Formoterol in the treatment of experimental cancer cachexia: effects on heart function

Miriam Toledo · Jochen Springer · Sílvia Busquets · Anika Tschirner · Francisco J. López-Soriano · Stefan D. Anker · Josep M. Argilés

Received: 11 February 2014 / Accepted: 2 June 2014 / Published online: 29 August 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract

Background and aims Formoterol is a highly potent β2-adrenoceptor-selective agonist, which is a muscle growth promoter in many animal species, resulting in skeletal muscle hypertrophy. Previous studies carried out in our laboratory have shown that formoterol treatment in tumour-bearing animals resulted in an amelioration of muscle loss through different mechanisms that include muscle apoptosis and proteolysis.

Methods The study presented involved rats bearing the Yoshida AH-130 ascites tumour model—which induces a high degree of cachexia—treated with the beta-2 agonist formoterol (0.3 mg/kg BW).

Results The administration of formoterol to cachectic tumour-bearing rats resulted in a significant reduction of muscle weight loss. The treatment also increased lean body mass and body water. The treatment, however, did not influence heart weight, which was much decreased as a result of tumour burden. Untreated tumour-bearing rats showed important changes in parameters related with heart function; left ventricle (LV) ejection fraction, fractional shortening, LV diameter and volume (diastolic) and LV stroke volume, LV mass and posterior wall thickness (PWT) (both systolic and diastolic). The administration of formoterol affected LV diameter and volume, LV stroke volume and LV mass.

Conclusions The results suggest that formoterol treatment, in addition to reducing muscle wasting, does not negatively alter heart function—in fact, some cardiac parameters are improved—in animals affected by cancer cachexia.

Keywords Cancer · Cachexia · Muscle wasting · Heart function · Formoterol

1 Introduction

Cachexia is a multifactorial syndrome and appears usually in advanced cancer. It occurs in the last stages in the majority of cancer patients being responsible for a 22% of their deaths [1]. The degree of cachexia is inversely correlated with the expected survival time and always prognoses a poor outcome [2]. Cancer cachexia features anorexia; weight loss; muscle loss and atrophy; anaemia; and alterations in carbohydrate, lipid and protein metabolism [3].

Recent murine studies indicate that skeletal muscle and cardiac muscle wasting in cancer cachexia are associated [4–8]. The decrease in heart weight is accompanied by changes in the cardiac function, which are suggestive of congestive heart failure [4–8]. According to Schünemann, “cancer fatigue syndrome reflects clinically non-overt heart failure”, which clearly gives heart abnormalities part of the blame in the fatigue of cancer patient [9]. Tian et al. suggested that cardiac alterations in a mouse cancer cachexia model include as follows: marked fibrosis, disrupted myocardial ultrastructure and altered composition of contractile proteins such as tropo-in I and myosin heavy chain [7]. Similarly, Mühlfeld et al., in
the cachectic tumour rodent model Lewis lung carcinoma, observed a reduction of the total number of axons in the left ventricle as a consequence of tumour burden [8]. This altered innervation came along with a reduced expression of nerve growth factor [8]. The challenged heart function observed in tumour-bearing animals seems to be specifically related to cardiac alterations [10]. The heart atrophy seems to be linked to increased cardiac muscle proteolysis, shown through elevated protein ubiquitinization and expression of MURF-1 and atrogin-1 [10]. However, Cosper and Leinwand suggest that to increased cardiac muscle proteolysis, shown through elevated protein ubiquitinization and expression of MURF-1 and atrogin-1 [10]. However, Cosper and Leinwand suggest that the cardiac proteolysis is rather caused by increased autophagy [11], contrary to what happens in skeletal muscle. Surprisingly, in experimental animal, the inhibition of NF-κB protects against tumour-induced cardiac atrophy [12]. Drott and Lundholm also observed an increase in oxygen consumption (as well as cardiac atrophy)—most likely related to the anaemia so common in cancer patients—in the heart of an experimental cancer rodent model [13]. Important ultrastructural changes also occurred such as an increased ratio of myofibrils/mitochondria and also sarcomeric alterations, consistent with those observed during cardiac failure. The higher oxygen consumption can be caused by increased energy expenditure, converting the heart in an additional organ involved in generating energy inefficiently. Indeed, the heart rate seems to be elevated in cancer patients [14]. The heart rate seems to be a very effective measure of cancer death risk, although the association between these two parameters is still unclear. The increased heart rate might be a marker of chronic stress and anxiety: a natural consequence of the disease.

