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REVERSAL OF ADVANCED DIGOXIN INTOXICATION WITH FAB FRAGMENTS OF DIGOXIN-SPECIFIC ANTIBODIES

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Abstract Purified Fab fragments of ovine digoxin-specific antibodies reversed severe digoxin intoxication in a patient who had taken 22.5 mg of the drug with suicidal intent. Atrioventricular block with extreme bradycardia was temporarily managed by pacing, but progressive, intractable hyperkalemia (serum potassium of 8.7 meq per liter) with increasing pacing threshold and progressive intraventricular conduction delay was controlled only after infusion of 1100 mg of Fab. Sinus rhythm returned 10 minutes after completion of Fab infusion. Within five hours, the serum potassium concentration fell to 4.0 meq per liter. Free digoxin concentrations in serum fell sharply to undetectable levels, whereas total serum digoxin concentration concomitantly increased 12-fold. Renal excretion of digoxin bound to Fab was documented. Reversal of toxicity was not accompanied by hemodynamic instability, and antibodies to sheep Fab fragments were not detected in the patient's serum after treatment. Thus, purified digoxin-specific Fab fragments are capable of rapid reversal of advanced digoxin toxicity. (N Engl J Med 294:797-800, 1976)

ALTHOUGH exogenous antibodies have been used for therapeutic purposes for several decades,[1] the concept of use of hapten-specific antibodies to reverse toxic effects of a drug has been advanced only recently.[2-5] Severe, life-threatening digitalis toxicity unresponsive to conventional measures, although not common, is occasionally encountered and presents a therapeutic challenge.[6,7] Our prior studies have demonstrated the ability of digoxin-specific antibodies and their Fab fragments to reverse established toxic effects of digoxin in experimental models both in vitro and in vivo.[4] We now report the initial clinical use of purified digoxin-specific Fab fragments to reverse advanced digoxin intoxication in man.

MATERIALS AND METHODS

Preparation of purified Fab fragments of digoxin-specific antibodies. Sheep were immunized with a digoxin-serum albumin conjugate[8] as previously described.[3] Blood was collected in Fenwal bags by venipuncture, and plasma separated from formed elements by centrifugation. The γ-globulin fraction of sheep anti-digoxin antiserum was digested with papain as previously described.[3,9] Digoxin-specific Fab fragments were purified by affinity chromatography,[3] with maintenance of sterile technic throughout. Purified material was tested to document sterility and lack of pyrogenicity, stored at −20°C, and passed through a sterile 0.22-μm Millipore filter just before use.

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Measurement of digoxin concentrations. Serum digoxin concentrations before administration of specific Fab fragments were measured by radioimmunoassay as previously described,[10,11] with use of dilutions as necessary to place unknown values on the optimal portion of the standard curve.

For determination of total digoxin concentration after Fab administration, serum samples were diluted fivefold with physiologic saline and heated to 100°C in a boiling-water bath for one hour to destroy binding activity. Coagulated protein was removed by centrifugation at 10,000 × g for 20 minutes, and digoxin concentration in aliquots of the clear supernatant phase determined by radioimmunoassay. Excess digoxin-specific Fab fragments were added to standard serum samples containing known amounts of digoxin, and the specimens were simultaneously subjected to the identical sequence of steps. Recovery of digoxin varied from 90 to 95 per cent (mean of 93 per cent) by this method, and the results reported have not been corrected for this loss of 5 to 10 per cent.

Free digoxin concentrations in serum after Fab administration were determined by equilibrium dialysis. Aliquots of serum obtained at various times before and after Fab administration were dialyzed against equal volumes of normal serum with use of dialysis cells with 1-ml capacity on either side of the dialysis membrane, as previously described.[8] After equilibration for 20 hours at 22°C, radioimmunoassay for total digoxin content on each side of the dialysis membrane was carried out as described above. Equal digoxin concentrations were present in both chambers after dialysis of samples obtained prior to Fab administration.

