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# Neuropsychological and Neurophysiological Assessment of the Central Effects of Interleukin-2 Administration

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**Neuropsychiatric disturbances may occur following interleukin-2 (IL2) administration. We studied the effects of IL2 infusion on cerebral functions in 7 patients with neuropsychological tests and event-related evoked potentials (P300). We observed a failure in the cognitive performances, an increase in latency, and a decrease in amplitude of P300. These effects followed IL2 administration and were reversible.**
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## INTRODUCTION

THE CLINICAL use of recombinant interleukin-2 (rIL2) with autologous *in vitro* rIL2 activated lymphocytes (LAK cells), or tumour infiltrating lymphocytes (TIL), represents an innovative approach to the treatment of cancer. Clinical studies with rIL2, LAK and TIL in advanced and heavily pretreated patients have produced significant clinical responses in melanoma and renal cancer [1]. Treatment-related toxicity, however, is quite frequent and dose-dependent. Fluid retention is a serious side-effect of therapy and usually requires careful intensive management. Neuropsychiatric side-effects were also found to be among the most serious disturbances in this form of adoptive immunotherapy, leading sometimes to treatment withdrawal [2, 3]. Several higher brain functions such as arousal, thinking and behaviour were affected [2]. We report the results of monitoring central nervous system (CNS) toxicity in a continuous intravenous (i.v.) IL2 infusion protocol with neuropsychological tests and event-related evoked potentials (ERP) recording. The P300 component of the acoustic ERP was chosen since this potential has been recently applied to the assessment of confusional states, dementia of various origin, and amnestic states [4, 5].

The main goal of our study was to determine whether cognitive impairment following continuous IL2 infusion can be quantified using clinical and neurophysiological methods, and whether such effects are reversible.

## PATIENTS AND METHODS

*Patients*

7 patients affected by metastatic melanoma were studied. In all cases a computerised tomography (CT) scan was performed to exclude the presence of brain metastases. The study included 6 male patients and 1 female, ranging between 31 and 56 years of age (mean ± S.D. = 42.4 ± 9.2).

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In vitro *culture of tumour infiltrating lymphocytes*

Each patient was submitted to surgical excision of a suitable metastatic melanoma mass. From this specimen, TIL were separated and cultured *in vitro* in the presence of rIL2. TIL were infused subsequently.

*Treatment*

Before starting TIL and rIL2 dosing, each patient received $3 \times 10^6$ U of interferon intramuscularly (i.m.) for 3 consecutive days and a bolus i.v. of 350 mg/mq of cyclophosphamide. Immediately after i.v. TIL infusion (lasting 90 min), i.v. rIL2 infusion was started and continued for 5 days. A second course of rIL2 infusion was performed after a further 5–7 days. Additional cycles were considered in cases of objective response. Patients underwent a total of 17 treatment courses (5 patients two courses, 1 patient three courses and 1 patient four courses). The amount of TIL infused ranged from 0.175 to $7.25 \times 10^9$ per cycle (mean ± S.D. = $2.95 \pm 2.06 \times 10^9$), while rIL2 doses ranged from 8 to $19 \times 10^6$ CU (Cetus Units) per course (mean ± S.D. = $12.9 \pm 3.5 \times 10^6$).

*Clinical evaluation*

Patient evaluation followed the schedule shown in Fig. 1 for 13 out of the 17 courses performed. Daily interviews with the patients during IL2 administration explored the onset of

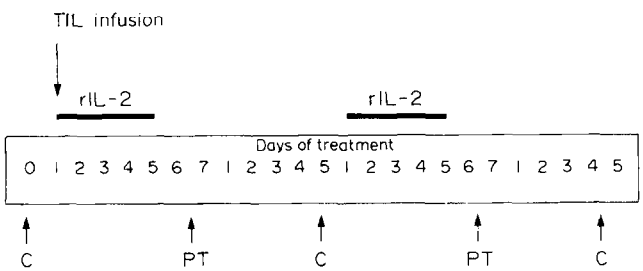


**Fig. 1. Schedule of IL2 administration and neuropsychological evaluations in a patient undergoing two IL2 courses. After the basal evaluation (day 0, C = control evaluation, tested and evoked potential recording), the infusion lasted 5 days and the evaluation was repeated the 2nd day after the end of each course (post-treatment evaluation = PT). Another control (C) was performed 5 days after the end of each course.**

drowsiness, fatigue, sleep–wakefulness cycle alterations, confusion or delusions. A formal neuropsychological evaluation was performed before starting every treatment course, 24–48 h after the end of the infusion and was repeated 5 days after the last course was administered (Fig. 1). The tests were not administered in the presence of excessive fatigue, fever, confusion, or with a mini-mental state examination (MMSE) score of $\leq 23$. Neurological objectivity and psychiatric history were also taken during the first interview. During data analysis the mean values of all the post-treatment evaluations were compared with those of the scores obtained in the control evaluations.

