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Review

Antibodies as Carrier Proteins

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Pre-existing antibodies against a drug substance can significantly alter the pharmacokinetic profile of the drug in the circulation. Rapid clearance, mediated by complement or Fc receptors, occurs for crosslinked immune complexes, but not for complexes containing only one or two antibodies. With antibodies functioning as carrier proteins, monovalent antigens may enjoy a prolonged circulatory half-life, as observed in the case of digoxin, insulin, and various interleukins. While such an effect should be highly sensitive to fluctuations in antibody affinity and titer, it may present a means of extending the circulation of potent but rapidly cleared therapeutic agents. This mini-review attempts to delineate the causal relation between the factors influencing antibody binding and the circulatory life of a therapeutic agent, be it a small drug or a macromolecule.

KEY WORDS: antibodies; drug clearance; multivalency; affinity; titer.

INTRODUCTION

One of the key determinants of the circulatory half-life, as well as the activity of a drug, is the extent to which it binds proteins in the blood. While bound to protein, a drug molecule is generally unavailable to the site of action and to the excretion mechanisms of the liver and kidney (1). The extent of protein binding is determined by the concentration in the blood of the binding protein, the number of binding sites per protein molecule, and the affinity of each binding site for the drug molecule (2). Although protein binding is usually associated with albumin and other inert blood proteins, antibodies specific to a drug can provide an interesting opportunity for protein binding with very high affinity and low capacity. Such binding has a number of implications in pharmacy and the pharmaceutical sciences.

Repeated exposure to a drug having the appropriate physicochemical properties and route of administration may initiate an immune response that leads to production of antibodies against the drug. Whether such antibodies are intentionally elicited or are an undesired side effect of therapy, they are said to arise from active immunization, since the host's own immune system is triggered to produce them. Antibodies can also be produced outside the host and infused into the bloodstream in a process called passive immunization. The results of passive immunization differ significantly from those of active immunization, in part because exogenous antibodies are likely to have shorter half-lives, may distribute differently in the body, are not regenerated, and may themselves be immunogenic (3).

Endogenous antibodies enjoy long half-lives in the circulation. Three of the four subclasses of IgG have half-lives of about 20 days in humans, while the half-life for the remaining subclass, IgG3, is 7 days, similar to that of IgM (10 days) and IgA (6 days) (4). IgE, the immunoglobulin class responsible for allergic reactions, has a serum half-life of only 2 days, and with a serum concentration of about 50 ng/mL, is unlikely to play a significant role as a carrier protein. IgG contains two antigen-binding sites per molecule while dimeric IgA contains four and pentameric IgM contains 10 binding sites per molecule. B cells that secrete IgG and IgA are fully differentiated and with time undergo the process of affinity maturation which involves somatic hypermutation of antibody genes and selection of clones based on antibody affinity (5). For this reason, the average affinity of IgG or IgA for antigen is likely to increase over the course of months or even years after exposure to an antigen. While IgM can obtain high avidity for a polyvalent antigen through multiple binding interactions, it is produced early in an immune response, prior to affinity maturation, and its individual binding sites generally have considerably lower affinity for antigen than those of IgG and IgA.

A unique characteristic of antibodies as compared to other serum proteins that can bind drugs is that antibodies may specifically function to neutralize ligands to which they bind in addition to initiating processes designed to sequester or remove foreign materials. Thus antibodies have the potential to speed elimination and negate the activity of drugs, as well as to prolong their circulation and pharmacological effect. The picture that emerges from a variety of studies in disparate disciplines is one in which antibody binding can have a wide range of effects depending upon the size of the drug, the number of binding sites it has for antibody, and the concentration and affinity of the antibody. This mini-review attempts to summarize and discuss the biopharmaceutical implications of data on drug-antibody interactions. This data has been gathered in a wide

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variety of experimental systems, involving both active and passive immunization both in patients and in animal models.

