The Use of Antibodies As Therapeutic Agents in Toxicology

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ABSTRACT Antivenoms were first used in animals as immunotoxicotherapy in 1887 and were proposed for humans in 1908. But until the 1970's, immunoglobulins and purified antibodies were essentially used for research and diagnostic purposes. The treatment of a life-threatening digoxin poisoning in 1976 was the first documented clinical benefit of (ovine) Fab fragments. Presently extensive work has been directed towards tricyclic antidepressant drugs (murine antibodies), colchicine (caprine antibodies) and diverse snake envenimations. The basic mechanism of detoxification after administration of specific antibody is known : sequestration, extraction and elimination. The Fab fragment is the most frequently proposed antidotal binding site because of its diffusion properties in the peripheral compartments and its renal excretion by glomerular filtration. A limiting factor is the irreversible binding of the toxin and kinetics of toxin release from the receptor : negative results have been instructive in that sense such as for paraquat. Another limiting factor is the dose of antibody that might be required : equimolar administration is possible for toxins dangerous at the scale of milligrams (such as digoxin or colchicine). Most drugs in clinical use have toxic ranges 10 to 100 times higher and much larger doses would be needed. The administration of such large doses of drug-specific antibodies or their fragments has no clinical or experimental precedent. This approach may prove to be feasible but the efficacy 1/2 to 1/10 molar doses also warrants testing. Calculation of the amount of infused antibody should actually be derived from the slope of the dose-effect curve rather than stoichiometrically. Other improvements will stem from advances in immunologic methodology. Monoclonal and chimeric antibodies are new tools that should help to resolve the clinical problems of immunogenicity and adverse reactions associated with polyclonal antidotal binding sites.

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HISTORICAL DEVELOPMENT OF IMMUNOTOXICOTHERAPY

The history of immunotherapy is a colorful one, dating from 1796, when Jenner actively immunized a healthy 8-year-old boy against variolous using a cowpox vaccine. Confidently, he subsequently inoculated the child with variolus virous to test the

efficacy of his vaccine!¹⁾ This concept, greeted with great skepticism by the medical public through subsequent refinements led to the disease's effective eradication. The next major breakthrough, came almost 90 years later when Pasteur, a chemist whose theories were also contested by colleagues in the medical community, successfully vaccinated Joseph Meister against rabies after infection in 1885¹⁾. This famous case likely spurred immunotherapy research in fields outside the domain of infectious disease.

Only two years later, in 1887, Sewall reported that pigeons could be actively immunized with preventive inoculation of rattlesnake venom²⁾. Calmette³⁾ further developed this idea, showing that envenomated animals could be treated using the serum of another animal which had been immunized to the venom. The concept of passive immunotoxicother-

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apy (ITT) was thus born. The lack of awareness by these early investigators of the existence of antibodies and of the high specificity of antigen-antibody binding led to misconceptions. Calmette initially believed his cobra antiserum would cure all snakebites, but was soon proven in error when Brazil revealed that Calmette's antiserum did not protect against crotalid vipers⁴). In 1900, Erlich proposed the concept of antibodies⁵), making it possible to conceive how an antiserum could protect the recipient.

Though the use of certain antivenoms has become commonplace since these early works, major progress in the practical use of immunoglobulins and purified antibodies against nonmicrobial toxins was quite limited until the late 1960's, when another breakthrough was to occur, in the form of development of toxin-specific antibody fragments. This exemplary story unfolded in two steps: 1) active immunization and 2) passive immunization with whole antibody, and subsequently with antibody fragments.

In 1967, Butler and Chen reported that coupling of the digoxin hapten to serum albumin resulted in an immunogenic conjugate, allowing the production of anti-digitalis specific antibodies in the rabbit⁶). In 1969, digoxin antibodies were used in radioimmunologic quantification of therapeutic and toxic concentrations of digoxin⁷), and one year later, of digitoxin⁸). In 1970, an important advancement was made, with the isolation and characterization of the antibodies and control of their specificity⁹). This progress led to elimination of cross reactions with similar structures, such as steroid hormones.

Schmidt, in 1971, brought about the first therapeutic use of digoxin antibodies, demonstrating that digoxin toxicity could be prevented by active immunization in the rabbit¹⁰). Reversal of digoxin toxicity by antibody therapy (passive immunotherapy) was reported that same year in dogs^{11,12}).

