Formoterol and cancer muscle wasting in rats: Effects on muscle force and total physical activity

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Abstract. Cancer cachexia occurs in the majority of cancer patients before death, and it is responsible for the death of 22% of cancer patients. One of the most relevant characteristics of cachexia is that of asthenia, which reflects significant muscle wasting noted in cachectic cancer patients The aim of the present study was to assess whether the β_2-adrenergic agonist formoterol is associated with an improvement in physiological parameters such as grip force and total physical activity in cachetic rats. Administration of the β_2-agonist formoterol (0.3 mg/kg for 7 days) in rats bearing Yoshida AH-130 ascites hepatoma tumors, a model which induces a strong loss of both body and muscle weight, resulted in a significant reversal of the muscle wasting process, as reflected by individual muscle weights. The anti-wasting effects of the drug were also observed in terms of total physical activity and grip force, thus resulting in an improvement in physical performance in cachectic tumor-bearing rats.

Introduction

Cancer cachexia occurs in the majority of cancer patients before death, and it is responsible for the death of 22% of cancer patients (1). Abnormalities associated with cancer cachexia include anorexia, weight loss, muscle loss and atrophy, anemia and alterations in carbohydrate, lipid and protein metabolism (2,3). The degree of cachexia is inversely correlated with the survival time of the patient and is always indicative of a poor patient prognosis (4-6). Perhaps one of the most relevant characteristics of cachexia is that of asthenia, which reflects the significant muscle wasting that occurs in the cachectic cancer patient (7). Deplection of lean body mass is one of the main consequences of cachexia which involves not only skeletal muscle but also affects cardiac proteins, resulting in alterations in heart performance. In addition to the increased muscle protein degradation found during cancer growth, the presence of the tumor also induces an increased rate of DNA fragmentation in skeletal muscle in both rats and mice (8).

β_2-adrenergic agonists are potent muscle growth promoters in many animal species (9,10). Treatment with β_2-adrenergic agonists results in skeletal muscle hypertrophy (11-14), while they cause a reduction in the body fat content (15,16). Formoterol, one of these compounds, is a highly potent β_2-adrenoceptor-selective agonist which combines the clinical advantages of rapid onset of action with duration of action. This compound is currently in use in humans for the treatment of bronchospasm associated with asthma. *In vitro*, formoterol is a potent airway smooth muscle relaxant with high efficacy and high affinity and selectivity for the β_2-adrenoceptor (17). Moreover, formoterol relaxes bronchial smooth muscle and also provides important clinical benefits in symptomatic patients with chronic obstructive pulmonary disease (18).

Previous studies carried out in our laboratory demonstrated that formoterol treatment in tumor-bearing animals resulted in an amelioration of muscle loss through different mechanisms that include muscle apoptosis and proteolysis (19). In light of these findings, the aim of the present investigation was to determine the influence of the cachectic state on the physical performance of rats, and to assess whether the β_2-adrenergic agonist formoterol is associated with an improvement in physiological parameters such as grip force and total physical activity.

Materials and methods

Animals. Male Wistar rats (Interfauna, Barcelona, Spain), 5 weeks of age, were used in the different experiments. The animals were maintained at 22±2˚C under a regular light-dark cycle (lights on from 08:00 a.m. to 08:00 p.m.) and had free

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Table I. Food intake, body weight and muscle weight in tumor-bearing rats.

	C	C+F	T	T+F
Food intake	106±2.0	112±2.5	78±4.4[a]	85±2.1[a]
Body weight				
Initial	128±2.4	122±3.5	127±4.4	125±2.2
Final	164±4.3	159±3.5	123±7.2[a]	126±2.8[a]
Difference	36±2.2	37±0.9	-4.1±4.8[a]	1.6±2.0[a]
Muscle weight				
Gastrocnemius	663±16	725±20[d]	545±8.5[a]	627±9.6[a,f]
Tibialis	215±5.7	238±3.8[e]	188±3.6[b]	208±4.8[a,d]
EDL	51±1.9	60±2.5[d]	41±2.0[c]	48±1.3[b]
Diaphragm	279±14	362±14[f]	117±11[a]	131±7.3[a,d]
Heart	412±16	493±14[e]	318±8.1[a]	367±8.8[a,e]
Carcass weight	90±1.8	91±2.5	72±1.1[a]	78±0.7[a,f]
Tumor cell content	-	-	3638±179	3752±558

