

## L-methionine as immune supportive supplement: a clinical evaluation

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**Summary.** The objective of the study was to test L-methionine as a possible immune supportive supplement in HIV infected patients by means of a clinical study. A double-blind, placebo-controlled study was designed. The patients ( $n=253$ ) from four different trial centres were randomly divided into two groups, active and placebo, and regularly assessed by clinical and safety parameters. After six months from commencement, clinically and statistically significant differences were observed. The females of the active treatment group presented with a decreased level of decline in their CD4 counts ( $p=0.0027$ ), so also the patients of Centre 1 ( $p=0.0377$ ). All patients were placed onto active treatment after 12 months and were followed up for 48 months after the trial started. The same tendencies could be observed in the group as a whole, with no serious side effects directly associated to treatment. The study confirmed the supportive role of L-methionine in immune-compromised or deficient patients.

**Keywords:** L-methionine – Immune support – Supplement – HIV

### Introduction

L-methionine is an essential amino acid and the initial metabolite in processes including, transmethylation and trans-sulphuration (Finkelstein, 1990). S-adenosyl-L-methionine (SAM) is the product of methionine adenylation and can provide a methyl group to a variety of substances (Mudd et al., 1995). This methylation plays an important role in, amongst others, the immune system. In certain genetic cases of severe combined immunodeficiencies (SCID), the mechanism could partly be due to a lack of methylation capacity (Hershfield and Mitchell, 1995). In a deficiency of SAM or a low ratio of SAM with respect to S-adenosyl-homocysteine (SAH), a T-cell deficiency and in more severe cases a combined (T- and B-cells) immunodeficiency can develop (Hershfield and Mitchell, 1995; Surtees et al., 1990). Supplementation of L-methionine, significantly increases circulating SAM levels in humans (Lagendijk, 1992) and subsequently the overall methylation capacity.

Glutathione, a metabolic product of L-methionine, has been found to be important as a mediator of normal immune responsiveness and to have antiviral activity (Ho and Douglas, 1992; Kalebic et al., 1991; Palamara et al., 1996). Absorption of oral glutathione is poor (Witschi et al., 1992), probably due to hydrolysis of glutathione by intestinal and hepatic glutamyltransferase. Supplementation of L-methionine (and L-cysteine) has, however, been shown to increase intracellular glutathione by as much as two fold (Wang et al., 1997).

Glutathione levels are decreased in HIV patients (Buhl et al., 1989; Buhl, 1994), and play an important role in the regulation of their immune system (Skurnick et al., 1996; Delmas-Beauvieux et al., 1996; Barbaro et al., 1996; Aukrust et al., 1996). Low plasma glutathione levels in children with HIV were found to be associated with low CD4 cell count values and increased viral loads (Rodriguez et al., 1998). Herzenberg even implicates glutathione levels as predictive of survival in HIV patients (Herzenberg et al., 1997). Results suggest that glutathione could inhibit the reverse transcriptase process of HIV-1 type 1 and so directly influence virus levels (Kameoka et al., 1996; Sen and Packer, 1996). Glutathione was effective in reducing the proviral DNA load in the first period of infection in murine AIDS (Palamara et al., 1996; Rossi et al., 1996). It could also be shown that exogenous glutathione strongly suppresses the production of p24gag protein, as well as the virus infectivity (Palamara et al., 1996).

Depletion of glutathione also leads to methionine depletion, which damages the methylation processes (Lertratanangkoon et al., 1996). This was confirmed by a study which indicated low concentrations of

methionine in the plasma of HIV infected patients (Muller et al., 1996).

Elevated homocysteine levels, an intermediate of methionine metabolism, might contribute to the risk of coronary heart disease (Ueland and Refsum, 1989; Ubbink et al., 1991). Vitamin supplementation (containing vitamin B6, folic acid and vitamin B12) normalise elevated circulating homocysteine levels (Ubbink et al., 1993; Brattstrom et al., 1988). Magnesium is also an essential cofactor for the enzyme methionine adenosyl transferase. In the study reported here, the effect of L-methionine supplementation in combination with the cofactors; vitamin B6, vitamin B12, folic acid and magnesium was investigated. Specifically, the possible beneficial effect of this combination on the immune system of immune compromised or deficient patients, was researched.

