. Treatment decisions should be based on empirical grounds, with initial therapy consisting of 10 to 20 vials. Subsequent doses can be based on clinical response.

SUMMARY

Digoxin-specific antibody fragments have dramatically advanced the care of patients poisoned with cardioactive steroids. In the more than 30 years since the release DSFab, a lethal overdose has become manageable allowing the clinician ease in treatment with almost no risk to the patient.

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