Sonderdruck aus :

Deutsche Zeitschrift für
Onkologie 6
Journal of Oncology

26. Jahrgang
Dezember 1994
Dtsch. Zschr. Onkol.
ISSN 0931-0037

vfm
Verlag für Medizin
Dr. Ewald Fischer
69121 Heidelberg

Tolerance and Feasibility of a 12-Month Therapy Using the Antiretroviral Agent PB 100 in AIDS-Related Complex Patients

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Zusammenfassung

PB 100, ein pflanzliches Alkaloid, das in vitro die Vermehrung des humanen Immundefizienz-Virus 1 (HIV 1) stark hemmt, wurde in einer Phase 2 Pilotstudie bei einer homogenen Gruppe von 10 ARC-Kranken mit wenigen oder keinen Symptomen, aber großem kurzfristigen Risiko einer AIDS-Erkrankung angewandt, um die Toleranz und Durchführbarkeit einer 12monatigen oralen Behandlung mit 1 g/Tag festzustellen. Unverträglichkeit oder Nebenwirkungen waren bei den vierteljährlichen Untersuchungen nicht festzustellen. Eine gleichzeitige Kontrolle mit Aids-spezifischen Markern zeigte, daß die durchschnittliche Zahl der CD 4, anfangs zwischen 200-400 Zellen/mm^3, um 100 Zellen/mm^3 stieg, während sich das Verhältnis CD 4/CD 8 erhöhte. Die Konzentrationen des viralen Antigens p 24 blieben bei den 8 Kranken, bei denen sie anfangs negativ waren, gleich. Einer der restlichen 2 Fälle mit anfangs positiven p 24-Konzentrationen wurde negativ, bei dem anderen Fall blieben die Konzentrationen gleich. Die Konzentration von β2-Microglobulin, anfangs zwischen 3-4 mg/l, blieb immer unter 5 mg/l. Bei den 9 Patienten, die wir regelmäßig überwachen konnten, traten weder opportunistische Krankheiten noch HIV-begleitende Tumoren auf. Zusammenfassend kann festgestellt werden, daß keine Resistenz gegen das Mittel entwickelt wurde. PB 100 kann deshalb als ein vielversprechendes, neues Mittel bei der Behandlung von HIV-Infektionen betrachtet werden.

Schlüsselwörter

AIDS-ARC Kranke, CD 4, CD 8, Lymphozyten, antiretrovirales Agens PB 100.

Summary

PB 100, a plant-derived alkaloid which potently inhibits in vitro replication of human immunodeficiency virus 1 (HIV 1), was used in a phase 2 pilot study to evaluate tolerance and feasibility of a 12 month, 1g/day oral treatment, in a homogeneous group of

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10 ARC patients with few or no symptoms, but at high short-term risk of AIDS. Quarterly examinations revealed no sign of intolerance or adverse side effects. Concomitant checking of prospective AIDS markers showed that mean CD 4 count, initially from 200 to 400/mm^3, improved by 100 cells/mm^3, while CD 4/CD 8 ratios were raised. Concentrations of p24 viral antigen did not change in the 8 patients where they initially negative; in one of the two remaining cases, initially positive p24 concentration became negative and, in the other, it remained stable. β2-microglobulin, initially 3-4 mg/l, always remained below 5 mg/l. In the 9 patients who could be regularly followed, no opportunistic disease nor HIV-associated tumor appeared. Taken together, these results indicate that no drug resistance developed. PB 100 may thus be considered as a promising new drug for the treatment of HIV 1 infection.

Keywords

AIDS-related complex Patients, CD 4, CD 8, lymphocytes, antiretroviral PB 100.

The novel antiviral agent PB 100, a plant-derived β-carboline alkaloid extracted from Pao pereira, is a potent inhibitor of HIV 1 replication in vitro [1]. It was designed to selectively attack the virus without harming uninfected cells. Demonstration of PB 100 antiretroviral activity, which is mediated by reverse transcriptase inhibition, as well as evidence of the drug's lack of toxicity in laboratory animals and in man *, were deemed adequate to warrant the present study.

