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Fab Antibody Fragments
Some Applications in Clinical Toxicology

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Abstract

This review provides current information on the use of antigen-binding fragments (Fab) from cleaved antibodies to treat poisoning with digoxin and other potent, low formula mass poisons, such as colchicine and tricyclic antidepressants. Anti-digoxin Fab fragments have been used successfully for many years in the management of severe poisoning with digoxin, digitoxin, and a range of other structurally related compounds, including cardiotoxins from *Nerium* and *Thevetia* sp. (oleander) and *Bufo* sp. (toads). However, their main use remains treating digoxin poisoning.

Equimolar doses of anti-digoxin Fab fragments completely bind digoxin *in vivo*. The approximate dose of Fab fragments (mg) is 80 times the digoxin body burden (mg). If neither the dose ingested nor the plasma digoxin/digitoxin concentration is known, in an adult 380mg of anti-digoxin Fab fragments should be given. The dose for elderly patients or those with renal impairment should be similar to that for those with normal renal function. Fab fragments have a plasma half-life of 12–20 hours, but this can be prolonged in patients with renal impairment. Analysis of serum ultrafiltrate using an immunoassay shown not to have matrix bias remains the most accurate approach to measuring free digoxin in the presence of anti-digoxin Fab fragments.

The antibody fragments are given intravenously over 15–30 minutes after dilution to at least 250mL with plasma protein solution, 0.9% (w/v) sodium chloride, or deionised water, except in infants where the volume infused can be reduced. Factors limiting the efficacy of Fab fragments are the dose, the duration of the infusion and any delay in administration. Guidelines for Fab fragment

administration in children include (i) dilution to a final Fab concentration of 10 g/L in either 5% (w/v) dextrose or 0.9% (w/v) sodium chloride; (ii) infusion through a 0.22μm filter; (iii) administration of the total dose over a minimum of 30 minutes; and (iv) avoiding coadministration of other drugs and/or electrolyte solutions. Fab fragments are generally well tolerated. Adverse effects attributable to Fab treatment include hypokalaemia and exacerbation of congestive cardiac failure; renal function could be impaired in some patients.

Fab fragment preparations for treating acute colchicine and tricyclic antidepressant poisoning have been developed, but are not available commercially. Colchicine poisoning is rare in Western countries, and pharmacological management together with supportive care is usually effective even in severe tricyclic antidepressant overdosage. Attempts have been made to produce anti-paraquat antibodies capable of enhancing paraquat elimination from the lung, but thus far all such attempts have proved unsuccessful.

Nowadays, antidotal therapy is used most commonly in the treatment of carbon monoxide, opioid, and paracetamol (acetaminophen) poisoning, but there remain a wide range of circumstances where the availability and appropriate use of an antidote can dramatically reduce morbidity and mortality.[1] This review aims to give current information on one such area of clinical toxicology, namely the use of antigen-binding fragments (Fab) from cleaved antibodies to sequester *in vivo* low formula mass poisons with high intrinsic toxicity, such as digoxin and colchicine, thereby mitigating or abolishing toxicity. Issues such as antidote efficacy, cost and availability are discussed as appropriate. However, the use of antibody fragments to treat snake envenomation etc. is not covered in detail.

Antivenins have been used to treat bites or stings from snakes, spiders, lizards and jellyfish for many years.[1-5] Anti-toxins are also employed to counteract the neuromuscular effects of botulinum and tetanus toxins. Most antivenins and antitoxins employ immunoglobulin G (IgG) preparations to bind and inactivate proteinaceous poisons and other toxins, and they can dramatically decrease morbidity and mortality if administered appropriately. Unfortunately, IgG itself is antigenic and has been associated with severe and sometimes fatal acute (anaphylactic) and chronic (serum sickness) immune reactions in patients.[6] In addition, IgG is a large molecule (relative formula mass approximately 150 000) and is not readily eliminated. Degradation of the circulating antibody-poison complex could eventually release large quantities of poison back into the circulation.[7]

Cleavage of the IgG molecule with papain (figure 1) yields two identical Fab fragments (relative formula mass approximately 50 000) and one crystalloid fragment (Fc). The Fab fragments contain antigen binding sites, but the Fc fraction does not bind antigen. Use of pepsin splits off the Fc fragment, but leaves the two Fab fragments joined to give a 'divalent' fragment $(Fab)_2$. Purification of the Fab fraction gives a preparation that can bind antigen and is not only less immunogenic than IgG, but also can diffuse from blood into some tissue compartments and is readily eliminated via glomerular filtration.[8] Fab fragments also tend to be more stable than IgG in storage.[9] Single chain variable fragments (Fv) consist of the variable regions of the heavy (V_H) and light (V_L) chains linked via a peptide spacer.[10] The linkage overcomes the dissociation of noncovalently associated Fv fragments that may occur at low concentrations.[11] The subject of 'antibody engineering' has been reviewed recently.[12]

The ability to prepare antisera to low formula mass drugs and other poisons suggested that 'immunotoxicotherapy' might be used to similarly inactivate such compounds *in vivo*. The aim of immunotoxicotherapy is to sequester, extract or redistribute, and eliminate toxins by the administration of antibodies or antibody fragments that possess specific active binding sites for the poison in question. For immunotoxicotherapy to be possible and cost

