

IGF-1 treatment reduces weight loss and improves outcome in a rat model of cancer cachexia

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Abstract

Background A hallmark symptom of cancer cachexia is the loss of skeletal muscle. This is at least partially due to a deregulation of the growth hormone/IGF-1 axis and a subsequently impaired protein synthesis in skeletal muscle. Here, we investigated the effect of IGF-1 supplementation in a rat model of cancer cachexia.

Methods Juvenile rats were inoculated with the Yoshida AH-130 hepatoma and treated once daily with 0.3 mg kg⁻¹ day⁻¹ (low dose) or 3 mg kg⁻¹ day⁻¹ (high dose) IGF-1 or placebo for a period of maximal 16 days. Body weight and body composition (by NMR) were assessed at baseline and at the end of the study or day of death. Locomotor activity and food intake were assessed at baseline and day 10/11 after tumour inoculation for 24 h.

Results Untreated tumour-bearing rats lost 55.3±2.14 g body weight, which was reduced by low-dose to -39.6±11.1 g ($p=0.0434$) and high-dose IGF-1 to -42.7±8.8 g ($p=0.057$). Placebo-treated rats lost 41.4±2.0-g lean mass, which was attenuated by low-dose IGF-1 (-28.8±8.3 g, $p=0.041$) and high-dose IGF-1 (-30.9±7.4, $p=0.067$). Spontaneous activity and food intake were improved by low-dose IGF-1 only. No effect on fat mass was observed. Low-dose IGF-1 significantly reduced mortality (HR=0.45, 95%CI=0.21–0.93, $p=0.0315$), whilst the high dose did not reach significance (HR=0.68, 95%CI=0.26–1.74, $p=0.42$).

Conclusion Low-dose IGF-1 reduced mortality and attenuated loss of body weight as well as muscle mass in the Yoshida hepatoma rat model. Moreover, an improved quality of life was observed in these animals. Further experiments using different doses are necessary.

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Insulin like growth factor-1 · Survival · Body composition

1 Introduction

Patients who suffer from cancer cachexia have a poor quality of life, show increased rates of morbidity and mortality, as well as a poorer responsiveness to antineoplastic therapies [1]. It is estimated that 25% of all cancer patients die as a result of cachexia, which is a multifactorial process [2, 3]. In these patients, both proteins and fat are lost due to wasting. Skeletal muscle accounts for approximately 50% of the body's protein; hence, it is severely affected by the condition [4]. At the same time, the major intrinsic anabolic factor insulin-like growth factor 1 (IGF-1), which enhances skeletal muscle protein synthesis and proliferation and differentiation of satellite cells, is reduced

cancer cachexia [5]. The effects of IGF-1 on skeletal muscle are mediated mainly by the PI3K/Akt pathway leading to the downstream activation of protein synthesis [6] and inhibition of FOXO-1, which regulates the expression of the rate-limiting ubiquitin E3-ligases Murf-1 and MAFbx, thereby reducing proteosomal protein degradation [7, 8]. Recombinant IGF-1 has clinically been used in growth hormone-resistant growth defects in children [9]. However, there have been concerns about side effects that include arthralgia and hyperplasia of lymph nodes, and an increased risk for malignancy has been discussed [10]. Interestingly, a study in children with growth impairment describes the profound effect of IGF-1 leading to increased BMI and fat mass [11].

Anti-cachectic therapy in patients with cancer cachexia is so far limited to anabolic steroids, which have not been very successful and may have serious side effects such as kidney failure [12]; therefore, treatment alternatives are urgently needed. Here, the effects of IGF-1 treatment on body weight, body composition and survival were assessed in the rat Yoshida hepatoma cancer cachexia model.

2 Methods

2.1 Animal model

Juvenile Wistar Han rats (weight approx. 200 g) were injected with 10^8 AH-130 cells into the peritoneum, and the animals were monitored over a period of maximal 16 days. Before tumour inoculation, the baseline weight, body composition, quality of life indicators (spontaneous activity and food intake) were assessed. Rats were randomized into placebo ($n=37$), 0.3 mg kg⁻¹ day⁻¹ IGF-1 ($n=14$) and 3 mg kg⁻¹ day⁻¹ IGF-1 ($n=15$). IGF-1 was obtained from the National Hormone & Peptide Program (Harbor-UCLA Medical Center, Torrance, USA). IGF-1 or placebo (saline) was injected subcutaneously once daily. Body composition and weight were recorded on day 16 or the respective days of killing, if an animal was killed prematurely due to reaching ethical endpoints. Locomotor activity and food intake were determined on day 10/11. At the end of the study, plasma was collected and the organs were removed and weighed. Tumour cell number was determined using a Neubauer chamber.

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 [13].

