Progesterone improves survival in hepatoma cachexia rat model

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Abstract

Background Medroxyprogesterone and megestrol acetate are synthetic progesterone derivatives. Progestagen is an approved drug for cancer cachexia in the USA and in some European countries. These agents have been described to increase appetite and to lead to weight gain. However, the effects on survival are still unknown. The aim of this study was to evaluate the effects of progesterone on survival, cardiac function, and appetite and body weight in the Yoshida hepatoma AH-130 rat cancer cachexia model.

Methods and Results In this study, the effects of progesterone were tested in cachectic tumour-bearing rats. Rats were treated with 0.5, 5 or 50 mg/kg/day, respectively, or placebo daily, starting 1 day after tumour inoculation for a period of 16 days. Cardiac function was analysed by echocardiography at baseline and at day 11. Food intake was assessed before tumour inoculation and at day 11. Body weight and body composition were evaluated at the beginning and the end of study or the day of euthanasia. Survival was significantly improved by 5 mg/kg/day (hazard ratio: 0.48, 95% confidence interval: 0.24–0.95, P = 0.0356). However, there was no significant difference between the progesterone treatment groups compared with placebo in body weight change and body composition, as well as food intake on day 11. Cardiac function also showed no significant difference compared with placebo.

Conclusions Progesterone improves survival but has no beneficial effects on cardiac function, body weight, and food intake in this aggressive hepatoma cancer cachexia rat model. Further studies are needed to elucidate the mechanism of the survival benefit.

Keywords Progesterone; Appetite; Body weight

Introduction

Cachexia describes a serious clinical syndrome accompanied by weight loss, muscle wasting, and appetite loss.¹ Cancer cachexia occurs in over 50% of cancer patients, and 22% of deaths are attributed to cancer cachexia.² The existence of cachexia leads to poor quality of life and high mortality.³⁵ Although pharmacological treatments including anabolic steroids, recombinant growth factor, ghrelin, and progesterone therapy were reported, there were no consistent results.⁶,⁷ The 2010 European Palliative Care Research Collaborative Care Research Collaborative Cachexia guidelines recommended that progesterone therapies should be considered for patients who suffer from refractory cachexia and anorexia in patients with cancer.⁸ Since 1993, megestrol acetate (MA), a progesterone derivative, has been approved by Food and Drug Administration for the treatment of anorexia, cachexia, or unexplained significant weight loss in patients with AIDS. Our group has already published the effect of MA in cancer cachexia showing...
improved survival and reduced wasting through a down-regulation of autophagy in both skeletal muscle and cardiac muscle. Meanwhile, medroxyprogesterone (MPA), which is a steroidal progestin, a synthetic variant of human hormone progesterone, has been described to improve appetite and body weight in a cancer trial. Its anti-cachexic action has yet to be fully established. Some reports suggest that MPA may lead to the suppression of some cytokines such as TNF-α and IL-6. Moreover, it is known that MPA can stimulate appetite through neuropeptide Y in the hypothalamus. MPA has been described to reduce the in vitro production of serotonin and cytokines such as IL-1, IL-6, and TNF-α. MA also has similarity to increase the appetite and food intake.

In a placebo control trial, MPA increased weight gain and improve the quality of life. However, the weight gain was mainly due to increased body fat. The aim of this study was to assess the effects of MPA on cardiac function, body composition, and survival in cancer cachexia rat model.

Material and methods

Male Wistar Han rats (Harlan Laboratories, Rossdorf, Germany) of 8 weeks of age and weight of 199.2 ± 1.15 g were kept under standard laboratory conditions in a specific pathogen-free animal facility and maintained at 22 ± 2°C with alternating 12 h light dark cycle and free access to food and water. All the experimental procedures were performed in accordance with the permission of the LaGeSo Berlin.

Study design

Rats were randomized into two groups to be intraperitoneally injected with either Yoshida 10⁸ AH-130 hepatoma cells (n = 101) or saline (n = 10, sham). Tumour-bearing rats were further randomized to be treated with placebo (n = 55) or 0.5, 5, and 50 mg/kg/day progesterone, respectively. All treatments were given in a blinded fashion via gavage once daily over a period of maximum 16 days. Treatment with MPA or placebo started 1 day after tumour inoculation.

