Growth Hormone Administration Preserves Lean Body Mass in Sarcoma-bearing Rats Treated with Doxorubicin

Bruce Ng, Ronald F. Wolf, Benny Weksler, Murray F. Brennan, and Michael Burt
Surgical Metabolism Laboratory, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

ABSTRACT

Cachexia and malnutrition play a significant role in the morbidity and mortality associated with antineoplastic chemotherapy regimens. Conventional nutritional support during cancer therapy has shown little benefit in terms of reducing morbidity and mortality. We examined the anabolic properties of growth hormone (GH) that preserve normal body composition in sarcoma-bearing animals treated with doxorubicin.

On day 0, 40 male Fischer 344 rats were inoculated with \(10^5\) methylcholanthrene-induced sarcoma cells s.c. in the left flank. On day 9, animals were randomized into 3 groups: control (CTL, \(n = 13\)); doxorubicin (DOX, \(n = 13\)); and doxorubicin plus GH (DOX-GH, \(n = 14\)). Two CTL animals were excluded due to tumor invasion into the peritoneal cavity. From day 9 to day 23, the DOX-GH group received daily s.c. recombinant rat GH injection (1 mg/kg). On day 13, DOX and DOX-GH groups received 7 mg/kg of DOX i.v. while the CTL group received sham i.v. sterile saline injection. Body weight and tumor dimensions were measured daily. On day 23, all animals were weighed and sacrificed. Tumors were resected and weighed. Body composition analysis was performed. Plasma GH levels were measured by radioimmunoassay and insulin growth factor 1 levels were measured by chondrocyte proliferation bioassay.

The DOX-GH group had a significantly higher mean body weight, carcass weight, total fat free body mass, insulin growth factor 1 levels and GH plasma levels as compared to the DOX group. No difference in total food intake was observed between the DOX and DOX-GH groups. There was no difference in final tumor weight and tumor growth rate between DOX and DOX-GH groups.

Exogenous growth hormone can attenuate weight loss and preserve host body composition in tumor-bearing rats treated with doxorubicin without stimulating tumor growth.

INTRODUCTION

Cachexia and malnutrition are among the major causes of morbidity and mortality in patients with cancer. Anorexia and progressive weight loss are commonplace. Studies have reported that a net loss of protein occurs in cancer-bearing hosts as measured by whole-body nitrogen kinetics (1, 2) and body composition analysis (3). In addition, cancer therapy such as chemotherapy, radiotherapy, and surgery unavoidably contribute to the loss of muscle and fat mass. However, most cancer patients undergoing treatment do not benefit from routine nutritional support (4, 5). The lack of efficacy of nutritional therapy in these patients may be secondary to decreased utilization of nutrients resulting from abnormalities in host metabolism (6).

We have been investigating the potential anabolic effect of recombinant GH in preserving host composition in the cancer-bearing host. Exogenous GH has been demonstrated to increase whole-body and skeletal muscle protein synthesis (7–9). We have previously reported improvements in whole-body protein net balance in cachectic cancer patients receiving GH (10). However, it is uncertain if these anabolic properties can be extended to cachectic or chemotherapy-treated tumor-bearing hosts. Furthermore, possible mitogenic influences of GH such as those observed in lymphoid malignancies (11, 12) could preclude the use of GH in cancer-bearing hosts. Recently, GH administered to experimental tumor models has not shown obvious enhancement of malignant activity as reflected by its effect on the number of tumor nodules (13, 14).

This study was designed to characterize the effect of exogenous GH on preservation of host body composition as well as its influence on tumor growth in a model of sarcoma-bearing rats receiving doxorubicin.

MATERIALS AND METHODS

Animal and Tumor Models. Forty adult male Fischer 344 rats weighing 200–225 g (Charles River Breeding Laboratories, Kingston, NY) were used in this study. Animals were treated in accordance with the Animal Welfare Act and the NIH Guide for the Care and Use of Laboratory Animals. All experiments were approved by the Institutional Animal Care and Use Committee, Memorial Sloan-Kettering Cancer Center. Animals were housed in individual hanging cages. They were fed standard laboratory rat chow (Purina Rodent Chow 5021; Purina, Inc., St. Louis, MO) and water ad libitum and subjected to alternate 12-h periods of light/dark.

A transplanted methylcholanthrene-induced sarcoma was used in this study. This tumor model has been used by our laboratory and elsewhere to study tumor-host metabolic interaction (15). This tumor cell line is fast growing and locally invasive, rarely metastasizes, and has not shown evidence of spontaneous regression. Tumors were harvested fresh from tumor-bearing rats that underwent serial tumor passage in our laboratory. Single cell suspension of tumor was prepared by mincing viable tumor fragments and then incubating tumor pieces in Hanks’ balanced saline solution with 1% collagenase (Worthington Biochemical Corp., Freehold, NJ) at 37°C for 45 min, followed by passage of digested tumor through a 45-μm pore size metal screen. Cell viability was determined by the trypan blue exclusion method.

