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Aspidosperma subincanum II. Usefulness of uleine and ribonucleic fragments in the treatment of AIDS patients

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ABSTRACT

Aids patients were treated during a year with three different food supplements commercially available: para-pau-aspido (*Aspidosperma subincanum* Mart. ex A. DC., Apocynaceae); 2Leid (nucleic acids and cytokines); and Para Immuno (propolis, pollen and royal jelly). All foods, given either alone or in combination, proved useful to all AIDS patients who received the supplements, be these under tri-therapy (Triomine: stavudine, lamivudine, névirapine) or left unattended.

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Introduction

The treatment of AIDS patients rests on the administration of three chemicals designed to reduce the viral load. The HIV is immunodepressive but no effort is made during chemical treatment to stimulate the multiplication and activity of lymphocytes. This stimulation would however benefit the patients because some drugs are known to be immunosuppressive, which add to the immunodepression induced by the pathogen. Means exist to stimulate the immune response, either by inducing the synthesis of nitric oxide by lymphocytes and other cells, or else by promoting the multiplication of lymphocytes.

Uleine (**1**), present in *Himatanthus lancifolius* and *Aspidosperma subincanum* Mart. ex A. DC., Apocynaceae, have been extensively studied (Seidl et al., 2010). Its capacity to promote the synthesis of nitric oxide (Souza et al., 2007) makes of these plants candidates for use as an adjuvant in the treatment of patients suffering from an immune-depression. The bark of *Aspidosperma subincanum* has been purified and the food supplement rich in uleine so obtained has been demonstrated innocuous (Federlin et al., 2014).

Nucleic acid oligomers have been shown to inhibit *in vivo* Shope fibroma and vaccinia viruses (Beljanski et al., 1975). This effect was traced to a stimulation of the multiplication of lymphocytes and platelets (Beljanski and Plawecki, 1979). Lymphocyte populations depleted *in vivo* by cyclophosphamide responded to a stimulus of ribo-oligo-nucleotides (Beljanski et al., 1983). This positive effect of regeneration of lymphocytes by nucleic acids observed *in vivo* was verified on two malignant lymphoma cases (Donadio et al., 1991).

These food supplements would appear useful to help the immune system of immuno depressed patients as tuberculosis and Aids patients to counter the immunodepressive activity of the pathogens as well as the immunodepressive activity of some drugs used to eliminate the pathogen. This hypothesis was verified by administering the supplements during a year to AIDS patients. In addition, a third food supplement consisting of propolis, royal jelly and pollen, known to stimulate the immune system, was also offered to the clinician, enabling her to make a complete study of available immuno stimulators.

The clinician focused on the improvement of the health of her patients by verifying the activity of a combination of food supplements given to patients either under chemical treatment or else, due to lack of resources, left unattended. Lack of resources restricted the monitoring in the course of time of the effects of the treatments, to weight and lymphocyte numbers.

The detailed raw results (66 pages) are available (Bouillon and Ndayikunda, 2006). The authors analysed and exploited them.

Materials and Methods

Uleine source and the immunostimulant

Uleine (**1**) was extracted from the plant *Aspidosperma subincanum* Mart. ex A. DC., Apocynaceae, and presented as the food supplement "Para-pau-aspido" by the Company Parabolic Biologicals (Beauvechain, Belgium) under two different concentrations: PP20 (20 mg total alkaloids) and PP 80 (80 mg total alkaloids).

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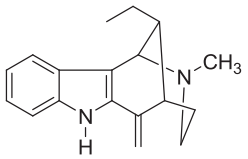
A supply of the products sufficient to cover a year of treatment was donated. The food supplement "Para Immuno" elaborated by the Company Parabolic biologicals was made of propolis extracted from bee-hives, Royal Jelly and pollen. This product was donated in amounts sufficient to cover a year of treatment.



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2Leid is prepared by the laboratory "Laboratoire Labo-Life". This immunostimulant to be taken sub-lingually is made of cytokines, ribonucleic acids, deoxyribonucleic acids and TGFbeta, and the product was donated in amounts sufficient to cover a year of treatment.

