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ORIGINAL ARTICLE

Ursodeoxycholic acid treatment in a rat model of cancer cachexia

Anika Tschirner · Stephan von Haehling · Sandra Palus · Wolfram Doehner · Stefan D. Anker · Jochen Springer

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Abstract
Background Cancer cachexia is characterized by loss of both adipose and skeletal muscle tissue and by an increased production of proinflammatory cytokines. Ursodeoxycholic acid (UDCA), a bile acid used for centuries in the treatment of liver disease, is known to confer anti-inflammatory and anti-apoptotic effects as well as beneficial effects on mitochondrial integrity and cell signaling. We hypothesized that UDCA ameliorates the wasting process in the Yoshida hepatoma tumor model. In addition, we sought to establish if UDCA exerts beneficial effects on survival in this model.
Methods and results Forty-seven male rats were inoculated intraperitoneally with 10^8 Yoshida hepatoma AH-130 cells and treated with placebo or one of two different doses of UDCA, 25 or 100 mg/kg daily. Body weight, body composition, and activity indicators were measured over the course of study up to day 16. UDCA treatment had no effect on tumor growth, loss of body weight, and loss of fat mass. Compared with placebo, low-dose UDCA improved tissue loss in the lung (p=0.022) and tended to reduce tissue loss in brown adipocytes (p=0.06), gastrocnemius muscle (p=0.06), extensor digitorum longus muscle (p=0.09), and soleus muscle (p=0.07). Compared with placebo, high-dose UDCA tended to reduce the loss of lean body mass (p=0.06), lung tissue (p=0.1), white adipose tissue (p=0.11), and gastrocnemius muscle (p=0.11). The activity and food intake were not altered in tumor-bearing rats by either dose of UDCA. Both doses tended to decrease the mortality rate in tumor-bearing rats, (hazard ratio (HR), 0.42; 95% confidence interval (CI), 0.17–1.04; p=0.061 for low-dose UDCA; HR, 0.44; 95% CI, 0.18–1.05; p=0.065 for high-dose UDCA).
Conclusion UDCA treatment in the Yoshida hepatoma model showed a trend towards attenuation of tissue loss in animals with progressive weight loss in cancer cachexia. Tumor growth and activity indicators were not altered. Both doses of UDCA tended to reduce the mortality rates in tumor-bearing animals. Larger studies with longer follow-up are required to verify these findings.

Keywords Cancer cachexia · Ursodeoxycholic acid · Wasting · Yoshida hepatoma animal model

A. Tschirner · S. von Haehling · S. Palus · W. Doehner · S. D. Anker · J. Springer
Applied Cachexia Research, Department of Cardiology, Charité Medical School, Berlin, Germany

A. Tschirner (✉) · S. von Haehling · S. Palus · J. Springer
Center for Cardiovascular Research, Charité Medical School, Campus Mitte, Hessische Str. 3-4, 10115 Berlin, Germany
e-mail: anika.tschirner@charite.de

W. Doehner
Center for Stroke Research Berlin, Charité Medical School, Berlin, Germany

S. D. Anker
Centre for Clinical and Basic Research, IRCCS San Raffaele, Rome, Italy

J. Springer
Norwich Medical School, University of East Anglia, Norwich, UK

1 Introduction

Alterations in body composition are a frequent clinical finding in patients with malignant cancer. Muscle loss with ensuing decrease in strength may develop even before weight loss becomes evident [1]. Cachexia as a

terminal stage of body wasting is defined as a "complex metabolic syndrome associated with underlying illness and characterized by" weight loss as a consequence of muscle loss with or without loss of fat mass [2]. The prevalence of cachexia among patients with malignant cancer ranges between 20% and 80% during advanced disease stages, dependent on the primary tumor site, comorbidites, and the presence or absence of metastases [3]. Furthermore, cachexia is associated with anorexia, systemic inflammation, and a reduced quality of life as a consequence of restricted mobility and a higher morbidity [2, 4, 5]. Muscle wasting occurs by activation of proteolytic systems, mainly the ubiquitin proteasome pathway, and apoptosis [4].