Study Design. After 5 days of acclimation to the animal facility, all rats were inoculated s.c. with \(10^5\) viable sarcoma cells in the left flank. On day 9 post tumor inoculation, animals were randomized into 3 groups: CTL (\(n = 13\)); DOX (\(n = 13\)); and DOX-GH (\(n = 14\)). Two animals from the CTL group were excluded due to early tumor invasion into the peritoneal cavity. From days 9 to 23, the DOX-GH group received daily s.c. recombinant rat GH injections (Bio-technology General Corp., New York, NY) at a dose of 1 mg/kg body weight. On day 13, all animals in the DOX and DOX-GH groups received an i.v. injection of DOX (7 mg/kg body weight) via an aseptic right external jugular catheter. The CTL group underwent sham i.v. saline injection (1.0 ml/kg). Body weight and food intake were measured daily. Tumors were measured in three dimensions with a caliper. On day 23, all animals were weighed and sacrificed. Tumors were resected and weighed. From the ratio of the final tumor weight and tumor measurements, daily tumor weights were extrapolated (16). Body composition analysis was performed on all animals.

Plasma GH levels were measured by radioimmunoassay, and IGF-1 was measured by chondrocyte proliferation bioassay.

Body Composition Analysis. Body composition analysis was performed as described previously (17). Animals were anesthetized with pentobarbital (50 mg/kg), and a midline laparotomy was performed. The animals were exsanguinated by direct aortic puncture. Blood samples were centrifuged, and plasma was frozen at −80°C for subsequent analysis. The intestine was opened and emptied of stool and undigested food and returned to the carcass. The pelvis was removed from the animal. Carcass and pelvis were frozen in liquid nitrogen at −180°C. The frozen pelvis and carcass were ground with 200 ml of water in an industrial meat grinder and were further homogenized in a blender for 5 min. Careful weights were obtained at each step and all tissue was retrieved.
GH ON BODY COMPOSITION OF DOX-TREATED TUMOR-BEARING RATS

RESULTS

The DOX-GH group maintained a significantly higher body weight and carcass weight compared to the DOX group (Table 1). The CTL group had a significantly higher body weight and carcass weight than both DOX and DOX-GH groups. Mean carcass weight during the experimental period was significantly higher than both the DOX and DOX-GH groups. There was no difference observed in tumor weight between the groups. Total fat-free mass is equal to the product of body mass in doxorubicin-treated animals; (b) GH and IGF-1 plasma levels are significantly elevated in the DOX-GH group compared to the DOX and CTL groups.

Table 1 Mean body weight, tumor weight, carcass weight, and total food intake

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body wt (g)</th>
<th>Tumor wt (g)</th>
<th>Carcass wt (g)</th>
<th>Total food intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL (n = 11)</td>
<td>232 ± 6a</td>
<td>268 ± 5</td>
<td>19.9 ± 1.2</td>
<td>248 ± 3</td>
</tr>
<tr>
<td>DOX (n = 13)</td>
<td>231 ± 6b</td>
<td>213 ± 2b</td>
<td>24 ± 0.5b</td>
<td>210 ± 2b</td>
</tr>
<tr>
<td>DOX-GH (n = 14)</td>
<td>234 ± 8c</td>
<td>234 ± 2b</td>
<td>24 ± 0.4b</td>
<td>232 ± 2b</td>
</tr>
</tbody>
</table>

* Mean ± SD.
  a P < 0.05 compared to CTL.
  b P < 0.05 compared to DOX.

Table 2 Body composition analysis

<table>
<thead>
<tr>
<th>Groups</th>
<th>% of Water</th>
<th>% of Fat</th>
<th>% of Fat-free mass</th>
<th>Total Fat-free mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL (n = 11)</td>
<td>66.7 ± 0.5b</td>
<td>11.2 ± 0.5</td>
<td>22.1 ± 0.5</td>
<td>54.9 ± 1.6</td>
</tr>
<tr>
<td>DOX (n = 13)</td>
<td>70.0 ± 0.3c</td>
<td>7.3 ± 0.4c</td>
<td>22.7 ± 0.3</td>
<td>47.7 ± 0.9</td>
</tr>
<tr>
<td>DOX-GH (n = 14)</td>
<td>69.3 ± 0.3c</td>
<td>7.7 ± 0.3c</td>
<td>23.0 ± 0.3</td>
<td>53.1 ± 0.9</td>
</tr>
</tbody>
</table>

* Index for total lean body mass.
  a Mean ± SD.
  b P < 0.05 compared to CTL.
  c P < 0.05 compared to DOX.

DISCUSSION

This study demonstrates that GH treatment in sarcoma-bearing rats treated with doxorubicin preserves lean body mass. Several observations were made: (a) GH attenuated the weight loss and preserved lean body mass in doxorubicin-treated animals; (b) GH and IGF-1 plasma levels were elevated in GH-treated animals; and (c) GH treatment did not affect final tumor weight and tumor growth rate.

Cancer cachexia, characterized by anorexia, debilitation, and severe weight loss, often develops in tumor-bearing hosts and has been significantly correlated with adverse prognosis (18, 19). A character-
istic lean body mass depletion is well documented (20). In addition, a failure in protein conservation mechanisms appears to develop in the tumor-bearing state in contrast to those responses appropriately seen in adaptation to simple starvation (21).

The anabolic properties of GH have been demonstrated in a variety of clinical and physiological studies. Improvements in nitrogen retention and total parenteral nutrition. Surgery (St. Louis), 103: 148—155, 1988.


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