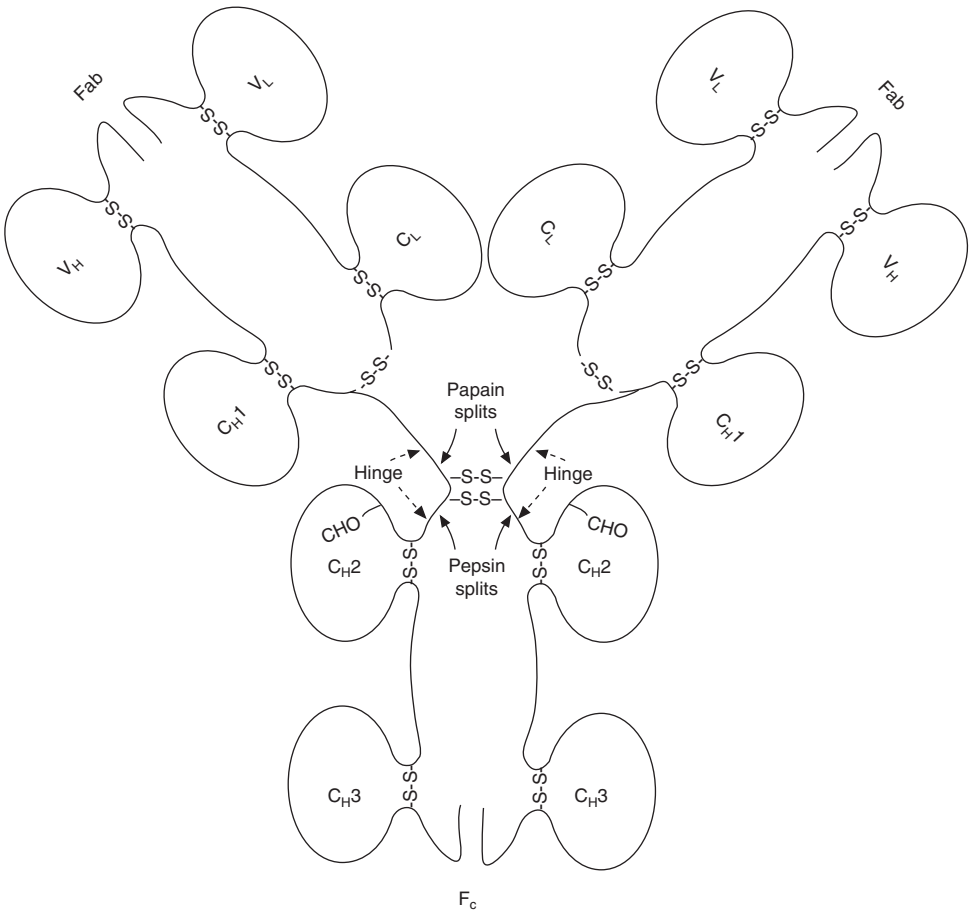

Fig. 1. Schematic diagram of the human immunoglobulin G (IgG) molecule (reproduced from Kabat,[13] with permission). The molecule is composed of two 'heavy' and two 'light' polypeptide chains and has two antigen binding sites (on the parts of the molecule which give rise to antigen-binding fragments [Fab] after papain digestion) and a complement fixing site (Fc). V_L and V_H denote light- and heavy-chain variable regions, respectively. C_H1, C_H2 and C_H3 are 'constant' regions on the 'heavy' chain; C_L is a 'constant' region of the 'light' chain; -CHO represents carbohydrate moieties linked to the Fc fragment.

effective, the toxin must be able to (i) give rise to antibodies and (ii) carry a high risk of causing death either by acute toxicity as with tricyclic antidepressants or pose long-term risks associated with tissue accumulation, such as hexachlorobiphenyl compounds.[14] However, the need to manufacture an antibody with the necessary affinity for the poison and to administer purified Fab fragments in a timely manner in the quantities needed to fully reverse toxicity have so far limited the practical development of this approach to treating severe poisoning with digoxin and related compounds and, to an extent, with colchicine.[15]

Administration of Fab fragments may result in extremely rapid reversal of toxicity, which is especially valuable if the severity of poisoning is immediately life threatening, as with severe digoxin poisoning. Disadvantages of the use of Fab fragments are their shorter plasma half-lives when compared with IgG, which may require repeated administration to prevent recurrence of toxicity.[5,9,16] Additionally, because Fab fragments are eliminated by glomerular filtration, patients with renal insufficiency may not excrete the Fab fragment-poison complex promptly, with the risk that the poison may be released back into the circulation.

Most antibody fragments used in immunotoxicotherapy are polyclonal and have broad specificity, which permits successful treatment of poisoning with a range of structurally related compounds.[14] The dose of Fab fragments required is based on the amount of poison present and not on the weight of the patient. Unfortunately, in many cases (snake bite, ingestion of poisonous plants) the amount of poison in the patient is not known and empirical dosage schedules have to be used together with close monitoring of the patient to determine whether additional Fab administration is required. Stoichiometric neutralisation with antibodies is effective against toxins present in microgram quantities, such as cardiac glycosides. Fortunately, for some toxins taken in doses that are 100- to 1000-times higher, such as tricyclic antidepressants and colchicine, partial neutralisation is effective since the cost of full stoichiometric neutralisation would be prohibitive.[17]

Recent years have brought about dramatic innovations in the safety and efficacy of immunotoxicotherapy. Fab fragments have been developed against a range of poisons, including colchicine, crotalid snake venoms, digoxin, paraquat, phencyclidine, and tricyclic antidepressants. However, despite the successes seen in digitalis and, more recently, in colchicine poisoning and crotalid envenomation, it must be emphasised that immunotoxicotherapy is only an adjunct to the intensive supportive care of patients who have been poisoned. The limitations of immunotoxicotherapy are its high costs and, at present, restricted availability and limitation to drugs and other poisons having toxic doses in the low milligram range. The major failure of this approach has been the inability to produce anti-paraquat antibody fragments capable of increasing paraquat excretion from the lung.

1. Anti-Digoxin Fab Fragments

1.1 Treatment of Poisoning with Digitalis Glycosides

Mortality is high in severe digoxin poisoning. The development of anti-digoxin antibodies for use in measuring plasma concentrations of this drug, and the subsequent use of the antibodies to abolish digoxin toxicity in animals, were important advances.[18] Unfortunately, whole antibodies are themselves immunogenic by virtue of the presence of the Fc fragment and they are also too large to be readily excreted in urine. The use of anti-digoxin Fab fragments derived from antibodies raised in sheep and purified by affinity chromatography overcame these problems.[19,20] Fab fragments have reduced immunogenicity compared with IgG and are small enough to diffuse into the interstitial space and then be readily excreted in urine.

The fragmentation of antibodies limits the risk of anaphylaxis, but reduces the stability of the antibody-toxin complex. The plasma half-life of anti-digoxin Fab fragments in the baboon is 9–13 hours and that of the parent IgG antibody is 61 hours; the total volume of distribution (Vd) of Fab fragments in the baboon is 8.7 times greater than that of IgG.[21] The affinity constant of anti-digoxin IgG and Fab fragments for digoxin is high (of the order of 10^{10} mol^{-1}),[21,22] and greater than that of digoxin for its receptor (Na^+K^+-activated ATPase) implicated in the development of digoxin toxicity. The affinity constant of the antibody fragment for digitoxin is also high (10^9 mol^{-1}). Administered intravenously, the fragments bind to circulating glycoside, forming relatively stable complexes that are unable to bind to tissue digitalis receptors. In animals, Fab fragments reverse digoxin effects much more quickly than does IgG. There is a suggestion that reversal of inotropy lags behind reversal of electrical arrhythmic effects.[23]

1.1.1 Clinical Use of Anti-Digoxin Fab Fragments

The use of antibody fragments in man was first reported in 1976,[19] and many patients have now been so treated.[2,3,17,24-28] Digibind®[1] (GlaxoSmithKline) is generally available in North America and in the UK, and Digitalis Antidot BM® (Boeringher Mannheim) has been released in Europe.[29] Both show some cross-reaction with other cardiac glycosides (see figure 2), such as digitoxin[29-32] and lanatoside C.[33] A further preparation, DigiFab® (originally DigiTab;® Protherics), has recently received US FDA approval for treating digoxin toxici-

1 The use of trade names is for product identification purposes only and does not imply endorsement.

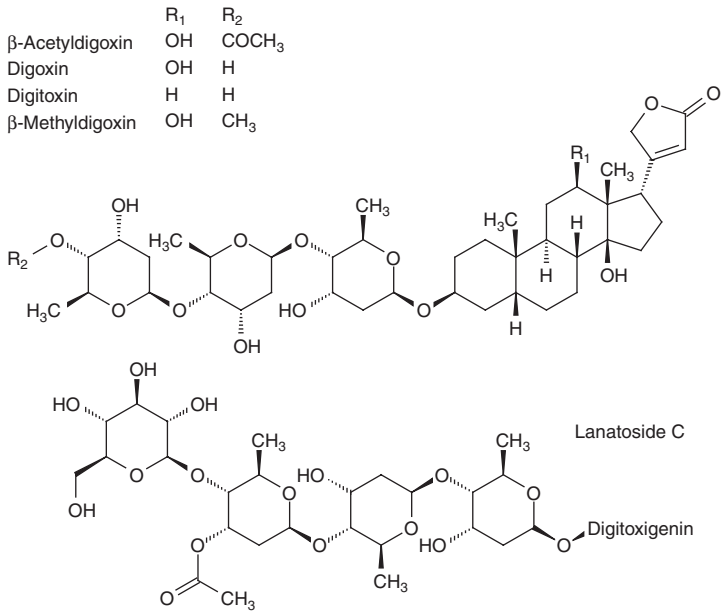

Fig. 2. Structural formulae of digoxin and some related cardiac glycosides (digitoxigenin is the aglycone derived from digoxin).

ty. It is likely that anti-digoxin Fab fragments would also reverse arrhythmias induced by β-methyldigoxin (metildigoxin, medigoxin) and by β-acetyldigoxin. Unfortunately, all antibody fragment preparations are very expensive and their use is normally only contemplated when other treatment options seem likely to fail.[34] Anti-digoxin antibody fragments should be stored in a refrigerator (2–8ºC) or in a freezer (–20ºC or below). Their efficacy is not impaired by brief freezing and thawing before use.[35] Indications for use of Fab fragments for digoxin poisoning are shown in table I.