*Neuropsychological tests*

MMSE was chosen as an index of global cognitive performance to rule out gross mental impairment [6]. The digit span subtest from the Wechsler adult intelligence scale, the Corsi's cubes test, and the verbal span test from the Italian standardised neuropsychological tests [7] were also incorporated. The Rene Zazzo's attention test [8] was used according to Girotti *et al.* [9]. The test is composed of a page of 40 lines, each consisting of 25 signs for a total of 1000 signs of eight different categories. The models of the two signs to be barred are printed at the top of the page, and the subject was asked to cross all the signs corresponding to the models. The test lasted 2 min. Two measures of performance were computed: speed of execution and inaccuracy of barring. Since the test is sensitive to a training effect [8], patients had to repeat it at least twice before basal values were determined. With this method we noticed a learning plateau.

*Neurophysiological evaluation*

The classic auditory odd-ball paradigm was used for P300 recordings. Ag/AgCl electrodes were used with the vertex derivation (Cz, 10–20 International System) as the active site referenced to linked mastoids. Impedence was kept below 3 Khm. The amplifier band width was 30–1 Hz. A prestimulus of 300 msec and a post-stimulus sweep of 700 msec was averaged using a Nicolet-cd-4. Binaural stimuli of 70 dB SL were presented with a rate of 0.7/sec. Target tones (2000 Hz) and non-target tones (750 Hz) were presented in a pseudo-random order. The probability of target versus non-target stimuli was 1–4. The subject was instructed to ignore non-target tones and to pay attention to and mentally count target ones. The potentials were averaged separately over a total of 300 stimuli corresponding approximately to 60 target tones.

P300 latency was measured at the peak of the positive component, appearing after N2 only with target stimuli, whereas P1, N1, P2 were recorded also with non-target stimuli [4]. P3a and P3b components of the late positive potential evoked by target tones were measured separately when possible [4]. P3b amplitude was measured from the P3b peak to the following negativity.

*Statistical analysis*

Thirteen control and 13 post-treatment evaluations were compared for neuropsychological and neurophysiological performance (Table 1). Mann–Whitney test for non-parametric data was used.

## RESULTS

*Clinical data*

For all patients, a reduction of arousal was observed during the course of treatment and was generally associated with a modified sleep–wakefulness cycle. Fatigue and mood alteration were found in all cases, which tended to worsen during the last days of each course and in subsequent courses. In most cases behavioural changes such as apathy, irritability, and also aggressiveness were observed. Delusion and confusional states were observed in only 2 patients (in each case on the fourth day of infusion). The complications in both cases were mild, lasting a short period of time and requiring no specific treatment. General toxicity was low and did not exceed grade II of the WHO toxicity rating scale. In one case, however, treatment was stopped due to gastrointestinal toxicity.

*Table 1. Neuropsychological evaluation*

| | Control | Post-treatment | *P* |
|---|---|---|---|
| MMSE | 28.7 (1.2) | 27.4 (1.6) | <0.01 |
| Verbal span | 4.8 (0.8) | 4.6 (0.5) | N.S. |
| Digit span forward recall | 6.0 (0.9) | 5.5 (0.7) | N.S. |
| Digit span backward recall | 4.9 (1.2) | 3.6 (1.0) | <0.01 |
| Corsi's spatial test, forward recall | 5.3 (1.1) | 4.5 (0.5) | <0.01 |
| Corsi's spatial test, backward recall | 4.8 (0.8) | 3.9 (0.4) | <0.01 |
| Zazzo's attention test speed | 30.2 (4.1) | 25.5 (1.6) | <0.01 |
| Zazzo's attention test inaccuracy | 5.0 (4.4) | 8.6 (2.5) | <0.01 |

Mean (S.D.), Mann–Whitney test.

