Metabolism of Human Carcinoembryonic Antigen in Xenogeneic Animals

J. Shuster, M. Silverman, and P. Gold

Division of Clinical Immunology and Allergy, and McGill University Medical Clinic of the Montreal General Hospital, Montreal, Quebec, Canada

SUMMARY

The metabolism of radiolabeled carcinoembryonic antigen (CEA) has been studied in rabbits and dogs. About 70% of injected CEA disappears from the circulation of these animals in 1 hr. The liver is the primary site of accumulation of the material. Sequential injection of the CEA into rabbits and dogs suggests that, by the metabolic parameters studied, the CEA is biologically heterogeneous, even in preparations that appear physicochemically and immunochemically homogeneous.

INTRODUCTION

The CEA of the human digestive system is a glycoprotein that has been isolated from adenocarcinomas of the entodermally derived digestive system epithelium (2, 3). By radioimmunoassay methods (10), the CEA has been demonstrated in the circulation of the majority of patients with colorectal cancer. However, the level of circulating CEA that has been isolated from adenocarcinomas of the entodermally derived digestive system epithelium (2, 3). By radioimmunoassay methods (10), the CEA has been demonstrated in the circulation of the majority of patients with colorectal cancer. However, the level of circulating CEA that has been isolated from adenocarcinomas of the entodermally derived digestive system epithelium (2, 3). By radioimmunoassay methods (10), the CEA has been demonstrated in the circulation of the majority of patients with colorectal cancer.

Recently, the metabolism of a number of radiolabeled serum glycoproteins has been studied in xenogeneic species (7, 9). These investigations revealed that most serum glycoproteins are rapidly removed from the circulation by the liver and that the rate of removal is closely related to the sialic acid content of the molecule under consideration. The purpose of this series of experiments was to study the metabolism of CEA in xenogeneic animals before similar studies are considered in humans.

MATERIALS AND METHODS

Fasting mongrel dogs were anesthetized with sodium pentobarbital (25 mg/kg), and catheters were inserted into the femoral vein (injection port) and femoral artery (sampling port). Both ureters and the bile duct were cannulated through an abdominal incision which was subsequently resutured. Solutions of 10% mannitol and 2% sodium taurocholate were administered i.v. to increase urinary output and bile flow, respectively.

A single preparation of purified CEA (6), labeled with $^{125}$I or $^{131}$I by the chloramine-T method (4), was used throughout the experiments. Dogs were given injections of 10 to 300 X $10^6$ cpm of the radiolabeled CEA, at a specific activity of 10 to 25 $\mu$Ci/µg, diluted in 20 ml autologous plasma. Timed serial samples of blood, urine, and bile were collected, and the radioactivity was measured in a Nuclear-Chicago $\gamma$ radiation spectrometer. Several samples of urine and bile, collected at specific intervals during the experiments, were chromatographed on calibrated Sephadex G-200 columns and assayed for their capacity to bind to goat anti-CEA antibody by the ammonium sulfate coprecipitation technique (1). In a number of experiments, the liver, kidneys, lungs, spleen, heart, thyroid, and intestines of the dogs were removed, weighed, and analyzed for accumulated radioactivity.

Similar investigations were performed in rabbits in which the plasma disappearance of similar quantities of radiolabeled CEA was studied for 1 hr. In addition, a number of studies were carried out in which the metabolism of the same CEA preparation was examined sequentially in rabbits and dogs. The experimental protocols used are shown in Chart 1.

The CEA used in these experiments was shown to be free of aggregates and homogeneous by chromatography on a calibrated Sephadex G-200 column (Chart 2). None of the animals used in these experiments demonstrated anti-CEA activity in their serum.

RESULTS

Plasma Disappearance of CEA in Dogs. A typical pattern of the disappearance of aggregate-free radiolabeled CEA from the plasma of dogs is shown in Chart 3. Within 5 min of its injection, 50% of the CEA was removed from the blood. The plasma disappearance of the radiolabeled material then continued at a slower rate for the next 25 min, at which time about 30% of the injected sample remained in the circulation. Thereafter, CEA disappeared very slowly from the blood so that, at the end of 1 hr, about 25% of the injected CEA was still in the circulation.

