Sterols and sterolins: new drugs for the immune system?

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Since the discovery of glucocorticoids, we have had a single strategy for manipulating the immune system in cases of destructive diseases mediated by uncontrolled immune responses. However, long-term use of immunosuppressive drugs can lead to the threat of opportunistic infections and malignancies. As we learn more about regulatory subsets of T lymphocytes and their cytokine profiles, the thrust has been on developing new ligands that ultimately give us more site-specific control. Our group has developed a patented mixture of plant sterols and sterolins that has anti-inflammatory properties and profound immune modulating effects on subsets of CD4+ T cells. We have tested this mixture in several clinical entities and we believe that it has wide applications in reverting immune abnormalities.

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▼ Since the introduction of glucocorticoids into our armory of drugs for the control of immune responses, little has changed in the way we approach the management of chronic inflammatory conditions and allergic conditions [1]. The use of anti-inflammatory drugs inhibits the immune cells and the factors they secrete that perpetuate the inflammation. However, the lymphoid component remains relatively uncontrolled except with the use of immunosuppressive agents such as cortisone, azathioprin, methotrexate, and so on. The long-term use of such medication is not without risks: there exists the possibility of recurrent infections, gastrointestinal illness and even the risk of the tumour development. Yet, our knowledge of the immune cells and their various subsets has undergone an explosion in the past 10 years. We now know and understand how the immune response is controlled by delicate balances between two distinct subsets of $CD4^+$ lymphocytes, the Th1 versus Th2 subsets, based on their cytokine profile [2]. We have now begun to classify certain immune pathologies into categories depending on the phenotypic presence of these different cells. Armed with this knowledge, we can now target specific subsets and hopefully restore the delicate balance desperately required to bring relief to chronic conditions.

Plant sterols: new kids on the block

β-Sitosterol (BSS), the major plant sterol, was first described in 1922 and has only enjoyed biological importance as a natural approach to control cholesterol plasma levels [3]. The glucoside of sterols, especially β-sitosterol glucoside (BSSG), has had even less biological attention because of the presence of extremely low levels of these molecules in the plasma of humans [4]. For this reason, plant sterols have only been considered medically important because of their ability to lower cholesterol levels when ingested in gram quantities. Yet, the scientific literature is scattered with studies showing other important biological activities of these molecules.

For instance, it was reported that sterols fed to rabbits could inhibit the inflammatory response of the animals when induced by carrigenan (a synthetic compound used to induce inflammation in animal models) [5]. The same molecule was later described as having insulin-releasing properties in animal models of diabetes [6]. Their potential as cancer-fighting molecules was first described in epidemiological studies conducted in populations that consume diets rich in vegetables and fruits [7]. Such studies could not explain the mechanisms of action of these plant sterols.

In 1996, our group was the first to ascribe immunomodulating properties to these underestimated natural molecules (Fig. 1). We showed that a proprietary mixture of the sterols and sterolins (BSS–BSSG mixture) has the ability to enhance the cellular response of T lymphocytes both *in vitro* and *in vivo*. In the same study, we also showed that this mixture could

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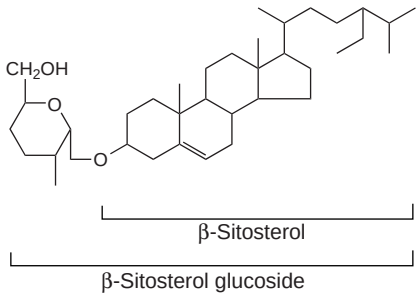

Figure 1. Molecular structures of β-sitosterol and β-sitosterol glucoside, the mixture reported to have immune-modulating properties.

enhance the cytotoxic ability of natural killer (NK) cells against the target cancer cell line NK562 [8]. Data from a representative experiment are shown in Fig. 2.

On examination of the profile of cytokines released by co-cultured T cells, the specificity of the sterols–sterolin mixture became clear: this mixture preferentially enhanced the activity of CD4 cells belonging to the Th1 phenotype. This implied that the BSS–BSSG mixture could be used to reinstate a balance between the TH1–Th2 cells, a delicate balance that determines the final outcome of an immune response. Often this balance is biased towards a Th2 phenotype with a resultant inefficient cellular response (as evidenced in chronic viral infections, including HIV infection [9]) and an overt humoral response (such as in allergic conditions and some autoimmune diseases). Simultaneously, it was shown that the same mixture could inhibit the release of pro-inflammatory cytokines from endotoxin-activated monocytes: interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) secretion could be inhibited by 98% at physiological concentrations [10]. Early reports from several authors had already shown that the extracts of leaves or other parts of plants had anti-inflammatory properties when tested in animal models, and some of these extracts compared favorably with hydrocortisone, a known anti-inflammatory compound [11,12]. These extracts, when analyzed, were found to be rich in the glucoside form of the sterol [11]. These studies did not provide any immunological mechanism for sterol mediated inhibition of inflammation. More recently, Navarro *et al.* showed that an acetone extract of *Sideritis foetens* Clem containing 61% BSS could inhibit neutrophil infiltration into tissues, but had no significant activity on mast degranulation [13].