2.3 Quality of life indicators

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

2.4 Statistics

Data were analysed using GraphPad PRISM 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). The results are shown as mean±SEM. The data were tested for normal distribution with the Kolmogorov–Smirnov test. Data with normal distribution were analysed with ANOVA followed by Student's *t* test, whilst data without normal distribution were analysed by Kruskal–Wallis and Mann–Whitney *U* test. Survival was assessed by Cox proportional hazard analysis. A *p* value of <0.05 was considered significant.

3 Results

No effect of IGF-1 treatment was observed on tumour growth; total cell numbers at the end of the study were $2.83\pm 0.42\times 10^9$ and $2.50\pm 0.28\times 10^9$ cells for 0.3 and 3 mg kg⁻¹ day⁻¹ IGF-1, respectively, whilst untreated rats had a mean of $2.21\pm 0.23\times 10^9$ cells ($p>0.2$ vs. both IGF-1 doses). No signs of other malignancies were observed during necropsy.

3.1 Body weight and body composition

The overall weight loss at the end of the study or day of death was highest in the placebo group (-55.3 ± 2.14 g) and was reduced by both low-dose (-39.6 ± 11.1 g, $p=0.0434$) and high-dose IGF-1 (-42.7 ± 8.8 g, $p=0.057$). This is also seen when the average weight loss per day alive is calculated (Fig. 1a). As expected, treatment with IGF-1 reduced the wasting of lean body mass, i.e. skeletal muscle. Placebo-treated rats lost 41.4 ± 2.0 g lean mass, which was attenuated by low-dose IGF-1 (-28.8 ± 8.3 g, $p=0.041$) and high-dose IGF-1 (-30.9 ± 7.4 , $p=0.067$), which is also observed in loss of lean mass per day alive (Fig. 1b). Treatment had no effect on overall loss of fat mass (placebo, -11.5 ± 0.5 g; low-dose IGF-1, -12.4 ± 2 g; high-dose IGF-1, -11.0 ± 1.3 g, all $p>0.1$) or the loss of fat mass per day alive (Fig. 1c).

3.2 Organ weights

As expected, a strong effect on tumour burden was seen on organ weight. The weight of the liver, spleen, kidney, brown adipose tissue (BAT), M. extensor digitalis longus

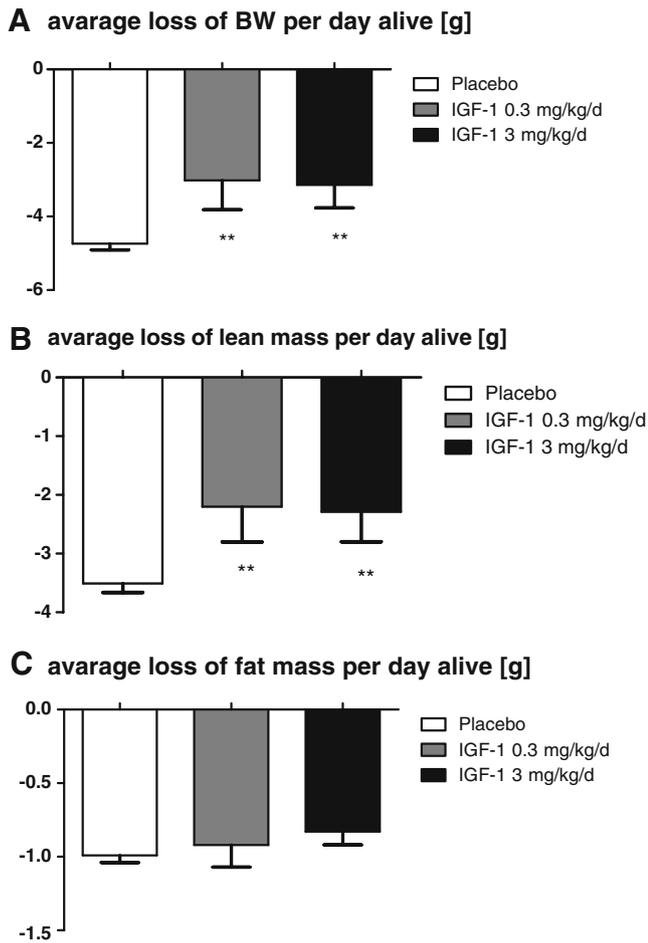


Fig. 1 Average loss of body weight (a), lean body mass (b) and fat mass (c). Data were calculated as change per day alive. IGF-1 reduced loss of body weight and lean mass, but had no effect on fat mass. ** $p < 0.01$

(EDL), M. tibialis and M. gastrocnemius (Table 1 and Fig. 2) was improved by low-dose IGF-1. The high dose only improved the weight of BAT, EDL, M. tibialis and M. gastrocnemius (Table 1 and Fig. 2). Heart, lung, white adipose tissue (WAT, epididymal fat pad) and M. soleus did not show any changes in weight (Table 1).