Anti-digoxin Fab fragments can reverse ventricular arrhythmias within 2 minutes, with persistence of ventricular extrasystoles for a further 15 minutes, and in general, rhythm and conduction disturbances are settled within 30 minutes.[20] The rate at which the fragments are distributed is the key to efficacy. Immediately following intravenous administration, anti-digoxin Fab antibodies bind intravascular free digoxin and then diffuse into the interstitial space, binding free digoxin there.[36] A concentration gradient is thus established that facilitates movement of the free intracellular digoxin and digoxin that is dissociated from its binding sites (the external surface of Na^+/K^+ ATPase in the heart) into the interstitial or intravascular spaces.

Table I. Indications for use of Fab fragments in digoxin poisoning[20]

Cardiovascular shock
Heart rhythm disturbances including any degree of heart block or ventricular arrhythmias
History of large ingested dose, confirmed by very high plasma digoxin concentrations
Hyperkalaemia (serum potassium ≥5 mmol/L)
Use early if patient is >55 years of age or has pre-existing cardiovascular disease

Sixty-three patients with severe digitalis toxicity, i.e. with life-threatening arrhythmias or hyperkalaemia, or both, were given intravenous Digibind® over 15–30 minutes. They ranged in age from a few days to 85 years and included 28 patients who had taken massive overdoses. Fifty-three of the 56 patients suitable for analysis recovered completely.[30] In a trial of Digitalis Antidote BM®, 32 of 34 patients were successfully treated.[29] Of 29 children and adolescents (aged up to 18 years) with severe digoxin poisoning treated with Digibind®, digoxin toxicity was resolved in 27 patients; 3 patients required additional Fab treatment.[37] In no series could deaths be attributed to failure to reverse toxicity.

In some cases, the rate of reversal of toxicity has been dramatic: gastrointestinal features of toxicity disappeared almost immediately and hyperkalaemia was corrected within 30–60 minutes of ending the infusion.[38] Arrhythmias were corrected equally quickly in some patients,[38] but more slowly (up to 13 hours; mean 3.2 hours) in others.[29] Even digitoxin-induced thrombocytopenia was considerably improved within hours.[31]

Published values for the elimination half-life of digoxin after the administration of Fab fragments are conflicting. Elimination half-lives have been reported as 16–20 hours,[30] and 20–30 hours compared with 160 hours for spontaneous elimination.[29] During the first 12 hours after infusion of Fab fragments all of the free digoxin in serum was bound to the fragments and, therefore, rendered inactive.[19] Treatment with Fab fragments increases the renal clearance of digoxin by 20–30%.[22] Thus Fab fragments for digoxin have both toxicokinetic and toxicodynamic actions.

Anti-digoxin Fab fragments have a Vd (0.40 L/kg) that slightly exceeds extracellular volume, suggesting that a proportion may enter cells.[17,36] IgG and $(Fab)_2$ fragments equilibrate with interstitial fluid within 12–24 hours, whereas Fab fragments equilibrate within 2–4 hours.[39] Circulating Fab fragments are filtered through the glomeruli and are thought to be rapidly and extensively reabsorbed in the proximal tubules.[21] Their plasma half-life in man is about 12–20 hours, which is in good agreement with results predicted from animal studies,[36,40] but this can be prolonged by up to 10-fold in patients with renal impairment.[36,39] Up to 60–70% of Fab total body clearance may be nonrenal, however.[15] The gastrointestinal tract, kidney, liver, spleen and lymph nodes are amongst the potential sites of catabolism of Fab fragments.

Infusion of Fab fragments is generally well tolerated.[41,42] The fragments should be used with caution in patients known to be allergic to ovine protein or to papain/pepsin. Cases of erythema at the injection site and of urticaria have been noted. Six of 717 adults had an allergic reaction to Digibind® in one survey,[41] but in a further study, 13 of 57 patients given DigiFab® showed similar reactions, which responded promptly to treatment.[42] These differences in reported rates of allergic reactions were probably due to both more careful observation in the latter study and to the much higher Fab fragment doses used – all patients received 30 vials of Fab fragments. Much smaller quantities were used by Hickey et al.,[41] some patients receiving just 3 vials. No delayed hypersensitivity reactions have been reported. The risk of anaphylaxis on repeated administration of Fab fragments has not been evaluated formally in man, although one patient received Digibind® on three separate occasions over the course of 1 year for multiple suicide attempts, with no adverse effects.[43] Adverse effects attributable to Fab treatment, notably hypokalaemia and exacerbation of congestive cardiac failure, have been recorded,[29,30,41,44] and there is concern that renal function could be impaired in some patients. One study has shown that Fab fragments reduce the glomerular filtration rate in the rabbit.[45]

1.1.2 Use of Anti-Digoxin Fab Fragments in Renal Failure

Elimination kinetics of Fab-bound digoxin are dependent on renal function and the patient's capacity for renal and nonrenal elimination. If elimination of the Fab-digoxin complex is delayed, the complex may break down and intoxication may recur. A study of renal elimination based on incomplete urine collection in patients with normal renal function suggested that digoxin was excreted only in the bound form during the first 6 hours, but by 30 hours after Fab administration all plasma digoxin was free digoxin. Median total body anti-digoxin Fab clearance in 11 patients was 24.5 mL/min, of which 13.6 mL/min was renal clearance.[17]

In renal failure the half-life of Fab fragments is prolonged 10-fold with no change in the apparent Vd.[36] Fab fragments remain detectable in plasma for 2–3 weeks after administration. Total plasma digoxin concentrations usually follow Fab fragment concentrations. There is no evidence for dissociation of Fab fragment-digoxin complexes with time.[46] If the correct dose of anti-digoxin Fab fragments is administered, the free plasma digoxin concentrations should be near zero. However, there is a rebound in free digoxin concentrations that appears up to 130 hours post-administration in patients with renal dysfunction compared with 12–24 hours in patients with normal renal function, presumably secondary to prolonged distribution and elimination

phases. Free plasma digoxin measurements are particularly useful in patients with severe renal dysfunction. In patients with renal failure the patient's cardiac status must be carefully monitored for signs of recurrent toxicity.