IMMUNE COMPLEXES AND PHARMACOKINETICS

When multivalent antigens and antibodies are combined at stoichiometric ratios of about 1:1, they tend to form large, extensively crosslinked complexes that precipitate from solution. If the antigen provides only a few binding sites for antibody or if either antibody or antigen is in a large molar excess, then smaller and less extensively crosslinked soluble immune complexes are formed (6). When opsonized by complement, these soluble immune complexes can deposit themselves, via complement receptor 1, on the surface of erythrocytes, which carry them to the liver and spleen to be phagocytosed by fixed macrophages (7,8). In the absence of complement, the complexes can interact with macrophages, monocytes, neutrophils, and other hematopoietic cells via Fcγ receptors that recognize the constant region of IgG (9,10). Although complement may play a role in preventing precipitation and tissue deposition of immune complexes (8), clearance of soluble but sufficiently crosslinked immune complexes is rapid whether mediated by complement or by Fcγ receptors (11).

However, as part of the delicate balance the immune system maintains between acceptance of self and destruction of non-self, multiple binding interactions are required to trigger the mechanisms of immune clearance. While a single molecule of IgM can activate the complement cascade, the antibody must bind multiple epitopes on its target in order to do so. Likewise, several molecules of IgG must be held in close proximity via crosslinking by a multivalent target in order to activate complement. Two of the Fcγ receptors, FcγRII and FcγRIII, have low affinity for monomeric IgG such that detectable binding requires the concurrent binding of two or more receptors to two or more molecules of IgG linked together in an immune complex (12). The remaining class of Fc receptor, FcγRI, binds monomeric IgG efficiently, but as with FcγRII and FcγRIII, its effector functions are activated by the crosslinking of receptors on the cell surface (12). This crosslinking is achieved by the binding of several receptors to the same immune complex. In short, both complement-dependent and complement-independent clearance mechanisms for soluble immune complexes require crosslinking of immunoglobulin molecules by multivalent antigens.

An antigen with only one combining site for antibody is incapable of crosslinking antibodies and hence would not be subject to the normal immune clearance mechanisms. Mannik *et al.* (13) found that immune complexes containing one or two IgG persisted in circulation while those with more than two IgG molecules were cleared rapidly. Skogh *et al.* (14) conjugated human serum albumin (HSA) to different extents with dinitrophenyl (DNP) groups and injected the conjugates into mice immunized against DNP. Clearance of the conjugates increased with DNP substitution level. For instance, those conjugates averaging only three DNP groups per molecule were cleared no faster than unconjugated HSA. If binding of a drug to antibody does not trigger clearance, then the antibody should act as a carrier protein for the drug, similar to any other protein in the blood that happens to bind the drug. Observations made in such a disparity of disciplines as toxicology, diabetes, and cytokine therapy show this to be the case.

Antibodies in the Treatment of Poisoning

Antibodies and Fab fragments with specificity for various toxic compounds are finding increasing use in the treatment of poisoning. When infused, these antibodies or fragments bind tightly to their target compound, rendering it unavailable to its normal site of toxicity. Such binding has been found to have profound impact on the disposition and clearance of these agents. Schmidt *et al.* (15) reported in 1974 that active immunization of rabbits against digoxin led to an increase in the β-phase half-life of total digoxin from 2.8 days to 51 days, and digoxin could be detected in immunized animals up to a year after a single dose. Significant but slightly less dramatic increases in digoxin half-life were also seen in dogs (16) and mice (17) passively immunized with sheep anti-digoxin antibodies. Fab fragments tended to be less effective in prolonging digoxin half-life (16,17), presumably because they are small enough to be subject to renal excretion (18). Interestingly, Griffiths *et al.* (19) found that in rabbits given digoxin 6 weeks after active immunization, the drug had a slightly prolonged half-life of 4.1 days, while digoxin given 44 weeks after immunization, showed a half-life of 25 days. Since digoxin-specific antibody titers were about the same at 6 and 44 weeks after immunization, this effect was apparently due to increased antibody affinity as a result of affinity maturation.

While sequestration of digoxin by specific antibodies dramatically reduces the clearance of the drug, it also pulls digoxin out of tissue and into the central compartment, dramatically reducing the apparent volume of distribution (Vd) (18). Thus in animals and humans that have been dosed with digoxin, the total plasma concentration of digoxin rises several-fold upon administration of antibodies or Fab fragments specific for digoxin (16,20). Since digoxin half-life increases with increasing Vd and decreases with increasing clearance, these two effects of antibody binding should oppose each other. The balance between the two pharmacokinetic parameters may determine whether and to what extent drug elimination is delayed by antibody treatment. It has been reported, in fact, that treatment of phencyclidine (PCP) toxicity with anti-PCP Fab fragments led to no significant change in terminal elimination half-life. This finding was attributed to decreases in clearance and Vd that canceled each other out (21). In rabbits actively immunized against colchicine, on the other hand, an 85% decrease in Vd was overwhelmed by a 99.6% decline in clearance, leading to a β phase half-life increase from 12 hours for non-immunized animals to 430 hours for immunized ones (22).