In 1973, another breakthrough was announced by Butler and colleagues¹³). Separation and purification of ovine specific antibody Fab fragments was achieved, offering numerous advantages over the whole antibody: a smaller foreign protein load translated into diminution in the risk of anaphylaxis and serum sickness. Furthermore, the smaller Fab fragment (MW=50,000) formed an Fab-digoxin complex which could be rapidly eliminated by the kidney.

The first human intoxication treated with digoxin Fab fragments came in 1976, resulting in reversal of a grave poisoning¹⁴). In 1977, Fab fragments were shown to have cross reactivity for digitoxin reversing serious toxicity in the dog¹⁵). Hess and colleagues reported in 1979 a similar efficacy of Fab fragments

for lanatoside toxicity¹⁶). Finally, in 1980, Fab fragments were shown to be useful in the treatment of digitoxin poisoning in man¹⁷).

The tremendous progress made in immunotoxicotherapy against digoxin has spurred researchers to exploit this technology against other toxins. The principles for development of new ITT molecules and a discussion of the successes, failures, and hopes in immunotoxicotherapy follows.

DEVELOPMENTAL REQUISITES FOR IMMUNOTOXICOTHERAPY (ITT)

Potentially among the most powerful detoxification procedures currently available¹⁸), the aim of immunotoxicotherapy is to simultaneously sequester, extract or redistribute, and eventually eliminate the toxin by using specific active binding sites (SABS), which may be derived from antibodies or fragments of varying molecular weight (**Table 1**). A number of requisites for successful immunotherapy exist, some depending on the toxin involved, and others on the SABS.

Toxin-dependent requisites

For ITT to prove maximally beneficial in relation to its high cost, the toxin should have the following characteristics:

1) high risk of mortality and short-term effects (digoxin, tricyclic antidepressants, colchicine, paraquat, snakes or scorpion venoms) or with long-term effects and risk of tissue accumulation (environmental contaminants such as hexachlorobiphenyl compounds).

2) capability of producing an antibody response in the host, either in its native form or after conjugation to a protein. The immunological response is dependent on the chemical structure and molecular weight (haptens to macromolecules) of the toxin.

3) availability for antibody binding. If the apparent volume of distribution of the toxin is much greater than that of the antibody or fragment (whole antibodies, for example, being restricted to the vascular compartment), the efficacy of the antibody is dependent on the degree and rapidity of redistribution of the toxin into the compartment occupied by the antibody or fragment.

4) reversibility of effects (functional toxin). There is no evidence that therapeutic antibodies can reverse structural lesions. This, in large part, explains their limited utility against paraquat. On the other hand, antibodies can neutralize residual unbound toxin, even if a great part of the toxin has already damaged the organism, thus potentially averting a

Table 1. Toxins and their antibodies.

Toxin	Antibody	Molecular weight
gram-negative bacterial lipopolysaccharide	IgM	~600,000
tetanus	IgG	~180,000
viper or scorpion venoms	Fab2, Fab'2	~100,000
digoxin, colchicine	Fab, Fab'	~ 50,000
tricyclic antidepressants	sFv	~ 22,000

lethal outcome.

5) toxicity after absorption in the milligram range (cardiac glycosides, colchicine). Stoichiometric neutralization with antibodies has proven to be effective against these toxins. Other severe intoxications, however, have toxic dose ranges 10 to 100 times higher (tricyclic antidepressants and chloroquine). The administration of full neutralizing doses of antibodies in such cases evokes significant risk-benefit and economic reservations. Fortunately, partial neutralization may prove to be effective.

Antibody-dependent requisites

The choice of antibody or fragment size (Table 1) depends, in part, on toxin distribution. For toxins with essentially vascular distribution, IgM or IgG antibodies are effective, though they carry a greater risk of sensitization. For those with significant extravascular distribution, fragmentation is appropriate.

To form stable toxin antibody complexes, antibody binding sites must have high affinity for the toxin. For example, the success of antibody fragments for digoxin and colchicine is largely related to their affinities of greater than 10^9 M^{-1} . This value seems to be the minimal limit to develop efficient antibody for immunotherapy¹⁸⁾.

The fragmentation of antibodies¹⁹⁾, and specifically the removal of Fc fragments from IgG, increases the safety of antibodies, limiting the risks of hypersensitivity reactions and of serum sickness¹⁸⁾. While useful against haptens ($<1,000$ daltons), fragmentation must be questioned when considering immunotherapy against high molecular weight toxins (most animal venoms), whose toxin-Fab complex may largely exceed 60,000 daltons, thus incapable of renal glomerular filtration. The advantages of fragmentation may be offset by the reduction of stability of the toxin-antibody complex, with the risk of the subsequent release of the toxin.