C, control; F, formoterol-treated; T, tumor-bearing rat group. EDL, extensor digitorum longus muscle. Results are represented as the mean ± SEM for 8 animals. Food intake is expressed in g/100 g initial body weight (ibw) and refers to the ingestion during the period of the experiment prior to sacrifice which took place 7 days after tumor inoculation. Carcass (body without organs or tumor) weight is expressed as g/100 g ibw. Final weight, body weight without tumor weight. Tumor cell content is expressed in millions of cells.Values significantly different from the non-tumor-bearing animal group are indicated by [a]$p<0.001$, [b]$p<0.01$, [c]$p<0.05$ (Student's t-test). Values significantly different from the non-treated animal groups are indicated by [d]$p<0.05$, [e]$p<0.01$, [f]$p<0.001$ (Student's t-test).

access to food and water. The food intake was measured daily. All animal manipulations were carried out in accordance with the European Commission guidelines for the use of laboratory animals.

Tumor inoculation. Rats were divided into two groups: controls and tumor-bearing hosts. The latter received an intraperitoneal inoculum of 10^8 Yoshida ascites AH-130 hepatoma cells obtained from cells exhibiting exponential growth as previously described (20). Both groups were further divided into treated and untreated groups, the former being administered a daily subcutaneous (s.c.) dose of formoterol [0.3 mg/kg body weight (bw) dissolved in physiological saline solution], and the latter a corresponding volume of solvent. On day 7 after tumor transplantation, the animals were weighed and anesthetized with an intraperitoneal injection (i.p.) of ketamine/xylazine mixture (3:1) (Imalgene® and Rompun®, respectively). Each tumor was harvested from the peritoneal cavity, and the volume and cellularity were evaluated. Tissues were rapidly excised, weighed and frozen in liquid nitrogen.

Total physical activity. Total physical activity was determined for 7 days in the control and tumor-bearing animals (non-treated and treated rats) using activity sensors (IR Actimeter System and Actitrak software from Panlab, Barcelona, Spain) that translate individual changes in an infrared pattern caused by movements of the animals into arbitrary activity counts. Data were collected for a total period of 24 h. In order to carry out the measurements, animals remained in their home cage, and a frame containing an infrared beam system was placed on the outside of the cage. This minimized stress to the animals.

Grip force assessement. Skeletal muscular strength in rats was quantified by the grip-strength test as previously described (21,22). The grip-strength device (Panlab-Harvard Apparatus) comprised a triangular pull bar connected to an isometric force transducer (dynamometer). In brief, the grip strength meter was positioned horizontally, and the rats were held by the tail and lowered towards the device. The animals were allowed to grasp the triangular pull bar and were then pulled backwards in a horizontal plane. The force applied to the bar just before the grip was lost was recorded as the peak tension. At least three measurements were taken per rat at baseline and on test days, and the results were averaged for analysis. This force was measured in grams.

Statistical analysis. Statistical analysis of the data was performed by means of the Student's t-test.

Results and Discussion

Implantation of the tumors resulted in a significant decrease in food intake (26%) of the rats (Table I). This was not reversed upon formoterol treatment, repudiating any possible implication of the β_2-agonist in the reversal of cancer-induced anorexia.

Seven days after tumor inoculation, a clear decrease in body weight associated with a significant decrease in muscle weight was noted (Table I). The decrease in body weight was attenuated by formoterol treatment; in fact, formoterol treatment resulted in significant increases in muscle weight in the tumor-bearing rats (Table I). This effect was observed in the gastrocnemius, tibialis and extensor digitorum longus

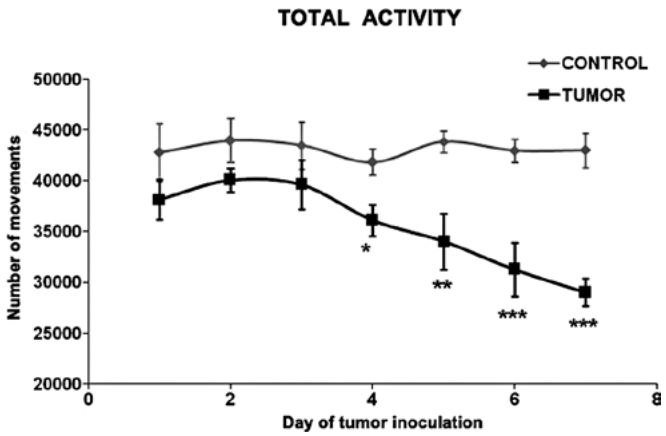

Figure 1. Total physical activity in cachectic tumor-bearing rats. Number of movements represent the total number of movements (locomotor and stereotyped movements) performed by the animals. Stereotyped movements were movements made without displacement (eating and cleaning movements) and locomotor movements were those with displacement. Results are represented as the mean ± SEM for 8 animals and are expressed as the number of movements during the 7-day period after tumor inoculation. Values significantly different from the non-tumor-bearing animal group are indicated by $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ (Student's t-test).