Ursodeoxycholic acid (UDCA) is a tertiary bile acid that has been used for centuries in the clinical treatment of gallstones, primary biliary cirrhosis, and primary sclerosing cholangitis [6–8]. It is known that UDCA suppresses secondary bile acids (cholic acid)-induced colonic carcinogenesis [9, 10]. UDCA exerts anti-inflammatory effects by inhibiting the NF-κB pathway and therefore reducing the expression of tumor necrosis factor alpha (TNF-α) [7, 11, 12]. In addition, UDCA has anti-apoptotic action mediated by preventing damage of the mitochondrial membrane, release of ROS and consequently inhibiting the induction of transcription factors responsible for proliferation and apoptosis [6, 12–15]. Here, we hypothesized that the effects of UDCA may translate into beneficial effects in an experimental cachexia model using the Yoshida hepatoma model.

2 Methods

2.1 Study design

Fifty-seven 7 weeks old male Wistar Han rats weighing approximately 200 g were used. Animals were housed in groups of three, 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, 47 rats were inoculated intraperitoneally with 10^8 exponentially growing Yoshida hepatoma AH-130 cells [16]. Blood samples and organ collection were scheduled for day 16 but had to be performed earlier in 35 animals because ethical standards (apathy, reduced body temperature, and disturbed blood flow) ruled out killing. All organs were weighted after killing the animals. In addition, we assessed body weight and body composition before tumor implantation and on the day of killing. Quality-of-life indicators (spontaneous activity and food and water intake) were measured on days 0 and 10/11. Tumor cells were counted on the last study day.

2.2 Treatment with UDCA

Rats were randomized to control (n=10), placebo (n=28), 25 mg kg^{-1} day^{-1} UDCA (n=9), and 100 mg kg^{-1} day^{-1} UDCA (n=10). UDCA was purchased as Ursofalk suspension from Dr. Falk Pharma GmbH, Freiburg, Germany. Treatment with UDCA or placebo started 1 day after tumor inoculation and was then given daily by gavage until the end of the study.

2.3 Body composition

Body composition (lean mass, muscle mass, water content, and fluids) was analyzed using an EchoMRI-700 (Echo Medical Systems, Houston, Texas, USA). The rat was put in a tube so that it could not move during the measurement which takes 90 s. The analysis of the body structures are based on nuclear magnetic resonance which measures the resonance of magnetic active nuclei in the tissue.

2.4 Quality-of-life indicators

Spontaneous activity and food intake are indicators for quality of life [17]. Animals were housed individually with 100 g of food, and their movement was measured by an infrared scanner over 24 h with the Supermex Locomotor System (Muromachi Kikai Co., Ltd., Tokyo, Japan).

2.5 Statistics

Data were analyzed with GraphPad PRISM 5.0 (GraphPad Software, Inc, La Jolla, CA, USA). Results are shown as mean±SEM. Data were analyzed for normal distribution using the Kolmogorov–Smirnov test. If there was a normal distribution, the data were analyzed with analysis of variance followed by Student's t test while data without normal distribution were analyzed by Kruskal–Wallis and Mann–Whitney U test. Survival was tested by Cox proportional hazard analysis and hazard ratio (HR) and 95% confidence interval (CI) are shown. A p value of <0.05 was considered significant.

3 Results

We studied 57 animals; 35 of them had to be killed before day 16 because they reached ethical endpoints (day 11, n=9; day 12, n=10; day 13, n=8; day 14, n=5; and day 15, n=3). UDCA treatment did not have an effect on the growth of the Yoshida hepatoma tumor. At the end of the study or earlier in cases that animals had to be killed, total tumor cell numbers were $2.04\pm0.26\times10^9$ cells in placebo-treated animals, $1.95\pm0.49\times10^9$ cells in animals that received 25 mg kg^{-1} day^{-1} of

UDCA and $2.08\pm0.46\times10^9$ cells in animals that received 100 mg kg^{-1} day^{-1} of UDCA ($p>0.5$ both doses UDCA vs. placebo). There were no observations of other malignancies during necropsy.

3.1 Body weight and body composition

Control animals gained body weight (+59.30±2.80 g), lean body mass (+40.70±2.61 g), and fat mass (+9.83±1.41 g). Average loss in body weight was highest in the placebo group (−53.36±3.06 g), which means 35.4% and was slightly but not statistically significantly reduced in both groups with UDCA treatment (−47.22±10.02 g for 25 mg kg^{-1} day^{-1} UDCA, 28.6%; −36.6±13.79 g for 100 mg kg^{-1} day^{-1} UDCA, 21.6%; Fig. 1a). The loss of lean mass was slightly reduced by giving high-dose UDCA (−27.53±9.73 g, $p=0.06$ vs. placebo; Fig. 1b), and the loss of fat mass was not statistically significantly altered by treating tumor-bearing animals with UDCA ($p>0.5$ both doses vs. placebo; Fig. 1c).

