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# Simvastatin reduces wasting and improves cardiac function as well as outcome in experimental cancer cachexia

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ABSTRACT

*Background:* Chronic inflammation is common in cancer cachexia (CC) and directly involved in the atrophy seen in this condition. Recently, several groups have described a form of cardiomyopathy in CC animal models. Hence, we investigated the effect of simvastatin with its known anti-inflammatory and cardioprotective effects in a rat model of CC.

*Methods:* Juvenile Wister Han rats (weight approx. 200 g) were inoculated with Yoshida AH-130 hepatoma cells and treated once daily with 0.1, 1, 10 or 20 mg/kg/d simvastatin or placebo for 14 days. Body weight and body composition (NMR) were assessed at baseline and at the end of the study. Cardiac function was analysed by echocardiography at baseline and day 11.

*Results:* Tumour-bearing, placebo-treated rats lost 47.9 ± 3.8 g of their initial body weight. Treatment with 0.1, 1, 10 or 20 mg/kg/d simvastatin significantly reduced wasting by 39.6%, 47.6%, 28.5% and 35.4%, respectively (all $p < 0.05$ vs. placebo). This was mainly due to reduced atrophy of lean mass, i.e. muscle mass. Cardiac function was significantly improved, e.g. cardiac output (untreated sham: 78.9 mL/min) was severely impaired in tumour-bearing rats (42.4 mL/min) and improved by 1, 10 or 20 mg/kg/d simvastatin (62.2, 59.0 and 57.0 mL/min, respectively, all $p < 0.05$ vs. placebo). Most importantly, 10 or 20 mg/kg/d simvastatin reduced mortality (HR:0.16, 95%CI:0.04–0.76, $p = 0.021$ and HR:0.16, 95%CI:0.03–0.72, $p = 0.017$ vs placebo, respectively).

*Conclusion:* Simvastatin attenuated loss of body weight as well as muscle mass and improved cardiac function leading to improved survival in this CC model. Simvastatin may be beneficial in a clinical setting to treat CC.



## 1. Introduction

Cachexia, i.e. involuntary weight loss, is a multifactorial process that represents a major unmet medical need particularly in patients with advanced cancer [1]. It is estimated that 25% of all cancer patients die as a result of cachexia [2]. Anti-cachectic therapy in patients with cancer cachexia is so far limited to nutritional support and anabolic steroids [3,4], the latter being associated with significant adverse effects [5]. Cachexia not only affects skeletal, but also cardiac muscle, which severely impairs cardiac function [6,7]. Likewise, fat tissue is wasted during the course of cachexia progression [8]. Drugs that prevent or slow down the development of cachexia may help to maintain quality of life and improve outcome.

For the past 20 years, 3-hydroxy-3methylglutaryl conenzyme A reductase inhibitors (statins) have been the drug of choice for the treatment of dyslipidemia [9]. It has been acknowledged for almost the same time span that statins possess effects beyond cholesterol reduction, so-called pleiotropic effects [10]. The latter effects make statins interesting candidates for the treatment of body wasting in the progression of cancer. Simvastatin is a particularly interesting candidate in this regard, as it was recently shown to decrease the development of matrix metalloproteinase-9, an effect that could be involved in reduced invasiveness of several cancer cell lines [11,12]. In addition, statins have been shown to reduce the activity of the proteasome [13], which is considered to be the main mechanism of muscle wasting in humans [14]. Simvastatin has also been shown to reduce inflammatory parameters like C-reactive protein and interleukin-1β in patients with cardiovascular diseases [9] or sepsis [15]. Moreover, statins have been described to reduce the activity of activator protein-1 (AP-1)

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**Table 1**
Total number of cancer cells at the end of the study. Baseline and end of study data on body weight (BW), lean mass and fat mass. Baseline and day 11 data on food intake and spontaneous locomotor activity.