The present tendency is to prepare polyclonal, rather than monoclonal, and ovine²⁰⁾ rather than equine antibodies. This appears to be based primar-

ily on problems of industrial manufacturing capacity. The risk of prions in ovine tissues may induce a rethinking of this tendency, however.

SUCCESSSES IN IMMUNOTOXICO-THERAPY

Digoxin Fab

Digitalis Fab fragments are obtained from sheep immunized with a digoxin-serum albumin conjugate. The IgG antibodies are separated, highly purified, digested by papain, then cleaved into one Fc and two Fab fragments, which are then isolated by affinity chromatography. The smaller molecular weight of Fab fragments (50,000 daltons) provides a more rapid onset of action than intact IgG due to enhanced volume of distribution, while permitting rapid renal excretion of the Fab-digitalis complex and decreased immunogenicity.

Fab fragments have both toxicokinetic and toxicodynamic actions²¹⁾. Administered intravenously, the fragments bind to circulating digitalis forming relatively stable complexes which are unable to bind to tissue digitalis receptors. Provided that a dose containing adequate binding sites has been administered, total digoxin concentrations increase 5 to 20 times, while free digitalis drops to zero within minutes. By mass action, free intracellular digitalis and receptor-bound digitalis are displaced and reactivation of membrane ATPases ensues. In patients with normal renal function, the Fab-digitalis complex elimination half-life is approximately 10 to 20 h, compared to spontaneous half-lives of 160 h for digitoxin and 39 h for digoxin²¹⁾.

The toxicodynamic action of Fab fragments begins to reverse the toxic clinical effects of digoxin and digitoxin within the first hour after administration.

In case series involving more than 1,000 patients, adverse effects of Fab fragments were rare²²⁻²⁴⁾. Hypersensitivity reactions were mild and occurred in only 0.8% of 717 adults²³⁾. Exacerbation of previous

heart failure is infrequent and difficult to prove. Recrudescence toxicity may occur some time after immunotherapy, corresponding to the free digitalis rebound (spontaneous redigitalization). The risk is exaggerated in persons receiving <50% of the estimated full neutralizing dose.

Because full neutralization is expensive, Fab administration is sometimes delayed until serious arrhythmia occurs. In this event, the benefit of immunotherapy is greatly reduced, ventricular fibrillation and asystole too often leading to a fatal outcome. Thus a "prophylactic" attitude should be considered and the risk of arrhythmia countered by early administration of a half equimolar quantity of Fab fragments²¹.

Patients with immediately life-threatening symptoms (ventricular tachycardia, ventricular fibrillation, sinus bradycardia ≤ 40 bpm refractory to 1 mg of atropine, second or third-degree atrioventricular blocks with slow ventricular rate or ventricular asystole, mesenteric infarction or cardiogenic shock) should immediately receive an equimolar dose of Fab.

In adult patients with potentially lethal intoxications (advanced age, serum potassium > 6 mmol/l, male sex, underlying cardiomyopathy, and/or bradycardia between 40 and 60 beats per minute unresponsive to atropine), it is recommended to give one half the neutralizing dose of Fab ("prophylactic" dose). If heart rate remains under 60 bpm, the other half of the dose should be administered. Fab fragment dosages are calculated from the body load of glycoside, which is estimated either from the ingested amount or from serum concentration^{21,22}.

In children, because of their low weight and because the clinical value of a half dose of Fab in this situation has not yet been assessed, equimolar neutralization is always chosen.

In cases of massive adult overdosage, when no data are available for the patient, a dose of 800 mg of Fab fragments (which neutralizes 10 mg of digitalis) is recommended. In our experience and that of others, 800 mg of Fab does not provide full neutralization of all intentional digitalis intoxications, since the ingested amount may be extreme. Thus, if 800 mg of Fab fails to reduce digitoxin-induced arrhythmias, the dose should be repeated.

In contrast, for chronic overdosage a mean dose of 80–160 mg of Fab is generally adequate.

