AIDS Bulletin

Immunomodulation in HIV/AIDS: The Tygerberg/Stellenbosch University experience

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Introduction

Since the initial reports of AIDS 15 years ago, our understanding of the disease process and the behaviour of the aetiological agent, the HI virus (HIV) have advanced substantially. Sensitive new tools are now available to monitor levels of HIV replication *in vivo* and have greatly illuminated the basic pathogenic mechanisms. These tools provide an effective means to guage risk of disease progression and to assess the efficacy of therapeutic regimens. Impressive advances in treatment have recently been realised with the development of more potent inhibitors of viral replication and, with the introduction of these antivirals, therapeutic strategies can be designed to accomplish lengthy and near complete suppression of virus replication in HIV-infected individuals.

Despite rapid advances and significant achievements in the area of antivirals, immune modulators which have been postulated since the early 1990s to provide therapeutic strategies that would reduce or prevent disease progression, remain the subject of philosophical editorials rather than of definitive efficacy studies.

We would like to share our experience with the use of phytosterols in a clinical trial which has been running at Tygerberg Hospital for the last 3 years. Our interest in the phytosterols arose from our original work on the extract from the Hypoxis plant (commonly called the African potato) which was being studied for its anecdotal anti-cancer properties. At the time, the patients participating on the cancer clinical trial were followed for any possible toxic side effects and immunological parameters were included in the panel of biochemical and cytological measurements made. To our surprise, many patients exhibited enhanced proliferative responses of their peripheral blood lymphocytes to mitogens *in vitro*. Testing of the purified sterols and sterolins showed that these plant fats were responsible for this enhanced lymphycytic activity.

Briefly, we showed that the sterols and sterolins could enhance the proliferation of T lymphocytes as well as the activity of NK cells *in vitro*. This observation was confirmed by conducting limited *in vivo* studies using healthy volunteers. It should however, be stated that the observed activity of the sterols/sterolins on T cells could not take place in the absence of a stimulus. In other words, the plant molecules could not induce a non-specific activation of the T cells. It was when we examined the secretion of cytokines in vitro that we realised the potential therapeutic application of the sterols/sterolins clinically: indeed, the sterols/sterolins mixture seem to enhance the TH_1 rather than the TH_2 or both arms of the T cell response. We showed that both the secretion of IL2 and IFN-y was greatly enhanced whereas the IL4 and IL6 secretion were in fact inhibited or untouched.

The dichotomy of the T cell response has been the focus of extensive research for the last 10 years following the observation of a separation of murine T helper cells into 2 distinct types according to the profile of cytokines secreted. The so-called TH_1 helper cells secrete IL2, IFN-*y* and IL12 whereas the TH_2 cells are characterised by their secretion of IL4, IL10, IL6 and others.In general, TH_1 cytokines promote cell-mediated responses and inhibit TH_2 activity (humoral activity) and vice versa. This dichotomy is not so clear cut in humans but it is generally assumed that it exists. It is for this reason that many authors have proposed the importance of this TH_1/TH_2 balance in diseases where it has been shown that a suboptimal TH_1 activity predominates, including HIV, Tuberculosis, and many others.

Results of pilot studies using the FIV model

We were able to obtain cats infected with the retrovirus FIV, which is considered the equivalent to HIV. The FIV induces the same pathogenic


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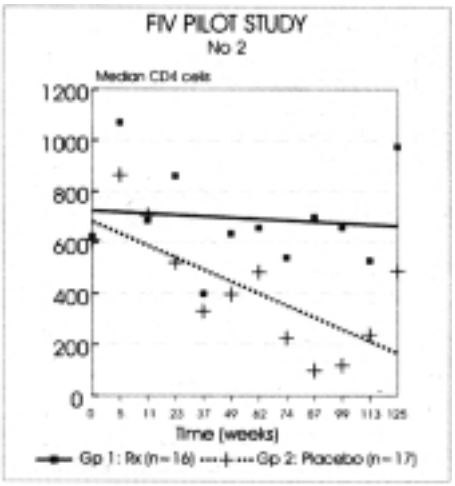
mechanisms (CD4 cell loss, opportunistic infections, etc.) and finally the infected cats succumb to the immunesuppressive state. This model was therefore appropriate for the study of the plant sterols/sterolins before its investigation in humans. The 5 cats were divided into 2 groups. One group received capsules containing the sterols/sterolins and the second group was not treated. The untreated cats died within 57 weeks whereas the treated cats are still alive and showing no signs of disease progression 168 weeks later. A second study was undertaken comprising a larger number of cats since we had no definite details of length of time since infection and this could have biased the observations. For the second study, we used a well-defined strain of the FIV, namely Petaluma. Group 1 (n=16) received active sterols/sterolins mixture and Group 2 (n=17) received placebo capsules containing the carrier. The CD4 cell numbers have been determined

over the last 168 weeks and the results (up to 125 weeks) are presented in Fig. 1. As seen, the regression line of the median

Figure 1



CD4 cell numbers of both group differ statistically and indicate that the treated cats exhibit stable CD4 cell numbers over this period whereas those of the untreated cats declined substantially. Also, more FIV-related deaths have occurred in the untreated group.