Therapeutic approaches to treat or prevent cachexia have not been very satisfactory, mainly because of the toxicity and side effects of the used drugs. The most common anti-cachexia treatments currently used are based on nutritional approaches, progestagens—such as megestrol acetate [15]—or glucocorticoids, the latter showing important adverse effects. Our laboratory introduced the use of β2-adrenergic agonists as possible drugs for the treatment of cachexia [16]. These agents are known as potent muscle growth promoters in many animal species, causing skeletal muscle hypertrophy [17–20], while they reduce the body fat content [21, 22], but unfortunately, β2-adrenergic agonists also show a certain cardiotoxicity [23–25]. Formoterol is a highly potent β2-adrenoceptor-selective agonist, which combines the clinical advantages of the rapid onset of action with a long lasting duration of action. This compound is already prescribed to humans for the treatment of bronchospasm associated with asthma. Our previous studies have shown that formoterol treatment in tumour-bearing animals improved muscle loss through different mechanisms such as muscle apoptosis and proteolysis [26]. A phase II clinical study involving formoterol has also shown positive results in patients with advanced cancer [27].

Bearing all this in mind, and since some β2-agonists are involved in cardiotoxicity, the aim of the present investigation was to analyse the effects of formoterol treatment on heart function in tumour-bearing cachectic rats.

2 Material and methods

2.1 Animals

Male Wistar rats (Harlan-Winkelman, Borchen, Germany) of 5 weeks of age were used in the different experiments. The animals were maintained at 22±2 °C with a regular light–dark cycle (light on from 08:00 a.m. to 08:00 p.m.) and had free access to food and water. The food intake was measured daily. All animal manipulations were made in accordance with the European Community guidelines for the use of laboratory animals.

2.2 Tumour inoculation and treatment

Rats were divided into two groups, namely controls (n=7) and tumour hosts (n=16). The latter received an intraperitoneal inoculum of 10⁸ AH-130 Yoshida ascites hepatoma cells obtained from exponential tumours [28]. The tumour group was further divided into treated (n=8) and untreated (n=8), the former being administered a daily intraperitoneal (i.p.) dose of formoterol (0.3 mg/kg body weight (bw)) and the latter a corresponding volume of solvent. On day 8 after tumour transplantation, the animals were weighed and anaesthetized with an i.p. injection of ketamine/xylazine mixture (3:1) (Imalgene and Rompun, respectively). The tumour was harvested from the peritoneal cavity and its volume and cellularity evaluated. Tissues were rapidly excised, weighted and frozen in liquid nitrogen.

2.3 Body composition analysis

A nuclear magnetic resonance spectroscopy device (EchoMRI-700™, Echo Medical Systems, Houston, TX) was used to assess body composition with a sensitivity of 2 g [29]. Total body fat, lean mass and body fluids can be measured by this system. In this study, body composition was analysed 1 day before starting the treatment and 1 day before sacrifice (8 days), and the results are expressed as the difference between both measurements.

2.4 Echocardiographic study

Rats were anaesthetized using 1.5 % isoflurane and laid in supine position on a platform with all legs taped to ECG electrodes for heart rate monitoring. Body temperature was monitored and maintained at 39 °C using a heating pad. All hair was removed from the chest. A high-resolution echocardiography system (Vevo 770; VisualSonics Inc, Toronto,
Canada) was used [29]. The following parameters were assessed using M-mode: the thickness of intraventricular septum (IVS), left ventricular (LV) posterior wall thickness (PWT), LV end-diastolic diameter (LVDd) and LV end-systolic diameter (LVDs). In this study, echocardiography was performed 1 day before starting the treatment (results not shown) and 1 day before sacrifice (8 days).

