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The erythropoietin-derived peptide ARA 284 reduces tissue wasting and improves survival in a rat model of cancer cachexia

Sandra Palus[1], Yulia Elkina[1], Tanja Braun[1], Stephan von Haehling[2], Wolfram Döhner[1,3], Stefan D. Anker[1,4], Anthony Cerami[5], Michael Brines[5] & Jochen Springer[1,4]*

[1]*Berlin Institute of Health Center for Regenerative Therapies (BCRT), Charité Universitätsmedizin Berlin, Berlin, Germany;* [2]*Department of Cardiology and Pneumology, University Medical Centre Göttingen, Göttingen, Germany;* [3]*Centre for Stroke Research Berlin, Charité Universitätsmedizin Berlin, Berlin, Germany;* [4]*German Centre for Cardiovascular Research (DZHK) partner site Berlin, Charité Universitätsmedizin Berlin, Berlin, Germany;* [5]*ARAIM Pharmaceuticals, Tarrytown, NY, USA*

Abstract

Background Cancer cachexia (CC) is a severe complication during the last stages of the disease, which is characterized by the substantial loss of muscle and fat mass. Currently, there is no effective treatment of CC. Erythropoietin plays tissue-protective role in different tissues. Based on the structure of erythropoietin, small non-erythropoietic peptides were synthesized, which activate tissue-protective signalling pathways.

Methods Here, we investigated the influence of the tissue-protective peptide ARA 284 on CC in rats using the Yoshida hepatoma model.

Results Treatment with ARA 284 (1.7 μg/kg/day) counteracted the loss of body weight (12.46 ± 4.82% ARA 284 vs. 26.85 ± 0.88% placebo, $P < 0.01$), fat mass ($P < 0.01$), and lean mass ($P < 0.01$). It improved spontaneous activity of ARA 284-treated animals. Further, gastrocnemius mass was increased (13.2% ARA 284 vs. placebo, $P < 0.01$) in association with induced p-Akt ($P < 0.01$) and decreased in p-p38 MAPK, GSK-3β, and myostatin (all $P < 0.01$), suggesting an induction of anabolic pathways. At the same time, we observed the significant increase in the survival of animals by high-dose ARA 284 treatment (hazard ratio: 0.46, 95% confidence interval: 0.23–0.94, $P = 0.0325$).

Conclusions Taken together these results suggest that ARA 284 can be considered beneficial in experimental CC and it remains to be seen, if it can have similar beneficial effects in CC patient.

Keywords Cancer cachexia; Tissue-protective molecule; Muscle wasting; Survival; Cardiac function

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**Correspondence to: Jochen Springer, Berlin Institute of Health Center for Regenerative Therapies (BCRT), Charité Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany. Phone: +49-30-450 539403; Fax: +49-30-450 553951. Email: jochen.springer@bih-charite.de*
SP and YE contributed equally to this manuscript.

Introduction

Cancer cachexia is a syndrome that significantly worsens the quality of life and outcomes in cancer patients. Its main feature is loss of more than 5% of body weight independent of fluid retention within 12 months or less, due to reduction in muscle and fat mass. Cancer cachexia is frequently associated with the development of fatigue, anorexia, abnormal biochemistry, and other clinical features.[1] Some types of cancer, for example, gastric or pancreatic cancer, can lead to cachexia in up to 85% of cases.[2] Unfortunately, no treatments have received approval for the treatment of cancer cachexia so far, and treatment approaches are urgently needed.

Erythropoietin (EPO) is a haematopoietic peptide hormone mainly released from peritubular capillary cells in the kidney.[3] It plays an important role in the development of erythropoietic progenitors in the bone marrow.[4] EPO can also be produced by many other cells of the body, for example, brain, kidney, or liver, in response to hypoxia or metabolic stress. In these tissues, EPO plays a pivotal role in tissue protection by preventing inflammation and apoptosis[5–7] and turning on repair of the damaged tissue. The receptor that conveys these functions is distinct from the one responsible for haematopoietic activity. In fact, the latter function is conveyed via the specific EPO-R homodimer receptor expressed by erythrocyte precursors. In contrast, tissue-protective effects are mediated via the innate repair receptor, which is composed of the EPO-R disulfide linked to CD131 commonly called β-common receptor.[8] Binding of EPO to this receptor complex activates a signalling cascade characteristic of other members of the type 1 cytokine receptor family, including activation of Janus tyrosine kinase 2, which subsequently activates distinct pathways[4] such as STAT, PI3K/Akt, and MAPK[9,10] depending upon the tissue. Activation of this receptor may open a therapeutic option for the treatment of cancer cachexia.

