EDITORIAL

Pharmacokinetics and Antibodies

Antibody molecules are very complex structures, grouped into five distinct classes; IgG, IgA, IgM, IgE, IgD. These classes differ from each other in size, charge, amino acid composition and carbohydrate content. In addition, the immunoglobulins within each class are very heterogeneous (*1*). Each molecule is bifunctional, one site binding to an antigen, the other to a host cell or complement. The now widely familiar basic structure consists of two identical heavy chains and two identical light chains and the chemical details of the subclasses are also largely known. But many of the physiological functions, for example that of IgD, are not yet fully understood.

The advent of hybridoma technology has made large amounts of monoclonal antibody available in the form of highly homogeneous specific reagents for clinical use. The challenge is to put these powerful biologic tools to work as useful diagnostic and therapeutic agents in man. One of the most exciting and promising areas of research involves using monoclonal antibodies for imaging and therapy of cancer. Since human monoclonal antibodies are not yet available this means developing effective methods for using radiolabeled mouse antibodies in humans. Because of the lack of knowledge about many aspects of the physiological function and metabolism of antibodies, advances have been mainly in radiochemistry and monoclonal antibody production, and much remains to be learned about their pharmacodynamics.

Factors Affecting Radiolabeled Antibody Accumulation in Tumor

Blood flow. Tumors grow radially from a central group of cells, so as the tumor enlarges, the dividing cells form a shell around a relatively hypoxic core. When these cells outgrow their blood supply, they die and form a necrotic central nest containing some viable cells that are very resistant to radiation. Blood flow in this situation is very low making delivery of drugs or radiopharmaceuticals through the circulation very difficult. In animal models such as the nude mouse the tumor often has a good blood supply and the tumor relatively speaking is huge. On a weight basis such tumors often are equivalent to 1 kg in a human. This partly explains tumor uptakes of 20–30% of the injected dose per gram in the mouse, compared to human studies where 1.0%/g is exceptionally high, 0.1–0.001%/g being more usual (*2*).

Permeability. Radiopharmaceuticals must reach the tumor through the circulation, crossing the capillary wall and diffusing throughout the interstitial fluid to reach the tumor cells. The large size of the antibody molecule slows the rate of diffusion across these barriers and becomes an important factor in the rate of uptake. This may be the principal reason that it takes radiolabeled antibody several days to attain a significant concentration in human scintigraphic studies. Antibody fragments such as Fab, and (Fab')2, ~⅓ and ⅔ the weight of whole antibody, have the ability to diffuse more rapidly into the tumor.

Concentration gradient. The driving force causing diffusion of labeled antibody from the vascular compartment into the tumor is the concentration gradient between the blood and the tumor. The higher the integral blood concentration with time, the higher the tumor concentration will be. Although the antibody fragments have a more rapid diffusion they also have a more rapid disappearance from the blood, and a lower integral blood concentration with time. Reports comparing whole antibody with its fragments show integral blood concentration to be more important than molecular weight since tumor concentration was highest with whole antibody, and lowest with Fab, with F(ab')2 intermediate (*3,4*). In nephrectomized mice, fragments reach the same concentrations as whole antibody, but the blood background is equally high.

Receptor (antigen) binding. The unique specificity of antibodies has been the most compelling reason for their application in radioimmunoimaging as the repertoire of targets is almost limitless. Binding to the tumor antigen is noncovalent, like the binding of certain drugs and small molecular weight radiopharmaceuticals to receptors on the cell membrane, and the same affinity rate constants apply. Thus increased binding of antibody to tumor target antigen will occur with high affinity for the antigen (Ka = 10^9), and with increased

antigen concentration (10^5/cell). At these levels of antigen concentration a one gram tumor with 10^9 cells would contain only 10^{14} antigen sites (~0.1 nmol). Since 1 mg of IgG = 6.7 nmol, ideally one would need high specific activity, in this case no more than 0.015 mg (0.1 nmol) antibody carrying ~1 mCi activity (67 μCi/μg). This will avoid saturating the target.