Triomine 530: stavudine 30 mg, lamivudine 150 mg, névirapine 20 mg, for patients whose weights was inferior to 60 kg.

Triomine 540: stavudine 40 mg, lamivudine 150 mg, névirapine 200 mg, for weights over 60 kg.

Clinical studies

Number of subjects treated was eighty, of whom sixty were included in this study. They were orphans at the orphanage "Maison Shalom", located in the Burundi.

The files of the patients were a year old when the study was initiated in 2006 and many showed an unfavourable clinical symptomatology and opportunist infections, *i.e.* chronic diarrhoea, persistent fever, generalised lymphadenopathies, diverse pneumopathies, mycoses, generalised dermatoses, zona, alteration of the general state, loss of weight and asthenia. Nearly all patients had conserved their symptomatology when the treatment with supplements started and some had worsened and developed additional opportunist infections.

Among the subjects under tri-therapy since six months, some showed no clinical change, others had developed new opportunist illnesses and others were intolerant to the tri-therapy. Those patients under tri-therapy who showed no improvement and who had developed opportunistic infections that were not observed before the chemical treatment, were included in the study.

CD4' were monitored at time one year before treatment (T-1) and at the initiation of the treatment (T0) but this monitoring could not be pursued. Weight and total lymphocyte numbers were monitored one year before treatment (T-1), at time 0 (T0), 3 months (T3) and 12 months (T12) after initiation of food supplement treatment. Treatment was stopped at T12.

The food supplement was approved by the Belgian Ministry of Health on June 23 2009 and does not require the approval of an Ethics committee for its use.

Results

Table 1 presents results of fourteen patients who were under conventional tri-therapy for about six months before addition of the food supplements to the treatment. Their state of illness was advanced. The regimen was given according to the medical imperatives defined by the treating physician. Three very ill patients under tri-therapy who died either at the beginning of the treatment or within the first three months of treatment were omitted because treatment with food supplements is a long-term process that could not have been effective at their stage of the illness. Table 2 shows the results of 46 AIDS people who were without specific medical treatment. Their state of illness was less severe than the patients under tri-therapy. The results obtained by patients under tri-therapy are presented at some length because these results are most interesting.

Patients under tri-therapy

P.P 80, 2Leid and P.I. and tri-therapy

The first four patients (Nbs. 1 to 4) were in a critical physical condition at time zero (T0) and received the combination [P.P 80 + 2Leid + P.I. + tri-therapy]. Patient 1 was a 19-year old girl. She weighed 30 kg at the beginning of the treatment (T0) and the number of CD4 cells had collapsed down to 6. Evidently, she did not benefit from the tri-therapy and seemed doomed. In three months of food supplements intake (T3), she gained 15 kg and her number of total lymphocytes increased from 212 to 2915. She was in good health at T12, after 12 months of the additional alternative treatment.

The tri-therapy followed from time T-1 to T0 appeared more beneficial to patients 2 to 4 because they maintained or even slightly increased in weight during that time and had retained their CD4 and lymphocyte levels, albeit in very low numbers. The food supplements [P.P 80 + 2Leid + P.I. and tri-therapy] caused a dramatic gain in weight. More importantly, there was a spectacular increase in the number of their total lymphocytes. All were in a satisfactory state of health after 12 months of food supplements intake.

P.P.80, 2Leid and tri-therapy

Patients 5 to 8 received only P.P.80 + 2Leid, in addition to the chemicals. They were in a very poor state of health before starting food supplementation: severe drops in CD4 counts, except in patient 8, low weight and low lymphocyte count. The tri-therapy had proven ineffective. After three months of food supplement intake [P.P.80 + 2Leid + tri-therapy], lymphocyte counts increased dramatically and 3 out of 4 patients had a significant gain in weight. Patient 6 also gained weight after 12 months of alternative therapy and all were in a satisfactory state of health at that moment (T12).

