INT J TUBERC LUNG DIS 1(5):518-522

A randomised placebo-controlled trial of the efficacy of beta-sitosterol and its glucoside as adjuvants in the treatment of pulmonary tuberculosis

P.R. Donald,* J.H. Lamprecht, M. Freestone, C.F. Albrecht, P.J.D. Bouic, D. Kotze†, P.P. van Jaarsveld

Departments of *Paediatrics and Child Health, Pharmacology and Medical Microbiology, Tygerberg Hospital and the University of Stellenbosch, †Department of Statistics, University of the Western Cape, South Africa.

SUMMARY

OBJECTIVE: To evaluate the adjuvant effect of beta-sitosterol and its glucoside in the treatment of culture proven pulmonary turberculosis (PTB).

DESIGN: A blinded randomised placebo-controlled trial in culture proven drug sensitive PTB. Patients were hospitalised for the duration of the treatment and evaluated at monthly intervals with regard to sputum culture positivity, chest radiography, weight gain, Mantoux test response, routine haematology and liver functions.

STATISTICAL EVALUATION: General linear models for repeated measures (SAS GLM package) compared the interaction effects, group effects and time effects of findings in 19 patients receiving sitosterols with those in 18 patients receiving a placebo (talcum powder). Absolute values and change from baseline values were evaluated, although only the latter were reported.

RESULTS: Weight gain was significantly greater in the sitosterol group (mean weight gain 8.9kg) than the placebo group (mean gain 6.1kg) (P = 0.0023 group effects; P = 0.0001 for time effects). Speed of achieving culture negativity; radiological improvement and induration on Mantoux testing was similar in the two groups. Change in lymphocyte counts from baseline was significantly higher in the sitosterol group (P = 0.0001 and P = 0.0001 for group and time effects) as was the increase in eosinophil counts (P = 0.0001 and P = 0.0137 for group and time effects).

CONCLUSION: The study has shown significantly improved weight gain and higher lymphocyte and eosinophil counts in PTB patients receiving sitosterols in addition to an efficacious antituberculosis regimen. Sitosterols and their possible mode of action should now be evaluated in larger numbers of tuberculosis patients and in diseases with a similar immunopathogenesis.

KEY WORDS: sitosterols; pulmonary tuberculosis; tuberculosis treatment

BETA-SITOSTEROL (BSS) and its glucoside (BSSG) are the most abundant sterols found in plants. In common with other phytosterols they are not endogenously synthesised in the human body and are derived exclusively from the diet.[1] Although they differ from cholesterol by only an extra ethyl group in the side chain, they show profound biological effects in a number of experimental animal models. These include, inter alia, reduction of carcinogen-induced colon cancer,[2] anti-inflammatory[3] and anti-complement activity.[4] In man they have been used to lower cholesterol[1] and in the management of benign prostatic hypertrophy[5] and more recently have also been shown to have an immuno-modulating effect.[6] In femtogram concentrations they stimulate proliferation of peripheral blood lymphocytes, probably by enhancing interleukin-2 (IL-2) and interferon-γ (IFN-γ) secretion.

The treatment of tuberculosis has now been refined to the point where more than 95% of patients who comply with therapy can be successfully treated with a 6-month regimen of a combination of isonazid, rifampicin and pyrazinamide.[7] The role of the different drugs in this 'short-course' chemotherapy has also been clarified.[8] Despite this, close to 25% of tuberculosis patients in developing countries may fail to complete their therapy, leading to high relapse rates and the danger of drug-resistant tuberculosis. In view of the considerable deficiencies of current antituberculosis regimens when used under operational conditions, it was therefore considered appropriate to undertake a pilot study of the effect of BSS/BSSG in pulmonary tuberculosis patients.

