THE USE OF AVIDIN TO ACCELERATE BLOOD CLEARANCE OF BIOTINYLATED IMMUNOGLOBULINS

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A new trend in the diagnosis and treatment of diseases connected with the use of antibodies specific for components of zones of pathological changes has developed rapidly in recent years [3, 5, 11]. Immunovisualization in the gamma-camera is used to determine the location of malignant tumors and also of zones of thrombosis. One factor reducing the efficiency of immunovisualization is a high background level of radioactivity in the blood, due to prolonged circulation of labeled antibodies, not bound with the target. Suggested ways of increasing target/blood contrast include lactosylation of antibodies to ensure rapid elimination through the liver [4] and postinjection of antispecific antibodies, conjugated with liposomes [2]. The first method ensures rapid removal of antibodies but does not enable a high concentration of them to be created in the target region. The use of the second method is impossible in practice, for it makes extremely high demands on the affinity and specificity of the antispecific antibodies.

This paper describes the development of a method of rapid clearance of exogenous antibodies from the blood, based on the use of an avidin-biotin ligand pair which, on the one hand, enables a high concentration of antibodies to be created in the target region, and on the other hand, enables their blood level to be reduced at any assigned moment due to uptake by the liver.

EXPERIMENTAL METHOD

The following reagents were used: the hydroxysuccinimide ester of biotin, 2,4,6-trinitrobenzenesulfonic acid, and iodogen were from "Pierce" biotin-sepharose was from "Sigma," human class G immunoglobulins were isolated from plasma by the method in [7]; avidin was isolated from hens' eggs by the method in [8]; BSA-sepharose, avidin-sepharose, and sepharose with immobilized monoclonal goat antibodies against human immunoglobulins were obtained by binding the proteins to CNBr-activated sepharose 4B. Monoclonal antibodies to human fibrinogen, capable of binding selectively with canine thrombus in vivo [1], were generously provided by N. V. Popov (All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR).

Human immunoglobins G and antibodies to fibrinogen were biotinylated by the reaction with the hydrosuccinimide ester of biotin [9]. The degree of modification was varied by changing the molar ratio of the reagents from 1:1 to 100:1. The preparations obtained were labeled with ^{125}I with the aid of iodogen with specific radioactivity of 500,000 cpm/μg protein and tested on microcolumns with affinity sorbents.

Preparations with a degree of modification of their amino groups of 15-20%, according to the results of titration with 2,4,6-trinitrobenzenesulfonic acid [6], were used for the subsequent experiments. Binding of biotin-IgG with avidin-sepharose in this case amounted to 94%, with the sorbent with immobilized goat antibodies against human immunoglobulins it was 90%, and in the control, binding with BSA-sepharose was 4%. In the case of antibodies to fibrinogen 98% of the radioactive label bound with avidin-sepharose, 75% with fibrinogen-sepharose, and 2% with BSA-sepharose.

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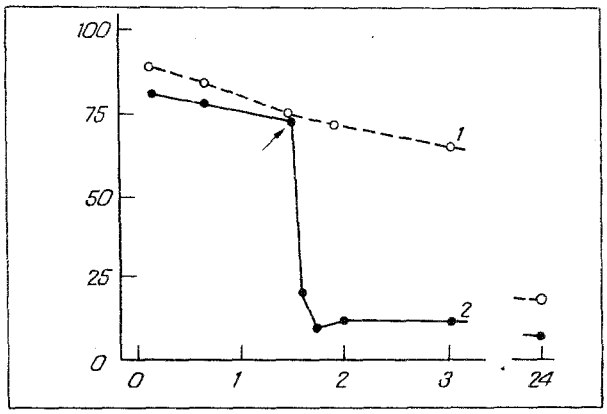

Fig. 1. Kinetics of elimination of biotin-IgG from rat's bloodstream. Abscissa, time (in h); ordinate, radioactivity in blood (in % of injected dose). 1) Kinetics of clearance without injection of avidin, 2) the same, with injection of avidin 90 min after injection of biotin-IgG. Here and in Fig. 3, arrow indicates time of injection of avidin.

