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The xanthine oxidase inhibitor oxypurinol reduces cancer cachexia-induced cardiomyopathy

Jochen Springer [a,b,c,*], Anika Tschirner [a,b], Kai Hartman [a,b], Stephan von Haehling [a,b], Stefan D. Anker [d,1], Wolfram Doehner [a,e,1]

[a] Applied Cachexia Research, Department of Cardiology, Charité Medical School, Berlin, Germany
[b] Center for Cardiovascular Research, Charite Medical School, Berlin, Germany
[c] Norwich Medical School, University of East Anglia, Norwich, UK
[d] Centre for Clinical and Basic Research, IRCCS San Raffaele, Rome, Italy
[e] Center for Stroke Research Berlin, CSB, Charite Medical School, Berlin, Germany

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ABSTRACT

Background: Cachexia is a common complication of cancer and may be responsible for 22% of all cancer-related deaths. The exact cause of death in cancer cachexia patients is unknown. Recently, atrophy of the heart has been described in cancer cachexia animal models, which resulted in impaired cardiac function and is likely to contribute to mortality. In cancer patients hyperuricaemia independent of tumour lysis syndrome is often associated with a worse prognosis. Xanthine oxidase (XO) metabolizes purines to uric acid and its inhibition has been shown to improve clinical outcome in patients with chronic heart failure.
Methods: The rat Yoshida AH-130 hepatoma cancer cachexia model was used in this study. Rats were treated with 4 or 40 mg/kg/d oxypurinol or placebo starting one day after tumour-inoculation for maximal 15 days. Cardiac function was analyzed by echocardiography on day 11.
Results: Here we show that inhibition of XO by oxypurinol significantly reduces wasting of the heart and preserves cardiac function. LVEF was higher in tumour-bearing rats treated with 4 mg/kg/d (61 ± 4%) or 40 mg/kg/d (64 ± 5%) oxypurinol vs placebo (51 ± 3%, both $p < 0.05$). Fractional shortening was improved by 4 mg/kg/d (43 ± 3%) oxypurinol vs placebo (30 ± 2, $p < 0.05$), while 40 mg/kg/d oxypurinol (41 ± 5%) did not reach statistical significance. Cardiac output was increased in the 4 mg/kg/d dose only (71 ± 11 mL/min vs placebo 38 ± 4 mL/min, $p < 0.01$).
Conclusion: Inhibition of XO with oxypurinol has beneficial effects on cardiac mass and function in a rat model of severe cancer cachexia, suggesting that XO might be a viable drug target in cancer cachexia.

1. Introduction

Depending on the type of cancer, cachexia is present in up to 80% of terminally-ill cancer patients [1]. Cachexia is characterized by progressive tissue wasting and weight loss, associated with a general hypercatabolism that results in reduced quality of life, impaired response towards anti-tumour therapy and ultimately a worse overall outcome. In fact, cancer cachexia is considered to be the cause of death in 22% of cancer patients [2]. However, the exact cause of death in cancer cachexia is unknown. Interestingly, hyperuricaemia is often observed in cancer patients, which seems to be independent from tumour lysis syndrome [3] and to be a negative prognostic marker in end-stage cancer patients [4].

Several lines of evidence, both clinical and experimental, have suggested that the inhibition of xanthine oxidase (XO) by allopurinol, oxypurinol or febuxostat has beneficial effects on cardiac function in heart failure [5–7]. The inclusion of uric acid assessment for risk stratification in heart failure patients has recently been suggested [8], as increased uric acid levels resulting form up-regulated XO activity have been shown to have predictive value for mortality in CHF [9]. However, lowering of uric acid serum levels alone without inhibiting XO did not have a positive effect on hemodynamic parameters in CHF patients [10]. Therefore, the inhibition of XO seems to be crucial in this context. This may be due to the production of large amounts of reactive oxygen species (ROS) as by-products during the metabolising of purine to uric acid by XO [11]. Recently, we showed increased uric acid levels and increased markers of oxidative stress in the Yoshida hepatoma cancer cachexia model, as well as an approximately 52-fold induction of XO-produced reactive oxygen species [12]. Inhibition of XO not only reduced uric acid levels and oxidative stress, but also wasting of body weight, muscle and fat mass, and significantly improved survival [12]. Interestingly, several groups have shown that experimental cancer

* Corresponding author at: Center for Cardiovascular Research, Charité, Campus Mitte, Hessische Str. 3–4, 10115 Berlin, Germany. Tel.: +49 30 450 525023; fax: +49 30 450 525978.
E-mail address: jochen.springer@charite.de (J. Springer).
[1] Equal contribution.

