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CLINICAL AND IMMUNOLOGICAL ASSESSMENT IN HIV+ SUBJECTS RECEIVING INOSINE-PRANOBEX. A RANDOMISED, MULTICENTRIC STUDY

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Inosine-pranobex (methisoprinol, isoprinosine; INPX) is the *p*-acetamidobenzoic salt of *N,N*-dimethylamino-2-propanol and inosine in a 3:1 molar ratio. In early studies, INPX was found to partially inhibit human immunodeficiency virus (HIV) and to increase the immunocompetence of HIV-infected subjects *in vitro*.

We report the results of a randomised, multicentric clinical trial carried out on 553 HIV+ patients. 261 individuals were treated with INPX (two 500 mg tablets every 6 h for 3 months) and the remaining 292 constituted the untreated control group. INPX treatment was associated with a slightly improved clinical condition or with a trend in that direction, as compared to the untreated group. A preservation of the CD4/CD8 cell ratio values, a decrease in the CD8+ cells and an increase in the Leu 2-7+ cell number better than in the untreated individuals was also observed in the patients taking INPX. No serious or adverse effects of INPX have been observed.

Key words: **AIDS, Isoprinosine, Immunotherapy.**

INTRODUCTION

Approaches to the treatment of AIDS (acquired immune deficiency syndrome) and related conditions (ARC = AIDS-related complex; PGL = persistent generalized lymphadenopathy; HIV+ = human immunodeficiency virus seropositivity) have paralleled the understanding of the disease. Initially, therapy was focused on various complications of the illness, e.g. *Pneumocystis carinii* pneumonia and Kaposi's sarcoma. With the identification of the aetiological agent of AIDS, an exogenous C-type retrovirus (HIV) great interest in anti-viral agents was stimulated. The observation that AIDS and AIDS-related conditions present multiple defects of the immune system, especially of the CD4+ cell compartment, justified the idea that immunostimulation had to be attempted. However, the potential risk of increasing viral replication, by the activation of the remaining CD4+ cells having integrated proviral RNA, restricts the range of biological response modifiers to include only those not enhancing HIV production.

Inosine-pranobex (INPX) is a derivative of inosine with both anti-viral and immunopotentiating activities. INPX enhances lymphocyte proliferation, macrophage activation, natural killer (NK) cell activity, interleukin (IL)-2 cell receptor expression and IL-1, IL-2 and gamma interferon (γ-IFN) production. The drug is not mitogenic for T-cells, it does not increase HIV production when used alone or in combination with zidovudine (3′-azido-3′-deoxythymidine; AZT) and at concentrations ≥ 200 μg/ml has an anti-HIV activity.[1] Wallace and Bekesi reported a double blind controlled-placebo study with INPX (3 g per day) given for 28 days to ARC patients.[2] An increase in NK cell activity, which persisted after termination of treatment, increased T-lymphocytes and CD4+ cells and normalisation of CD4+/CD8+ cell ratios were shown in the INPX-treated subjects. A study that followed the Wallace trial failed to confirm the

above findings.[3] Therefore, four multicenter studies in the U.S.A., Australia, Italy and in Scandinavia began in 1987 for HIV seropositive non-AIDS subjects. We report the preliminary data of the Italian multicenter study.

PATIENTS AND METHODS

553 patients with HIV infection completed the trial. These individuals were referred to the Infectious Diseases Hospital and/or University Clinics of Genova, Milan, Bologna, Faenza, Ancona, Roma, L'Aquila and Bari, Italy, by their physicians, specifically to be treated for HIV infection. An informed consent was obtained from all the persons participating in the study, which were treated as out-patients.

HIV-related diseases were classified clinically. PGL was defined as the presence of bilaterally enlarged lymph nodes in at least two extra-inguinal areas, in the absence of any other cause for lymphadenopathy. The ARC was diagnosed from symptoms (fever, weight loss, night sweats, persistent unexplained diarrhoea, overwhelming malaise), signs of lymphadenopathy, oral candidiasis or splenomegaly, and at least one abnormality of immunocompetence. None of the enrolled subjects, at the moment of the study, presented major infections from pyogenic pathogens or had a diagnosis of AIDS or Kaposi's sarcoma. Furthermore, patients who had used antiviral or immunomodulating drugs in the preceding 4 weeks and antibacterial or antimicotics in the 2 weeks before enrollment, were not admitted to the study. The criteria for eligibility also included a haemoglobin level of 13 g dl^{-1} or more, a total granulocyte count of 3500 per cubic millimetre (mm^3) or more and a platelet count of 160,000 per mm^3 or more. Subjects were asked not to take any medications without consulting the investigators.