Recombinant human EPO has been used clinically to treat anaemia in patients with advanced chronic kidney disease, but also during cancer chemotherapy.[11] Research focused on the mechanisms of tissue protection showed that this effect can be achieved only in the presence of comparatively high concentrations of EPO. Such high EPO doses, however, may provoke clinically significant side effects such as hypertension or thrombosis.[12,13] Additionally, decreased survival in cancer patients treated with EPO has been suggested to be the result of the stimulation of tumour growth.[14]

The use of peptide sequences based on the structure of EPO[15] has been found to offer advantages over the use of the full EPO molecule,[16] for example, providing anti-inflammation and tissue protection without stimulating erythropoiesis (reviewed in Brines and Cerami[17]). Recently, a number of peptides derived from helices A, B, or C or the AB loop have been evaluated by independent investigators and shown to provide potent anti-inflammation and tissue protection (reviewed in Ercan *et al*.[18]). For example, we have prepared a number of peptides derived from structure of helix B of EPO which interact with the innate repair receptor and not the homodimer of EPO-R, providing significant benefit.[17] In the current study, we have investigated the effects of a partially overlapping peptide also derived from helix A of EPO, ARA 284, on the progression of cancer cachexia, a condition driven in part by inflammatory processes, in a rat model of cancer cachexia. Notably, Colella *et al*. have previously shown that a partially overlapping helix A EPO peptide protects from light-induced and genetic photoreceptor degeneration[19] in rodents without stimulating erythropoiesis.

Materials and methods

Peptide

ARA 284 is a 15 amino acid peptide of mw 1708 corresponding to positions 14–28 of helix A of EPO (RYLLEAKEAENITTG). The peptide was synthesized by standard F-moc solid phase peptide synthesis, purified by HPLC and ion-exchange chromatography, and stored as a lyophilized powder. ARA 284 was confirmed to possess tissue-protective properties using a standardized *in vitro* screening assay employing human umbilical vein endothelial cells exposed to the toxin staurosporine (refer to Supporting Information, *Figure* S1 and methods). Additional *in vivo* assessments including protection from renal ischemia–reperfusion and sciatic nerve crush injuries (data not shown) further confirmed that ARA 284 was a tissue-protective molecule. Confirmation that ARA 284 is not erythropoietic was determined using an *in vivo* haematopoiesis rodent model (refer to *Figure* S2 and methods). ARAIM Pharmaceuticals provided ARA 284.

Study design

Male Wistar Han rats aged 8 weeks with a mean weight of 204 ± 6 g (range 190–222 g) were housed in groups of three or four at a constant temperature of 22°C and exposed to a 12 h light cycle. Animals had free access to food and water. On Day 1, rats were inoculated intraperitoneally with 10^8 Yoshida hepatoma AH-130 cells. Blood samples and organs were collected on Day 16 or the day of death, if rats had to be euthanized earlier to comply with ethical standards. All organs were weighed before freezing. In addition, body weight and body composition by nuclear magnetic resonance[20] were assessed before tumour inoculation and on the day of sacrifice. Cardiac function (echocardiography) and quality of life indicators (spontaneous activity and food intake) were measured on Days 0 and 10/11 (for schematic overview, refer to *Figure* S3). Tumour cells were counted at the end of the study on Day 16 or on the day tumour-bearing rats had to be euthanized due to reaching ethical endpoints. All analyses were performed by an investigator blinded to treatment groups.

Treatment with ARA 284

Dose selection was based on the results of previous *in vitro* studies that have shown that tissue-protective peptides activate the innate repair receptor with potency approximately equal to that of EPO (reviewed in Collino *et al*.[21]). Further, *in vivo* study using a stroke model in the rat has shown that effective doses of EPO range between 500 and 5000 IU/kg body weight.[22] Therefore, rats were randomly allocated to receive sham or tumour-injection, and both groups further

randomized to one of three subcutaneous treatment arms: (1) low-dose (0.17 μg/kg/day, equivalent to ~500 IU of EPO) ARA 284 (n = 12), (2) high-dose (1.7 μg/kg/day, equivalent to ~5000 IU of EPO) ARA 284 (n = 22), or (3) saline (placebo, n = 24).[23] The group size in the sham groups was n = 5, 4 or 5 for placebo and 0.17 or 1.7 μg/kg/day ARA 284, respectively. ARA 284 was provided by ARAIM Pharmaceuticals (Tarrytown, New York, USA). Treatment was started on Day 1 after tumour inoculation. All study personnel were blinded to treatment allocation. Sham animals (n = 5) were not inoculated with tumour. All procedures were approved by the local animal use and care committee (G 0114/08 LaGeSo, Berlin, Germany).

Body composition

Body composition of animals was analysed as described before[20] using NMR scans (EchoMRI-700, Echo Medical Systems, Houston, Texas, USA).