The usual specific activities of iodine-131- (^{131}I) labeled IgG are ~1–10 μCi/μg; less than ideal. In practice however, due to the large amount of nonspecific binding of antibodies through their Fc moiety or other mechanisms, labeled antibody is further diluted with cold antibody to saturate these sites. Up to 20–30 mg have been used with 1–10 mCi ^{131}I (0.05–0.34 μCi/μg). This compromise has been found necessary due to the overwhelming preponderance of nonspecific binding sites.

Factors Affecting Radiolabeled Antibody Background

Quantitatively over 90% of injected radiolabeled antibody remains in the background pool and 1% or less is in the target tumor. Since purity, affinity, radiolabeling and specificity of monoclonal antibodies has approached optimum, and most of the other factors affecting tumor uptake such as blood flow and the affinity of the antibody are fixed, for further improvements it may be important to consider the causes and ways of reducing the background.

Rate of excretion. Whole antibody is only very slowly excreted and blood levels stay high for many days. The most important route of metabolism with iodinated antibodies is deiodination in the liver. Using chelate labeled antibodies the rate of elimination is even slower, creating greater background, especially in the liver. The accumulation of indium-111 (^{111}In) in the liver may be due to metabolic trapping or possibly loss of ^{111}In from the chelate and sequestration of ^{111}In in liver lysosomes.

Metabolism. Practically every preparation of radiolabeled antibody is unique. Variables include specific activity (number of radiolabels per molecule of antibody), type of physical decay (gamma, beta), site of radiolabel on the antibody (hopefully not in the antigen binding site), antibody class (IgM, etc.), antibody isotype (IgG1, IgG2a, etc.), and each one of these characteristics can affect the biodistribution and metabolism. For example, heavily labeled antibody (many labels per molecule) causes rapid removal from the blood by the liver, and a completely different biodistribution. The introduction of an enzyme cleavable link between the chelate and the antibody results in more rapid metabolism and excretion of the chelate in the urine (*5*). This results in a lower whole body and blood background.

Nonspecific binding. The binding of antibody to Fc receptors located on cell membranes in the liver, blood, and bone marrow in most experimental mouse tumor models usually accounts for a larger total uptake than the tumor. As mentioned, in the liver this is particularly high with chelate conjugates. Fab fragments sequester in the kidney cortex and only ~50% is excreted in the urine, increasing background and radiation dose.

Antibody specificity. Proper screening of monoclonals and selection of antigen will virtually eliminate cross reactions. The use of monoclonal antibodies theoretically reduces nonreacting or poorly reacting molecules to a minimum.

Permeability. The blood brain barrier totally excludes molecules the size of antibodies. This breaks down in brain tumors which are easily imaged with radioiodinated serum albumin (RISA) (*6*). This is a unique feature of brain capillaries, and is not found anywhere else in the body. In all other sites the capillaries allow antibodies to pass just as readily as in tumors. This large nonspecific component has made imaging tumors with RISA difficult in areas other than the brain. It also makes control experiments with nonspecific antibody necessary to assess the fraction of the uptake due to specific interaction with the target.

Strategies to Lower Background

The problem of background reduction in radioimmunoimaging has been approached in several different ways: (a) computerized background subtraction after second and third radiopharmaceutical injection, (b) antibody fragments, (c) second antibody, (d) local delivery, lymphoscintigraphy etc., (e) metabolizable chelate linker, (f) reversible antibody hapten complexes, and (g) pre-targeted immunoscintigraphy.

Computerized background subtraction. To obtain images of suitable quality within 24 to

48 hr DeLand et al. (*7*) have developed background subtraction techniques which involve the injection of a second and even a third radiopharmaceutical to outline selectively the blood pool and other background areas which then may be subtracted from the tumor image. This increased the sensitivity, but may also introduce artifacts into the final image (*8,9*). Simultaneous recording of target and background radiopharmaceuticals eliminates patient motion artifact using dual isotope labeling. However artifacts due to differences in distribution and attenuation between the true and simulated background activity remain a problem.