3.3 Quality of life

Spontaneous activity and food intake are indicators for the quality of life of an animal. Baseline 24-h spontaneous activity and food intake were similar in all groups (all $p > 0.2$, data not shown). The activity was reduced by tumour burden in all groups on day 10/11. Low-dose IGF-1 attenuated the reduced movement (Fig. 3a), whilst high-dose IGF-1 was ineffective ($p > 0.1$). This was also observed for food intake in the high-dose group, whilst low-dose IGF-1 increased food intake vs. placebo (Fig. 3b).

3.4 Survival

The overall survival was 5% in the placebo group, 21% in the low-dose and 20% in the high-dose IGF-1 groups (Fig. 4). The median survival was 12 days in the placebo group, 14 days in the low-dose and 13 days in the high-dose IGF-1 groups. Only low-dose IGF-1 significantly reduced mortality (HR=0.45, 95%CI=0.21–0.93, $p = 0.0315$), whilst the high dose did not reach significance (HR=0.68, 95%CI=0.26–1.74, $p = 0.42$).

4 Discussion

We have shown that the tumour growth was not affected by IGF-1, but the loss of body weight was attenuated, the

Table 1 Organ and tissue weights at day 16 (end of study) or day of death

Organ weight (mg)	Placebo (n=37)	IGF-1 0.3 mg (n=14)	IGF-1 3 mg (n=15)
BW baseline (g)	210±2	213±3	211±3
BW end of study (g)	151±3	174±11**	168±10*
heart	505.21±14.00	539.56±31.57	528.51±25.81
lung	885.22±29.57	1,023.54±108.44	925.11±66.35
liver	6,093.39±227.81	7,399.51±440.45*	6,756.66±490.83
spleen	163.64±10.01	266.42±49.29**	217.97±41.39
kidney	698.41±16.58	794.21±24.74**	744.65±25.94
WAT	86.38±26.43	301.20±126.86	146.42±70.90
BAT	78.59±3.14	123.18±14.80***	109.00±14.39**
Gastrocnemius	713.22±14.07	847.58±51.37**	818.84±52.38*
Soleus	69.73±1.43	76.70±3.49	73.58±3.91
Tibialis	262.49±6.10	325.06±20.48***	295.74±20.11*
EDL	63.66±1.48	74.04±5.11*	71.98±4.24*

* $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$

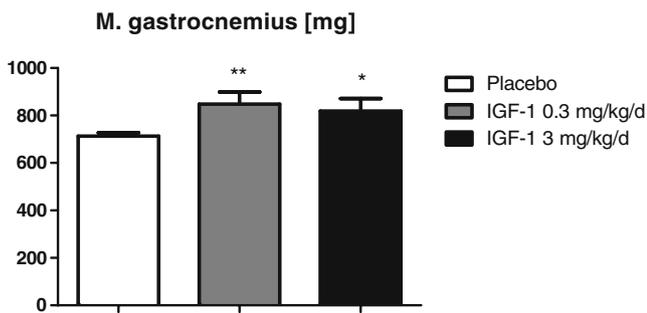


Fig. 2 Weight of the M. gastrocnemius at the end of the study or the day of death. IGF-1-treated groups have a higher muscle weight. $**p < 0.01$

quality of life was improved, and the survival was increased by treatment with IGF-1.

A previous study has shown that IGF-1 levels are lower in the liver and skeletal muscle of Yoshida AH-130 hepatoma bearing rats, suggesting that impaired IGF-1 signalling is involved in the pathogenesis of muscle wasting in tumour-bearing rats [5]. A recent publication using plasmid gene transfer of IGF-1 into M. tibialis of tumour-bearing mice and rats showed resistance to IGF-1, whilst the same gene transfer in young and old animals increased the fibre size [14]. Contrary to these results, a reduction of weight loss was seen in both Yoshida hepatoma-bearing groups treated with recombinant IGF-1 in the present study, where lean body mass loss was reduced. This finding is supported by the higher weights of the mixed fibre type