Spontaneous activity and food intake

Animals were housed individually with 100 g of food and 300 mL of water, and their movement was measured by an infrared scanner over 24 h using Supermex activity monitoring system (Muromachi Kikai Co., LTD., Tokyo, Japan).[24]

Cardiac function

Echocardiography was performed at baseline and Day 11, as described previously.[25] Briefly, rats were anaesthetised using 1.5% isoflurane. Body temperature was monitored and maintained at 36–38°C using a heating pad. All hair was removed from the chest using a chemical hair remover. The inlet and inferior boundary of the thorax were marked, and the midline of the chest was drawn and divided into three equal segments. A high-resolution echocardiography system (Vevo 770; VisualSonics Inc, Toronto, Canada) was used in these studies.

Enzyme activities of the 20S proteasome

One hundred and fifty micrograms of protein samples from *gastrocnemius muscle* were used to measure three enzyme activities of the 20S proteasome (*ZLLE-AMC* for PGPH activity, *Bz-Val-G-A-AMV* for Trypsin-like activity, and *Suc-LLVY-AMC* for chymotrypsin-like activity). Sample preparation and determination of fluorescence intensity was performed as previously described.[26]

Western blotting analysis

Gastrocnemius muscles of randomly chosen animals from each group were used to assess the change of protein markers (ARA 284LD n = 9, ARA 284HD n = 10, placebo n = 14, and sham n = 5). Total protein was isolated according to standard protocols, and protease inhibitors (Complete Mini; Boehringer Mannheim, Indianapolis, IN, USA) and phosphatase inhibitors (p2850, Sigma-Aldrich) were used. Primary antibodies against the following proteins were used: Akt, phospho-Akt (Ser473), GSK-3β, phospho-GSK-3β(Ser9), p38 MAPK, phospho-p38 MAPK (all Cell Signalling), GAPDH (Sigma), and myostatin (R&D Systems) as well as appropriate secondary antibodies. GAPDH was used as an internal loading control. The protein bands from each membrane were normalized to the mean value of sham samples (defined as 100%) from the same membrane.

Statistics

Data were analysed with GraphPad PRISM 8.0 (GraphPad Software, Inc, La Jolla, CA, USA). Results were shown as mean ± SEM. Normally distributed data were analysed using analysis of variance and Dunnett's multiple comparisons test, non-normally distributed data were analysed using Kruskal–Wallis and Dunn's multiple comparisons test within the sham groups or tumour-bearing groups. Survival was tested by Cox-proportional hazard analysis giving hazard ratios with 95% confidence interval. A P value < 0.05 (two tailed) was considered to be statistically significant.

Results

ARA 284 did not have significant effects on the tumour itself. Total cells number on the day of euthanasia were $1.92 \pm 0.20 \times 10^9$, $2.00 \pm 0.39 \times 10^9$, and $2.09 \pm 0.35 \times 10^9$ for placebo, 0.17 μg/kg/day ARA 284 and 1.7 μg/kg/day ARA 284, respectively. The ascites volumes were 116 ± 2, 106 ± 6, and 110 ± 8 mL for placebo, 0.17 μg/kg/day ARA 284 and 1.7 μg/kg/day ARA 284, respectively.

Body weight and body composition

The intraperitoneal injection of 10^8 Yoshida hepatoma cells produces a rapid loss of weight that averaged 26.85 ± 0.88% of body weight (*Figure* 1). Daily treatment with dose ARA 284 (1.7 μg/kg/day; equivalent on a molar basis to ~5000 IU/kg EPO) until the day of euthanasia led to a significant attenuation of weight loss (12.46 ± 4.82%,