2.5 Statistical analysis

Statistical analyses of the data were performed by two-way analysis of variance (ANOVA). Statistically significant differences by post hoc Duncan test. Different letters in superscript indicate significant differences between groups.

3 Results

Formoterol treatment resulted in significant increases in lean body mass and water both in controls and tumour-bearing rats as compared with untreated animals (Fig. 1). Tumour burden resulted in an important decrease not only in lean body mass but also in body fat; the loss of fat mass was unaltered by formoterol treatment (Fig. 1a). In a similar way, the beta-2 agonist did not inflict any changes in body fat in control animals (Fig. 1a). The increase in lean body mass was reflected by an increased weight of all analysed muscles (Fig. 2). In healthy control animals, formoterol treatment resulted in significant increases in gastrocnemius (10 %), tibialis (9 %) and EDL (13 %). In tumour-bearing animals, the treatment promoted an increase of gastrocnemius (15 %), soleus (11 %), EDL (14 %), tibialis (14 %) and diaphragm (23 %) (Fig. 2). Although the results did not reach statistical significance ($p=0.083$), there was a tendency for formoterol to increase heart weight (Fig. 2). Other studies have, however, clearly shown an increase in heart weight in formoterol-treated tumour-bearing animals [26, 27]. The observed effects of formoterol on muscle mass clearly agree with previous studies both in mice [26] and rats [26, 27].

Additionally, we decided to investigate the effects of formoterol on heart parameters in the experimental rat model used in our study. The results presented in Table 1 indicate that tumour-bearing animals displayed an overall deterioration of cardiac function: untreated tumour-bearing rats showed a significant impairment of the left ventricular ejection fraction and the fractional shortening being associated with a worse heart contractility. Also, the stroke volume (LVSV) and the end-diastolic volume (LV vol dia) were reduced in the tumour group compared to control rats, while the end-systolic volume was non-significantly increased.

The administration of formoterol prevented the loss of left ventricular diameter (LVD) (in diastole and systole) (Table 1); in fact, the loss of LV mass is correlated with survival, and its prevention after the treatment with drugs commonly used to treat heart failure improved survival [35]. Furthermore, formoterol could increase LV stroke volume (LVSV) and the end-diastolic and end-systolic volume (LV vol).

4 Discussion

Formoterol is able to ameliorate muscle wasting in tumour-bearing rats through different mechanisms that include decreased protein degradation [26], increased protein synthesis, decreased apoptosis [26] and increased muscle regeneration [30]. Formoterol also reduces oxidative stress associated with muscle wasting [31]. A factor to be taken into consideration is the presence of β-adrenoceptors in tissues other than skeletal muscle. From this point of view, any therapy involving the systemic administration of formoterol must take into account that it affects tissues other than the skeletal muscle.

![Fig. 1](https://example.com/fig1)

**Fig. 1** Body composition in tumour-bearing rats. Results are expressed as the difference between day 0 (tumour inoculation) and day 8; mean ± SEM. C control rats, C + F control rats treated with formoterol, T tumour-bearing rats, T + F tumour-bearing rats treated with formoterol. Statistical significance of the results by two-way analysis of variance (ANOVA). Statistically significant differences by post hoc Duncan test. Different letters in superscript indicate significant differences between groups.
particularly the heart. However, as promising as formoterol seems as a therapeutic drug for cancer-related muscle wasting and weakness (asthenia) through its effect on pathways that modulate skeletal muscle growth, the intracellular pathway involved—β-agonist signalling—has been well described and shows selective coupling to a heterotrimeric G-protein in order to initiate downstream signalling, traditionally believed to occur via the stimulatory Go subunit (Goα) coupling to adenylate cyclase (AC), and resulting in the conversion of ATP to cyclic AMP (cAMP) with the subsequent activation of protein kinase A (PKA) is highly susceptible to down regulation via chronic stimulation, with possible adverse effects if administration is discontinued.

Fig. 2 Skeletal muscles and heart weights in tumour-bearing rats. Results are mean ± SEM. Muscle weights are expressed as mg/100 g of initial body weight (IBW). C control rats, C + F control rats treated with formoterol, T tumour-bearing rats, T + F tumour-bearing rats treated with formoterol. Statistical significance of the results by two-way analysis of variance (ANOVA). Statistically significant differences by post hoc Dun-can test. Different letters in superscript indicate significant differences between groups.

