Pegylated interferon-α2b treatment in melanoma patients: influence on amino acids, 5-hydroxyindolacetic acid and pteridine plasma concentrations

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Our objective was to study the influence of pegylated interferon-α2b (PEG-IFN-α) on the metabolism of amino acids and pteridines. We used an exploratory study into plasma concentrations of large neutral amino acids, 5-hydroxyindolacetic acid (5-HIAA), total biopterin (BIOP) and neopterin (NEOP) in 40 high-risk melanoma patients. Patients were randomized to treatment with PEG-IFN-α once a week in a dose of 6 μg/kg/week s.c. during 8 weeks, followed by a maintenance treatment of 3 μg/kg/week s.c. or to observation only. We found that treatment with PEG-IFN-α decreases tryptophan (TRP) concentrations in the first 3 months of treatment to a maximum of 25.3% compared to controls [95% confidence interval (CI): 14.9 to 34.4]. The TRP:LNAA ratio, an index for the availability of TRP to the central nervous system (CNS), decreases during 6 months with 18.8% (95% CI: 11.9 to 25.2). Concentrations of NEOP rose; however, concentrations of BIOP, the sum of tetrahydrobiopterin [$BH_{(4)}$] and its oxidative products, did not decrease. The ratio of phenylalanine to tyrosine was increased with 11.7% (95% CI: 1.0 to 23.5) during 6 months. We conclude that, like conventional IFN-α, PEG-IFN-α lowers TRP concentrations and decreases the availability of TRP to the CNS. PEG-IFN-α has a similar influence on pteridine metabolism as standard IFN-α. If a lowered availability of TRP and a consequent decrease of serotonergic neurotransmission are indeed a mechanism underlying neuropsychiatric side-effects of IFN-α, patients on PEG-IFN-α are not at a lower risk of developing neuropsychiatric side-effects as patients on conventional IFN-α. *Anti-Cancer Drugs* 15:587–591 © 2004 Lippincott Williams & Wilkins.

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Introduction

Interferon (IFN)-α is used in a wide variety of diseases, amongst others renal cell carcinoma, melanoma, chronic myelogenous leukemia (CML), and chronic viral hepatitis B and C. The attainment of an optimal dose intensity is often blocked by side-effects. The side-effects could be related, in part, to the short half-life and the fluctuations in concentration of IFN-α. As a possible solution, PEG-IFN-α has been developed. Polyethylene glycol (PEG) is a linear, uncharged, hydrophobic and flexible polymer. Attached to IFN-α, it lengthens plasma half-life and reduces sensitivity to proteolysis, without compromising biological activity, as measured by assays of cytopathic effects (the ability to protect cells from virus-induced cytopathogenesis) and inhibition of cell replication. Indeed, with PEG-IFN-α, a higher dose intensity can be reached in CML patients [1]. Furthermore, it is more convenient for patients, as conventional IFN-α has to be administered 3–5 times a week and PEG-IFN-α only once a week.

Neuropsychiatric side-effects such as depression, anxiety and cognitive impairment are frequently induced by treatment with IFN-α, and often necessitate dose reduction or even treatment cessation [2,3]. Lowering of TRP availability to the central nervous system (CNS) by IFN-α has been hypothesized as one of the mechanisms underlying IFN-α-induced depression. At the blood–brain barrier, tryptophan (TRP) is competing for entry into the CNS with the other long neutral amino acids (LNAA), tyrosine (TYR), phenylalanine (PHE), isoleucine (ILE), leucine (LEU) and valine (VAL) [4]. A decrease of TRP relative to the other LNAA would set TRP at a disadvantage in entering the CNS. As TRP is the precursor of serotonin (5-HT) and the availability of TRP is the rate-limiting step in 5-HT synthesis by nerve cells, this in turn would lead to lower 5-HT levels in the CNS [5]. Derangements in 5-HT function play a role in depression. Treatment with IFN-α decreases TRP levels, both in the short and long term [6,7], possibly leading to CNS 5-HT deficiency.