Haemodialysis and charcoal haemoperfusion have no role in the management of digoxin poisoning. However, anti-digoxin Fab fragments are effective even in anephric patients, although features of toxicity may recur 7–14 days later, possibly indicating the need for a further dose of fragments. In one patient with end-stage renal disease and severe digoxin poisoning, anti-digoxin Fab fragment administration was followed by plasma exchange at 16 hours, and further Fab dosage was followed by plasma exchange at 38 and 86 hours. Cardiac abnormalities disappeared after Fab administration and there were apparently high total digoxin concentrations in the exchanged plasma, indicating effective complex elimination.[47] However, as valuable as this treatment appears, it has been suggested that the optimal time to perform plasma exchange is within 3 hours of Fab fragment administration. Even then the total amount of digoxin removed in the exchanged plasma was <1% of the total amount ingested.[48]

1.1.3 Anti-Digoxin Fab Fragments – Dose Calculation

The dose of Fab fragments required is governed by the need for equimolar neutralisation of the body load of digoxin or other cardiac glycoside.[20,49] The body burden of digoxin can be estimated in two ways. The first is from information on the amount acutely ingested multiplied by 0.8 to take account of digoxin bioavailability (80%). The second method can be used when the ingested dose is not known. Instead the body burden is derived from the plasma concentration in the quasi-steady state, which in practice is the plasma (or serum) digoxin concentration 4–6 hours or more post-dose. The plasma digoxin concentration (μg/L) is multiplied by 0.005 × bodyweight (kg) to obtain an estimate of total body burden in milligrams (figure 3). The plasma concentration factor is derived from the Vd (5 L/kg bodyweight) divided by 1000 to reduce the body load estimate to milligrams. The approximate formula mass ratio of Fab and digoxin is 80. Hence the approximate dose of Fab fragments (mg) required is 80 times the digoxin body burden (mg). There is no correction for percentage absorbed dose in digitoxin poisoning (bioavailability of digitoxin 100%).[35] However, the plasma concentration factor is 10-fold smaller in digitoxin poisoning compared with digoxin (figure 3) [digitoxin Vd 0.5 L/kg]. If neither the dose ingested nor the plasma digoxin concentration is known, it is conventional practice in an adult to infuse 380mg Fab fragments. The dose for elderly patients or those with renal impairment should be similar to that for younger patients with normal renal function.[50,51]

The fragments are given intravenously over 15–30 minutes after dilution to at least 250mL with plasma protein solution, 0.9% (w/v) sodium chloride, or deionised water according to the manufacturer's instructions. A longer duration of infusion diminishes the efficacy of the antidote.[20] Factors limiting the efficacy of Fab fragments are the dose given, the duration of the infusion, and any delay in administration.[52] In one case, where the dose given was insufficient, the patient died from recurrence of ventricular fibrillation.[44] Administration at too slow a rate (over 4 hours) in one case resulted in reduced efficacy.[29] Rapid infusion of Fab fragments seems necessary for them to 'catch' the glycoside in the extracellular compartment.

Guidelines for Fab fragment administration in children include (i) dilution to a final Fab concentration of 10 g/L in either 5% (w/v) dextrose or 0.9% (w/v) sodium chloride; (ii) infusion through a 0.22μm filter; (iii) administration of the total dose over a minimum of 30 minutes; and (iv) avoiding coadministration of other drugs and/or electrolyte solutions.[26,53]

1.1.4 Measurement of Plasma Digoxin after Fab Fragment Administration

Conventional serum immunoassays of glycoside concentration are no longer useful when the patient has been treated with Fab fragments, and equilibrium dialysis or ultrafiltration is required to measure free, pharmacologically active digoxin.[54-60] Digestion of the Fab fragment-digoxin complex using a proteolytic enzyme is also required before measurement of 'total' digoxin, as the affinity of the Fab fragment for digoxin may well be similar to or greater than the affinity of the antibody used in the immunoassay. Using these techniques it has been shown that plasma free digoxin falls to almost zero a

Estimation of body burden

Digoxin

1. If the dose (mg) ingested is known, multiply by 0.8 (oral bioavailability of digoxin 80%)

2. If the dose ingested is not known, the digoxin body burden (mg) is calculated thus:

$$\frac{\text{Plasma digoxin concentration}^{1}\ (\mu g/L) \times 5\ (L/kg) \times \text{bodyweight (kg)}}{1000}$$

Digitoxin

1. If the dose (mg) ingested is known, use this figure directly (oral bioavailability of digitoxin 100%)

2. If the dose ingested is not known, the digitoxin body burden (mg) is calculated thus:

$$\frac{\text{Plasma digitoxin concentration}^{1,2}\ (\mu g/L) \times 0.5\ (L/kg) \times \text{bodyweight (kg)}}{1000}$$

Calculation of dose of Fab fragments

Since 80mg Fab fragments will sequester approximately 1mg of digoxin or digitoxin, the dose of Fab fragments (mg) is calculated from:

Estimated digoxin body burden (mg) x 80

The number of vials of Fab fragments used (Digibind® [GlaxoSmithKline] vials each contain 38mg Fab fragments, DigiFab® [Protherics] vials contain 40mg fragments, Digitalis Antidot BM® [Boehringer Mannheim] vials contain 80mg fragments) should be rounded up to the nearest whole vial

Fig. 3. Estimation of dose of antigen-binding fragments (Fab) required for digoxin and digitoxin poisoning (reproduced from Flanagan and Jones,[1] with permission from CRC Press). Notes: **1** = Normally blood for therapeutic monitoring of cardiac glycosides is taken at least 6 hours post-dose to allow time for absorption and tissue distribution to be completed. However, after massive acute oral overdosage these processes may not be complete even at 6 hours. Moreover, the time of ingestion may not be known accurately. In these circumstances it may be appropriate to delay taking blood for plasma glycoside assay if the clinical condition of the patient and any accompanying history suggests that use of Fab fragments may be indicated. If the blood is taken before absorption/distribution is complete, use of the resulting plasma digoxin concentration in the above equation may lead to overestimation of the digoxin body burden and thus of the Fab fragment dose required. However, in a life-threatening situation this is preferable to undue delay. **2** = Plasma digitoxin assays are not widely available, but digitoxin cross-reacts on some digoxin assay kits and thus a plasma 'digitoxin' result can be obtained.

few minutes after Fab administration whilst the total digoxin rises rapidly to values approximately 10- to 30-fold above pre-treatment values.[24,61] Plasma digoxin measurements using conventional methodology may not be reliable for up to 2 weeks after treatment, especially in patients with impaired renal function.[62]

Jortani et al.[60] performed a detailed study of the factors affecting the reliability of results with four digoxin immunoassays after anti-digoxin Fab administration. Serum samples containing various concentrations of digoxin and Digibind® were prepared and analysed, before and after ultrafiltration. Four samples collected from Digibind®-treated patients were also analysed before and after ultrafiltration. The slopes and the *y*-intercepts of the measured versus the expected values for serum and its ultrafiltrate overlapped for the microparticle enzyme immunoassay (MEIA) digoxin assay (AxSYM®, Abbott). For the other three immunoassays tested (ACS:180® [Chiron], Stratus® [Dade Behring], and On-Line® [Roche]) either the slope or the intercept for measured versus the expected results for serum were significantly different than those for ultrafiltrate. Following the addition of digoxin and Digibind®, differences in results for serum analysed directly or after ultrafiltration were <0.50 μg/L. Comparable samples from digoxin-overdosed patients treated with Digibind® had differences of >1.0 μg/L. McMillin et al.[63] tested the effects of Digibind® and DigiFab® in 14 digoxin-competitive immunoassays. Although minimal interference was

observed with the AxSYM® method, analysis of serum ultrafiltrate using an immunoassay shown not to have matrix bias remained the most accurate approach to measuring free digoxin in the presence of anti-digoxin Fab fragments.