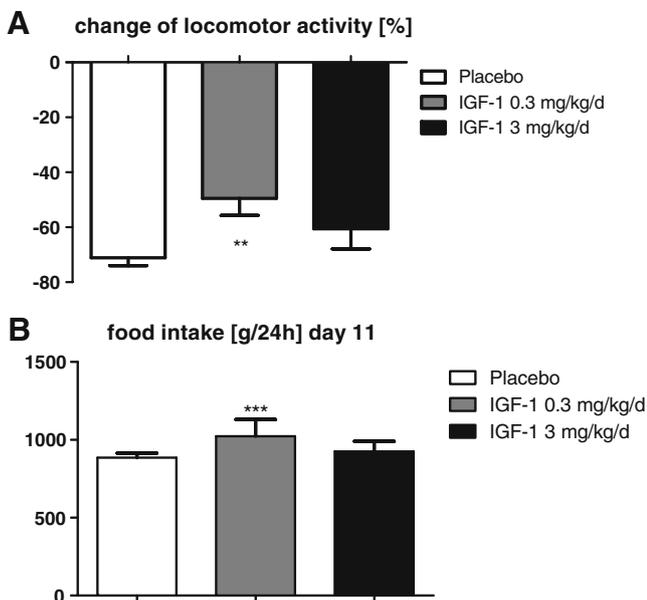


Fig. 3 Quality of life: 0.3-mg $\text{kg}^{-1} \text{day}^{-1}$ IGF-1 improved spontaneous activity, as shown by an attenuated reduction compared with baseline values (a) and total food intake per 24 h (b) on day 10/11 compared with placebo, whilst 3 mg $\text{kg}^{-1} \text{day}^{-1}$ was ineffective. $**p < 0.01$, $***p < 0.001$

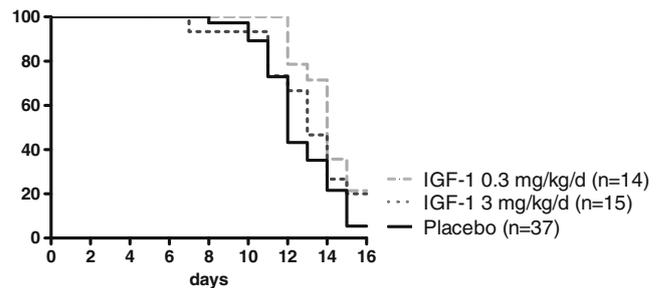


Fig. 4 Kaplan–Meier survival curves: 0.3-mg $\text{kg}^{-1} \text{day}^{-1}$ IGF-1 reduced mortality compared with placebo; 3 mg $\text{kg}^{-1} \text{day}^{-1}$ is ineffective

muscles M. gastrocnemius and M. tibialis, as well as the fast-twitch muscle EDL, whilst the weight of the slow-twitch M soleus was not increased. The contrary results to the study by Penna et al. [14] are likely due to the difference in IGF-1 administration and dose. However, our results also suggest that IGF-1 is not able to fully prevent skeletal muscle wasting in tumour-bearing rats and that increasing the dose does not produce better results as the 0.3-mg $\text{kg}^{-1} \text{day}^{-1}$ dose was superior to 3-mg $\text{kg}^{-1} \text{day}^{-1}$ IGF-1. Together with the lack of effect on the liver, kidney and spleen weight, this may point to the serious side effects of high-dose IGF-1, which do not seem to include increased tumour cell proliferation. Overall, the incomplete prevention of muscle atrophy may be due to the increased expression of negative regulators of muscle mass such as myostatin [15] or the inflammatory cytokines, which can impair IGF-1 bioavailability and signalling [16]. Furthermore, the half-life of IGF-1 is short [17], which limits its activity in our study. Ng et al. [18] used a continuous infusing of IGF-1 in Fischer 344 rats bearing a fibrosarcoma and showed a preservation of lean mass. In the present study, treatment had no effect on overall fat mass, supported by the unchanged weight of the epididymal fat pad, but the weight of brown adipose tissue was higher in IGF-treated rats, suggesting an improved thermogenesis [19].

Locomotor activity and food intake are considered quality of life indicators in animals [20]. Compared with baseline, a strong decrease in activity and food intake was seen in all groups 10 days after tumour inoculation, but the group treated with low-dose IGF-1 had significantly improved food intake and a higher activity compared with placebo, suggesting an improved quality of life. High-dose IGF-1 was ineffective.

The Yoshida hepatoma model has been known to have a high mortality rate in untreated tumour-bearing rats [21], which is in accordance with the results of this study. Both doses of IGF-1 reduced the risk of death (HR=0.45 and 0.68 for the low and high doses, respectively); however, only the low dose significantly improved outcome compared with placebo.

In the 0.3-mg kg⁻¹ day⁻¹ IGF-1 group, reduced mortality and attenuated loss of body weight, as well as muscle mass, were seen in the Yoshida hepatoma rat model. Moreover, an improved quality of life was observed in these animals. The 3-mg kg⁻¹ day⁻¹ IGF-1 dose was likely too high and had weaker effects compared with the lower dose. This may be due to a decreased sensitivity of target tissue or even general toxic effects. Therefore, further experiments using different doses are necessary.

In conclusion, IGF-1 attenuated the development of cachexia, improved quality of life and survival. The treatment had no effect on tumour growth. The therapeutic approach of IGF-1 for tumour-associated cachexia should be tested in further studies. IGF-1 may be an interesting candidate for a multidrug approach to treat cancer cachexia.

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Conflict of interest All authors declare that they have no conflict of interests regarding this present study.

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