FUCOSE BINDING BY SERUM ALBUMIN OF CANCER PATIENTS

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The writers reported previously that blood albumin undergoes structural changes in various diseases [7-9]. This protein reacts to pathology evidently earlier than other blood proteins, possibly in connection with its transport function [9]. When various substances bind with albumin, its conformation changes [3]. This structural modification is perhaps a signal for the albumin–metabolite complex to be removed from the blood stream [8].

The nature of these structural changes is not quite clear, but one cause of the modification may be acylation or glucosylation, processes which readily take place with proteins. In pathology both quantitative and qualitative changes in the bonding of ligands are possible. For example, pure albumin contains no carbohydrates, but in pathology carbohydrates appear in its composition. For instance, the binding of glucose with albumin has been reported in diabetes [12]. We have found an increase in the content of albumin-bound carbohydrates in various cancer patients [2].

It has recently been found that large quantities of an unusual enzyme, fucose transferase, which transfer fucose from an unknown donor [11], appear in the blood stream of patients with breast cancer.

It was accordingly decided to study the fucose content in albumin circulating in the blood of cancer patients.

EXPERIMENTAL METHOD

Blood of patients and healthy subjects was obtained from clinics of the Crimean Medical Institute (the diagnoses were supplied by the physicians in charge). Preparations of serum albumin were obtained by preparative electrophoresis in polyacrylamide gel [1]. The purity of the preparations was verified by analytical electrophoresis [6], by an immunochemical method with polyspecific serum against human blood proteins [5], by N-terminal analysis [10], and by determination of molecular weight [16].

Fucose was determined quantitatively by the method in [13] in the presence of a 3% solution of cysteine hydrochloride. Optical density was measured at 396 and 427 nm on the Specord UV-VIS spectrophotometer.

To analyze carbohydrate components of the serum albumin, these components were subjected to gas–liquid chromatography in the form of their trimethylsilyl derivatives [14].

In the control a group of eight blood donors was tested. Preparations of albumin isolated from the blood of 21 patients with malignant neoplasms of various situations and albumin from 10 patients with other diseases were studied in parallel series.

EXPERIMENTAL RESULTS

The results of quantitative analysis of carbohydrate components in serum albumin by gas–liquid chromatography are given in Table 1. They show that the serum albumin of patients with malignant tumors contained a considerable quantity of carbohydrate components, far more than the donors' albumin. The possibility of complex formation with α-globulins and other plasma glycoproteins which are carriers of carbohydrates was ruled out not only by the results of immunologic analysis, but also by the unchanged molecular weight and the N-terminal amino acid. The writers showed previously that the N-terminal amino acid in albumins from cancer patients undergoes modification of the glucosylation type [4]. This modification is reversible and disappears after treatment of the protein with formic acid.

It is a particularly interesting fact that no fucose was present in the serum albumin of the healthy subjects, although it was present in protein from the cancer patients. This was the reason why the investigation of fucose in albumin preparations from cancer patients was quantitative. In these studies the albumin preparations were divided into three groups: 1) albumins from patients with malignant liver tumors; 2) albumins from patients with malignant tumors of other organs (rectum, pancreas, stomach, breast); 3) albumins from patients with noncancerous diseases (cirrhosis of the liver, bronchitis,

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TABLE 1. Results of Quantitative Analysis of Carbohydrate Components in Human Serum Albumin (M ± m)

Albumin preparation	Carbohydrate component, nmoles/nmole albumin				
	fucose	mannose	galactose	glucosamine	neuraminic acid
From healthy subjects	Traces	2,4±0,8	2,4±0,5	1,8±0,5	3,4±0,4
From patients with malignant liver tumors	0,7±0,09	6,9±0,5	5,2±0,6	8,4±1,0	4,0±0,9
Patients with malignant tumors elsewhere	0,3±0,04	4,6±0,4	2,7±0,5	4,5±0,4	3,9±0,6

TABLE 2. Quantitative Analysis of Fucose in the Composition of Albumin

Group of albumin preparations	No. of preparations	Fucose, nmoles/nmole albumin
1	5	0,340±0,060
2	16	0,120±0,040
3	10	0,008±0,002
Control (healthy human albumin)	8	0,013±0,005

kidney diseases, tonsillitis). The content of fucose in the albumin of the patients of these three groups was compared with the control – albumin from healthy subjects. The results are given in Table 2.

