Prevention of Cancer Cachexia by a Novel Nuclear Factor κ-B Inhibitor in Prostate Cancer

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Abstract

Purpose: To investigate the association between serum interleukin-6 (IL-6) and cachexia in patients with prostate cancer and the inhibitory effect of a new nuclear factor κ-B (NF-κ-B) inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), on IL-6 production and cachexia in an animal model of hormone-refractory prostate cancer.

Experimental Design: The association between serum IL-6 levels and variables of cachexia was evaluated in 98 patients with prostate cancer. The inhibitory effects of DHMEQ on IL-6 secretion and cachexia were investigated in in vitro and in vivo studies using JCA-1 cells derived from human prostate cancer.

Results: Serum IL-6 levels were significantly elevated and cachexia developed in JCA-1 tumor-bearing mice as well as in prostate cancer patients with progressive disease. IL-6 secretion was significantly inhibited in JCA-1 cells exposed to DHMEQ. Intraperitoneal administration of DHMEQ (8 mg/kg) to tumor-bearing mice produced a significant amelioration of the reduction in body weight, epididymal fat weight, gastrocnemius muscle weight, hematocrit, and serum levels of triglyceride and albumin when compared with administration of DMSO or no treatment. DHMEQ caused a significant decrease of serum IL-6 level in JCA-1 tumor-bearing mice (all P < 0.05).

Conclusions: These results suggested an association between serum IL-6 and cachexia in patients with prostate cancer and in JCA-1 tumor-bearing mice and that a new NF-κ-B inhibitor, DHMEQ, could prevent the development of cachexia in JCA-1 tumor-bearing mice presumably through the inhibition of IL-6 secretion. DHMEQ seems to show promise as a novel and unique anticachectic agent in hormone-refractory prostate cancer.

Cancer cachexia, which features the loss of muscle and fatty tissue as well as anorexia, asthenia, and anemia (1, 2), makes therapeutic intervention difficult (3) and is an important cause of death in cancer patients (4). Although little is known about the detailed mechanisms of cachexia, recent studies have revealed that inappropriate production and release of cytokines such as interleukin-6 (IL-6) is involved in the induction of cachexia (5–8). Progressive prostate cancer is often associated with anorexia, weight loss, and accelerated malnutrition that lead to cachexia, even if metastases are confined to the bones. It has been reported that human prostate cancer cells produce IL-6 and that the serum level of IL-6 is elevated in patients with prostate cancer (9, 10). However, few studies have investigated the association between the serum IL-6 level and cachexia in patients with prostate cancer. Production of IL-6 is regulated by several transcription factors, among which nuclear factor κ-B (NF-κ-B) is one of the pivotal regulators of cytokine-inducible gene expression (11). Schwarz et al. (12) have suggested that suppression of NF-κ-B may result in the amelioration of cachexia in a mouse tumor model. It is also well known that NF-κ-B shows constitutive activation in hormone-refractory prostate cancer cells (13, 14). As far as we know, no investigators have explored a treatment strategy for cachexia based on the regulation of NF-κ-B by administration of a compound synthesized from a natural product. Recently, we have investigated the effectiveness of a new NF-κ-B inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), which is a 5-dehydroxymethyl derivative of epoxyquinomicin C that shows anti-NF-κ-B activity in cultured human leukemia Jurkat cells and inhibits type II collagen–induced rheumatoid arthritis in mice (15). The present study was undertaken to evaluate the association between IL-6 and cachexia in patients with prostate cancer, as well as the inhibitory effect of DHMEQ on IL-6 production and cachexia in an animal model of hormone-refractory prostate cancer.