Finally, it should be remembered that not all 'digoxin' immunoassays always measure simply digoxin, especially in samples from patients with renal or hepatic disease, or with diabetes.[64] Coadministration of a number of other drugs may give rise to falsely lowered 'digoxin' values.[65]

1.2 Other Uses of Anti-Digoxin Fab Fragments

Yew (*Taxus baccata* and other *Taxus* species) contains toxic alkaloids known as taxines (figure 4), in almost all parts of the plant. Although these alkaloids are arrhythmogenic they are structurally unrelated to the cardiac glycosides found in *Digitalis purpurea* (purple foxglove), principally digitoxin (figure 2), and it is unlikely that anti-digoxin Fab fragments have any role in treating poisoning with *Taxus* species.[66] On the other hand, anti-digoxin Fab fragments have been used to reverse toxicity from cardiac glycosides present in plants such as *Apocynum cannabinum* (Indian hemp), *D. purpurea* (admittedly with only transient benefit),[67] *Nerium oleander* (common or pink oleander) and *Thevetia peruviana* (yellow oleander).[68-72] Anti-digoxin Fab fragments have even been used speculatively and were associated with prompt resolution of toxicity in a patient who had ingested a herbal 'internal cleansing' preparation (possibly containing *D. lanata*) and who presented with clinical features suggestive of cardiac glycoside toxicity.[73] However, use of anti-digoxin Fab fragments had no effect in a patient thought to be poisoned with *D. lanata* ingested as a component of a further 'internal cleansing' preparation.[74] Details of some plants and animals that contain toxins likely to cross-react with anti-digoxin Fab fragments are given in table II. The structural formulae of oleandrin and its deglycosylated metabolite oleandrigenin (from *N. oleander*) are illustrated in figure 5.

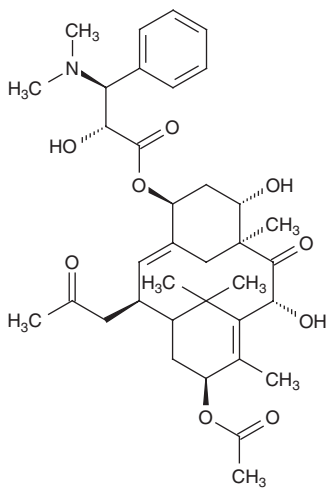

Fig. 4. Taxine A.

There has been one randomised, controlled trial of the use of anti-digoxin Fab fragments in yellow oleander poisoning, conducted in Sri Lanka. Sixty-six patients who presented to hospital with a serious oleander-induced arrhythmia were randomised to receive either 1200mg of anti-digoxin Fab fragments (n = 34) or a saline placebo (n = 32). The presenting arrhythmia had resolved completely after 2 hours in 15 antibody-treated patients and 2 controls; 24 and 5 patients, respectively, were in sinus rhythm at 8 hours. It was concluded that anti-digoxin Fab fragments are a safe and effective treatment for serious yellow oleander-induced cardiac arrhythmias. It was thought that their use in small rural hospitals should minimise costly transfer of patients and reduce the numbers of deaths. A detailed cost-benefit analysis of this approach has been presented.[78]

Anti-digoxin Fab fragments have also been used to treat poisoning with toad venom, the most toxic components of which are cardioactive sterols (bufadienolides, notably bufalin, cinobufotalin, and cinobufagin (figure 6),[79] which differ from cardenolides in the possession of a 6-membered unsaturated lactone moiety at position 17 of the sterol nucleus. Chan Su and Lu Shen Wu, traditional Chinese medications, and Love Stone, a topical aphrodisiac, are made from the dried venom of the toad *Bufo bufo gargarizans.* Not surprisingly, toxicity from toad venom is similar to digoxin toxicity and carries a high mortality rate. Severe bufalin toxicity after consumption of toad soup has even been reported.

Brubacher et al.[77] reported six previously healthy men who developed vomiting and brady-

Table II. Plants and animals containing toxins likely to cross-react with anti-digoxin Fab fragments[69,75-77]

Scientific name	Common name(s)	Principal cardiotoxin(s)
Plants		
Acokantha oppositifolia	Common poison bush	Acovenoside A, ouabain
Acokanthera oblongifolia (*A. spectabilis, Carissa acokanthera*)	Bushman's poison bush, dune poison bush, wintersweet	Ouabain (G-strophanthin, acocantherin)
Adenium honghel		Digitalin
Adonis microcarpa	Pheasant's eye	Adonin
Adonis vernalis		Vernadigin, adonitoxin, cymarin
Antiaris toxicara	Upas tree	Convallatoxin, α-antiarin
Antiaris welwitschia, A decipiens		Antioside, α-antiarin
Apocynum cannabinum	Indian hemp (US), dogbane	Strophanthidin
Asclepias curassavica	Blood flower, swallow wort, Indian root	Calactin
Asclepias eriocarpe, A. labriformis		Eriocarpin, labriformidin, labriformin
Asclepias physocarpa, *Asclepias* sp.	Balloon cotton bush	Asclepin
Bersama abyssinica		Hellebrigenin 3-acetate
Bryophyllum pinnatum		Bryophyllin A
Bryophyllum tubiflorum	Mother of millions	Bryotoxins B and C
Calotropis procera (*Asclepias procera*)	King's crown, rubber bush	Calatropin, usharidin, calactin
Castilla elastica	Black rubber tree	Cymarin
Cerbera floribunda, C. dilata		Cerbertin
Cerbera manghas	Sea mango	Cerberin (monoacetylneriifolin)
Cerbera odollam, Thevetia neriifolia		Cerberoside
Convallaria majalis	Lily of the valley	Convallerin, convallamarin, convallatoxin (strophanthidin α-L-rhamnoside)
Cryptostegia grandiflora	Rubber vine, India rubber vine, Palay rubbervine, purple allamanda	
Digitalis lanata	Yellow foxglove	Digoxin, digitoxin, digitonin, gitoxin, diginatin, lanatoside C
Digitalis orientalis		Digoxin
Digitalis purpurea	Purple foxglove	Digitoxin, digitonin, gitoxin, digitalin
Erysimum sp. (*E. helveticum, E. cheiranthoides, E. crepidifolium*)	Sibertian wallflower, treacle mustard	Helveticoside
Helleborus niger	Christmas rose	Helleborin, hellebrin and helleborein
Helleborus foetidus	Stinking hellebore	Helleborin, hellebrin and helleborein
Helleborus viridis	Green hellebore	Helleborin, hellebrin and helleborein
Kalanchoe lanceolata		Lanceotoxin A, lanceotoxin B
Nerium oleander	Oleander	Oleandrin, oleandrigenin, neriine, diginoside, digitoxigenin, adynerin
Ornithigolatum umbellatum	Star of Bethlehem	Convallatoxin
Rhodea japonica	Nippon lily	Rhodexin A
Strophanthus divaricatus		Musaroside, decoside, divaricoside, divostroside, sarmentoloside
Strophanthus gratus (*S. sarmentosus*)	Dogbane	Ouabain
Strophanthus kombe		Cymarin, K-strophanthoside
Strophanthus sarmentosis		Sarmentoloside, bipindoside
Strophanthus thollonii		Bipindoside