Laboratory monitoring was done before enrollment, at the beginning of the trial, and 3 months after. It included: measurement of haemoglobin, platelet count, white blood cell (WBC) count, lymphocyte count, erythrocyte sedimentation rate, serum glucose, blood urea nitrogen, bilirubin, serum aspartate aminotransferase, alkaline phosphatase and uric acid, serologic tests for hepatitis B, serum levels of IgA, IgG and IgM (Menarini Diagnostici, Firenze, Italy). Blood was also collected for detection of anti-HIV antibody and for measurement of serum p24 antigen levels by enzyme-linked immunosorbent assays (E.I. DuPont, Wilmington, and Abbott Laboratories, Chicago). T helper/inducer cells (Leu 3 = CD4+), T suppressor/cytotoxic cells (Leu 2 = CD8+) and natural killer cells (Leu 2-7+) were determined by direct immunofluorescence using monoclonal antibodies (Becton Dickinson, Mountain View, CA) and two-color flow cytometry (FACSCAN, Becton Dickinson) on whole blood. All blood samples were taken between 8.30 and 9.30 a.m. to avoid bias through diurnal variation.

Patients were randomized by computer-generated random numbers. 553 patients completed the 3 month clinical trial; 261 (47%) individuals (group I) were treated with inosine pranobex (methisoprinol, isoprinosine, INPX, Viruxan™, Sigma Tau, Pomezia, Italy) — two 500 mg tablets of INPX every 6 h for 3 months — and the remaining 292 (53%; group II) constituted the untreated control group.

The two groups were compared using both the non-parametric Mann–Whitney U-test and the Student's *t*-test, which gave comparable significance levels.

Group I, INPX-treated, was constituted by 174 males (66%) and 87 females (34%). 189 individuals were drug addicts (72%), 36 (14%) homosexuals and the remaining 36 (14%) haemophiliacs and heterosexuals. The mean age of group I patients was 28 ± 3 yr. The control group, group II, was made up by 202 males (69%) and 90 females (31%). Also in this group the vast majority of the participants were drug addicts (213 = 73%), 39 (13%) were homosexuals and 40 (14%) haemophiliacs and heterosexuals. The mean age of group II patients was 27 ± 2 yr. Antibodies directed against hepatitis B surface antigen (HBsAg) were found in 14 individuals (5%) of group I and in 16 (5%) of group II.

According to the CDC classification system for HIV infection,[4] 163 (62%) individuals of group I were classified as CDC group II (asymptomatic infection). 83 (32%) subjects presented PGL (CDC group III) and 15 subjects resulted in the CDC group IV, subgroup C2, since they showed oral hairy leukoplakia and oral candidiasis. The clinical examination of these subjects also revealed lymphnodes in 93 (37%) individuals, fever in 7 (2.7%), diarrhoea in 10 (3.8%), weight loss in 9 (3.4%), night sweats in 14 (5.3%) and minor infections (impetigo, cutaneous abscesses, otitis, sinusitis, urethritis) in 11 (4.2%) patients.

In group II, 178 (61%) individuals were classified as CDC group II, 87 (30%) as CDC group III, and the remaining 27 (9%) as CDC group IV, subgroup C2. 95 (32%) patients were found to have lymphnodes, ten (3.4%) fever, nine (3%) diarrhoea, eight (2.7%) weight loss, 15 (5.1%) night sweats and two (0.68%) minor infections.

The subjects with a number of CD4+ cells > 900 per mm^3 were 28 (11%) in group I and 41 (14%) in group II. 73 (28%) individuals of group I and 98

(33%) of group II had 600 to 900 CD4+ cells per mm^3; 85 (32%) persons of group I and 91 (31%) of group II had 200 to 400 CD4+ cells per mm^3; 65 (25%) patients of group I and 55 (19%) of group II had 200 to 400 CD4+ cells per mm^3. Less than 200 CD4+ cells per mm^3 were observed in ten (4%) patients of group I and in seven (3%) patients of group II.

WBC counts haemoglobin level, platelet count, serum IgA, IgG and IgM values, the absolute amounts of CD4+, CD8+, Leu 2-7+ cells, and the CD4/CD8 cell ratio of the patients from both groups are reported in Table 1.