These results gave us the impetus to conduct a clinical trial in HIV-infected individuals for several reasons:

- The sterols/sterolins have been used clinically in Germany for the treatment of men with benign prostate hypertrophy (BPH) with no adverse or toxic side effects reported over the last 20 years.
- Sterols/sterolins had a favourable non-toxic profile in all the pre-registration studies conducted in Germany and since we knew that the doses used clinically were 5000 times less than those used in the pre-clinical studies we were reassured of their safety.
- Immunomodulation had become a reality for the treatment of HIV/AIDS patients since trials using recombinant IL2 were planned and initiated by the NIH. The initial results hold promise in the management of HIV/AIDS patients.
- In parallel, a clinical trial (double blind and placebo controlled) using the sterols/sterolins as adjuvant therapy in pulmonary tuberculosis patients showed that the treated patients derived more benefit when compared to their placebo-treated patients: higher weight gain, clearly rapid recovery from their tuberculosis, more rapid normalisation of the hematological parameters, etc.

For these reasons, permission was granted by the ethics committee of our university as well as that of the Medicines Control Council to conduct a trial in HIV-infected patients. This trial was open labelled and not placebo controlled (for ethical reasons). The exclusion criteria included pregnancy and children.

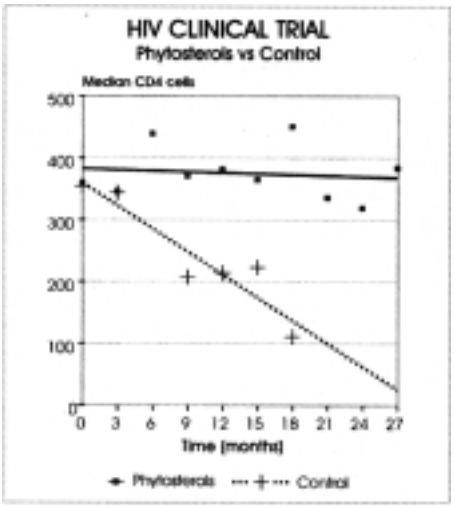
Results of HIV clinical trial using sterols/ sterolins

Eighty patients were entered on this clinical trial over 36 months with clinical follow up at 3-monthly intervals. Blood parameters include the routine CD4 cell determination, degree of apoptosis in the lymphocyte population, serum cytokine (measurement of the pro-inflammatory IL6 which was shown to activate viral replication in latently infected cells) and plasma RNA viral load as determined by quantitative PCR methods.

- **CD4 cell numbers with extended use of the sterols/sterolins:**

  To date, we have complete data for the last 27 months and several patients have been followed up for longer time periods (up to 37 months). Analysis of the CD4 cell numbers shows a stable median count for the group as a whole with no significant decrease in these cells over the 27 months. Fig. 2 shows the median CD4 cell numbers of this group of patients compared to a "control" group of patients who were followed at the Infectious Clinic at Tygerberg Hospital but who elected not to participate in the trial. The median CD4 cell numbers of these patients exhibited the classical decline over 18 months. (Fig. 2).

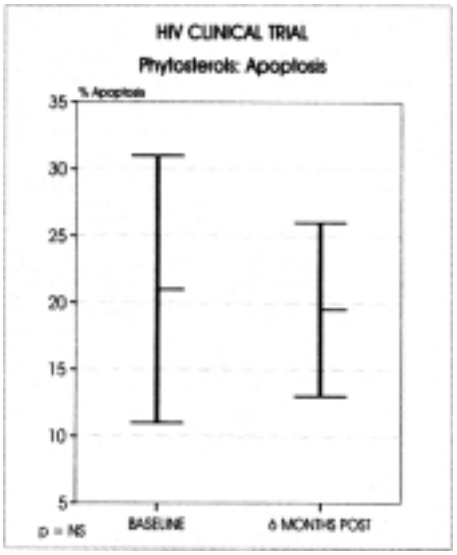
Figure 2



Analysis of the data indicates that there is no statistical difference between the baseline (entry) CD4 cell count and the same parameter at other time points

