Rapid Blood Clearance of Biotinylated IgG After Infusion of Avidin

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The techniques of immunotherapy and radioimmunoimaging suffer from the problem of background: intravenously injected antibodies remain in the circulation much longer than it is necessary for effective binding to the target. Various approaches, including the postinjection of second antibodies, were explored to overcome the problem with some success. The phenomenon of a 100-fold more rapid blood clearance of biotinylated immunoglobulins after postinjection of an equivalent dose of avidin is described. The concentration of ^{125}I-labeled biotinylated IgG in the circulation of rats slowly decreased to 20% of initial in 24 hr. Avidin injection at any interval during this period induced 90–95% reduction of radioactivity in blood in 15 min. Up to 70% of the radioactivity was recovered in the liver. Avidin-induced blood clearance of biotinylated immunoglobulins may find applications in immunotherapy and radio- or nuclear magnetic resonance immunoimaging.

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A general solution for the problem of targeting of intravenously injected substances to the region of a pathologic process has been found with the advent of monoclonal antibodies. Various immunoderivatives (immuno-liposomes, -erythrocytes, -enzyme complexes, radioactive or photoactive immunoconjugates), chimeric antibodies or antibodies with direct effect on cell receptors are extensively being applied in clinical and experimental work (*1–3*). Nevertheless, the associated problem of background still remains unsolved: an excess of the infused reagent would circulate in the blood thus obscuring visualization of the sites with specific accumulation of antibodies. The contrast between target and blood increases concomitantly with clearance of unbound antibodies but natural blood clearance of injected immunoglobulins is a slow process (*4*). Therefore, various methods of enhanced blood clearance have been developed. Lactosamination of antibodies causes their rapid hepatic uptake (*5*), but the "time-concentration" conditions for the specific binding are proportionally impaired. Postinjection of an excess of second antibodies or their conjugates with liposomes produce immune complexes that are preferentially retained by the liver (*6*). However, strict demands on the specificity and affinity of second antibodies make this approach rather impractical. In this paper we describe a new method which utilizes effect of enhanced liver uptake of iodine-125- (^{125}I) labeled biotinylated IgG after postinfusion of avidin, a cationic (pI > 10) glycoprotein with an extremely high affinity for biotin (kD $\approx 10^{-15}M$) (*7*).

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MATERIALS AND METHODS

The substances used were: N-hydroxysuccinimidobiotin (NHS-biotin), 2,4,6 trinitrobensolsulphoacid (TNBS), Iodogen (Pierce Chemical Co., Rockford, IL), biotin-Sepharose (Sigma Chemical Corporation, St. Louis, MO), Sephacryl S-400, human IgG, isolated from plasma (*8*); and avidin, isolated from hen egg (*7*). BSA-Sepharose, avidin-Sepharose, and Sepharose with goat antibodies against human immunoglobulins were obtained by protein immobilization on CNBr-activated Sepharose 4B (Pharmacia).

Biotinylation of Human IgG

Human IgG was treated with NHS-biotin (*9*). The NHS-biotin/IgG molar ratio varied from 1:1 to 100:1, thus samples with different degrees of modification of protein aminogroups were obtained. The biotin-IgG (biIgG) were radiolabeled with ^{125}I, and their sorption on avidin-Sepharose was determined. More than 90% of radioactivity was retained on the sorbent when NHS-biotin/IgG ratio exceeded 20:1.

For further experiments, samples obtained at NHS-biotin/IgG molar ratio 25:1 were used. Titration with TNBS (*10*) revealed that ~15% of aminogroups were modified. At least 90% of [^{125}I]biIgG preparation bound to goat-antihuman IgG antibodies immobilized on Sepharose, while binding to BSA-Sepharose (control) was < 5%.

Radioactive Labeling of Proteins

Biotinylated human IgG were labeled with ^{125}I using Iodogen (*11*). Specific activity 10^5 cpm/μg of protein was obtained. Avidin was modified by Bolton-Hunter reagent (*12*), and then labeled with ^{125}I using Iodogen. Modification of avidin aminogroups did not exceed 10% according to TNBS titration, specific activity was 5×10^5 cpm/μg of avidin. About 95% of labeled avidin bound to biotin-Sepharose while <5% adhered nonspecifically to control BSA-Sepharose.

