CHEMICAL MODIFICATION OF IMMUNOGLOBULINS TO ACCELERATE THEIR CLEARANCE FROM THE BLOOD STREAM DURING RADIOIMMUNODIAGNOSIS

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A promising trend in the diagnosis of tumors in recent years is immunoscintigraphy, based on selective accumulation of polyclonal or monoclonal antibodies to tumor antigens, labeled with γ-isotopes and injected into the blood stream, in the region of the tumor. However, this method has an important drawback: only about 1% of the antibodies reaches the target tumor, and over 90% creates a background pool [5].

Several different approaches have been made to the solution of this problem, with the aim of reducing background radioactivity: the use, not of native antibodies, but of their fragments [13, 14], injection of second antibodies [1], local delivery of labeled samples [3], and consecutive injection of unlabeled antibodies and of the radioactive label [6]. In this last version, the use of an avidin-biotin system, enabling the total quantity of antibodies injected to be reduced, has proved sufficiently effective [9].

Avidin possesses nonspecific affinity for various tissues and organs, notably the liver [2]. With this in mind we suggested that consecutive injections of biotinylated samples of immunoglobulins and of their conjugates with polymers and of avidin, would lead to a fall of the level of labeled preparations circulating in the blood stream as a result of their uptake by organs of the reticuloendothelial system.

EXPERIMENTAL METHOD

1. The total immunoglobulin fraction was isolated from human serum by the standard method [10]. The fraction eluted with starting buffer (10 mM NaCl, 50 Na_2HPO_4, pH 7.5) from D-52-cellulose was used. Purity of the protein preparation was verified by SDS-polyacrylamide gel electrophoresis [12], and the resulting sample contained 90% of total protein as IgG.

2a. IgG was modified by the standard method with N-hydroxysulfosuccinimidyl-biotinamidohexanoate (OSSI-CAP-Biotin, from "Pierce"). The efficacy of modification was estimated by titration of NH_2-groups with 2,4,6-trinitrobenzenesulfonate (TNBS).

2b. The sample of native IgG was treated with the cyclic anhydride of diethylenetriaminepenta-acetic acid (ca-DTPA), dissolved in dimethyl sulfoxide (DMSO). The reaction was carried out under standard conditions [9]. The resulting preparation was further modified with OSSI-CAP-Biotin by the technique mentioned above. The number of modified protein NH_2-groups was determined by the reaction with TMBS.

2c. 5 mg of poly-L-lysine hydrobromide (PL-HBr) with molecular weight of 18 kD was dissolved in 1 ml of 0.1 M carbonate buffer, pH 8.0. 1.8 mg of OSSI-CAP-Biotin was dissolved in 200 µl of DMSO and mixed with a solution of PL-HBr. The mixture was incubated for 1 h at room temperature, after which 8 mg of ca-DTPA, dissolved in 200 µl of DMSO, was added drop by drop. Next, 50 mg of succinic anhydride ("Sigma") was gradually added, keeping the pH constant at 8.0. The sample was lyophilized.

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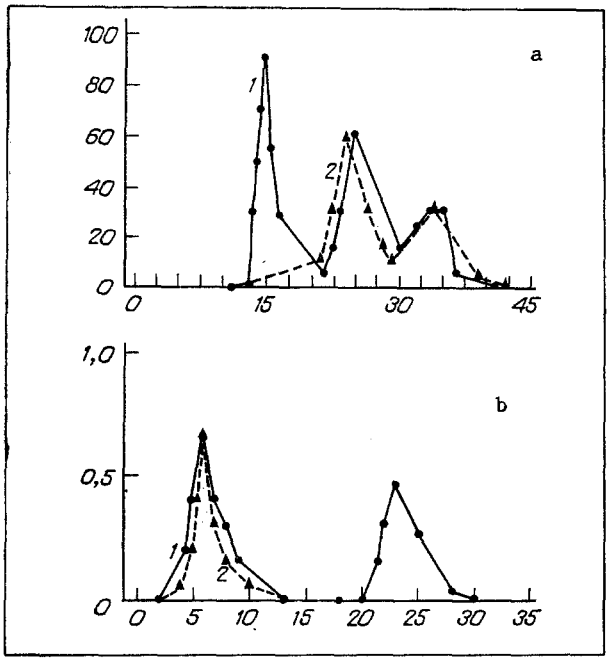

Fig. 1. Elution profile from gel-filtration column. a) Sample containing IgG and biotin-PL-DTPA; b) Sample containing biotin-PL-DTPA. Abscissa, Nos. of fractions; ordinate, radioactivity (in relative units).

TABLE 1. Changes in Ratio of Radioactivity of Organs to That of Blood after Injection of Avidin

Organ	^{125}I-IgG-biotin		^{111}In-DTPA-IgG-biotin		^{111}In-DTPA-(IgG)PL-biotin	
	without avidin	with avidin	without avidin	with avidin	without avidin	with avidin
Brain	0,03±0,01	0,1±0,02	0,04±0,01	0,08±0,00	0,17±0,06	0,14±0,09
Liver	0,19±0,01	2,4±1,05	0,82±0,10	5,53±0,20	2,42±0,14	9,85±1,22
Spleen	0,18±0,00	2,51±1,05	0,26±0,04	1,06±0,03	0,79±0,16	2,36±0,57
Blood	1,00±0,00	1,00±0,00	1,00±0,00	1,00±0,00	1,00±0,00	1,00±0,00
Kidneys	0,35±0,05	1,45±0,51	0,67±0,08	1,49±0,18	7,19±1,08	22,8±2,84
Lungs	0,42±0,21	1,38±0,60	0,25±0,05	0,65±0,26	0,58±0,08	0,74±0,10