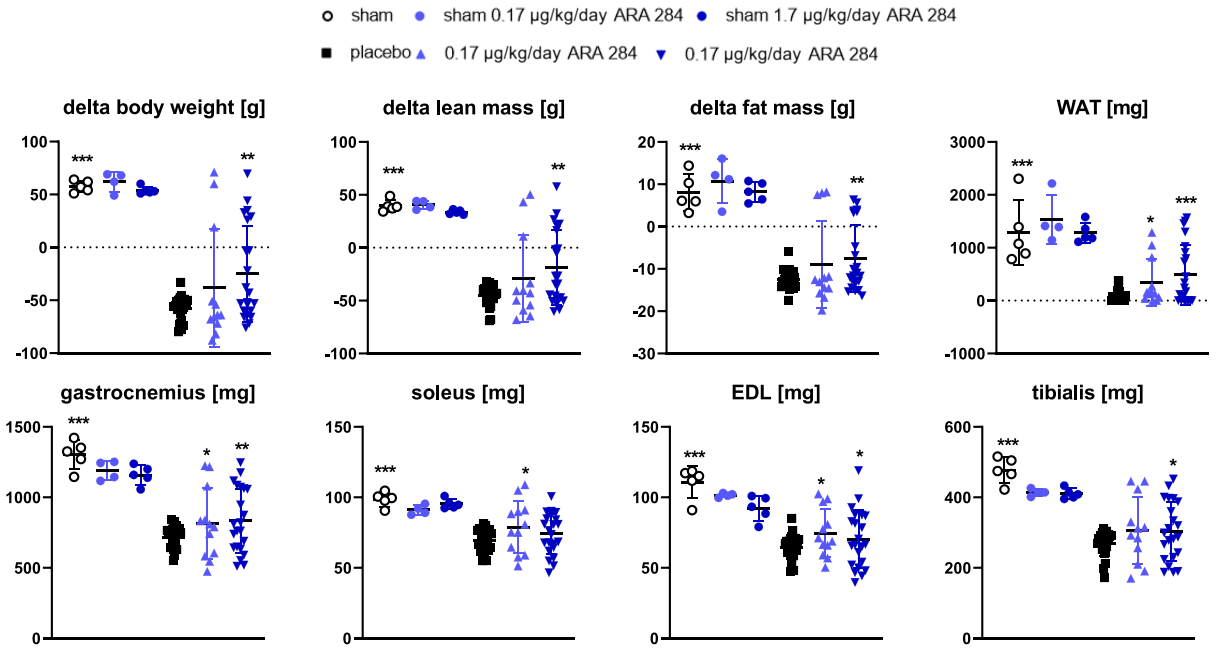


Figure 1 ARA 284 preserves body composition and muscle mass in tumour-bearing rats. Animals were treated daily starting 24 h after tumour-inoculation until the day of euthanasia with the low (LD) and high doses (HD) of the compound. The loss of the total weight, lean mass, and fat mass are presented as the difference between the mass at the beginning of the experiment and after euthanasia (max. 16 days). The mass of the gastrocnemius, soleus, extensor digitalis longus (EDL), tibialis, and white adipose tissue (WAT = epididymal fat) was measured after necropsy. No significant differences were seen between sham placebo and sham-treated groups. The data are normalized to sham and presented as mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with placebo animals. Sham: $n = 5$, placebo: $n = = 24$, 0.17 µg/kg/day ARA 284: $n = 12$, 1.7 µg/kg/day ARA 284: $n = 22$.

$P < 0.01$ vs. placebo). Likewise, treatment with low dose of ARA 284 (0.17 µg/kg/day) yielded less weight loss, but the effect was less pronounced than with the high dose of the substance (19.12 ± 8.0%, $P = 0.044$ vs. placebo), yet it was not significantly different to placebo or high-dose ARA 284.

Treatment with high-dose ARA 284 showed a protective effect for both lean body mass ($P < 0.01$ vs. placebo, *Figure* 1) and body fat mass ($P < 0.01$ vs. placebo) (*Figure* 1). The low dose had no significant effect (*Figure* 1) or a less pronounced effect compared with high-dose ARA 284 (*Figure* 1). The weight of the gastrocnemius muscle was also maximal in animals treated with the high dose of ARA 284 (*Figure* 1; $P < 0.01$), and low dose also improved gastrocnemius weight ($P < 0.05$). A similar effect was seen for the weights of the extensor digitalis longus muscle (both doses $P < 0.05$), while only low dose improved soleus weight and only high-dose ARA 284 improved tibialis weight (*Figure* 1).

Sham animals receiving ARA 284 did not show any differences in changes of body weight, lean, and fat mass compared with untreated sham animals. Moreover, the tissue weight of the whit adipose tissue, gastrocnemius, soleus, extensor digitalis longus, and tibialis was similar in all sham groups (*Figure* 1).

Spontaneous activity and food intake on Day 10/11

Treating sham animals with ARA 284 did not have an effect on food intake, while it did significantly increase the spontaneous activity on Day 10/11. Interestingly, the high-dose ARA 284 showed a significantly lower activity at baseline (*Figure* 2). High-dose ARA 284 resulted in an increased spontaneous activity compared with placebo ($P < 0.05$), while low-dose ARA 284 had no significant effect ($P = 0.58$) (*Figure* 2). Food intake was significantly increased only in the high-dose group of ARA 284 ($P < 0.01$).

Cardiac function on Day 11

As expected, treating sham animal with ARA 284 did not have an effect on heart weight and left ventricular (LV) mass, as well as changes in heart function (*Figure* 3). However, heart weight was significantly lower in untreated tumour-bearing rats compared with sham on the day of euthanasia (*Figure* 3). High-dose ARA 284 increased heart weight compared with placebo. LV mass, determined by echocardiography, was significantly increased by both ARA 284 doses on Day 11, while the change in LV mass from