## Materials and methods

### Patients

The study was conducted in accordance with good clinical practice (GCP) as laid out in the "Good Clinical Practice for Trials on Medical Products in the European Community" (GPMP) guidelines. Compliance with these requirements also constitutes conformity with the ethical principles of the Declaration of Helsinki. The clinical study protocol, informed consent document(s), and any other appropriate study-related documents were reviewed and approved by MASA Research Ethics Committee (MREC), Clindepharm International Pharma-Ethics ethical committee, as well as the Research Protocol and Ethics Committee of Pretoria Academic Hospital and Tshwane University of Technology Ethics Committee. Informed consent was obtained prior to the conduct of any study-related procedures.

The population ( $n = 253$ ) were HIV positive patients with a CD4 count of between 200 and 500 cmm. They were divided into Group 1 ( $n = 124$ ) and Group 2 ( $n = 129$ ). Patients were from Gauteng and included private and state patients from four different centres; Centre 1 ( $n = 57$ ), Centre 2 ( $n = 88$ ), Centre 3 ( $n = 31$ ) and Centre 4 ( $n = 77$ ). The average age was 34.5 years with 142 male patients and 111 female patients.

The inclusion criteria were:

- Age > 18 years
- Laboratory confirmation of HIV positivity
- CD4 count at entry <500 and >200 cmm
- Prophylactic treatment of any possible secondary problem
- Patients giving their written consent and agreeing to the described protocol.

The exclusion criteria were:

- Pregnancy
- Women who refuse to use contraception
- Underlying/concomitant renal disease
- Concomitant significant hepatic disease (liver enzymes >3 times upper limit of normal)
- Life expectancy on clinical grounds <6 months
- Known inborn error(s) of metabolism
- Concomitant use of other N-acetyl cysteine (NAC) or glutathione products
- Hemoglobin < 8 g/dl, WCC < 750/ml at entry

- Concomitant use of corticosteroids or other potentially immunosuppressive agents, e.g. adriamycin, atoposide, doxorubicin, cyclophosphamide
- Concomitant use of interferon, or any other immunological agents
- Any patient who, on clinical grounds, needed or would, within the next six months, need other antiviral treatment.

### Experimental design

The trial was a randomised, double blind, placebo controlled study. For six weeks, prior to the start of the trial, the patients were not allowed to receive any other antiviral or HIV treatment. Treatment was given over a period of 48 months.

All patients ( $n = 253$ ), received three colourless capsules, filled with fine, cream to pale yellow powder and one cherry red, biconvex, round, film-coated tablet twice daily on an empty stomach, i.e. every morning and evening for 48 months. Group 1 ( $n = 124$ ) received a placebo (Treatment 1) for the first 12 months of the study period and then started to take the active treatment (Treatment 2) as from month 12 for the remainder of the trial. Group 2 ( $n = 129$ ) received the active treatment (Treatment 2) for the full 48 months.

Treatment 1	
Capsule content:	
Starch	601 mg
Magnesium carbonate	18 mg
Treatment 2	
Capsule content:	
L-methionine	467.5 mg
Vitamin B6	1.8 mg
Vitamin B12	0.036 mg
Folic acid	1.44 mg
Tablet content:	
Magnesium chloride	535 mg

Adverse events were listed on an individual basis, and summarised by the body system.

## Results

A total of 162 patients, 64% ( $n = 92$ ) of Group 1 and 60.5% ( $n = 70$ ) of Group 2 withdrew before the end of the 48-month study period mainly as a result of not keeping scheduled appointments. One patient withdrew because of an adverse event and two patients fell pregnant. Three patients died during the 48-month study period: One patient (Group 2) died of respiratory failure and one patient (Group 1) died of pneumocystic pneumonia. The reason for death of the third patient (Group 2) is unknown.