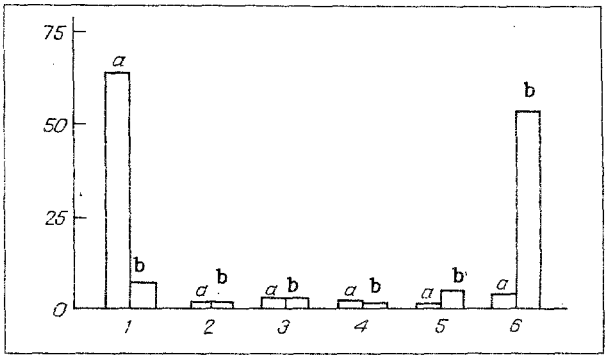

Fig. 2. Biodistribution of biotin-IgG among rat's organs. Ordinate, accumulation of radioactivity in organs 2 h after injection of biotin-IgG (in % of injected radioactivity). 1) Blood, 2) heart, 3) kidneys, 4) lungs, 5) spleen, 6) liver; a) without injection of avidin, b) 30 min after injection of avidin.

Clearance of the biotinylated immunoglobulins from the blood and their accumulation in the organs were studied on male Wistar rats weighing 250-300 g. Under pentobarbital (50 mg/kg) anesthesia, a catheter was introduced into the rats' femoral artery. When the rats recovered from the anesthetic they were given an injection of 20 μg of biotinylated immunoglobulins, of which 4 μg was labeled with ^{125}I. The kinetics of their elimination was then recorded by withdrawing a sample of 300 μl of blood through the catheter and measuring its specific radioactivity. In the study of rapid clearance, 90 min after injection of 20 μg of biotin-IgG (of which 4 μg was labeled with ^{125}I) into the rats, 3-200 μg avidin was injected through the catheter, after which the kinetics of the fall of specific radioactivity of the blood was recorded as described above. Organs for testing for their content of radioactive label were taken 2 h after injection of biotin-IgG (the experiment without avidin) and 30 min after injection of avidin (i.e., 2 h after injection of biotin-IgG) in the rapid clearance experiments. All experiments were carried out in three parallel determinations and the results of one typical experiment are shown on the graphs.

The experiments on dogs followed the same routine. The dose of monoclonal antibodies to fibrinogen was 40 μg/kg body weight and rapid clearance was induced by injecting avidin in a dose of 200 μg/kg body weight.

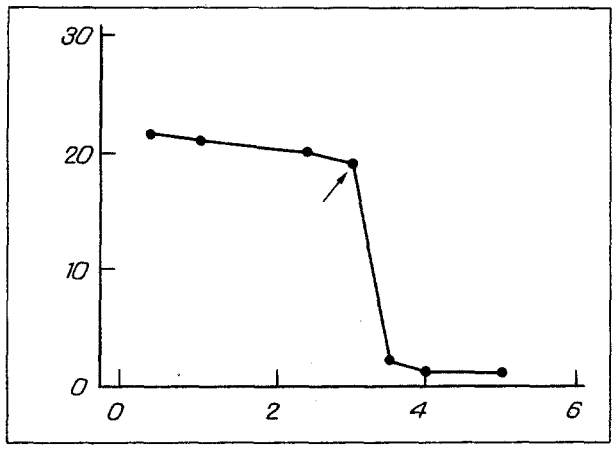

Fig. 3. Kinetics of elimination of biotinylated monoclonal antibodies to human fibrinogen from dog's bloodstream. Abscissa, time (in h); ordinate, specific radioactivity in blood ($\times 10^{-3}$ cpm/ml).

EXPERIMENTAL RESULTS

Clearance of the weakly biotinylated (degree of modification of the amino groups not more than 20%) human IgG introduced into the rat's bloodstream was virtually identical with clearance of native IgG. A decrease of 50% in the specific radioactivity of the blood after injection of 20 μg biotin-IgG took place after 10 h, and a fall of 80% after 24 h (Fig. 1; see [10]). However, on subsequent injection of avidin (at any time in the course of these 24 h) the rate of clearance increased by two orders of magnitude. The concentration of biotin-IgG in the blood fell sharply, flattening out on a plateau in the course of 15-20 min (Fig. 1). The level to which their concentration could fall depended on the dose of avidin injected, had the minimal dose causing specific radioactivity in the blood to fall below 10% of the initial level was 100 μg. Repeated injections of small doses of avidin (3-30 mg) had an additive effect and caused rapid clearance of the corresponding portions of biotin-IgG.