Table 2 shows that healthy human albumin contains only traces of fucose whereas albumin from patients with cancer contains considerable quantities of it. The fucose content in albumin from paitents with other diseases was virtually identical with the control. The data in Tables 1 and 2 show that albumin isolated from blood serum by electrophoresis contains a certain quantity of monosaccharides, but practically no fucose. If a developed tumor is present fucose appears, although in normal subjects and patients with other diseases it is present only in traces. The high fucose content in liver cancer will be noted.

Thus although the appearance of albumin with conformational changes in pathology was described by the writers previously as a nonspecific reaction, the character of bonding of the ligand in this case may prove to be specific. Interest in this phenomenon is associated not only with the actual appearance of fucose transferase, but also with the role of fucose in processes of transformation and growth [15].

It is suggested that determination of fucosyl transferase activity may facilitate the diagnosis and assessment of the results of chemotherapy and radiotherapy [11].

Determination of fucuse in the composition of albumin is possibly an easier test than determination of fucosyl transferase activity. The results are evidence that the fucose content is higher in liver cancer, whereas in breast cancer, in which increased fucosyl transferase activity was found, it is much lower (group 2).

Further investigations of fucosylated blood proteins in cancer patients may be important not only for specific diagnosis, but also for the study of the pathogenesis of these diseases.

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ANTITUMOR AND TOXIC PROPERTIES OF LIPOSOMES CONTAINING cis-DICHLORODIAMINOPLATINUM

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Several complex compounds of platinum, including cis-dichlorodiaminoplatinum (CDP), possess marked antitumor activity, probably due to their interaction with DNA [3, 4, 9]. However, the practical application of platinum complexes is made difficult by their high toxicity; the principal factor limiting the use of effective doses of the compound is a disturbance of renal function [9]. It was shown recently [11] that the distribution of therapeutic substances in the organs and tissues of a recipient can be modified by incorporating them into phospholipid vesicles or liposomes.

It was decided to study how the antitumor and toxic properties of platinum complexes were modified as a result of their incorporation into liposomes. For this purpose liposomes of different phospholipid composition, containing CDP, were obtained and their action tested on mice with a solid Crocker's sarcoma.

EXPERIMENTAL METHOD

Phosphatidylcholine isolated from egg yolk by the method [7], phosphatidylserine from bovine brain [2], lysophosphatidylcholine obtained from egg phosphatidylcholine with the aid of phospholipase A_2 [8], and cholesterol recrystallized from absolute ethanol were used. The purity of the phospholipids was verified immediately before the experiment by thin-layer chromatography on silica-gel in a chloroform–methanol–water mixture (65:25:4) [1]. Lipid phosphorus was determined spectrometrically [10].

Liposomes were obtained from pure phosphatidylcholine and from mixture of phosphatidylcholine with phosphatidylserine (85:15), phosphatidylcholine with lysophosphatidylcholine (93:7), or phosphatidylcholine with cholesterol (93:7); all ratios are molar.

To prepare liposomes a solution of phospholipids in an organic solvent (benzene or chloroform–methanol in the ratio of 2:1) was evaporated to dryness on a rotary vaporizer. The lipid film was dispersed in physiological saline containing a saturated solution of CDP. The dispersion was sonicated for 1 min by means of the UZDN-1 generator (output frequency 22 kHz). The resulting solution was centrifuged at 500g for 10 min to remove solid particles and subjected to exhaustive dialysis against physiological saline to remove any CDP not taken up. The total concentration of lipids in the dispersion was 10%. The resulting CDP liposomes contained 10 μg platinum/mg lipid phosphorus.

Crocker's sarcoma (S-180) was transplanted subcutaneously into noninbred albino mice weighing 18-20 g. The CDP preparations were injected intravenously as a single dose into the caudal vein of the mice 48 h after transplantation of the tumor. The animals were killed by decapitation on the 4th or 12th days after injection of the preparation. The percentage inhibition of tumor growth, determined relative to the mean weight of the tumors, was used as criterion of antitumor activity of the preparations; their toxic action was assessed by the change in body weight of the animals, changes in the spleen, and the total peripheral blood leukocyte count.

To study the distribution of CDP in the experimental animals, tissues (spleen, liver, kidney, muscle) were removed and dried and ground into a fine powder. Platinum was determined by Bankovskii's method.

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