Materials and Methods

Patients. The association between serum IL-6 and cachexia in patients with prostate cancer was evaluated in this retrospective study. Ethics approval was obtained from our institutional ethics committee. Ninety-eight archival serum samples from patients with histologically...
confirmed prostate cancer were examined. There were 55 patients with untreated disease, 23 patients in remission after endocrine therapy, and 20 patients with relapse. The definitions of remission and tumor progression were previously reported (8). Remission included any of the following: (a) reduction or disappearance of tumor masses; (b) a decrease in the number, size, or intensity of lesions on successive bone scans; or (c) a significant decrease of serum prostate-specific antigen. In addition, there had to be no new lesions and no deterioration of symptoms or performance status. Any of the following events was considered evidence of tumor progression: (a) the appearance of any new metastasis; (b) an increase in the number, size, or intensity of lesions on successive bone scans; or (c) significant cancer-related deterioration of symptoms or performance status. Classification of the patients with prostate cancer was done in accordance with Modified Jewett Stageing System (16). Serum levels of IL-6 were measured using the Quantikine enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN) according to the instructions of the manufacturer. Laboratory tests included analysis of serum albumin and hematocrit. Performance status was assessed in accordance with the Eastern Cooperative Oncology Group scale, in which 0 indicated that the patient had no symptoms; 1, the patient had symptoms but was ambulatory; 2, the patient was bedridden less than half the day; 3, the patient was bedridden half the day or longer; and 4, the patient was chronically bedridden and required assistance with activities of daily living. Body mass index (BMI) was calculated by the following formula: weight (kg)/height² (m²).

Cell line. JCA-1 cells derived from human prostate cancer (17) were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 μg/mL streptomycin (Life Technologies, Inc., Grand Island, NY), and 100 IU/mL penicillin (Life Technologies).

Chemicals. DHMEQ was synthesized in our laboratory (15). We also referred to the article by Suzuki et al. (18) in which the molecular structure was shown. It was dissolved in DMSO to prepare a 10 mg/mL solution and was subsequently diluted in culture medium to a final DMSO concentration of <0.1%.

In vitro interleukin-6 assay. JCA-1 cells (1 × 10⁶) were seeded in a total volume of 1 mL of medium in each well of 24-well tissue culture plates and allowed to grow overnight. Then cells were treated with 1.0 or 1.5 μg/mL of DHMEQ, whereas other cells treated with the same concentrations of DMSO served as controls. After 48 hours of incubation, the supernatant of each well was collected and stored at −80°C until assay, and the number of viable cells was determined by trypsin blue dye exclusion. The IL-6 concentration was measured using an enzyme immunoassay specific for human IL-6 (R&D Systems QuantiGlo Human IL-6 Immunoassay kit) according to the instructions of the manufacturer.

Animal model. All procedures involving animals and their care in this study were approved by the animal care committee of our institution in accordance with institutional and Japanese government guidelines for animal experiments. Male Balb/C- nu/nu mice were obtained from Sankyo Lab Service Corp. (Tokyo, Japan). The mice were housed at a constant temperature and humidity and received a standard diet and water. JCA-1 cells (1 × 10⁶) were inoculated s.c. into the right flank of each mouse. When the tumors reached 10 mm in diameter, mice were randomly assigned to three groups. DHMEQ (8 mg/kg) was administered i.p. in a volume of 0.2 mL once daily for 25 days to group 2 (n = 12). This group was labeled Tumor (+), No drug. As a healthy control, age-matched mice were observed without any treatment [group 4, n = 11; this group was labeled Tumor (+), No drug]. As a healthy control, age-matched mice were observed without any treatment [group 1, n = 14; this group was labeled Tumor (−)]. No drug. As a healthy control, age-matched mice were observed without any treatment [group 1, n = 14; this group was labeled Tumor (−)]. No drug. During the treatment period, mice were carefully monitored and body weight was measured every other day. At the time of sacrifice, the tumor, gastrocnemius muscle, and epididymal fat were dissected and weighed. Blood samples were collected into nonheparinized tubes, and serum was separated within 1 hour of sacrifice. The serum samples were stored at −80°C and thawed just before testing. Serum IL-6 activity was determined using an enzyme immunoassay specific for human IL-6 (R&D Systems Quantikine Human IL-6 Immunoassay kit) according to the instructions of the manufacturer. At the same time, the hematocrit and the serum levels of triglycerides and albumin were also measured in each mouse.

Statistical analysis. All values are expressed as the mean ± SE. Variables for different groups were compared using Student’s t test or ANOVA. P < 0.05 was considered statistically significant.