Urine samples were diluted 1:5 with physiologic saline and heated to 100°C for one hour, and total digoxin concentrations determined by radioimmunoassay as previously described.[12] Standard curves were constructed with identically handled control urine samples to which known amounts of digoxin had been added. To determine bound and free digoxin concentrations, urine samples were also subjected to equilibrium dialysis as described above, except that dialysis was against phosphate-buffered saline (sodium chloride, 150 mM, sodium phosphate, 0.01 M, pH 7.4). Radioimmunoassay was then carried out after heating as previously noted.

Measurement of Fab-fragment concentrations. Binding capacity of Fab-fragment preparations before administration was determined by ^{3}H-digoxin binding studies as previously described.[8]

For the determination of concentrations of sheep Fab fragments in serum and urine from the patient, rabbit anti-sheep γ-globulin serum was absorbed with human γ-globulin, and a globulin fraction[13] of the absorbed antiserum was coupled to bromoacetyl cellulose.[14] Then, using a solid-phase radioimmunoassay system,[15] we measured serum and urinary Fab concentrations by assessing the ability of serum and urine from the patient to compete with ^{125}I-labeled sheep Fab fragments for binding sites on the insolubilized bromoacetyl cellulose-antibody matrix.

Detection of anti-sheep Fab antibodies. The presence of antibodies to sheep Fab fragments in the patient's serum was tested for by the bisdiazotized benzidine hemagglutination method.[16]

Case Report

A 39-year-old man had ingested approximately 90 0.25-mg digoxin tablets two hours before arrival at another hospital. After initial evaluation and gastric lavage, he was transferred to the Massachusetts General Hospital. There was a history of rheumatic fever at the age of 10 years, with symptoms attributed to mitral stenosis and regurgitation first appearing 10 years before admission. Five years before admission he underwent mitral-valve replacement, with symptomatic improvement, but was subsequently maintained on digoxin because of recurrent atrial fibrillation. Other medications were warfarin and a thiazide diuretic. There was also a history of excessive alcohol consumption and emotional instability with prior suicide attempts.

On admission the patient was ethanol intoxicated, alternately combative and lethargic, complaining of nausea, and vomiting frequently. The temperature was 37°C, the blood pressure 110/70 mm Hg, and the pulse 40 to 60 per minute and irregularly irregular. The chest was clear. Jugular venous pressure was less than 5 cm of water, and the carotid pulses were normal. The apex impulse was not hyperdynamic, and the sounds from the mitral prosthetic valve were unremarkable. There was a soft systolic murmur at the apex and along the left sternal border. The abdomen and the extremities were unremarkable.

Initial laboratory data included a blood ethanol level of 190 mg per deciliter, a serum urea nitrogen concentration of 9 and a serum creatinine of 1.3 mg per deciliter. Electrolyte concentrations two hours after digoxin ingestion were sodium, 141, potassium, 4.6, chloride, 101, and bicarbonate, 29 meq per liter. An electrocardiogram showed atrial fibrillation with a relatively high degree of atrioventricular block and periods of a regular junctional mechanism at 50 per minute as well as occasional runs of atrial tachycardia with high grade atrioventricular block. The ventricular rate did not respond to a total of 3.0 mg of atropine given intravenously, and a temporary pervenous pacing catheter electrode was placed in the right ventricular apex, and pacing instituted at a rate of 60 per minute.

Despite vigorous supportive measures, the spontaneous ventricular rate progressively slowed (Fig. 1) concomitantly with rising serum potassium concentrations (Fig. 2) that were not controlled by frequent intravenous administration of glucose and insulin or of sodium bicarbonate, or by polystyrene sulfonate resin (Kayexalate) given by a retention enema. Twelve hours after ingestion of the digoxin the serum potassium concentration had risen to 8.7 meq per