METHODS

The study was undertaken at the DP Marais tuberculosis hospital of the South African National Tuberculosis Association (SANTA) at Westlake near Cape Town. This area has a particularly high incidence of tuberculosis (>500/100 000 in 1993).[9] Adult male

Correspondence to: Professor P R Donald, Department of Paediatrics and Child Health, University of Stellenbosch, PO Box 19063, Tygerberg 7505, South Africa. Tel: (+27) 21 938-9138. Fax: (+27) 21 938-9138 or 938-9122.
Article submitted 12 June 1996. Final version accepted 3 July 1997.

pulmonary tuberculosis patients admitted to the hospital with pulmonary tuberculosis confirmed by sputum smear microscopy for acid fast bacilli and culture for *Mycobacterium tuberculosis* were randomised to receive either sitosterols or a placebo. The allocation of the patients to the different treatment groups was unknown to those conducting the trial. The sitosterols and placebo were supplied as identical capsules by Essential Sterolin Products (Pty) Ltd (Gauteng, South Africa). The active capsules contained 0.2 mg BSSG, 20 mg BSS plus 200 mg talcum. The placebo capsules contained only 200 mg talcum. Patients received one capsule three times daily together with their standard antituberculosis regimen.

Clinical Evaluation

Patients were evaluated on admission and monthly with regard to weight gain and radiological improvement, sputum culture, full blood count, differential white cell count, sedimentation rate and liver function tests. A Mantoux test was carried out on admission with 5 units purified protein derivative (PPD) (Japan) and monthly thereafter. Patients' participation in the study for at least 4 months was required for their inclusion in the final data analysis. Patients with drug resistant tuberculosis were excluded from the analysis.

Chest radiographs were assessed independently by a physician and a radiologist who were unaware of which treatment group the patients had been placed in. Because of considerable variation in radiological technique it proved possible only to identify the time at which the chest radiographs showed indisputable improvement.

Statistical Evaluation

Two data sets, the absolute values and the change from baseline derived from repeated measurement of the variables over the study period were evaluated. Many methods of analysis have been suggested for such data including simple *t*-tests at each separate time point and more complex designs.[10] The SAS General Linear Model (GLM) procedure,[11] as described by Milliken and Johnson,[12] when usual assumptions do not hold, was used for this analysis to determine if the interaction effects and main effects (group and time) were significantly different between the sitosterol and placebo groups.

For the first set of results obtained on entry to the study, t-tests were performed between the absolute values at baseline for the two groups (sitosterol and placebo) to determine significant difference. When no significant differences were determined at baseline, GLM repeated measures analyses were performed to investigate differences between groups (group effects).

For the second approach a new set of values was calculated, namely 'change from baseline', and analysed for significant group and time effects by means of the same SAS GLM repeated measures. This resulted in a different measurement ('change from the baseline') compared to the first approach (absolute data) and the statements which are tested (the hypothesis of no interaction or the hypothesis of equal main effects) for the two approaches differ with respect to the actual measure utilised. Only the results for the 'change from baseline' are reported since these changes identify the treatment response more effectively.

The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Stellenbosch, the South African Medicines Control Council and the Management Committee of the DP Marais Hospital. Written informed consent was obtained from patients for their participation in the study.

RESULTS

Twenty-three patients were included in the active group which received sitosterols. Four of these were subsequently excluded - one died after 3 months of treatment, one was found to have multidrug-resistant tuberculosis (resistance to isoniazid and rifampicin), one was HIV-positive and one patient absconded after one month's treatment. The mean age of the 19 remaining patients was 43.4 years and all had fully drug sensitive *M. tuberculosis* isolated from their sputum. Their mean weight was 49.4 kg (±6.4 kg) and 9 (47%) had previously been treated for tuberculosis.

Twenty-four patients were entered in the placebo group from which six were excluded: three absconded (two after 3 month's therapy and one after 1 month's therapy), one had multidrug-resistant tuberculosis, one was HIV-positive and one requested discharge for home treatment after 2 month's hospitalisation. The mean age of the 18 patients remaining in the placebo group was 36.6 years, their mean weight 50.5 kg (±8.8 kg) and seven (39%) had previously been treated for tuberculosis. All of the patients evaluated had fully drug sensitive *M. tuberculosis* isolated from their sputum.

No significant differences existed between the variables of each group evaluated at the time of entry to the study ($P > 0.05$ in all instances).