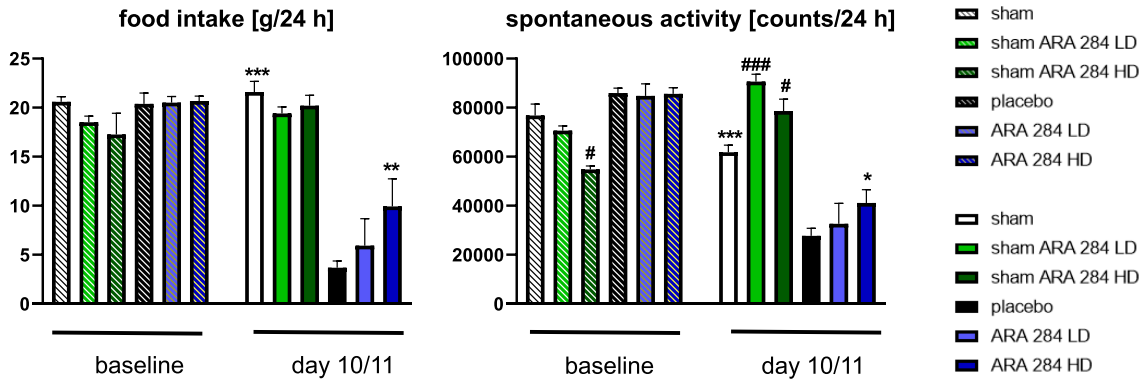


Figure 2 ARA 284 improves activity and food intake. Baseline data show no difference in food intake and spontaneous activity between the groups. Only the higher dose of ARA 284 increased spontaneous activity and food intake of tumour-bearing rats. In sham animals, both doses increased spontaneous activity. The data are normalized to sham and presented as mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. placebo animals. Sham: $n = 5$, placebo: $n = 24$, 0.17 μg/kg/day ARA 284: $n = 12$, 1.7 μg/kg/day ARA 284: $n = 22$.

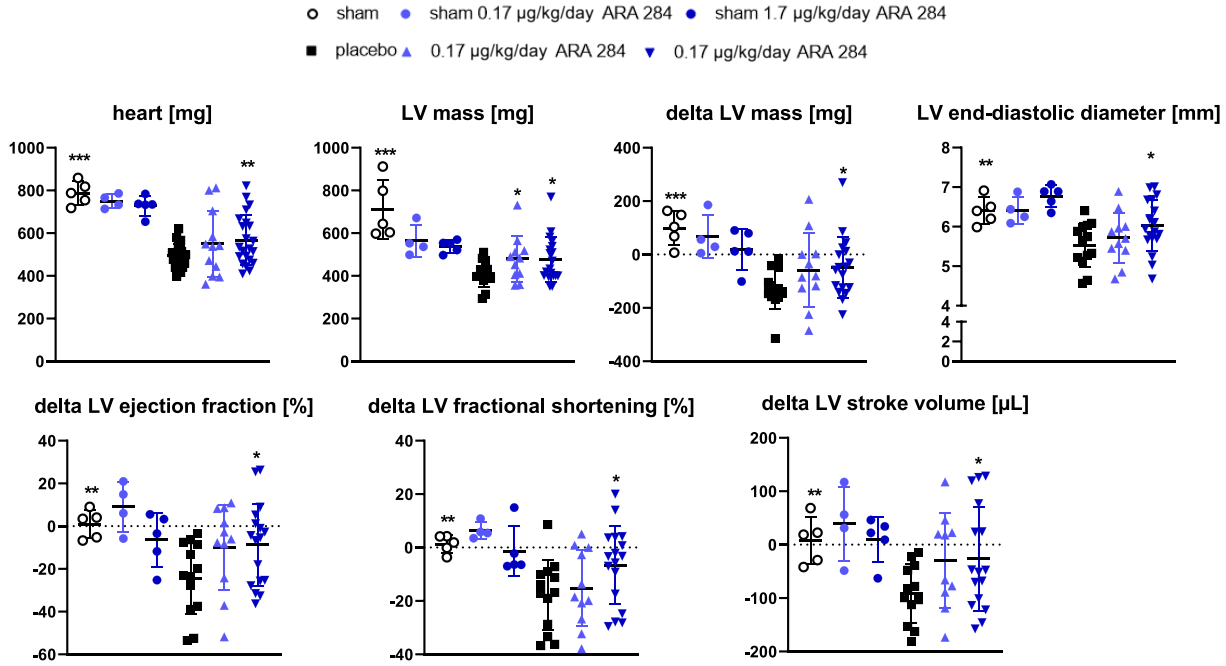


Figure 3 ARA 284 improves cardiac mass and function. The weight of the heart is strongly decreased in tumour-bearing rats compared with sham animals at euthanasia. High-dose ARA 284 improved heart weight vs. placebo. This was also seen at Day 11, where an increased left ventricular (LV) mass by both ARA 284 doses mass determined by echocardiography, whereas only the high dose had a significant effect on delta LV mass between baseline and Day 11. The heart of tumour-bearing rats becomes smaller, as evidenced by the reduction in LV end-diastolic diameter, which was attenuated by high-dose ARA 284. The change in LV ejection fraction, LV fractional shortening, and LV stroke volume shows a clear reduction in the placebo group and was improved by high-dose ARA 284. No significant differences were seen between sham placebo and sham-treated groups. The data are normalized to sham and presented as mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. placebo animals. Sham: $n = 5$, placebo: $n = 14$–24, 0.17 μg/kg/day ARA 284: $n = 11$–12, 1.7 μg/kg/day ARA 284: $n = 16$–22. Echocardiography could not be performed on all animals due to ascites volume and general weakness that made anaesthesia problematic at best in some animals.