3.2 Organ weights

Tumor-bearing rats lost significantly weight in nearly all analyzed organs compared with control animals. Treatment with both doses of UDCA did not attenuate the weight loss in the heart, liver, spleen, kidney, fat tissue, and tibialis muscle (Table 1). Treatment of cachectic animals with 25 mg kg^{-1} day^{-1} UDCA improved the weight of the lung significantly ($p=0.022$ vs. placebo; Fig. 1d), and there was a trend towards an increase in the weight of brown adipose tissue (BAT), gastrocnemius muscle (GC; Fig. 1e), extensor digitorum longus muscle (EDL), and soleus muscle ($p=0.06$ for BAT and GC, $p=0.09$ for EDL, and $p=0.07$ for soleus muscle; low-dose UDCA vs. placebo; Table 1). High-dose UDCA attenuated somewhat but not significantly the weight loss of the lung, white adipose tissue (WAT) and GC ($p=0.1$ for lung, $p=0.11$ for WAT and GC vs. placebo; Table 1 and Fig. 1e).

3.3 Quality of life

Indicators for quality of life are spontaneous activity and food intake. The activity was decreased in tumor-bearing animals, and there were no effects with either dose of UDCA (Fig. 1f). Food intake was reduced in the placebo group and UDCA treatment of the tumor-bearing animals did not increase food intake (Fig. 1g).

3.4 Survival

On day 16 following tumor inoculation, the survival rates in the placebo, high-, and low-dose UDCA groups were 11%, 40%, and 44%, respectively (Fig. 2). Median survival in the

