Contrasting Rates of Reversal of Digoxin Toxicity by Digoxin-Specific IgG and Fab Fragments

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SUMMARY Both heterologous IgG and Fab fragments of appropriate affinity and specificity have been shown capable of reversing advanced cardiac glycoside toxicity. Fab fragments are more rapidly excreted and theoretically have a smaller risk of unwanted immunologic effects, but relative rates of toxicity reversal have not been established. Rates of reversal of advanced digoxin toxicity by digoxin-specific IgG and Fab fragments were therefore compared in a dog model of advanced digoxin intoxication. Initial studies confirmed more rapid distribution of sheep Fab fragments (M.W. 50,000) than of the parent IgG molecule (M.W. 150,000) after intravenous injection. Twenty-five pentobarbital-anesthetized dogs were given 0.3 mg/kg digoxin intravenously, resulting in rapid onset of ventricular tachycardia in all animals. Eight dogs subsequently given nonspecific IgG or Fab died in asystole or ventricular fibrillation an average of 55 minutes after digoxin administration. Ten of 11 dogs given 1.33 moles of binding sites per mole of digoxin as intact IgG returned to sinus rhythm at a mean time of 85 minutes after antibody infusion. In contrast, six of six dogs given an equivalent dose of specific Fab fragments returned to sinus rhythm in a significantly shorter mean time of 36 minutes (P < 0.01). Variability of time to arrhythmia reversion was less in Fab-treated dogs. These data demonstrate a decisive advantage of specific Fab fragments over intact IgG for potential clinical use in advanced, life-threatening digoxin intoxication.

THE ADMINISTRATION of heterologous antibodies has a long history in the treatment of tetanus, diphtheria and rabies.1,2,3 Recently, attention has been directed to the potential use of specific antibodies in other conditions in man which currently lack a specific treatment, including digitalis intoxication.4 Experimental studies have demonstrated that digoxin-specific antibodies or their Fab fragments reverse pharmacologic and toxic effects of cardiac glycosides in both in vitro and in vivo model systems.4,5,6 Purified digoxin-specific Fab fragments have been used successfully clinically to reverse digoxin-induced intractable hyperkalemia and advanced atrioventricular block following massive suicidal ingestion.9 Fab fragments were selected for clinical use in preference to the intact parent IgG molecule since they are excreted more rapidly10 and on theoretical grounds would be expected to be of lesser immunologic risk.

The speed with which an immunoglobulin molecule or fragment with specific binding properties is able to reverse an established toxic effect is of both theoretical and practical importance. We therefore undertook the present studies to test the hypothesis that the smaller size of the Fab fragment compared to its parent IgG molecule would permit more rapid distribution to the interstitial space, and that this would be accompanied by more rapid reversal of advanced digoxin toxicity. In addition, we determined for the first time the efficacy of high-affinity digoxin-specific antibodies and Fab fragments for the reversal of advanced digoxin toxicity after a single bolus of an otherwise uniformly lethal dose of digoxin.

Methods

Immunologic Methods

Digoxin-specific IgG and Fab fragments were prepared as described previously11-13 with maintenance of sterile technique throughout. Sheep were immunized with digoxin-serum albumin conjugates.11 Antisera from serial bleedings from a single animal with average intrinsic affinity constants in the 7.0 X 10⁹ to 1.5 X 10¹⁰ M⁻¹ range were pooled. Aliquots of the IgG fraction were digested with papain15,16 and passed over a Sephadex G-75 (Pharmacia Fine Chemicals, Piscataway, NJ) column to remove traces of undigested IgG. Following passage through a sterile 0.22 μm Millipore filter (Millipore Corp., Bedford, MA), concentrations of digoxin-specific IgG and Fab were determined by ³H-digoxin binding.17 Preparations were stored at −20° until administration. Nonspecific sheep IgG and Fab were identically prepared for use in control experiments.