Table 1 Effect of formoterol on cardiac function

| Experimental group          | C       | C + F   | T       | T + F   | ANOVA   |
|-----------------------------|---------|---------|---------|---------|---------|
|                            | A       | B       | A+B     |         |
| LV ejection fraction (%)    | 77±0.7  | 73±0.6  | 68±2.4  | 67±2.3  | 0.000 ns |
| Fractional shortening (%)   | 47±0.7  | 44±0.5  | 38±1.8  | 38±1.7  | 0.000 ns |
| LVD dia (mm)                | 6.3±0.1 | 6.8±0.1 | 5.4±0.2 | 5.8±0.1 | 0.000 0.000 ns |
| LVD sys (mm)                | 3.4±0.0 | 3.9±0.0 | 3.3±0.1 | 3.6±0.1 | ns 0.000 ns |
| PWT dia (mm)                | 1.7±0.1 | 1.7±0.1 | 1.3±0.1 | 1.2±0.1 | 0.000 ns |
| PWT sys (mm)                | 2.7±0.1 | 2.5±0.0 | 2.0±0.1 | 1.8±0.1 | 0.000 0.043 ns |
| LV vol dia (μl)             | 201±5   | 258±6   | 136±5   | 156±7   | 0.000 0.000 0.007 |
| LV vol sys (μl)             | 38±2.2  | 57±2.2  | 43±2.8  | 51±3.3  | ns 0.000 ns |
| LVSV (μl)                   | 163±6   | 201±6   | 93±5    | 105±7   | 0.000 0.000 ns |
| LVmass (mg)                 | 453±15  | 515±17  | 292±23  | 303±8   | 0.000 0.032 ns |
| Cardiac output (mL/min)     | 71±3    | 83±3    | 35±3    | 35±2    | 0.000 ns 0.048 |

Echocardiographic data at day 8 of non-tumour-bearing rats (C), non-tumour-bearing rats treated with formoterol (C + F), tumour-bearing rats (T) and tumour-bearing rats treated with formoterol (T + F). Results are mean ± SEM. LV ejection fraction (LV vol dia-LV vol sys)/LV vol dia; fractional shortening (LVD dia-LVD sys)/LVD sys; left ventricle diameter in diastole (LVD dia); left ventricle diameter in systole (LVD sys); posterior wall thickness in diastole (PWT dia); posterior wall thickness in systole (PWT sys); left ventricle volume in diastole (LV vol dia); left ventricle volume in systole (LV vol sys); left ventricle stroke volume (LVSV) (LV vol dia-LV vol sys); left ventricle mass (LV mass) (expressed as mg/100 g of initial body weight); cardiac output (expressed as mL/min). Statistical significance of the results by two-way analysis of variance (ANOVA); ns non-significant differences. A (tumour effect); B (treatment effect); A*B (interaction effect of tumour and treatment)
Based on a clinical study with over 4,000 autopsy reports, Houten and Reilley [32] suggest that at least 11% of cancer deaths are due to heart problems. In fact, these data could be underestimated since a high percentage of deaths are actually attributed to infections, drug-induced toxicity and alterations in the osmotic balance, which are directly related to heart problems. McBride et al. [33] observed that more than 50% of multiple myeloma patients suffered cardiac failure in the cause of a neoplastic process. Our own data also indicate that in experimental animals, tumour implantation resulted in a lower heart weight [5]. Drott and Lundholm [13] observed a heart-related increase in oxygen consumption in an experimental cancer model. Furthermore, several studies also reported important ultrastructural changes characterized by an increase in the ratio of myofibrils/mitochondria and sarcomeric alterations as also observed during cardiac failure [7]. Moreover, a clear systolic dysfunction is associated with tumour growth [4], and, in mice, cancer induces cardiomyocyte remodelling and hypoinnervation in the left ventricle [8]. The cardiac parameters analysed in the present study are signs for a myocardial dysfunction induced after tumour inoculation. In addition, the reduction of the posterior wall thickness (PWT) suggests atrophy of the myocardium as shown by a reduced LV mass and heart weight in the tumour-bearing rats (Table 1). All these results are similar to the previously reported results by Springer et al. [34, 35]. The authors reported that there was an impairment of the heart function at day 7 due to an increased proteolysis, decreased anabolism and elevated rate of autophagy in the heart in tumour-bearing rats [35].