Plasma Clearance and Organ Uptake of Biotinylated IgG

Wistar male rats, 250–300 g were fitted with femoral artery catheters under pentobarbital anesthesia (50 mg/kg). After the animals had recovered from the anesthesia, 20 μg of biotinylated labeled IgG were injected via the catheter. The kinetics of blood clearance was measured, blood samples (≈300 μl) being obtained from the catheter and counted in a gamma counter. For study of the accelerated clearance, animals were injected with 3-200 μg avidin 90 min after initial injection of biotinylated IgG. Subsequently, the kinetics of radioactivity blood clearance was measured as described above. Five parallel experiments were made; the results of a typical experiment are presented in the figures.

For analysis of organ uptake animals were anesthetized and killed 2 hr after [^{125}I]biIgG injection (experiments without avidin), or 30 min after avidin was injected at 1.5 hr point. Organs were rinsed in saline, weighed, and counted in the gamma counter.

Studies with ^{125}I-labeled avidin were performed as described above. A portion of 100 μg avidin (4 μg ^{125}I-labeled avidin) was injected into each of the rats.

Analytical Gelfiltration

Approximately 1 μg of labeled biotinylated immunoglobulin G was added to 1 ml of citrated rat plasma. When needed, 5 μg of avidin were added afterwards at vigorous stirring. Gel filtration of 800 μl plasma was performed on a column with 50 ml (1.6 × 25 cm) Sephacryl S-400. The column was calibrated with Blue dextran and mixture of human IgG and IgM.

RESULTS

Biotinylation of human IgG did not change the rate of their clearance significantly. Amount of radiolabel in blood samples gradually decreased and reached at 10 and 24 hr, respectively, 50% and 20% of initial 2×10^6 cpm (20 μg [^{125}I]biIgG), injected in a rat (Fig. 1). The clearance rate of [^{125}]biIgG increased 100-fold when 0.1 mg of avidin was postinjected at any time within this 24 hr. The concentration of [^{125}I]biIgG in the blood lowered rapidly and reached a plateau of 5–10% of the initial level in 5–15 min (Fig. 1). The plateau level was dose depended in the range 3 to 100 μg of avidin (Fig. 2). Higher doses produced same effect as 100 μg. Repeated injections of a smaller dose were additive with respect to plateau level within 15 min.

Biodistribution of ^{125}I-biIgG without avidin and after avidin-induced clearance was compared at 2 hr point. Without avidin, the radioactivity was accumulated mostly in the blood (≈80%). Up to 70% of the radioactivity cleared by avidin from the blood was found in the liver; significant increase was also detected in the spleen (Fig. 3). No difference in the bound radioactivity was detected in the heart, lungs, kidneys, other organs, and tissues.

Two reasons for the ability of avidin to induce blood clearance of biotinylated IgG are possible. First, large

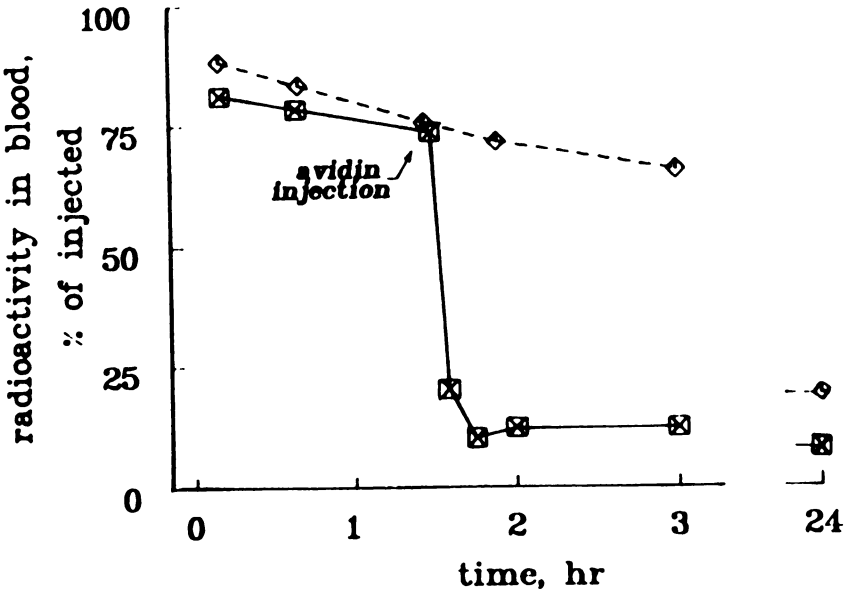

FIGURE 1
The kinetics of the plasma clearance of biotinylated human IgG with no avidin injected < ◇ >, with avidin introduced 60 min after immunoglobulins injection < ⊠ >.