Antibody fragments. Antibody fragments such as Fab and F(ab')2 disappear more rapidly from the blood than whole antibody. Their use may significantly decrease the time required for sufficiently high target to background ratios to be reached for imaging. Wilbanks et al. (*3*) compared the in vivo distribution of whole IgG and Fab fragments prepared from the same antisera specific for mouse mammary epithelial cells. Simulated mammary tumor metastases and normal tissue were measured 24 hr after injection. The data showed higher tumor to organ ratios with Fab fragments but the absolute concentration in the tumor was about three times higher with whole antibody. Similar results have been reported by Wahl et al. (*4*) using monoclonal anti CEA whole antibody, F(ab')2, and Fab fragments. They showed F(ab')2 fragments were the best compromise between the rapidly cleared Fab fragments and the slowly cleared whole antibody. The absolute concentration of whole antibody in these experiments was 2.4 times the F(ab')2 concentration and 26 times the Fab concentration at 48 hr. We have demonstrated the importance of prolonged high blood levels as a major factor determining the final tumor concentration of nonspecific proteins in an experiment in which second antibody was given at various times to lower circulating IgG or transferrin quickly to 10–15% of its former level in 15 min (*10*). When transferrin was allowed to circulate 18 hr it reached tumor concentration of 15%/g at 20 hr, but when circulating transferrin was reduced 2 hr following its injection the 20 hr tumor concentration only reached 5%/g.

Second antibody. Begent and co-workers (*11*) suggested the use of liposomally entrapped second antibody (LESA) for lowering blood levels of anti-CEA antibody. They demonstrated enhanced clearance of anti-CEA in patients with gastric cancer 24 hr after i.v. LESA administration. Subsequently it has been shown that liposome entrapment is not necessary as antibody alone works very efficiently. It is interesting that polyclonal antibody works better than monoclonal antibody in transporting circulating antibody to the liver, possibly because it is more efficient in crosslinking and lattice formation. A disadvantage of this approach is the concomitant rise in liver background, resulting in increased radiation dose and decreased contrast between tumor metastases and normal liver tissue. Using radioiodinated antibody deiodinases in the liver may accelerate the excretion of iodide that is split off the antibody.

Local delivery. Antibody may be injected locally, for example subcutaneously, to increase the concentrations presented to the target (regional lymph nodes) (*12*). Other sites that have been studied include intraperitoneal (colonic and ovarian metastases), intrapleural, intrapericardial (malignant effusions) (*13*), and intraarterial (hepatic metastases). While this method gives higher concentrations to the regional target, for example regional lymph nodes, it is limited to accessible areas and does not lower the radiation exposure.

Metabolizable chelate linker. The insertion of a metabolizable ester chelate link between the chelate and protein was shown to increase the excretion and decrease the blood background in tumor mice injected with chelate labeled anti-idiotype to 38C 13 mouse lymphoma (*5*). This improved tumor to background ratios with a modest drop in absolute tumor concentrations. Several other types of linkers are currently undergoing investigation. Endogenous enzymes such as esterases present in various organs especially the liver are capable of attacking these bonds and cleaving off the chelate moiety. The small charged hydrophillic chelate is then rapidly excreted in the urine.

Reversible antibody hapten complexes. A novel method for radiopharmaceutical delivery is the use of monoclonal antibody hapten complexes wherein a small rapidly excreted chelate is the haptenic group (*14*). The pharmacokinetics of ^{111}In-labeled chelates is profoundly altered by first complexing them with antibody. Instead of rapid excretion in minutes, the chelate antibody complexes circulate for days just as if the antibody had been covalently labeled. The complexes differ however from covalently labeled antibodies in that the label is reversible. This reversible equilibrium binding of haptens by antibodies allows competitive

inhibition by the injection of a nonradioactive hapten (chase) at a predetermined time. We have shown in the mouse tumor model that the complex attains high tumor concentrations nonspecifically at 24 hr and that blood and whole-body background can be reduced 40% in 3 hr with chase while tumor concentrations were less affected, increasing tumor/organ ratios. Introduction of the chase at 24 hr reduced the integral radiation dose up to 80–90% (*15*). In these experiments it was possible to carry haptenic groups with molecular weights up to 50 kD (Fab fragments) and to chase these groups at a predetermined time.

