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Erythropoietin improves cardiac wasting and outcomes in a rat model of liver cancer cachexia

Masakazu Saitoh [a,*], Michiyoshi Hatanaka [a,b], Masaaki Konishi [a], Junichi Ishida [a], Sandra Palus [a], Nicole Ebner [a], Wolfram Döhner [c], Stephan von Haehling [a], Stefan D. Anker [a], Jochen Springer [a]

[a] Innovative Clinical Trials, Department of Cardiology and Pneumology, University Medical Center Göttingen, Göttingen, Germany
[b] Medicinal Research Laboratories, Shionogi & Co., Ltd., Osaka, Japan
[c] Center for Stroke Research Berlin, Charite´ Medical School, Berlin, Germany

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ABSTRACT

Background: Erythropoietin administration, which is clinically used in cancer patients with cancer-induced anemia, has also potentially beneficial effects on nonhematopoietic organs. We assessed the effects of erythropoietin on cancer cachexia progression and cardiac wasting compared with placebo using the Yoshida hepatoma model.
Methods: Wistar rats were divided in a sham group (n = 10) and a tumor-bearing group (n = 60). The tumor-bearing group was further randomized to placebo (n = 28), 500 Unit/kg/day (n = 16) or 5000 Unit/kg/day of erythropoietin (n = 16). Body composition was measured using nuclear magnetic resonance spectroscopy, cardiac function using echocardiography, physical activity using infrared monitoring system.
Results: Tumor-bearing rats with high dose erythropoietin led to a significant improvement on survival compared with placebo (hazard ratio: 0.43, 95%CI: 0.20–0.92, p = 0.030), though low dose erythropoietin did not reach significance (hazard ratio: 0.46, 95%CI: 0.22–1.02, p = 0.056). Loss of body weight, wasting of lean mass, fat mass, and reduced physical activity were ameliorated in rats treated with both low and high doses of erythropoietin (p < 0.05, all). Moreover, reduced left ventricular mass and left ventricular systolic function were also ameliorated in rats treated with low and high doses of erythropoietin (p < 0.05, respectively).
Conclusions: Overall, the present data support that cardiac wasting induced by cancer cachexia plays an important role which leads to impaired survival, provided that the erythropoietin could be an effective therapeutic approach for cancer cachexia progression and cardiac wasting.

1. Introduction

Cancer cachexia is a multifactorial syndrome of involuntary weight loss, fat or muscle loss and poor quality of life, and correlates with high mortality rate [1]. Moreover, it has been revealed that cachexia related clinical manifestation also includes wasting or dysfunction of tissue/organ such as brain, liver, gut and heart [2]. It has been reported that cardiac muscle wasting is associated with cancer cachexia leading to impaired cardiac function in animal models [3] and humans [4]. Previously our group showed that cardiac wasting was improved by treatments with either the β-blocker bisoprolol or the aldosterone antagonist spironolactone, resulting in improved quality of life and survival in tumor-bearing rats [5].

Cancer related clinical manifestations also include anemia, which contribute to fatigue, impaired quality of life, and poor survival [6–8]. Erythropoietin administration, which is clinically used in patients with cancer-induced anemia [9,10], has also potentially beneficial effects on nonhematopoietic organs. Although specific receptor of erythropoietin is present in skeletal muscle, adipose tissue, and heart in murine models [11–13], beneficial effects of erythropoietin on cardiac wasting are not well known in cancer cachexia model.

The Yoshida hepatoma model is well-established cancer cachexia model, is known to have cardiac wasting and high mortality rate [5,14]. In this study, we investigate the effects of erythropoietin on cancer cachexia progression, cardiac wasting, and physical performance status using the Yoshida hepatoma model.

2. Materials and methods

2.1. Animals and cachexia model

Male Wistar-Han rats were housed in a specific-pathogen-free facility under a 12 h light/dark cycle with food and water provided ad libitum. On the first day of experiment (D0), rats were injected intraperitoneally with 10^8 growing Yoshida AH-130 tumor cells, as described

* Corresponding author at: Innovative Clinical Trials, Department of Cardiology and Pneumology, University Medical Centre Göttingen, Robert-Koch-Str. 40, 37075 Göttingen, Germany.
E-mail address: msaitoh@shi.heart.or.jp (M. Saitoh).

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previously [15]. After 5 days, rats developed large tumors, and after 10 days, rats were severely cachectic.

2.2. Experimental design

Rats were divided into two groups: sham group (n = 10) and tumor-bearing rats (n = 60). The tumor-bearing rats were further randomized to placebo (n = 28), 500 Units/day of erythropoietin (500 U EPO, n = 16) or 5000 Units/day erythropoietin (5000 U EPO, n = 16). 500 Units/kg/day or 5000 Units/kg/day were administered s.c. daily. Erythropoietin administration began on the next day of tumor inoculation. The placebo group received saline injections.

All animal manipulations were made in accordance with the European Community guidelines for the use of laboratory animals. Study protocol and study procedures were approved by the local animal ethics committee.

2.3. Body composition

On D0 and day 16 or day of animals' euthanasia (D16), nuclear magnetic resonance spectroscopy (EchoMRI-700TM, Echo Medical Systems, Houston, TX) was performed to assess body composition of each rat [16]. Body composition was recorded after removal of the tumor, or the respective days of killing, if rats had to be euthanized earlier due to reaching ethical endpoints. Weight of heart was recorded, and tumor cells were counted using a Neubauer chamber.

