Serum Ceruloplasmin Concentrations in Rats with Primary and Transplanted Sarcomas Induced by Nickel Subsulfide*

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ABSTRACT

Ceruloplasmin (CPN) concentrations were measured by p-phenylenediamine oxidase assay in serums from (a) 30 control Fischer rats; (b) 5 rats with primary sarcomas induced by i.m. injection of nickel subsulfide ($\alpha$Ni$_3$S$_2$), and (c) 12 rats at intervals up to six weeks after s.c. transplantation of four $\alpha$Ni$_3$S$_2$-induced sarcomas. Serum CPN concentrations were not significantly increased in rats with primary sarcomas (mean = 0.38 g per liter) (S.D. ± 0.05), versus 0.35 g per liter (S.D. ± 0.04) in controls. In contrast, serum CPN concentrations were increased within 11 to 21 days in all rats with transplanted sarcomas. Maximum concentrations of serum CPN occurred at 31 to 34 days after tumor transplantation, (mean = 0.56 ± 0.05 g per liter), equivalent to 1.6 ± 0.2 times the initial CPN concentrations in serums obtained prior to treatment ($P < 0.001$). The development of hyperceruloplasminemia in rats with transplanted sarcomas and not in rats with primary sarcomas is attributed to greatly enhanced growth-rates of the transplanted neoplasms.

Introduction

Serum ceruloplasmin (CPN) is a Cu-metalloenzyme which contains approximately 95 percent of total serum copper.42

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Hyperceruloplasminemia and concomitant hypercupremia are found in many patients with malignant tumors and are also observed in patients during pregnancy, during administration of estrogens and diphenylhydantoin and during the "acute phase reaction" that is induced by trauma or infections.11, 23, 42, 54 Measurements of serum CPN or Cu concentrations are not reliable screening tests for cancer, owing to clinical nonspecificity.14, 18, 20, 31, 54 However, concentrations of serum CPN or Cu are valuable prognostic indices in patients with leukemias,4, 8, 15, 18, 19, 22, 23, 46, 48
lymphomas, sarcomas, melanomas and carcinomas of the lung, larynx, breast, ovary, uterus, kidney and bladder. The clinical applications of serum CPN and Cu assays to monitor tumor progression and therapeutic responses in patients with leukemias and lymphomas are analogous to the clinical uses of measurements of tumor-markers such as serum carcinoembryonic antigen, α-fetoprotein, placental lactogen, chorionic gonadotrophin, acid phosphatase and Regan isoenzyme of alkaline phosphatase in patients with other specific varieties of malignant tumors. In untreated patients with lymphomas and acute leukemias, increased concentrations of serum CPN and Cu are almost invariably observed; diminutions of serum CPN and Cu concentrations are often the earliest clinical signs of favorable response to chemotherapy or radiation therapy; and renewed increases of serum CPN and Cu concentrations are often the first indications of tumor recurrence.

Materials and Methods

The experimental animals were (a) adult male rats of the Fischer-344 strain with primary sarcomas that were induced by i.m. injection of 1.25 or 2.5 mg of nickel subsulfide (αNi₃S₂) in the course of an investigation that has been described previously, and (b) adult female rats of the Fischer-344 strain.* The rats were housed, fed, weighed, examined, killed and autopsied as described in previous reports. Tumor transplants were performed by mincing tiny portions of sarcomas (±0.1 g) in 0.5 ml of sterile NaCl solution (8.5 g per liter) and injecting the finely minced suspensions s.c. into the lower abdominal wall by use of a syringe with 16-gauge trochar. Blood samples (±0.02 ml) were collected from the rats' tail veins. Serum CPN concentrations were measured by the p-phenylenediamine oxidase assay of Sunderman and Nomoto, under conditions that are optimal for rat serum (pH 5.2; 37°C; acetate buffer).

Results

A frequency distribution graph of CPN concentrations in sera of 30 untreated rats is shown in figure 1. Serum CPN concentrations in the control rats averaged 0.35 (S.D. ± 0.04) g per liter, (median = 0.35 g per liter; range = 0.27 to 0.49 g per liter).