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Tetrahydrobiopterin [$BH_{(4)}$] is a necessary cofactor for various enzymes, amongst others for the enzymes which hydroxylate TRP, TYR and PHE. These enzymes are the rate-limiting steps in the biosynthesis of 5-HT, dopamine and noradrenaline, respectively [8]. Treatment with IFN-α is associated with an impressive and lasting increase in neopterin (NEOP) concentrations [7,9]. Theoretically, by shunting away precursors of $BH_{(4)}$, this increased production of NEOP could lead to $BH_{(4)}$ deficiency, and thereby hamper the synthesis of 5-HT, dopamine and noradrenaline, which could cause psychiatric disturbance.

At present, we are unaware of comparable data on PEG-IFN-α in relation to amino acid and pteridine metabolism. Does PEG-IFN-α affect the activity of the enzymes involved in amino acid and pteridine metabolism, and to what extent? As remarked, one of the expectations of the introduction of PEG-IFN-α was a reduction of side-effects. We therefore decided to perform an exploratory study into the long-term effects of treatment with PEG-IFN-α on plasma concentrations of amino acids, the 5-HT metabolite 5-hydroxyindolacetic acid (5-HIAA) and pteridines in melanoma patients, compared to controls.

Materials and methods

Samples were obtained from patients participating in a trial of the European Organization for Research and Treatment of Cancer (EORTC 18991), evaluating the efficacy and toxicity of PEG-IFN-α-2b versus controls in high-risk melanoma patients. Patients underwent resection of a thick primary melanoma or regional lymph node metastases, in the absence of distant metastases. Patients were randomized in a 1:1 ratio to the treatment arm (8 weeks induction with 6 μg/kg/week s.c., followed by 5 years maintenance with 3 μg/kg/week s.c.) or observation only. The study protocol excluded patients with organic mental disorders, with psychiatric disorders at baseline that could be exacerbated by PEG-IFN-α (e.g. depression) and with alcohol abuse. In addition, we excluded samples from patients on antidepressant, antipsychotic or antiepileptic drugs. Samples were considered ineligible after PEG-IFN-α was stopped. No samples were analyzed from patients with distant metastatic disease and a bad general condition. This report concerns the first 50 patients enrolled in the EORTC study in our center. Ten subjects had to be excluded, seven patients because no samples were taken at all and three because of psychotropic drug use. Of the remaining 40 patients, 22 (11 males, 11 females, mean age 45.7 years, range 32–68) belonged to the treatment arm, 18 (11 males, seven females, mean age 46.3 years, range 26–67) to the observation group. Samples were missing due to administrative failure and, moreover, the number of available samples diminished in the course of time due to attrition (because of recurrent disease or treatment cessation). Mean doses (SD) of PEG-IFN-α used were: at 4 weeks 5.70 (0.92), at 8 weeks 5.0 (1.4), at 3 months 2.9 (0.54) and at 6–8 months 2.9 (0.3) μg/kg/week.

Blood samples were taken at baseline, at 4 and 8 weeks during the induction phase, and at 6–8 months. For practical reasons, it was not possible to obtain blood samples at fixed times or under fasting conditions. The study was approved by the local ethical committee and patients gave informed consent.

Procedures

EDTA blood was obtained by venipuncture, and after immediate centrifugation (10 min at 1000 *g*) plasma was separated and frozen at −80°C. All assays were performed within a period of 1–3 years after blood sampling. Amino acids were determined as previously described [10]. The TRP:LNAA ratio was calculated by dividing 100 times the plasma concentration of TRP by the sum of the other LNAA, e.g. TYR, PHE, VAL, LEU and ILE. The TYR:LNAA ratio was calculated in the same manner, substituting TRP for TYR and vice versa. 5-HIAA and pteridines were determined as previously described [11,12].

