Immunomodulatory Effects of Maharishi Amrit Kalash 4 and 5 in Mice

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Abstract To evaluate the immunomodulatory effects of two kinds of Ayurvedic food supplements (Maharishi Amrit Kalash 4 and Maharishi Amrit Kalash 5, M-4 and M-5), superoxide anion (O\textsuperscript{2-}) production of peritoneal macrophages and the response of spleen cells to concanavalin A (Con A) were examined in mice given an aqueous emulsion of M-4 and M-5 p. o. at doses of 50 and 100 mg/kg for 10 days. O\textsuperscript{2-} production of peritoneal macrophages in the M-5 (50 mg/kg)-treated group was significantly higher than that in the control group. The indices of stimulation of spleen cells by Con A were significantly (3 to 4 times) higher in groups treated with M-4 and M-5 at all doses than in the control group. These results indicate that M-4 enhances lymphocyte responsiveness and M-5 enhances not only lymphocyte responsiveness but also macrophage function. It is also suggested in this study that M-4 and M-5 have mitogenic effects on lymphocytes.

Key words: Maharishi Amrit Kalash, Immunomodulatory effect, Macrophage, Splenocyte, Mouse

Introduction

Ayurveda is the oldest medical system in the world. It originated in India at around 6,000 B. C. The system is presently in practice and the World Health Organization has approved its potency\textsuperscript{1}. The ancient Ayurvedic system of medicine has provided a large number of useful medical procedures and pharmaceuticals. This system provides an approach to prevention and cure of diseases and emphasizes the philosophy of maintaining excellent health by normalizing the cellular functions and improving the inherent vitality of tissues.

The Ayurvedic physician prescribes herbal food supplements called Rasayana. Maharishi Amrit Kalash is a commercially available Rasayana prepared according to the ancient Ayurvedic formulation\textsuperscript{2}. This preparation, as well as other Rasayanas is believed to enhance the body’s resistance to infections and diseases, and enhance longevity\textsuperscript{2}. It is possible that such medicines have immunomodulatory potency. However, there has been only one paper reporting the immunomodulatory effects of Maharishi Amrit Kalash\textsuperscript{3}. The purpose of this study is to evaluate the in vivo immunomodulatory effects of two kinds of Maharishi Amrit Kalash (M-4 and M-5). They are composed of a variety of herbs, minerals and dairy products\textsuperscript{3}.

Materials and Methods

Animals and treatment

Fifty three male ddY mice (10 weeks old, initial weights of 33-35g) were purchased from Japan SLC (Hamamatsu). They were randomly divided into 5 groups, and maintained at a temperature of 21-23°C, a humidity of 60±5% and under an artificial light-dark cycle (light: 0800-2000). They were acclimated for 1 week prior to the experiment. Food (CE-2, Nihon Clea, Tokyo, Japan) and water were supplied ad libitum.
M-4 and M-5 were obtained from Maharishi Ayurveda Products International (Lancaster, USA). The ingredients in M-4 are: Indian gallnut, Indian gooseberry, dried catkins, Indian pennywort, nutgrass, white sandalwood, evalyulus alsinooides, embella, aloewood, licorice, cardamom, cinnamon, cyperus, turmeric, honey, raw sugar and ghee (clarified butter). The ingredients in M-5 are: gymnema aurentiacum, black musale, heart-leaved moonseed, sphaeranthus indicus, butterfly pea, licorice, vanda spatulatum, elephant creeper and Indian wild pepper. The exact amounts of various ingredients in M-4 and M-5 were not disclosed by the supplier, but the quality control (e.g., minimal variation from batch to batch) was assured.

Mice were given a p.o. aqueous emulsion of M-4 and M-5 (50mg/kg or 100mg/kg) once daily for 10 days. Each group consisted of 10 or 11 mice. Mice in the control group received water (0.1ml/10g body weight). The animals were killed by bleeding 48 hours after the final administration under ether anesthesia for the following experiments.

Isolation of macrophages and determination of superoxided anion (O$_2^-$) production

Peritoneal exudate cells (PECs) were collected from the sacrificed mice using 5ml of phosphate-buffered saline (PBS (-)). The PECs obtained from 6 or 7 mice of each group were pooled. Macrophages in the PEC suspension were isolated by the cell adhesion method. The recovered cells were washed twice in Hanks’ solution (Nissui Seiyaku Co. Ltd., Tokyo, Japan) by centrifugation at 1,000 rpm for 5 min, and resuspended in 2ml of RPMI 1640 medium (Nissui Seiyaku Co., Ltd, Tokyo, Japan) containing 10% heat-inactivated fetal calf serum (FCS-RPMI 1640) (Gibco Laboratories Life Technologies, Inc., USA). The viability of peritoneal macrophages determined by the trypan blue dye exclusion test was more than 98%.

O$_2^-$ production of macrophages was assayed by the nitro blue tetrazolium (NBT) (Nacalai Tesque, Inc., Kyoto, Japan) reduction method. Two hundred microliters (µl) of macrophage suspension (2.0×10^6 cells/ml) was incubated for 2 hours. After aspiration of the solution, 100 µl of NBT (4 mg/ml) and 100 µl of phorbol 12-myristate 13-acetate (0.3 µg/ml) (PMA) (Shigma Chemical Co.) solutions in FCS-RPMI 1640 were added to wells of a 96-well flat-bottomed microplate (Corning Laboratory Sciences Co., New York, USA). After 30 min incubation in 5% CO$_2$ at 37°C, the medium was removed and the cells in the well were fixed by 2.0 ml of 70% ethyl alcohol. After removal of the alcohol, the fixed cells were dissolved by 0.1ml 2N KOH and 0.1ml dimethyl sulfoxide. Optical density of the solution at 630 nm was measured using a microplate reader (Corona Electric Co. Ltd., MTP 120, Tokyo, Japan) for use as the index of the amount of O$_2^-$ produced by the activated macrophages. The results are presented as the mean±SE of triplicate sets.