It is unclear from the literature cited whether the sustained presence of digoxin or colchicine in the blood results in prolonged toxicity or pharmacological effect. The extent and intensity of any such prolongation is likely to be a function of several factors, including drug dose, antibody titer, and the relative affinity for drug of antibodies versus drug receptors. In the case of digoxin, a commercial Fab preparation has been shown to efficiently compete with the cardiac receptor (Na^+/K^+ ATPase) for drug binding, suggesting that these antibody fragments have a higher affinity for drug than does the receptor. Given sufficient titers of such antibodies or fragments, the amount of drug bound

to receptors would likely be too low to have a measurable toxic effect.

Insulin Antibodies

Antibody binding has been shown to prolong the half-life of insulin in insulin-dependent diabetics. Within several months of starting subcutaneous insulin therapy, the majority of diabetics develop antibodies against insulin, and about a quarter develop high titers, as determined by the percentage of insulin bound by serum antibodies (23). Bolli *et al.* (24) found that, although all diabetics experienced prolonged hypoglycemia after an insulin injection, those with high titers of anti-insulin antibodies remained hypoglycemic for over 12 hours whereas those with low titers experienced about 6 hours of hypoglycemia that was followed by severe rebound hyperglycemia. The relatively prolonged hypoglycemia in diabetics with high antibody titers was attributed to sustained total plasma insulin levels not apparent in patients with low antibody titers; a strong correlation ($r = 0.94$) was indeed found between the extent of insulin antibody binding and plasma insulin level. Pharmacokinetic parameters for insulin such as time to peak, area under the concentration vs. time curve (AUC), and steady state concentration during continuous infusion have been correlated to total antibody binding as well as to association constants and binding capacities for high-affinity antibodies (23,25). In contrast to the effects of digoxin-specific antibodies, high titers of antibodies against insulin have been reported to increase both Vd and metabolic clearance of insulin, with the overall effect still being an increase in insulin half-life (25,26). Although unbound insulin has a much lower Vd than such drugs as digoxin and colchicine, it is not altogether clear why its Vd should increase upon antibody binding. Nor has the reason for its increased metabolic clearance been adequately addressed in the literature. In light of the fact that insulin is a sufficiently large molecule to crosslink antibodies, immune clearance by phagocytic cells cannot be ruled out.

Anti-Cytokine Antibodies

Some researchers attempting to use antibodies to neutralize undesirable cytokines were surprised to find that the biological activity of the cytokines often increased with the antibody therapy. Although anti-cytokine antibodies with sufficiently high avidity ($Ka > 10^{10}$–10^{12}) have been reported to be capable of neutralizing cytokine activity (27), numerous reports indicate that similar antibodies, presumably of lower avidity, can actually enhance the overall effect of their corresponding cytokines. Antibodies against IL-2 (28,29), IL-3 (30), IL-4 (31), IL-6 (32,33), and IL-7 (31) have been found to prolong the circulation and pharmacological effect of these cytokines, apparently because the antibodies act as carrier proteins. Jones and Ziltner found that complexing of IL-3 with its antibodies extended the β-phase half-life from 10 to 72.5 minutes with little change in Vd. They observed a 7-fold increase in biological effect when IL-3 was administered in a complex with its antibody (30). Martens *et al.* passively immunized mice with monoclonal antibodies (mAb) against IL-6 then challenged them with lipopolysaccharides (LPS) to induce IL-6 production. Immunized mice achieved a 10–30 fold higher level of biologically active IL-6 than mice treated with LPS alone (32). Finkelman *et al.* observed similar increases in activity in administering IL-3, IL-4, and IL-7 with their respective mAb (31). Courtney *et al.* (28) observed a substantially prolonged half-life for recombinant IL-2 when it was administered with its mAb and found that the IL-2-antibody complex was more effective than IL-2 alone in delaying growth of explanted tumors. Although no side effect/toxicity data was presented, the authors suggest that administration of IL-2 in a complex with its antibody might reduce the toxic side effects of the interleukin, since a lower dose could be given and a more even free-drug level would be maintained.