Investigation of the radioactive label showed that biotin-IgG eliminated from the bloodstream with the aid of avidin accumulate in the liver (up to 70%) and in the spleen. Accumulation of radioactivity in the other organs studied could not be observed, by contrast with the control (experiments without avidin) (Fig. 2).

The method of rapid elimination of exogenous antibodies from the bloodstream developed above is potentially promising as a means of increasing target/blood contrast during immunovisualization of tumors and thrombi. Accordingly, we tested it on dogs, using monoclonal antibodies to human fibrinogen, accumulating in thrombi in experiments on dogs in vivo [1]. Biotinylation did not lead to a decrease in antibody activity (see: Experimental Method). The kinetic curve of elimination of these biotin-antibodies from the dog's bloodstream (40 μg/kg body weight) before and after injection of 200 μg avidin/kg body weight is shown in Fig. 3. The results confirm the universal applicability of the suggested method and its potential value in practical immunodiagnosis.

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BENZ(a)ANTHRACENE AND 2,3,7,8-TETRACHLORODIBENZO(*p*)DIOXIN MODULATE MITOGEN-STIMULATED LYMPHOCYTE PROLIFERATION

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KEY WORDS: lymphocytes; proliferation; xenobiotics; benzpyrene hydroxylase

Polycyclic aromatic hydrocarbons and polyhalogenated aromatic hydrocarbons are the xenobiotics most dangerous for human health [5]. In this investigation we studied the functional properties of human peripheral blood mononuclear cells (MC) exposed to the action of benz(a)anthracene (BA) and 2,3,7,8-tetrachlorodibenzo(*p*)dioxin (TCDD) under different conditions of incubation of lymphocytes.

EXPERIMENTAL METHOD

MC were isolated by centrifugation of heparinized blood from healthy blood donors in a Verografin–Ficoll density gradient [4]. MC harvested from the interphase were washed three times with buffered physiological saline, after which the cell suspension was transferred into RPMI-1640 culture medium. Populations of T lymphocytes were identified by methods of rosette formation with different species of test erythrocytes. The total number of T cells was determined by the method of rosette formation with sheep's erythrocytes (E-RFC [1]). The number of active (early) E-rosette-forming cells ($E\text{-}RFC_{ac}$) was determined by the method [8]. To determine the relative number of lymphocytes forming rosettes with autologous erythrocytes the method in [7] was used. A lymphocyte population enriched with T-cells was obtained by sedimentation of E-RFC in a Verografin–Ficoll gradient, followed by lysis of the erythrocytes by hypoosmolar shock. To assess the effect of xenobiotics on the subpopulation structure and functional properties of the lymphocytes, the MC were preincubated for 24 h in culture medium consisting of medium RPMI-1640, 20% of serum from group IV blood donors heated to 56°C, 2 mM glutamine, and 40 μg/ml gentamicin. The cells were incubated in the presence of 10 μM BA or 10 nM TCDD, generously provided by Dr. D. Nebert (National Institutes of Health, USA). After washing three times to remove the xenobiotics, the functional properties of the treated lymphocytes were estimated. Activity of natural killer (NK) cells was determined by their ability to lyse the target cell (K-562 [3]). To estimate the proliferative activity of MC, lymphocytes numbering 10^6 per well were stimulated with concanavalin A (con A) in a concentration of 25 μg/ml in round-bottomed planchets for immunologic research. The intensity from incorporation of ^{3}H-thymidine.

In some experiments, as regulators of lymphocyte proliferation we used MC (10^6 per well), treated for 45 min at 37°C with mitomycin C (mit C) in a concentration of 35 μg/ml. To assess benzpyrene hydroxylase activity, freshly isolated cells or cells stimulated for 72 h with phytohemagglutinin (PHA), in a concentration of 10 μg/ml, were treated with BA, as inducer of the monooxygenase system, in a concentration of 10 μA. Activity of benzpyrene hydroxylase was determined from the quantity of

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