PP 80 and tri-therapy

Patients 9 to 12 received P.P.80 in addition to the tri-therapy. Patients 9 and 10 were clinically speaking in a "stable state", with relatively high CD4 and lymphocyte counts at time T0. The weight of patient 9 was not measured before the start of the study, but patient 10 had a stable weight. By contrast, patients 11 and 12 had low CD4 counts despite tri-therapy and had lost weight. The tri-therapy had proven inefficient. Three months after they started to take the P.P.80 food supplement, their weight and their lymphocyte counts had increased. After twelve months of treatment with the food supplement, their health was good.

PP20, Para Immuno and tri-therapy

This regimen was given to patient 13, a 15-years old girl weighing 24 kg at the time of her admission in the orphans' home, under chemo-therapy but with diarrhea, fever and pneumopathy and having lost ¾ of her CD4's during chemical treatment, recovered when P.P.20 and P.I. was given during three months. The use of P.P.20 instead of P.P.80, a 4-fold lower dose in active principles, resulted in a similar weight gain and lymphocyte count recovery as with the previous 12 patients, indicating that this lower dosage was just as efficacious, at least in a teenager.

Patient 14, under tri-therapy and receiving only 2Leid as food supplement had a moderate CD4 cell count but was losing weight before joining the study. Three months later, she had not only recovered but gained weight and her lymphocyte count had

Table 1
Fourteen AIDS patients under conventional tri-therapy. These patients were advanced in disease, with only #9 and #10 in a conserved state. For this reason, they were chemically-treated but the treatment sometimes worsened their condition, shown by a loss in weight (e.g. #2, 11, and 14, or else a status quo: e.g. #5, #6, #10). Some underwent a severe loss of CD4 during the treatment: e.g. #6, #7, #11, #12). All gained in lymphocytes numbers during food supplement treatment.

	Patient			Treatment			Symptoms	Weight				Viral L	CD4		Lymphocytes		
N°	File	Age	Sex	PP	2Leid	P.I.	T0	T-1	T0	T3	T12	T-1	T-1	T0	T0	T3	T12
1	LB 42	19	F	80	yes	yes	3, 5, 16, 17		30	45	51			6	212	2915	3600
2	LA02	26	F	80	yes	yes	3,6,5	55	50	62	69	120000	84	80	131	2760	
3	LA49	34	F	80	yes	yes	very bad	46	51	62	70	125000	25	24	802	2615	
4	LA50	36	F	80	yes	yes	very bad	56	62	72	83	125000	62	56	904	2464	
5	LA18	20	F	80	yes		3,4, very bad	44	45	60	80	140000	34	2	151	1768	2916
6	LB41	20	M	80	yes		3,4, very bad	60	60	61	66	0	611	90	402	1915	2690
7	LA32	32	F	80	yes		3,4, very bad	44	46	56	60	98650	245	40	806	2900	3050
8	LA35	37	F	80	yes		3,4, very bad	47	55	72	79	150000	16	127	679	2464	
9	LB33	12	F	80			Conserved		30	32	53			191	720	1920	4500
10	La15	21	F	80			conserved state	59	60	65	76	20000	200	204	1504	2115	3915
11	LA06	30	F	80			2,3, 5, 6	46	40	53	67	120000	95	59	300	1584	3900
12	LA23	31	F	80			3, 13,6,5,	55	50	66	75	135000	50	60	800	2160	3916
13	LA31	15	F	20		yes	3, 6, 5	24	26	33	40	0	435	100	802	2005	3050
14	LA07	23	F		yes		6, 3, 11	45	40	52	64	10 000	300		1401	2500	3900

Viral L: viral load.
Treatments: PP: para aspido, 20 or 80 units; 2Leid: cytokines and nucleic acids; PI: royal jelly, propolis, pollen. Tritherapy: stavudine, lamivudine, nevirapine
Symptoms: 1. congenital; 2. adenopathy; 3. diarrhea; 4. dermatitis; 5. pneumopathy; 6. hyperthermy; 7. Inappetence; 8. shingles; 9. otitis; 10. pharyngitis; 11. coughing; 12. psychomotor delay; 13. lymphadenopathy; 14. candidiasis; 15. vomiting; 16. hepatitis; 17. sarcoma.

doubled. These results are comparable to those observed in the other 13 patients.