Its purposes were to evaluate tolerance and feasibility of a 12-month PB 100 therapy in HIV 1-infected, AIDS-related complex (ARC) patients with yet few or no symptoms, but, judging from their prospective cytological and biological markers, at high risk of short-term onset of AIDS. Study protocol involved monitoring of these markers, as well as of disease symptoms, and their satisfactory evolution concomitantly demonstrated the efficacy of PB 100 as an antiretroviral agent in AIDS therapy.

* PB 100 (flavopereirine) was supplied by Cerbiol, Saint Prim, France (Dr. *M. Beljanski*) and preliminary toxicity studies were carried out by Institut de Recherches S. I. R. International (Prof. *Jean Cahn*) Montrouge, France.

Patients and Methods

This phase 2 pilot study (phase 1 and 2 having now been merged where anti-HIV drugs are concerned) was carried out at Lapeyronie Hospital in Montpellier, France. Ten outpatient volunteers were selected for their closely matching characteristics, so as to form a homogeneous group. All these young heterosexual men and women, aged 24 to 36, were former heroin addicts, yet had abstained from this drug for at least four years. HIV 1 infection had been diagnosed from 3 to 6 years prior to entering the study, but seroconversion might have taken place quite earlier. Patients had been followed but had received no treatment against HIV. Their CD4 count ranged from 400 to 200/mm^3; slope of yearly decrease was known. On entering the study, 5 of the ten patients showed no symptoms of disease, while the others suffered from minor mucosal and cutaneous infections and/or limited adenopathies. Clinically, they belonged to the A2 and B2 categories of the 1993 CDC classification (formerly II, III, IV C2 and IV E), i. e., they were ARC patients at high risk of short-term AIDS onset [2]. Additional conditions for entering the study consisted in having a Karnofsky index equal to or higher than 90%, over 11 g/dl hemoglobin, over 1500/mm^3 granulocytes, over 80×10^3/mm^3 platelets, less than 120 mg/l serum creatinine, less than 120 IU/l serum ASAT and ALAT. Informed consent was obtained from the patients, and the study conformed to French public health regulations regarding experimentation in man.

PB 100 * was orally administered, thrice daily, in capsules containing 250 mg of the agent: two in the morning, one at noon and one at night, amounting to a total daily dose of 1 g. Patients were examined on entering the study (Month 0), and subsequently every three months (Months 3, 6, 9 and 12), for signs of gastric, pancreatic, hepatic, neurologic and hematologic intolerance or adverse side effects; serum creatinine and transaminase levels were measured. In the checklist were also included clinical symptoms classified by the CDC as AIDS-relevant, and AIDS biological markers: CD4, CD8 and lymphocyte subset counts were measured, as well as amounts of P 24 viral antigen and β2-microglobulin. To avoid chronobiological variations [3], blood samples were always drawn between 8 and 9 AM; they were analysed on the same day (Laboratoires de Chimiobiologie et d'Immunologie du CHU and Laboratoire de Cytologie du CRTS, Montpellier). Samples were also used to set up a serum library.

Student's paired t test ($p \leq 0.05$) was used both to evaluate final results by comparing parameter values at inception and conclusion of study, and to follow intermediate changes by comparing quarterly values. A slight bias was introduced at Month 9 by the absence of patient n°9, who failed to report at that date and presumably missed several weeks of treatment.

