Abstract. Rats bearing the Yoshida AH-130 ascites hepatoma are subjected to substantial weight loss, which is accompanied by anorexia at the end of the tumour cycle. Total physical activity (measured using the IR Actimeter system and Actitrack software) was determined during 11 days in control and tumour-bearing animals, skeletal muscle strength being also by the grip-strength test. The results presented clearly show that the presence of the tumour induces an earlier decrease in physical performance, which affects both skeletal muscle force and physical activity (both locomotor movements and stereotyped movements and distance travelled, among others parameters).

Introduction

The Yoshida AH-130 ascites hepatoma is a highly cachectic rat tumour of rapid growth and poorly differentiated cells. This tumour is characterized by a relatively short doubling time of one day (1,2) and is widely used in experimental studies (3,4). The implantation of the tumour leads to a very fast weight loss, which is accompanied by anorexia in the terminal state. The weight loss is associated with both fat and skeletal muscle wasting, as has been shown previously by our research group (5-7).

These abnormalities together associated with weight loss, muscle loss and atrophy, anaemia and alterations in carbohydrate, lipid and protein metabolism (8-10) are the main characteristics of the cancer cachexia syndrome. The degree of cachexia is inversely correlated with the survival time of the patient and it always implies a poor prognosis (11-13). Perhaps one of the most relevant characteristics of cachexia is that of asthenia, which reflects the substantial muscle waste that takes place in the cachectic cancer patient (14). Lean body mass depletion is one of the main trends of cachexia and it involves not only skeletal muscle but it also affects cardiac proteins, resulting in considerable alterations in heart performance. In addition to the increased muscle protein degradation found during cancer growth, the presence of the tumour also induces an increased rate of DNA fragmentation in skeletal muscle in both rats and mice (15). All these mechanisms contribute to a marked muscle wasting.

The aim of the present investigation was to determine the influence of the cachectic state on the physical performance of the animals.

Materials and methods

Animals. Male Wistar rats (Interfauna, Barcelona, Spain), five 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 body weight and food intake was measured daily. All animal manipulations were made in accordance with the European Community guidelines for the use of laboratory animals.

Tumour inoculation. Rats were divided into two groups, namely controls and tumour hosts. The latter received an intraperitoneal inoculum of 10⁸ AH-130 Yoshida ascites hepatoma cells obtained from exponential tumours (16). On different days 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, weighed, and frozen in liquid nitrogen.

Total physical activity. Total physical activity (IR Actimeter system and Actitrack software from Panlab, Barcelona) was determined during 11 days in control and tumour-bearing animals using activity sensors that translate individual changes in the infrared pattern caused by movements of the animals.
into arbitrary activity counts. Data were collected for a total period of 24 h separated into 12-h periods (dark and light phases). For the measures, animals remained in their home cage, a frame containing an infra-red beam system was placed on the outside of the cage. This minimised stress to the animals.

**Grip force assessment.** Skeletal muscular strength in rats was quantified by the grip-strength test (17,18). The grip-strength device (Panlab-Harvard Apparatus, Spain) comprised a triangular pull bar connected to an isometric force transducer (dynamometer). Basically, the grip strength meter was positioned horizontally and the rats are held by the tail and lowered towards the device. The animals are allowed to grasp the triangular pull bar and were then pulled backwards in the horizontal plane. The force applied to the bar just before it lost grip was recorded as the peak tension. At least three measurements were taken per rat on both baseline and test days, and the results were averaged for analysis. This force was measured in grams/grams initial body weight.

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

**Results and Discussion**

As can be seen in Fig. 1, the growth of the tumour has two clear phases. In the first one, following a certain lag, the tumour grows exponentially until day 7 after tumour inoculation, when it enters the second phase, which is clearly stationary, since the number of doubling cells equals that of the dying ones. This fast tumour growth induces weight loss from day 4 onwards with a maximum of about 22% (Fig. 1). Interestingly, as can be seen in Fig. 1, no anorexia exists until about day 4 when the animals progressively stop eating. At day 10 after tumour inoculation, the reduction in food intake accounts for 87%. Previous studies carried out in our laboratory have shown that weight loss in this animal model is associated with both adipose and skeletal muscle wasting (6,7).

The muscle loss is due to several factors that include increased proteolysis (5), increased apoptosis (15), increased amino acid oxidation (19,20) and decreased muscle regeneration (21,22).

Bearing all this in mind, the objective of the present investigation was to see to what extent the loss of weight, particularly muscle, is reflected into the physical performance of the animals. As can be seen in Fig. 2, tumour growth is associated with a decreased total physical activity as measured by the total number of movements undertaken by the animals for a period of 24 h. The decrease is already significant at day 4. Interestingly, when total activity is separated into the two light phases, the differences induced by the tumour burden are more marked during the dark phase (night). It is well known that rodents are normally more active during this phase (23). The decrease in physical activity affects both locomotor (movements with displacement) and stereotyped (movements without displacement: eating and cleaning) movements. Again, the decreased activity is more marked for both types of movements during the dark phase (Fig. 3). In Fig. 4, the distance covered by the tumour-bearing rats is depicted. The distance decreases as tumour burden increases and again it seems to be affected more
severely during the dark phase. Interestingly, in addition to the distance travelled by the animals, the velocity of the displacement is also decreased as a result of tumour inoculation (Fig. 5). Again, the presence of the tumour seems...
to influence the velocity of the animals to a higher extent during the dark phase. Finally, as expected, the time involved in physical activity is also influenced by tumour-burden. Indeed, as can be seen in Fig. 6, resting time was increased with tumour burden in the light and dark phases. Conversely, slow and fast movement times were significantly decreased as tumour presence increased, the difference being more marked in the dark phase. Similar results have been described in humans under cancer cachexia (24,25). In experimental animals, similar results have also been found using the C26 adenocarcinoma model (26).

Interestingly, as can be seen in Fig. 7, tumour inoculation also resulted in decreased muscle force as measured by the gripping power of the animals.

From the results presented here, it can be concluded that muscle waste associated with experimental cancer leads to substantial decrease in physical performance.

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