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Preliminary Report on PEG-Interferon-Induced Depressive- and Anxiety-like Responses in Mice

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Abstract

Interferon therapy is well-known for its association with medication or treatment-induced depressive symptoms, which limits its clinical efficacy for the treatment of hepatitis, cancer, etc. In humans, conventional interferons and pegylated (PEG) interferons both demonstrated therapeutic efficacy and similar side effect profiles. However, in the preclinical model, while direct injection of conventional formulations is well established to induce depressive-like responses in rodents, the depressive-inducing effect of PEG-interferon is still debatable. Previous studies showed that PEG-interferon is ineffective; in this study, we adjusted the dosing and frequency of PEG-interferon treatment and have demonstrated encouraging preliminary results. In comparison with control mice, the PEG-interferon-treated mice showed elevated immobility time in the tail suspension test, lower interaction time in social interaction, and reduced locomotor activity in the open field test. All these data suggest that PEG-interferon, with an adjusted dose and frequency relevant to its clinical therapeutic regime, can cause significant depressive and anxiety-like responses and thus may be a potential model of interferon therapy-induced depression.

Keywords: Interferon, depression, anxiety, psychological wellbeing, preclinical model

Introduction

Interferon (IFN) is a superfamily of cytokines generated endogenously by the body in response to various stimuli, such as the presence of foreign bacteria or viruses(1). The predominant components of type I IFN are IFN-α and IFN-β, which are produced by leukocytes and fibroblasts. IFN-γ is classified as type II IFN and is typically secreted by T-cells and natural killer cells. Because of its immunomodulatory, antiproliferative, and antiviral properties, it is extensively used to treat cancers such as leukaemia, non-Hodgkin's lymphoma, and hepatitis B and C infections.

Due to its high susceptibility to proteolytic degradation in the gastrointestinal tract following oral administration, IFN is typically administered subcutaneously or intramuscularly from a pharmacokinetic standpoint. Tmax for type I IFN ranges from 1 to 8 hours, with concentrations remaining detectable for 4 to 24 hours. The incorporation of polyethylene glycol (PEG) or albumin into IFN- increases its efficacy and half-life by decreasing renal clearance and increasing molecular size(2, 3).

Since IFNs are highly involved in the regulation of the immune system, endocrine system, and neuronal pathway, depression is one of the most notable side effects of IFN therapy. Dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis plays a crucial role in this phenomenon by elevating corticotropin-releasing hormone (CRH), which stimulates the adrenal gland excessively and decreases serotonin and norepinephrine in a specific brain region (4). In addition, it is a powerful

proinflammatory cytokine that induces multiple pathways, including JAK-STAT, CRK, and MAPK. p38, which induces the activation of multiple protein kinases and leads to the expression of the glucocorticoid beta isoform receptor, is one of the MAPK downstream components(5). This results in the loss of glucocorticoid receptors' initial active function to inhibit proinflammatory cytokine release and regulate CRH release.

Despite the common reports of depression cases in patients treated with conventional interferon and PEG-interferon(6), previous animal studies showed opposite findings. While conventional interferon causes depressive- and anxiety-like responses in rodents(7), PEG-interferon was reported otherwise(8). In the present study, we adjusted the dosing and treatment frequency of PEG-interferon to mimic the clinical treatment regime and tested whether depressive- and/or anxiety-like responses were present.

Material and Methods

Chemicals and Reagents

PEG-interferonwas purchased from Roche Pharmaceuticals under the brand name Pegasys® (Basel, Switzerland).

Animals

Eighteen male Institute of Cancer Research (ICR) mice,6-7weeks old, weighing 30g ± 7g, were housed in a temperature (25°C), humidity (72%) and light (12-hour light/dark cycle) controlled cages with food and water*ad libitum*. The mice were allowed to acclimatise to the laboratory environment for at least 7 days before the experiment.

Experimental protocol

Each mouse in the PEG group (n=9) was given intraperitoneal (i.p.) injection of PEG-interferon (36 µg/kg/day) for 14 days. On the other hand, the mice in the negative control (CTRL, n=9) group received vehicle Behaviouralexperiments were conducted on the 15th day.

Open Field Test

A large open-field testing apparatus is separated into four 50 x 50 cm quadrants. One mouse was placed at the centre of each quadrant, and its exploratory behaviours were recorded for 30 minutes with a recorder fixed on top of the open-field testing apparatus. The locomotion wasanalysed, which represents anxiety-like behaviour in rodents. The apparatus was treated with a 75% ethanol solution after each batch of testing. The open fied test was conducted based on previous studies, with minor change(9, 10).

Social Interaction Test

The social interaction test was conducted on the same apparatus as the open field test, as previously described with minor modifications (9, 11). A strange mouse is enclosed in a perforated plastic cup on one side of each chamber. An empty enclosure was fixed opposite the strange mouse. The active interaction between the subject mouse and the strange mouse is recorded by the webcam mounted on top of the apparatus. The social preferences of the subject mouse were analysed.

Tail Suspension Test

The mouse's tail was fixed to the end of the table with adhesive tape and the whole body of the mouse was freely suspended in the air.118 Black cardboard was used as a background to provide contrast. The test was carried out and recorded for a duration of 6 minutes. The immobile time of each mouse was measured and serves as an indicator for depressive-like responses in rodents(12).