No overt differences were observed between the two groups with regard to the safety variables except for above mentioned events. In general, there were no relevant changes in the clinical chemistry variables. One-hundred-and-seventeen LPCAs (last evaluation, pre-defined change abnormal) occurred at 48 months, 53 in Group 1 and 64 in Group 2.

With regard to the CD4 cell count, the two treatment groups compared well at baseline (Entry), with mean CD4

**Table 1.** Difference in change of CD4 count in female patients (Group 2 – Group 1)

Variable	Stats	Base	Change 6 weeks-base	Change 3 months-base	Change 6 months-base	Change 12 months-base	Change 18 months-base	Change 24 months-base	Change 36 months-base	Change 48 months-base
Group 2	N	59	54	51	45	35	20	18	9	5
	Mean	338.9	-8.8	-6.8	-8.5	27.6	14.9	6.8	10.7	55.6
	SD	109.4	69.3	72.6	65.1	102.0	126.1	110.0	154.0	222.1
*Group 1	N	52	37	34	24	19	15	12	6	4
	Mean	359.6	-19.5	-35.7	-67.9	-6.6	84.7	-25.8	90.1	96.9
	SD	99.3	87.0	62.3	91.9	124.7	187.6	92.1	177.4	239.5
<i>p</i> -value			0.5166	0.0604	0.0027	0.2817	0.1964	0.4054	0.3735	0.7965
Diff			10.69	28.96	59.43	34.21	-69.88	32.53	-79.36	-41.28
95% Conf			-21.93 to 43.32	-1.29 to 59.20	21.39 to 97.46	-28.90 to 97.31	-177.7 to 37.95	-46.34 to 111.40	-265.4 to 106.65	-405.7 to 323.13

*p*-value (ANCOVA); 95% confidence interval. Difference in change from baseline (Group 2 – Group 1); \* Group 1 received active treatment after 12 months

**Table 2.** Difference in change of CD4 count in patients of Centre 1 (Group 2 – Group 1)

Variable	Stats	Base	Change 6 weeks-base	Change 3 months-base	Change 6 months-base	Change 12 months-base	Change 18 months-base	Change 24 months-base	Change 48 months-base
Group 2	N	29	29	24	18	16	13	11	3
	Mean	343.0	-17.8	-15.7	-13.7	46.0	20.1	-23.9	-62.7
	SD	123.2	60.8	78.1	41.2	94.1	106.8	99.0	63.5
*Group 1	N	28	19	20	16	12	10	10	3
	Mean	378.5	15.2	-27.8	-58.8	1.9	12.1	-52.7	87.5
	SD	92.6	106.7	83.5	76.8	69.0	174.3	61.0	74.8
<i>p</i> -value			0.1779	0.6230	0.0377	0.1822	0.8929	0.4383	0.0570
Diff			-33.06	12.09	45.08	44.16	8.02	28.79	-150.2
95% Conf			-81.71 to 15.58	-37.17 to 61.36	2.72 to 87.45	-22.07 to 110.38	-114.3 to 130.32	-47.32 to 104.90	7.5 to 7.17

*p*-value (ANCOVA); 95% confidence interval. Difference in change from baseline (Group 2 – Group 1); \* Group 1 received active treatment after 12 months