Five rats with primary sarcomas induced by i.m. injection of αNi₃S₂ were included in this investigation. The sarcomas comprised two fibrosarcomas, two liposarcomas and one rhabdomyosarcoma. The latent period between i.m. injection of 1.25 or 2.5 mg of αNi₃S₂ and initial palpation of these tumors as peab-sized nodules at the injection site averaged 60 (S.D. ± 14) weeks; (median = 61 weeks; range = 44 to 81 weeks). The interval between initial palpation of these tumors and death of the rats averaged 16.4 (S.D. ± 4.9) weeks; (median = 19 weeks;

* Purchased from Charles River Breeding Laboratories, North Wilmington, MA.
TABLE I  
Serum CPN Concentrations in Rats with Transplanted αNi₃S₂-Induced Sarcomas

<table>
<thead>
<tr>
<th>Group</th>
<th>Transplanted Sarcomas</th>
<th>No. of Rats</th>
<th>Initial Serum CPN Concentration (g/liter; mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>01 to 14 days</td>
</tr>
<tr>
<td>A</td>
<td>Rhabdomyosarcoma†</td>
<td>3</td>
<td>0.36±0.05</td>
</tr>
<tr>
<td>B</td>
<td>Liposarcoma‡</td>
<td>1</td>
<td>0.35</td>
</tr>
<tr>
<td>C</td>
<td>Fibrosarcoma‡</td>
<td>4</td>
<td>0.35±0.02</td>
</tr>
<tr>
<td>D</td>
<td>Fibrosarcoma‡</td>
<td>4</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>A-D</td>
<td>All Sarcomas</td>
<td>12</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>E</td>
<td>Vehicle Controls</td>
<td>8</td>
<td>0.36±0.04</td>
</tr>
</tbody>
</table>

* Serums obtained 1 to 11 days before treatment.  
† Transplant of primary sarcoma.  
‡ Fifth passage of sarcoma.  
§ P < 0.05, †† P < 0.01, ** P < 0.001 vs initial CPN concentration in each rat, computed by paired-sample t test.  

range = 9 to 21 weeks). At autopsy, the tumor diameters averaged 5.7 (S.D. ± 0.9) cm; (median = 5.3 cm; range = 5 to 7 cm). No distant metastases were observed. CPN concentrations in serums obtained from these rats within four days before death averaged 0.38 (S.D. ± 0.05) g per liter (median = 0.37 g per liter; range = 0.32 to 0.46 g per liter). These values did not differ significantly from the CPN concentrations in serums from the untreated control rats (P >0.1, computed by Student’s “t” test).  

Three of the primary sarcomas were transplanted s.c. into rats of Groups A, B, and C, as specified in table I. Rats in Group D received s.c. implants of a fibrosarcoma that had originally been induced by i.m. injection of 1.25 mg of αNi₃S₂ and had subsequently been propagated by four serial transplantations in Fischer rats at monthly intervals. In Groups A to D, the period between s.c. implantation of the tumors and death of rats averaged 5.7 (S.D. ± 0.6) weeks; (median = 5.9 weeks; range = 4.5 to 6.3 weeks). At autopsy, the tumor diameters averaged 8.1 (S.D. ± 1.6) cm; (median = 7.8 cm; range = 7 to 9 cm). No distant metastases were observed. Control rats in Group E were killed six weeks after s.c. injection of the NaCl vehicle. Serum CPN concentrations in rats of Groups A to E are listed in table I. Increased concentrations of serum CPN were first detected in rats in Groups A to D from 11 to 21 days following implantation of αNi₃S₂-induced sarcomas (figure 2). Hyperceruloplasminemia developed concurrently or a few days before tumors could be palpated as pea-sized nodules at the sites of implantation. The maximum concentration of serum CPN in rats of Groups A to D occurred at 31 to 34 days after implantation and averaged 1.6 (S.D. ± 0.2) times the corresponding initial CPN concentrations; (median = 1.5-fold increase; range = 1.2 to 1.9-fold increase). In contrast, the concentration of serum CPN at 32 days in control rats of Group E averaged 1.03 (S.D. ± 0.03) times the corresponding initial CPN concentrations in serums that were obtained prior to s.c. injection of the NaCl vehicle (median = 1.03-fold increase; range = 0.99 to 1.06).
Discussion