To assess the therapeutic relevance of a 3 month treatment with INPX, the following parameters were evaluated: (1) overall clinical status; (2) symptom variations; (3) diarrhoea; (4) chronic or recurrent fevers; (5) minor infections; (6) immunocompetence; (7) drug tolerance and side effects.

RESULTS

After 3 months, none of the patients who completed the clinical trial were diagnosed as having full-blown AIDS or developed serious infections, i.e.

Table 1. Effect of INPX on clinical and immunological parameters

	Group I, INPX		Group II, control	
Total cohort	261		292	
Age	28 ± 3		27 ± 3	
Males	174		202	
Females	87		90	
Drug addicts	189		213	
Homosexuals	36		39	
Haemophiliacs and heterosexuals	36		40	
	Day 0	Day 90	Day 0	Day 90
HBsAg+	14	14	16	16
p24+	9	9	9	9
CDC group II	163	136	178	146
CDC group III	83	90	87	96
CDC group IV, C2	15	35	27	50
Lymph nodes	93	95	95	93
Fever	7	6	10	15
Diarrhoea	10	7	9	11
Weight loss	9	8	8	7
Night sweats	14	8	15	17
Minor infections	11	12	2	11†
CD4 > 900/mm^3	28	22	41	34
CD4 600–900	73	61	98	69
CD4 400–600	85	78	91	101
CD4 200–400	65	79	55	74
CD4 < 200	10	21	7	14
Leucocytes/mm^3	5295 ± 542	5539 ± 581	5889 ± 206	6103 ± 265
Lymphocytes/mm^3	2086 ± 128	1992 ± 192	2143 ± 97	2204 ± 220
Haemoglobin g dl^{-1}	14.8 ± 0.7	14.6 ± 0.9	15.1 ± 0.5	14.8 ± 0.7
Platelets/mm^3 ($\times 10^3$)	196 ± 21	192 ± 19	194 ± 26	192 ± 24
IgA mg dl^{-1}	206 ± 41	223 ± 43	183 ± 28	188 ± 30
IgG mg dl^{-1}	1969 ± 207	2009 ± 203	1982 ± 195	2049 ± 159
IgM mg dl^{-1}	226 ± 31	232 ± 36	213 ± 42	225 ± 44
CD4/mm^3	538 ± 109	503 ± 141	651 ± 119	618 ± 108
CD8/mm^3	992 ± 109	881 ± 232*	1008 ± 159	1103 ± 136
CD4/CD8	0.55 ± 0.15	0.60 ± 0.21	0.64 ± 0.10	0.56 ± 0.09
Leu 2-7+/mm^3	201 ± 54	278 ± 114*	234 ± 98	240 ± 138

*$P < 0.01$; †$P < 0.02$.

Pneumocystiis carinii pneumonia. In the INPX-treated group the number of individuals complaining about chronic diarrhoea decreased from ten (3.83%) to seven (2.68%). In the untreated group, the percentage of subjects with diarrhoea, after 3 months, rose from nine (3%) to 11 (3.77%). In group II, also, the number of patients presenting chronic or recurrent fevers augmented from ten (3.4%) to 15 (5.1%) while in group I a decrease from seven individuals (2.7%) to six (2.3%) was observed. A significant increase ($P < 0.02$) in the number of subjects with minor infections, from two (0.7%) to 11 (3.7%), was reported among the untreated patients, while only a 0.40% increase (from 11 to 12 individuals) was observed in the INPX-treated group. However, when the CDC classification system was employed to assess the disease progression, a 10% progression from CDC group II toward more advanced forms of HIV infection — CDC group III and CDC group IV — was observed in both groups.

Serum levels of IgA, IgG and IgM showed a trend toward an increase, irrespective of the group considered (Table 1). On the other hand, a statistically significant difference was found in evaluating the CD8+ cell number and Leu 2-7+ cell values ($P < 0.01$). Both groups showed a decreased number of CD4+ cells (about 5%) after the 3 month interval but, while in the untreated patients this decrease was accompanied by an increase of CD8+ cells (about 8%), a reduced number (13%) of CD8+ was present in the INPX-treated individuals. Therefore, the CD4+/CD8+ cell ratio was increased (7%) in the group I patients and decreased (−14%) in the group II individuals and the net difference between the two groups is 21%. The Leu 2-7+ cell subset determinations have been performed only on a part of the patients who completed the trial for technical reasons, i.e. on 179 of the INPX-treated individuals and 165 of the untreated patients. However, the data show that INPX administration is accompanied by increased levels ($P < 0.01$) of the Leu 2-7+ cells in the patients taking INPX. No significant modifications were observed in the untreated subjects.