Interestingly, the prolongation of pharmacological effect appears to depend on the binding of antibody to the receptor-combining site of the cytokine, presumably because this protects the combining site from proteolytic degradation (31). Despite blocking the active portion of the cytokine, some of the antibodies studied had to be in a molar excess upward of 10,000 before they significantly blocked cytokine activity (28,31,32). This is presumably due to the very high affinity these cytokines have for their receptors, with association constants in the range of 10^{10} to 10^{12} M^{-1} (34). Association constants for antibody-antigen interactions can also be in this range, but they are likely to be of lower magnitude in the absence of long-term affinity maturation. It appears that antibodies in these cases bind the cytokines strongly enough to effectively compete with clearance and degradation mechanisms but not strongly enough to block binding to the cytokine receptors.

Two groups showed that immune clearance of the interleukin/antibody complexes could be brought about by artificially crosslinking the complexes or by increasing the number of antibodies binding to a single cytokine molecule. Finkelman *et al.* (31) conjugated their anti-IL-4 antibody with biotin then induced crosslinking by administering avidin. The biotin-conjugated antibodies were active in sustaining IL-4 activity, but when administered with avidin, this activity was lost, presumably due to immune clearance of the complex. Montero-Julian *et al.* (35) found that when IL-6 was administered with either one or two distinct mAb its clearance decreased from 2.37 (no antibody) to 0.16 (one mAb) or 0.23 (two mAb) mL/h. By contrast, the clearance increased to 1.6 mL/h when three distinct mAb were used. The complexes with one or two mAb did not bind significantly to macrophage Fcγ receptors while complexes with three antibodies did.

Antibody Redirection

Relying on the sustained circulation of small immune complexes, a novel technology was proposed to redirect endogenous antibodies to tumors and other target tissues. Shokat and Schultz in 1991 (36) proposed the use of small molecular conjugates capable of binding pre-existing antibody on one end and target cells on the other. Such a molecule would theoretically bind to antibody in the bloodstream and circulate with the antibody until it reached the target tissue. In a manner similar to that of a bispecific antibody, the conjugate would effectively serve as a bridge between the target tissue and the antibody, redirecting the antibody from its original epitope to an epitope unique to or overexpressed on the target tissue. Thus this approach, giving rise to a surrogate bispecific antibody, depends upon an immune response being triggered by conjugate-mediated binding of antibody to target cells but not by the initial binding of antibody

to conjugate. Using this technology, Lussow *et al.* (37) have shown that a fluorescein/IL-2 conjugate can be used to down-modulate IL-2 receptor positive T-cells in animals passively immunized with anti-fluorescein antibodies.

CONCLUSIONS

Experience acquired in such divergent disciplines as toxicology, diabetes, and cytokine research suggest that antibodies to drugs present both pitfalls and opportunities. As we progress into the era of macromolecular drugs (38), immune responses against drugs are likely to be increasingly commonplace. During the progression of such immune responses, both the serum concentration of the antibodies produced and their affinity for drug are likely to change. The pharmacokinetics as well as the efficacy of a drug could be altered considerably over the course of treatment. On the other hand, with improvements in the engineering of humanized monoclonal antibodies, it may be possible to utilize antibodies or their fragments as specific carriers for highly potent but rapidly cleared drugs, such as the recombinant cytokines. A number of observations can be made based on the data reviewed in this article:

1. Antibody binding can have a large impact on the distribution of a drug as well as on its clearance and bio-availability. A theoretical schematic representation of drug-antibody interactions in the bloodstream is shown in Fig. 1.

2. Binding to an antibody can either extend or abruptly curtail the circulation of a drug, depending on whether or not the drug can crosslink antibodies. Drugs with only one or two combining sites per molecule should enjoy sustained circulation as a result of antibody-binding. This should be commonplace for low molecular weight compounds.

