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Perspective

Taltirelin alleviates fatigue-like behavior in mouse models of cancer-related fatigue

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ABSTRACT

Fatigue affects most cancer patients and has numerous potential causes, including cancer itself and cancer treatment. Cancer-related fatigue (CRF) is not relieved by rest, can decrease quality of life, and has no FDA-approved therapy. Thyrotropin-releasing hormone (TRH) has been proposed as a potential novel treatment for CRF, but its efficacy against CRF remains largely untested. Thus, we tested the TRH analog, taltirelin (TAL), in mouse models of CRF. To model fatigue, we used a mouse model of chemotherapy, a mouse model of radiation therapy, and mice bearing colon 26 carcinoma tumors. We used the treadmill fatigue test to assess fatigue-like behavior after treatment with TAL. Additionally, we used wild-type and TRH receptor knockout mice to determine which TRH receptor was necessary for the actions of TAL. Tumor-bearing mice displayed muscle wasting and all models caused fatigue-like behavior, with mice running a shorter distance in the treadmill fatigue test than controls. TAL reversed fatigue-like behavior in all three models and the mouse TRH_1 receptor was necessary for the effects of TAL. These data suggest that TAL may be useful in alleviating fatigue in all cancer patients and provide further support for evaluating TAL as a potential therapy for CRF in humans.

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1. Introduction

Cancer-related fatigue (CRF) is a significant problem. It is prevalent among cancer patients, is not relieved by rest, can significantly reduce quality of life, and may be caused by any combination of the cancer itself, or its treatments (for review, see Refs. [1,2]). Consequently, CRF can result in decreased patient compliance and overall worse clinical outcomes. To treat fatigue, non-pharmacological (e.g., physical exercise and cognitive behavioral therapy) and pharmacological interventions (e.g., methylphenidate [MPH] or modafinil) have been tested. Whereas non-pharmacological interventions may effectively alleviate CRF in many patients, the current drug treatment options may only be effective in specific subsets of patients [3–6]. Indeed, a recent meta-analysis examining the efficacy of clinical trials of non-pharmacological and drug interventions for CRF found that, while the non-pharmacological interventions were often efficacious, drug treatments were not [7]. Non-pharmacological interventions, however, are not feasible or effective for all patients. Moreover, despite research efforts to understand its pathogenesis and develop more effective treatment options, CRF remains undertreated and poorly understood. Given the unmet need, new, effective CRF treatments are needed.

Thyrotropin-releasing hormone (TRH), a tripeptide neurohormone/neuromodulator, showed some promise as a potential novel treatment for CRF [8]. This study was limited, however, by its small sample size of eight participants and by its use of TRH, which degrades quickly in the blood [9] and has low oral bioavailability and poor blood-brain barrier penetration, thus requiring parenteral administration of large doses. In contrast, taltirelin (TAL), an analog of TRH, has greater stability than TRH *in vivo* [10] and a comparable

Abbreviations: 5-FU, 5-fluorouracil; ANOVA, analysis of variance; C26, colon 26 carcinoma cells; CRF, cancer-related fatigue; MPH, methylphenidate; PBS, phosphate-buffered saline; TAL, taltirelin; TFT, treadmill fatigue test; TRH, thyrotropin-releasing hormone.

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in vitro efficacy [11]. Additionally, it is orally bioavailable and efficacious in rodents [12,13]. As such, TAL provides a stronger candidate for exploring the potential for TRH receptor agonism as a treatment for CRF.

Only one human TRH receptor has been identified. The mouse has two subtypes, the TRH_1 and TRH_2 receptor. The mouse TRH_1 receptor is orthologous to the human TRH receptor and their amino acid sequences share 94.6% identity [14]. Moreover, the TRH_1 receptor serves the same endocrine role as the human TRH receptor (i.e., stimulating release of thyroid-stimulating hormone, or TSH) and is highly expressed in the anterior pituitary of the rat [15,16]. The TRH_2 receptor, in contrast, only shares 56.8% amino acid sequence identity with the human TRH receptor [14] has no known endocrine role, and, in rats, is most highly expressed in the thalamus, brainstem, and spinal cord [15]. Given the subtype differences, it may be important to know which TRH receptor may mediate the effect(s) in the mouse before any observations from mice treated with TRH agonists can be definitively translated to other animals or humans.

Numerous mouse models have been developed and used to recapitulate and better understand CRF produced by various causes, including chemotherapy [17–19], radiation [20], and cancer and cancer cachexia [21–23]. Given the many causes of CRF and the possibility that the underlying mechanisms of CRF may vary depending on the cause, it is important to determine if TAL has anti-fatigue effects in multiple models of CRF. Thus, the current study used three mouse models of CRF. The first was a model of chemotherapy-induced fatigue, adapted from a published model [19]. The second was a mouse model of peripheral irradiation that causes fatigue-like behavior by mimicking the peripheral radiation exposure of radiation therapy [20]. The third was a well-established and widely-studied model of cancer cachexia [24–26] in which mice develop tumors that cause fatigue-like behavior [21], muscle wasting, and weight loss [25,27]. Once fatigue was induced, we tested if TAL alleviated fatigue-like behavior in these models. Additionally, we used TRH receptor knockout mice to determine whether the actions of TAL were specifically mediated by a TRH receptor and to identify the responsible receptor(s).