**Table 3.** Difference in change of CD4 count in all patients (Group 2 – Group 1)

Variable	Stats	Base	Change 6 weeks-base	Change 3 months-base	Change 6 months-base	Change 12 months-base	Change 18 months-base	Change 24 months-base	Change 36 months-base	Change 48 months-base
Group 2	N	129	115	119	119	119	119	119	119	119
	Mean	340.3	-8.9	-7.6	-8.0	7.2	5.9	-10.5	-4.2	1.1
	SD	98.5	66.2	73.4	69.6	92.5	106.4	105.2	119.0	118.2
*Group 1	N	124	97	102	102	102	102	102	102	102
	Mean	342.6	-12.4	-19.2	-26.6	-6.1	6.1	-6.6	3.9	13.8
	SD	91.5	82.9	74.4	103.4	112.9	134.2	121.2	146.5	148.0
<i>p</i> -value			0.7335	0.2450	0.1141	0.3387	0.9877	0.7992	0.6512	0.4796
Diff			3.49	11.62	18.58	13.25	-0.25	-3.88	-8.09	-12.69
95% Conf			-16.70 to 23.68	-8.02 to 31.26	-4.50 to 41.66	-13.98 to 40.49	-32.17 to 31.67	-33.90 to 26.14	43.30 to 27.12	-48.00 to 22.62

*p*-value (ANCOVA); 95% confidence interval. Difference in change from baseline (Group 2 – Group 1); \* Group 1 received active treatment after 12 months

**Table 4.** Difference in change of total lymphocyte count in all patients (Group 2 – Group 1)

Variable	Stats	Base	Change 6 weeks-base	Change 3 months-base	Change 6 months-base	Change 12 months-base	Change 18 months-base	Change 24 months-base	Change 36 months-base	Change 48 months-base
Group 2	N	129	115	119	119	119	119	119	119	119
	Mean	1789.5	-30.3	40.2	28.1	161.7	191.2	140.6	140.3	107.5
	SD	632.4	434.3	405.9	427.9	575.7	600.5	580.6	586.5	669.8
*Group 1	N	124	97	102	102	102	102	102	102	102
	Mean	1782.2	-111.4	-112.8	-143.3	-3.4	47.0	9.0	14.5	-11.4
	SD	545.5	459.2	516.5	523.0	585.9	612.1	610.6	612.9	664.1
<i>p</i> -value			0.1883	0.0145	0.0079	0.0362	0.0791	0.1024	0.1207	0.1881
Diff			81.14	152.96	171.40	165.10	144.23	131.62	125.86	118.87
95% Conf			-40.04 to 202.31	30.57 to 275.36	45.31 to 297.49	10.74 to 319.45	-16.89 to 305.35	-26.51 to 289.76	-33.38 to 285.09	-58.56 to 296.31

*p*-value (ANCOVA); 95% confidence interval. Difference in change from baseline (Group 2 – Group 1); \* Group 1 received active treatment after 12 months

**Table 5.** Other secondary variables for 12 months

	Group 1			Group 2		
	Before (entry)	After (12 months)	Change ('after' – 'before')	Before (entry)	After (12 months)	Change ('after' – 'before')
CD4 cell percentage (%)	20.5	19.2	–1.3	20.6	19.5	–0.9
CD8 cell count	1047.0	1075.1	15.3	1035.8	1179.4	126.7
CD8 cell percentage (%)	58.3	57.0	–2.2	59.4	56.8	–3.0
CD4 + CD8 cell ratio	0.4	0.4	0.0	0.4	0.4	–0.1
Erythrocyte count (/pl)	4.9	4.6	–0.3	5.0	4.6	–0.4
Haemoglobin (g/dl)	14.3	13.5	0.2	14.4	13.5	–1.0
Platelet count (/nl)	218.9	215.9	–2.8	222.9	220.8	–0.9
Leukocyte count (/nl)	4.7	4.9	0.4	4.06	4.8	0.2
Weight (kg)	69.8	70.4	0.7	67.9	69.1	0.8

cell counts of 342.6 and 340.3 cmm for Groups 1 and 2 respectively.

Clinical and statistical significant differences were already found after six months in two subgroups; the female group ( $n = 111$ ) and Centre 1 ( $n = 57$ ). The decision to switch Group 1 from placebo (Treatment 1) to active treatment (Treatment 2) at the 12-month stage, as pre-defined in the protocol, was based on these results. The results of the other three centres and the male subgroup reflected the same tendency, but were not statistically significant.