Radioiodination of IgG and Fab

For kinetic studies, digoxin-specific IgG and Fab fragments were radiolabeled with ¹²⁵I by the lactoperoxidase method of Marchalonis.18 The final reaction mixture in each instance was passed over a 1 x 10 cm column of Sephadex G-75 over which had been previously passed 10 mg of unlabeled Fab or IgG. The first ¹²⁵I-containing material eluted from this column was collected, dialyzed against three changes of two liters of phosphate buffered saline solution (0.15 M NaCl, 0.01 M Na₂HPO₄, pH 7.4) at 4°, then stored in aliquots at −20°. Greater than 98% of the ¹²⁵I counts in both ¹²⁵I-Fab and ¹²⁵I-IgG preparations were precipitable by trichloroacetic acid (TCA). These
preparations were further characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to the method of Weber and Osborn, except that the 2-mercaptoethanol step was omitted. Gels were stained with Coomassie brilliant blue and scanned for quantitation of protein bands. Matched gels were sliced in 2 mm slices, each of which was counted in a gamma well scintillation counter. This analysis showed that more than 95% of both protein and $^{125}$I counts were localized in the appropriate bands corresponding to molecular weights of 50,000 and 150,000 for Fab and IgG respectively. The remaining counts trailed diffusely.

**Fab and IgG Kinetic Studies**

That the smaller molecular weight of Fab compared with IgG is associated with more rapid distribution in the body is suggested by data from earlier studies in species other than the dog. We confirmed that such differences between Fab and IgG distribution were present in the anesthetized dog model used in the present experiments. Five mongrel dogs (12-22 kg) were studied initially to determine Fab kinetics and then, after a period of at least one week, to determine IgG kinetics. Plasma samples obtained before Fab administration and just prior to IgG administration were tested for presence of antibody to the previously administered Fab according to the method of Cerottini.

$^{125}$I-Fab was mixed with 1:10 dilutions of the dog plasma to be tested. After incubation for 18 hours at 4°C, the globulin fraction was precipitated by adding an equal volume of cold ammonium sulfate at 80% saturation. After 30 minutes at 4°C, the precipitate was separated by centrifugation for 20 minutes at 5,000 g, washed with 3 ml of cold, 40% saturated ammonium sulfate, and counted in a gamma well scintillation counter. $^{125}$I counts in the precipitates from plasma obtained one week after Fab administration did not differ from control values for plasma obtained prior to Fab administration, confirming the absence of detectable anti-Fab antibody formation.

After sodium pentobarbital (30 mg/kg I.V.) anesthesia, intravenous catheters were positioned in a forelimb and in the external jugular vein for administration of $^{125}$I-Fab (5 mg/kg, 5 μCi) or $^{125}$I-IgG (5 mg/kg, 5 μCi) and for withdrawal of blood samples at the completion of infusion and 5, 15 and 30 minutes and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24 and 48 hours later. After IgG, further samples were collected at 72 and 96 hours. Plasma aliquots were mixed with an equal volume of 12% trichloroacetic acid (TCA), cooled on ice for 20 minutes, centrifuged (5,000 x g) for 20 minutes and the supernatant was removed. Total and TCA-precipitable counts were measured in a gamma well scintillation counter. Plasma Fab and IgG concentration-time curves were fitted, by means of computer program, to a biexponential equation and distribution and elimination phase half-lives obtained.

**Digoxin Toxicity Reversal**

Toxicity studies in 25 pentobarbital-anesthetized dogs were carried out as previously described, except that digoxin 0.3 mg/kg (Lanoxin, Burroughs Wellcome) was administered intravenously over one minute. At the onset of overt digoxin toxicity, manifest as sustained ventricular tachycardia, dogs were given one of the following intravenous infusions:

1. Digoxin-specific IgG in a 3-minute infusion containing a number of digoxin binding sites equal in molar terms to the digoxin dose, followed by one-third of this dose as a continuous infusion over the subsequent 30 minutes (11 dogs);
2. Digoxin-specific Fab fragments by a regimen exactly analogous to 1) above (six dogs); or
3. Control nonspecific IgG (four dogs) or Fab fragments (four dogs) in a total protein dose and time schedule equivalent to 1) or 2) above.

Venous blood samples were collected at the onset of toxicity, and plasma digoxin concentrations measured by radioimmunoassay as previously described. Times of digoxin administration, development of toxicity (defined as onset of sustained ventricular tachycardia), and reversal of toxicity (defined as return of stable normal sinus rhythm without ventricular ectopic activity) or development of fatal arrhythmia were recorded.

