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Research report

Interferon-alpha treatment induces depression-like behaviour accompanied by elevated hippocampal quinolinic acid levels in rats

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HIGHLIGHTS

- One week administration of IFN-α induces depression-like behaviour in the rat forced swim test.
- IFN-α treatment alters brain tryptophan levels and downstream kynurenine metabolites.
- Imipramine reverses IFN-α-induced depression-like behaviour.
- Celecoxib shows potential for antidepressant activity possibly mediated via the tryptophan–kynurenine pathway.

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ABSTRACT

Immunotherapy with the cytokine interferon-alpha (IFN-α) can induce symptoms of depression, and it is likely that the tryptophan–kynurenine pathway may be involved in this regard. In this study we investigated the effects of IFN-α on depression-like behaviour and central metabolites of the tryptophan–kynurenine pathway in rats. Secondly, we explored the modulating effects of an antidepressant (imipramine) and anti-inflammatory drug (celecoxib) on IFN-α-induced behavioural and pathophysiological changes in the brain. The following treatment groups were used: Control (saline), IFN-α (6×10^4 IU/kg s.c.), IFN-α + imipramine or IFN-α + celecoxib. Drugs were administered daily for 1 week. IFN-α treatment induced depression-like behaviour by increasing immobility in the forced swim test (FST), and decreased tryptophan levels in the brain. There was a trend for an increased kynurenine/tryptophan ratio, indicative of indoleamine 2,3-dioxygenase (IDO) activation, and increased quinolinic acid in the hippocampus. Imipramine decreased immobility in the FST, but did not reverse the IFN-α-induced changes in the tryptophan–kynurenine pathway. There was a trend for celecoxib to decrease immobility and to reverse the IFN-α-induced increase in the kynurenine/tryptophan ratio. Thus, our study provides further evidence for IFN-α-induced depression-like behaviour through central changes of the tryptophan–kynurenine pathway.

1. Introduction

Immunotherapy with interferon-alpha (IFN-α) is commonly used for chronic hepatitis C and several types of malignancies due to its antiviral, antiproliferative, and immunoregulatory effects [7]. However, IFN-α therapy causes serious side effects; early signs include neurovegetative symptoms (anorexia, fever and fatigue) [3], while prolonged therapy causes symptoms of depression in up to 45% of patients leading to discontinuation of the therapy [22,23,2]. Similar observations have been made in animal studies following IFN-α treatment, such as early signs of sickness (similar to the neurovegetative symptoms in humans) [6,30,] and depression-like behaviour following chronic IFN-α treatment [19,20,25,].

A number of findings suggest that the neuropsychiatric side effects during IFN-α therapy may be linked to aberrations in the tryptophan–kynurenine pathway [2,34]. Clinical studies have found that IFN-α therapy reduces plasma tryptophan and serotonin levels [2] and increases kynurenine levels in plasma and CSF

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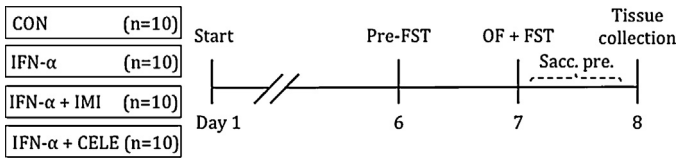

Fig. 1. Experimental design illustrating treatment groups and behavioural tests: The pre-forced swim test (pre-FST) was performed on day 6, the open field (OF) and FST performed on day 7, and the saccharin preference (Sacc. pre) test was performed the following night. Animals were euthanized and tissue samples collected on day 8.

[2,4,27]. In addition, the kynurenine/tryptophan ratio, an index of indoleamine 2,3-dioxygenase (IDO) activity, is increased in patients receiving IFN-α therapy [2,4,5,35,].

IDO is an extrahepatic enzyme that catalyses the conversion of tryptophan into kynurenine [12] which may lead to the production of neuroactive metabolites such as kynurenic acid, 3-hydroxykynurenine and quinolinic acid. Interestingly, quinolinic acid levels in CSF have been found to correlate with the severity of depression symptoms [27], and post-mortem studies have shown increased microglia quinolinic acid levels in subregions of the frontal cortex from severe depressed patients [31].

However, changes in the brain levels of kynurenine metabolites have not yet been determined following IFN-α administration, as well as the modulating action of antidepressants on these effects. The aim of this study was therefore to investigate the effects of chronic IFN-α administration on depression-like behaviour and brain tryptophan–kynurenine metabolites in rats. In addition, we studied whether co-administration of the antidepressant imipramine or the anti-inflammatory agent celecoxib can reverse the potential IFN-α-induced behavioural and physiological effects.