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cachexia does not only lead to skeletal muscle atrophy, but also to a strong reduction of cardiac mass [13–15]. This cardiac atrophy in cancer cachexia has been described to result in an impaired cardiac function [14]. The magnitude of these effects on the heart were also shown to be gender specific, with male mice being more affected [15].

Given the above described positive effects of XO-inhibition on cardiac function in CHF-patients with increased uric acid levels and the published data on a cardiomyopathy in experimental cancer cachexia, we hypothesised that the inhibition of XO by oxypurinol may reduce cardiac atrophy and have beneficial effects on cardiac function in the Yoshida AH-130 hepatoma cancer cachexia model.

2. Methods

2.1. Tumour model

Male Wister rats (mean weight 208 ± 1 g) were injected intra-peritoneally with 10^8 Yoshida AH 130 tumour cells as described previously [16] and randomized into the following groups: placebo (n = 22), 4 mg/kg/d oxypurinol (n = 12) or 40 mg/kg/d oxypurinol (n = 11). Additionally, three groups were injected with saline (=sham, n = 10, oxypurinol 4 or 40 mg/kg/d (both n = 4)) and used as a reference. The procedures were approved by the local animal ethics committee (G 0114/08, LaGeSo Berlin, Germany). Animals were injected with the tumour cells or saline and housed in groups of three under SPF conditions. Treatment with placebo or active compound, given per gavage, started one day after tumour inoculation. Animals were monitored twice daily and were euthanized maximal 16 days after tumour inoculation. Several animals had to be euthanized prematurely for ethical reasons according to prospectively defined criteria of disease burden [12]. The final assessment of body weight was performed after removal of the tumour and the average daily change of body weight was calculated.

2.2. Uric acid in plasma

At the end of the study plasma levels of uric acid were measured by a validated laboratory (Labor28, Berlin, Germany).

2.3. Echocardiography

Echocardiography using the high resolution Vevo 770 (Visual Sonics, Toronto, Canada) was performed as described before [17]. Briefly, rats were anesthetized using a 1.5% isoflurane and laid in supine position on a platform with legs attached to ECG electrodes for heart rate monitoring. Body temperature was monitored and maintained at 36–38 °C using a heating pad. All hair was removed from the left chest using a chemical hair remover. 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. The cross-sectional area (CSA) of the aortic annulus is derived from the annulus diameter (D) measured in the parasternal long axis view as: $CSA = D^2 x\pi/4$, and cardiac output (CO) was calculated by CSA x Aortic velocity-time integral x heart rate. LVmass was calculated using a 2D area-length method and the following equation: $LVmass = 1.05[5/6(A_1(L + t) - 5/6(A_2xL)]$. The baseline echo was performed one day before tumour inoculation and repeated on day 11 of the protocol.

2.4. Statistics

Data were analyzed using GraphPad PRISM 5.0 (GraphPad Software, Inc, La Jolla, CA, USA). Results are shown as mean ± SEM. All data have been tested for normal distribution using the D'Agostino & Pearson omnibus normality test. Between group comparison was performed for data with normal distribution using ANOVA followed by Tukey's tests; data with skewed distribution were analysed by Kruskal-Wallace and Dunn's test. All statistical tests were two sided and a p-value <0.05 was considered significant.

