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Tandospirone reduces wasting and improves cardiac function in experimental cancer cachexia

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ABSTRACT

Background: Cancer cachexia is thought to be the cause of >20% of cancer related deaths. Symptoms of cancer cachexia patients include depression and anorexia significantly worsening their quality of life. Moreover, in rodent models of cancer cachexia atrophy of the heart has been shown to impair cardiac function. Here, we characterize the effects of the antidepressant and anxiolytic drug tandospirone on wasting, cardiac function and survival in experimental cancer cachexia.

Methods: The well-established Yoshida hepatoma rat model was used and tumor-bearing rats were treated with 1 mg/kg/d (LD), 10 mg/kg/d (HD) tandospirone or placebo. Weight, body composition (NMR), cardiac function (echocardiography), activity and food intake were assessed. Noradrenalin and cortisol were measured in plasma and caspase activity in skeletal muscle.

Results: Ten mg/kg/d tandospirone decreased the loss of body weight ($p = 0.0003$) compared to placebo animals, mainly due to preservation of muscle mass ($p<0.001$), while 1 mg/kg/d tandospirone was not effective. Locomotor activity ($p = 0.0007$) and food intake ($p = 0.0001$) were increased by HD tandospirone. The weight ($p = 0.0277$) and function of heart (left ventricular mass, fractional shortening, stroke volume, ejection fraction, all $p < 0.05$) were significantly improved. In the HD tandospirone group, plasma levels of noradrenalin and cortisol were significantly reduced by 49% and 52%, respectively, which may have contributed to the lower caspase activity in the gastrocnemius muscle. Most importantly, HD tandospirone significantly improved survival compared to placebo rats (HR: 0.34; 95% CI: 0.13–0.86; $p = 0.0495$).

Conclusion: Tandospirone showed significant beneficial effects in the Yoshida hepatoma cancer cachexia model and should be further examined as a prospective drug for this syndrome.

1. Introduction

Cancer cachexia is a complication occurring at the last stages of the disease. Cachexia is characterized by a minimum of 5% loss of body weight in one year or less in the presence of an underlying disease. Typical symptoms of cachexia are secondary anorexia, fatigue and decreased muscle strength [1]. The prognosis of cachectic cancer patients is very poor and in more than 20% of cases cachexia is considered to be the cause of death rather than the tumor itself [2].

Weight loss in cachexia is due to the reduction of muscle mass accompanied by the loss of fat tissue [1]. However, most research is focused on stopping or reversing muscle wasting. There is general consensus that of the underlying mechanisms of loss of muscle mass is mainly characterized by disbalance of protein synthesis and protein degradation [3].

It is thought that essentially all organs and tissues are targeted by cachexia, but this has not received much attention and the mechanisms underlying these changes are somewhat unclear [4]. It was shown that hearts suffer from atrophy and dysfunction in murine cancer cachexia models [5–7]. The connection between heart and muscle wasting is not completely understood, but the limited data suggests that molecular

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markers and signaling pathways to those activated in cachectic skeletal muscle are involved in the atrophy of cardiac tissue [8,9]. Moreover, treatment with an anti-myostatin compound mainly developed to protect skeletal muscle also preserved cardiac mass [5].

Aside from profound pathophysiological alterations on the molecular level in various tissues, the quality of life of patients with cancer cachexia is of major importance. Pain, unfavorable prognosis and harsh treatment regimes lead to stress, weakness and ultimately depression in this patients population reviewed in [10]. It was shown in both clinical trials and animal models that depression can promote disease progression, metastasis formation, and decreased immune response [11] and may lower the effectiveness of chemotherapy [12]. Furthermore, depression is linked to suppressed appetite, i.e. secondary anorexia common in cancer cachexia [13]. This is attributed to decreased release of serotonin, which further complicates the psychological state of patients. Interestingly, one of the major side effects of antidepressant drugs is the increase in appetite reviewed in [14], which could have positive effects in the treatment of cachectic cancer patients. Particularly, members of azapirone family that act as selective agonists of 5-hydroxytryptamine 1A (5-HT1A) receptor, a serotonin receptor, and show anxiolytic as well as antidepressant effects could be of interest [15].

In this study we investigated the effect of tandospirone (metanopirone) on the development of cancer cachexia and on heart function using the AH-130 Yoshida hepatoma rat model. Tandospirone is a selective 5-HT1A receptor partial agonist (approx. 55–85% intrinsic activity) [16], which is clinically used in Japan and China. Aside from overall body weight and body composition (fat and lean body mass), cardiac function, quality of life markers and survival were assessed.

2. Materials and methods

2.1. Study design

Male Wistar Han rats at the age of 8 weeks and approximate weight of approx. 200 g were housed in groups of three or four in our SPF-animal facility at a constant temperature of 22 °C and exposed to a 12 hour light cycle. Rats had free access to food and water. On day 1 the rats were inoculated intraperitoneally with 10^8 Yoshida hepatoma AH-130 cells. Organs were weighed on day 16 or the day of death if rats had to be sacrificed earlier due to reaching ethical endpoints [17]. In addition, body weight and body composition were assessed before tumor inoculation and on the day of sacrifice. Spontaneous activity and food and water intake were measured over a period of 24 h on day −1/0 and on day 10/11. Echocardiography was performed on day 0 and day 11. Tumor volume and total cell number were assessed at the day of euthanasia.

2.2. Treatment with tandospirone

Groups of randomly chosen rats were treated with low dose (1 mg/kg/d, n = 9), high dose (10 mg/kg/d, n = 10) of tandospirone or saline (placebo, n = 23). Animals were treated by gavage (200 μL per 200 g rat) once daily and treatment with tandospirone or placebo started 24 h after tumor inoculation. All personnel involved in the study was blinded to treatment allocation. All procedures had been approved by the local animal's ethics committee.