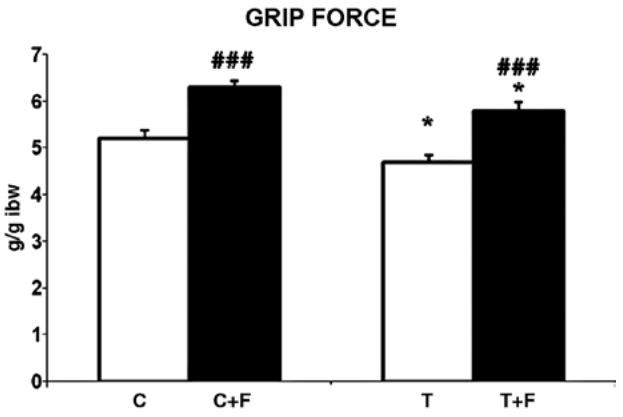

Figure 2. Effects of formoterol on grip force in the cachectic tumor-bearing rats. For more details refer to the Material and methods section. Results are represented as the mean ± SEM for 8 animals. Results are expressed as g/g initial body weight (ibw). C, control; F, formoterol-treated; T, tumor-bearing group. Values significantly different from the non-tumor-bearing animal group are indicated by $^{*}p<0.05$ (Student's t-test). Values significantly different from the non-treated animal groups are indicated by $^{###}p<0.001$ (Student's t-test).

(EDL) muscles and also in the heart. Similar results were previously described by our research group (19,23). Indeed, formoterol and other β_2-agonists such as clenbuterol were found to be effective in ameliorating muscle weight loss during wasting (19,23,24).

At the biochemical level, the mechanisms underlying the effects of the β_2-agonist are complex. Formoterol was found to decrease protein degradation in skeletal muscle by inhibiting the ubiquitin-proteasome pathway (19). In addition, formoterol was found to decrease the enhanced apoptosis observed in skeletal muscle during cancer cachexia (19). Thirdly, at least *in vitro* formoterol increased protein synthesis in skeletal muscle (19). Notably, these effects of the β_2-agonist appear to be associated with an increased muscle regeneration capacity (25).

In spite of these previously demonstrated positive effects of the β_2-agonist at the biochemical level, no measures of physical performance associated with formoterol treatment during cancer cachexia have been reported. Therefore, the aim of the present investigation was to assess whether formoterol, in addition to improving physical and biochemical parameters in an experimental model of cancer cachexia, also affects various factors involved in improving quality of life such as total physical activity and muscle force. In fact, previous investigations with β_2-agonists and muscle strength have lead to controversial results. Lanigan *et al* assessed limb muscle strength and endurance following administration of β_2-agonists and found no beneficial effects on muscle performance (26). Conversely, Signorile *et al* reported that, at least in patients with muscular atrophy following spinal cord injury, β_2-adrenergic agonist treatment resulted in an improvement in muscle strength (27).

In the present study, tumor burden significantly affected the total physical activity in the rats bearing the Yoshida AH-130 ascites hepatoma cell tumors (Fig. 1). As early as 4 days after