The efficacy of binding of PL-HBr with OSSI-CAP-Biotin and with ca-DTPA was estimated by the reaction with TMBS. To 0.7 mg of biotin-PL-DTPA, dissolved in water (6 mg/ml) was added 1.14 mg of the sodium salt of N-hydroxysulfosuccinimide (OSSI, "Pierce"), the mixture was incubated for 5 min with continuous mixing, after which 0.475 mg of 1-ethyl(3'-dimethylaminopropyl)carbodi-imide (EDC, "Sigma") was added with mixing in the course of 5-10 min. The resulting solution was mixed with 2.1 mg of native IgG and incubated for 2 h at room temperature. By subsequent gel-filtration on a column with Sephadex G-100, the biotin-PL-DTPA complex bound with IgG could be separated from the unreacted complex. Fractions of the first peak containing conjugate and native IgG were selected for ion-exchange chromatography on DE-cellulose, conducted on a gradient form 150 mM NaCl, 50 mM Na_2HPO_4, pH 7.5 to 1M NaCl, 50 mM Na_2HPO_4, pH 7.5.

The biotinylated samples of IgG were labeled with ^{125}I with the aid of Iodo-Gen [4]. The specific radioactivity of the resulting sample was 0.2 μCi/mg.

Preparations of DTPA-IgG-biotin and DTPA-(IgG)PL-biotin were labeled with ^{111}In in 0.01M citrate buffer, pH 6.2. The specific radioactivity of the preparations was 0.15 μCi/μg.

The distribution of labeled samples among the organs was studied in male Wistar rats weighing 200-300 g. Preparations containing 20 μg protein in a volume of 0.5 ml of physiological saline were injected into the caudal vein. After 2 h, 0.5 ml physiological saline

was injected into the control animals and 270 μg of avidin, dissolved in 0.5 ml physiological saline, into the experimental animals. The animals were decapitated under ether anesthesia 30 min later. Blood was collected and the organ biopsied. Accumulation of radioactive label in the tissues was then determined on a "Compugamma" counter (LKB, Sweden).

EXPERIMENTAL RESULTS

Titration of NH_2-groups of IgG and PL with TNBS showed that, first, modification of IgG by OSSI-CAP-Biotin amounted to 30%, i.e., 9.6 moles of biotin to 1 mole of protein. Second, consecutive treatment of native IgG with ca-DTPA and OSSI-CAP-Biotin resulted in 20% of NH_2-groups being bound with ca-DTPA and 20% with OSSI-CAP-Biotin, i.e, 6.4 moles of biotin to 1 mole of protein. Third, after the reaction of PL-HBr with OSSI-CAP-Biotin, 30% of the NH_2-groups of poly-L-lysine were modified. There were thus 60 moles of biotin to 1 mole of the polymer. The gel-chromatogram of the sample containing IgG and biotin-PL-DTPA showed three peaks (Fig. 1a). The first peak corresponds to a compound with mol. wt. of 150-160 kV. This is the DTPA-(IgG)PL-biotin conjugate and unbound IgG. The second peak corresponds to the biotin-PL-DTPA complex, with mol. wt of about 25 kD, and the third peak corresponds to unbound ^{111}In. The ratio of radioactivity in the three peaks suggests that 40% of the polymer complex was bound with protein. The ion-exchange chromatography profile consists of two peaks of optical density, measured at a wavelength of 280 nm (Fig. 1b). Preliminary control chromatography of the native IgG indicates that the peak in the starting buffer corresponds to unbound protein, the second peak to the DTPA-(IgG)PL-biotin conjugate. The second peak contains 46% of total protein added to the reaction mixture. Consequently, there are 1.8 moles PL and, correspondingly, 109 moles of biotin to 1 mole of IgG.

The data on the biodistribution of the above-named preparation and their redistribution among the organs as a result of injection of avidin are given in Table 1. They show that the quantity of labeled protein circulating freely in the blood stream was reduced in each series of experiments after injection of avidin. This is confirmed by an increase in the organ:blood ratio. However, the character of the change in this parameter for each organ depends on the type of biotinylated sample injected. Preparations of IgG-biotin and DTPA-IgG-biotin accumulated mainly in the liver and spleen, and binding of chelating agents with the IgG-biotin complex, in the molar ratio chosen, evidently did not affect its pharmacokinetic properties. DTPA-(IgG)PL-biotin accumulates in the kidneys. This is probably due to the further addition of polylysine to the complex. The writers showed previously that this modified polymer accumulates in the kidneys. The reasons for this are not fully clear, for we know that positively charged polymers, which pass easily through the glomerular membranes, which carry a negative charge [11], have a tendency to accumulate in the kidneys. However, the modified polylysine which we used has a sufficiently high negative charge. The transport properties of the conjugate are evidently determined not only by the size of the electrostatic charge, but also, perhaps, by the presence of physiological activity of the polymer itself.

By modifying samples of IgG and, in more concrete cases, by modifying specific antibodies we can vary the pathways of elimination of unbound labeled proteins, circulating in the blood stream and giving rise to background radioactivity.

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