Impact of Insulin on Doxorubicin-induced Rat Host Toxicity and Tumor Regression

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ABSTRACT

To test whether the anorexia and host depletion following doxorubicin chemotherapy can be improved by concomitant insulin therapy, 70 F344 rats were divided equally between tumor-bearing (TB) and non-tumor-bearing (NTB) groups and studied for food intake, host weight, and tumor size changes. Sarcoma fragments were implanted s.c. in TB rats and 18 days later all rats received an i.v. dose of doxorubicin (8 mg/kg). The following day TB and NTB rats were randomized to receive neutral protamine hagedorn insulin (2 units/100 g/24 h) or normal saline until food intake returned to normal. Following doxorubicin administration food intake and host weight declined in an identical pattern in both NTB and TB rats treated with saline. However, beginning on day 6 insulin-treated TB and NTB rats ate significantly more than saline-treated controls. Insulin-treated animals returned to normal food intake levels in 50% less time than controls. This improved food intake resulted in an improved host mass beginning also on day 6 for both TB and NTB rats. In addition, it appeared that insulin treatment significantly improved the tumor shrinkage initiated by doxorubicin. Following doxorubicin, insulin-treated TB rats had a greater reduction of tumor size (10.6 ± 1.2 cm³) compared to saline-treated rats (6.6 ± 0.8 cm³, P < 0.01). To further characterize the effect of insulin and/or doxorubicin on tumor growth, the experiment was repeated in the same manner except for two additional TB groups: saline- and insulin-treated tumor bearers with treatment beginning 19 days after tumor implant. Rats treated with doxorubicin had a significant reduction in tumor size compared to rats not treated with doxorubicin (P < 0.001). Insulin alone did not affect tumor growth, but insulin plus doxorubicin significantly decreased tumor size compared to doxorubicin alone (P < 0.01). In a second experiment using 80 rats insulin treatment had no apparent effect on reduction of peripheral blood counts including white blood cells, neutrophils, lymphocytes, platelets, and hematocrit induced by doxorubicin in either NTB or TB rats. Insulin given 24 h previously had minimal effect on plasma glucose. The marked improvement in food intake and host weight, as well as additional tumor reduction with exogenous insulin following doxorubicin, suggests that insulin may have a role in reversal of doxorubicin host nutritional toxicity and perhaps improvement of antitumor efficacy.

INTRODUCTION

The complications of high-dose aggressive chemotherapy can be a major barrier to effective treatment of cancer. Anorexia with subsequent weight loss is one of the most threatening of these complications (1). Weight loss has been identified as a significant factor in prognosis of cancer patients undergoing chemotherapy (2). However, attempts to replenish cancer patients with dietary supplements and parenteral nutrition have not improved outcome (3). Recent reports suggest that nutritional support is neither indicated nor helpful in cancer patients receiving anticancer therapy (4, 5).

Exogenous insulin treatment has increased food intake of normal and tumor-bearing rats (6, 7). This increased food intake has resulted in increased host mass, which has retained normal body composition of both fat and protein (8). Insulin reversal of cancer cachexia appears reasonable because its effects appear to directly benefit the host. It increases host protein synthesis, reduces host protein breakdown, increases host fat synthesis, reduces host lipolysis, and inhibits host gluconeogenesis (9). Cancer cachexia appears to be at least partially mediated by the exact opposite mechanisms of insulin. In addition, despite its potent anabolic host effect, insulin has not appeared to stimulate sarcoma growth (7).

In this study we investigated the use of exogenous insulin to reverse the anorexia and host tissue depletion found with administration of doxorubicin chemotherapy to both tumor-bearing and control rats. We also evaluated tumor response to doxorubicin with and without insulin therapy. Finally, we quantitated host bone marrow response to doxorubicin in order to evaluate whether insulin might lessen bone marrow toxicity through increased nutrient transport protein synthesis within stem cells.

MATERIALS AND METHODS

General. The work was performed on 190 male Fischer 344 rats weighing between 150 and 200 g. In experiment 1, rats were individually housed in wire-bottomed cages within a temperature- and humidity-controlled room. In experiment 2, rats were housed in the same environment (4 animals per cage). A 12-h light/dark cycle was maintained. In experiment 1 rats were allowed a casein-based, semisynthetic diet (C-21; ICN Nutritional Biochemicals, Cleveland, OH). C-21 is a nonscatterable paste diet which allows accurate measurement of daily food intake. In experiment 1 food intake was measured daily. In experiment 2 rats were fed standard rat chow (Ralston-Purina). Rats in all experiments were allowed water ad libitum.