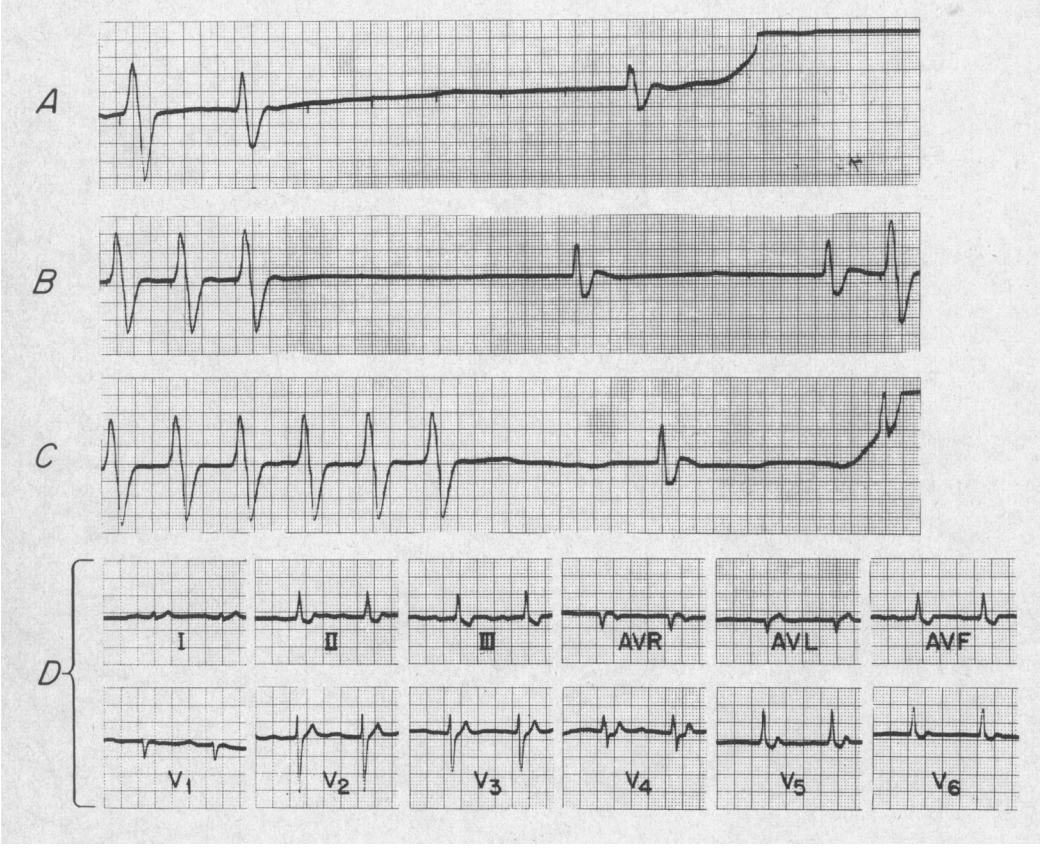

Figure 1. Sequential Electrocardiograms Recorded before, during, and after Treatment with Digoxin-Specific Fab Fragments.

In A, the tracing recorded immediately before the start of Fab infusion, serum potassium is 8.7 meq per liter; the escape interval when pacer stimulus is reduced below threshold is 4.60 seconds. In B, the tracing recorded 15 minutes after the start of Fab infusion, serum potassium is 8.0 meq per liter; the escape interval is 3.96 seconds. In C, the tracing recorded 30 minutes after the start of Fab infusion, the escape interval is 2.76 seconds. In D, the tracing recorded two hours after the start of Fab infusion, serum potassium is 7.4 meq per liter; a sinus mechanism is present at a rate of 75 per minute, with first-degree atrioventricular block (PR interval of 0.24 second).