Statistical analysis

All data was presented as mean ± standard error of mean (SEM). Student's t-test were employed to analyse the statistical difference between groups. Statistical differences were considered significant if $P < 0.05$.

Results and Discussion

In the present study, the mice received repeated injections of PEG-interferone, which

is known to cause oxidative stress and neuroinflammation in the limbic system, leading to neuropsychological disorders(13).

Figure 1 demonstrates the distance travelled(m) by the subjects in the open field test.There is a significant decrease in distance travelled in PEG-interferon-treated group as compared to the control group.The open field test is a technique widely used in rodent behavioural studies where the rodent's exploratory behaviour is quantified and assessed under anxiety-related stress conditions(14).Low activity showed in highly emotional mice and vice versa in low emotional mice. Stimulus from the open field test can be assumed as predator challenges, anxious and stressed mice will have "freezing" action to disguise themselves with surrounding and give up on struggling. A healthy mouse is highly active due to its exploration habits tending to move around with long distance travelling(15). injected with vehicle (CTRL). In this study, the mice which received PEG-interferon exhibited a higher level of anxiogenic-like behaviour than their counterparts administered with the vehicle, instead of PEG-interferon.

Next, we examined whether PEG-interferon treated mice exhibit social withdrawal, one of the common indicators of depression and/or anxiety(16).The main principle of SIT is to assess the social preference or social behaviour of rodents towards a standard tester. The variable that is being quantified is the time spent by the subject mice in social interaction(17). The anxiogenic effect can be seen through a sociability decrease in the subject mice and vice versa, an increase in social interaction time is indicative of an anxiolytic effect. Figure 2 shows that the PEG-interferon treated mice has significantly higher tendency to spend their time alone while in contrast, the control group has the higher interaction time with stranger mice.

The tail suspension test is a method used to assess the ability of the animal to cope with moderately stressful conditions by establishing an inescapable or helpless situation where the tail of mice is suspended for a duration of 6 minutes(18). Observation of the rodent's escape-oriented movement or agitation followed by immobility is being used to evaluate the depressive-like effect of PEG-IFN. The action of immobility in tail suspension test is believed to be the disruption in the mice's behaviour to be able to persistently escape the uncontrollable stress situation(18).The data obtained from our results (Figure 3) shows that the mice receiving PEG-interferon therapy demonstrated significantly higher immobile time than the control group. Those mice were perceived to establish a despair behaviour towards the

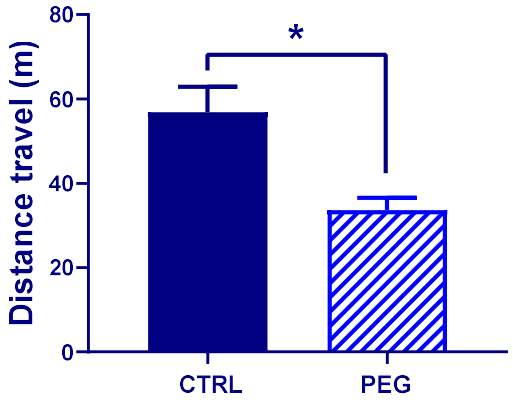

Figure: 1: Open fieldtest

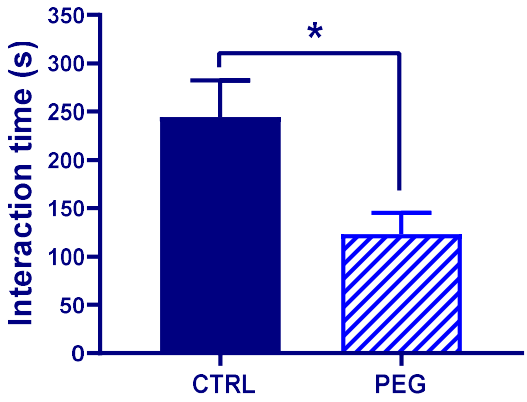

Figure: 2: Social interaction test

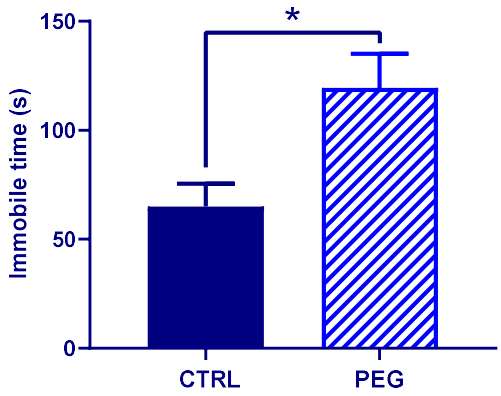

Figure: 3: Tail suspension test

unfavourable condition due to a lack of struggling or escape-oriented behaviour which serves as a symptom of depression.

Conclusion

In the present study, we have demonstrated a model that utilised PEG-interferon as inducer that may mimic PEG-interferon associated depressive- and anxiety disorders in clinical settings. This preclinical model has the potential to be applied in future studies to discover novel pharmacotherapiesfor these conditions, as an alternative to the conventional interferon formulation.

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Conflict of Interest

The authors declare no conflict of interest.

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