Results

The serum IL-6 level of 20 patients with relapse was significantly higher than that of 55 untreated patients or that of 23 patients in remission (Table 1). Serum levels of prostate-specific antigen were significantly higher in patients with relapse than in untreated patients or patients with remission. Serum albumin levels, hematocrit, and BMI were significantly lower in patients with relapse than in untreated patients or patients in remission (Table 1). The performance status was also significantly worse in patients with relapse when compared with untreated patients or patients in remission (Table 1). The serum albumin level and hematocrit were significantly lower (all P < 0.05) in patients with serum IL-6 level ≥ 7 pg/mL than in patients with serum IL-6 level < 7 pg/mL (data not shown). BMI was significantly lower in patients with serum IL-6 level ≥ 7 pg/mL (19.38 ± 0.41 kg/m²) than in patients with serum IL-6 level < 7 pg/mL (22.94 ± 0.28 kg/m²; P < 0.0001; Fig. 1).

The IL-6 level in the culture medium of JCA-1 cells treated with DHMEQ at 1.0 or 1.5 μg/mL for 48 hours was 4.03 ± 0.35 and 2.98 ± 0.17 pg/mL/10⁶ cells, respectively, being significantly lower than when cells were treated with DMSO alone (10.54 ± 3.73 pg/mL/10⁵ cells; P = 0.031 and P = 0.015, respectively; Fig. 2).

When mice had developed tumors ~ 10 mm in diameter after inoculation of JCA-1 cells, treatment was initiated and the day when treatment was started was designated as day 0. At the end of experiments, the mean weight of tumors of DHMEQ-treated mice was 3.02 ± 0.54 g, which was smaller than that of DMSO-treated mice (4.16 ± 0.81 g) and untreated mice (4.15 ± 1.10 g), but the differences were not statistically significant. The body weight, epididymal fat weight, gastrocnemius muscle weight, hematocrit, and serum levels of triglyceride and albumin were significantly lower in untreated JCA-1 tumor–bearing mice (group 4) than in healthy control mice without tumors (group 1), and serum IL-6 levels were significantly elevated in group 4 mice at the time of sacrifice (Figs. 3 and 4; Table 2). Although the body weight of untreated tumor-bearing mice and tumor-bearing mice treated with DMSO decreased in a time-dependent manner, the weight of JCA-1 tumor–bearing mice treated with DHMEQ did not decline significantly (Fig. 3). On day 26, body weight (28.24 ± 1.44 g), epididymal fat weight (197.11 ± 31.67 mg), and gastrocnemius muscle weight (499.27 ± 30.26 mg) were significantly greater in DHMEQ-treated mice (group 2) than in mice treated with DMSO alone (group 3; 24.09 ± 1.30 g, 117.12 ± 19.10 mg, and 306.28 ± 24.46 mg; P = 0.018, P = 0.044, and P < 0.001, respectively) or untreated mice (group 4; 21.46 ± 1.08 g, 43.48 ± 2.97 mg, and 261.13 ± 14.54 mg; P = 0.002, P = 0.001, and P < 0.001, respectively; Table 2).

In addition, the hematocrit (41.89 ± 1.54%) and the serum triglyceride level (60.63 ± 7.36 mg/dL) were significantly higher in group 2 mice than in group 3 mice (36.71 ± 1.81% and...
with serum IL-6 levels <7 pg/mL (22.94 ± 0.41 kg/m²) than in patients with serum IL-6 level <7 pg/mL (22.23 ± 0.28 kg/m²; P = 0.0001). *Significantly different from the mean value of patients with serum IL-6 level <7 pg/mL.