All operators involved in the study were blinded to treatment allocation. One day before tumour inoculation, baseline body weight, body composition, and echocardiographic analysis were assessed. Cardiac function was re-analysed on day 11. Body composition and body weight were recorded on 16 or the day of the euthanasia if the animals had to be sacrificed for ethical reasons. At the end of the study and for each tumour-bearing animals, the tumour was harvested from the peritoneum, and its volume was evaluated. Tumour cell number was determined using a Neubauer chamber. Organs and tissues were rapidly removed and weighed.

Body composition analysis

Total body fat and lean mass were measured using the nuclear magnetic resonance spectroscopy device EchoMRI-700™ (Echo Medical System, Huston, TX, USA) as described before.

Spontaneous activity and food intake

Animals were housed individually, and spontaneous movement was recorded by an infrared monitoring system (Supermax, Muromachi, Tokyo, Japan) over a 24 h period as described before. Food intake was also recorded during this period.

Echocardiographic analysis

Echocardiographic analyses were performed using the high resolution Vevo 770 system (Visual Sonics Inc., Tronto, Canada), which was described previously. Briefly, rats were anaesthetized with 1.5% isoflurane and laid in a supine position on a heated surface to maintain body temperature and with all legs taped to electrocardiogram electrodes. All hair was removed from the left part of the chest. Recordings were made in B-mode and M-mode to assess functional parameters and dimensions. Cardiac function was analysed by echocardiography at baseline and at day 11.

Statistical analysis

Data were analysed with GraphPad PRISM 6.0 (GraphPad Software, Inc, La Jolla, CA, USA). Results are shown as mean ± SEM. Normality was tested using D’Agostino Pearson’s test. Normally distributed data were analysed by one-way ANOVA followed by Tukey’s test, while data without normal distribution were analysed using Kruskal–Wallis analysis of variance and subsequent Dunn’s tests. Survival was tested by Cox-proportional hazard analysis, hazard ratio, and 95% confidence interval. A P value of <0.05 was considered significant.

Results

Tumour-bearing rats treated with 5 mg/kg/day progesterone showed a better prognosis compared with placebo (hazard ratio 0.48, 95% confidence interval: 0.24–0.95, P = 0.0356) despite the lack of a difference in both tumour weight and cell number between placebo and progesterone treatment groups (Figure 1 and Table 1).
In this analysis, we found a significant body weight loss in progesterone 5 mg group compared with placebo group (136.7 ± 2.97 vs. 162.5 ± 4.92 g, \(P < 0.01\)). Lean mass was also reduced in progesterone 5 mg treatment group compared with placebo group (108.5 ± 2.72 vs. 120.1 ± 2.38 g, \(P < 0.05\)). However, fat mass showed no significant difference among all groups. Muscle analysis showed a significant reduction of muscle weight in gastrocnemius and tibialis with progesterone 5 mg treatment group compared with placebo group (gastrocnemius 699.3 ± 25.4 vs. 864 ± 28.4 mg; tibialis 234.9 ± 10.8 vs. 305.1 ± 10.4 mg, respectively) (Table 2).

### Table 1  Tumour weight and total cell count at the end of the study

|                      | Placebo        | Progesterone (0.5 mg) | Progesterone (5 mg) | Progesterone (50 mg) | \(P\) value |
|----------------------|----------------|-----------------------|---------------------|----------------------|-------------|
| Tumour weight, g     | 108.6 ± 3.74   | 110.6 ± 4.4           | 109 ± 4.69          | 108.2 ± 6.13         | 0.992       |
| Tumour cell total    | 3868 ± 271.1   | 3696 ± 245.2          | 3389 ± 303.4        | 3245 ± 195.9         | 0.452       |