A new advance : Anticolchicine antibodies

Until recently there was no successful specific therapy for colchicine intoxication, which is associated with a high mortality rate (90% at ingested doses above 0.8 mg/kg body weight). Patients with

early hemodynamic collapse due to colchicine overdose have particularly poor prognosis. Although intracellular binding of colchicine was expected to limit the efficacy of immunotoxicotherapy for colchicine poisoning, a reversible effect on microtubules was found *in vitro*²³. A study in mice showed that administration of specific anticolchicine immunoglobulin G (IgG), even after the colchicine distribution phase, significantly decreased the mortality rate^{26,27}. In further studies²⁸, IgG colchicine antibodies were produced in goats and given to mice which had received an LD 50 of colchicine intraperitoneally. Despite a relatively low dose of antibody, a beneficial effect was shown and colchicine pharmacokinetic parameters were altered favorably. The steady-state volume of distribution decreased significantly in the treated mice. There was sequestration of colchicine in the intravascular space and a decrease in colchicine concentration in most tissues of antidote-treated mice, indicating a tissue extraction process.

Active immunization for colchicine poisoning has also been examined by administering 3 mg/kg of colchicine (above LD 50) to previously immunized rabbits²⁹. The protective effects depend on the anticolchicine antibody titer.

Rouan and colleagues³⁰ prepared a specific high-affinity colchicine-binding monoclonal antibody and investigated its ability to reverse *in vitro* the effects of colchicine on Chinese hamster ovary cells. Colchicine-induced polyploidy and chromosomal aberrations were reversible even when the antibody was administered up to 6 hours after colchicine exposure.

The first clinical use of ITT in human colchicine poisoning was reported in 1995³¹. Recounting in brief, a 25-year-old female presented 24 hours after ingestion of 1 mg/kg of colchicine with vomiting, diarrhea, hypotension and severe DIC. On transfer to our hospital 12 hours later, she was in cardiac collapse with oliguria and ARDS. Prognostic features implied a 99% chance of death in less than 24 hours. With her consent, goat colchicine-specific antibodies at a dose of 0.125 molar ratio of ingested dose or about 0.4 molar ratio of persistent body burden were administered. The patient began to respond to inotropic agents during antibody infusion. Despite cardiogenic shock, bone marrow aplasia, bacteremia, complete hair loss, and transient peripheral neuropathy, this patient survived without sequelae.

In addition to the toxicodynamic effects of colchicine Fab fragments toxicokinetics were significantly altered, resulting in a 6-fold increase of total colchicine concentrations 10 min after starting Fab infusion. Free colchicine concentrations fell to un-

detectable levels over a 7 h period. A 4.4-fold decrease of colchicine distribution volume (2.9 l/kg) under Fab treatment indicated that substantial amounts of colchicine were being removed from peripheral sites and redistributed into the extracellular space, corresponding to the distribution volume of Fab fragments. Fab infusion increased urinary colchicine excretion rate 6-fold (24 and 134 $\mu\text{g/h}$, respectively, before and after Fab administration). Colchicine was renally excreted, bound at 98% to Fab fragments over a 16 h period. The amount of Fab-bound colchicine renally excreted was equal to the binding capacity of the Fab fragment amount recovered in urine. Thus, the neutralization yield of Fab fragments was near 100%. The amount of neutralized colchicine was estimated at 3.7 mg among 9 mg of colchicine present in the body prior to Fab dosing.

DISAPPOINTMENT IN IMMUNOTOXICOTHERAPY

An unrealized hope: The anti-endotoxin monoclonal antibodies

Antibodies were proposed in the treatment of gram-negative sepsis due to the poor outcome of such patients treated with current antimicrobial agents. Morbidity and mortality might be diminished, in theory, by interrupting the inflammation cascade initiated in sepsis. The toxic manifestations of gram-negative bacteria appear to be triggered by lipopolysaccharide (LPS), a component of the outer cell membrane of the bacteria. Lipid A, the toxic moiety of LPS, is present in many bacterial species. Because of this, two monoclonal antibodies directed against lipid A were developed^{32,33}.

E5 is a murine immunoglobulin M (IgM) monoclonal antibody that binds to an epitope on lipid A. HA-1A is a human IgM monoclonal antibody which binds to lipid A. Both E5 and HA-1A have been tested in placebo-controlled clinical trials, but neither has consistently altered mortality in patients with sepsis, and neither therefore, has been approved by the Food and Drug Administration (FDA).

A recent multi-center trial found that Fc fragments against tumor necrosis factor (TNF) did not reduce sepsis mortality³⁴. Work continues, however, to find an effective immunotherapy against septic shock.