**Results**

**IgG and Fab Kinetics**

Table 1 summarizes the results of studies of plasma IgG and Fab kinetics in five dogs. The mean Fab distribution-phase half-life ($t_{1/2d}$) was 0.54 hours and was substantially shorter than the corresponding value of 2.28 hours for IgG ($P < 0.025$ by paired $t$ test). These data are in general agreement with prior studies of IgG and Fab distribution rates in other species. The mean elimination-phase half-life of 17.1 hours was considerably shorter than that for IgG (51.0 hr, $P < 0.02$ by paired $t$ test), also confirming prior observations.

**Digoxin Toxicity Reversal**

Time intervals between digoxin administration and onset of toxicity for each group of dogs studied are

<table>
<thead>
<tr>
<th>Dog</th>
<th>$t_{1/2a}$ (hr)</th>
<th>$t_{1/2d}$ (hr)</th>
<th>Mean</th>
<th>$t_{1/2a}$ (hr)</th>
<th>$t_{1/2d}$ (hr)</th>
<th>Mean</th>
</tr>
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<tr>
<td>1</td>
<td>0.90</td>
<td>45.0</td>
<td>0.50</td>
<td>15.4</td>
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<tr>
<td>2</td>
<td>2.20</td>
<td>84.3</td>
<td>0.39</td>
<td>12.3</td>
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<td></td>
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<tr>
<td>3</td>
<td>1.22</td>
<td>39.8</td>
<td>0.70</td>
<td>17.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.59</td>
<td>32.9</td>
<td>0.79</td>
<td>23.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.50</td>
<td>53.0</td>
<td>0.34</td>
<td>17.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.28</td>
<td>51.0</td>
<td>0.54*</td>
<td>17.1†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different from $t_{1/2a}$ mean for IgG ($P < 0.05$); paired $t$ test for individual animals confirms significant difference ($P < 0.025$).
†Significantly different from $t_{1/2a}$ mean for IgG ($P < 0.01$), confirmed by paired $t$ test for individual animals ($P < 0.02$).
summarized in Table 2. Onset of ventricular tachycardia occurred on average of 13.3 minutes after digoxin administration in the eight control dogs studied. Toxicity occurred at similar times in six dogs subsequently treated with digoxin-specific Fab (16.5 minutes) and in 10 dogs subsequently treated with digoxin-specific IgG (12.7 min). Mean plasma digoxin concentrations in each group of dogs at development of toxicity (Table 2) were also similar.

All dogs treated with nonspecific IgG or Fab fragments died in ventricular fibrillation (five animals) or asystole (three animals). The mean time of death was 55 minutes after digoxin administration. Ten of 11 dogs treated with digoxin-specific IgG reverted to sinus rhythm an average of 84.9 minutes after IgG administration (Table 2). In contrast, six dogs treated with digoxin-specific Fab fragments reverted to sinus rhythm in a significantly shorter interval of 36.1 minutes (P < 0.01) after Fab infusion. Also noted was a substantially greater consistency of the time course of toxicity reversal among Fab-treated dogs, which showed a coefficient of variation for time to arrhythmia reversion of 10%, in contrast to a value of 34.2% for IgG-treated animals. The remaining dog treated with digoxin-specific IgG, but not included in the statistical evaluation summarized in Table 2, failed to revert to sinus rhythm and died in ventricular fibrillation 31 minutes after IgG infusion.

Electrocardiograms recorded after 18-24 hours in all nine dogs treated with digoxin-specific antibody (five with IgG, four with Fab) so studied demonstrated persistence of stable sinus rhythm without evidence of ventricular ectopic activity or conduction disturbances.

**Discussion**

Little attention has been directed toward heterologous Fab fragments as therapeutic agents in immune deficiency states since their clearance from the body has generally been considered too rapid to be clinically useful. Recently the theoretical advantage of relatively rapid clearance of Fab fragments has been suggested for treatment of advanced digitalis intoxication in man and purified sheep digoxin-specific Fab fragments have been successfully used clinically for this purpose. This therapeutic approach is based on similar hapten-binding properties of Fab compared with IgG and absence of antigenic and complement-binding determinants of the Fc fragment. Further, the smaller size of Fab fragments has been shown to result in relatively rapid excretion of intact Fab via glomerular filtration, diminishing the probability of unwanted immunologic responses while providing a basis for enhanced glycoside elimination.

