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Pegylated human interferon alpha 2a does not induce depression-associated changes in mice

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ABSTRACT

Interferon (IFN) alpha proteins are proinflammatory cytokines having immunomodulating and antiviral properties. States during which cytokine systems are activated (e.g., during viral infection or during treatment of chronic hepatitis C and various malignancies with IFN alpha, etc.) can be associated with depression-like syndromes or even full-blown depressive episodes. Therefore, the role of IFN alpha and other cytokines in the pathogenesis of depressive disorder ("cytokine hypothesis of depression") has been assessed for many years with contradictory results. We have investigated whether intraperitoneal administration of high doses (up to 600 µg/kg body weight) of pegylated, recombinant human IFN alpha 2a in mice induces changes known to be associated with depression using three different readouts: behavior in a model of despair (Porsolt swim test), presence of anhedonia (sucrose preference test), and sensitivity of the hypothalamic–pituitary–adrenal system (dexamethasone suppression test). We also assessed potential IFN-induced changes in gene expression in the liver. In none of the performed experiments, depression-associated effects could be found despite very high serum levels of IFN-induced antiviral activity compared to levels measured in hepatitis C virus (HCV) patients treated routinely with pegylated recombinant human IFN alpha 2a. The lack of such expected effects is probably due to the fact that pegylated human recombinant IFN alpha 2a does not activate the murine class I IFN receptor. Our results do not support the hypothesis that administration of recombinant pegylated human IFN alpha to mice produces a robust model of depression.

1. Introduction

Polyethylene glycosylated interferon (PEG-IFN)-alpha2a or PEG-IFN-alpha2b and ribavirin is currently the standard therapy for chronic hepatitis C (Deutsch and Hadziyannis, 2008). PEG-IFN has improved pharmacokinetic and antiviral properties compared to non-pegylated IFN. One cluster of side effects of IFN treatment, depression-associated symptoms, occurs in 21–58% of patients receiving IFN alpha (Raison et al., 2005). Neurobehavioural symptoms experienced during IFN alpha therapy include depressed and anxious mood, anhedonia, suicidal thoughts, tension, loss of concentration, fatigue, loss of energy, sleep disturbances, psychomotor retardation, abnormal appetite, pain, etc. overlapping greatly with symptoms of depression (Raison et al., 2005, 2006).

Symptoms of depression are mediated centrally. Most cytokines are relatively large, hydrophilic molecules. They may be passively transported from the blood to the brain parenchyma at sites where the blood-brain barrier is absent or less restrictive. An indirect route for cytokine-mediated immune-to-brain communication may be provided by signaling via the vagus nerve (Schiepers et al., 2005). IFN alpha exerts its effects through binding to the type I IFN receptor which is also found in the central nervous system. This leads to activation of transcription of different genes involving the JAK-STAT pathway (Pestka et al., 2004). Some data indicate, however, that IFN alpha might induce behavioral effects by binding to opioid receptors (Makino et al., 2000a). IFN alpha has been shown to activate corticotropin-releasing factor (CRF) production and/or release in animals, to induce Adrenocorticotropic hormone (ACTH) and cortisol in IFN-treated patients developing depression, and to alter serotonin and dopamine metabolism, key pathophysiological mechanisms involved in depression (Capuron and Miller, 2004; Raison et al., 2006).

In several studies, the induction of symptoms or changes known to be associated with depression has been studied by administering IFN in rodents peripherally or centrally. Observed parameters include the forced swim test, a model of behavioral despair with a high validity and specificity to test antidepressant efficacy of drugs (Porsolt, 2000), locomotor activity, reward behavior, neuroimmune and

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neuroendocrine activation, activation of IFN-stimulated genes in the central nervous system and others (Cryan et al., 2002; De La Garza et al., 2005; Loftis et al., 2006; Wang et al., 2008). Experimental settings vary greatly between the studies. Pharmacological parameters (dose, place of administration, frequency of administration, and total number of doses) and time course of the experiments differ. IFNs of different species (rodents and human) and classes have been tested in mice and rats. Therefore, results are not homogeneous and even contradictory and at the moment, no paradigm of IFN alpha-induced depression in rodents has been clearly established (see also discussion).

