THE HEPATIC CLEARANCE OF CIRCULATING NATIVE AND ASIALO CARCINOEMBRYONIC ANTIGEN BY THE RAT

Peter Thomas*
Chester Beatty Research Institute, Institute of Cancer Research:
Royal Cancer Hospital, Fulham Road, London SW3 6JB, U.K.

and

Douglas A. Hems
Department of Biochemistry, Imperial College of Science and
Technology, London SW7 2AZ, U.K.

Received October 10,1975

SUMMARY

Native human carcinoembryonic antigen has a circulating half life of less than 5 min when injected intravenously into rats. The intact rat accumulated carcinoembryonic antigen almost exclusively in the liver. Trace amounts were found in spleen and lung. The half life of the native glycoprotein in a rat liver perfusion system was 28 min while that of the asialo glycoprotein was 11 min. In both cases the time course for removal of glycoprotein from the perfusate was first order. After perfusion (90 min), about 10% of the glycoprotein was found in the bile. The rapid uptake of the native glycoprotein suggests that the recognition of terminal galactose may not be the only factor involved in determining the hepatic assimilation of glycoproteins.

The c learance of glycoproteins from the circulation of rats and rabbits following intravenous injection has been well documented (1). In general asialo glycoproteins are removed from the circulation by the liver at a very much faster rate than are the corresponding native glycoproteins (1). The major factors determining this rapid uptake of asialo glycoproteins appear to be the exposure of terminal galactose residues on removal of sialic acid and the presence of specific galactose receptors on the surface of the liver parenchymal cell (1).

Carcinoembryonic antigen (CEA)+ is a glycoprotein of mol. wt. approx. 200000 present in human colonic carcinomas and foetal digestive tract (2,3). The glycoprotein contains approx. 60% of carbohydrate which is made up of galactose, mannose, N-acetyl glucosamine (2-acetamido-2-deoxy-D-glucose), fucose and sialic acid units (4). Recent work has shown that the monitoring of plasma CEA levels is useful in relation to the early detection of recurrent or metastatic colorectal carcinomas (5).

* Alexander Keiller Fellow
+ CEA = Carcinoembryonic antigen

Shuster et al, (6) have investigated the fate of circulating CEA in the rabbit and dog, while Primus et al. (7) have studied the metabolism in hamsters bearing the CEA producing GW-39 tumours. Both groups of workers used tracer amounts of iodine radiolabelled glycoprotein. We now report on the fate of larger concentrations of circulating native and asialo-CEA, in the intact rat and the perfused rat liver, in experiments where the changes in immunologically active glycoprotein were followed with a radioimmunoassay (rather than with iodine-labelled material).

MATERIALS AND METHODS

CEA was isolated from perchloric acid extracts of human colonic carcinoma metastatic to the liver (8) and assayed using a double antibody radioimmunoassay (9). Asialo-CEA was prepared by treatment with neuraminidase (vibrio cholerae) (4). Terminal galactose residues in both native and asialo glycoproteins were determined using the galactose oxidase, sodium borotritiide method (4,10).

Livers of female Sprague-Dawley rats (wt. approx. 200 gm) were perfused as described by Hems et al. (11), except that diethyl ether was the anaesthetic. The glucose content of the perfusion medium was about 10 mM. Bile was collected by cannulation of the duodenum. Livers were perfused for approx. 30 min before the addition of the CEA, dissolved in 1 ml of bicarbonate-saline. Samples (1 ml) of perfusion fluid were removed at time intervals for analysis, centrifuged to remove red cells and stored at -20°. The CEA content was later determined by radioimmunoassay (9).

For the in vivo experiments female Sprague-Dawley rats (200 gm) under nembutal anaesthesia were injected with a solution of CEA (90 μg) in isotonic saline (0.2 ml) via the tail vein. Samples (0.2 ml) of blood were taken from the tail vein at intervals, centrifuged and the CEA content of the plasma determined. The animals were killed after 15 min and the organs were removed and stored at -30°C. Subsequently the organs were thawed, homogenised with 2 vols of water and extracted with an equal volume of 2M perchloric acid. Precipitated material was removed by centrifugation and the supernatant dialysed against water to neutral pH. The non dialysable material was freeze dried, the residue dissolved in isotonic saline and its CEA content determined by radioimmunoassay. At the end of the perfusion experiments, the livers were treated in an identical manner.