Results

Evaluation after twelve months of 1 g/day PB 100 oral administration yielded the following results. There were no signs of intolerance or adverse side effects of any kind, and no alteration of serum creatinine and transaminase levels. Mean erythrocyte and platelet counts (respectively 4.8 millions/mm^3 and 161,700/mm^3) did not vary significantly. Patients' general condition remained good, with, on the whole, a 100% Karnofsky index, and weight variation of no more than 2 kg above or below initial values at month 0 (except for dropout patient n°9, who suffered at month 12 from an opportunistic infection and lost 12 kg). Mycoses and dermatitis which had been present at the outset disappeared in the course of PB 100 treatment in 6 out of 10 patients and, in the others, did not develop further. Signs of HIV focal infection were remarkably few, limited to minor adenopathies (less than 2 cm in diameter) which regressed in the course of therapy. In 9 out of 10 patients, i. e. in all those who could be regularly followed, no opportunistic disease occurred during PB 100 treatment. When he entered the study, patient n° 8 suffered from a lung disease; it was subsequently cured within a month using sulfamide + trimethoprim (Bactrim®) and PB 100. Patient n° 2's recent tuberculosis did not reappear. As for patient n° 9, missing at month 9, he was seen to suffer at month 12 from a mycobacterial infection of the gastric mucosa; after completion of the study, he was switched to zidovudine therapy for ethical reasons. It must be underlined that no AIDS-

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associated tumor appeared in any of the patients during PB 100 treatment. Evolution of prospective AIDS biological markers was also satisfactory. An overall increase of CD4 was observed (fig. 1). Statistical comparison of the slopes of CD4 decrease during the year prior to the study with the slopes during PB 100 therapy clearly shows a reversal: mean slope is decreased by 58.2 % (fig. 2). Initial 200-400/mm^3 CD4 count, prospective of a 46 % risk of AIDS, had risen above 400/mm^3 at month 12 in 5 out of 10 patients, thereby decreasing AIDS risk to 16 % [4] [5]. Although still below its normal value of 1, CD4/CD8 ratio had gone up from 0.35 to 0.5, mainly due to CD4 increase (fig. 3). Total lymphocyte count was also raised (fig. 4). Though not predictive of AIDS disease onset, counts of CD19 and CD2 cells, which are thought to play a part in immune response stimulation, were also seen to increase. Mean monocyte count (initially 338/mm^3) exhibited a slightly significant increase at month 12. In all but two of the patients, P24 antigen concentrations were negative upon entering the study and remained so during PB 100 therapy; patient N°8's initially positive P24 concentration remained stable and patient N°9's became negative at month 12. Although initial β2-microglobulin concentrations slightly rose above their initial 3-4 mg/l values, considered prospective of a 33 % risk of AIDS [6], they never reached the threshold value of 5 mg/l which is prospective of a 69 % risk of AIDS; variation was not statistically significant, except for patient n°9.

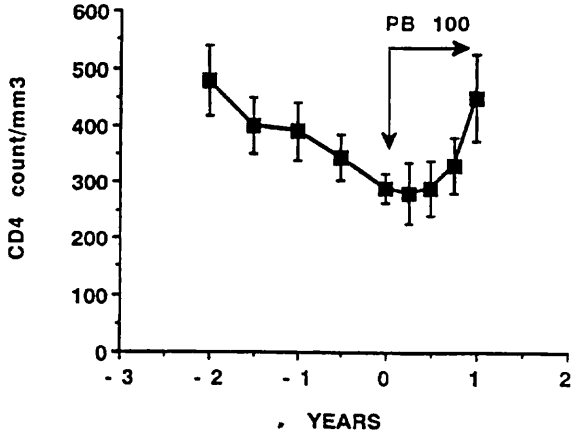

Fig. 1: Evolution of CD4 count over 3 years in 10 patients (± SD), prior to and during PB 100 therapy.