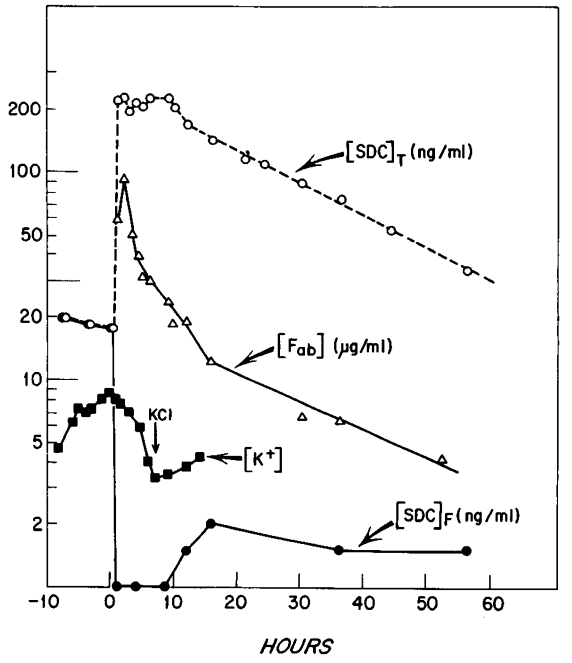

Figure 2. Time Course of Serum Potassium Concentration in meq/liter, [K+] (■—■), Total Serum Digoxin Concentration $[SDC]_T$ (O----O), Free Serum Digoxin Concentration $[SDC]_F$ (●—●), and Serum Concentration of Sheep Digoxin-Specific Fab Fragments [Fab] (△—△).

The scale on the vertical axis is logarithmic. On the horizontal axis, 0 denotes the time at which administration of digoxin-specific Fab fragments was started.

liter (Fig. 2), and the pacing threshold had increased from less than 1 mA to 2 mA. The interval from last paced beat to an escape beat when pacing was briefly interrupted had risen from 1.8 to 4.6 seconds, corresponding to a spontaneous heart rate of 14 per minute (Fig. 1). There was marked widening of the QRS complex.

In view of the grave prognosis of advanced digoxin ingestion accompanied by heart block, extreme bradycardia and intractable hyperkalemia,[6,7,17] informed consent was obtained from the patient and his family, and treatment with Fab fragments of digoxin-specific antibodies was begun. After preliminary skin testing by serial intradermal injection of 5, 50, and 100 μg of specific Fab fragments in 0.1 ml of saline at five-minute intervals with no response, a test dose of 2 mg of Fab in 1 ml of saline was given intravenously, with careful observation of vital signs. Five minutes later therapy with digoxin-specific Fab fragments was begun and carried out over approximately two hours. It was estimated that the patient had absorbed approximately 80 per cent, or 18 mg, of the 22.5 mg that he had ingested. Accordingly, an equimolar amount of specific Fab fragments (1100 mg) was administered in 600 ml of physiologic saline over the two-hour period. Temperature, blood pressure, and respiratory rate remained constant, and there were no evident allergic or toxic manifestations.

One hour after the start of the Fab-fragment infusion the escape interval had fallen to 2 seconds, and at 90 minutes atrial fibrillation with a ventricular response of 40 to 50 per minute was observed. Ten minutes after the end of Fab administration and two hours after its inception, sinus rhythm at a rate of 75/minute was observed (Fig. 1). The PR interval was 0.24 second, and there were ST-segment and T-wave abnormalities consistent with marked digitalis effect. The serum potassium concentration had fallen to 7.4 meq and continued to fall steadily, reaching a nadir of 3.4 meq per liter seven hours after the onset of Fab administration (Fig. 2). Over the subsequent six hours 120 meq of potassium chloride was given by mouth to maintain the serum potassium at about 4 meq per liter.

On the following day the rhythm reverted to atrial fibrillation, and occasional ventricular ectopic beats were noted. These ectopic beats were easily suppressed by brief intravenous administration of lidocaine and were not subsequently observed. Serial urinalyses did not demonstrate proteinuria or other evidence of glomerulitis, and creatinine clearance remained stable at between 80 and 100 ml per minute.

Results

Laboratory Studies

Figure 2 plots the time course of total serum digoxin concentration, free serum digoxin concentration, and total serum concentration of sheep Fab fragments. Free digoxin concentration in serum fell precipitously to undetectable levels (less than 1 ng per milliliter) by the time the first post-treatment serum sample was obtained at one hour, and remained at levels below 1 ng per milliliter through nine hours, after which levels of 1.5, 2.0, 1.5, and 1.5 were present at 12, 16, 36, and 56 hours respectively.

A sharp rise occurred in total serum digoxin concentration, from 17.6 ng per milliliter immediately before infusion to 223 ng per milliliter one hour after the Fab infusion was started. Despite a continuing rise in sheep Fab-fragment concentration from 60 μg per milliliter at one hour to 98 μg per milliliter at two hours, total serum digoxin plateaued at values of 223 and 226 ng per milliliter respectively and remained at about that level until 12 hours after infusion, when an exponential decline with a half-life of 20 hours began and extended through 56 hours.