| | Sham | | | | | Tumour-bearing | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Placebo | Simvastatin | | | | Placebo | Simvastatin | | | |
| Dose | | 0.1 mg/kg/d | 1 mg/kg/d | 10 mg/kg/d | 20 mg/kg/d | | 0.1 mg/kg/d | 1 mg/kg/d | 10 mg/kg/d | 20 mg/kg/d |
| n | 8 | 8 | 8 | 8 | 8 | 23 | 15 | 16 | 15 | 16 |
| Cells [$\times 10^9$] | | | | | | 6.32 ± 0.54 | 6.29 ± 0.71 | 6.56 ± 0.46 | 7.59 ± 0.55 | 7.80 ± 0.42 |
| BW day 0 [g] | 196 ± 5 | 201 ± 3 | 196 ± 4 | 195 ± 7 | 199 ± 8 | 197 ± 2 | 203 ± 3 | 198 ± 3 | 198 ± 1 | 201 ± 3 |
| BW day 14 [g] | 257 ± 5 | 255 ± 3 | 259 ± 4 | 250 ± 9 | 257 ± 6 | 150 ± 4 | 174 ± 7 | 173 ± 9 | 164 ± 3 | 170 ± 4 |
| Lean day 0 [g] | 153 ± 4 | 157 ± 3 | 152 ± 3 | 153 ± 5 | 153 ± 6 | 152 ± 1 | 157 ± 2 | 154 ± 2 | 152 ± 1 | 155 ± 2 |
| Lean day 14 [g] | 195 ± 5 | 197 ± 3 | 194 ± 4 | 190 ± 7 | 195 ± 5 | 117 ± 4 | 137 ± 6 | 137 ± 7 | 127 ± 3 | 133 ± 3 |
| Fat day 0 [g] | 15.1 ± 0.8 | 14.8 ± 0.8 | 14.3 ± 0.6 | 15.4 ± 0.7 | 16.8 ± 0.7 | 16.3 ± 0.5 | 15.4 ± 0.3 | 15.5 ± 0.8 | 17.2 ± 0.6 | 15.9 ± 0.5 |
| Fat day 14 [g] | 24.3 ± 0.8 | 22.0 ± 0.8 | 19.6 ± 0.8 | 24.1 ± 1.5 | 23.6 ± 0.7 | 5.5 ± 0.6 | 6.6 ± 1.3 | 6.7 ± 0.9 | 7.3 ± 0.9 | 6.9 ± 0.6 |
| Food day 0 [g/24 h] | 19.0 ± 0.5 | 20.5 ± 0.5 | 18.0 ± 3.3 | 20.1 ± 1.2 | 19.5 ± 1.9 | 18.8 ± 0.6 | 19.3 ± 0.6 | 18.6 ± 0.6 | 20.5 ± 0.9 | 18.6 ± 0.5 |
| Food day 11 [g/24 h] | 22.2 ± 0.8 | 19.3 ± 3.3 | 21.0 ± 1.1 | 20.1 ± 0.7 | 21.3 ± 1.0 | 6.4 ± 0.8 | 10.6 ± 1.1 | 11.4 ± 2.1 | 8.7 ± 1.5 | 8.8 ± 0.9 |
| Activity day 0 counts/24 h] | 79,382 ± 7410 | 82,277 ± 7038 | 83,418 ± 5147 | 74,623 ± 3904 | 76,789 ± 4383 | 74,985 ± 2707 | 77,205 ± 3485 | 79,085 ± 3432 | 79,132 ± 2547 | 76,318 ± 4896 |
| Activity day 11 [counts/24 h] | 74,779 ± 3677 | 69,441 ± 5142 | 73,862 ± 4979 | 63,199 ± 3393 | 71,775 ± 2798 | 38,557 ± 2521 | 49,178 ± 2838 | 49,059 ± 2929 | 41,808 ± 2567 | 40,590 ± 3067 |

and Nuclear Factor kappa B (NFκB), two transcription factors with prominent roles in inflammation [9]. These effects render simvastatin an interesting candidate for the treatment of cachexia in cancer.