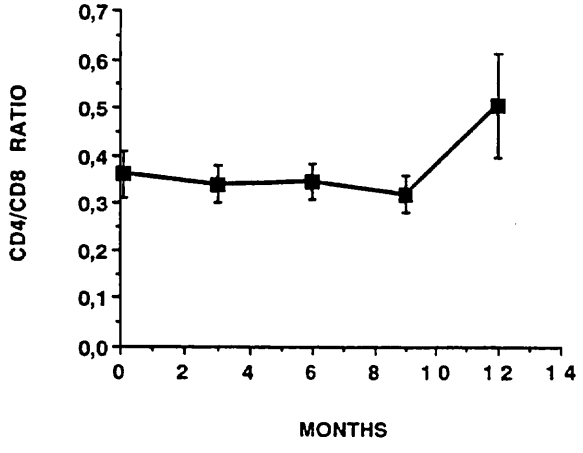

Fig. 3: Evolution of CD4/CD8 ratio in 10 patients (± SD) during a 12-month PB 100 therapy.

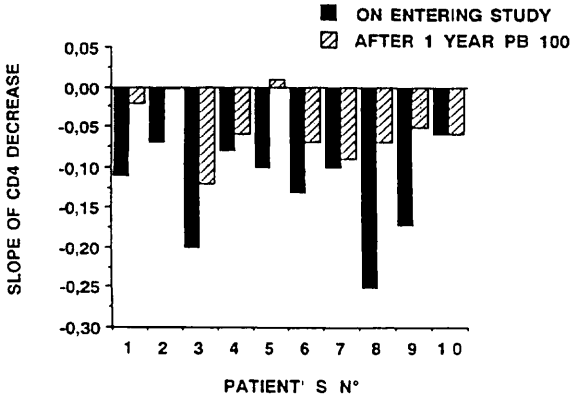

Fig. 2: Evolution of the slope of yearly CD4 decrease, in 10 patients (± SD) prior to and after a 12-month PB 100 therapy.

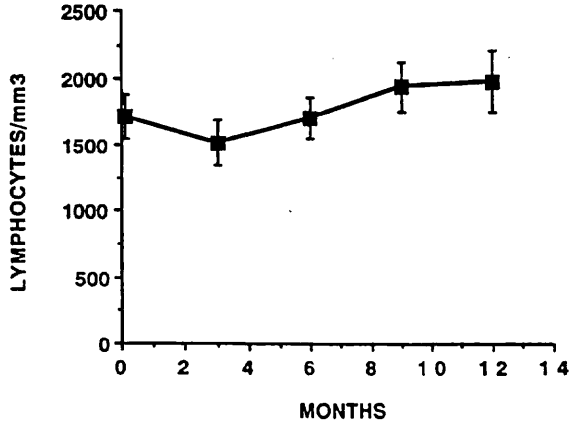

Fig. 4: Evolution of total lymphocyte count in 10 patients (± SD) during a 12-month PB 100 therapy.

Discussion

Aimed at assessing tolerance and feasibility of PB 100 therapy in a homogeneous group of young adult ARC patients displaying few or no clinical symptoms, but at high short-term risk of AIDS, this year-long phase 2 pilot study not only demonstrated the innocuity of this novel antiretroviral agent, but also revealed its high efficacy in checking HIV infection. An oral dose of 1g/day was very well tolerated. At quarterly examinations of patients, special attention was paid to signs of gastric intolerance and/or adverse side effects on the pancreas, liver, nervous system or blood cells, which are a well-known major drawback of most present AIDS treatments. No such effects were noted using therapeutic doses of PB 100, for which there exists, moreover, a wide safety margin. Toxicity studies carried out in rodents by the oral route (Institut de Recherches S. I. R. International, Montrouge, France) showed DL50 in the rat to be as high as 10.45 g/kg, while no alterations of either anatomical or biochemical nature were apparent in the interval between low dose (1/20 DL50 per day) and high dose (1/5 DL50 per day). In the present study, 1 g per day proved sufficient for a 60 kg human patient.

At completion of the study, patients' condition had in no way deteriorated. No major opportunistic infection had occurred (except in patient n°9, who did not report for examination at month 9 and presumably did not properly follow treatment, as indicated in "Results"). No HIV-associated tumor developed.