Table 1. Disease status and variables

<table>
<thead>
<tr>
<th>No. patients</th>
<th>Serum levels of IL-6 (pg/mL)</th>
<th>Rate of serum IL-6 levels of ≥7 pg/mL (%)</th>
<th>Prostate-specific antigen (ng/mL)</th>
<th>Albumin (g/dL)</th>
<th>Hematocrit (%)</th>
<th>BMI (kg/m²)</th>
<th>Performance status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Untreated patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>0.69-111.06</td>
<td>5.21 ± 2.06</td>
<td>10.9 (6/55)</td>
<td>171.09 ± 58.73</td>
<td>3.58 ± 0.06</td>
<td>36.26 ± 0.70</td>
</tr>
<tr>
<td>Stage A</td>
<td>3</td>
<td>0.86-1.6</td>
<td>1.20 ± 0.22</td>
<td>0 (0/3)</td>
<td>2.27 ± 0.71</td>
<td>3.57 ± 0.23</td>
<td>41.73 ± 3.35</td>
</tr>
<tr>
<td>Stage B</td>
<td>15</td>
<td>0.69-5.65</td>
<td>2.45 ± 0.40</td>
<td>0 (0/15)</td>
<td>42.52 ± 19.18</td>
<td>3.57 ± 0.09</td>
<td>38.27 ± 1.26</td>
</tr>
<tr>
<td>Stage C</td>
<td>11</td>
<td>1.07-7.45</td>
<td>2.16 ± 0.45</td>
<td>9.1 (1/11)</td>
<td>50.94 ± 14.90</td>
<td>3.76 ± 0.06</td>
<td>38.16 ± 1.07</td>
</tr>
<tr>
<td>Stage D</td>
<td>26</td>
<td>1.08-111.06</td>
<td>8.55 ± 4.30</td>
<td>19.2 (5/26)</td>
<td>315.57 ± 118.37</td>
<td>3.52 ± 0.11</td>
<td>33.67 ± 0.94</td>
</tr>
<tr>
<td>Patients with remission as a result of endocrine therapy</td>
<td>23</td>
<td>0.26-4.77</td>
<td>2.45 ± 0.26</td>
<td>0 (0/23)</td>
<td>79.34 ± 53.48</td>
<td>3.78 ± 0.09</td>
<td>36.60 ± 1.03</td>
</tr>
<tr>
<td>Patients with relapsed bone metastatic disease after endocrine therapy</td>
<td>20</td>
<td>4.46-135.53</td>
<td>41.58 ± 8.07</td>
<td>15.0 (17/20)</td>
<td>14.35 ± 1210.34</td>
<td>2.78 ± 0.08</td>
<td>31.00 ± 1.37</td>
</tr>
</tbody>
</table>

NOTE: The untreated patients were separated into subgroups of stage A to D in accordance with Modified Jewett Staging System. Performance status was assessed in accordance with the Eastern Cooperative Oncology Group scale.

Discussion

It has been reported that elevation of the serum levels of IL-6 is strongly associated with cachexia in patients with various types of advanced cancer. Serum cytokine levels are increased in prostate cancer patients who have weight loss when compared with those who show no weight loss (8, 19). In the present study, prostate cancer patients with relapse showed more severe cachexia and had higher serum IL-6 levels than untreated patients or patients in remission. In addition, the prostate cancer patients with higher serum IL-6 levels were more cachectic than those with lower IL-6 levels. Wallenius et al. investigated the effect of IL-6 on the physique in mice lacking the gene encoding IL-6 (IL6−/− mice) and found that they developed mature-onset obesity that was partly reversed by IL-6 replacement. Taken together, these results suggest a strong relationship between the serum level of IL-6 and weight loss (20). In the present study, DHMEQ produced a significant decrease in the IL-6 level and a significant improvement in the body weight of tumor-bearing mice.

Alexandrakis et al. (21) showed that IL-6 was significantly higher and hemoglobin was significantly lower in patients with multiple myeloma than in the controls, and a significant decrease in hemoglobin concentration and hematocrit was also found in patients with higher serum IL-6 levels. Ishiko et al.

![Fig. 1. Relationship between IL-6 and BMI. BMI was significantly lower in patients with serum IL-6 level ≥7 pg/mL (19.3 ± 0.41 kg/m²) than in patients with serum IL-6 level <7 pg/mL (22.3 ± 0.28 kg/m²; P < 0.0001). *Significantly different from the mean value of patients with serum IL-6 level <7 pg/mL.](image1)

![Fig. 2. Effect of DHMEQ on IL-6 secretion by JCA-1 cells. At concentrations of 1.0 and 1.5 μg/mL, DHMEQ significantly inhibited IL-6 secretion by JCA-1 cells. Columns, mean value of samples; bars, SE. *, significantly different from control (DMSO alone; P = 0.031). **, significantly different from control (DMSO alone; P = 0.015).](image2)
(22) showed that there is severe anemia in cancer-bearing rabbits, whereas the mean hemoglobin value of normal rabbits was much higher. In our study, the serum IL-6 level was significantly lower in Tumor (+), DHMEQ mice than in Tumor (+), DMSO mice or Tumor (+), No drug mice. Columns, mean IL-6 levels of samples; bars, SE. * significantly different from Tumor (+), DMSO (P = 0.030) and Tumor (+), No drug (P = 0.006).