### Table 2  Body composition and muscle measurement

|                  | Sham          | Placebo       | Progesterone (0.5 mg) | Progesterone (5 mg) | Progesterone (50 mg) |
|------------------|---------------|---------------|-----------------------|---------------------|----------------------|
| BW, g            | 278 ± 6.57    | 162.5 ± 4.92  | 144.0 ± 4.08          | 136.7 ± 2.97**      | 146.4 ± 2.46         |
| Lean, g          | 210.7 ± 3.86  | 120.1 ± 2.38  | 114.2 ± 3.85          | 108.5 ± 2.72*       | 117.0 ± 2.20         |
| Fat, g           | 27.9 ± 1.92   | 5.52 ± 0.49   | 4.89 ± 0.40           | 4.91 ± 0.74         | 6.19 ± 0.67          |
| Gastrocnemius, mg| 1426 ± 27.4   | 864 ± 28.4    | 767.6 ± 36.5          | 699.3 ± 25.4**      | 834.9 ± 28.0         |
| EDL, mg          | 115.1 ± 6.3   | 74.8 ± 2.5    | 63.9 ± 3.4            | 61.1 ± 2.8          | 74.5 ± 2.2           |
| Soleus, mg       | 120.9 ± 5.6   | 81.5 ± 2.4    | 75.1 ± 2.8            | 70.5 ± 2.4          | 83.9 ± 5.0           |
| Tibialis, mg     | 501.3 ± 7.4   | 305.1 ± 10.4  | 238.0 ± 21.6**        | 234.9 ± 10.8***     | 287.1 ± 9.3          |
| BAT, mg          | 311.1 ± 43.1  | 114.7 ± 7.7   | 101.1 ± 5.9           | 95.7 ± 6.3          | 112.1 ± 8.1          |
| WAT, mg          | 1281 ± 173.4  | 278.2 ± 82.2  | 26.6 ± 15.3           | 64.0 ± 31.7         | 35.2 ± 22.4          |

BAT, brown adipose tissue; BW, body weight; EDL, extensor digitalis longus; WAT, white adipose tissue.

Mean \(\pm\) SE.

*\(P < 0.05\).

**\(P < 0.01\).

***\(P < 0.001\) vs. placebo.
Figure 2 The effect of progesterone on cardiac function and heart wasting.

A  Heart rate (bpm)

B  Ejection fraction (%)

C  Fractional shortening (%)

D  Stroke volume (µl)

E  LVESV : Left Ventricular end systolic volume (µl)

F  LVEDV : Left Ventricular end diastolic volume (µl)

G:  Left ventricular mass (mg)
systole (mm)

H:  LVID s Left ventricular diameter in
**Food intake**

Tumour-bearing rats showed a decreased food intake in spite of treatment allocation. In placebo group, a severe reduction of food intake was observed (6.52 ± 0.96 vs. 23.66 ± 0.83 g/24 h, placebo vs. sham, \( P < 0.001 \)). However, there was no improvement in food intake with progesterone treatment compared with placebo (4.98 ± 1.39, 5.19 ± 1.82, 3.55 ± 1.17 g/24 h, the group treated with progesterone 0.5, 5, 50 mg, respectively).

**Cardiac function analysis**

The baseline characteristics of echocardiogram were described in Table 3. We found a significant difference in progesterone 5 mg group concerning ejection fraction and left ventricular end-systolic volume at the baseline (80.63 ± 0.87 vs. 84.13 ± 0.59\% in ejection fraction; 47.21 ± 3.29 vs. 36.65 ± 1.03 in left ventricular end-systolic volume, \( P < 0.001 \)). At the assessment of day 11, progesterone group showed no difference compared with other groups (Figure 2 and Table 4). Both ejection fraction and fractional shortening were not improved by progesterone treatment. Moreover, with respect to the structure of heart including left ventricular muscle mass, left ventricular end-diastolic volume, and left end-systolic volume, progesterone had no effect preventing left ventricular remodelling.