The tumor was a transplantable methylcholanthrene-induced sarcoma. The tumor was transplanted in the s.c. space by a 1 x 2-mm pellet under i.p. pentobarbital (50 mg/kg) anesthesia. Non-tumor-bearing control animals were anesthetized and underwent similar sham transplantation. The sarcoma grows uniformly and the natural history has been previously reported (10). It is locally invasive, does not metastasize, and kills its host 30-35 days following transplantation. Three orthogonal linear dimensions of the tumor were measured with calipers every other day from the time the tumor was first palpable. Tumor mass was estimated from these measurements using a calculation based on the final excised tumor weight. Host weight is total body weight in a non-tumor-bearing animal and total body weight minus the extrapolated tumor weight in a tumor-bearing animal (11).

Doxorubicin (Adriamycin; Adria Laboratories Inc., Columbus, OH) was administered under ether anesthesia to all animals (8 mg/kg) by penile vein. Previous studies have documented a 13% 30-day mortality in non-tumor-bearing rats following this dose of doxorubicin (12).

NPH3 insulin (Iletin I; Eli Lilly and Co., Indianapolis, IN) was given s.c. to TB and NTB rats at a dose of 2 units/100 g total body weight. This dose had previously produced significant hypoglycemia (blood sugar = 60 mg/dl) in both TB and NTB rats with hyperphagia and no treatment deaths (7, 8). Animals not receiving insulin received an injection of normal saline at a similar volume. All injections were given between 11 a.m. and 12 noon.

Experiment 1. A total of 70 rats became accustomed to C-21 diet. A tumor was implanted in each of 35 animals and 35 underwent sham procedures on day 0. On day 18 following tumor implant all rats were given doxorubicin. On the next day rats were randomized to receive insulin or saline treatment. Treatment was continued for 20 days following doxorubicin administration or until food intake returned to

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2 The abbreviations used are: NPH, neutral protamine hagedorn; TB, tumor-bearing; NTB, non-tumor-bearing.
normal. Four groups were evaluated: NTB-saline (n = 17), TB-saline (n = 17), NTB-insulin (n = 18), and TB-insulin (n = 18). Food intake, total body weight, tumor size, and host weight were measured daily. Tumor response to doxorubicin ± insulin was determined by the maximum change in tumor size for each individual tumor-bearing rat following treatment. Experiment 1 was repeated as above in 20 rats: 10 NTB and 10 TB. In addition, to determine the effect of insulin and/or doxorubicin on tumor growth, 20 other TB rats were randomized to insulin or saline 19 days after tumor implant and injections were continued for 20 days or until death.

Experiment 2. A total of 80 rats were randomized to receive tumor implant or sham tumor implant. At day 18 following tumor implant 8 TB and 8 NTB rats were sacrificed and aortic blood was drawn as described below. Doxorubicin was administered to the remaining 64 animals: 32 TB and 32 NTB. On the following day TB and NTB rats were randomized to receive insulin or saline. Treatments were continued throughout the entire experimental period. On days 3, 6, 9, and 13 following doxorubicin administration, aortic blood was collected under pentobarbital anesthesia (50 mg/kg i.p.) and the animals were sacrificed. At each of the above time points 4 animals from each group were bled (NTB-saline, NTB-insulin, TB-saline, TB-insulin). Whole blood was collected for white blood count, differential white cell count, platelet count, and hematocrit. Plasma was collected for glucose determination by the glucose oxidase method. All of these values were determined by a blinded investigator using standard methods.

Statistics. Statistical analyses of data were performed by independent Student's t-test for parametric data, Wilcoxon rank sum test for non-parametric data, and $\chi^2$ test. All results are expressed as mean ± SE.

RESULTS

Experiment 1

Food Intake. Fig. 1 demonstrates the pattern of food intake among insulin- and saline-treated non-tumor-bearing and tumor-bearing rats following i.v. injection of doxorubicin. Prior to doxorubicin treatment food intake was constant at approximately 15 g per day in both NTB and TB groups. On the day doxorubicin was administered a rapid decline of food intake occurred in all animals. Between days 3 and 6 following doxorubicin all rats started to recover almost 50% of normal food intake, with no differences noted between insulin- and saline-treated groups. Seven days following doxorubicin injection a second phase of more severe anorexia began in the salinetreated control groups of both TB and NTB animals. However, both NTB and TB insulin-treated rats avoided this second phase of anorexia and continued regaining normal food consumption.