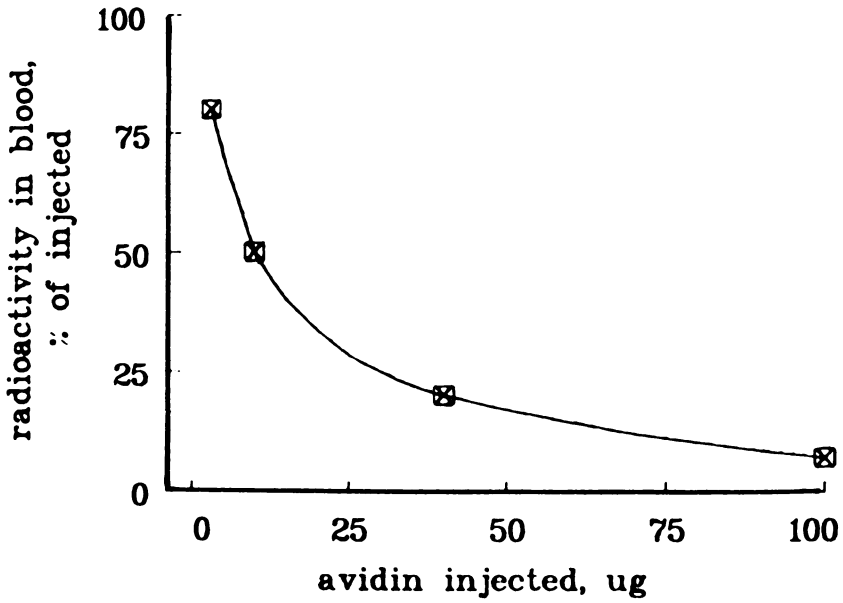

FIGURE 2
Clearance effect versus injected avidin dose.

immune complexes might be produced because avidin has four biotin-binding sites and biotinylation of IgG molecules was more than 1:1. Then, avidin itself could be entrapped by liver cells as a cationic glycoprotein thus providing specific liver uptake of the complexes. To check the latter, ^{125}I-labeled avidin was infused into rats. Its clearance kinetics exactly corresponded to the blood depletion of [^{125}I]biIgG plus avidin (data not shown). Less than 10% of avidin remained in the blood 15 min after injection. About 70% of the label was found in the liver. On the other hand, analytic gelfiltration of rat plasma samples containing [^{125}I]biIgG revealed the shift of radioactivity peak from position, corresponding to IgG (160 kD), to > 900 kD position after avidin addition (Fig. 4). It should be noted that neither labeled avidin nor labeled biIgG-avidin complex was associated with blood cells, but the radioactivity was always recovered in plasma. Therefore, both causes are realizable in vivo with a similar general effect.

DISCUSSION

Avidin-induced blood clearance of biotinylated immunoglobulins may find wide applications in immunotherapy and radio- or NMR immunoimaging. It provides extremely rapid (5–15 min) and efficient (up to 20 times) increase of the contrast between blood and target tissue (except liver and spleen). The clearance may be started at any chosen time thus providing an opportunity to maintain an increased concentration of immunoreagents around the target and when necessary reduce it. The method is universal because any sort of immunoglobulins can be easily biotinylated. It is important that the effect is achieved even with slightly modified proteins (1–2 biotin molecules per antibody).