All of the patients included in the final analysis had fully drug sensitive organisms and received the standard regimen of isoniazid, rafampicin and pyrazinamide for 6 months as used by the South African National Tuberculoses Control Programme at that time.

By the end of 1 month of treatment 11 patients in both the sitosterol group (58%) and the placebo group (61%) were still sputum culture positive; two patients in each group (11%) were still positive after 2 months of treatment. No patient in either group had a positive sputum culture thereafter.

One set of chest radiographs from a patient in each

Table Mean values (standard deviation) of investigations in pulmonary tuberculosis patients receiving sitosterol or placebo

	Month of treatment							Significance of deviation from baseline group effect (time effect)
	0	1	2	3	4	5	6	
Body mass (kg)								
Sitosterol (SD)	49.4 (6.4)	50.6 (6.7)	52.2 (7.7)	54.9 (8.0)	56.6 (8.3)	58.1 (8.1)	58.3 (9.2)	0.0023 (0.0001)
Placebo (SD)	50.5 (8.8)	52.3 (9.1)	53.9 (9.7)	54.9 (9.9)	56.0 (9.8)	55.8 (7.6)	56.7 (10.3)	
Lymphocyte count ($x10^9$/ l)								
Sitosterol (SD)	1.49 (0.80)	2.08 (0.71)*	2.15 (0.69)*	2.18 (0.74)*	2.18 (0.64)	24.2 (0.62)	2.27 (0.65)*	0.0001 (0.0001)
Placebo (SD)	1.24 (0.60)	1.62 (0.57)	1.69 (0.66)	1.75 (0.57)	2.00 (0.60)	2.00 (0.71)	1.86 (0.76)	
Monocyte count ($x10^9$/ l)								
Sitosterol (SD)	0.68 (0.36)	0.66 (0.34)	0.65 (0.34)*	0.58 (0.23)	0.55 (0.22)*	0.53 (0.19)	0.50 (0.17)	0.0053 (0.0001)†
Placebo (SD)	0.67 (0.27)	0.62 (0.22)	0.59 (0.22)	0.56 (0.24)	0.45 (0.16)	0.52 (0.17)	0.50 (0.18)	
Eosinophil count ($x10^9$/ l)								
Sitosterol (SD)	0.13 (0.13)	0.40 (0.28)*	0.50 (0.56)	0.42 (0.30)	0.46 (0.33)	0.45 (0.46)	0.46 (0.43)*	0.0001 (0.0137)
Placebo (SD)	0.12 (0.14)	0.29 (0.23)	0.53 (0.24)	0.36 (0.34)	0.33 (0.22)	0.33 (0.19)	0.23 (0.16)	
Platelet count ($x10^9$/ l)								
Sitosterol (SD)	477 (139)	430 (193)*	395 (101)*	338 (134)*	342 (122)*	292 (77)*	277 (74)*	0.0001 (0.0001)†
Placebo (SD)	430 (135)	409 (132)	349 (146)	340 (114)	304 (104)	226 (84)	280 (84)	
Sedimentation rate (mm/hour)								
Sitosterol (SD)	63 (32)	69 (27)*	68 (25)*	62 (23)*	58 (29)*	48 (16)*	37 (20)*	0.0001 (0.0001)†
Placebo (SD)	71 (34)	74 (33)	61 (23)	53 (24)	46 (28)	46 (32)	48 (27)	
Serum albumin (g/ l)								
Sitosterol (SD)	34.8 (6.0)	38.9 (4.5)	41.4 (4.5)	42.6 (4.2)	43.4 (3.7)	43.3 (2.7)	43.1 (4.5)	0.0052 (0.0001)
Placebo (SD)	36.5 (4.4)	40.1 (4.7)	41.9 (6.1)	42.5 (4.2)	44.3 (2.9)	43.3 (2.8)	43.5 (3.2)	

* Indicates significant difference (P > 0.05) between treatment and placebo group for change from baseline valuesat time point.
† Significant interaction repeated measures factor (time) is different for the groups at each level.

group was inadvertently sent to a peripheral clinic upon the patient's discharge and could not be recovered. Radiological improvement amongst the remaining chest radiographs was not significantly different between the two groups. There were three patients in the sitosterol group and one in the placebo group who at the end of 6 months of treatment showed no signs of radiological improvement, although their sputum cultures were negative.