Mitogen-induced proliferation of splenic lymphocytes

Single cell spleen cell suspensions were prepared from individual mice by pressing the spleen between two glass slides. After washing in Hanks’ solution, the cells were suspended in FCS-RPMI 1640 (4×10^6 cells/ml). The viability of splenocytes determined by the trypan blue dye exclusion test was more than 98%.

The cell suspension (50 µl) and Concanavalin A solution (25 µl, Con A 12 µg/ml) in FCS-RPMI 1640 in wells of a 96-well flat-bottomed microplate (Corning Laboratory Sciences Co., New York, USA) were incubated for 72 hr in 5% CO$_2$ at 37°C. The proliferation of spleen cells was assayed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT)9. Six hours before the end of the incubation, 10ul of 0.5% MTT (Shigma Chemical Co.) dissolved in PBS(−) was added to the well. After the incubation, 150 µl 0.04N HCl-isopropanol was added to each well. Then the optical density at 570 nm was measured with a microplate reader.
Electric Co. Ltd., MTP 120, Tokyo, Japan). The experiments were done in triplicate. The stimulation index (S. I.) was calculated according to the following equation.

\[
S. \text{I.} = \frac{\text{mean optical density of the cells stimulated with Con A}}{\text{mean optical density of the cells not stimulated with Con A}}
\]

Statistics

The statistical significances of difference among values was tested using one-way analysis of variance followed by Scheffe’s multiple comparison. The level of significance was set at \(p<0.05\).

Results

Table 1 shows the effects of M-4 and M-5 on the body weight and the body weight gain for 10 days in the mice. There were no significant differences among the M-4 and M-5 treated groups and the control group in body weight and body weight gain.

Figure 1 shows the effects of M-4 and M-5 on superoxide anion (\(O_2^-\)) production of peritoneal macrophages in mice. \(O_2^-\) production was significantly high only in the M-5-(50 mg/kg) treated group compared with that in the control group (\(p<0.05\)).

Table 2 and Fig. 2 show the effects of M-4 and M-5 on the proliferation of splenic lymphocytes stimulated with Con A in the mice. The OD values in the non-stimulated cells of both M-4-(50 and 100 mg/kg) and M-5-(50 and 100 mg/kg) treated groups were significantly (about 2 times) higher than that of the control group (\(p<0.01\)). The OD values in the Con A-stimulated cells of both M-4-(50 and 100 mg/kg) and M-5-(50 and 100 mg/kg) treated groups were significantly (3 to 4 times) higher than that of the control group (\(p<0.01\)). Stimulation indices of both M-4-(50 and 100 mg/kg) and M-5-(50 and 100 mg/kg) treated groups were significantly (about 2 times) higher than that of the control group (\(p<0.01\)).


Table 2 Effects of M-4 and M-5 on proliferation of splenocytes induced by Con A in mice.

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<th>Group</th>
<th>Absorbance (570 nm)</th>
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<td>Control</td>
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Each value represents the mean ± SE.
Significantly different from control at **p<0.01.
M-4, Maharishi Amrit Kalash 4; M-5, Maharishi Amrit Kalash 5. N, Number of mice used.

Discussion

In the present study, we examined the effects of M-4 and M-5 on $O_2^-$ production of peritoneal macrophages as an indicator of macrophage functions and on then Con A-induced mitogenic response of spleen cells as an indicator of lymphocyte functions in mice.

We found that the ability of macrophage to generate $O_2^-$ in mice was not affected significantly by daily oral treatment with M-4 at any doses but was increased significantly by M-5 at 50 mg/kg. Dileepan et al.\(^3\) reported that macrophage $O_2^-$ production of rats (weighing 210-225 g) was not affected by treatment with M-5 at a dose of 50 mg/rat/day for 10 or 20 days. The discrepancy of the results may be attributable to the difference of animal species. There is another possibility, however. The dose used by Dileepan et al.\(^3\) was about 2-4 times higher than that used in the present study. Such high doses might exceed the optimal dose to enhance the macrophage $O_2^-$ production.

Dileepan et al.\(^3\) reported that M-5 did not enhance spontaneous splenic lymphocyte proliferation in rats. However, the net OD values of non-stimulated spleen cells in the MTT assay were increased significantly by the treatment with M-4 and M-5 at all doses in mice, suggesting that both M-4 and M-5 may be mitogenic to murine lymphocytes.

In addition, the index for stimulation of spleen cells by Con A was significantly higher in groups treated with M-4 and M-5 at all doses than in the control group. Lymphocyte proliferation is a complex event involving, among other things, the participation of interleukins 1 and 2 and interleukin-2 receptor expression\(^{10}\). It is possible that both M-4 and M-5 ingestion enhance the production of various cytokines and/or T cell receptor expression. Further studies are needed to clarify the mechanism of the immunomodulatory actions of M-4 and M-5.

In conclusion, M-4 enhances only lymphocyte responsiveness and M-5 enhances not only lymphocyte responsiveness but also macrophage function. There is a possibility that M-4 and M-5 have mitogenic effects on lymphocytes.
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References


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