*Neuropsychological tests*

The results of post-treatment neuropsychological evaluation are shown in Table 1 and an apparent decline in some performance criteria was observed following IL2 administration. The MMSE score was lower following IL2 infusion although this remained within the normal range [6]. In a comparison of the scores at the first evaluation with those obtained a week after the last treatment course, we did not find any variation among any of the 7 subjects studied. Compare Table 2 with the first column of Table 1.

*Evoked potentials*

Evoked potential recordings showed a significant increase in the latency of the P3b component and a decrease in P3b amplitude while the earlier components (N1, P2, N2, P3a) were not affected by treatment (Table 3 and Fig. 2). Patients' performance in counting target tones was never less than 98%, both before and after IL2 administration. P3b latency and amplitude had recovered in all patients to pretreatment values at the last recording performed 5 days after the last treatment course (P3b latency mean = 315 msec ± 13.5 msec, amplitude = 12.3 ± 3.3 μV).

*Table 2. Results of neuropsychological test at the end of therapy*

| | |
|---|---|
| MMSE | 29.3 (0.9) |
| Verbal span | 4.5 (0.7) |
| Digit span, forward recall | 6.1 (0.6) |
| Digit span, backward recall | 5.0 (0.8) |
| Corsi's spatial span, forward | 5.8 (0.6) |
| Corsi's spatial span, backward | 5.1 (0.6) |
| Zazzo's attention test, speed | 30.0 (3.3) |
| Zazzo's attention test, inaccuracy | 3.6 (1.7) |

7 patients, means (S.D.).

*Table 3. Event-related evoked potentials components*

| Latency (msec) | N1 | P2 | N2 | P3a | P3b |
|---|---|---|---|---|---|
| Control | 97.4 (8.3) | 167.3 (19.3) | 209.8 (25.7) | 253.1 (30.2) | 318.0 (21.2)* |
| Post-treatment | 96.1 (13.9) | 169.3 (14.2) | 215.5 (23.4) | 272.7 (34.8) | 344.0 (21.4)* |
| Amplitude (uv) | N1P2 | P3b | | | |
| Control | 9.2 (3.0) | 13.9 (4.3)* | | | |
| Post-treatment | 10.1 (4.3) | 8.9 (2.7)* | | | |

*$P < 0.01$, Mann–Whitney test.

## DISCUSSION

The clinical signs observed during IL2 treatment could be related to a mild mental status alteration, almost always without fulfilling DSM III criteria for delirium. However, this occurred in 2 out of 13 treatment courses. The doses used in our protocol were lower and administered via continuous i.v. infusion if compared with the much higher doses administered via i.v. boli by other authors reporting more severe neuropsychiatric complications [2].

The results of neuropsychological testing showed a cognitive failure after the end of each course that was more evident in the performances requiring a higher level of attention, namely the reverse recall of memories, and the Corsi's test, where a spatially oriented immediate memory effort is needed. This finding is in agreement with the deficits shown in the Zazzo's test.

P300 results paralleled those of neuropsychological testing. There have been previous reports of correlations among fluctuations of various confusional states and P300 latency in metabolic and toxic encephalopathies [5]. During chronic renal failure, a significant increase in P300 latency was observed with no evidence of cognitive impairment. The authors concluded that P300 had a good potential for quantifying and monitoring the subclinical neuronal dysfunction of this clinical condition [10]. Recently, a progressive decrease in P300 latency was observed in patients with post-traumatic amnesia. This decrease paralleled improvements in neuropsychological tests [4]. As far as pharmacological effects are concerned, scopolamine abolished P300 while physostigmine restored the wave form [11].

Latency and amplitude changes in P300 components were reported with $NO_2$ and nitrogen narcosis [12, 13] and P300 recording was also recently proposed as a method for monitoring consciousness level during surgical anaesthesia [14]. It can be concluded that different events globally affecting higher brain functions, ranging in severity from subclinical dysfunction to frank delirium, can in some way be studied with the aid of P300 recording. Our results agree with this hypothesis. In fact, CNS toxicity is a recognised but poorly understood side-effect of cytokines, since both interferons and interleukins showed potential acute and chronic cerebral toxicity [15–17].