We aimed to assess the effects of simvastatin on body weight, body composition, cardiac function, and quality of life as well as survival in a rat Yoshida AH-130 hepatoma cancer cachexia model.

## 2. Methods

### 2.1. Animal model

Juvenile Wistar Han rats (n = 129, body weight: 199.3 ± 1.1 g) were first randomized to be injected with either Yoshida $10^8$ AH-130 hepatoma cells or saline (=sham) into the peritoneum and the animals were monitored over a period of maximal 14 days. This randomization was aimed to ensure an equal distribution of rats based on their initial body weight into sham or tumour-bearing groups. The Yoshida hepatoma model was chosen, because the tumour growth is very well characterized and the tumour does not develop metastases. The tumour causes the formation of ascites fluid in the peritoneum, in which the tumour cells grow free-floating. The growth itself is linear until day 7, when the growth rate slows and it plateaus around day 12 [16]. Before tumour inoculation, baseline weight, body composition and quality of life indicators (spontaneous activity and food intake) were assessed. Tumour-bearing rats were further randomized to be treated with placebo (n = 25), 0.1 mg/kg/d simvastatin (n = 16), 1 mg/kg/d simvastatin (n = 16), 10 mg/kg/d simvastatin (n = 16) or 20 mg/kg/d simvastatin (n = 16). Two rats randomized to the tumour placebo group, one to the 0.1 mg/kg/d and one to the 10 mg/kg/d simvastatin groups had to be excluded, because of development of subcutaneous solid tumours at the injection site. Sham rats were further randomized to be treated with placebo (n = 8), 0.1 mg/kg/d simvastatin (n = 8), 1 mg/kg/d simvastatin (n = 8), 10 mg/kg/d simvastatin (n = 8) or 20 mg/kg/d simvastatin (n = 8). All treatments were given per gavage once daily. A simvastatin suspension was prepared with sterile water for daily treatment, which was given per gavage to ensure the precise intake of simvastatin in each individual rat. Rats were housed in groups of two or three under standard laboratory conditions in a SPF-facility and given access to food and water ad-libitum. Body composition and weight were recorded on day 14 or the respective days of euthanasia, if an animal was euthanized before day 14 due to reaching ethical endpoints [17]. Locomotor activity and food intake were determined on day 10/11. Cardiac function was analysed on day 11. At the end of the study, organs were removed and weighed. Tumour cell number was determined using a Neubauer chamber at the end of the study. All staff handling the rats was blinded to treatment allocation.

### 2.2. Body composition

Body composition (fat and lean body mass) was analysed with an NMR spectroscopy device EchoMRI-700TM (Echo Medical Systems, Houston, TX) as described before [18].

### 2.3. Quality of life indicators

Animals were housed individually and spontaneous movement was recorded by an infrared monitoring system (Supermex, Muromachi, Tokyo, Japan) over a 24 h period. Food intake was recorded during this period [19].

### 2.4. Echocardiography

Echocardiography using the high resolution Vevo770 system (Visual Sonics, Toronto, Canada) was performed as described before [20]. Briefly, rats were anaesthetized with 1.5% isofluran, laid in supine position and the hair was removed from the left chest. Recordings were made in B- and M-mode to calculate functional parameters and determine cardiac dimensions.

### 2.5. Statistics

Data were analysed using GraphPad PRISM 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Results are shown as mean ± standard error of mean (SEM). Data of rats that were euthanized before day 14 were included all calculations and the bias they represent was considered to be acceptable without correcting for day of death. Data were tested for normal distribution with the Kolmogorov Smirnov test. Data with normal distribution were analysed by analysis of variance (ANOVA) followed by Tukey's test, while data without normal distribution were analysed by Kruskal–Wallace and Dunns test. A p-value of $<0.05$ was considered significant.