Continued next page

Table II. Contd

Scientific name	Common name(s)	Principal cardiotoxin(s)
Strophanthus spp.		Lokundjoside
Thevetia peruviana (*T. neriifolia, T. thevetioides*, *Cascabela thevetia, C. thevetioides*)	Yellow oleander, be-still tree, tiger apple, luckynut	Thevetin A and B, thevetoxin, digitoxigenin, neriifolin
Urginea (*Scilla*) *maritima*	Red squill, sea onion	Scillaren A and B, scilliroside
Animals		
Bufo bufo gargarizans, B. marinus, B. alvarius and other *Bufo* spp.	Chinese toad, marine toad, Colorado river toad	Bufalin, cinobufotalin, cinobufagin

cardia after ingesting a purported topical aphrodisiac. Each patient had positive apparent digoxin concentrations on immunoassay. Four patients died as a result of cardiac dysrhythmias, but two of three patients treated with anti-digoxin Fab fragments recovered. A subsequent study has shown that anti-digoxin Fab fragments are effective in treating Chan Su poisoning in mice.[80] Monoclonal digoxin immunoassays may fail to cross-react with the cardioactive sterols and thus should not be relied upon to confirm exposure.[76]

Oleander glycosides and bufalin/cinobufotalin can be detected in blood by the fluorescence polarisation immunoassay (Abbott TD_x®) for digitoxin (polyclonal rabbit antibody). The cross-reactivities of these compounds in the digoxin assay were much lower. For example, when a drug-free serum was supplemented with 10 mg/L of oleandrin, 127.7 μg/L of digitoxin equivalent was observed (digitoxin therapeutic range 20–35 μg/L), but only 2.4 μg/L of digoxin equivalent (digoxin therapeutic range 0.5–2 μg/L).[75] Digibind® neutralised all cardiac toxins studied as evidenced by a significant fall in free concentrations in experiments *in vitro* and *in vivo* in mice.[81]

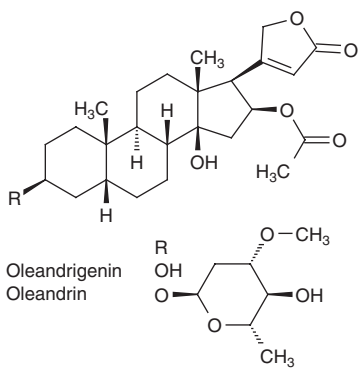

Fig. 5. Oleandrin and its aglycone.

Finally, *in vitro* trials have been undertaken of the possible use of anti-digoxin Fab fragments in treating poisoning due to the rodenticide scilliroside, one of the cardiac glycosides present in the bulb of red squill (sea onion, the red variety of *Urginea [Scilla] maritime*), and proscillaridin (a drug derived from scilliroside which has been used to treat cardiac failure in patients with poor renal function) [figure 6].[82] Digidot® (Boehringer Mannheim) antibody fragments were used. The affinity constants for the fragments for scilliroside and proscillaridin were 80- and 500-fold weaker, respectively, than for digoxin. Nevertheless, it was thought that sufficient binding to give clinical benefit might be achieved in treating poisoned patients.

2. Other Potential Applications of Fab Fragments

Infusion of anti-colchicine, anti-desipramine, and anti-phencyclidine Fab fragments causes redistribution of these drugs into plasma in animals.[83-88] There is also *in vitro* evidence of a protective effect of a verapamil-specific IgG (V-IgG) on verapamil toxicity.[89] V-IgG plus verapamil treatment had a mean reduction in developed tension of 14.1% (SD 12.2) in left ventricular papillary muscle strips from male rats compared with 36.2% (SD 14.9) for a nonspecific IgG (N-IgG) plus verapamil and 34.9% (SD 8.1) for verapamil alone ($p < 0.05$). There was no significant difference between the two control groups.

2.1 Colchicine Poisoning

Acute colchicine (figure 7) poisoning is rare, but is associated with a mortality of approximately 90%

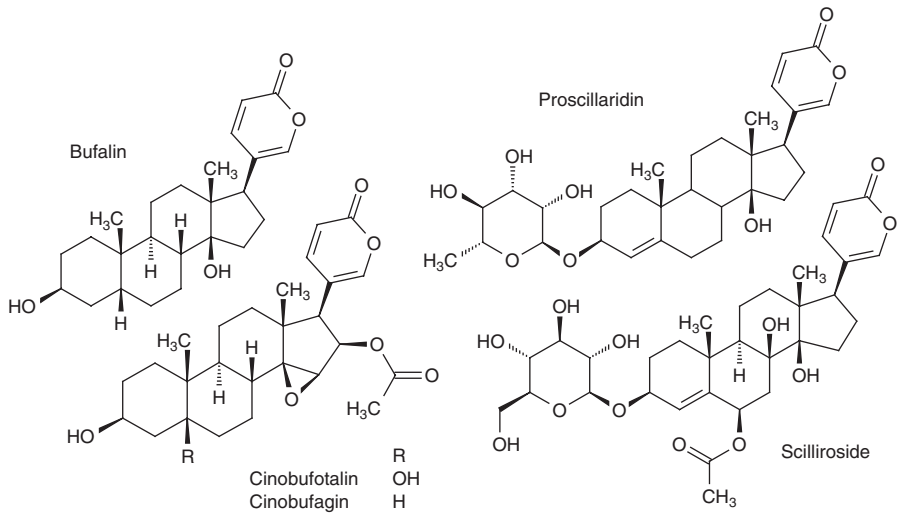

Fig. 6. Some bufadien- and bufatrien-olides.

at doses of 0.8 mg/kg or more. Exposure may occur by ingestion of tablets or intravenous dosage,[90] as well as by eating meadow saffron (*Colchicum autumnale*) leaves or flowers[91,92] or glory lily (*Gloriosa superba*) tubers.[93,94] Colchicine poisoning typically exhibits three phases: initially gastrointestinal symptoms predominate, whilst in the second phase multiorgan failure may lead to death. In the recovery phase patients often present with hair loss. Since haemodialysis and haemoperfusion are not effective measures because of the high Vd of the poison, aggressive decontamination with gastric lavage and oral activated charcoal is indicated as early as possible after ingestion. Until recently there was no successful specific therapy for colchicine poisoning, although the use of granulocyte colony-stimulating factor (G-CSF) may help if the patient survives the initial stages of the episode.[95]

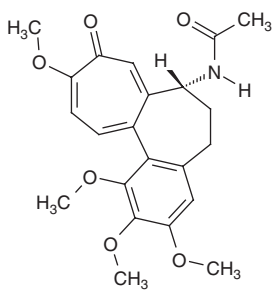

Fig. 7. Structural formula of colchicine.