Evolution of prospective markers was most encouraging, with a mean increase of 100 CD4/mm^3, a significantly increased CD4/CD8 ratio (though not yet up to normal) and, most meaningful from a prospective viewpoint, reversal (+58.2 %) of the mean slope of CD4 decrease, which, in the years prior to this study, had been clearly conducive to AIDS.

Other prospective markers such as concentrations of P24 viral antigen (initially negative in 8 out of the 10 patients) and β2-microglobulin (always below 5 mg/ml) remained on the whole unchanged. In addition, from month 9 on, an increase was noted in total lymphocyte count and in the amounts of CD19, CD2, monocytes, and also erythrocytes.

These distinctly positive results may be better understood if one considers the drug's fundamental properties. In vitro experiments on cultures of HIV 1-infected CD4 cells show that PB100 very efficiently inhibits retroviral replication, in a dose dependent way and at doses far below cytotoxicity [1]. In one series of these in vitro tests, over 99% inhibition of HIV 1 replication was obtained using 30 µg/ml or 60 µg/ml PB100. Syncytium formation, a major cytological aspect of AIDS, is prevented by the drug. Initial experiments using erythroblastosis retrovirus reverse transcriptase [7] demonstrated that PB100 inhibits the activity of this enzyme, thereby preventing replication.

Experiments with CD4 cultures also reveal that, after addition of PB100, HIV-infected cell multiplication sharply drops, whereas uninfected cell multiplication proceeds normally [1]. This selective activity on the virus and virus-infected cells is an outstanding property of PB100. Paradoxically, this might explain the outbreak of minor adenopathies in some of the patients in the course of the study, and the subsequent disappearance of these infections at month 12. PB100 does not affect an infected cell as long as the virus within it does not replicate. It may be presumed that the observed adenopathies originated from cells in which a previously quiescent virus started to replicate; this then enabled the drug to act. The lack of toxicity exhibited by PB100 is essentially dependent on its selectivity, that is, its intrinsic property of being specifically targeted to viral replication and virus-infected cell multiplication.

It may be deduced from patients' satisfactory general condition, from the overall absence of outbreaks of opportunistic infections or AIDS-associated tumors, that over a full year of PB100 therapy, no viral resistance developed. Other workers' experience (personal communications) shows that no drug resistance develops even after several years of PB100 therapy. HIV resistance is a central impediment with most antiviral agents in use today, and efforts to obviate this have required adoption of multidrug therapies. Resistance sometimes develops as early as after 27 weeks of treatment [8]. It has been traced to mutations that occur within the reverse transcriptase gene as the HIV provirus replicates [9]. PB100 may show the way out of this predicament. Its efficient inhibition of reverse transcriptase activity and HIV replication leaves the virus no chance to mutate.

It must also be stressed that PB100 crosses the blood-brain barrier, a useful quality in case of HIV cerebral infections. It also exhibits several other interesting properties which are beyond the scope of this report. Based on an entirely novel concept, this therapeutic drug should become an asset for AIDS therapy.