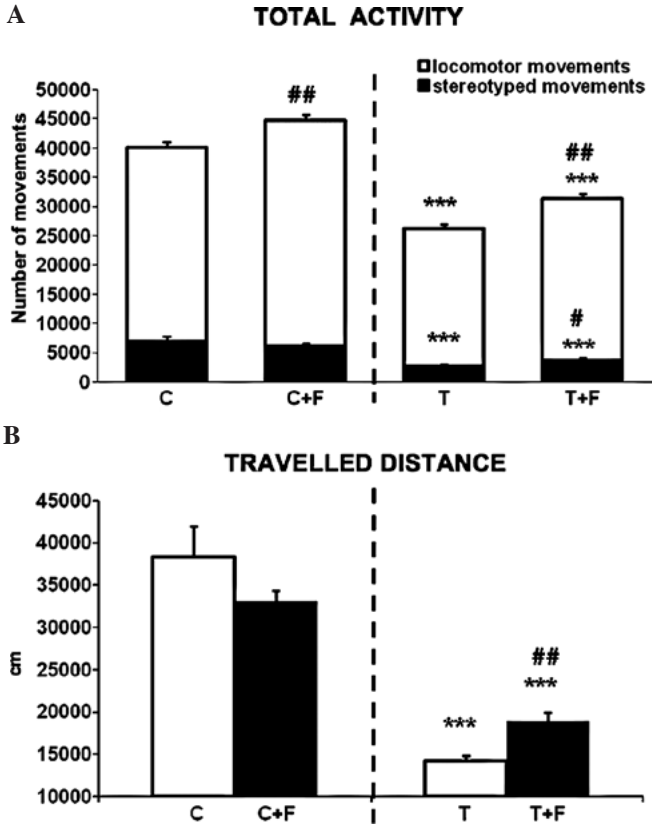

Figure 3. Effects of formoterol on physical activity and distance travelled in the cachectic tumor-bearing rats. (A) Total activity (24 h) was measured as the number of movements that included the total number of movements (locomotor and stereotyped movements) performed by the animals. Stereotyped movements were movements without displacement such as eating and cleaning movements and locomotor movements were movements with displacement. (B) Total travelled distance (cm). Parameters were monitored during the last 24 h before sacrifice (day 7 after tumor inoculation). Results are represented as the mean ± SEM for 8 animals and are expressed as the number of movements. C, control; F, formoterol-treated; T, tumor-bearing group. Values significantly different from the non-tumor-bearing animal group are indicated by $^{***}p<0.001$ (Student's t-test). Values significantly different from the non-treated animal groups are indicated by $^{#}p<0.05$, $^{##}p<0.01$ (Student's t-test).

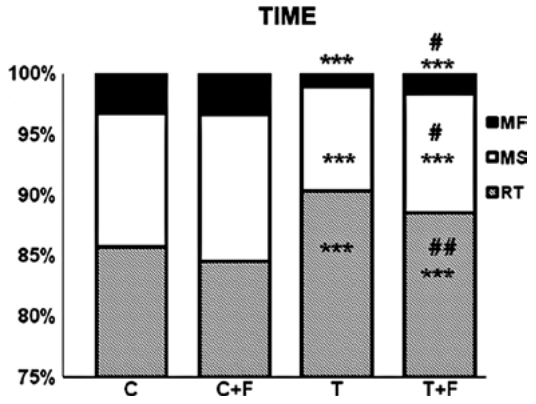

Figure 4. Effects of formoterol on time distribution in cachectic tumor-bearing rats. For more details refer to the Material and methods section. Results are represented as the mean ± SEM for 8 animals. Results are expressed as the percentage of total time. RT, time involving resting (sleeping, cleaning and eating time): 0-2 cm/sec; MS, time involving slow movements: >2-5 cm/sec; MF, time involving fast movements: >5 cm/sec. C, control; F, formoterol-treated; T, tumor-bearing group. Values significantly different from time 0 are indicated by $^{***}p<0.001$ (Student's t-test). Values significantly different from the non-treated animal groups are indicated by $^{\#}p<0.05$, $^{\#\#}p<0.01$ (Student's t-test).

tumor implantation – at which point body and muscle weight loss are already apparent (28) – a significant decrease in physical activity was observed. The decrease continued up until day 7 after tumor inoculation. Similar results have been previously reported using the same tumor model (29). Tumor burden causes a reduction in total physical activity through the activation of muscle wasting either via the release of tumor factors (30) or alternatively through changes in circulating and tissular cytokines or cytokine receptors (31,32).

We demonstrated that formoterol treatment significantly improved grip force in the tumor-bearing rats (23%) (Fig. 2). This correlated with an increase in muscle weight as shown in Table I. Therefore, the β_2-agonist clearly acts at the biochemical level, and its action is reflected in a physiological parameter, grip force, in this case. Notably, formoterol also improved the physical performance of the animals. Total physical activity and total distance travelled by the rats were significantly increased by treatment with formoterol (19 and 33% respectively) (Fig. 3). Moreover, resting time, which was increased in the tumor-bearing rats, was decreased by formoterol treatment. Conversely, slow and fast movement times, which decreased in the tumor-bearing rats, increased in the formoterol-treated rats (Fig. 4).

Collectively, the results presented here allow us to conclude that the treatment of tumor-bearing animals with the β_2-agonist formoterol clearly resulted in an improvement in both muscle force and total physical performance. This, together with previous results obtained by our research group (19), clearly indicate that formoterol may be a good candidate drug for the treatment of muscle wasting associated with cancer cachexia. Further preclinical studies are therefore warranted.

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