2. Methods

2.1. Animals

Adult male Sprague-Dawley rats ($n = 40$), weighing 295.5 g ± 2.4 g at the beginning of the experiment, were purchased from Taconic (A/S Denmark), and allowed one week for habituation. Animals were housed in pairs with water and food ad libitum, in a temperature controlled room (22 ± 1 °C) and a 12-h light dark cycle (light on at 6 AM). All experimental procedures were approved by the Danish National Committee for Ethics in Animal Experimentation (permission id 2012-15-2934-00254, C5).

2.2. Experimental design

Animals were randomly divided into four groups: control (CON), IFN-α treatment (IFN-α), IFN-α and imipramine (IFN-α + IMI) or IFN-α and celecoxib (IFN-α + CELE), with $n = 10$/group. Drugs were administered daily for 7 days. Depression-like behaviour was assessed on day 7, and the experiment was terminated 24 h after the 7th injection on day 8 (Fig. 1).

2.3. Drugs and treatments

IFN-α (recombinant human Interferon Alpha A/D, R&D Systems) was administered by daily subcutaneous injections at a dose of 6×10^4 IU/kg/day. IFN-α was dissolved in phosphate buffered saline (PBS, pH 7.4) containing 0.1% Bovine Serum Albumin (BSA). Control animals received PBS. All injections were given at approximately 9 AM. The IFN-α type was chosen on the basis that it has been previously shown to be behavioural active in rodents Dunn et al. [8], and the dosage choice was based on previous studies showing a depression-like phenotype in rats [20]. Celecoxib was chosen because a recent review found that it have beneficial effects on symptoms of depression in humans [14], and the dose (16 mg/kg/day, celebrex®, Pfizer, Germany) was based on previous studies showing an anti-inflammatory and antidepressant effect in rats [13]. The antidepressant imipramine (Sigma UK) was chosen because of its strong antidepressant actions in the forced swim test [15], and the dose (10 mg/kg/day) was selected based on previous studies showing antidepressant effects following cytokine administration in rodents [19]. Celecoxib and imipramine were mixed into food pellets at aforementioned dosages (16 mg/kg/day and 1 mg/kg/day, respectively), with the exact dose received being calculated by daily weighing the amount of food consumed. As the animals were housed in pairs, the daily amount of food was divided by two to assess the intake by the individual animal.

2.4. Evaluation of sickness behaviour

Body weight, food and water intake were measured daily before drug administration. Locomotor activity was assessed in the open field test 24 h after the 6th injection, immediately prior to the forced swim test (FST). The apparatus was a square arena (100 cm × 100 cm) with dark walls and floor. Rats were placed in a corner and allowed to explore the area for 5 min. The session was recorded digitally and scored using EthoVision version 8 (Noldus Information Technology).

2.5. Depression-like behaviour

Depression-like behaviour was evaluated using the FST and the saccharin preference test to evaluate behavioural despair and anhedonia, respectively.

2.5.1. Forced Swim test (FST)

The modified FST was conducted with a 15 min initial swim session, followed by a 7 min test trial 24 h later on day 7 [18,26]. Briefly, rats were placed in a cylinder (*h*: 60 cm, *d*: 18 cm) containing 40 cm deep water at 24 °C ± 1 °C for 15 or 7 min. The trials were recorded and the first 5 min scored blindly by an experienced observer. Depression-like behaviour was assessed as the time spent immobile, namely when the rats only make the necessary movements to keep their heads above the water.

2.5.2. Saccharin preference

The saccharin preference test was conducted overnight after the 7th injection. Rats had access to two bottles, one containing a saccharin solution (0.1%) and the other tap water. Fluid consumption was obtained by weighing the bottles before and after each test session, and saccharin preference was calculated as the percentage of saccharin compared to the total amount of liquid consumed, and normalised to baseline saccharin consumption. Baseline saccharin intake was obtained during the week preceding the experiment onset to ensure that rats were habituated to the taste of saccharin. Four trials with the two-bottle choice similar to the trial session were used for habituation.

2.6. Tissue collection and homogenization

Twenty-four hours after the 7th injection, animals were euthanized by decapitation. Brains were quickly removed frontal cortex, hippocampus and hypothalamus were dissected, frozen on dry ice and stored at −80 °C until later analysis.

Right frontal cortex, right hippocampus and hypothalamus were weighed and rinsed from blood using Cell Wash Buffer (Bio-Rad). Tissue was homogenized in 5x volume of Cell Lysis buffer (Bio-Plex™ Cell Lysis Kit, Bio Rad) containing protease inhibitors and a