Behavioural Brain Research 294 (2015) 25–35

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Depressive-like behaviours and decreased dendritic branching in the medial prefrontal cortex of mice with tumors: A novel validated model of cancer-induced depression

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HIGHLIGHTS

- The onset of depression in cancer patients is biologically mediated.
- We propose a subcutaneous tumor model of cancer-induced depression (CID).
- A positive control model of corticosterone-induced depressive state is used.
- Tumor mice exhibited anhedonia, behavioural despair, and mPFC dendritic atrophy.
- We report the first validated animal model of CID to be used in mechanistic studies.

ARTICLE INFO

Article history:
Received 19 May 2015
Received in revised form 23 June 2015
Accepted 16 July 2015
Available online 26 July 2015

Keywords:
Cancer
Depression
Animal model
Anhedonia
Behavioural despair
Medial prefrontal cortex

ABSTRACT

Depression is commonly comorbid in cancer patients and has detrimental effects on disease progression. Evidence suggests that biological mechanisms may induce the onset of cancer-induced depression (CID). The present investigation aims to establish a validated preclinical animal model of CID. Female BALB/c mice were allocated to four groups: control ($n = 12$), chronic oral exposure to corticosterone (CORT) ($n = 12$), CORT exposure followed by chronic low dose fluoxetine (FLX) treatment ($n = 12$), and subcutaneous inoculation of 4T1 mammary carcinoma cells ($n = 13$). Anhedonia was evaluated using the sucrose preference test (SPT), and behavioural despair was evaluated using the forced swim test (FST) and tail suspension test (TST). Sholl analyses were used to examine the dendritic morphology of Golgi-Cox impregnated neurons from the medial prefrontal cortex (mPFC). CORT exposure and tumor burden were both associated with decreased sucrose preference, increased FST immobility, and decreased basilar and apical dendritic branching of neurons in the mPFC. CORT-induced behavioural and dendritic morphological changes were reversible by FLX. No differences in TST immobility were observed between groups. On the secondary TST outcome measure, CORT exposure and tumor burden were associated with a trend towards decreased power of movement. CORT exposure induced a positive control model of a depressive-like state, with FLX treatment confirming the predictive validity of the model. This verified the sensitivity of behavioural and histological tests, which were used to assess the CID model. The induction of a depressive-like state in this model represents the first successfully validated animal model of CID.

1. Introduction

Major depressive disorder (MDD) is one of the most commonly diagnosed psychiatric disorders in primary care settings. Its lifetime prevalence is ~8–12% [1], increasing to as high as 57% in breast cancer patients and a staggering 95% in high-grade glioma [2]. Depression in cancer patients has often been conceptualized as "reactive depression" due to the expected psychosocial influence

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http://dx.doi.org/10.1016/j.bbr.2015.07.040
0166-4328/

of a cancer diagnosis [3]. However, treating the onset of depression as purely reactive in cancer patients does not account for any possible biological influence of the cancer itself. There is considerable clinical evidence that psychological changes relating to depression actually precede the diagnosis of cancer [4–6]. Over 25% of women with breast cancer exhibit symptoms of depression prior to being informed of their cancer diagnosis [7], and depression symptoms appear to be predictive of a later cancer diagnosis [8]. These clinical findings suggest a biologically causative effect of cancer on the initiation of depressive symptoms, independent of confounding factors such as a patient's knowledge of a psychologically stressful cancer diagnosis.

A major impediment to the mechanistic study, drug development, and effective treatment of CID has been the lack of valid animal models that would facilitate preclinical research. The current investigation aims to address this need by developing a robust, validated mouse model of CID. To accomplish this, we first aimed to demonstrate that our chosen tests were sensitive to depressive-like behaviours induced by an existing, well-established animal model of non-cancer related depression-like state. Predictive validity of the model was also assessed by intervention with a clinical antidepressant, fluoxetine (FLX). Anhedonia, the diminished ability to experience pleasure, is considered a core symptom of MDD [9,10]. In the present investigation, anhedonia was assessed with the sucrose preference test (SPT), which is based on the neurobiological assumption that intake of sucrose-sweetened water is a valid measure of sensitivity to reward [11]. Another common measure of depressive-like behaviour in animals is "behavioural despair", which we assessed using the forced swim test (FST) [12] and tail suspension test (TST) [13]. In these paradigms, despair is characterized by the duration of immobility (i.e. lack of escape behaviour) when exposed to forced swimming or tail suspension. To induce a positive control model of a depressive-like state, we exposed mice chronically to corticosterone (CORT), which provides a well-established preclinical model of depressive-like behaviours [14–21]. FLX, a selective serotonin reuptake inhibitor (SSRI), was used at a low chronic dose to reverse CORT-induced behavioural changes [22,23]. CORT-induced depressive-like behaviours and their reversal by FLX established the sensitivity of the behavioural tests. In parallel with this, a group of animals were inoculated with 4T1 mammary carcinoma cells subcutaneously to determine if a depressive-like state could be induced by cancer alone, and if this model could conform to the requirements of a robust preclinical animal model.