- **Measurement of apoptosis in lymphocyte population:**
  The measurement of apoptosis in the peripheral blood lymphocyte population in these patients participating on the trial over a 6-month period showed no significant change in this phenomenon, (Fig. 3)

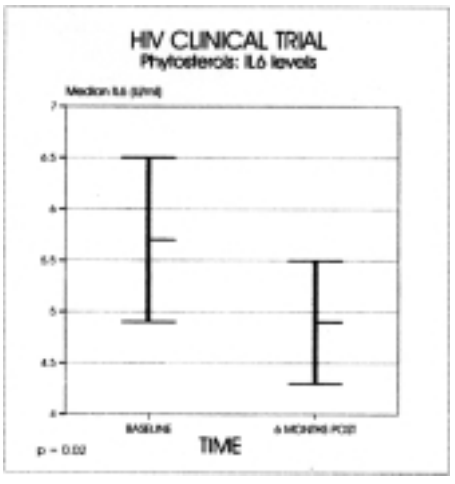
Figure 3



This programmed cell death has been proposed to account for the excessive CD4 cell loss in HIV-infected individuals and increases with disease progression. As shown in our patients, and although not statistically significant, the degree of apoptotic events in the blood of the patients showed a slight decline over the 6-month period.

- **Serum IL6 levels in trial patients:**
  This pro-inflammatory monokine has been implicated in the induction of viral replication in latently infected cells. It is for this reason that several authors have proposed the use of anti-oxidants to help inhibit this process especially in individuals who are co-infected with other pathogents which ultimately lead to the activation of the monocytic cells. As shown in Fig. 4, the median serum IL6 levels decreased significantly over a 6-months period.

Figure 4



- **Plasma viral RNA levels:**
  Considering that the sterols/sterolins do not have direct antiviral activities (tested *in vitro*) it is not surprising that the viral

loads of the patients did not drop after 3-6 months of sterol/sterolins intake. Analysis of the viral loads show that the mean decrease in the loads was 0.05log but that this change increased over a longer time period: -0.23log at 12 months. Unfortunately, we could not conduct viral loads on all the patients since this marker was introduced into the trial at a late stage (due to the availability of the commercial tests in SA) and hence we only have follow-up loads on 42 of the patients. Nevertheless, we feel confident that the sterols/sterolins would eventually lead to a decrease in the viral loads (indirectly via the immune system) but that this decrease would not be as dramatic as that induced with the current antivirals.

Discussion

The sterols/sterolins have been known since 1922 when they were first chemically identified and their structure elucidated. However, their only clinical use has been as a cholesterol lowering agent due to their similarity in structure. For the last 20 years the sponsoring company has been producing and exporting the sterols/sterolins to Germany where they are registered as a treatment for BPH. It is only since 1987 that work conducted at Tygerberg Hospital has conclusively shown that these plant fats have immunomodulatory activities.

We have subsequently shown that the sterols/sterolins can be used as adjuvants in the treatment of pulmonary tuberculosis patients and that their use in the management of HIV-infected patients holds promising results especially in patients who do not have easy access to registered anti-virals. During the recent visit to South Africa by Prof. Luc Montagnier when we had the honour of sharing our trial results with him, it was made quite clear to our research group that the sterols/sterolins hold an important place in the therapy of HIV-infected patients since it is an affordable, non toxic immunomodulator which could be used in conjunction with antivirals which could possibly be decreased in dosage to prevent side effects and costs.

Our results to date indicate that the immune cells of the patients are stabilised over an extended period of time (27 months and longer in certain cases) while the decrease in the serum levels of the pro-inflammatory monokines possible favours decreased viral replication. We are now awaiting the results of a new trial which will be initiated under the auspices of Prof. Montagnier in Paris whereby the patients will be treated (either pulse mode or continuously) with the sterols/sterolins together with lower doses of antivirals. In South Africa, new trials using immunomodulatory plant preparations (e.g. Mistletoe extract) and recombinant IL2 are currently being conducted. This shows that patient management has entered a new phase whereby antivirals will be used to decrease or even stop viral replication and these immunomodulators will be used to maintain or rebuild a failing immune system.

It seems incredible that the sterols/sterolins plant fats have such potent immunomodulatory activities in both humans and animals. But then, to think that these molecules are totally absent from our basic foodstuffs due to the refinement processes applied today (e.g. oils, maize, flour, etc.) makes one want to almost propose that our foods should be enriched with these amazing phytochemicals.

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