Table 1 presents the food intake response in terms of time to recovery of normal food intake. Normal food intake is arbitrarily defined as least 75% of baseline food intake prior to doxorubicin treatment. In insulin-treated TB rats the time to recovery of normal food intake was significantly reduced from 14.0 ± 0.4 days to 7.6 ± 0.6 days ($P < 0.001$). In addition, all insulin-treated TB rats regained normal intake during the experimental period while only 5 of 17 saline-treated rats recovered normal food intake within the experimental period ($\chi^2, P < 0.00001$).

Host Weight. A significant improvement of host weight was documented in insulin-treated TB and NTB animals compared to saline-treated controls as illustrated in Fig. 2. These changes appeared to correspond closely to the pattern of food intake following doxorubicin administration. A steady decline in host weight of control animals occurred immediately following doxorubicin treatment. This decline was especially rapid between days 5 and 10, correlating with the most severe phase of anorexia (Fig. 1). Insulin treatment had no effect on the initial phase of weight loss, but a profound reversal of host weight loss occurred after day 7. By the end of the experimental period, mean host weight of insulin-treated TB and NTB rats returned to predoxorubicin levels, while mean host weight of saline-treated TB and NTB animals was reduced 30% below predoxorubicin treatment levels (Fig. 2).

Tumor Growth. Tumor size reduction in response to doxorubicin was nearly 40% greater in insulin-treated rats compared to saline-treated rats (Fig. 3; Table 1). Analysis of tumor response as absolute reduction in size demonstrated significantly greater tumor regression in TB rats receiving insulin in the postdoxorubicin injection period. Fig. 3 is a plot of the absolute reduction in tumor size which occurred in each individual rat.

Repetition of experiment 1 produced similar significant results in food intake, host weight, and tumor response following doxorubicin. Food intake and host weight data are not shown. A significant reduction in tumor size of rats treated with doxorubicin plus insulin was again demonstrated compared to rats treated with doxorubicin alone ($P < 0.01$) (Fig. 4). Insulin itself had no effect on tumor growth as previously demonstrated

| Table 1 Comparison of food intake, host weight, and tumor response between insulin- and saline-treated TB rats following doxorubicin administration |
|---------------------------------|-----------------|-----------------|
|                                 | Saline          | Insulin         |
| Time to return of normal food intake (days) | 14.0 ± 0.4      | 7.6 ± 0.6*      |
| Initial host weight (g)         | 247 ± 4         | 246 ± 4         |
| Final host weight (g)           | 202 ± 3         | 251 ± 5*        |
| Host weight changes (g)         | -47 ± 4         | +5.3 ± 9*       |
| Initial tumor size (cm$^3$)     | 19.6 ± 2.0      | 23.9 ± 2.2      |
| Tumor size change (cm$^3$)      | -6.6 ± 0.8      | -10.6 ± 1.2*    |

* Data are mean ± SE.  
* $P < 0.001$ versus saline.  
* $P < 0.01$ versus saline.
INSULIN REVERSAL OF DOXORUBICIN NUTRITIONAL TOXICITY

but doxorubicin significantly reduced tumor growth ($P < 0.001$) (Fig. 4).

Experiment 2

Glucose and Bone Marrow Toxicity. Table 2 presents results of peripheral blood counts and plasma glucose in saline- and insulin-treated non-tumor-bearing rats. Samples were taken immediately prior to doxorubicin administration and every 3 days thereafter. White blood cell, platelet, neutrophil, and total lymphocyte counts all reached their lowest level at 6 days following doxorubicin. These lowest values represented a 60% reduction from normal levels and were similar to previous results using doxorubicin in rats (12). On days 9 and 13 following doxorubicin a recovery and rebound leukocytosis and thrombocytosis occurred. The only significant differences between insulin- and saline-treated groups were at day 13, when platelet counts were greater in insulin-treated NTB rats. Also, a significant reduction in hematocrit occurred at days 9 and 13 following doxorubicin injection in insulin-treated NTB rats compared to saline control.