The peak concentration of sheep Fab fragments in the patient's serum occurred at the completion of the infusion and declined rapidly at first, reflecting distribution through the extracellular space, then more slowly, presumably reflecting both excretion and catabolism. No detectable sheep Fab fragment concentrations were present in serum samples obtained nine and 21 days after treatment. Antibodies to sheep Fab fragments could not be detected by a sensitive hemagglutination method in samples of the patient's serum obtained one, three, and four weeks after treatment.

Incomplete urine collections precluded accurate quantitation of total renal excretion of digoxin and Fab fragments. Qualitatively, however, substantial renal digoxin excretion was documented, with urinary total digoxin concentrations as high as 960 ng per milliliter during the initial 24 hours of observation. Three urine samples collected between the beginning of Fab infusion and six hours later contained undetectable (less than 1 per cent) amounts of digoxin in the free state, despite total digoxin concentrations ranging from 155 to 660 ng per milliliter, indicating that digoxin was excreted by the kidney bound to specific Fab fragments during this period. Subsequently, the fraction of total urinary digoxin present in the free state increased gradually to 100 per cent by 30 hours after Fab infusion. These data are consistent with the time course of Fab excretion in urine as measured by radioimmunoassay. Initial urinary Fab concentrations after administration were as high as 42.5 μg per milliliter, falling to less than 2 μg per milliliter in a urine sample collected 30 to 52 hours after Fab infusion.

Discussion

Digoxin-specific antibodies of high affinity and specificity can be elicited by immunization of animals with digoxin covalently linked to suitable carrier proteins.[2,8] Such antibodies have found widespread use in the determination of clinically relevant concentrations of cardiac glycosides in serum, urine, and other biologic fluids.[10,18] We have also shown that cardiac glycoside-specific antibodies can reverse established toxic phenomena in several in vitro model systems, including erythrocyte monovalent cation transport,[3,19] isolated cardiac Purkinje fiber and atrioventricular-node preparations,[20] isolated papillary muscles[21] and atrial strips,[3] and isolated cardiac $(Na^+ + K^+)-ATPase$ activity.[4,22] These observations have been extended to reversal of advanced digoxin toxicity in intact dogs.[3,5]

The potential clinical advantages of reduced exposure to foreign protein and more rapid excretion[23] have led us to develop methods for the purification of digoxin-specific Fab fragments by an affinity chromatographic approach.[3] The present report demonstrates that these purified digoxin-specific Fab fragments are capable of rapid reversal of advanced digoxin toxicity in man, without demonstrable ill effects. Our previous experience with digitalis intoxication of this magnitude,[6,24] together with that of others,[17] leads us to believe that the present case would have had a fatal outcome without this intervention.

The rapid fall in free digoxin concentration in serum to near-zero levels after Fab administration was expected from previous observations in vitro.[25] Since the interaction of cardiac glycosides with their cellular receptors is a reversible process,[26] this reduction in free digoxin concentration results in progressive removal of drug from receptor sites as the drug-receptor equilibrium is displaced in the direction of dissociation.[4] The striking rise in total digoxin concentration in serum, previously observed in animal experimental studies,[23] occurs as a result of displacement of digoxin from tissue compartments to plasma and other extracellular compartments within which the Fab fragments are distributed. The 50,000-dalton molecular weight of Fab fragments permits glomerular filtration and relatively rapid renal excretion of both Fab fragments[27,28] and bound digoxin, in contrast to the very slow excretion kinetics of digoxin bound to intact γ-globulin before the immune degradation phase.[23,29,30]

On the basis of this initial clinical experience, we are cautiously optimistic about the potential of purified Fab fragments of digoxin-specific antibodies in the management of advanced, life-threatening toxicity unresponsive to conventional therapeutic measures. It may be that this represents but one example of a number of therapeutic opportunities in which the specific inactivation by antibody of an exogenous drug or an endogenous hormone may be undertaken, particularly if non-immunogenic antibody preparations of desired specificity can be obtained.[4]

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