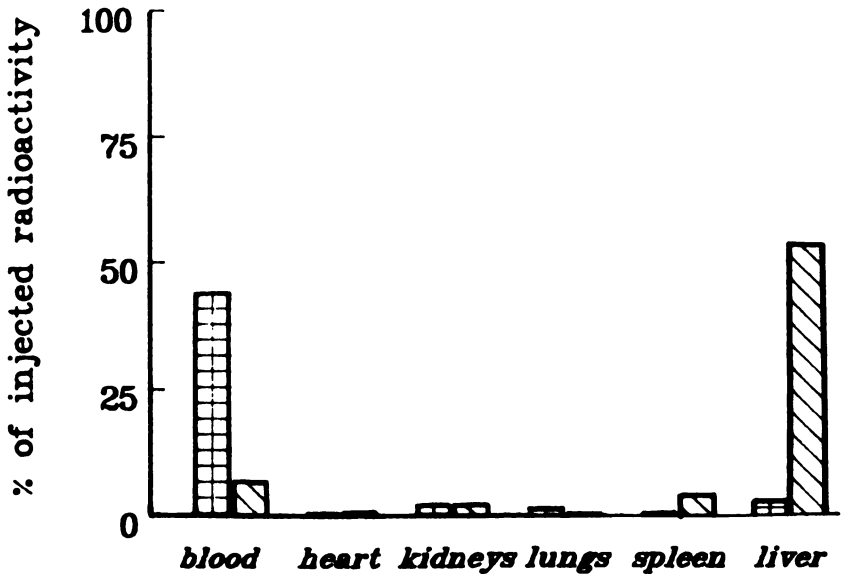

FIGURE 3
Biotinylated human IgG distribution in rat organs without avidin < ⊞ >, with avidin injected < ▨ >.

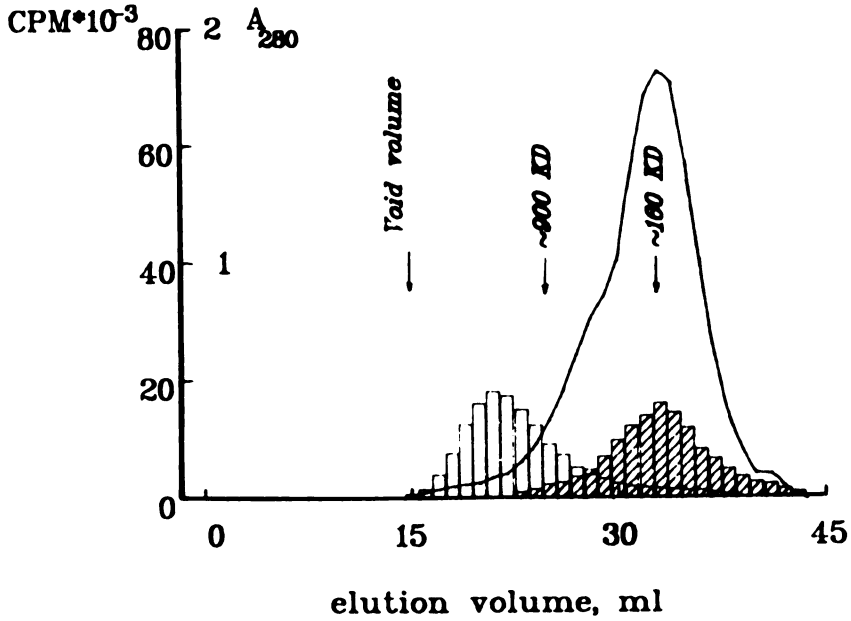

FIGURE 4
Gel filtration of plasma containing (A) biotinylated human IgG, (B) avidin-biotinylated IgG complexes < ⊓ > - peak of radioactivity, <——> - peak of optical density.

As known, a more severe modification does not change affinity properties of polyclonal antibodies (*13*). With monoclonal antibodies individual sensitivity may occur. In our experience with a panel of monoclonal antibodies to human fibrinogen it is usually possible to prepare their biotinylated derivatives which retain high specificity and affinity (unpublished data).

Drug targeting is a very promising approach in therapy. Affinity of avidin or avidin-bearing complexes to liver may be exploited for this purpose as well. However, more investigations are needed to follow the fate of entrapped complexes and to check possible side effects. Special attention should be paid to a probable increase in immunogenicity of immunoglobulins complexed with avidin. With the avidin doses used in our experiments we did not observe any side effects in the animals. Other reports have confirmed this finding (*14*). The presence of minor amounts of biotin in the blood (10 n*M*) (*15*) is easily overcome by using more than 10 μg avidin per 1 ml of blood.

It is noteworthy that the method developed in rats could have applications in other animals. Avidin induced 90% clearance of anti-fibrinogen monoclonal antibodies infused into dogs (unpublished data).

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