The results presented here suggest that formoterol may inhibit, to some extent, atrophic mechanisms in the heart, therefore improving heart function. It should be taken into consideration, however, that the improvement in ventricle diameter could also be due to larger chambers. If this were the case, it would suggest heart dilation and incoming failure. Furthermore, the posterior wall is not getting thinner what is typical for dilation. The beneficial effect of formoterol could be explained by the fact that the activation of beta-2-adrenergic receptors can induce anti-apoptotic signalling [26, 36, 37]. Some authors have suggested that inhaled formoterol administration does not show negative effects on healthy subjects [38]. Moreover, formoterol could have some beneficial effects on isolated rat hearts where it improves contractility and thus heart rate [39].

The results suggest that formoterol treatment, in addition to reducing muscle wasting, does not negatively alter heart function in animals affected by cancer cachexia; to the contrary, some cardiac parameters are indeed improved by the β2-adrenoceptor-selective agonist. Future anti-cachectic multi-modal treatment including formoterol may, thus, contribute to decrease cardiomyopathy associated with cancer cachexia.

Acknowledgments This work was supported by a grant from the Ministerio de Ciencia y Tecnología (SAF-26091-2011). The authors certify that they comply with the ethical guidelines for authorship and publishing of the Journal of Cachexia, Sarcopenia and Muscle (von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia and Muscle. J Cachexia Sarcopenia Muscle. 2010;1:7–8.).

Conflict of interest statement and statement of authorship Each author has participated sufficiently, intellectually or practically in the work to take public responsibility for the content of the article, including the conception, design and for data interpretation. All authors have read and approved the final manuscript. All authors of this research have no conflict of interest related with employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations and grants or other funding: Miriam Toledo, Jochen Springer, Silvia Busquets, Anika Tschirner, Francisco J. López-Soriano, Stefan D. Anker and Josep M Argilés declares that they have no conflict of interest.

References

1. Warren S. The immediate cause of death in cancer. Am J Med Sci. 1932;184:610–3.
2. Argilés JM, Moore-Carrasco R, Fuster G, Busquets S, López-Soriano FJ. Cancer cachexia: the molecular mechanisms. Int J Biochem Cell Biol. 2003;35:405–9.
3. Argilés JM, Alvarez B, Lopez-Soriano FJ. The metabolic basis of cancer cachexia. Med Res Rev. 1997;17:477–98.
4. Xu H, Crawford D, Hutchinson KR, Youtz DJ, Lucchesi PA, Velten M, et al. Myocardial dysfunction in an animal model of cancer cachexia. Life Sci. 2011;88:406–10.
5. Olivan M, Springer J, Busquets S, Tschirner A, Figueras M, Toledo M, et al. Theophylline is able to partially revert cachexia in tumour-bearing rats. Nutr Metab. 2012;9:76.
6. Der-Torossian H, Gourin CG, Couch ME. Translational implications of novel findings in cancer cachexia: the use of metabolomics and the potential of cardiac malfunction. Curr Opin Support Palliat Care. 2012;6:446–50.
7. Tian M, Nishijima Y, Asp ML, Stout MB, Reiser PJ, Belury MA. Cardiac alterations in cancer-induced cachexia in mice. Int J Oncol. 2010;37:347–53.
8. Mühlfeld C, Das SK, Heinzel FR, Schmidt A, Post H, Schauer S, et al. Cancer induces cardiomyocyte remodeling and hypoinnervation in the left ventricle of the mouse heart. PLoS One. 2011;6:e20424.
9. Schünemann M, Anker SD, Rauchhaus M. Cancer fatigue syndrome reflects clinically non-overt heart failure: an approach towards onco-cardiology. Nat Clin Pr Oncol. 2008;5:632–3.
10. Tian M, Asp ML, Nishijima Y, Belury MA. Evidence for cardiac atrophic remodeling in cancer-induced cachexia in mice. Int J Oncol. 2011;39:1321–6.
11. Cooper PF, Leinwand LA. Cancer causes cardiac atrophy and autophagy in a sexually dimorphic manner. Cancer Res. 2011;71:1710–20.
12. Wysong A, Couch M, Shafiar S, Li L, Rodriguez JE, Asher S, et al. NF-κB inhibition protects against tumor-induced cardiac atrophy in vivo. Am J Pathol. 2011;178:1059–68.
13. Drott C, Lundholm K. Glucose uptake and amino acid metabolism in perfused hearts from tumor-bearing rats. J Surg Res. 1990;49:62–8.
14. Hyltander A, Drott C, Körner U, Sandström R, Lundholm K. Elevated energy expenditure in cancer patients with solid tumours. Eur J Cancer. 1991;27:9–15.
27. Greig CA, Johns N, Gray C, Macdonald A, Stephens NA, Skipworth RJE, et al. Phase I/II trial of formoterol fumarate combined with megestrol acetate in cachectic patients with advanced malignancy. Support Care Cancer. 2014;22:1269–75.