We examined whether it is possible to induce behaviors associated with depression using pegylated human IFN alpha in mice at conditions assuring high levels of IFN-induced antiviral activity in serum. We assessed three different readouts of depression-related measures (forced swim test, sucrose preference, and dexamethasone challenge) and changes in gene expression characteristic of an IFN challenge by i.p. administration of pegylated recombinant human IFN alpha 2a (Pegasys®).

2. Methods

This study was conducted in Bonn and Heidelberg, Germany, in accordance with the ethical principles for medical research involving human subjects (World Medical Association, 2002) and local legislation concerning human and animal research and according to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Pegylated IFN alpha 2a (Pegasys®) was a gift of Roche Germany.

2.1. Biological activity of pegylated IFN alpha 2a in humans

We assessed first the level of antiviral activity in humans suffering from chronic hepatitis C and undergoing standardized treatment with Pegasys® (weekly subcutaneous injection of 180 µg). In seven patients 9 ml of venous blood was collected in potassium-ethylenediaminetetraacetic acid (EDTA)-containing tubes (one sample/patient, one as a negative control before the start of IFN therapy, the six others after 4–5 weeks of IFN treatment). Collection occurred always immediately before the subsequent weekly injection. Serum was prepared according to standard procedures and kept at −20 °C until assessment of the antiviral activity.

Biological activity of IFN in serum samples was measured in a standard cytopathic effect inhibition assay using the human lung carcinoma cell line A549 (ATCC CCL 185) cultured in DMEM containing 10% FCS. Monolayers of 30,000 cells/well in flat-bottom microtiter plates were incubated overnight with twofold dilutions of samples. The liquid was then removed. The cells were washed and infected with Mouse Encephalomyocarditis-Virus (EMCV) at a multiplicity of 0.5. After 26–30 h, when a pronounced cytopathic effect had developed in untreated control cultures, the suspension was removed and the cell monolayers were fixed with 2% glutaraldehyde solution for 3–5 min. The fixative was then removed, and the plates were washed with water to remove all debris of cells lysed by viral infection. Subsequently, the remaining protected cells were stained with 1% crystal violet for 5 min. After thorough washing and drying of the plates the dye was solubilized in 33% aqueous glacial acetic acid and the OD of the eluate was quantified in an enzyme-linked immunosorbent assay (ELISA) reader at 570 nm. A twofold dilution of an internal laboratory standard preparation of human IFN based on the NIH reference IFN alpha preparation (cat. no. Gxa01-901-535) was included in each test. One laboratory unit corresponded to one international unit (IU) defined as the concentration resulting in a 50% protection against viral lysis. A 50% protective effect was assumed at half maximal OD570 values. Titers of the antiviral bioassay are given as IU.

2.2. Animal experiments

These experiments were carried out in two parts. The aim of the first experiment was to find a treatment schedule, which produces similar INF-alpha serum activity as observed in human patients. We then used the identified condition to conduct the second part (dexamethasone suppression, glucose preference and Porsolt tests as well as collection of liver samples).

CD1 male mice (22–26 g body weight at the beginning of the experiment) were kept in groups of three in the first and single housed in the second set of experiments. The animals had free access to food or drinking solution.

In the first animal experiment, we assessed the level and evolution of IFN-induced antiviral activity after the weekly i.p. administration of IFN alpha 2a up to 4 weeks. Mice were injected 0 (control), 6, 60 or 600 µg/kg body weight of IFN alpha 2a in a volume of 10 ml/kg PBS (phosphate buffered saline). At day 1 (first injection), the control triplet was injected saline and 3×6 triplets were injected 6, 60 or 600 µg/kg body weight of IFN alpha 2a, respectively (57 mice in total). The control triplet was sacrificed 24 h later. In each of the treatment groups, one triplet was sacrificed after 24, 72, and 168 h. 1 week after the first injection, the three remaining triplets of each treatment group were injected the second dose and one triplet of each group was sacrificed at day 14, whereas the remaining two triplets of each group were administered the third dose at that moment. This procedure was repeated at day 21. The three last triplets were sacrificed at day 28. Blood was collected in ice-cooled Eppendorf tubes, centrifuged for 10 min with 4000 *g*, the serum was frozen and used for the determination of antiviral activity. Changes in IFN titer were compared using two-way analysis of variance (ANOVA) (between factor: dose; within factor: time) followed by LSD test.