3. A single monoclonal antibody is likely to be less effective than polyclonal antibodies in inducing rapid clearance of a moderate sized antigen, since the monoclonal can bind to only one type of antigenic site and is hence less likely to be crosslinked by the antigen.

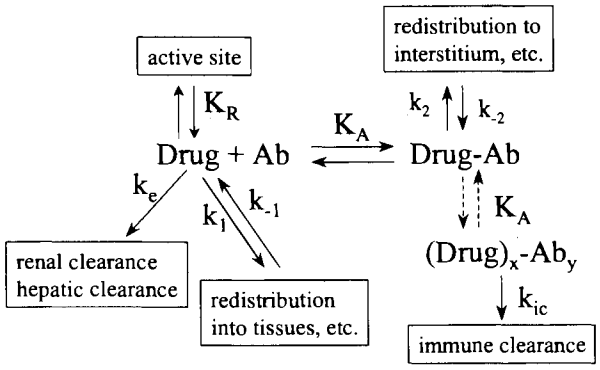

Fig. 1. A theoretical schematic representation of drug-antibody interaction in the bloodstream. Free drug binds to its receptor with affinity constant K_R and is subject to normal clearance mechanisms with rate constant k_e. Drug binds circulating antibodies (Ab) to an extent determined by the affinity constant K_A. The drug may be subject to redistribution to other compartments, as may be the complex. If a drug is capable of crosslinking antibodies to form a large immune complex ($(Drug)_x$-Ab_y), then rapid immune clearance, governed by rate constant k_{ic} will occur.

4. When antibody binding leads to retention of a drug in circulation, the drug may or may not retain its activity. Antibodies that have a greater affinity for a drug than its receptor are likely to neutralize the drug while those with weaker affinity are more likely to prolong the drug's efficacy.

5. Since immune response varies from patient to patient, a substantial intersubject variability in drug disposition and pharmacokinetics is expected for antibody-binding compounds when antibodies result from active immunization. In the case of passive immunization, intersubject variability might arise from patient to patient differences in the clearance of exogenous antibodies.

6. Over the course of a response to active immunization, both the concentration and affinity of antibodies can change significantly. In the case of passive immunization, the antibody affinity is unlikely to change, but concentration may decline over a relatively short time frame due to accelerated clearance and lack of replenishment of the exogenous antibodies. Table I provides a mathematical analysis of the drug-antibody interaction, showing how changes in affinity and concentration of binding sites could impact the extent of drug binding. For example, a 33% decline in the concentration of high affinity ($K_A = 10^{10}$) antibodies is predicted to cause a 50 fold increase in the serum concentration of unbound drug, substantially altering the pharmacokinetics and efficacy of the drug. Likewise, an order of magnitude increase in antibody affinity might lead to a 90% decrease in free drug concentration. It should be noted, however, that in the complex realm of antibody binding, predictions from mathematical and *in vitro* models do not always correlate well with *in vivo* results (39).

7. Because antibodies have only two combining sites per 150,000 daltons of molecular weight, the molar concentration of antigen-binding sites remains relatively low. Even if 10% of all circulating IgG were specific for a drug, it could only carry about 20 micromolar drug. Considering that serum free drug concentrations will be even lower, a drug must be very potent to be efficacious in the concentration range in which antibody binding is relevant. Since high potency is usually associated with a narrow therapeutic window, the sensitivity of free-drug concentrations to fluctuations in antibody concentration and binding site affinity could be especially worrisome.

Table I. Effect of Antibody Concentration and Affinity on Free Drug[a]

Affinity (M^{-1})	Antibody Binding Site Conc. (μM)		
	0.75	1.0	1.5
10^6	69%	62%	50%
10^7	40%	27%	14%
10^8	28%	9.5%	1.9%
10^9	25%	3.1%	0.2%
10^{10}	25%	1.0%	0.02%

[a] Calculations are based on the equation for the association constant ($K_A = [D_b]/([D_f][Ab_f])$, where $[D_b]$ and $[D_f]$ are bound and free drug concentrations and $[Ab_f]$ is the concentration of unoccupied antibody binding sites. The percentage of unbound drug is given for a hypothetical total drug concentration of 1 μM (10^{-3} mg/mL for a drug with molecular weight of 1000 daltons or 0.02 mg/mL for a 20 kilodalton protein).

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