Although there was a decrease in CD4 count of the females in both groups at six months, the decrease in Group 2 was significant less than the decrease in Group 1 ( $p = 0.0027$ , using no LOCF; last observation carried forward). The difference in change (Group 2 – Group 1) of 59.43 cmm (95% CI from 21.39 to 97.46), was of clinical importance, as pre-defined in the protocol (Table 1). Entry values were used as baseline in all figures. After six months, the Group 2 patients of Centre 1 showed a statistically significant ( $p = 0.0377$ ) smaller decrease in the CD4 count compared to the patients of Group 1, with a difference in change (Group 2 – Group 1) of 45.08 cmm with 95% CI from 2.72 to 87.43 (Table 2).

The mean of the total Group 2 at 12 months was 346.3 cmm and the mean of Group 1 was 336.8 cmm. Relative to the baseline (Entry), there was an increase in the CD4 cell count of Group 2, but a decrease in the CD4 cell count of Group 1. The difference in change from baseline (Group 2 – Group 1), was 13.25 cmm (ANCOVA: 13.1 cmm), with the 95% CI from –13.98 to 40.49 cmm (ANCOVA: –14.18 to 40.38 cmm), showing a tendency towards higher values in Group 2 (Table 3).

The mean of Group 2 regarding the total lymphocyte count at 12 months was 1972.2 cmm and the mean of

Group was 1794.6 cmm. Relative to baseline (Entry), there was an increase in the total lymphocyte count of Group 2 and a slight decrease in the total lymphocyte count of Group 1. The difference in change from baseline (Group 2 – Group 1) was 165.0 cmm ( $p = 0.0362$ ; 95% CI [10.74 cmm; 319.45 cmm]), indicating a statistically significant difference in the change between the two groups (Table 4). The changes seen in HIV levels were unfortunately not clinically or statistically significant.

The question “Compared to one year ago, how would you rate your health in general now?” had the following trend for the response “Much better than one year ago”: At entry, less than 6.2% of patients in Group 2 and 8.1% in Group 1 felt much better than one year ago.

At 12 months, 27.6% of the patients in Group 2 and only 18.3% in Group 1 reacted favourably. Group 1 received treatment from this time point onwards and after being on Treatment 2 for 12 months, 31% of patients in this group felt much better than 1 year ago, comparing well with Group 2 at 12 months.

After being on treatment for 24 months, 38.5% of patients in Group 2 and 34.8% Group 1 were feeling much better than one year ago. The trend continued until the final time point.

The results obtained for other secondary efficacy variables at 12 months were also recorded (Table 5).

## Discussion

The objective of this double blind placebo controlled study was to test the essential amino acid L-methionine as a possible immune supportive supplement, specifically by means of a clinical study in HIV infected and AIDS patients. This was the first time that L-methionine was considered for its possible immune supportive function.

Within six months of the study, clinically and statistically significant differences were observed, specifically the females on active treatment, which showed a statistically significant decreased level of decline in their CD4 counts ( $p = 0.0027$ ), so also the patients of Centre 1 subgroup ( $p = 0.0377$ ). As a result of this positive reaction and the ethical implications thereof, all patients were at the end of the 12-month period placed onto active treatment and followed up until the 48-month endpoint.

Although not all results were statistically significant, the same tendencies observed in these two subgroups could be seen in the group as a whole, thus a general improvement in CD4 count, total lymphocyte count, as well as general improvement in quality of life. After 12 months, the differences between the two groups, now both on active treatment, slowly diminished. This serves to confirm the positive results detected during the first 12 months, as the difference could have been expected to be decreased and/or eliminated after all patients were placed on active treatment. No serious side effects directly associated with treatment were observed. Although the active group showed a slightly higher incidence of adverse events compared to the placebo group, most of these were resolved within the treatment period, or were shown not to be related to the treatment.

This study confirmed the possible positive role of L-methionine in the supportive treatment of immune compromised or deficient patients. It presents a relatively safe and affordable option in the supportive treatment of these patients.

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