RESULTS AND DISCUSSION

The clearance rates of native and asialo-CEA by the rat liver perfusion system are shown in Fig. 1. Secondary plots of log concentration against time showed that the rate of clearance for each glycoprotein was first order. The native glycoprotein in these conditions was cleared more slowly (half time 28 min) than the asialo glycoprotein (half time 11 min). However, this rate of clearance for a native glycoprotein is unusually rapid. For example, in perfusion experiments to determine the clearance rate of erythropoietin, Briggs et al. (12) showed a rapid clearance of the asialo-glycoprotein while the native glycoprotein remained in the perfusion medium for several hours. Control experiments showed that this rapid uptake of native CEA was

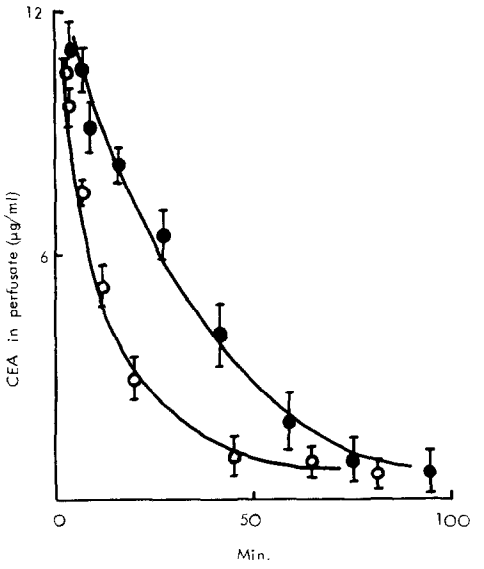

Fig. 1. Disappearance of CEA in the perfused liver. Livers were perfused with (initially) about 11 µg/ml of CEA; native (●) or asialo- (o). The average liver weight was 7.8 gm. and perfusion volume 65 ml. Details are in the text. Results (± S.E.M.)are each from three perfusions.

due to the liver (rather than a nonspecific binding to the apparatus). As it is thought that the rate of clearance of glycoproteins is determined by an hepatic receptor recognising terminal galactose residues it seemed possible that the rapid clearance of native carcinoembryonic antigen could be explained if exposed galactose residues were present on the molecule. Regoeczi et al. (13) have shown that native α_1 acid glycoprotein is rapidly removed from the circulation of the rabbit, but estimated that the molecule contained 2.6 terminal galactosyl groups while human α_1 acid glycoprotein contained 0.97 galactosyl groups per molecule and was eliminated from the circulation of the rabbit at a slow rate. However the native CEA used in these studies contained only 0.5 ($\pm$ 0.3) exposed galactose units per molecule whereas the corresponding number for asialo CEA is 15 ($\pm$ 1.2). Thus the rapid uptake of native CEA, which was even more marked in the in vivo experiments cannot be explained on the basis of the peripheral galactose units. When native CEA was injected via the tail vein into rats 70% of the dose had been cleared from the circulation after 7 min and after 15 min CEA could no longer be detected by radioimmunoassay. Thus in vivo the half life of native CEA in rats is less than 5 min. These results provide the first reported

Organ	CEA Content (μg)	Percent Recovery
Liver	68 (63-71)	76
Kidney	0	-
Spleen	2 (0.5-3)	2
Gut	trace	-
Lung	3 (2-5)	3
Heart	0	-
Skin	0	-

Table 1

Recovery of immunologically identifiable CEA from the organs of the rat 15 min after injection of 90 μg of CEA via the tail vein. Results are expressed as the mean values from 4 experiments; ranges are given in parenthesis. Experimental details are described in the text.

instance of rapid removal by the liver of a circulating glycoprotein high in carbohydrate but low in terminal galactose.