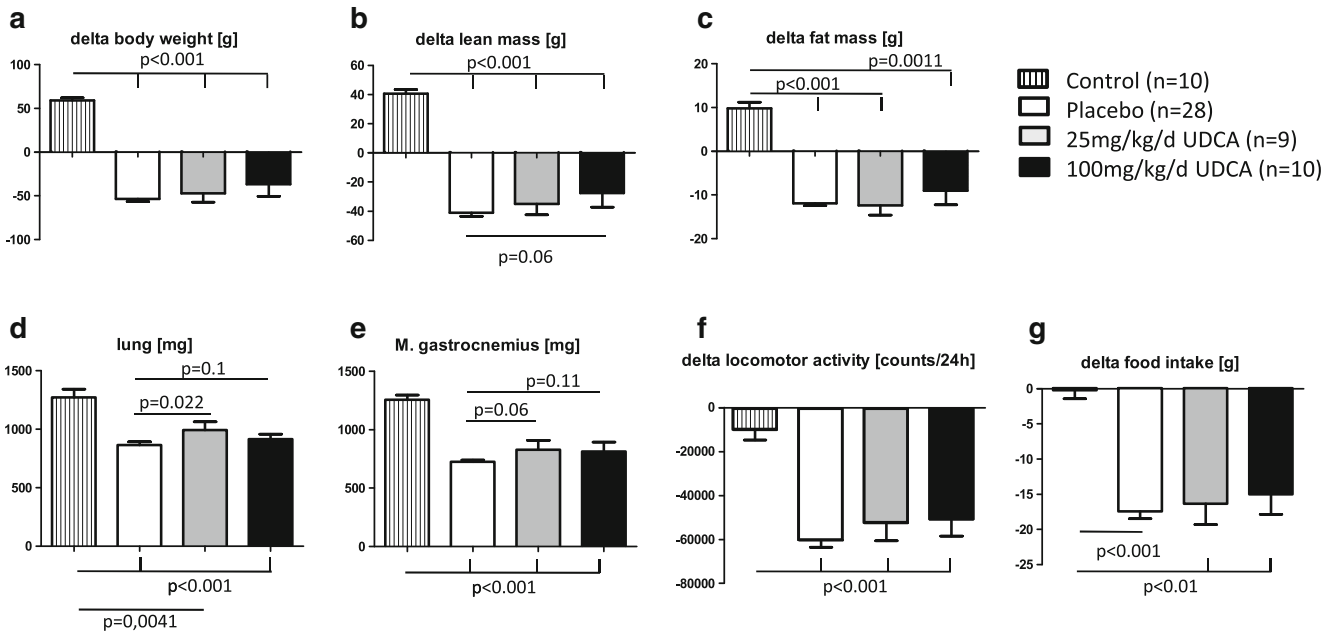

Fig. 1 Change of body weight (**a**), lean body mass (**b**), and fat mass (**c**): control animals gained weight and placebo animals lost significantly body weight, lean body mass, and fat mass ($p<0.001$ vs. control); 100 mg kg^{-1} day^{-1} UDCA reduced loss of body weight and fat mass but not significantly. Furthermore, the high dose of UDCA prevents slightly the loss of lean mass ($p=0.06$ vs. placebo). Weight of lung (**d**) and GC (**e**): 25 mg kg^{-1} day^{-1} UDCA improved significantly the weight of the lung ($p=0.022$ vs. placebo). The same dose improved the weight of the GC, but not significantly ($p=0.06$ vs. placebo). Quality-of-life indicators: both doses of UDCA did not have an effect on spontaneous activity (**f**) and food intake (**g**)

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

Organ weight (mg)	Control (n=10)	Placebo (n=28)	25 mg kg^{-1} day^{-1} UDCA (n=9)	100 mg kg^{-1} day^{-1} UDCA (n=10)
Heart	781±16.5	520±17.2*	509±21*	529±33*
Lung	1,273±69	864±27*	992±71**, ***	916±43*, ****
Liver	10,600±393	6,325±265*	6,798±345*	6,265±684*
Spleen	641±30	189±30*	206±34*	222±57*
Kidney left	1,126±31	708±19.3*	690±32*	717±45*
Kidney right	1,162±39	735±22*	724±33*	729±42*
WAT	1,309±151	87±24*	301±155*	438±241***, ****
BAT	220±12.1	83±4.77*	103±8.6*, ****	96±14.0*
M. gastrocnemius	1,255±42	725±16.0*	828±81*, ****	814±81*, ****
M. tibialis	457±12.4	268±7.10*	296±23*	300±29*
M. EDL	107±3.66	64±1.51*	71±5.4*, ****	68±6.3*
M. soleus	98±2.97	71±1.60*	79±5.2***, ****	69±6.4*

*p<0.001 vs. control; **p<0.05 vs. placebo; ***p<0.01 vs. control; ****p<0.12 vs. placebo

placebo group was 12 days, 13 days for low-dose UDCA, and 13.5 days for high-dose UDCA. Treatment with 25 and 100 mg kg^{-1} day^{-1} UDCA showed a trend towards reducing mortality of the tumor-bearing animals (HR, 0.42; 95% CI, 0.17–1.04; p=0.061 for low-dose UDCA; HR, 0.44; 95% CI, 0.18–1.05; p=0.065 for high-dose UDCA). Combining both UDCA-treated animal groups for the survival analysis increased the statistical power and significantly decreased mortality of tumor-bearing animals (HR, 0.40; 95% CI, 0.19–0.84; p=0.016 for UDCA combined vs. placebo).

4 Discussion

In our study, UDCA treatment of tumor-bearing animals had no effect on the growth of the tumor, loss of body weight, and loss of fat mass. Low-dose UDCA attenuated significantly the weight loss of the lung and slightly the weight loss of BAT and skeletal muscles compared with placebo. High-dose UDCA increased the lean body mass, the weight of the lung, WAT, and gastrocnemius muscle (GC). Both doses of UDCA had no effect on spontaneous activity and food intake and tended to decrease mortality rates in tumor-bearing animals.

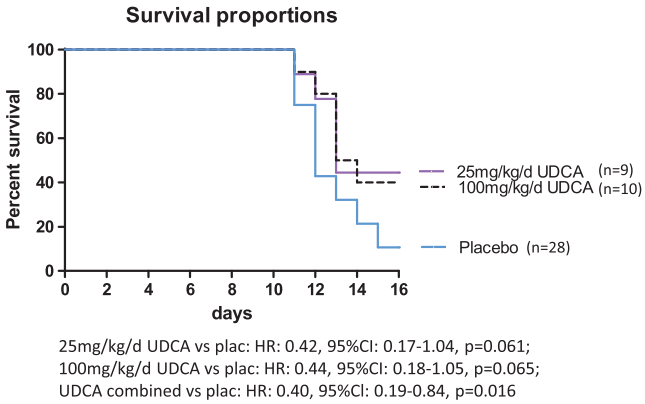

Fig. 2 Kaplan–Meier survival curves, both doses UDCA slightly reduce mortality compared with placebo