The results of investigations showing a significant group effect are summarised in the Table. Changes from baseline values for haemoglobin, haematocrit, neutrohpil count, serum globulin, creatinine and urea did not differ between the sitosterol and placebo groups. Induration on Mantroux testing also did not differ between the two treatment groups. Weight gain was, however, significantly greater in the sitosterol group (mean gain 8.9 kg) than in the placebo group (mean gain 6.1 kg) (group effect $P = 0.0023$; time effect $P = 0.0001$). Lymphocyte counts were higher in the sitosterol group (group effect $P = 0.0001$; time effect $P = 0.0001$), as were eosinophil counts (group effect $P = 0.0001$; time effect $P = 0.0137$). Significant group and time effects were also found for monocyte counts, platelet counts and sedimentation rate, but a significant interaction of group and time complicates the interpretation of the main effects.

DISCUSSION

Following the initiation of an efficacious antituberculosis regimen it is to be expected that gain in weight will occur, that the majority of sputum cultures will be negative by the end of 2 months of therapy and that radiological resolution of the majority of tuberculosis lesions will occur by the end of 6 months therapy. In both groups of patients evaluated a considerable gain in weight occurred, but gain in weight was significantly greater in the sitosterol group (group effect $P = 0.0023$; time effect $P = 0.0001$). No patient in either group had a positive sputum culture after the second month of treatment and the radiological response to treatment was similar in both groups.

The haematological findings in pulmonary tuberculosis patients have been documented on a number of occasions, as has the response of the various parameters to therapy. Thus the occurrence anemia, lymphopaenia, neutrophil leucocytosis, monocytosis, eosinopaenia and thrombocytosis is well established,[13] and was found in our patients. The institution of appropriate chemo-therapy will usually lead to a resolution of the anaemia, a rise in the lymphocyte count, a fall in the polymorphonuclear leucocyte count, a fall in the polymorphonuclear leucocyte count and the monocyte count, a rise in the eosinophil count and a fall in the platelet count. This course of events

was evident in our patients, but it is noteworthy that the increase in lymphocytes and eosinophils, above baseline was significantly greater in patients receiving sitosterols (for lymphocyte count group and time effects $P = 0.0001$ and 0.0001 respectively, and for eosinophils $P = 0.0001$ and 0.0137 respectively).

Since the sitosterols enhance T-cell proliferative responses both in vitro and in vivo this response was perhaps not unexpected.[6] However, we do not know what subset of lymphocytes the increased counts represent. In previous studies of the pattern of lymphocyte response in sputum smear positive pulmonary tuberculosis, a reduction in total T-cell and CD4 counts and an increase in CD8 counts before the start of therapy was followd by an increase in the number of CD4 cells and a decrease in CD8 counts after the start of treatment.[14] We can therefore only speculate that a similar pattern is likely to have been found in both groups of patients, since flow cytometric analysis of lymphocyte subsets was not conducted in these patients. Unpublished data from other trials evaluating the use of sitosterols indicates a preferential increase in CD4 positive lymphocytes over CD8 positive cells.

The interpretation of the significantly greater increase in eosinophils in the sitosterol group is also not clear. On the one hand it could be interpreted as indicating an allergic response to sitosterols. There is, however, very little indication of any allergic phenomena in previous studies.[5] A comparable commercially available sitosterol product (HarzolR) has a long history of use for benign prostatic hyper-trophy in Germany without producing any recognizable allergic phenomena. IL-4 and IL-5 of the Th2 family of cytokines are also known to promote eosinophilia.[15] It is also of interest that a close relationship between eosinophil counts and CD4 + lymphocyte counts has been found in patients with HIV-infection and tuberculosis.[16]