baseline to Day 11 was only improved by high-dose ARA 284. We have previously shown that the heart becomes smaller in tumour-bearing animals,[27] which was also observed in the present study and improved by high-dose ARA 284. The change in LV ejection fraction and fractional shortening were reduced by the tumour compared with the sham group and preserved by high-dose ARA 284, which also improved the LV stroke volume (*Figure* 3).

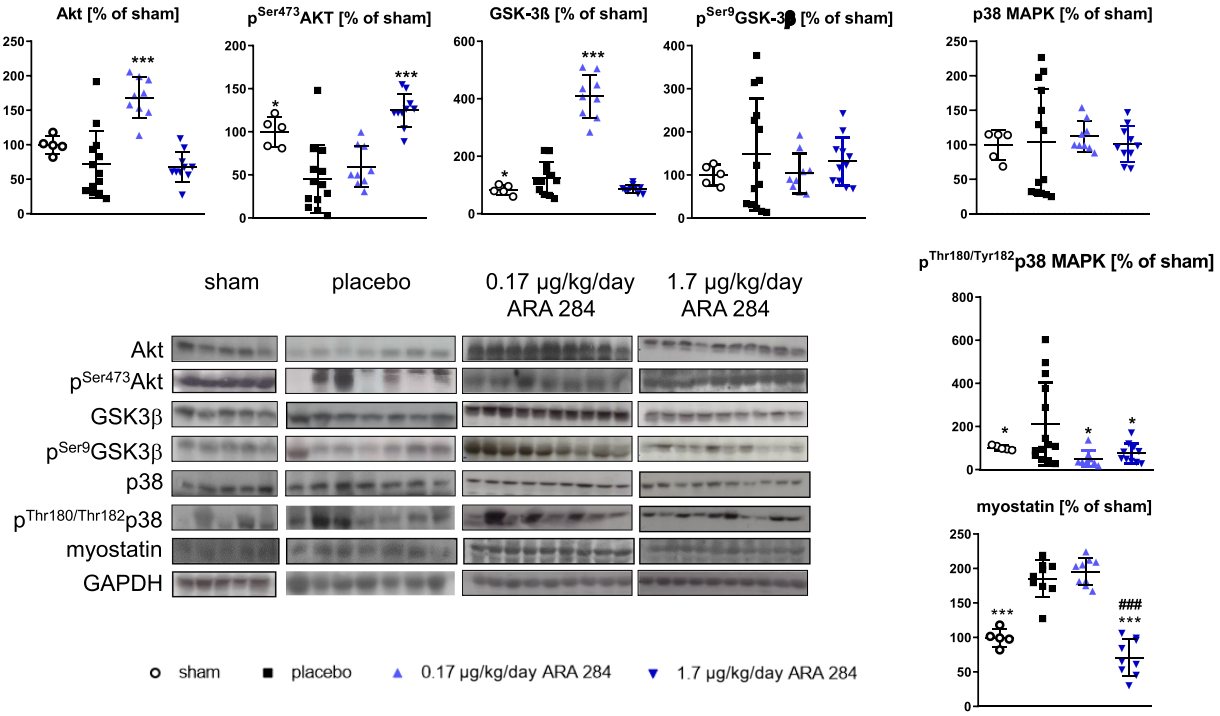


Figure 4 ARA 284 modulates molecular markers consistent with muscle growth. The level of pAkt (Ser473) in gastrocnemius muscles of tumour-bearing rats treated with the higher dose of ARA 284 was increased in comparison with placebo. The level of total GSK-3β and pGSK-3β (Ser9) in gastrocnemius muscles of tumour-bearing rats treated with different doses of ARA 284 in comparison with placebo show that there is no significant change in phosphorylation. The levels of p-p38 MAPK (Thr180/Tyr182) and uncleaved myostatin in gastrocnemius muscles of tumour-bearing rats treated with ARA 284. Reduced levels of phosphorylated p38 MAPK and myostatin are consistent with a better maintenance of muscle mass. The data are normalized to sham and presented as mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. placebo animals; $\#\#\#P < 0.001$ vs. low-dose ARA284. Sham: $n = 5$, placebo: $n = 14$, 0.17 µg/kg/day ARA 284: $n = 9$, 1.7 µg/kg/day ARA 284: $n = 10$ (samples were randomly chosen).