2.3. Body composition

Body composition of animals was analyzed using the Nuclear Magnetic Resonance devise EchoMRI-700 (Echo Medical Systems, Houston, Texas, USA), as described before [18].

2.4. Spontaneous activity and food intake

Animals were housed individually over 24 h and were given 100 g food. Their spontaneous movement was measured by using Supermex activity monitoring system (Muromachi Kikai Co., LTD., Tokyo, Japan), as described before [19].

2.5. Echocardiography

For the longitudinal measurement of cardiac diameter and function a high resolution echocardiography system (Vevo 770; VisualSonics Inc., Toronto, Canada) was used, as described before [20]. Briefly, rats were anesthetized using a 1.5% isoflurane and laid in supine position on a heated platform with legs attached to ECG electrodes for heart rate monitoring. All hair was removed from the left chest using a depilatory cream. The following parameters were assessed using M-mode: left ventricular (LV) posterior wall thickness (LVPW), LV end-diastolic diameter (LVEDD) and LV end-systolic diameter (LVESD). The LV end-diastolic volume (LVEDV) and end-systolic volume (LVESV) were calculated from B-mode images. LVmass was calculated using the following equation: $\text{LVmass} = 0.8\{1.04[([\text{LVEDD} + \text{IVSd} + \text{PWd}]^3 - \text{LVEDD}^3)]\} + 0.6$.

2.6. Biomarkers and clinical parameters

Plasma levels of cortisol, and noradrenaline were measured by commercial ELISA-kits (Asbach Medical Products, Germany) according to manufacturer's protocols. Results were calculated using 4 parameter logistics.

2.7. Caspase activity

The gastrocnemius muscle of placebo and high dose tandospirone were homogenized in ice-cold lysis-buffer (100 mM HEPES pH 7.5, 10% sucrose, 0.1% tergitol-type NP-40 (NP-40), 10 mM DTT and complete mini protease inhibitor cocktail, Boehringer, Germany). The homogenate was frozen in liquid nitrogen and heated to 37 °C for 3 cycles. After centrifugation (20,000 *g* for 30 min) 100 μg protein was used for the caspase-3 and caspase-6 activity assessment. The protein was pre-incubated in assay buffer (100 mM HEPES pH 7.5, 10% sucrose, 0.1% CHAPS, 2% DMSO, 10 mM DTT with or without 50 μM caspase-3 (Ac-DEVD-CHO) or caspase-6 inhibitor (Ac-VEID-CHO)) at 37 °C for 30 min. The fluorogenic substrate (50 μM) Ac-DEVD-AMC was added for caspase-3 and Ac-VEID-AMC for caspase-6. The change fluorescence intensity was measured with a fluorometer (Twinkle LB 970, Berthold, Bad Wildbad, Germany) at 360 nm excitation and 460 nm emission in 5 min intervals for 1 h. The activity, expressed as nmol/mg/min, was calculated by using free amidomethylcoumarin (AMC) as working standard.

2.8. Statistics

Data were analyzed with GraphPad PRISM 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Results are shown as mean ± SEM. Normally distributed data were analyzed by one way ANOVA followed by Tukey's test, while data without normal distribution were analyzed using Kruskal–Wallis and subsequent Dunn's tests. Survival was tested by Cox-proportional hazard analysis and hazard ratio (HR) and 95% confidence interval (CI). A p-value of <0.05 was considered significant.

3. Results

3.1. Tandospirone reduces wasting in cancer cachexia

The loss of the total body weight in the placebo group reached 27.3 ± 1.2% suggesting the development of severe cachexia (Fig. 1A). This was significantly reduced by 10 mg/kg/d tandospirone to a loss of body weight of 8.3 ± 6.0% ($p = 0.0003$), but not by 1 mg/kg/d (Fig. 1A). As body wasting consists of the loss of fat as well as lean body mass, body composition was analyzed (Fig. 1B,C). Similar to the overall weight loss, changes in lean mass were similar in placebo and LD groups (26.5 ± 1.6% and 24.7 ± 2.6%, respectively) while HD tandospirone effectively protected lean mass (−7.6 ± 5.9%, $p = 0.0005$; Fig. 1B). The weight of mixed fiber type gastrocnemius muscle (Fig. 4A) was significantly higher in groups treated with tandospirone compared to placebo animals ($p = 0.0108$ for LD, $p < 0.0001$ for HD). Similarly the weight of fast-twitch muscle extensor digitorum longus (EDL) significantly changed in both tandospirone-treated groups (LD: $p = 0.02$, HD: $p = 0.0008$, vs placebo; Fig. 4B). For the slow-twitch soleus muscle a significant weight increase was observed only in HD group ($p = 0.0002$ vs placebo; Fig. 4C). Together with the data on lean mass, these results point to a positive effect of tandospirone on the prevention of the muscle mass loss during cancer cachexia.

As expected, there was a considerable loss of fat mass in untreated tumor-bearing rats, which was reduced by HD tandospirone only ($p = 0.0134$; Fig. 1C). However, HD tandospirone treated rats had increased weight of epididymal white and intrascapular brown fat ($p = 0.0017$ and $p = 0.0340$ for white epididymal and brown intrascapular fat vs placebo, respectively; Fig. 2A,B).

3.2. Quality of life markers

Patients with cancer cachexia have a drastically reduced quality of life being evident by decreased physical activity and poor appetite [1]. Tumor-bearing rats showed a marked decrease in both food intake