Nitric oxide involvement in depression during interferon-alpha therapy

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Abstract
It has been postulated that interferon induces depression via a nitric oxide-related system. The purpose of this study was to test whether there was a difference in the effects of interferon-alpha (IFN-α) on nitric oxide production between patients with and without interferon-induced depression. The subjects had chronic hepatitis C and were being treated with IFN-α. We measured plasma nitrate, a marker of nitric oxide production in vivo, before, during, and after interferon therapy. Of 146 patients, 9 developed depression within the first 4 wk of interferon therapy, and 8 developed depression later. In the former group, a significant plasma nitrate increase was observed during therapy, followed by a decrease to baseline after discontinuation. This, however, was not the case with the latter group or those who had no psychiatric symptoms. These results suggest nitric oxide involvement in at least some forms of IFN-α-induced depression.

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Introduction
Recently, the availability of interferon (IFN) has been expanding despite reports of adverse effects, with depression being the most common (Dieperink et al., 2000). Although IFN-induced depression is reportedly accompanied by abnormalities of both the serotonergic system (Bonaccorso et al., 2002) and the hypothalamic–pituitary–adrenal (HPA) axis (Maes et al., 1996, 2001), theories on the underlying mechanism are at present mainly speculative (Dieperink et al., 2000). Moreover, the cytokine network, which is activated by IFNs, is reportedly involved in the aetiology of IFN-induced depression (Bonaccorso et al., 2001; Maes et al., 1996, 2001).

It is well established that various cytokines induce nitric oxide (NO), which is now recognized as a neuronal messenger (Snyder and Bredt, 1991). NO can act as a modulator of the secretion of HPA axis-related hormones (Schmidt and Walter, 1994), as well as both the release and reuptake of monoaminergic neurotransmitters (Lorrain and Hull, 1993; Pögörn et al., 1994; Seilicovich et al., 1995; Zhu and Luo, 1992). Therefore, it has been speculated that NO elicited by cytokines is involved in IFN-induced depression (Hurlock, 2001).

The aim of this study is to assess the difference in NO system response to IFN between patients suffering from IFN-α-induced depression and those without such symptoms. This was done by measuring nitrite (NO₂⁻) and nitrate (NO₃⁻), two final NO oxidation products measurable in vivo, in patient plasma, since NO is rapidly converted into oxidative products that are used as surrogate markers of NO production (Moncada and Higgs, 1993).

Methods
The subjects had chronic hepatitis C (n = 146). Patients who had a history of psychiatric symptoms prior to IFN therapy were excluded from this study. We carried out the study in accordance with the latest version of the Declaration of Helsinki, and informed consent was obtained from each patient through prior notification. A total of 5–10 million units of IFN-α were injected into the muscle daily for the first 2 wk, and then three times a week for the next 22 wk. During the course of the research period, spanning from 2 wk prior to IFN therapy to 1 yr after its completion, an experienced psychiatrist met each patient every 2 wk.
Depression was diagnosed according to the DSM-IV criteria for major depressive disorder excluding item D. Patients who developed depressive symptoms were prescribed antidepressants, and their liver functions were carefully monitored. The psychiatrist chose the drug and its dosage according to each patient’s symptoms. From among the patients not showing psychiatric symptoms, 10 were randomly selected (5 females) for examination of blood samples (control group).

Since there were two onset time-peaks, we divided the patients with depression into the following two groups: the early-onset (EO) group, which consisted of patients whose symptoms appeared within 4 wk after starting IFN therapy; and the late-onset (LO) group, those who showed symptoms at 4 wk or later. Another group of patients, who exhibited psychotic symptoms, was termed the psychotic (P) group.

We were not able to collect blood samples on a fixed time schedule, as these collections were constrained by patient cooperation and convenience. Thus, blood was drawn either once or twice, during the 2–8 wk before starting IFN, and 1–3 times at least 4 wk after starting IFN, then 1–5 times at least 4 wk after completion of IFN. When blood collection was carried out more than once in the same period, the average value was adopted for statistical analysis. Plasma was obtained and pNO\textsubscript{2}−, pNO\textsubscript{3}− levels were measured as described previously (Leone et al., 1994; Suzuki et al., 2001).

