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 β₂-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 β₂-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). Depletion 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).

β₂-adrenergic agonists are potent muscle growth promoters in many animal species (9,10). Treatment with β₂-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 β₂-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 β₂-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 β₂-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...
The outside of the cage. This minimized stress to the animals. and a frame containing an infrared beam system was placed on the measurements, animals remained in their home cage, Data were collected for a total period of 24 h. In order to carry by movements of the animals into arbitrary activity counts. System and Actitrak software from Panlab, Barcelona, Spain) treated and treated rats) using activity sensors (IR Actimeter for 7 days in the control and tumor-bearing animals (non-

total physical activity. Tumor inoculation. Rats were divided into two groups: controls and tumor-bearing hosts. The latter received an intraperitoneal inoculum of 10⁸ 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 assessment. 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 β₂-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 muscles.
(EDL) muscles and also in the heart. Similar results were previously described by our research group (19,23). Indeed, formoterol and other $\beta_2$-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 $\beta_2$-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 $\beta_2$-agonist appear to be associated with an increased muscle regeneration capacity (25).

In spite of these previously demonstrated positive effects of the $\beta_2$-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 $\beta_2$-agonists and muscle strength have lead to controversial results. Lanigan et al assessed limb muscle strength and endurance following administration of $\beta_2$-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, $\beta_2$-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
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-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-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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