Table 3 summarizes similar data for tumor-bearing rats receiving doxorubicin on day 0. As in the NTB population insulin had no apparent effect on the time to recovery of normal peripheral blood counts. Platelet counts in insulin-treated TB rats, however, maintained a significantly highest lowest level (day 6) than saline-treated controls. Also, the rebound leukocytosis was significantly greater in TB rats treated with insulin rather than saline (day 13). Time to return to normal (predoxorubicin) levels of blood cell counts in both NTB and TB rats treated with insulin or saline following doxorubicin administration were not different (Tables 2 and 3).
INSULIN REVERSAL OF DOXORUBICIN NUTRITIONAL TOXICITY

Table 2 Peripheral blood counts and plasma glucose of insulin- and saline-treated NTB rats before (day 0) and after doxorubicin administration

<table>
<thead>
<tr>
<th></th>
<th>Day 0*</th>
<th>Day 3*</th>
<th>Day 6*</th>
<th>Day 9*</th>
<th>Day 13*</th>
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<tbody>
<tr>
<td>White blood cells (cells/mm³)</td>
<td></td>
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<tr>
<td>Saline</td>
<td>6170 ± 559</td>
<td>2375 ± 570</td>
<td>1787 ± 248</td>
<td>3467 ± 192</td>
<td>7975 ± 1612</td>
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<tr>
<td>Insulin</td>
<td>2762 ± 360</td>
<td>1530 ± 278</td>
<td>4212 ± 443</td>
<td>7133 ± 2193</td>
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<tr>
<td>Platelets (× 10⁹/mm³)</td>
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<tr>
<td>Saline</td>
<td>597 ± 50</td>
<td>764 ± 142</td>
<td>194 ± 23</td>
<td>408 ± 53</td>
<td>1166 ± 41</td>
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<tr>
<td>Insulin</td>
<td>692 ± 38</td>
<td>309 ± 74</td>
<td>277 ± 74</td>
<td>1617 ± 81</td>
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<td>Neutrophils (cells/mm³)</td>
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<tr>
<td>Saline</td>
<td>992 ± 179</td>
<td>436 ± 144</td>
<td>42 ± 32</td>
<td>747 ± 192</td>
<td>5653 ± 1150</td>
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<tr>
<td>Insulin</td>
<td>535 ± 328</td>
<td>35 ± 35</td>
<td>1291 ± 355</td>
<td>5487 ± 2031</td>
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<td>Lymphocytes (cells/mm³)</td>
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<tr>
<td>Saline</td>
<td>5034 ± 425</td>
<td>1939 ± 706</td>
<td>1745 ± 270</td>
<td>2719 ± 172</td>
<td>2271 ± 553</td>
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<tr>
<td>Insulin</td>
<td>2233 ± 404</td>
<td>2233 ± 404</td>
<td>1315 ± 278</td>
<td>2921 ± 246</td>
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<tr>
<td>Hematocrit (%)</td>
<td></td>
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<tr>
<td>Saline</td>
<td>48.2 ± 0.5</td>
<td>46.5 ± 0.6</td>
<td>47.6 ± 0.8</td>
<td>47.0 ± 1.2</td>
<td>41.8 ± 1.3</td>
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<tr>
<td>Insulin</td>
<td>43.3 ± 3.2</td>
<td>47.0 ± 0.9</td>
<td>43.4 ± 0.7</td>
<td>33.8 ± 3.9</td>
<td></td>
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<tr>
<td>Glucose (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Saline</td>
<td>184 ± 7</td>
<td>159 ± 8</td>
<td>162 ± 10</td>
<td>163 ± 15</td>
<td>195 ± 12</td>
</tr>
<tr>
<td>Insulin</td>
<td>172 ± 15</td>
<td>153 ± 12</td>
<td>159 ± 12</td>
<td>236 ± 14</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SE of 8 rats sampled prior to randomization and treatment.
* Mean ± SE of 4 rats.
* P < 0.005 versus saline.
* P < 0.05 versus saline.
* P < 0.05 versus TB day 0 (see Table 3).

Plasma glucose prior to doxorubicin was significantly lower in tumor-bearing rats compared to NTB controls (P < 0.05); however, the reduction was only 12% (Tables 2 and 3). Glucose levels determined approximately 24 h following a saline or insulin dose were not different in TB and NTB rats treated previously with doxorubicin, except in NTB rats treated for 12 days with insulin following doxorubicin (Table 2). These animals demonstrated mild hyperglycemia compared to controls (P < 0.05).

DISCUSSION

Anorexia and subsequent weight loss are common complications of systemic chemotherapy. They adversely affect quality of life and restrict responses to chemotherapy as well as the amount which may be delivered. The impact of anorexia and accompanying malnutrition has been well documented (13). Attempts to overcome anorexia with parenteral nutrition during aggressive chemotherapeutic trials have not produced satisfactory results, and many oncologists question the use of nutritional support (4, 5). This study was designed to test the role of exogenous insulin, a powerful anabolic hormone, in amelioration of anorexia and host tissue wasting associated with administration of doxorubicin to rats.