It has been described that UDCA treatment can prevent chemically induced colonic tumorigenesis in experimental animal models and human colorectal cancer by inhibiting proliferation of tumor cells [9, 10, 12, 18, 19]. In the present study utilizing the Yoshida hepatoma animal model, we could not confirm this, because the treatment with UDCA had no effect on tumor growth. The reason therefore that UDCA was not so beneficial in our tumor model could be the type of the tumor (chemically induced tumor vs. injected tumor cells) and the duration of the treatment. Kohno et al. showed a prevention of colon carcinogenesis in mice by giving UDCA (long-term) for 17 weeks after chemical tumor induction [11]. Furthermore, Narisawa et al. treated rats with colon cancer with UDCA for 27 weeks and showed a suppression of cancer progression [9]. In our case, long-term studies of rats inoculated with Yoshida hepatoma cells are not feasible because the tumor cells are too aggressive and hence UDCA treatment of 15 days may be too short to shown any beneficial effect. Moreover, we did not see an anti-cancer effect because it could be that we did not assess the anticarcinogenic concentration of UDCA in this tumor model and hence, more dose–response studies are needed.

The body weight and the fat mass were unaltered in UDCA-treated tumor-bearing rats compared with placebo. Only high-dose UDCA affected the lean body mass; 25 mg kg^{-1} day^{-1} UDCA significantly increased the weight of the lung and showed a trend towards increasing the

weight of BAT, GC, EDL, and soleus muscle ($p<0.12$ vs. placebo) while high-dose UDCA improved the weight of the lung, WAT, and GC ($p<0.12$ vs. placebo). This effect may be mediated by UDCA reported to be anti-inflammatory and anti-apoptotic. In vitro and in vivo cancer studies showed that UDCA treatment inhibits the NF-κB pathway leading to the expression of the proinflammatory cytokine TNF-α from which is known that it is associated with wasting in cancer cachexia [7, 11, 12, 20]. A possible mechanism explaining the anti-inflammatory and anti-apoptotic effect could be that UDCA blocks the cancer induced generation of reactive oxygen species and subsequently inhibiting the transcription factors AP-1, and NF-κB important for carcinogenesis through the PKC signaling pathway, established from other studies [7, 12, 21–24].

It was seen that high dose of UDCA did not exert improved effects on body wasting than low-dose UDCA, so there was no dose-dependent correlation. This may be mediated by the fact that UDCA has side-effects like diarrhea, and therefore UDCA is less absorbed.

The locomotor activity and food intake were unchanged at day 10 after tumor inoculation compared with placebo showing no effect of UDCA on improving quality of life. The mortality of tumor-bearing animals treated with both doses UDCA was reduced compared with placebo but did reach significance when analyzing the combined UDCA doses with placebo because of an increased statistical power.

The results indicate that a low (25 mg kg^{-1} day^{-1}) and high (100 mg kg^{-1} day^{-1}) dosage of UDCA had no effect on tumor growth but slightly on body wasting and survival in an experimental Yoshida hepatoma animal model. This could be due to the kind of tumor, the fast tumor progression, the unknown efficient UDCA concentration and because of the short treatment time of UDCA. More studies are needed to show an anti-cancer effect and a more ameliorated cachexia to identify effects of higher doses or longer treated periods.

4.1 Study limitations

The primary endpoint of this study was to investigate if UDCA treatment could reduce body wasting in an experimental cancer cachexia model. Due to the small overall effects of UDCA treatment on body weight, we did not analyze signaling mechanisms including TNF-α measurement. The study was not powered for a survival endpoint, but a combination of both treated groups resulted in improved outcome. This suggests that a study powered for survival should be performed. However, since the effects on body weight and body composition were minimal, it may not be relevant to patient's health.

A second limitation of the study is that we have used the fast-growing and aggressive tumor AH-130 as a model for cachexia in rats, which may be the reason that UDCA had limited effects. However, further experiments could include UDCA treatment in a less aggressive and therefore long-term cancer cachexia animal model followed by measurement of proinflammatory cytokines.

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Conflict of interest The authors declare that they have no conflict of interest.

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