Recoveries of CEA from the organs of the rat are shown in Table I, and are in general agreement with the results obtained in the dog, rabbit and guinea pig (6,7) in that the liver was the major site of its accumulation and catabolism, though the recoveries reported here are higher than those obtained previously (6,7). This may reflect our use of native CEA at higher concentrations, equivalent to those found in patients with advanced metastatic disease rather than radiolabelled material in tracer amounts. Use of radioiodine labelled proteins can for example present problems from self decomposition. Thus it has been shown that up to 20% of the label in CEA can be due to low molecular weight contamination after only relatively short periods of storage (unpublished results).

Bile collected during the perfusion experiments (approx. 1.5 ml in 2 h) contained large amounts (6-10% of the dose) of immunologically active CEA, and would suggest that CEA can both enter and leave the liver without major alteration. The integrity of the protein component and the six disulphide bridges must be preserved for retention of immunoactivity (4, 14) though at least some of the external monosaccharide units may be removed without effect (15, 16). The fate of these units in the liver remains to be clarified.

The removal of circulating CEA by the liver into the bile is of interest in that high concentrations (up to 10 μg/ml) have been found in the bile of patients with various types of cancer (unpublished results) and may also explain at least in part the presence of CEA in faeces (17), and the increase of plasma CEA levels in biliary tract obstruction (18).

This investigation has been supported in part by Grant (G973/785/K) from the Medical Research Council. Professors A.B.Foster, A.M.Neville and Dr J.H. Westwood are thanked for their interest and Dr C.Turberville for gifts of CEA.

REFERENCES

1. Ashwell, G., and Morell, A.G. (1974) Advances Enzymol., 41, 99-128.
2. Gold, P., and Freedman, S.O. (1965) J.Exp. Med., 121, 439-462.
3. Gold, P., and Freedman, S.O. (1965) J.Exp. Med., 122, 467-481.
4. Westwood, J.H., Bessell, E.M., Bukhari, M.A., Thomas, P., and Walker, J.M. (1974) Immunochemistry, 11, 811-818.
5. Mackay, A.M., Patel, S., Carter, S., Stevens, U., Laurence, D.J.R., Cooper, E.H., and Neville, A.M. (1974) Br. med. J., 4, 382-385.
6. Shuster, J., Silverman, M., and Gold, P. (1973) Cancer Res., 33, 65-68.
7. Primus, F.J., Hansen, H.J., and Golden, D.M. (1974) Nature, 249, 837-838.
8. Krupey, J., Wilson, R., Freedman, S.O., and Gold, P. (1972) Immunochemistry, 9, 617-622.
9. Laurence, D.J.R., Stevens, U., Bettelheim, R., Darcy, D., Leese, C., Turberville, C., Alexander, P., Johns, E.W., and Neville, A.M. (1972) Br. med. J., 3, 605-609.
10. Morell, A.G., Van den Hamer, C.J.A., Scheinberg, I.H., and Ashwell, G. (1966) J.Biol.Chem., 241, 3745-3749.
11. Hems, D.A., Whitton, P.D., and Taylor, E.A. (1972) Biochem. J., 129, 529-538.
12. Briggs, D.W., Fisher, J.W., and George, W.J. (1974) Am. J. Physiol., 227, 1385-1388.
13. Regoeczi, E., Hatton, M.W.C., and Charlwood, P.A. (1975) Nature, 254, 699-701.
14. Thomas, P., Westwood, J.H., and Foster, A.B. (1974) Biochem. Soc. Trans. 2, 1248-1249.
15. Westwood, J.H., Thomas, P., and Foster, A.B. (1974) Biochem. Soc. Trans. 2, 1250-1251.
16. Hammerstrom, S., Engvall, E., Johansson, B.G., Svensson, S., Sundblad, G., and Goldstein, I.J. (1975) Proc. Nat. Acad.Sci.USA., 72, 1528-1532.
17. Freed, D.L.J., and Taylor, G. (1972) Br. med. J., 1, 85-87.
18. Lurie, B.B., Lowenstein, M.S., and Zamcheck, N.D. (1975) J.A.M.A., 233, 326-330.