Anti-colchicine antibodies were prepared many years ago in rabbits,[96] and more recently Fab fragments derived from such antibodies produced in rabbits or in goats were shown to be effective in protecting against colchicine poisoning in mice,[97-99] and in rabbits.[100] Anti-colchicine antibody Fab fragments infused over 7 hours were used to treat a patient poisoned with colchicine[101] and a very successful example of the use of immunotoxicotherapy in colchicine poisoning using goat colchicine-specific Fab fragments was reported by Baud et al.[102] Despite cardiogenic shock, bone marrow aplasia, sepsis, alopecia, and transient peripheral neuropathy, the patient survived with no permanent physical sequelae. Substantial amounts of colchicine were removed from peripheral sites and redistributed into the extracellular space by Fab infusion. The urinary colchicine excretion rate increased 6-fold. There have been no further reports of the use of such fragments in humans. Unfortunately, anti-colchicine antibody Fab fragments are not available commercially, and even if they were it is hard to see how the logistical problems of prompt supply of the antidote could be overcome, especially in less developed countries where it is most needed.[103]

2.2 Tricyclic Antidepressant Poisoning

Tricyclic antidepressants are still one of the leading causes of morbidity and mortality from acute poisoning in developed countries.[104] High affinity tricyclic antidepressant-specific monoclonal Fab fragments have been prepared.[105] Monoclonal Fab or sheep polyclonal Fab fragments rapidly reverse the cardiovascular toxicity of tricyclic antidepressants in rats, prolonging survival.[106,107] The therapeutic effect occurred within minutes and was evident with Fab doses that were 10–30% of the stoichiometric dose. Strategies for reducing the required dose of Fab fragments are desirable to limit costs and ensure efficacy, as well as minimising the risk of Fab fragment-induced renal toxicity.[108] As an example, combining tricyclic antidepressant-specific Fab with sodium bicarbonate is more effective than either treatment alone.[106,108]

Similarly, anti-desipramine antibody Fab fragments administered in quantities equivalent to approximately 10, 20 and 30% of the desipramine dose caused dose-dependent amelioration in signs of toxicity (QRS interval, heart rate) in rats poisoned with desipramine.[108,109] Enhanced removal of desipramine from the serum of rats given anti-desipramine Fv fragments (a 26kD single chain fragment derived from a monoclonal anti-desipramine Fab fragment designated G5-scFv)[110,111] and of imipramine from the brain of rats given anti-tricyclic antidepressant antibody has also been reported.[112-114] There have been early anecdotal reports of the clinical value of this approach, for example Heard et al.[115] reported clinical improvement after use of an ovine antibody (Fab fragment) to tricyclic antidepressants for the treatment of toxic effects of amitriptyline on the CNS and heart in a 48-year-old man, but no reports of controlled studies have been published as yet.

Be all this as it may, TriTab® (Protherics) was an antibody product developed to treat tricyclic antidepressant poisoning. On the basis that preclinical tests demonstrated TriTab® to be effective in reversing tricyclic antidepressant toxicity, a phase II clinical study in ten patients indicated faster recovery in more severely affected cases. However, a large amount of Fab fragments was required to treat a patient with TriTab® and it was felt that the investment required to make TriTab® available for clinical use was not a commercial option.[116]

2.3 Paraquat Poisoning

Paraquat is sold as concentrates (up to 24% w/v) for professional use and as granules (2.5–8% w/w) for use in the garden. Poisoning has occurred by dermal absorption when concentrated spray solutions have leaked from backpacks, but in general paraquat is poorly absorbed through intact skin or from the respiratory tract. However, accidental or deliberate paraquat ingestion of concentrates especially has a high mortality rate. Paraquat is rapidly absorbed from the gastrointestinal tract and may become sequestered in the epithelial alveolar cells. There is thought to be a paraquat accumulation receptor on the outside of the alveolar epithelial cell membrane containing two negatively charged sites more than 0.5nm apart (actual distance unknown) – putrescine may be the natural substrate for this receptor (figure 8). Once in the lung, paraquat initiates a redox cycle involving molecular oxygen resulting in the production of superoxide radical anion and depletion of intracellular nicotinamide adenine dinucleotidephosphate (NADPH) [figure 9].[117] Detoxification of superoxide by superoxide dismutase results in the formation of hydrogen peroxide and this in turn may cause further NADPH depletion by reaction with reduced glutathione (GSH) catalysed by the selenium-containing enzyme glutathione peroxidase. Reduction of ferric iron to ferrous iron by superoxide and subsequent oxidation to ferric iron by reaction with hydrogen peroxide (Fenton reaction) can result in the production of hydroxyl radicals which are highly reactive and can cause lipid

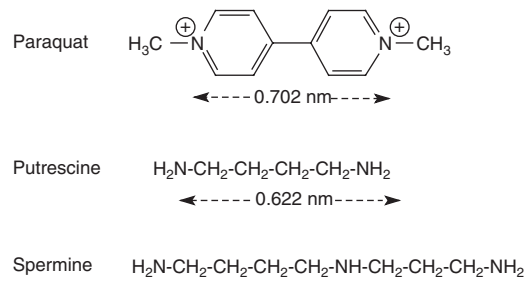

Fig. 8. Molecular formulae of paraquat, putrescine and spermine showing geometric standards of the distance between nitrogen atoms.[117]