Patients without tri-therapy

A larger number of patients (Table 2: Nb 15 to 60) received no tri-therapy. The clinician defined their health status at time zero by qualitative criteria (conserved, good, bad etc.) and took note of opportunist infections. The loss of weight in these AIDS patients from the moment of their admission in the orphans' home (T-1) to time zero (T0), *i.e.* a year later when food supplementation started, is an objective sign of their evolution, as is their viral load and the level of their CD4'. Some of them, especially young children, gained weight and increased the number of CD4 while others saw a decline in their CD4 between T-1 and T0, but only two of them (Nbs 29 and 41) had CD4 levels below the 100 mark at time T0.

Table 2 shows that, irrespective of the severity of their illness and the worsening of their physical condition during the year that preceded supplement treatment, all recovered and gained weight in addition to increasing their total lymphocyte count. All were in satisfactory or good health at the end of the twelve-month course of food-treatment. If the effects of this treatment are visible after three months in most of the treated AIDS people, some however needed more than three months of food supplement intake before showing clear evidence of recovering health (Nbs 21, 27, 56) even if an increase in numbers of total lymphocytes took already place at time T3.

Discussion

During the whole year of treatment with natural products, we deplored no collateral secondary effects except two patients on PP80, who signalled a nose blooding that disappeared after the end of the treatment. This evidence of innocuity corroborates the results obtained in animal studies (Federlin et al., 2014).

All applied protocols proved efficacious. The uleine boosts the synthesis of nitric oxide, the ribonucleotides stimulate the multiplication of lymphocytes and the Para Immuno (propolis, Royal jelly and pollen) stimulates the immune system. A combination of all three works best in as much that every facet of the global treatment was exploited. However, the single application of uleine to non-treated patients, be it at its highest or lowest concentration, yielded remarkable results by itself. Patients 26, 27 and 28, who were given PP80, showed a substantial improvement of their condition, at a par with that observed when complete regimens were given. This observation is valid also for patients 47 to 51, who received PP20: their condition improved at the same rate as patients handled with the two additional food supplements. The chemically unattended patients were generally in a better condition (i.e. said "conserved") than those who were under treatment. In this group, patients 9, 10, 11 and 12 gained no weight during chemical treatment or else lost weight. Yet, PP80 given alone to them in addition to tri-therapy improved considerably their health. An explanation for this remarkable effect may be found in the fact that uleine also interferes with the inflammatory response (Nardin et al., 2008) and regulates the immune system (Nardin et al., 2010). The substance possesses thus properties shared by the two other regimen proposed. One must note here that a reduction in the number of pills taken, and in the frequency of their administration, must be favoured in all cases not only for reasons of cost and burden but because it plays a considerable positive role on the psychology of the patients.

The progress registered initially on the clinical level by nearly all patients receiving food supplements was confirmed by a significant increase of total lymphocytes numbers and weight increase from the 3th month on. Patients under tri-therapy progressed most obviously at the level of secondary effects and opportunistic infections. In 2006, at the end of the supplement treatment, we registered no case of diarrhoea, no case of hyperthermia, no case of dermatose or other opportunistic illnesses. We had only three deaths by pneumocytosis, meningitis and bacterial pneumopathy but these were very advanced cases at the beginning of the treatment.

The patient under tri-therapy were given food supplements when they showed no improvement and/or developed opportunistic diseases that did not exist before the treatment with the anti-retrovirals. A tri-therapy based on stavudine, lamivudine and nevirapine is a heavy treatment that often causes secondary effects in the patient and can be a factor of health degradation in many treated subjects. Our results indicate that tri-therapy worsens the health of some AIDS patients instead of improving it, while taking food supplements not only does not cause the same negative effects but can help the patients get over those produced by conventional therapy and even cause partial recovery of AIDS patients not receiving it.

Table 2
46 patients receiving no standard chemical therapy. Some patients were with opportunistic diseases but a sizable number were in good state or "conserved". All of them saw the number of their lymphocytes increase during treatment.