Results of experiment 1 (Fig. 1) suggest that after administration of 60 and 600 µg/kg body weight of IFN alpha 2a IFN serum activity is maintained at least at the level found in humans during 7 days after IFN administration, even if this activity decreases rapidly during the days following IFN administration. Therefore, in the second set of experiments, we assessed the effect of 2 weekly i.p. injections of 60 and 600 µg/kg body weight of IFN alpha 2a as described above on depression-related behavior (sucrose preference, Porsolt forced swim test), hormonal response (dexamethasone suppression test), and IFN-associated gene expression in the liver. Using the treatment schedule displayed in Table 1, we wanted to avoid the continuous stressing of the animals with daily injections, which could significantly influence the stress and depression-related behavior of the animals. The treatments, dexamethasone suppression, glucose preference, and Porsolt tests were carried out between 10:00 and 14:00, in the active phase of the animals. Animals were separated at day 1 into the three treatment groups (control, IFN 60 and 600 µg/kg body weight, $n = 15$ for each). They were treated at days 5 and 12 with IFN alpha 2a or placebo. Activity of the animals in the forced swim test was determined the day before receiving the first injection of pegylated IFN alpha 2a or saline (day 4) and at day 20. The animals were challenged with dexamethasone at day 21. They were sacrificed 3 h after dexamethasone administration. Serum and a piece of liver were collected and frozen.

The mice had free access in the home cage to two bottles, one filled with water, the other with 0.1% sucrose. The position of the bottle was changed daily. The amount of liquids consumed was determined as change in bottle weights before the first treatment (at days 1 and 4) and then at days 7, 10, 13, 16, and 19. Preference was calculated as sucrose consumed/(sucrose + water consumed) × 100. Animals showing no preference for sucrose during the days before the treatments were excluded from the statistical evaluation in the sucrose preference test. Treatment groups were compared using two-way ANOVA (between factor: treatment, within factor: time).

To conduct the forced swim test, animals were placed in a Plexiglas cylinder (10 cm internal diameter, 50 cm high) filled with water (10 cm height) at 22–23 °C. Duration of the experiment was 6 min, the behavior of the animals was evaluated between the 2nd and 6th minutes, during 4 min. An observer using a stopwatch measured the immobility time. A mouse was judged to be immobile when it remained floating in the water, making only those movements necessary to keep its head above the water (Porsolt et al., 1977; Porsolt, 2000). The experiment was carried out before and after the IFN treatment regime. Immobility times were compared using paired Student's *t*-test in each treatment group separately. Change in immobility time was calculated as percent difference in immobility time between the first and second trial. Difference in change in immobility between the groups was calculated using one-way ANOVA.

To perform the dexamethasone suppression test the mice were treated with 50 µg dexamethasone i.p. diluted in PBS and placed back to their home cage. Three hours

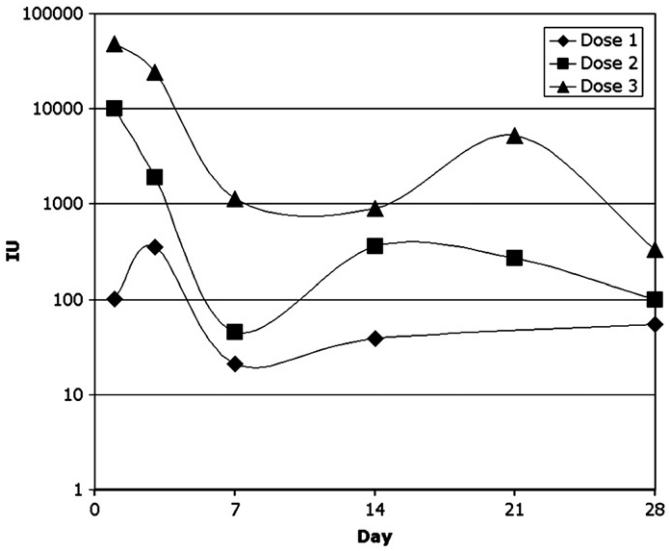

Fig. 1. Antiviral activity in mouse serum after intraperitoneal administration of IFN alpha 2a (6 (dose 1), 60 (dose 2), and 600 (dose 3) µg/kg body weight). Injections took place at days 0, 7, 14, and 21. Every point represents the mean of the double measure of serum of 1 to 3 sacrificed mice at days 1, 3, 7, 14, 21, and 28. At day 21, no measure did reach the level of quantification at dose 1. IU = international units.