The severity of depression was measured by the 17-item Hamilton Rating Scale for Depression (HAMD; Hamilton, 1960) at two time-points for each patient, at the diagnosis of and at confirmed recovery from depression.

To test whether antidepressant treatment affected pNO\textsubscript{2}− and pNO\textsubscript{3}− levels, eight sex- and age-matched healthy volunteers [4 females; mean ± standard deviation (s.d.) age = 37 ± 6.4 yr] took 25 mg/d imipramine for 2 wk and blood was collected from each volunteer before and 2 wk after imipramine treatment.

Data were expressed as means ± S.D. and subjected to a repeated-measure analysis of variance (ANOVA). Post-hoc analysis was performed with Fisher’s Protected Least Significant Difference (Fisher’s PLSD) test.

Results

During the study period, 17 patients were diagnosed with depression. The EO and LO group numbers were 9 (5 females) and 8 (5 females) respectively. Onset times in the former and latter groups were 10 ± 3 d and 100 ± 33 d respectively. Mean HAMD scores of the EO and LO groups were 24.3 ± 4.9 and 22.9 ± 5.2 respectively. In the EO group, within 8 wk the HAMD score fell to 13.3 ± 2.6, while in the LO group only 2 patients improved (more than a 10-point drop in score) within 8 wk and 4 patients experienced remission, although symptoms persisted for approx. 1 yr. Forty patients also complained of depressive mood and/or insomnia, though they did not meet the DSM-IV criteria. There were 3 patients in P group (2 females). The main psychotic manifestations were hallucinations, a delusional state and manic episodes. Average ages of the EO, LO, P and control groups were 43 ± 7.6, 39 ± 5.2, 45 ± 6.9 and 42 ± 3.2 yr respectively.

An antidepressant was administered to 7 patients in the EO group (imipramine, 2; amitriptyline, 2; clomipramine, 3) and 6 patients in the LO group (imipramine, 3; amitriptyline, 2; clomipramine, 1). No patients in the other groups took antidepressants. The imipramine equivalent doses (Ali, 1998) of the EO and LO groups were 22.1 ± 14.4 and 30.8 ± 15.9 mg/d respectively. Other patients in the EO and LO groups refused antidepressant administration during this study.

Among the EO, LO and control groups, there was a significant time-course-related difference in pNO\textsubscript{2}− level changes (F = 5.1, p < 0.01). In the EO group, during-IFN pNO\textsubscript{2}− levels were significantly higher than those of the pre-IFN (p < 0.001) and post-IFN (p < 0.001) periods, as shown in Figure 1. No significant differences among periods were observed in the other groups. We also analysed the pNO\textsubscript{3}− data, collected at only one time-point during IFN, 4–8 wk after starting IFN, but these did not substantially change the overall findings.

There were no significant correlations between absolute values of pNO\textsubscript{2}−, pNO\textsubscript{3}−, and HAMD scores at the time depression was diagnosed in either the EO or the LO group. In the EO group, pNO\textsubscript{2}− level changes (levels during-IFN minus post-IFN therapy) did not correlate with HAMD score changes (score during depression minus that after recovery).

Before and 2 wk after imipramine treatment, pNO\textsubscript{2}− and pNO\textsubscript{3}− levels of the healthy volunteers were 8.7 ± 6.3 and 79.9 ± 38.3 μM (before) and 9.5 ± 4.7 μM and 85.9 ± 28.5 μM (after) respectively. There was no significant difference in the level of pNO\textsubscript{2}− or pNO\textsubscript{3}− before vs. after imipramine treatment.

Discussion

The most important finding in this study was that, in the EO group, pNO\textsubscript{2}− levels increased significantly during-IFN therapy and decreased to baseline afterwards. We previously showed that ‘endogenous’
(non-organic) major depression is accompanied by higher pNO$_3^{-}$ levels than in healthy controls (Suzuki et al., 2001). Although it is unclear which occurs first, the pNO$_3^{-}$ elevation or the onset of depression, data from both this and the previous study suggest that NO is aetiologically involved in depression. However, pNO$_3^{-}$ levels in the EO group remained high during IFN therapy even after recovery from depression. This is not consistent with previous data which showed the pNO$_3^{-}$ levels of patients with ‘endogenous’ major depression to decrease to the control level upon recovery from depression (Suzuki et al., 2001). There is no evidence explaining why pNO$_3^{-}$ levels decreased after recovery in patients with ‘endogenous’ depression in our previous study but not in the EO group in this study, although we can raise the following possibility. NO acts potently in both the induction of depression and the activation of an unknown recovery system against depression. If there is latent dysfunction of the recovery system in patients with ‘endogenous’ depression, mood states associated with this form of depression may improve only after reduction of NO.