In 1966, Drs. William F. Enneking and Leo Flynn* were investigating the metabolic effects of intraosseous implantation of the VX-2 carcinoma in rabbits, and they noted that sera of tumor-bearing rabbits frequently were blue in color. They consulted one of the present authors (F.W.S., Jr.), who determined that the blue color was caused by hyperceruloplasminemia. Measurements in this author’s laboratory revealed progressive increases in the mean concentrations of serum CPN in a group of tumor-bearing rabbits, commencing at two weeks after implantation and reaching a 6-fold increase at six weeks. Enneking and Flynn administered $^{64}$Cu(II) to tumor-bearing rabbits by i.v. injection and found that $^{64}$Cu uptake was greater in the liver than in the tumor. Hence they inferred that the VX-2 carcinoma might stimulate the liver to synthesize CPN. During the past 12 years, one of the present authors, (F.W.S., Jr.) has maintained a tabulation of published measurements of serum ceruloplasmin and copper in experimental animals with malignant tumors (table II). To date, hyperceruloplasminemia and hypercupremia have been found in animals with neoplasms that were (a) spontaneous; (b) induced by oncogenic viruses; (c) induced by chemical carcinogens; and (d) induced by bone-seeking radioisotopes. The occurrence of hyperceruloplasminemia and hypercupremia during the growth of malignant neoplasms appears to be a general phenomenon in mammals, based upon observations in five species of experimental animals, as well as in man.

Four avenues of research have shown that serum CPN and Cu concentrations can be considered as biochemical markers of neoplasia in experimental animals.

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Changes in Serum CPN and/or Cu Concentrations in Tumor-Bearing Animals

<table>
<thead>
<tr>
<th>Species</th>
<th>Tumor</th>
<th>Primary (P) or Transplanted (T)</th>
<th>Serum CPN or Cu</th>
<th>Observed Change* (X-fold)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Ehrlich ascites tumor</td>
<td>T</td>
<td>CPN &amp; Cu</td>
<td>1.2-1.5</td>
<td>[13,36]</td>
</tr>
<tr>
<td></td>
<td>Rauscher viral leukemia</td>
<td>P</td>
<td>Cu</td>
<td>+</td>
<td>[35]</td>
</tr>
<tr>
<td></td>
<td>Moloney viral sarcoma</td>
<td>P</td>
<td>Cu</td>
<td>1.8</td>
<td>[14,34]</td>
</tr>
<tr>
<td></td>
<td>SJL/J lymphoma</td>
<td>T</td>
<td>Cu</td>
<td>1.0</td>
<td>[14]</td>
</tr>
<tr>
<td>Rat</td>
<td>MTX sarcoma</td>
<td>T</td>
<td>CPN</td>
<td>1.4</td>
<td>[36]</td>
</tr>
<tr>
<td></td>
<td>Jensen carcinoma</td>
<td>T</td>
<td>CPN</td>
<td>2.8</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td>O-Ya ascites tumor</td>
<td>T</td>
<td>CPN</td>
<td>1.5</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>Yoshida sarcoma</td>
<td>T</td>
<td>CPN &amp; Cu</td>
<td>2.0</td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td>Walker carcinosarcoma-256</td>
<td>T</td>
<td>CPN &amp; Cu</td>
<td>2.9</td>
<td>[57,60]</td>
</tr>
<tr>
<td></td>
<td>Svec's erythroleukemia</td>
<td>T</td>
<td>CPN &amp; Cu</td>
<td>2.6</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>Morris hepatomas 3683F, 7800, 512Stc, and 3924</td>
<td>T</td>
<td>CPN &amp; Cu</td>
<td>1.5-2.5</td>
<td>[5,24,60]</td>
</tr>
<tr>
<td></td>
<td>DMBA mammary tumors 5A &amp; 7A</td>
<td>T</td>
<td>CPN &amp; Cu</td>
<td>1.5-2.5</td>
<td>[5,24]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>VX-2 and VX-7 carcinomas</td>
<td>T</td>
<td>CPN</td>
<td>4-20</td>
<td>[9,10,39,55,56]</td>
</tr>
<tr>
<td></td>
<td>Brown-Pearce carcinoma</td>
<td>T</td>
<td>CPN</td>
<td>1.5-2.0</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>Shope papillocarcinoma</td>
<td>P</td>
<td>CPN &amp; Cu</td>
<td>2-6</td>
<td>[40]</td>
</tr>
<tr>
<td>Dog</td>
<td>Osteosarcomas (spontaneous, and induced by 90Sr and 226Ra)</td>
<td>P</td>
<td>CPN &amp; Cu</td>
<td>1.5</td>
<td>[11,12]</td>
</tr>
<tr>
<td>Monkey</td>
<td>Herpes saimiri sarcoma</td>
<td>P</td>
<td>Cu</td>
<td>+</td>
<td>[14]</td>
</tr>
</tbody>
</table>

* Changes in serum CPN or Cu concentrations are expressed as multiples of initial mean values in test animals or as multiples of comparable mean values in control animals.