28. Tessitore L, Costelli P, Bonetti G, Baccino FM. Cancer cachexia, malnutrition, and tissue protein turnover in experimental animals. Arch Biochem Biophys. 1993/10/01 ed. Dipartimento di Medicina ed Oncologia Sperimentale, Universita di Torino, Italy.; 1993;306:52–8.

29. Akashi YJ, Palus S, Datta R, Halem H, Taylor JE, Thoene-Reineke C, et al. No effects of human ghrelin on cardiac function despite profound effects on body composition in a rat model of heart failure. Int J Cardiol. 2009;137:267–75.

30. Ametller E, Busquets S, Fuster G, Figueras MT, Olivan M, de Oliveira CCF, et al. Formoterol may activate rat muscle regeneration during cancer cachexia. Insences J. 2011;1:1–17.

31. Montalban AM, Anzalone G, Albano GD, Di Sano C, Gagliardi R, Bonanno A, et al. Beclomethasone dipropionate and formoterol reduce oxidative/nitrosative stress generated by cigarette smoke extracts and IL-17A in human bronchial epithelial cells. Eur J Pharmacol. 2013;718:418–27.

32. Houten L, Reilley AA. An investigation of the cause of death from cancer. J Surg Oncol. 1980;13:111–6.

33. McBride W, Jackman JD, Gammon RS, Willerson JT. High-output cardiac failure in patients with multiple myeloma. N Engl J Med. 1988;319:1651–3.

34. Springer J, Tschirmer A, Hartman K, von Haehling S, Anker SD, Doehner W. The xanthine oxidase inhibitor oxypurinol reduces cancer cachexia-induced cardiomyopathy. Int J Cardiol. 2013;168:3527–31.

35. Springer J, Tschirmer A, Haghiakia A, von Haehling S, LalH, Grzesiak A, et al. Prevention of liver cancer cachexia-induced cardiac wasting and heart failure. Eur Heart J. 2013.

36. Communal C, Singh K, Sawyer DB, Colucci WS. Opposing effects of beta(1)- and beta(2)-adrenergic receptors on cardiac myocyte apoptosis: role of a pertussis toxin-sensitive G protein. Circulation. 1999;100:2210–2.

37. Shizukuda Y, Buttrick PM. Subtype specific roles of beta-adrenergic receptors in apoptosis of adult rat ventricular myocytes. J Mol Cell Cardiol. 2002;34:823–31.

38. De Mey C, Nassr N, Lahu G. No relevant cardiac, pharmacokinetic or safety interactions between roflumilast and inhaled formoterol in healthy subjects: an open-label, randomised, actively controlled study. BMC Clin Pharmacol. 2011;11:7.

39. Watson DC, Sargianou M, Leivaditis V, Anagnostopoulos C. Beta2-adrenergic activation via administration of atenolol/formoterol combination increases contractility and coronary blood flow in isolated rat hearts. Hell J Cardiol. 2013;54:341–7.