Similar plasma disappearance curves were obtained in a number of experiments in which dogs, given injections of different preparations of radiolabeled CEA, were studied for periods of up to 4 hr. In all of the experiments, from 40 to 50% of the CEA was removed from the blood within 5 min of

Received July 28, 1972; accepted October 4, 1972.
J. Shuster, M. Silverman, and P. Gold

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Injection Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CEA-^{125}I → Dog</td>
</tr>
<tr>
<td></td>
<td>CEA-^{131}I</td>
</tr>
<tr>
<td>2</td>
<td>CEA-^{125}I → Rabbit</td>
</tr>
<tr>
<td></td>
<td>CEA-^{131}I</td>
</tr>
</tbody>
</table>

Chart 1. Experimental protocol for the study of the metabolism of the iodine-radiolabeled CEA in dogs and rabbits.

In order further to examine the question of whether the plasma disappearance curves for the CEA reflected heterogeneity within the molecular population, we studied the sequential metabolism of CEA in 2 different animals by Experimental Protocol 2 (Chart 1). The sequence of injection from rabbit to dog was chosen for 2 reasons. First, this permitted the examination of a much larger fraction of the material, residual in the 1st animal at the end of 1 hr, without overloading the intravascular volume of the 2nd recipient. Second, the number of cpm of iodine transferred and the volumes of blood that could be taken from the dog allowed for more accurate determination of the disappearance of radioactivity from the circulation of the 2nd animal. It was on injection, and only 20 to 40% of the CEA remained in the circulation after 1 hr. In dogs studied for up to 170 hr after injection of CEA, about 1% of the material could still be detected in the blood at the end of 72 hr (Chart 3).

In order to determine whether the pattern of disappearance of CEA from plasma was associated with a saturation phenomenon, we performed a series of experiments in which dogs were given injections first of CEA-^{125}I and 1 hr later of a comparable quantity of CEA-^{131}I. The rates of removal of the 2 preparations were virtually identical.

Organ Accumulation of Radioactive Material in Dogs. Parallel to the rapid disappearance of CEA from the circulation was the equally rapid accumulation of the radiolabel in the liver. At the end of 1 hr, 55% of the injected radioactivity was found in the liver. In this interval, however, only a trace of radioactivity appeared in the bile, and less than 1% of the injected material was detected in each of the kidneys, lungs, spleen, or heart. There was a negligible accumulation of radioactivity in the thyroid and in the small or large bowel.

Plasma Disappearance of CEA in Rabbits. Studies in rabbits, similar to those described above for dogs, revealed that at the end of 1 hr there was a mean disappearance of 72% of either CEA-^{125}I or CEA-^{131}I from the blood. These values are identical to those obtained in dogs. In rabbits as in dogs, 50% of the injected material was sequestered in the liver 1 hr after the injection of the radiolabeled CEA.

In order further to examine the question of whether the plasma disappearance curves for the CEA reflected heterogeneity within the molecular population, we studied the sequential metabolism of CEA in 2 different animals by Experimental Protocol 2 (Chart 1). The sequence of injection from rabbit to dog was chosen for 2 reasons. First, this permitted the examination of a much larger fraction of the material, residual in the 1st animal at the end of 1 hr, without overloading the intravascular volume of the 2nd recipient. Second, the number of cpm of iodine transferred and the volumes of blood that could be taken from the dog allowed for more accurate determination of the disappearance of radioactivity from the circulation of the 2nd animal. It was on
the basis of these considerations that the interspecies transfer design was chosen.

When the radiolabeled CEA was administered to rabbits and the material remaining in the circulation of the rabbit after 1 hr was then administered to dogs, the early rapid phase of CEA disappearance was not observed. Instead, the rate of removal of the radiolabel from the plasma during the 1st 5 min in the dog was approximately one-third of that usually attained (Chart 4).

Properties of Circulating Radiolabeled Material in Dogs. The chromatographic characteristics, on calibrated Sephadex G-200 columns, of the radioactive material obtained from dog plasma between 1 min and 24 hr after CEA injection were identical to those of the preinjection radiolabeled CEA (Chart 2). For the 1st 24 hr, the circulating CEA displayed binding characteristics to goat anti-CEA antibodies that were quantitatively identical to those demonstrated by the purified preinjection material. In the zone of antibody excess, 70 to 75% of the preinjection sample and circulating radiolabeled material was specifically bound to anti-CEA antibody. Thus, the data suggest that at least 98% of the injected CEA was immunoreactive or immunochemically homogeneous.