HOPES FOR THE FUTURE IN IMMUNOTOXICOTHERAPY

Anti-tricyclic antidepressants immunotherapy

Tricyclic antidepressants compose the drug class most often responsible for death from intentional overdose in the United States³⁵. Thus, ITT is under study. The lethal dose of tricyclic antidepressants (TCAs) is 10 to 100 times greater than the drugs studied before, however, and will presumably require correspondingly higher doses of antibodies (up to several g/kg) to reverse toxicity. Preliminary studies with TCA-specific antibodies suggest that the administration of effective doses may be feasible.

High affinity TCA-specific monoclonal Fab' or sheep polyclonal Fab fragments rapidly reverse the cardiovascular toxicity of the TCA desipramine (DMI) in rats, and prolong survival^{36,37}. Polyclonal fragments may, in fact, offer an advantage in being less specific (thus cross-reacting with other drugs of the same class). Therapeutic effect occurs within minutes and is evident with relatively low Fab or Fab' doses (10 to 30% of the molar DMI dose), suggesting that these antibody fragments preferentially redistribute DMI out of a rapidly equilibrating compartment.

TCA-specific Fab' or Fab is generally well tolerated in rats, but doses several times higher than those anticipated for human use may lead to cardiovascular deterioration or death. 1/8 of the rats receiving the larger doses of TCA specific Fab died (2 g/kg representing a molar ratio of TFab to DMI of 0.22). This death may relate to the rapid rate of infusion of relatively large amounts of Fab³⁸. Strategies for further minimizing the required dose are desirable. Combining TCA-specific Fab with sodium bicarbonate (standard therapy for TCA overdose) is more effective than either treatment alone and thus represents one such strategy³⁹. Even smaller antibody fragments may offer improved therapeutic properties. A recombinant single chain Fv fragment (sFv), one half the size of Fab, has been cloned which retains a high affinity for DMI and is able to alter DMI distribution *in vivo*. Because sFv has a shorter elimination half-life and more extensive renal excretion than Fab, it may have therapeutic advantages⁴⁰. Moreover, this fragment can potentially be engineered to improve its therapeutic properties.

Thus, the use of drug-specific antibody fragments may represent a viable general strategy for the treatment of human tricyclic antidepressant overdose.

Snake and scorpion antivenoms

In an attempt to reduce the problem of sensitization associated with polyvalent IgG crotalid antivenom, an ovine Fab fraction specific for 3 *Crotalus* and 1 *Agkistrodon* species has recently been tested in mice, in comparison with the classic crotalid polyvalent equine antivenom. The Fab fragments were 3.0–11.7 times as effective as the whole IgG product, with an average potency ratio of 5.2. Thus, the new antivenom Fab product may be effective at a much smaller dose, with a smaller risk of sensitization²⁰. Studies of equine Fab2' fragments against vipers (*Viperidae* species)⁴¹ and scorpions (*Buthus* species)⁴² and ovine Fab fragments against vipers (*Viperidae* species)⁴³ are ongoing in Europe. Antivenom against Centruroides scorpions has been demonstrated safe and effective⁴⁴ but is not generally available.

Antibodies against drugs of abuse

In 1972, evidence for active immunization against morphine was demonstrated in mice⁴⁵. This therapy has not been applied to man, naloxone having obviated the problem of reversal of narcotic effects.

Carrera and colleagues⁴⁶ have recently reported on a new development in ITT with a different goal in mind. Active immunization with a cocaine conjugate results in decreased cerebellar and striatal concentrations of cocaine subsequently injected in rats. The authors' aim in this case is not, for the moment, the reduction of toxic effects (though this may prove to be possible as well), but to decrease the 'desirable' effects of cocaine abuse, thus decreasing the impetus for its use among chronic abusers. While substitution of other stimulants or increasing doses of cocaine might limit the utility of such therapy in prevention, passive immunization to reverse the visceral toxicity (such as cocaine-induced chest pain) would be of great potential interest.

CONCLUSION

Immunotoxicotherapy (ITT) has proven efficacious in snake envenomation for almost a century. Recent years have brought about dramatic innovations in the safety and efficacy of immunotherapy, highlighted by the successes seen in digitalis and colchicine poisoning. There is hope that successful immunotoxicotherapy against other highly toxic drugs, such as cyclic antidepressants and cocaine, may be on the horizon. Refinement of older therapies, such as snake antivenom, is also underway.

ITT is not a replacement for, but an adjunct to intensive, supportive care of poisoned patients. The

toxicity associated with modern antibody fragment therapy appears to be rare and minor. The limitations of ITT are its high cost and its restriction, for the moment, to drugs having low toxic doses (in the range of a few mg).

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