Depression has been associated with dendritic atrophy and reduced neuronal activity in specific brain regions, particularly the hippocampus and medial prefrontal cortex (mPFC) [24–28]. Therefore, a histological analysis of dendritic branching of pyramidal neurons in the mPFC was performed to investigate structural evidence of an induced depressive-like state and corroborate results from the behavioural analyses.

We hypothesized that the behavioural tests and the dendritic analyses would reliably identify depressive-like behaviours and structural changes in the mPFC in the CORT model of a depressive-like state, and that these changes would be reversible by chronic administration of FLX. We further hypothesized that our subcutaneous tumor model would exhibit depressive-like behaviours and dendritic atrophy using the same analyses, thereby validating an animal model of CID for further use in mechanistic studies and pharmaceutical development.

In the current investigation, reversal of CORT-induced behavioural and dendritic changes by FLX confirmed the predictive validity of this model and verified the sensitivity of the behavioural and histological tests. Using these tests, tumor burden was shown to be associated with depressive-like behaviours and decreased dendritic branching in the mPFC, demonstrating a physiological association between cancer and a depressive-like state, and validating this animal model of CID.

2. Materials and methods

2.1. Mice

Forty-nine female BALB/c mice aged 4–6 weeks were obtained from Charles River Laboratories (St. Constant, QC, Canada). Female mice were selected as male mice appear to more frequently gnaw and bite at tumor sites [29]. Mice were single-housed in sterile cages maintained at 24 °C with a 12-h light/dark cycle, and were provided ad libitum access to autoclaved food and water. All procedures were performed according to guidelines established by the Canadian Council on Animal Care under a protocol reviewed and approved by the *Animal Research Ethics Board* of McMaster University.

2.2. Drug treatments

To induce a positive control model of a depressive-like state, mice were administered chronic oral CORT as outlined previously [15]. Briefly, the pH of sterile water was increased to 12–13 using 10 N NaOH and CORT hemisuccinate (Steraloids, Newport, RI, USA) was added and allowed to dissolve overnight at 4 °C to slow its decay. Once dissolved, the pH was neutralized to 7.0–7.5 using 10 N HCl. The resulting CORT solution (35 μg/mL) was administered ad libitum to mice in place of their normal drinking water over a 21-day period. Mean dose was calculated to be 6.53 ± 0.16 mg/kg/day in these animals.

For the reversal of CORT-induced depressive-like behaviours, CORT treatment in drinking water was stopped, and the SSRI FLX was administered ad libitum in drinking water for a 21-day period. FLX hydrochloride (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in sterile drinking water at a concentration of 150 μg/mL to obtain dosages between 10 and 18 mg/kg/day [23]. The mean dose of FLX consumed was 16.46 ± 0.16 mg/kg/day. FLX was administered in opaque bottles to protect it from light.

2.3. Tumor cell inoculation

Murine 4T1 mammary carcinoma cells are derived from a spontaneously arising mammary tumor in BALB/c mice [30,31]. 4T1 cells (American Type Culture Collection, Manassas, VA, USA) were maintained in culture according to supplier specifications. Mice were anesthetized by isoflurane inhalation and inoculated with 15,000 4T1 cells in serum-free RPMI 1640 media (Life Technologies, Burlington, ON, Canada) subcutaneously on the right side of their lower backs. This tumor site was selected instead of the orthotopic site to avoid movement constraints and potential confounding behaviours in the FST and TST. All other mice that did not receive 4T1 injections were sham-inoculated with serum-free RPMI 1640 media. Tumor size was monitored using a digital caliper every 3–4 days once tumors became palpable at day 8. Mice were weighed weekly and overall health status was monitored every 3–4 days initially, and then daily during the final week prior to endpoint. All mice were euthanized 28 days after the mice in the tumor group were inoculated with cancer cells.

2.4. Behavioural analyses

Sucrose preference was evaluated as a measure of anhedonia. Mice were first habituated to 3% sucrose over a 72-h period. During this period, regular drinking water was replaced with 3% sucrose solution. This concentration of sucrose has been shown in our preliminary experiments to provide a robust sucrose preference