**Discussion**

The main finding in present study was that MPA improved survival in cancer cachexia model. However, echo cardiac evaluation showed no beneficial effect on cardiac function. We also found that progesterone had no beneficial effects on both muscle mass and fat mass wasting. As we reported before, megace improved survival and reduced wasting through a down-regulation of autophagy process in both skeletal muscle and cardiac muscle.\(^9\) We estimated reasons why progesterone did not show some effect on body composition, cardiac function, and appetite unlike megase. At first, from the action of a gender hormone point of view, MPA is a full agonist of the androgen receptor (AR), while MA is an agonist of the progesterone receptor.\(^{23,24}\) The most significant difference between the two hormones is that MA has anti-androgenic activity, while MPA does not have.\(^{25}\) Therefore, MPA has anti-gonadotropic effect, which play major beneficial role in the female hormone dominant cancer such as

**Table 3** The baseline characteristics of cardiac function and structure

|               | Sham       | Placebo    | Progesterone (0.5 mg) | Progesterone (25 mg) | Progesterone (50 mg) |
|---------------|------------|------------|-----------------------|----------------------|----------------------|
| EF, %         | 84.89 ± 0.45 | 84.13 ± 0.59 | 85.06 ± 0.62          | 80.63 ± 0.87***      | 82.86 ± 0.81         |
| FS, %         | 49.07 ± 0.72 | 50.85 ± 0.73 | 50.17 ± 1.18          | 47.31 ± 1.13         | 49.82 ± 1.05         |
| SV, μL        | 205.2 ± 6.87 | 197.7 ± 3.72 | 205.3 ± 5.97          | 194.9 ± 7.24         | 197.0 ± 4.162        |
| LVID, days    | 6.48 ± 0.16  | 6.47 ± 0.06  | 6.76 ± 0.07           | 6.74 ± 0.12          | 6.72 ± 0.12          |
| LVEDV, μL     | 241.6 ± 7.32 | 235.4 ± 3.91 | 241.3 ± 6.56          | 242.1 ± 9.441        | 237.7 ± 4.37         |
| LVESV, μL     | 36.41 ± 1.99 | 36.65 ± 1.03 | 35.98 ± 1.63          | 47.21 ± 3.29***      | 40.72 ± 2.04         |
| LVID, mg      | 478.5 ± 21.31 | 499.9 ± 9.81 | 533.4 ± 16.62         | 534.5 ± 17.96        | 504.2 ± 15.64        |
| PWT sys, mm   | 2.69 ± 0.04  | 2.68 ± 0.03  | 2.71 ± 0.07           | 2.60 ± 0.06          | 2.69 ± 0.06          |
| HR, b.p.m.    | 424.5 ± 13.63 | 425.1 ± 3.56 | 436.7 ± 6.52          | 422.9 ± 6.95         | 410.4 ± 11.06        |

EF, ejection fraction; FS, fractional shortening; HR, heart rate; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; SV, stroke volume.

Mean ± SE.

\(^*P < 0.05.\)

\(^{**}P < 0.01.\)

\(^{***}P < 0.001\) vs. placebo.

**Table 4** The effect of progesterone on cardiac function and heart wasting

|               | Sham       | Placebo    | Progesterone (0.5 mg) | Progesterone (25 mg) | Progesterone (50 mg) |
|---------------|------------|------------|-----------------------|----------------------|----------------------|
| EF, %         | 81.65 ± 1.78 | 69.55 ± 1.75 | 73.96 ± 2.68          | 68.8 ± 2.58          | 76.76 ± 1.70         |
| FS, %         | 50.42 ± 1.52 | 39.64 ± 1.34 | 46.12 ± 1.92          | 39.97 ± 2.40         | 44.65 ± 2.16         |
| SV, μL        | 210.8 ± 9.87 | 117.7 ± 8.47 | 103.1 ± 11.84         | 116.2 ± 10.61        | 113.0 ± 20.66        |
| LVID, days    | 6.93 ± 0.08  | 5.62 ± 0.11  | 5.10 ± 0.17           | 5.80 ± 0.21          | 5.20 ± 0.35          |
| LVEDV, μL     | 258.4 ± 10.68 | 164.9 ± 9.30 | 136.6 ± 13.35         | 171.5 ± 15.43        | 148.3 ± 27.29        |
| LVESV, μL     | 47.52 ± 5.79 | 47.59 ± 3.05 | 33.55 ± 3.56          | 55.26 ± 7.34         | 35.32 ± 7.02         |
| LV mass, mg   | 568.2 ± 26.69 | 429.4 ± 11.61 | 441.7 ± 19.21         | 469.4 ± 27.03        | 436.8 ± 18.23        |
| PWT sys, mm   | 2.76 ± 0.04  | 2.27 ± 0.04  | 2.49 ± 0.09           | 2.40 ± 0.10          | 2.33 ± 0.06          |
| HR, b.p.m.    | 415.6 ± 12.62 | 339.1 ± 9.34 | 349.4 ± 20.77         | 352.1 ± 12.33        | 291.6 ± 21.92        |

EF, ejection fraction; FS, fractional shortening; HR, heart rate; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; SV, stroke volume.