	Patient			Treatment			Symptoms	Weight				Viral L.	CD4		lymphocytes		
N°	File	Age	Sex	P.P.	2Leid	P.I.	T0	T-1	T0	T3	T12	T-1	T-1	T0	T0	T3	T12
15	LB52	15	F	80	yes	yes	3, 6, bad	45	30	35	45	0	610		924	1802	2720
16	LC03	17	F	80	yes	yes	conserved		44	54	60				1121	3915	
17	LB64	19	F	80	yes	yes	conserved		58	69	73				1215	2280	
18	LB60	20	F	80	yes	yes	good state	59	50	60	65	0	488		1320	3916	4200
19	LA21	23	F	80	yes	yes	good state	51	54	60	66	75000	95	420	1420	2850	3915
20	LA36	31	F	80	yes	yes	good state	60	65	73	81	0	500	318	1412	4080	
21	LC02	35	F	80	yes	yes	3,6, 14, bad		40	42	76				902		
22	LB23	20	F	80	yes		conserved	60	65	70	74	6000	296	315	1390	2500	3920
23	LA20	21	F	80	yes		+/-' good	48	52	58	64	122000	50	105	1240	1539	2620
24	LA39	35	F	80	yes		conserved	40	45	65	75	120000	25	312	1219	2900	3620
25	LC04		M	80	yes		3, 6, 17		50	60	65				502	2108	
26	LB30	20	F	80			conserved	35	50	61	66	10000	205	310	1311	2530	3910
27	LB19	20	F	80			good state	60	65	66	75	0	391	320	1301	1320	3900
28	LA33	30	F	80			conserved	46	50	60	65	100050	250	360	1250	2500	3200
29	LA13	3	F	20	yes	yes	3, 6, 5, 12	6	7	12	18	130 000	80	60	1201	2100	4610
30	LA45	3	F	20	yes	yes	good state	7	12	17	23	125000	25	391	1210	4608	5105
31	LA26	9	M	20	yes	yes	good state	14	16	20	36	0	420	402	1240	2100	4112
32	LA08	10	F	20	yes	yes	6, 3, 7, 4, 9	21	21	30	46	6 000	300	277	1314	3915	4904
33	LA44	10	F	20	yes	yes	good state	14	20	30	37	125000	85	420	1311	3016	3620
34	LA05	12	F	20	yes	yes	1, 6, 3, 8	28	29	35	51	12 000	250	277	1360	3005	4915
35	LA09	12	F	20	yes	yes	1, 9, 4	25	25	38	45	0	350	244	1901	2615	3916
36	LB53	13	M	20	yes	yes	very bad		13	20	25				612	3360	4600
37	LA04	14	F	20	yes	yes	5, 8	31	28	35	43	.50.000	140	130	1211	2900	4190
38	LA40	14	F	20	yes	yes	good state	16	20	30	46	53500	250	305	1229	3050	4215
39	LA01	11	M	20		yes	2,3, 4, 5, 10	26	24	30	35	80 500	120	100	711	2110	
40	LB46	21	F	20		yes	conserved		59	65	75			226	1247	3024	4500
41	LA27	4	M	20	yes		good	11	11	15	21	0	500	305	1321	1910	2915
43	LA41	10	M	20	yes		good	16	20	26	40	25000	350	315	1319	3600	4250
44	LA17	11	M	20	yes		+/-' good	15	20	25	35	130000	100	60	405	1120	2905
45	LA30	13	M	20	yes		conserved	19	21	31	36	0	600	372	1120	2820	3912
46	LA03	15	F	20	yes		3,7	45	41	50	60	110 000	91	300	1707	2910	4210
47	LA43	9	F	20			good	13	20	25	36	15000	350	416	1620	2600	3950
48	LA29	11	M	20			conserved	24	25	30	36	0	480	450	1200	2912	4045
49	LB35	12	F	20			conserved	19	22	29	35	0	412	383	827	1460	2920
50	LB32	13	F	20			Conserved	30	32	40	45	5000	490	259	561	1768	2900
51	LB13	19	M	20			good state	60	62	75	79	0	526	620	1302	4901	
52	LA10	21	F		yes	yes		60	55	61	72	30 000	200		1500	2500	4600
53	LB04	3	F		yes		3	12	12	15	26	0	710		1400	3500	
54	LB02	4	F		yes		3,6, delayed growth	12	13	18	24	0	420		1830	2930	
55	LB17	5	F		yes		3, 15, 14	18	18	25	30	0	518		1150	3900	4600
56	LB08	7	F		yes		6,14	18	18	25	37	0	520		1200	4500	
57	LB21	21	F		yes		3, 6, 7	60	42	45	50	0	620		1950	2500	3600
58	LA25	25	F		yes		3,4,14	44	45	60	65	10000	360		1300	3900	4115
59	LA22	26			yes		conserved state	55	50	61	67	8000	300		1200	2700	4105
60	LA47	33	F		yes		3, 6, 5 tuberc.	52	51	61	71	5500	350		1250	2950	3920
	LA38	33	M		yes		conserved state	46	46	55	66	60000	300		1220	2915	2400