None of the INPX-treated patients had to interrupt the therapy for side effects or toxicity related to the drug administration. In general, the tolerance was good and no modifications of the clinical chemistry parameters that could be reasonably attributed to the drug administration were observed.

DISCUSSION

HIV is at present a world-wide problem and presumably millions of people are affected. One of the main unsolved problems is that AZT, at present the only effective antiviral drug for the treatment of HIV infection, is associated with side effects such as anaemia, leukopenia, neutropenia and bone marrow dysfunction, requiring blood transfusions and/or reduction of the dose of AZT.[5] Moreover, it is very expensive. For these reasons, AZT administration has been restricted to the most advanced forms of HIV infection (AIDS, ARC). On the other hand, according to the available data, HIV+ persons have shown a slow but clear disease progression, with deterioration of basic immunological parameters. Apart from the clinical status assessment, the number of CD8+ lymphocytes may be an early indicator, whereas a decrease in the number of CD4+ cells is particularly indicative in the late course of the disease. Progression is also associated with a decreased CD4+/CD8+ cell ratio, with anaemia, with increased IgA serum levels, but not IgM or IgG, and with p24 antigenemia.[6–8] Recently, De Simone has shown that the clearance rate of AZT is affected by INPX in AIDS patients, and that there are several advantages in employing the combined AZT–INPX treatment, such as lower doses of AZT to maintain a therapeutic anti-retroviral level, longer interval periods between AZT treatments, the potentiality to enhance the immunological response resulting from INPX treatment.[9] The administration of INPX in the early stages of HIV infection, therefore, could be envisaged as a means of improvement of the host's immunocompetence which can delay the disease progression and constitute the basis for the gradual administration of AZT, when necessary.

To verify the first two points — the delay of disease progression and the host's immunoreconstitution — preliminary studies have been done in the U.S.A. However, mainly due to the small number of subjects enrolled, their results were considered questionable. To confirm the observed amelioration of the NK cells activity and the normalisation of the CD4/CD8+ cell ratio[2], a multicentre study was begun in Italy.

The majority of HIV+ individuals are drug addicts. About 40% of the enrolled individuals were excluded because they disregarded the protocol or stopped the INPX, or self-administered other compounds.

INPX proved to be a safe drug which is well tolerated by the HIV-infected patients. The p24 antigen levels, the immunological and laboratory parameters, and the clinical evolution confirm the experimental data showing that INPX does not increase HIV activation.[10–12] INPX treatment was apparently associated with an improved clinical

condition or with a trend in that direction (reduced number of patients with chronic diarrhoea, chronic or recurrent fevers, minor infections) with respect to the untreated group. Moreover, an increase in the Leu2-7+ cell number and a better preservation of the CD4/CD8 cell ratio values than in the untreated individuals was observed in the subjects taking INPX. We failed to show any difference in the disease progression for both groups, by employing the CDC staging classification.[13] We now plan to reconsider the patient population according to a different staging classification (i.e. the Walter Reed staging classification[14]).

According to the results, the rationale for INPX use in the early stages of HIV infection could be summarized by four items: (1) INPX is a thymomimetic drug, enhancing Leu 2-7+ levels and delaying the decrease of CD4/CD8 cells ratio.[2] A modulatory effect of the drug on the NK cells has been already reported *in vivo* and *in vitro*, as well as an activity on the activated suppressor T-cells of AIDS patients;[15] (2) INPX does not increase the HIV production by activating CD4+ lymphocytes with integrated proviral RNA, as demonstrated *in vitro* by experimental studies.[10–12] When used in conjunction with AZT, INPX increases the AZT serum levels and mean half-life through a competing mechanism for glucuronidation and excretion;[9] (3) INPX may improve the clinical condition of the patients. A previous study has shown a reduced *cytomegalovirus* shedding in semen from HIV-infected patients,[16] and in the broader context of subjects with secondary non-virally induced immunodeficiencies, INPX treatment was associated with a reduced incidence of opportunistic infections; (4) INPX is well tolerated by HIV-infected patients and can be administered *per os*.

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