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breast cancer treatment. On the other hand, the prevalence of hepatoma has been described more prevalent among men than among women (range 2.5:1 to 7:1) in clinical settings. Oestrogen or oestrogen receptors may suppress hepatocarcinogenesis through the regulation of MyD88-dependent IL-6 production and trigger cellular innate immunity. Thus, the roles of female hormones that play roles in hepatoma development have been established, while the roles of male hormone are still unknown.

Although AR seems to promote hepatocarcinogenesis in the early stage, there is less evidence describing a direct link between androgen or AR signalling and hepatoma progression in advanced stage. Moreover, it remains unclear whether AR expression can show the progression of hepatoma. Feng et al. reported that the AR can enhance the hepatocarcinogenesis by modulating cell cycle-related kinase-β-catenin activation signalling. Ma et al. reported that the incidence of carcinogen inducing hepatoma in AR-lacking mice was lower than in wild type mice despite serum testosterone concentration showed little difference. These findings may suggest AR rather than androgens play major roles in hepatocarcinogenesis. Moreover, Tavian et al. reported down-regulation of AR-mRNA in poorly differentiated hepatoma lesions. Other reports described that AR was up-regulated only in hepatoma <3 cm, while little expression of AR was seen in severe hepatoma. AR could control intrahepatic signalling. These findings seem that AR might be related to hepatocarcinogenesis in the early phase of hepatoma.

On the other hand, AR enhanced hepatoma cell anoikis by suppressing p38 phosphorylation according to Ma et al. This suggested that AR might be a suppressor of hepatocellular invasion in late stage of hepatoma. These opposite roles of AR in hepatoma might explain the controversial results in the previous reports. In this way, MPA may enhance AR that leads to the apoptosis in hepatoma model. When it comes to dose of progesterone, the reason why only 5 mg treatment group showed a better prognosis can be explained that MPA rarely cause any androgenic effect in precocious puberty, even at very high dose. In the present study, we used all male rats of 8 weeks of age. It may suggest that 50 mg high dose of progesterone did not express its androgenic effects. In this respect, MPA might play as a suppressor of hepatoma through an apoptosis in our study. However, the liver AR expression on rat models was not detected after progesterone treatment in our present study. Second, we showed that MPA has beneficial effects on neither body weight loss nor appetite loss. Some studies suggested that MPA or MA has effect on improving appetite loss and body weight loss. This may be because our cancer cachexia model showed severe condition of late phase of hepatoma in experiment animal model. In fact, Tessitore et al. suggested that an injected daily 5 mg of MPA could not prevent body weight loss, appetite, and tumour mass in AH-130 model rats as same as our model.

Third, one of the mechanisms related with appetite might be cytokines. In general, MPA has been described as an agent that can improve the appetite. Its effect is based on the reduction of cytokines or serotonin. Combined drug therapy including celecoxib and dietary intervention with MPA may improve outcomes in systemic immune metabolic syndrome, which are frequently present in advanced cancer. In randomized phase III clinical trial, combination of MPA or MA with eicosapentaenoic acid, L-carnitine, and thalidomide group showed significant improvement in the resting energy expenditure and reduction of interleukin-6 and increased appetite. However, because we have no data concerning cytokines, we could not conclude whether progesterone had effect on the reduction of cytokines or not. Further studies will be needed to elucidate the mechanism of the survival benefit.

Conclusions

Progesterone improves survival but has no beneficial effects on cardiac function, body weight, and food intake in this aggressive hepatoma cancer cachexia rat model.

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Conflict of interest

The authors declare that they have no conflict of interest.

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