ELEVATED ACTIVITIES OF α-2- AND α-3-FUCOSYLTRANSFERASES IN HUMAN SERUM AS A NEW INDICATOR OF MALIGNANCY

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Received March 30,1977

SUMMARY

The activity of two different fucosyltransferases have been determined in human serum using either endogenous or exogenous acceptors. The results demonstrate that especially α-3-fucosyltransferase may be of value to facilitate the diagnosis of neoplasia and to control the success of chemotherapy or x-ray treatment. About 85 % of all serum samples exhibited elevated transferase levels. Cases of infectious hepatitis can be differentiated from cancer because of the extremely high activity towards endogenous acceptors and the low level of α-3-fucosyltransferase.

Glycosyltransferases have gained increasing interest during the last years (1-6) and at least two fucosyltransferases have been found in human serum. One enzyme transfers L-fucose from GDP-L-fucose to the terminal galactose residues of oligosaccharides or desialylated glycoproteins by forming (1→2) linkages (7,8). The other enzyme, the α-3-fucosyltransferase, catalyses the addition of L-fucose to the C-3 position of terminal

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This work was supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, Germany.

Abbreviations used: Control, healthy donor; Br.Ca., breast carcinoma; M., metastases; Ter., teratoma; CLL, chronic lymphocytic leukemia; CML, chronic myelocytic leukemia; Plas., plasmocytoma; LGR, lymphogranulomatosis; Ch., chemotherapy (or x-ray treatment); LD Inf., lymphoproliferative disease with infiltration of liver and lung; Oth.T., other tumors; Hep., infectious hepatitis.

ISSN 0006-291X

N-acetyl-D-glucosamine or D-glucose (7,8). Investigations of Pacuszka & Kóscielak (9) indicate that serum α-2- and α-3-fucosyltransferases are also active with glycolipid acceptors.

Previous studies in our laboratory have shown that in rapidly growing Morris hepatomas the GDP-fucose : glycoprotein α-2-fucosyltransferase activity was elevated two-to threefold (10). Therefore the present study was undertaken to determine serum fucosyltransferase levels of healthy donors and patients suffering from cancer. These comparative investigations may be of value to facilitate the diagnosis of neoplasia.

MATERIALS AND METHODS

GDP-L-[U-^{14}C]fucose was obtained from the Radiochemical Centre (Amersham, Bucks., U.K.). Fetuin from fetal calf serum was supplied by Sigma Chemical Co. (St. Louis, Mo., U.S.A.). All other chemicals of analytical grade were purchased from E. Merck AG (Darmstadt, Germany). Blood samples, withdrawn from the vena intermedia, were centrifuged for 5 min at 4 000 x g. The resultant serum fraction was allowed to stand for 4 h and was than used for the measurement of enzyme activity. The GDP-fucose : glycoprotein fucosyltransferase was determined essentially as described by Munro and Schachter (8) by measuring the incorporation of L-[U-^{14}C]fucose from GDP-L-[U-^{14}C]fucose into desialylated fetuin. Fetuin from which both N-acetylneuraminic acid and galactose had been removed according to the method of Spiro (11) was generally used as second acceptor. The total volume of the incubation mixture (pH 5.5) was 115 µl, containing MES (4 µmol), $MgCl_2$ (2 µmol), sodium azide (0.4 µmol), GDP-L-[U-^{14}C]fucose (0.5 nmol, acceptor (0.5 mg) and 30 µl of serum. Control incubation mixtures contained no fetuin. Incubation was carried out for 21 h at 37°C and protein-bound radioactivity was determined as described previously (6,10).

RESULTS

Red blood cells contain glycosyltransferases (12,13) which may be released into the serum, if the whole blood is allowed to stand for a longer period of time. Therefore fucosyltransferase activities have been determined at various times between the collection and centrifugation of blood samples. Ten min after blood drawing

Table 1. INCORPORATION OF [^{14}C]FUCOSE INTO ENDOGENOUS ACCEPTOR OF HUMAN SERUM

	n	10^{-3} x radioactivity (dpm/ml serum)
healthy donor	6	70 ± 15
breast carcinoma	9	69 ± 12
breast carcinoma with metastases	10	94 ± 45
chronic lymphocytic (or myelocytic) leukemia	5	102 ± 35
plasmocytoma	7	92 ± 38
lymphogranulomatosis	6	131 ± 29
lymphoproliferative disease with infiltration of liver & lung	1	190
other tumors	12	100 ± 46
infectious hepatitis	3	687 ± 76

Values given are the mean ± S.D. of duplicate determinations from 3 to 10 different serum samples; n = number of serum samples.

the total activity of α-2-fucosyltransferase towards endogenous and exogenous acceptor increased by 30 %, reaching a maximum at about 150 min (60 %) and gradually declining thereafter. Contrary to this finding the pattern of total α-3-fucosyltransferase activity showed only minor alterations. When calculating enzyme activity towards the exogenous acceptor alone (desialo- or desialo-degalactofetuin) both transferases reach constant activities within 1 h after blood drawing. It is worth mentioning that all serum samples contained a high level of endogenous acceptor (Table 1). Taken