2.4. Echocardiography

Rats were anesthetized using 1.5% isoflurane and laid in supine position on a heating pad to maintain body temperature at 39 °C. All hair was removed from the left chest. A high-resolution echocardiography system (Vevo 770; Visual Sonics Inc., Toronto, Canada) was used [17]. Echocardiography was performed in M-mode to measure cardiac function and dimensions, and in B-mode to calculate functional parameters. Echocardiography was performed 1 day before starting the treatment and day 11 (D11).

2.5. Proteasome activity

Proteasome activity was analyzed, as described previously [5]. Briefly, the gastrocnemius muscle was homogenized in an ice-cold buffer. Protein was incubated with fluorogenic substrates (benzoyl-Val-Gly-Arg-7-amidocoumarin for trypsin-like activity, succinyl-Leu-Leu-Val-Try-7-amido-4-methylcoumarin for chymotrypsin-like activity, and benzyloxycarbonyl-Leu-Leu-Glu-7-amido-4-methylcoumarin for peptidylglutamyl peptidase activity, Biomol, Hamburg, Germany). The fluorescence intensity was measured with a fluorometer (Twinkle LB 970, Berthold, Bad Wildbad, Germany) at 360 and 460 nm emission. The activity, expressed as nanomole per milligram per minute, was calculated by using free amidomethylcoumarin as a working standard.

2.6. Physical performance status

Food intake and spontaneous physical activity were recorded on D11. Spontaneous physical activity was measured by an infrared monitoring system (Supermex, Muromachi, Tokyo, Japan) over a 24 h period as described previously [18].

2.7. Statistical analysis

Data were analyzed with GraphPad PRISM 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Results are shown as mean ± standard error of mean. For the comparisons among groups, data were analyzed with analysis of variance followed by post hoc comparisons using Tukey's

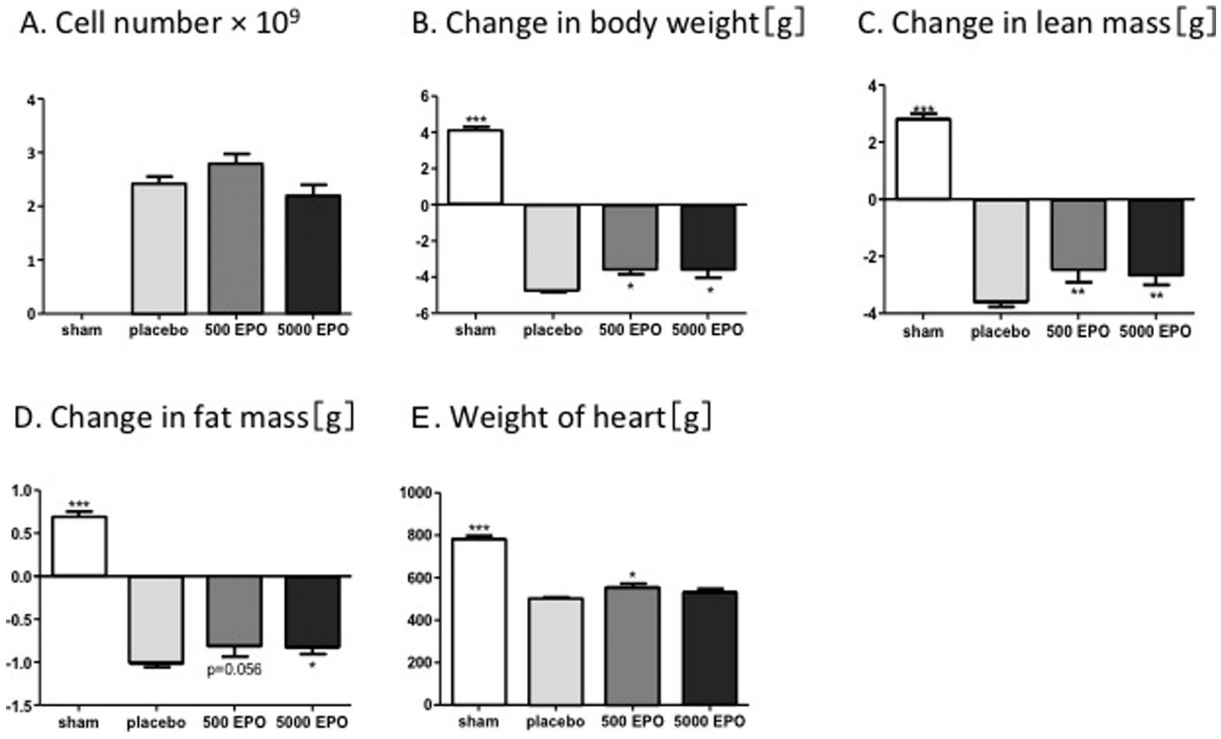

Fig. 1. Total tumor cell numbers × 10^9 (A): there was no significant difference in tumor cell numbers between rats with placebo and rats with EPO groups. Change in body weight (B), lean mass (C), fat mass (D): average loss of body weight, lean mass, and fat mass was higher in placebo group than both 500 U EPO group and 5000 U EPO group at the end of the study (500 U EPO: $p < 0.05$ for body weight, $p < 0.01$ for lean mass, and $p = 0.056$) and (5000 U EPO: $p < 0.05$ for body weight, $p < 0.01$ for lean mass, and $p < 0.05$ for fat mass). Weight of heart at the end of the study (E): weight of heart was higher in rats with 500 U EPO than in rats with placebo ($p < 0.05$). Abbreviation: Erythropoietin, EPO. *$p < 0.05$ vs. placebo, **$p < 0.01$ vs. placebo, and ***$p < 0.001$ vs. placebo.