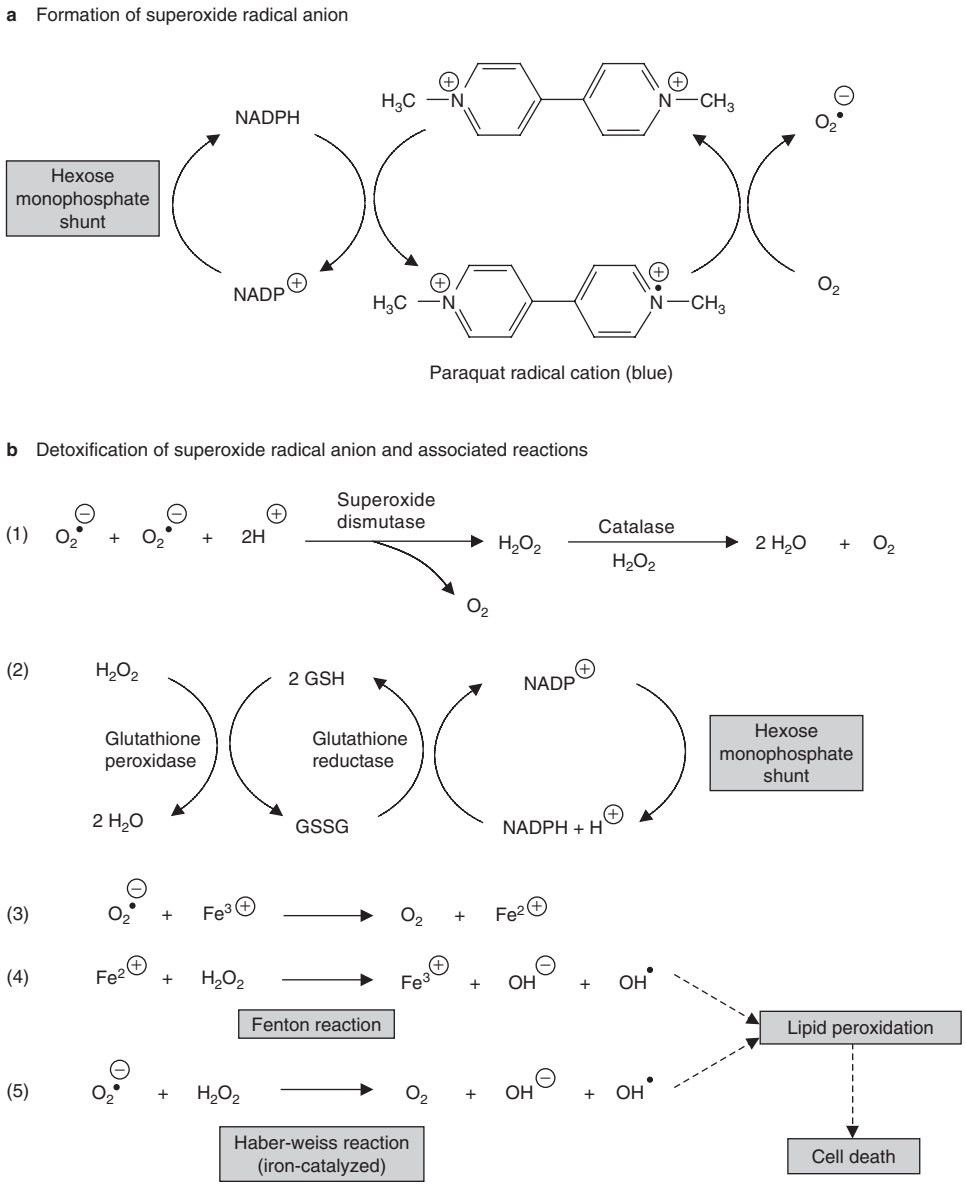

Fig. 9. Possible mechanisms by which paraquat causes depletion of lung reduced nicotinamide adenine dinucleotide phosphate (NADPH) and cell death.[117] **GSH** = reduced glutathione; **GSSG** = oxidised glutathione.

peroxidation, destruction of cell membranes, and ultimately cell death.

Clinically, an acute alveolitis develops causing haemorrhagic pulmonary oedema or adult respiratory distress syndrome. The lethal dose of paraquat for an adult is estimated to be 2–4g, i.e. 10–20mL of a 20% (w/v) solution. The features of upper gastrointestinal and respiratory tract damage reflect the corrosive nature of the solution swallowed while the systemic features are due more to the amount of paraquat ingested. The development of renal failure compromises the only efficient method of eliminat-

ing absorbed paraquat. Reduction of morbidity and mortality in paraquat poisoning relies at present on preventing absorption. Measures include limiting the supply of concentrates, the addition of stenching and emetic agents, and adequate labelling/public awareness campaigns. Despite much research into the mechanisms of paraquat poisoning and its potential treatment, only early gastrointestinal decontamination and supportive measures have an accepted therapeutic role.

The potential value of using anti-paraquat Fab fragments or some other paraquat-sequestering agent to remove paraquat from lung cells is clear. Antibodies from IgG- and IgM-secreting cell lines have been raised in murine hybridomas and show high selectivity and affinity for paraquat.[105,118] Paraquat-specific antibodies inhibit the uptake of paraquat by type II alveolar cells from the rat and reduce toxicity.[119,120] After intravenous injection of 0.1 mg/kg paraquat, the plasma paraquat concentration in rats pre-treated with anti-paraquat antibodies was increased and the amount excreted in the urine was significantly decreased compared with controls.[121] However, although using anti-paraquat antibodies can successfully sequester paraquat in the plasma compartment of rats and mice, it could not prevent paraquat from accumulating in tissues such as the lung.[121,122] Such *in vitro* and *in vivo* studies suggest that as the concentrations of paraquat in the lung are not changed, paraquat antibodies neither prevent paraquat uptake by the lung nor favour its release.

More recently a single chain Fv (scFv) fragment specific for paraquat was produced from hybridoma cells secreting a paraquat-specific murine monoclonal antibody, the aim being to produce a smaller molecule with high affinity for paraquat.[123] However, this scFv fragment was expressed in an insoluble form and only displayed moderate paraquat-binding affinity. Therefore, an attempt was made to produce a soluble scFv fragment and to increase its paraquat binding affinity. Unfortunately, it became clear that the supposed pH dependence of paraquat binding to the scFv fragment was due to tightly bound *tris*(hydroxymethyl)aminomethane (Tris) from the buffer used to purify the antibody.[124]

3. Cost and Availability of Fab Fragments

Despite much effort the only commercially available Fab fragment preparations (other than those used to treat snake envenomation) remain those designed to treat digoxin poisoning. The fact that there are three such preparations reflects the profit to be made – the current value of the US market for anti-digoxin Fab fragment preparations is estimated to be some $US30 million per annum.[125] In part this is due to its use in treating iatrogenic disease rather than deliberate self-poisoning and other factors such as length of stay in hospital have to be taken into account in addition to the cost of the Fab fragments themselves.

Mauskopf and Wenger[126] used data from uncontrolled studies of patients treated with anti-digoxin Fab fragments as well as clinical, medical care, and pharmacokinetic data from patients treated symptomatically to derive estimates of the difference in clinical outcome and medical care costs when using Fab fragments. Estimates were derived separately for patients with digoxin toxicity that was immediately life-threatening and patients whose manifestations were not immediately life-threatening. Treatment with Fab fragments reduced the probability of dying more for the seriously poisoned than for the less seriously poisoned patient. Such treatment was generally associated with an increase in total medical care costs for the serious cases because more of them survived the acute episode and required additional medical care before discharge from the hospital. For these patients, the estimated cost per life-year saved was between $US1900 and $US5400. When Fab fragments are used to treat less seriously poisoned patients, total medical care costs decreased because of an estimated decreased number of days in the coronary care unit and decreased use of pacemakers and other aggressive treatments.

More recently DiDomenico et al.[127] developed a computer-based decision analysis model to compare the treatment of non-life-threatening digoxin toxicity with either anti-digoxin Fab or standard therapy. Clinical variables (serum digoxin concentration, creatinine clearance, and bodyweight), event probabilities, and other model-specific variables were varied in univariate and multivariate sensitivi-

ty analyses. Use of Fab fragments was associated with an incremental cost of $US54 compared with standard therapy ($US2784 versus $US2730, respectively) but reduced length of stay in hospital from 3 to 1.5 days. Sensitivity analyses show that Fab was less costly at a serum digoxin concentration of >3.5 μg/L and creatinine clearance of <22 mL/min. Fab reduced length of hospital stay at a serum digoxin concentration of >2.3 μg/L. Monte Carlo simulation revealed that Fab was less costly in 37% of cases and reduced length of stay in hospital 72% of the time compared with standard therapy.

Economic considerations apply with much more force in less developed countries. Anti-digoxin Fab fragments are highly effective for oleander poisoning, for example in the study by Eddleston et al.,[42] but their use is limited by cost, a problem common to many antivenoms and other drugs in such countries.[128] It has been suggested that antidotes should be included in campaigns to increase the availability of affordable treatments for the developing world.[78]

4. Conclusions

Despite much effort the only commercially available Fab fragment preparations used to treat poisoning with low molecular weight compounds remain those designed to treat digoxin poisoning. However, the high cost of these preparations means that their use is largely restricted to North America, Europe and other developed countries despite being of proven value in treating severe poisoning with digoxin and related cardiac glycosides in other parts of the world. It seems unlikely that their use will be extended unless manufacturing costs can be reduced substantially and shelf-life improved. Similarly, the commercial production and use of Fab fragment preparations designed to treat acute poisoning with colchicine or tricyclic antidepressants seems unlikely at present. Colchicine poisoning is very rare in Western countries and pharmacological management together with supportive care is usually effective in the case of even severe tricyclic antidepressant overdosage. With paraquat, however, use of effective Fab fragments would clearly be of great benefit although all efforts to develop such a material have thus far proved unsuccessful.

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