The effect of ARA 284 on molecular signalling in the gastrocnemius

Administration of both concentrations of ARA 284 did not change the level of total Akt compared with placebo (*Figure* 3B). Active Akt, that is, Akt phosphorylated at position Ser473 (pAkt (Ser473)) was significantly decreased in the placebo group (*Figure* 4). Levels of pAkt (Ser473) were significantly increased in the group that received treatment with high-dose ARA 284, while low-dose ARA 284 had a significant intermediate effect on the phosphorylation levels of Akt. Low-dose ARA 284 increased the expression of GSK-3β (*Figure* 4). Neither dose changed the phosphorylation level of GSK-3β (pGSK-3β(Ser9)) (*Figure* 4).

While we did not observe changes in total p38 MAPK expression, higher levels of p-p38 MAPK (Thr180/Tyr182) in gastrocnemius muscles of placebo-treated animals were observed. Both doses of ARA 284 reduced the level of p-p38 MAPK ($P < 0.05$) with low dose being more effective suggesting their inhibitory effect on protein degradation through this pathway (*Figure* 4). A significant reduction of myostatin levels was found in animals treated with high-dose ARA 284 ($P < 0.0001$ vs. placebo). Low-dose ARA 284 had no effect in myostatin expression (*Figure* 4). The trypsin-like and PGPH-like activities of the proteasome were significantly reduced, while the chymotrypsin-like activity was significantly increased compared with placebo (*Figure* 5).

Survival

The survival, that is, reaching ethical endpoints, of animals treated with high dose of ARA 284 was significantly improved compared with the placebo group, whereas the low dose of ARA 284 only showed a weaker effect (*Figure* 6).

Discussion

We characterized tissue-protective effects of a non-haematopoietic, tissue-protective EPO derivative,[23] ARA 284, in a rat model of cancer cachexia. EPO has been shown to be effective in reducing muscle atrophy and loss of fat

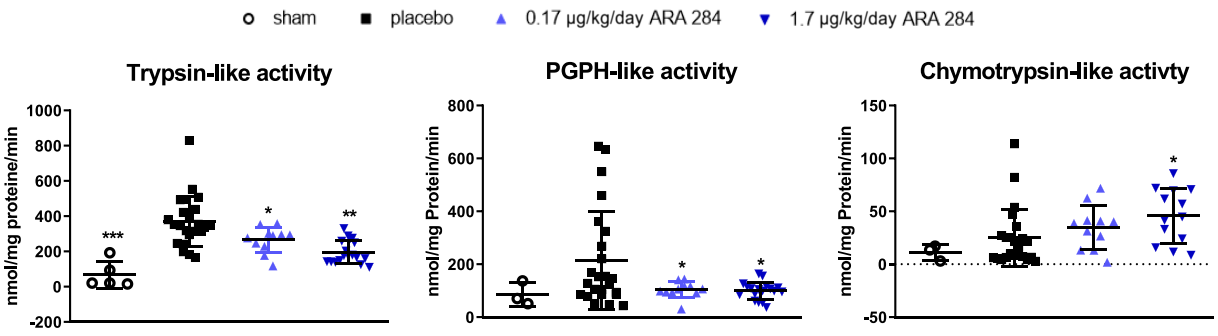
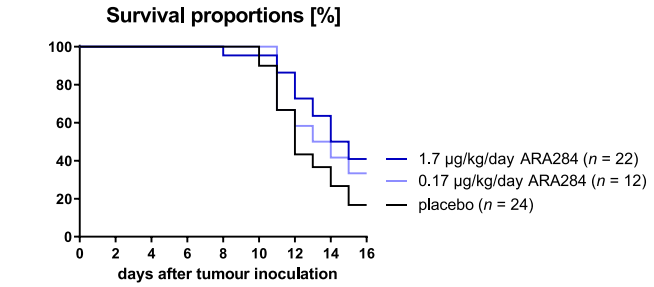


Figure 5 ARA 284 modulates the enzymatic activity of the proteasome. The activity of three enzymatic activities of the proteasome was measured in gastrocnemius tissue in a kinetic experiment. Both doses of ARA 284 decreased the trypsin-like and PGPH-like activities, while the chymotrypsin-like activity was not changed by low dose and increased by high-dose ARA 284 compared with placebo. The data are normalized to sham and presented as mean ± SEM, $*P < 0.05$, $***P < 0.001$ vs. placebo animals. Sham: n = 3–5, placebo: n = 23–24, 0.17 µg/kg/day ARA 284: n = 11, 1.7 µg/kg/day ARA 284: n = 13–18 (samples were randomly chosen).