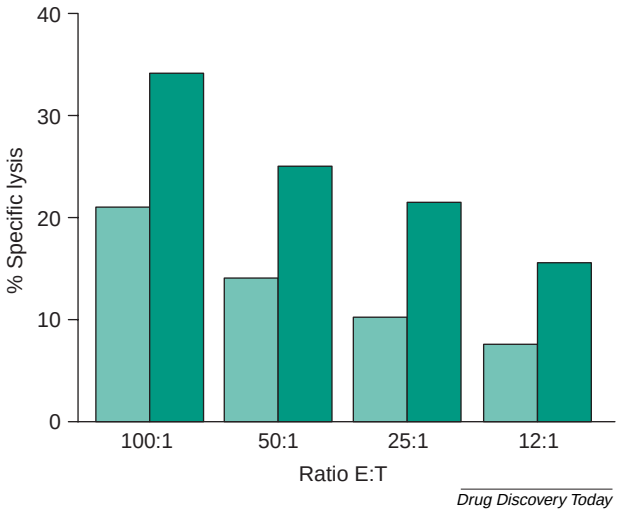

Figure 2. Natural killer (NK) cell lytic activity to NK 562 cell line in the presence (dark green) or absence (light green) of the sterol–sterolin mixture. The effector to target (E:T) ratios are indicated.

These preclinical results and knowledge of the safety and non-toxic profile of the plant molecules has enabled clinical testing of the sterol mixture. On the one hand, the mixture could be used for enhancing the cellular immune response because of the enhancement of cytokines that drive this arm of immunity. On the other hand, the anti-inflammatory properties of the mixture also indicated that they could be useful in the management of chronic conditions typified by inflammation.

Clinical studies showing the efficacy of plant sterols/sterolins *in vivo*

Adjuvant activities in pulmonary tuberculosis

One of the first studies undertaken examined the sterol mixture as an adjuvant in the treatment of pulmonary tuberculosis, a condition known to involve cellular immunity for clearance of the causative mycobacterium. This double-blind placebo-controlled study was the first to show clinical efficacy of the plant mixture: faster recovery from the infection, less inflammation and improved weight gain of patients [14].

Inhibition of immune stress in marathon runners

It is known that endurance sport such as marathon running and cycling leads to transient immune suppression, possibly caused by hormone-induced redistribution of immune cells as well as a decline in the functionality of these cells. This model of immune stress was used to investigate the potential of the BSS–BSSG mixture in preventing immune suppression. This double-blind placebo-controlled study showed clear differences in immune parameters such as neutrophilia, leucocytosis and lymphopaenia that are normally experienced by runners post-event [15]. The inversion of CD4:CD8

ratios was inhibited, indicating that immunosuppression related to the endurance event was abrogated by the ingestion of the plant sterol–sterolin mixture. An interesting observation made during this study was the maintenance of a pre-event cortisol:dehydroepiandrosterone sulfate (DHEA-S) hormone ratio, implying that the plant sterol mixture could have adrenal activity.

Plant sterol mixture in the management of HIV-infected patients

An open-labeled study in HIV-infected patients to whom no anti-retroviral drugs were prescribed was initiated because access to such drugs is denied to many patients because it is too expensive. Patients enrolled with intact immune systems (CD4 cell count >500 cells μl^{-1} blood) showed a significant decrease in their plasma viral loads and stable CD4 cell counts over a period of 40 months [16]. Analysis of the CD4 cell-type (Th1 versus Th2-type) showed that those receiving the BSS–BSSG mixture maintained a favorable Th1 response, which implies that their cell-mediated response was possibly responsible for the viral control and inhibition of CD4 cell loss in the absence of anti-retroviral drugs [17].

Phytosterol mixture and rheumatoid arthritis

A double-blind placebo-controlled study conducted in patients with active rheumatoid arthritis (RA) showed that the attenuation of disease activity [criteria according to the American College of Rheumatology (Atlanta, GA, USA)] was achievable in patients receiving the sterol mixture. Because of the potent anti-inflammatory properties of the mixture, patients with this chronic condition should benefit from the intake of the natural immune modulator. Markers of activity included the erythrocyte sedimentation rate (ESR), swollen joint count, tender joint counts, and the clinician's assessment of disease activity [18].