† Increased serum Cu concentrations; magnitude not specified.

and the specific activities of 3H-thymidine incorporated into tumor DNA.\(^{57,59,60}\) Moreover, the present study showed that serum CPN concentrations were not significantly increased in rats with slowly growing primary sarcomas, but were considerably increased in rats with rapidly growing transplants of some of the same sarcomas. Second, surgical ablation of tumors is attended by diminution of hyperceruloplasminemia and hypercupremia. In dogs with radiation-induced osteosarcomas and in rabbits with VX-2 carcinoma, amputation of the affected limb has resulted in return of serum CPN or Cu concentrations to near-normal levels.\(^{10,11,12}\) Third, chemotherapy or immunotherapy of tumors causes amelioration of hyperceruloplasminemia and hypercupremia. In mice with Ehrlich ascites tumor, chemotherapy with alkylating agents, 8-azaguaine, or 6-mercaptopurine restored elevated concentrations of serum Cu to normal.\(^{13}\) In rabbits with VX-2 carcinoma, successful immunotherapy by second innoculation of tumor cells was attended by amelioration of hyperceruloplasminemia.\(^{55}\)

Fourth, spontaneous regression of tumors is associated with normalization of serum CPN and Cu concentrations. In mice with Moloney viral sarcomas and in rabbits with VX-2 carcinomas, elevated serum CPN or Cu concentrations returned to normal in those animals whose tumors underwent spontaneous regression.\(^{14,55}\) On the basis of all of these studies, it appears that measurements of serum CPN and Cu can serve as indices of tumor progression or response to therapy in animals with rapidly growing malignant neoplasms.

The pathophysiologic mechanisms that are responsible for hyperceruloplasmi-
nemia in tumor-bearing animals are not clearly understood. However, several investigations have partially elucidated this phenomenon. Linder et al.\(^4\) employed double isotope labelling to demonstrate that the rate of CPN synthesis is increased and the rate of CPN degradation is decreased in rats after implantation of Morris hepatoma 3683F. They also observed that (a) plasma CPN and Cu concentrations are increased proportionately in tumor-bearing rats; (b) increased plasma CPN concentrations occur even when tumors comprise only 0.25 percent of body weight; and (c) elevations of plasma CPN concentrations are not correlated with tumor size.\(^2\)\(^4\)

Cohen et al.\(^5\) administered \(^64\)Cu(II) by gastric intubation to rats with transplanted DMBA-5A mammary tumor. They found substantial uptake of \(^64\)Cu into tumor tissue. For example, at two hours after intubation, the uptake of \(^64\)Cu per gram of tumor tissue was 15 to 20 percent of that in liver. Seto et al.\(^40\) fractionated serum CPN by polyacrylamide gel electrophoresis. In sera from normal and pregnant rabbits, only a single band of CPN activity was observed. In contrast, in sera from rabbits with primary Shope papillocarcinomas or transplanted VX-2 carcinomas, two distinct bands of CPN activity were consistently detected.\(^40\) Seto et al.\(^40\) did not include the possibility that one of the CPN bands might represent a degraded product or a complex of native CPN. However, they suggested that the two bands of CPN activity might represent CPN isoenzymes, and they noted that the two bands differed in regard to heat stability, pH optima and susceptibility to inhibition by sodium azide.\(^40\)

Wolfe et al.\(^62\) and Voelkel et al.\(^56\) showed that the VX-2 carcinoma produces and secretes large amounts of prostaglandin E\(_2\). In rabbits bearing the VX-2 carcinoma, close correlation was observed between plasma concentrations of CPN and PGE-M\(_2\), a major metabolite of prostaglandin E\(_2\).\(^56\) Administration of indomethacin to rabbits from the time of implantation of the VX-2 carcinoma suppressed the development of hypercerculo­plasminemia.\(^56\) Since indomethacin is an inhibitor of prostaglandin synthesis, Voelkel et al.\(^56\) hypothesized that prostaglandin E\(_2\) or its metabolites may be responsible for stimulation of hepatic synthesis of CPN in tumor-bearing animals.

References


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