Urinary and Biliary Excretion of Radiolabeled Material in Dogs. At the end of 1 hr, urinary excretion accounted for not more than 0.5% of the injected radioactive material. Sephadex G-200 chromatography revealed that the urinary constituents consisted exclusively of degradation products of CEA. With the exception of the urine specimens collected during the 1st 10 min after injection of the radiolabeled CEA, the excreted radioactive material consisted of low-molecular-weight polypeptides, eluted at the total column volume of Sephadex G-200 (Chart 5). On the other hand, the radioactive components of the urine, collected during the 1st 10 min of the experiments, displayed a polydisperse elution profile in which about 30% of the material had a molecular weight in the range of 20,000 to 50,000 (Chart 5). This initial urine sample contained a small amount of immunoreactive material, as demonstrated by binding to goat anti-CEA antibodies.

The small quantity of radioactive products that appeared in the bile during the 1st hr was eluted at the column volume and showed no immunoreactivity.

DISCUSSION

The results obtained in both the dog and rabbit experiments suggest that human CEA in these species is rapidly removed from the circulation by the liver. The plasmas of the animals used in this investigation did not contain antibodies or any other protein constituent that could bind to native CEA. Thus, despite the fact that xenogeneic animals were involved, the time course of the plasma disappearance of CEA was such that immune elimination could not explain the observed phenomena. Furthermore, if the lung and the spleen are considered to be representative areas of reticuloendothelial activity, then the relatively minute uptake of CEA by these organs constitutes strong evidence against either immune elimination or reticuloendothelial uptake as a basis for the accumulation of CEA in the liver. The most probable explanation for the findings is that the hepatocyte is the site of accumulation of the radiolabeled material (7).

These observations on the metabolism of CEA are very similar to those obtained with other serum glycoproteins. It will be apparent that glycoproteins in general, with the exception of transferrin, have a similar pattern of plasma disappearance that is largely dependent on their sialic acid content (7). Desialylated serum glycoproteins are rapidly removed from the circulation and are then recovered in the hepatocyte rather...
than the Kupffer cells of the liver. In addition, recent evidence suggests that the receptor involved in the hepatic recognition of desialylated glycoproteins is a sialyl transferase (9).

In previous studies of the chemical composition of purified CEA, it was shown that sialic acid is the constituent that shows the greatest quantitative variation between preparations obtained from different tumors (5). It has also been demonstrated that two groups of CEA molecules can be separated on mixed-bed ion-exchange resins and that in one group, the sialic acid content is 5 times greater than in the other (8). Thus, the small quantitative variation in plasma disappearance curves between different animals in the present experiments may, at least in part, be a reflection of the variation in sialic acid content of individual CEA molecules.

The data obtained in these studies are also of interest because they provide a means of assessing the biological homogeneity of the CEA molecule(s). The CEA prepared in this laboratory has previously been reported as demonstrating molecular homogeneity by the criteria of immunoelectrophoresis, molecular sieve chromatography, and analytical ultracentrifugation (6). Additional evidence obtained by radioimmunoelectrophoresis and the Farr ammonium sulfate coprecipitation technique supported this impression (6, 10).

However, the shape of the plasma disappearance curves obtained in the present experiments is highly suggestive of biological heterogeneity of the CEA molecule(s). It is possible that multicompartmental distribution and handling of CEA might have accounted for this pattern, even with a completely homogeneous molecular species. Against the latter explanation is the observation that both rabbits and dogs metabolized CEA at much more slowly. The metabolic differentiation between the two molecular species with respect to its biological recognition and metabolism. One of these species may be rapidly removed from the circulation by the liver, while the other is handled much more slowly. The metabolic differentiation between the 2 groups of molecules is further supported by the different immunoreactive and chromatographic characteristics of the early and late urinary excretion products.

ACKNOWLEDGMENTS

The authors are indebted to Mrs. M. Kennedy, Mrs. V. Buttrum, and Mr. T. Wilson for excellent technical assistance; and to Dr. S. O. Freedman for his invaluable advice in the preparation of the manuscript.

REFERENCES

Metabolism of Human Carcinoembryonic Antigen in Xenogeneic Animals

J. Shuster, M. Silverman and P. Gold


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/33/1/65

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.