This randomised double-blind placebo controlled trial of the use of sitosterols in a relatively small number of patients with pulmonary tuberculosis has shown several significant differences between those individuals who did and those who did not receive sitosterols. Patients receiving sitosterols had improved weight gain and a greater increase in peripheral blood lymphocyte and eosinophil counts. While these differences may be chance findings they could be cautiously interpreted as evidence of a possible beneficial effect ascribable to the sitosterols. This was unfortunately not reflected in the small number of patients receiving an efficacious antituberculosis regimen by either more rapid sputum culture negativity of be speed of radiological resolution of disease. There is, however, now good reason for an in-depth evaluation of sitosterols and their possible mode of action in larger numbers of patients and in conditions other than tuberculosis, but with a comparable immunopathogenesis.

Acknowledgments

This study was initiated and sponsored by Essential Sterolin Products (Pty) Ltd according to an agreement entered into with the University of Stellenbosch.

References

1 Ling W H, Jones P J H. Dietary phytosterols: a review of metabolism, benefits and side effects. Life Sciences 1995; 57: 195-206.

2 Raicht R F, Cohen B I, Fazzini E P, Sarwal A N, Takahashi M. Protective effect of plant sterols against chemically induced colon tumors in rats. Cancer Res 1980; 4: 403-405.

3 Yamamoto M, Matsui T, Sugiyama K, Yakota M, Nakagomi K, Nakazawa H. Anti-inflammatory active constituents of Aloe arborescens Miller. Agric Biol Chem 1991; 55: 1627-1629.

4 Yamada H, Yoshino M, Matsumoto T, et al. Effects of phytosterols on anti-complementary activity. Chem Pharm Bull 1987; 35: 4851-4855.

5 Berges R R, Windeler J, Trampisch H J, et al. Randomised, placebo-controlled, double blind clinical trial of B-sitosterol in patients with benign prostatic hyperplasia. Lancet 1995; 345: 1529-1532.

6 Bouic P J D, Etsebeth S, Liebenberg R W, Albrecht C F, Pegel K, Van Jaarsveld P P. Beta-sitosterol and beta-sitosterol glucoside stimulate peripheral blood lymphocyte proliferation: implications for their use as an immunomodulatory vitamin combination. Int J Immunopharmacol 1996; 18: 693-700.

7 Iseman M D, Sbarbaro J A. Short-course chemotherapy of tuberculosis. Am Rev Respir Dis 1991; 143: 697-698.

8 Mitchison D A. The action of antituberculosis drugs in short-course chemotherapy. Tubercle 1985; 66: 219-225.

9 Medical Officer of Health, Annual Report of Department of Health Services, Western Cape Regional Health Services 1992; 42-58.

10 Everitt B S. The analysis of repeated measures: a practical review with examples. The Statistician 1995; 44: 113-135.

11 Hatcher L, Stepanski E. A step-by-step approach to using the SAS system for univariate and multivariate statistics. Cary, NC: SAS Institute Inc, 1984.

12 Milliken G A, Johnson D E. Analysis of messy data. New York: Van Nostrand Reinhold Company. 1984. Vol 1.

13 Knox-Macaulay H H M. Tuberculosis and the haemopoietic system. Bailliere's Clinical Haematology 1992; 5: 101-129.

14. Singhal M, Banavalikar J N, Sharma S, Saha K. Peripheral blood T lymphocyte subpopulations in patients with tuberculosis and the effect of chemotherapy. Tubercle 1989; 70: 171-178.

15 Swain S L, McKenzie D T, Dutton R W, Tonkonogy S L, English M. The role of IL-4 and IL-5: characterization of a distinct helper T-cell subset that makes IL4 and IL5 (Th2) and requires priming before induction of lyphokine secretion. Immunol Rev 1988; 102: 77-105.

16 Ledru E, Diagbouga S, Tranchot-Diallo J, et al. Eosinophils: a putative marker of immunodepression in HIV-infected African patients with tuberculosis. Trans Royal Soc Trop Med Hyg 1994; 88: 117-118.