0.17 µg/kg/day ARA284 vs. placebo:	HR: 0.64	95%CI: 0.28-1.46	$P = 0.38$
1.7 µg/kg/day ARA284 vs. placebo:	HR: 0.46	95%CI: 0.23-0.94	$P = 0.0325$
0.17 ARA284 vs 1.7 ARA284 :	HR: 1.29	95%CI: 0.52-3.23	$P = 0.54$

Figure 6 ARA 284 improves survival in a dose-dependent manner. Kaplan–Meier survival curves and statistical analysis of survival of animals treated with ARA 284 and placebo show that the higher dose of ARA 284 is associated with increased survival.

mass and being cardio-protective in experimental cancer cachexia[28–30]; however, the haematopoietic effects of EPO could lead to complications. We show here dose-dependent improvements in body weight, particularly as a result of less reduction in muscle and fat mass in animals treated with ARA 284 in comparison with placebo. Spontaneous activity of the animals also was improved. Cardiac mass was reduced, and cardiac function was severely impaired in tumour-bearing animals, which was observed previously in mice,[31] rats,[27] and humans.[32,33] The reduction of LV fractional shortening points to fibrosis of the left ventricle, which was described before in rodents and humans.[27,31] High-dose ARA 284 preserved both cardiac mass and function compared with placebo, which may have contributed to the improved survival of ARA 284-treated animals in comparison with placebo.

We used gastrocnemius muscle to investigate possible biochemical mechanisms participating in tissue protection. The results showed an activation of Akt, that is, pathways of protein synthesis. In addition, we noted a down-regulation of regulators of catabolic processes, in particular of activated p38 MAPK and myostatin.

We analysed the level of expression and phosphorylation of regulatory proteins in gastrocnemius muscle to determine the effect of ARA 284 at the molecular level. Of the studied proteins, Akt plays a central role in the regulation of protein synthesis.[34] Increased Akt activity is associated with protein synthesis and at the same time to inactivation of proteins responsible for protein degradation and inhibition of myogenesis.[34,35] Phosphorylation of Akt at the position Ser473 is necessary for its activation.[36] Therefore, increased pAkt (Ser473) phosphorylation seen in animals treated with the higher dose of ARA 284 suggests an increased rate of protein synthesis. This is consistent with the higher weight of the gastrocnemius muscle that was observed of these animals. Akt also phosphorylates and thereby inactivates GSK-3β,[37] which is involved in negative regulation of muscle growth via arrest of cell cycle and reduction of protein synthesis.[38] Our results show the decrease in total GSK-3β expression for the higher dose of ARA 284. We did not observe any significant changes in the phosphorylated form of GSK-3β by treatment.

Under pathologic conditions, p38 MAPK can be activated by cytokines including TNF-α, which leads to activation of

protein degradation via up-regulation of the E3 ubiquitin ligases MAFbx and MuRF-1.[14] The level of phosphorylated form of p38 MAPK (Thr180/Tyr182) has been shown to increase during muscle wasting associated with several diseases (e.g. acute quadriplegic myopathy and diabetes type II) and ageing.[39–42] In our experiment, the level of p-p38 MAPK increased in placebo animals suggesting a role in muscle wasting in cancer cachexia. Both doses of ARA 284 significantly decreased the level of p38 MAPK phosphorylation indicating a reduction of atrophy mechanisms. Moreover, p38 MAPK is one of the downstream targets of myostatin leading to muscle wasting.[43] Myostatin is known as a negative regulator of muscle growth[44] and can activate several signalling cascades in the skeletal muscle cells.[45,46] Myostatin levels were significantly reduced by treatment with high-dose ARA 284.

This study has some limitations. During the *in vivo* phase of the experiment, no muscle function was assessed, and no tissue was fixed for histological analyses of muscle fibre number and distribution, and capillary density. Additionally, we did not measure cytokines.

Taken together, high-dose ARA 284 seems to down-regulate several pathways leading to muscle wasting (down-regulation of GSK-3β and myostatin expression, and inactivation of p38 MAPK) and activation of the signalling cascades leading to muscle growth (phosphorylation of Akt). For some proteins, treatment with low dose of ARA 284 did not result in any significant change (such as pAkt (Ser473) and myostatin), for others even showed an opposite effect when compared with the animal groups treated with high concentrations (such as GSK3β). Altogether, the results suggest that higher doses of ARA 284 may be more effective in the treatment of cancer cachexia, as shown here by the reduced wasting of body weight (both doses) as well as lean body mass (i.e. muscle) and fat tissue (high dose).

Currently, there are no approved drugs for the effective treatment of cancer cachexia. Further pre-clinical studies of ARA 284 are necessary, but clearly warranted because the observed effects of ARA 284 in experimental cancer cachexia are promising.

Acknowledgements

The authors of this manuscript certify that they comply with the ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle*.[47]

Conflict of interest

A.C. is a chief executive officer, and M.B. is a Chief Scientific and Medical officer of ARAIM Pharmaceuticals, and both have stock or options in the company. J.S. is a paid consultant for Actimed Therapeutics and Pephexia Therapeutics and received grant support from Boehringer Ingelheim. All other authors have no disclosures with the subject matter of this manuscript.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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