The pathophysiology of the CNS effects of IL2 administration is still unclear although several mechanisms are possible. IL2 produced alterations in neuroendocrine secretion with increased beta endorphins and cortisol, and decreased melatonin plasma levels [18]. Brain electrical activity and behavioural changes were described after IL1 [19] and interferon [20] administration, while an enhanced transport to the CNS of active substances, such as other lymphokines (interferon, tumour necrosis factor, IL1) may follow the production of these substances by activated lymphocytes. An increase in the permeability of the blood–brain barrier with potential brain oedema, as demonstrated in animal models [21] and in patients [22], may be an alternative or concomitant pathogenic factor. The possibility, however, that metabolic changes related to IL2 administration may contribute to CNS toxicity should be disregarded in our cases due to the low grade of general toxicity observed.

P300 sensitivity to IL2 treatment poses some questions that are worth researching: damage to the blood–brain barrier at the level of the reticular activating system may affect some of the structures that are thought to play a role in the generation of P300 [23]; an inhibitory effect of IL2 on catecholaminergic central pathways has been raised in discussions on the pathogenesis of IL2 neuroendocrine effects, and it could be related to the catecholaminergic hypothesis of P300 generation [18, 23]. In conclusion we suggest that P300 may be used, together with other CNS functional indexes, in evaluating the pathogenic hypotheses of CNS IL2 toxicity.

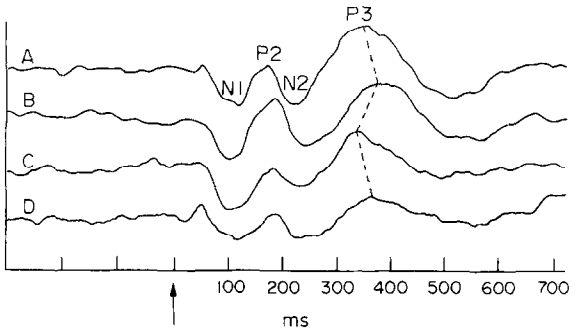


**Fig. 2. Latency shift of P300 component in a patient undergoing two IL2 courses. Traces B and D show two post-treatment recordings, A and C are control recordings. On the abscissa each division corresponds to 100 msec, the arrow to rare stimulus delivery. A prestimulus interval of 300 msec is shown.**

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# Karyotype in Multiple Myeloma and Plasma Cell Leukaemia

**Hans Josef Weh, Kay Gutensohn, Johannes Selbach, Rolf Kruse, Georg Wacker-Backhaus, Doris Seeger, Walter Fiedler, Werner Fett and Dieter Kurt Hossfeld**

**Between October 1988 and October 1991, 104 patients with multiple myeloma and 6 with plasma cell leukaemia were studied cytogenetically. Abnormal karyotypes were found in bone marrow cells of 33 patients (30%). Most pathological karyotypes were complex with numerous modal and structural anomalies. Numerical anomalies most frequently involved chromosome 11 and structural aberrations occurred most often in chromosomes 1, 11 and 14. The most consistent structural aberration was a 14q+ chromosome (10 patients) resulting from a t(11;14)(q13;q32) in 4 patients and a t(8;14)(q24;q32) in 1 patient. Sequential cytogenetic studies were performed in 15 patients. In 5 of 8 cases with a normal karyotype at diagnosis, chromosomal anomalies were detected when disease progressed. In concomitant cytogenetic/cytological studies it was found that in the majority of patients with normal karyotype the mitoses originated from contaminating normal bone marrow cells. Pathological karyotypes were detected more frequently in pretreated than in untreated patients, in patients with plasma cell leukaemia than in patients with multiple myeloma, in patients with stage III and dense bone marrow infiltration than in patients with stage I. Patients with abnormal karyotype, irrespective if pretreated or not, had a significantly shorter median survival than those with normal karyotype. These findings suggest that karyotype is an independent prognostic factor in multiple myeloma.**
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## INTRODUCTION

INVESTIGATIONS INCORPORATING chromosome analyses have been relatively rare in multiple myeloma and plasma cell leukaemia as compared with chronic myeloid and acute myeloid and lymphatic leukaemias. The main reason for this lack of cytogenetic information lies in the low proliferation rate of plasma cells *in vitro* and hence in a low number of mitoses that can be analysed. In the few reported studies [1–8] the chromosomal aberration rate was about 40% with t(8;14)(q24;q32) and t(11;14)(q13;q32) being the most frequent