Data were stored using SPPS software and analyzed using SAS software. Estimates of changes of parameters at follow-up compared to baseline were obtained using mixed model analysis of variance (ANOVA) after log transformation of the outcome variables. Effects of group (two levels), time (four levels) and their interaction on the change from baseline of the outcome variables were tested. Group by time effects with a statistical significance of $p > 0.10$ were eliminated from the model. Adjustment was made for the baseline measurement of the outcome variable at hand, sex and age, by including them as covariates in the model. No structure was imposed on the correlation of the residuals.

Results

As shown in Table 1, TRP concentrations in the treatment group decreased significantly at 4 weeks, 8 weeks and 3 months, both compared to baseline and compared to control patients. At 6 months no differences were observed. The TRP:LNAA ratio (an index for the availability of TRP to the CNS) was decreased at all points in time, both compared to baseline and compared to controls. Concentrations of 5-HIAA were decreased at three points in time (4 weeks, 8 weeks and 6 months) in the treatment group when compared to baseline, but only once (at 8 weeks) when compared to controls.

Concentrations of TYR (the precursor of dopamine) did not change significantly compared to baseline in the treatment group (data not shown). Concentrations of TYR were increased in the control group when compared

Table 1

	Group (32 samples)	Mean concentration (SD) in 10^{-6} mol/l at baseline		Estimations of percent changes (95% CI) during follow-up compared to geometric mean of treatment and control group at baseline (adjusted for baseline value, sex and age; baseline = 100%)			
				4 weeks (27 samples)	8 weeks (25 samples)	3 months (26 samples)	6 months (17 samples)
TRP	PEG-IFN-α	38.4 (7.5)	compared to baseline	−13.9[b] −21.8/−5.1	−14.8[a] −24.1/−4.5	−10.4[a] −18.4/−1.7	−5.5[NS] −15.1/5.2
	control	35.8 (5.6)	compared to baseline	15.3[b] 5.6/26.0	11.8[NS] −1.5/27.0	16.5[b] 7.7/25.9	4.5[NS] −4.8/14.8
			PEG-IFN-α versus controls	−25.3[c] −34.4/−14.9	−23.8[b] −35.9/−9.5	−23.1[c] −31.9/−13.1	−9.6[NS] −21.7/4.3
PHE	PEG-IFN-α	49.6 (10.0)	compared to baseline	12.7[a] 1.2/25.4	17.1[b] 7.8/27.3	13.8[NS] −0.1/29.7	26.1[b] 9.2/45.6
	control	50.6 (7.9)	compared to baseline	18.6[b] 7.8/30.5	11.7[a] 1.6/22.9	19.9[b] 7.9/33.2	2.1[NS] −9.8/15.6
			PEG-IFN-α versus controls	−5.0[NS] −17.6/9.6	4.8[NS] −7.6/18.0	−5.0[NS] −19.8/12.4	23.5[a] 2.0/49.5
5-HIAA	PEG-IFN-α	30.2 (13.4)	compared to baseline	−29.3[b] −43.8/−11.0	−33.0[b] −46.4/−16.3	5.3[NS] −21.2/40.9	−24.4[c] −32.7/−15.1
	control	37.2 (44.3)	compared to baseline	−8.3[NS] −25.5/12.8	−5.8[NS] −23.1/15.2	−8.5[NS] −27.5/15.1	−18.6[c] −25.4/−11.1
			PEG-IFN-α versus controls	−22.8[NS] −43.5/5.2	−28.9[a] −47.4/−3.8	15.1[NS] −20.6/66.9	−7.2[NS] −19.8/7.5
TRP:LNAA ratio	PEG-IFN-α	8.34 (1.22)	compared to baseline	−17.7[c] −22.7/−11.2	−15.1[c] −21.3/−8.3	−15.2[c] −21.6/−8.2	−14.6[b] −21.7/−6.8
	control	7.26 (1.08)	compared to baseline	2.0[NS] −4.2/8.7	4.6[NS] −3.2/13.1	4.4[NS] −3.0/12.4	5.2[NS] −3.1/14.2
			PEG-IFN-α versus controls	−18.8[c] −25.2/−11.9	−18.8[c] −25.2/−11.9	−18.8[c] −25.2/−11.9	−18.8[c] −25.2/−11.9
NEOP	PEG-IFN-α	11.73 (3.51)	compared to baseline	313.8[c] 225.3/426.5	383.4[c] 298.9/485.7	314.5[c] 233.7/484.2	454.9[c] 320.3/632.5
	control	13.16 (9.03)	compared to baseline	16.1[NS] −6.5/44.1	10.2[NS] −9.6/44.1	−5.4[NS] −25.8/20.5	−14.4[NS] −32.5/8.7
			PEG-IFN-α versus controls	256.4[c] 157.8/393.0	338.6[c] 233.4/477.0	366.9[c] 222.6/575.8	547.9[c] 349.8/833.2
BIOP	PEG-IFN-α	6.00 (1.78)	compared to baseline	18.0[a] 2.7/35.5	11.2[NS] −2.2/27.1	21.6[a] 5.2/40.5	11.0[NS] −2.0/25.8
	control	7.17 (2.09)	compared to baseline	10.6[NS] −2.2/24.9	4.5[NS] −8.6/19.5	16.4[a] 0.4/35.0	3.6[NS] −7.7/16.4
			PEG-IFN-α versus controls	6.6[NS] −8.4/24.4	6.6[NS] −8.4/24.4	6.6[NS] −8.4/24.4	6.6[NS] −8.4/24.4