On the other hand, in the case of IFN-induced depression, the recovery system begins to function in response to the sudden excess NO production, thereby diminishing the symptoms despite continuous excess NO production. Recently, we found that antidepressants activate the NO system in the brain (Suzuki et al., 2002, In Press), suggesting the NO system to also be involved in the mechanism of recovery from depression.

Although there was no significant difference in HAMD scores between the EO and LO groups, symptoms worsened more rapidly and the duration of depression was shorter in the former group. Onset was early in the EO group, which is consistent with previous studies (Capuron and Ravaud, 1999). Neurovegetative and somatic symptoms reportedly appear early during IFN-α therapy, whereas mood and cognitive symptoms develop later (Capuron et al., 2002). Similarly, in the EO group, neurovegetative and somatic symptoms were the most common initial symptoms. On the other hand, for most patients in the LO group, depressive mood was prominent. Even when
IFN therapy was completed, liver functions of 4 LO group patients did not improve. Their depressive mood was apparently attributable to disappointment at the ineffectiveness of IFN on their hepatitis. Moreover, in the LO group, there were no remarkable changes in pNO$^\cdot$ levels in any of the patients, in spite of changes in mood state. Thus, we speculate that psychological factors may be involved in the more marked onset of a depressive mood in the LO group compared to the EO group.

Although we confirmed that a low dose of imipramine does not significantly change pNO$^\cdot$ levels in healthy volunteers, since hepatitis patients have liver dysfunction, the possibility that antidepressant effects on patients differ from those on healthy volunteers remains. However, pNO$^\cdot$ levels in the LO group patients, who also took antidepressants and had liver dysfunction, did not change significantly during IFN therapy (data not shown). Moreover, paroxetine reportedly decreases serum NO$^\cdot$ plus NO$^\cdot$ levels in ischaemic heart disease patients (Finkel et al., 1996). Taken together, these observations suggest that the pNO$^\cdot$ elevation in the EO group is unlikely to have been induced by the administration of antidepressants. However, a limitation of this study is that the type, dosage, and duration of antidepressant treatment were not fixed, making it difficult to assess the actual effects of antidepressants on pNO$^\cdot$ levels.

We found no relationship between pNO$^\cdot$ levels and HAMD scores. However, it is noteworthy that the two patients with the highest and second highest pNO$^\cdot$ levels during IFN therapy confessed to having considered committing suicide. It is also noteworthy that the initial pNO$^\cdot$ levels of the P group tended to be high. Further examination is required, but pretreatment pNO$^\cdot$ levels may become a factor in predicting IFN-induced psychotic symptoms.

During the study period, pNO$^\cdot$ levels did not change significantly in any group (data not shown). As noted in a previous report (Leone et al., 1994), absolute pNO$^\cdot$ values were generally more than 10-fold higher than those of pNO$^\cdot$, possibly due to the conversion of NO$^\cdot$ to NO$^\cdot$ by haemoglobin (Doyle et al., 1985). Thus, we presume that the changes in NO production were not reflected as clearly by pNO$^\cdot$ levels as by pNO$^\cdot$ levels.

Whether IFN-α-induced excess NO production occurs centrally or peripherally remains unclear. However, there are reports of IFN affecting the brain both directly and indirectly via the cytokine network (Dieperink et al., 2000; Hurlock, 2001). In addition, if excess NO is produced in vessels by endogenous-type nitric oxide synthase (NOS), it may penetrate the blood–brain barrier and affect brain functions. The number of NOS-immunoreactive post-mortem paraventricular neurons is reportedly smaller in depressed patients than in normal cases (Bernstein et al., 1998). We infer from the present results that the hypothalamus was continuously exposed to excess NO, since NO suppresses transcriptional induction of NOS (Park et al., 1994).

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