The magnitude of the efficacy of the BSS–BSSG mixture is worthy of mention. Both the placebo group of RA patients and the treated group were given non-steroidal anti-inflammatories (diclofenac) as well as analgesics (paracetamol). The placebo group showed an average response of 21% compared with an average of 48% in the treated group (according to the American College of Rheumatology), therefore, it is evident that the BSS–BSSG mixture has notable pharmacological activity. In fact, most patients using the capsules containing the mixture used less of the aforementioned prescription drugs (determined by pill counting at each clinic visit), implying that their quality of life improved during the trial period and that they had less recourse to pain-controlling medication.

In previous years, a German drug (Flemun®; Firma Hoyer, Dusseldorf, Germany) shown to reduce cyclooxygenase and lipoxygenase activity *in vitro* and *in vivo* had shown some promise when tested in 154 patients with chronic polyarthritis [19]. However, this natural therapy is no longer available (the reason for this is unknown to the author), although it had shown promise in clinical trials.

Plant sterol mixture for FIV-infected domestic cats – a model of HIV

Feline immunodeficiency virus, the feline equivalent of HIV, exhibits the same immunopathogenic processes as those revealed in humans, and the infected domestic cats finally succumb to the disease with overt immune suppression. We have used this animal model to test the mixture of sterols–sterolins before beginning the human studies mentioned previously. Treated cats maintained stable CD4 cell counts over extended periods of time [20], and when mortality is used as the end-point of the study, the results are significantly different between the groups of cats [21].

Allergic rhinitis and sinusitis management

Because the phytosterol mixture changes the cytokine profile of T cells to a predominant Th1-type, it seemed applicable to test the mixture as a management strategy in allergic conditions whereby the underlying immune abnormalities described fit into a Th2-predominant profile (excessive IL-4 secretion and hence IgE synthesis). A group of 24 individuals was treated with the mixture over a period of 12 weeks and end-points included both clinical and laboratory markers of efficacy, including IgE levels. Statistically significant changes that occurred included less rhinonorea, less turbinate hypertrophy, less post-nasal drip symptoms and lower IgE levels. Subjective reporting from the patients also recorded symptomatic relief. This clinical study corroborated a previous *ex vivo* study conducted on the lymphocytes from allergic individuals ingesting the phytosterol mixture capsules [22].

Conclusions

To date, we have only known how to suppress an immune system. This approach was adopted to control symptoms arising from uncontrolled immune responses and especially to control the damage caused by chronic inflammation. We stand on the brink of new knowledge concerning the immune system, especially knowing that subsets of lymphocytes regulate an outcome by means of soluble messengers. As our scientific knowledge expands, so must our battery of molecules able to target specific sites in our immune system.

We are aware of many natural preparations containing sterols and/or sterolins (such as extracts of cat's claw and *Gingko biloba*). There are many other plant extracts that have been used in clinical studies without the active principles

known at present. The preparation used by our group is a standardized mixture of BSS and BSSG in a specific ratio of 100:1 because we found this to be optimal for both *in vitro* and *in vivo* activity. All of the reported clinical activities cited here have made use of this formulation and no adverse event has been reported by the patients and/or clinicians.

The plant sterols and sterolins (the sterols–sterolins mixture, as described here) are possibly the first such molecules that have been investigated in relation to immune regulatory subsets. The clinical results are promising and exciting. These molecules have been overlooked for too long: first described in 1922, they have only known one medical use so far: to lower endogenous cholesterol levels when taken in gram quantities. The immunomodulating properties of this mixture are active at extremely low concentrations, therefore we do not need much to benefit from their effects. This is further reinforced by others who have demonstrated their importance in human and animal nutrition to prevent chronic diseases [4,23].

However, many questions remain unanswered. For instance, what is the cellular target of these molecules? Some studies would indicate cytoplasmic receptors that are unidentified at present, but certainly gene regulation. Some *in vitro* studies conducted on cancer cell lines would implicate the sphingomyelin cycle and the activation of the phosphatase A2 pathway as targets [24]. It is still early days, but we are certain that new studies will yield conclusive results. We are also aware of a new synthetic steroidal molecule (IPL576092; Inflazyme Pharmaceuticals, Richmond, Canada) derived from marine organisms. This molecule has potent anti-inflammatory properties but it does not act via the glucocorticoid receptor like dexamethasone, which is exciting because it implies that this new molecule might have less side effects in patients; it is currently in clinical trials with asthma patients.

Nature has provided us with many molecules as therapeutics: antibiotics, statins and immune suppressives to name but a few. Perhaps the sterols and sterolins might enter this new century as the new therapeutics for targeting specific sites in the immune system. We need to undergo a paradigm shift in the way we manage patients with immune dysfunctions: it might no longer be necessary to simply suppress the symptoms of diseases, but rather to try to correct the underlying mechanistic abnormalities. Natural remedies should be revisited as important sources of novel ligands capable of targeting specific cellular receptors.

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