Exogenous insulin administered by long-acting NPH insulin preparations in dosages used in this study produced mild hypoglycemia (blood sugars = 60 mg/dl) in previous studies in both TB and NTB rats treated previously with doxorubicin, except in NTB rats treated for 12 days with insulin following doxorubicin (Table 2). These animals demonstrated mild hyperglycemia compared to controls (P < 0.05).
Blood glucose levels measured 24 h after a previous NPH insulin dose were for the most part unchanged but higher than expected (Tables 2 and 3). Elevated glucose concentrations observed might be secondary to pentobarbital, which was used to anesthetize animals for aortic puncture. Blood glucose levels were mildly reduced in tumor-bearing rats compared to NTB control rats (Tables 2 and 3), and similar tumor-bearing rats also have been shown to have abnormally elevated glucagon (14).

Previous studies provide clear evidence that insulin treatment can reverse the anorexia associated with cachexia and tumor-bearing conditions (6, 7). The result of insulin treatment of cachectic TB animals is an improvement in host lean tissue and fat mass (8), not solely water weight, which previously had been the result of hyperalimentation of TB rats (15, 16). Thus, insulin therapy can reverse cachexia in TB rats and provide vital cellular mass to the host without promoting tumor growth (6–8). No agent has been described that can reverse the anorexia and host weight loss induced by a systemic antineoplastic agent such as doxorubicin. The impact of insulin on chemotherapy-induced nutritional toxicity has not been investigated previously.

Our studies indicate that the pattern of anorexia following i.v. administration of doxorubicin to normal rats and rats bearing a malignant sarcoma is biphasic in nature, as presented in Fig. 1. The first phase, or early phase, is sudden and occurs between days 1 and 3 following treatment. Most likely this phase represents an acute toxicity illness secondary to anesthetia and the i.v. injection. A recovery of nearly 50% of normal food intake occurs from days 3 through 6. Then a second phase of more severe anorexia begins. This later decline in food intake appears responsive to insulin treatment whereas insulin has no effect on early anorexia. Insulin-treated TB and NTB animals demonstrate no decline in food intake following day 6 as opposed to control animals which decline to nearly 0 g of daily food intake. Insulin-treated TB and NTB rats also derive a significant host weight advantage (Fig. 2) as a result of both increased food intake and the anabolic properties of this hormone. Host benefits of exogenous insulin are more than simple increased food intake. Insulin promotes host protein synthesis and inhibits protein catabolism. Insulin increases host lipogenesis and decreases lipolysis (9). These other metabolic effects of insulin promote host tissue mass and lend further validity to the use of insulin to support the host.

Doxorubicin markedly inhibits tumor growth, and in two experiments insulin appears to enhance the antitumor effects of doxorubicin (Figs. 3 and 4). Doxorubicin is a potent DNA binder and subsequent inhibitor of DNA synthesis and mitotic activity. Most available evidence suggests that DNA binding is central to the antitumor activity of doxorubicin (17). Cells in resting phase, therefore, are resistant to its activity. Whether this apparent additional antitumor effect of added insulin to doxorubicin is mediated by increasing tumor cell growth, making it more susceptible to doxorubicin, or by possible host effects that are able to potentiate host antitumor response is impossible to distinguish from this current study. The mechanism of this tumor reduction effect should be investigated in additional studies.

The potential for exogenous insulin to hasten bone marrow recovery following doxorubicin treatment and allow greater doses of antineoplastic agents was investigated in the second experiment. The treatment of non-tumor-bearing and tumor-bearing rats with doxorubicin produced significant falls in WBC, platelet, neutrophil, and lymphocyte counts similar to previous observations (12). The administration of insulin in the postchemotherapy injection period could not shorten the interval to return of normal peripheral blood counts. The lowest platelet counts in TB rats were substantially improved by insulin administration. Other bone marrow parameters were unaffected by insulin treatment. Overall, these results indicate that insulin was unable to significantly stimulate bone marrow stem cells in the face of doxorubicin toxicity.

In summary, these experiments demonstrate the profound anorexia and host tissue depletion associated with high-dose doxorubicin treatment in both NTB and TB rats. Exogenous insulin administered in the immediate postchemotherapy period greatly stimulated food intake and resulted in improved host weight deposition. In addition, insulin therapy appeared to increase tumor regression following doxorubicin, but it had no apparent effect on host bone marrow recovery. We conclude that insulin may have a role in improving host nutritional tolerance and response to doxorubicin chemotherapy.

REFERENCES

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