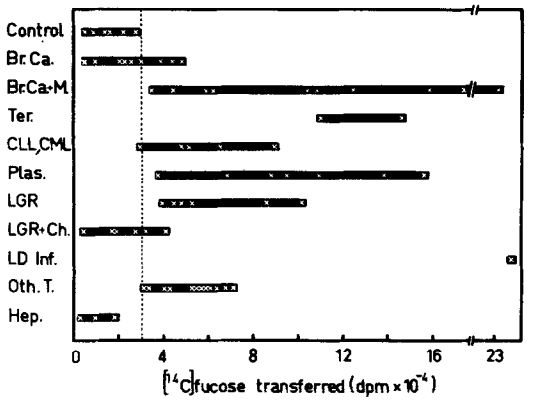

Figure 1: Serum levels of α-3-fucosyltransferase. Assays were carried out as described in Materials and Methods. Each cross represents the mean value of duplicate determinations using the exogenous acceptor desialo-degalacto-fetuin

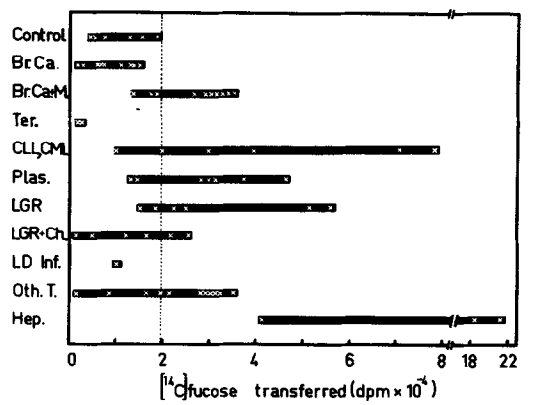

Figure 2: Serum levels of α-2-fucosyltransferase activity towards the exogenous acceptor desialo-fetuin.

together the fucosyltransferase activity towards the endogenous acceptor was more or less in the same range, except for certain types of neoplasia. However, sera from patients with infectious hepatitis exhibited a most striking increase in endogenous activity, a finding which will be discussed below.

As shown in Fig. 1 the activity of α-3-fucosyl-

transferase is substantially higher in most of the serum samples investigated. Especially high levels of activity were measured in the serum of those patients suffering from a highly malignant or metastatic tumor. However, even more conspicious is the finding that both transferases do not show uniform behaviour. Serum samples from patients with teratoma or lymphoproliferative disease exhibit a 8 to 10fold higher level of α-3-fucosyl-transferase when compared with healthy donors, whereas the activity of α-2-fucosyltransferase is within normal range (Fig. 2). In general lowest values were found in breast carcinoma without detectable metastases. Finally it should be pointed out that x-ray treatment or chemotherapy reduces considerably serum transferase levels, but insufficient data have been collected to allow any generalization at this point.

DISCUSSION

Results clearly demonstrate that during tumor progression and the development of metastases serum fucosyltransferase activities increase substantially. The origin of this enzymatic activity is still unclear, but studies on plasma sialyltransferase suggest that this enzyme is either secreted by neoplastic cells or is released by the degradation of cells (14,15). However, it cannot be excluded that at least in part liver (16,17) and even the blood cells (12) are the source for glycosyltransferases. We assume that a significant increase of serum glycosyltransferases is intimately connected with rapid proliferation and secretory processes of neoplastic (or normal) tissue. This assumption is supported by the observation of elevated rat serum sialyltransferase activity after partial hepatectomy (18). Within 36 h after operation the specific activity of the liver enzyme increased by 200 % and it is quite remarkable that this elevation was accompanied by a nearly twofold higher level of serum sialyltransferase. Therefore the elevated activity of glycosyltransferases in the serum should be

mainly due to an increased synthesis and/or secretion; the release of glycosyltransferases from dying cells is probably of minor importance. While these studies were completed an abstract appeared, describing measurements of fucosyltransferases in plasma and tissue of cancer patients (19). The authors found that α_1-acid glycoprotein is responsible for the high level of endogenous acceptor. It is well known that the concentration of this glycoprotein increases in the blood during inflammatory and neoplastic processes. Consequently an α_1-acid glycoprotein with free acceptor sites for N-acetylneuraminic acid may also serve as a suitable substrate for fucosyltransferases. However, it should be pointed out that further acceptors are expected to be present in human serum, because in most types of infectious hepatitis the level of α_1-acid glycoprotein does not increase, but decreases.

From the present data it can be concluded that there exists a correlation between serum fucosyltransferase activities, malignancy and the occurrence of metastases. This finding might be of value for the diagnosis of cancer and the control of chemotherapy and x-ray treatment. Cases of infectious hepatitis can be differentiated from neoplasia because of the extremely high endogenous acceptor activity and the low level of α-3-fucosyltransferase.

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