3. Results

As described before, inhibition of XO by oxypurinol significantly reduced mortality in this rat model of cancer cachexia [12]. In untreated rats uric acid was significantly increased in tumour-bearing rats compared to sham (Fig. 1). Treatment with oxypurinol reduced uric acid levels not only in tumour-bearing rats, but also in sham rats. However, no statistical significant difference was seen between the two doses of oxypurinol (Fig. 1). Also, the average loss of body weight was reduced by treatment with 4 mg/kg/d oxypurinol compared to placebo-treated tumour-bearing rats, while treatment of sham animals had no effect on weight gain compared to placebo-treated sham rats (Fig. 2A). The heart weight was significantly lower in placebo-treated tumour-bearing rats

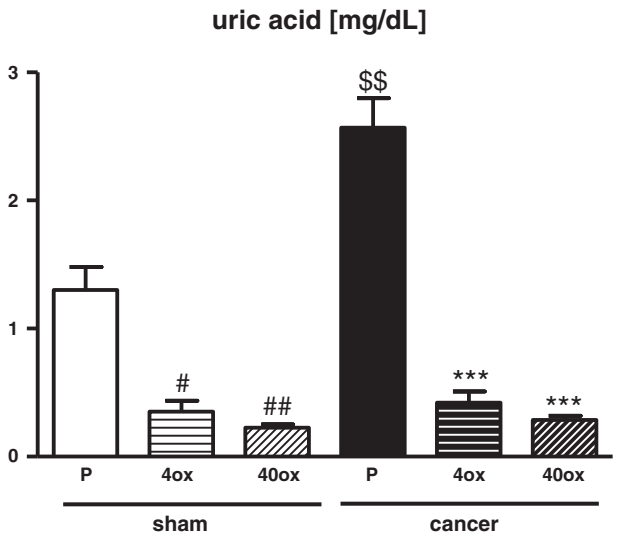

Fig. 1. Uric acid levels were increased in tumour-bearing rats compared to sham. Oxypurinol reduced uric acid levels in both sham and tumour-bearing animals compared to their respective placebo groups. P: placebo, 4ox: 4 mg/kg/d oxypurinol, 40ox: 40 mg/kg/d oxypurinol; ***: $p < 0.001$ vs placebo tumour-bearing groups; #: $p < 0.05$, ##: $p < 0.01$ vs sham placebo, $$: $p < 0.01$ placebo tumour-bearing vs placebo sham.

compared to sham. Treatment with 4 mg/kg/d oxypurinol increased total heart weight compared to placebo-treated tumour-bearing rats (Fig. 2B) This effect was also seen by echocardiographic assessment of LV mass on day 11 (Fig. 2C), although the change in LV mass compared to baseline did not reach significance (Fig. 2D).

4. Cardiac function

Baseline echocardiography showed no differences between groups (data not shown). On day 11 of the protocol, left ventricular (LV) ejection fraction (LVEF) and LV fractional shortening (LVFS) were reduced (by 32% and 43%, respectively) in placebo tumour bearing rats compared to sham and treatment with 4 or 40 mg/kg/d oxypurinol significantly increased both parameters in tumour-bearing rats (Fig. 3A and B). The LV end-diastolic diameter (LVEDD) was significantly lower in placebo tumour-bearing animals (−12%, Fig. 3C) and the LV end-systolic diameter (LVESD) was significantly larger in placebo-treated tumour-bearing rats compared to sham (+33%, Fig. 3D). While treatment with oxypurinol had no significant effect on LVEDD, LVESD was reduced to sham values by oxypurinol treatment (Fig. 3D). Consistent with these results, the LV posterior wall thickness (LVPW) was similar in all tumour groups in diastole (Fig. 4A), but significantly reduced in the placebo-treated tumour-bearing group compared to sham (−30%) or oxypurinol-treated tumour-bearing rats in systole (Fig. 4B).

The LV end-diastolic volume (LVEDV) was reduced by tumour-burden (−32% vs sham), but to a lesser extent in rats treated with 4 mg/kg/d oxypurinol (Fig. 5A). Interestingly, the LV stroke volume (LVSV) was strongly reduced in placebo tumour-bearing rats compared to sham (−51%) and was increased by 4 mg/kg/d oxypurinol, but did not reach statistical significance for 40 mg/kg/d oxypurinol (Fig. 5B). Heart rate (HR) was reduced in placebo tumour-bearing animals (−52% vs sham), which was improved by both treatment doses (Fig. 5C), with the 4 mg/kg/d dose being more effective compared to the high dose. Together with the higher LVSV this resulted in a higher cardiac output (CO) in the 4 mg/kg/d oxypurinol group (Fig. 5D).

5. Discussion

The main finding of this study is that pharmacological inhibition of XO by oxypurinol resulted in a reduced loss of body weight and improved cardiac function. Moreover, 4 mg/kg/d oxypurinol reduced