2. Materials and methods

2.1. Animals

Adult male or female C57BL/6NCr mice (7–8 weeks old at the start of experiments) and female Balb/cAnNCr mice (9 weeks old at the start of experiments) were obtained from Charles River Laboratories (Frederick, MD). Adult male and female wild-type (WT) and mice lacking the TRH_1 (R1KO), TRH_2 (R2KO), or both TRH_1 and TRH_2 (R1R2KO) receptor (10–11 weeks old at the start of experiments) were used for the indicated treadmill fatigue test (TFT) and serum hormone analysis. The TRH receptor knockout and WT mice were bred in our colony for these studies and have been previously characterized [11,28,29]. Female C57BL/6 mice were used for chemotherapy-induced fatigue experiments, which used 5-fluorouracil (5-FU) to induce fatigue, as this strain and sex was used to establish the TFT [30] and in other studies using 5-FU [19,31]. Male C57BL/6 mice were used for the radiation-induced fatigue experiment as this strain and sex was used to establish this model [20]. Balb/c mice were used for the colon 26 carcinoma (C26) model as the cells used to produce tumors were originally derived from this strain [24]. Male C57BL/6 mice and the Balb/c mice were individually housed for the duration of the studies. Mice were kept on a 12 h:12 h light cycle (lights off at 6PM). Food and water were provided ad libitum. All experiments were performed with prior approval of the NIDDK or NHLBI Institutional Animal Care and Use Committees.

2.2. Drugs

TAL was purchased from Tocris (Minneapolis, MN) and Santa Cruz Biotechnology (Dallas, TX), MPH was generously provided by Dr. Jonathan Katz of the National Institute on Drug Abuse, 5-FU was purchased from Fresenius Kabi (Lake Zurich, IL), and TRH was purchased from Sigma-Aldrich (St. Louis, MO). Ketamine was purchased from Lloyd Laboratories (Shenandoah, IA) and xylazine was purchased from Putney (Portland, ME).

For i.p. injection or oral gavage administration, drugs were prepared for a volume of 6 or 10 μL/g mouse body weight, respectively. Each day of treatment, 5-FU was diluted with PBS to the necessary concentration. MPH was prepared fresh daily in PBS. TAL was dissolved in PBS and aliquots were stored at −20°C. TAL aliquots were thawed and brought to room temperature prior to injection. TRH was prepared, stored, and thawed in the same manner as TAL.

2.3. Drug treatment

To study alleviation of fatigue, we administered one of three interventions: TAL, MPH, or PBS. TAL was injected i.p. at 1 mg/kg based on previous studies [11,32]. To adjust for lower oral bioavailability, a higher dose (10 mg/kg) was used to test the oral efficacy of TAL. MPH was injected i.p. at 6 mg/kg based upon pilot studies. In pilot studies testing intervention dosing regimens, we found b.i.d. dosing (between 9 and 11 AM and 3 and 5 PM) the day prior to the test, followed by a single dose 30 min prior to testing was effective. Thus, we used this regimen.

2.4. Treadmill fatigue test (TFT)

The TFT was performed per our published method [30]. An Exer-3/6 Treadmill (Columbus Instruments, Columbus, OH), set at a 10° incline with the shock grid delivering 1.22 mA electric shocks at 2 Hz, was used for training and testing. The experimenter did not interact with mice until they met the criterion for fatigue-like behavior, defined as remaining in the designated fatigue zone of the treadmill for 5 consecutive seconds. The fatigue zone encompassed the rear of the treadmill belt and the shock grid. Once fatigued, mice were promptly removed and returned to their home cages. Distance run was the primary measure of performance.

For C57BL/6 mice, the TFT was performed as previously described [30]. Balb/c mice were physically larger and, in pilot studies, displayed greater facility for treadmill running than C57BL/6 mice, so we increased the size of the fatigue zone (fatigue zone for C57BL/6: 20 cm; Balb/c: 22 cm) and tested Balb/c mice using a modified treadmill testing speed protocol to achieve higher testing speeds faster (Table 1).

2.5. Treadmill location

As a secondary measure of performance during the TFT, the location of each mouse was recorded at select times throughout each test. The treadmill was visually divided into three regions, the "top" (~16 cm of the treadmill belt, furthest from the shock grid), "bottom" (~20 or 22 cm [for C57BL/6 or Balb/c, respectively] in length, including the shock grid), and "middle" (~20 or 18 cm [for C57BL/6 or Balb/c, respectively] between the top and bottom). The location of each mouse running was recorded every 60 s for min 1–15, every 5 min for min 20–40, and every 10 min from min 50 through the end of testing. A score of 1 was given for each location recording in the bottom region, 2 for the middle, and 3 for the top. Scores for each mouse in a given test were averaged and the average location scores