[a] $0.005 < p < 0.05$.
[b] $0.0005 < p < 0.005$.
[c] $p < 0.0005$.

to baseline at 4 weeks and 3 months [21.0%, 95% confidence interval (CI): 7.8 to 35.7; $p = 0.0022$, respectively, 15.3%, 95% CI: 1.1 to 31.6; $p = 0.0348$], but were lower in the treated group compared to controls only at 4 weeks (−18.4%, 95% CI: −31.2 to −3.3; $p = 0.0209$). In all comparisons, the TYR:LNAA ratio did not change significantly in the two groups (data not shown). The concentrations of PHE increased at three of the four points in time in the treated and in the control group (Table 1). The PHE:TYR ratio, an index for the activity of the enzyme phenylalanine hydroxylase, did not change in the control group, but was increased in the treatment group compared to baseline at 4 weeks (10.9%, 95% CI: 1.0 to 21.8; $p = 0.0307$), at 8 weeks (14.6%, 95% CI: 4.6 to 25.6; $p = 0.0052$) and at 6 months (9.7%, 95% CI: 0.1 to 20.4; $p = 0.0490$). Moreover, the PHE:TYR ratio was increased in the treatment group compared to control at all points in time (11.7%, 95% CI: 1.0 to 23.5; $p = 0.0322$). The concentrations of ILE, LEU and VAL did not change significantly, with the exception of a slight increase of VAL in the control group at 8 weeks (2.4, 95% CI: 0.9 to 12.6; $p = 0.0242$) compared to baseline.

An impressive increase of up to 454.9% of NEOP was observed in the group treated with PEG-IFN-α. No significant changes of NEOP were seen in the control group (Table 1). The increase of NEOP was not accompanied by a decrease in BIOP.

Discussion

To our knowledge and in contrast to data on conventional IFN-α, no data on amino acid and pteridine metabolism in patients treated with PEG-IFN-α are available up till now. In this group of melanoma patients, treatment with PEG-IFN-α decreases peripheral TRP concentrations in