Table 2. Effects of DHMEQ on epididymal fat weight, gastrocnemius muscle weight, hematocrit, and serum levels of triglyceride and albumin

<table>
<thead>
<tr>
<th></th>
<th>Tumor (−), No drug</th>
<th>Tumor (+), DHMEQ</th>
<th>Tumor (+), DMSO</th>
<th>Tumor (+), No drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epididymal fat weight (mg)</td>
<td>314.40 ± 40.23</td>
<td>197.11 ± 31.67*</td>
<td>117.12 ± 19.10</td>
<td>43.48 ± 2.97</td>
</tr>
<tr>
<td>Gastrocnemius muscle weight (mg)</td>
<td>558.66 ± 19.62</td>
<td>499.27 ± 30.26*</td>
<td>306.28 ± 24.46</td>
<td>261.13 ± 14.54</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.14 ± 1.07</td>
<td>41.89 ± 1.54*</td>
<td>36.71 ± 1.81</td>
<td>34.70 ± 1.80</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>80.82 ± 13.77</td>
<td>60.63 ± 7.36*</td>
<td>36.50 ± 6.16</td>
<td>32.00 ± 2.91</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.45 ± 0.09</td>
<td>2.04 ± 0.07</td>
<td>1.95 ± 0.08</td>
<td>1.77 ± 0.08</td>
</tr>
</tbody>
</table>

*Significantly different from the group of Tumor (+), DMSO and Tumor (+), No drug.

Table 2. Effects of DHMEQ on epididymal fat weight, gastrocnemius muscle weight, hematocrit, and serum levels of triglyceride and albumin
the stable form (Iβα) induces apoptosis of cancer cells showing constitutive NF-κB activity in vitro (33). Kawamura et al. developed synthetic double-stranded oligodeoxynucleotides for use as “decoy” cis elements that block the binding of nuclear factors to the promoter regions of target genes. They injected decoy oligodeoxynucleotide targeting NF-κB directly into adenocarcinoma colon 26 tumors in mice to examine whether or not cachexia was alleviated by inhibiting the action of cytokines, and their results suggested that cytokines regulated by NF-κB may play a pivotal role in the induction of cachexia in the colon 26 model (34). However, the clinical feasibility of gene therapy for inhibiting NF-κB is limited by the need for intratumoral delivery of a vector that expresses the NF-κB inhibitor, and few studies have assessed the usefulness of this strategy with in vivo models. In contrast, we assessed a novel agent for inhibiting the activity of NF-κB. There have been no previous reports about the therapeutic effect of an agent synthesized from a natural product on cachexia mediated through the regulation of cytokines by NF-κB.

We have previously reported that DHMEQ produces a significant decrease in NF-κB activity in JCA-1 cells with constitutive NF-κB activation (35). Because of these encouraging in vitro findings, we investigated the effect of DHMEQ on cachexia induced by JCA-1 tumor secreting IL-6. We found that DHMEQ significantly inhibited IL-6 production and significantly prevented the development of cachexia in a JCA-1 tumor model.

In conclusion, we showed a significant association between IL-6 and cachexia in patients with progressive prostate cancer, as well as in JCA-1 tumor-bearing mice, and we showed that DHMEQ inhibits NF-κB and thus prevents the development of cachexia induced by prostate cancer in an animal model. Prevention of the complex syndrome of cachexia will improve the quality of life for cancer patients. The new NF-κB inhibitor, DHMEQ, seems to be a promising novel anti-cachetic agent for the treatment of hormone-resistant prostate cancer.

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References

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