Analysis of plasma IgG and Fab elimination curves in earlier studies in experimental animals and in man indicate that IgG and Fab fragments differ in their distribution in the body. During the initial or distribution phase, plasma Fab levels fall more rapidly and to lower levels than IgG. Arend and Silverblatt found in rats that the distribution phase half-life of Fab was 0.25 hour compared with 2.2 hours for IgG in agreement with the observations reported here.

In prior studies of antibody reversal of established digoxin toxicity in intact dogs, Schmidt and Butler used a three-day digoxin dosing regimen to produce overt digoxin toxicity, which was then successfully reversed with antidigoxin antiserum. Curt et al. gave sublethal doses of digoxin sufficient to produce ventricular tachycardia, then demonstrated reversal of the arrhythmia with digoxin-specific Fab fragments to be substantially more rapid than when nonspecific (control) Fab fragments were given. Neither of these studies compared relative rates of toxicity reversal by digoxin-specific Fab fragments and the intact parent IgG molecule. The present results demonstrate for the first time that high-affinity digoxin-specific antibodies and Fab fragments can reverse advanced digoxin toxicity after a single bolus of an otherwise uniformly lethal dose of digoxin. Since acute ingestion of massive doses of digoxin accidentally or with suicidal intent is not a rare event, these results may be clinically important.

Of additional interest is the observation that digoxin-specific Fab fragments reverse potentially lethal digoxin-induced cardiac arrhythmias substantially more rapidly than equivalent doses of digoxin-specific IgG. Furthermore, they do so with less variability in the time course of toxicity reversal. Since the equilibrium binding properties of cardiac glycoside-specific IgG and Fab fragments are similar and since the magnitude of second-order forward rate constants for binding of hapten to IgG or Fab are such that the reaction is completed within seconds, it seems likely that the differences in toxicity reversal times are related to the observed differences in distribution.

**Table 2. Immunologic Reversal of Digoxin Toxicity in Dogs**

<table>
<thead>
<tr>
<th>Antibody Type</th>
<th>Time to onset of toxicity</th>
<th>Plasma digoxin concentration at onset of toxicity</th>
<th>Time to reversion to sinus rhythm*</th>
<th>Coefficient of variation of reversal time</th>
<th>Time to death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonspecific antibody</td>
<td>13.3 ± 2.1</td>
<td>183.4 ± 37.7</td>
<td></td>
<td></td>
<td>54.7 ± 11.5</td>
</tr>
<tr>
<td>Digoxin-specific antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG (n = 10)</td>
<td>12.7 ± 1.5</td>
<td>149.8 ± 40.5</td>
<td>84.9 ± 11.2</td>
<td>34.2†</td>
<td></td>
</tr>
<tr>
<td>Fab (n = 6)</td>
<td>16.5 ± 2.0</td>
<td>177.5 ± 60.6</td>
<td>36.1 ± 3.6</td>
<td>10.0</td>
<td></td>
</tr>
</tbody>
</table>

*Indicates time from administration of IgG or Fab. Significance of differences from corresponding value for Fab: † = P < 0.01; ‡ = P < 0.001 by F test. Values shown are means ± SEM.
between IgG and Fab. Thus, more rapid arrival of specific Fab fragments than of IgG in the myocardial interstitial space may result in more rapid sequestration of digoxin in an inactive form as the drug dissociates from myocardial receptor sites. Although such a mechanism seems plausible, the present data do not permit more than speculation regarding this issue. The reversal time was not further diminished by administration of a nearly three-fold greater dose of specific Fab in 3 additional dogs so studied (data not shown), further substantiating that the dose of antibody selected for clinical use need not exceed levels approximately equimolar with the amount of digoxin to be counteracted, thus avoiding the added immunologic risk of larger doses.

We conclude that, in addition to their more rapid distribution and elimination, specific Fab fragments have the distinct advantage over IgG of more rapid and uniform reversal of advanced digoxin intoxication.

References

Contrasting rates of reversal of digoxin toxicity by digoxin-specific IgG and Fab fragments.

B L Lloyd and T W Smith

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