The ability to eliminate blood background shortly before imaging should improve the target to background ratios especially over vascular areas such as the heart when imaging clots or myocardial infarcts with antibodies. With this technique it is necessary to label the complexes with radionuclides having half-lives matched to the optimum imaging time for antibodies which diffuse slowly over 24–48 hr. Therefore, like covalently labeled antibodies, ^{131}I or ^{111}In must be used as the label. Other labels more attractive for single photon imaging (technetium-99m), and PET imaging, (gallium-68), have too brief a half-life for use in this approach.

Pretargeted immunoscintigraphy. We have recently proposed a method in which the antibody and the radiolabel are administered separately (*16*). Nonradioactive antibody is given first (pretargeted) and allowed time to reach maximum tumor concentration, usually at least one day. At the time of maximum tumor concentration of nonradioactive antibody, the blood is quickly cleared of excess circulating nonradioactive antibody using a special intravenous "chase". Shortly after (30–60 min) the radiolabel is given, and images made in 1–3 hr. This method avoids some of the previously mentioned problems such as prolonged radiation exposure to the patient, the need for long lived tracers (^{131}I, ^{111}In) and high blood and liver background.

An obvious improvement in this system is the development of bifunctional antibodies that could bind both a chelate and a tumor antigen (*17,18,19*). Either hybrid antibodies or antibody conjugates could be used for this application. Improved images have been obtained using two Fab′ fragments coupled at the SH groups via a heterobifunctional crosslinker. One Fab was specific for the radio labeled chelate, the other for CEA (*18*).

A potential limitation to this technique is the relatively large amount of antibody needed to bind the tracer in the target. This is inversely related to the binding constant of the antibody which is usually $\sim 1 \times 10^9$. In our experiments 50–100 μg were needed in the mouse which on a body weight basis is equivalent to 100–200 mg for a human. Only small improvements can be expected in the Kb for antibodies: possibly to 10^{10} and therefore it would be convenient to have a chelate binding substance with a much higher affinity.

Fortunately other noncovalent systems do exist that have affinities much higher than antibodies. One example is the avidin biotin system with an affinity (Kb) of 10^{15}. Hnatowitch et al. report in this issue some of the ways in which avidin (streptavidin) and biotin may be used to advantage in imaging applications in a mouse system. Chelate labeled biotin bound rapidly and quantitatively to avidin and streptavidin, was stable in serum, and bond formation occurred in vivo when the avidin and streptavidin were given separately. Our application of this system was to pretarget the subject with tumor specific antibody that had been conjugated to avidin. At the time of maximum uptake of antibody conjugate a chase of biotinylated transferrin was given to clear the blood of excess circulating conjugate. Shortly after, a chelate conjugate of biotin was given and tumor and organ distribution measured 3 hr later when the unbound radiolabeled biotin had been excreted by the kidney (*17*).

In their report, Hnatowitch et al. (*19*) show greatly improved images in mice using avidinated antibody against protein A beads in the mouse peritoneum, followed at various times by ^{111}In-labeled DTPA biotin conjugate. The advantage of low body background with target to nontarget ratios improved by more than two orders of magnitude compared to covalently radiolabeled antibody is striking. This result was better than the alternate arrangement with the biotin on the antibody and the radioactive chelate on the avidin, because the radioactive avidin did not clear as rapidly as biotin and the background was high.

It is clear that research into methods of controlling antibody background has produced many promising leads. Methods of background reduction will be as important to the eventual

successful clinical application of antibodies, as are improvements in antibody affinity, specificity and decreased antigenicity.

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