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Patient N°	Patient Date of HIV detection Stage on entering study	Antecedents 3 years prior to study	Month 0	Month 3	Month 6	Month 9	Month 12
1	M 36 yr HIV+ 1985 Stage IVC2 (B2)	Chronic hepatitis C Hairy leucoplasia	Hairy leucoplasia CD4 = 293 Agp24=0 βmgl=3.23	Hairy leucoplasia CD4=226 Agp24=0 βmgl=3.33	Adenopathies CD4=298 Agp24=0 βmgl=4.45	Seborrheic dermatitis CD4=396 Agp24=0 βmgl=3.62	NS CD4=562 Agp24=0 βmgl=4.55
2	M 34 yr HIV+ 1987 Stage IVC2 (B2)	Primary tuberculosis infection	NS CD4=271 Agp24=0 βmgl=3.14	NS CD4=379 Agp24=0 βmgl=3.33	Adenopathies CD4=341 Agp24=99 βmgl=4.57	NS CD4=354 Agp24=122 βmgl=3.55	Hairy leucoplasia CD4=445 Agp24=0 βmgl=3.8
3	F 33 yr HIV+ 1985 Stage IVC2 (B2)	Herpes zoster on thorax and abdomen Hepatitis HBS HVC	NS CD4=361 Agp24=0 βmgl=3.31	Adenopathies CD4=267 Agp24=0 βmgl=3.97	Adenopathies Skin lesion CD4=260 Agp24=0 βmgl=3.89	Adenopathies CD4=409 Agp24=0 βmgl=3.85	NS CD4=533 Agp24=0 βmgl=4.69
4	M 33 yr HIV+ 1985 stage II (A2)	NS	NS CD4=305 Agp24=0 βmgl=3.11	NS CD4=287 Agp24=0 βmgl=3.17	Adenopathies CD4=189 Agp24=0 βmgl=2.81	NS CD4=304 Agp24=0 βmgl=2.25	NS CD4=309 Agp24=0 βmgl=3.99
5	F 32 yr HIV+ 1986 Stage III (A2)	Adenopathies	Adenopathies Seborrheic dermatitis CD4=312 Agp24=0 βmgl=3.37	Adenopathies Seborrheic dermatitis CD4=400 Agp24=0 βmgl=4.06	Adenopathies Seborrheic dermatitis CD4=564 Agp24=0 βmgl=4.43	Seborrheic dermatitis Acne rosacea CD4=487 Agp24=0 βmgl=2.91	Seborrheic Dermatitis Acne rosacea CD4=522 Agp24=0 βmgl=4.25
6	M 26 HIV+ 1986 Stage IV (B2)	Thrombopoenia HIV (Splenectomy)	Hairy leucoplasia CD4=273 Agp24=0 βmgl=3.04	Hairy leucoplasia CD4=185 Agp24=0 βmgl=3.24	Hairy leucoplasia Adenopathies CD4=245 Agp24=0 βmgl=3.24	Hairy leucoplasia Seborrheic dermatitis CD4=279 Agp24=0 βmgl=2.54	NS CD4=412 agp24=0 βmgl=4.09
7	M 30 yr HIV+ 1985 Stage II (A2)	Hepatitis B	NS CD4=326 Agp24=0 βmgl=2.97	NS CD4=228 Agp24=0 βmgl=2.83	Adenopathies CD4=338 Agp24=0 βmgl=4.21	Adenopathies CD4=253 Agp24=0 βmgl=2.3	NS CD4=345 Agp24=0 βmgl=3.94
8	M 26 yr HIV+ 1985 Stage IVE (B2)	Ongoing interstitial lymphoid pneumopathy	Interstitial lymphoid pneumopathy adenopathies CD4=258 Agp24=120 βmgl=3.96	Adenopathies CD4=194 Agp24=231 βmgl=4.47	Adenopathies Oral candidosis CD4=274 Agp24=74 βmgl=4.5	Adenopathies Hairy leucoplasia CD4=283 Agp24=160 βmgl=4.3	Adenopathies Seborrheic dermatitis CD4=260 Agp24=180 βmgl=4.72
9	M 33 yr HIV+ 1984 Stage IVC2	NS	Adenopathies Oral candidosis CD4=376 Agp24=652 βmgl=4.18	Oral candidosis CD4=492 Agp24=1098 βmgl=5.77	NS CD4=363 Agp24=653 βmgl=6	Not seen	Atypical gastric mycobacterium CD4=551 Agp24=0 βmgl=9.51
10	F 31 yr HIV+ 1984 Stage II (C2)	NS	NS CD4=373 Agp24=0 βmgl=2.98	NS CD4=338 Agp24=0 βmgl=3.47	NS CD4=362 Agp24=0 βmgl=3.02	NS CD4=362 Agp24=0 βmgl=3.02	NS CD4=300 Agp24=0 βmgl=3.62

βmgl : β2-microglobuline
NS : nothing special