Viral L: viral load.
Treatments: PP: para aspido, 20 or 80 units; 2Leid: cytokines and nucleic acids; PI: royal jelly, propolis, pollen. Tritherapy: stavudine, lamivudine, nevirapine
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Authors' contributions

DM contributed in the fabrication of the food supplements, delivery and follow up; and RM analysed the raw data, exploited them and wrote the manuscript. Both authors have read and approved the paper for submission.

Conflict of interest

All authors have none to declare

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References

Beljanski, M., Chaumont, L., Bonissol, C., 1975. RNA fragments, in vivo inhibitors of Shope fibroma and vaccinia virus multiplication. C. R. Acad. Sci. Hebd. Seances Acad. Sci. D. 280, 783–789.
Beljanski, M., Plawecki, M., 1979. Particular RNA fragments as promoters of leukocyte and platelet formation in rabbits. Exp. Cell Biol. 47, 218–225.
Beljanski, M., Plawecki, M., Bourgarel, P., Beljanski, M., 1983. Leukocyte recovery with short-chain RNA fragments in cyclophosphamide-treated rabbits. Cancer Treat. Rep. 67, 611–619.
Bouillon, C., Ndayikunda, C., 2006. Traitement complémentaire du sida par un apport naturel et traditionnel. Chalets du bonheur a.s.b. l – Belgique, 56 p (Document is available by the corresponding author.
Donadio, D., Lorho, R., Causse, J.E., Nawrocki, T., Beljanski, M., 1991. RNA fragments and tolerance of cytostatic treatments in haematology: a preliminary study about two non-Hodgkin malignant lymphoma cases. Deutsch. Zschr. Onkol. 23, 33–35.
Federlin, J.D., Maes, D., Maes, R., 2014. Aspidosperma subincanum I. Characterisation, extraction of an uleine-enriched fraction and potential health hazard due to the contaminant ellipticine. Rev. Bras. Farmacogn. 24, 293–297.

Nardin, J.M., Lima, M.P., Machado, G.C., Hilst, L.F., Santos, C.A.M., Weffort-Santos, A.M., 2010. The uleine-rich fraction of *Himathantus lancifolius* blocks proliferative responses of human lymphoid cells. Planta Med. 76, 697–700.

Nardin, J.M., de Souza, W.M., Lopes, J.F., Florão, A., de Moraes Santos, C.A., Weffort-Santos, A.M., 2008. Effects of Himatanthus lancifolius on human leukocyte chemotaxis and their adhesion to integrins. Planta Med. 74, 1253–1258.

Seidl, C., Correia, B.L., Stinghen, A.E.M., Santos, C.A.M., 2010. Acetylcholinesterase inhibitory activity of uleine from *Himatanthus lancifolius*. Z. Naturforsch C 65, 440–444.

Souza, W.M., Brehmer, F., Nakao, L.S., Stinghen, A.E.M., Santos, C.A.M., 2007. Ação da uleína sobre a produção de óxido nítrico em células RAEC e B16F10. Rev. Bras. Farmacogn. 17, 191–196.