the first 3 months of treatment and results in a nearly 20% decrease of the TRP:LNAA ratio, an index for the availability of TRP to the CNS, during the whole study period of 6 months. Probably, PEG-IFN-α-based immunotherapy, like conventional IFN-α, stimulates the activity of the enzymes tryptophan dioxygenase (TD) and indoleamine 2,3-dioxygenase (IDO). Both enzymes degrade TRP, leading to lower blood concentrations and lowered availability of TRP to the CNS, which in turn could lead to decreased 5-HT synthesis and the emergence of depression. In research in cancer and hepatitis patients treated with conventional IFN-α, changes in concentrations of peripheral TRP, peripheral 5-HT and kynurenin (another metabolite of TRP) were found to correlate with changes in depression scores, suggestive for a causal link between lowering of tryptophan availability and depression [4,13,14]. We also observed a decrease in 5-HIAA (the breakdown product of 5-HT) at several points in time, possibly reflecting diminished 5-HT levels. However, for levels of 5-HIAA, the 95% CIs were wide. Apart from that, plasma 5-HIAA is for the most part derived from peripheral serotonin and therefore not considered an index for central 5-HT levels.

In our previous research in two groups melanoma patients treated with standard, short-acting IFN-α, we observed a decrease of the TRP:LNAA ratio of 11.2% (95% CI: 6.4 to 15.7), respectively, 5.8% (95% CI: 1.1 to 10.2) after the induction phase (4 weeks 50 MIU IFN-α/week). Six months maintenance treatment with a dose of 30 MIU/week resulted in a decrease in the TRP:LNAA ratio of 9.1% (95% CI: 3.6 to 14.2). When 15 MIU/week was given, no significant changes in the TRP:LNAA ratio were observed at 6 months [7]. Capuron *et al.* [4] observed a 25% decrease in TRP availability after 4 weeks in 16 oncology patients treated with four different regimens of (conventional) IFN-α, IL-2 or both. When IFN-α was part of the treatment ($n = 11$), doses of IFN-α of 100 MIU/week i.v., 54 MIU/week s.c. or 18 MIU/week s.c. were given. IL-2 was administered at a dose of 18 MIU/m^2/day at 5 consecutive days per week. With some reservation (amongst others because of the wide confidence intervals in our study) we speculate that PEG-IFN-α at a dose of 6 μg/kg/week s.c. takes an intermediate position as inhibitor of TD and IDO, between 50 MIU/week of conventional, short-acting IFN-α, on the one hand, and the high-dose regimens as reported on by Capuron *et al.* [4], on the other hand.

Furthermore, we were not able to replicate all increases in the concentrations of the LNAA in the control group from our earlier work. Most notably, concentrations of ILE, LEU and VAL did not increase consistently. However, the increases in control patients of TRP and TYR (at two points in time) and of PHE (at three points in time) underscore the need for a control group in this kind of research.

Like standard IFN-α, treatment with PEG-IFN-α gives rise to strong increases of NEOP. Theoretically, this rise could compromise $BH_{(4)}$ synthesis, but consistent with our earlier research, we found no change in total BIOP, the sum of $BH_{(4)}$ and its oxidative products, dihydrobiopterin and biopterin. However, we cannot exclude a shift, within total BIOP, from $BH_{(4)}$ to these oxidative products [15]. Again in accordance with our earlier data in IFN-α, the increased PHE:TYR ratio of the treated group versus controls in turn points to a lower activity of the enzyme phenylalanine hydroxylase, for which $BH_{(4)}$ is also a cofactor.

As no standardized psychiatric measurements were included in our study, we cannot directly relate the observed changes in amino acids metabolism to the development of psychopathology. However, we observed that of 27 patients (of the first 50 patients that were enrolled in our center) that were randomized to receive treatment with PEG-IFN-α, five patients were referred for psychiatric consultation. Four of them were diagnosed with an IFN-α-induced depression, and one with IFN-α-induced concentration difficulties and irritability. We conclude that PEG-IFN-α induces similar changes in amino acids and, more specifically, TRP metabolism, and in the metabolism of pteridines, as regular IFN-α. Comparing the data on TRP availability to the CNS of regimens of regular IFN-α and PEG-IFN-α, the influence of the PEG-IFN-α regimen may well be greater. If a lower availability of peripheral TRP to the brain is indeed a mechanism underlying the neuropsychiatric side-effects of treatment with IFN-α, patients on PEG-IFN-α are not at a lower risk of developing neuropsychiatric side-effects as patients on regular IFN-α.

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