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The effect of mangan and lithium on peripheral blood mononuclear cell viability after exposure of narrowband ultraviolet B in psoriasis patients

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ABSTRACT

Psoriasis is a chronic and recurrent inflammation skin disease characterized with hyperproliferation of keratinocyte mediated by T cell lymphocytes. Climatotherapy (bathing and sunbathing) at Dead Sea is model therapy which is effective for moderate and severe psoriasis. However there are group of psoriasis patients who are not responsive to climatotherapy. The success of the model therapy was based on the influence of ultraviolet irradiation which causes depletion of lymphocytes, macrophages, and dendritic cells of the skin. Patients who are not respond to climatotherapy shown to have higher level of mangan (Mn) and lithium (Li) serum than responsive patients. Mangan and Li were suspected influenced the viability of peripheral blood mononuclear cells (PBMC). The aim of the study is to evaluate the effect of Mn and Li levels on the viability of PBMC in the serum of psoriasis patient after exposure of narrowband UVB (NB-UVB). The PBMC were isolated from 6 psoriasis patients using Histopaque. Then the cells isolate was incubated in RPMI medium with 0.02 to 0.08 μmol/L Mn and 0.08 to 0.1 μmol/L Li for 3 days. After that the cells were irradiated once with 1/3 to 2 minimal erythem dose (MED) of NB-UVB. The PBMC viability was measured 3 hours after irradiation with MTT assay and read with ELISA plate reader. The results showed that increased levels of Mn and Li or combination did not affect on the viability of PBMC at 1/3 to 1 MED UV-UVB. But at higher irradiation dose (2 MED), the higher level of Mn and Li had negative affect in the viability of PBMC after irradiation. It could be concluded that the Mn and Li level in the serum did not affect the viability of PBMC after irradiation of NB-UVB. It was suggest that other cellular component that involved in the development of psoriasis lesions, such as dendritic cells, fibroblasts, macrophages, and keratinocyte, were associated with Mn and Li levels.

Key words: psoriasis – mangan – lithium - peripheral blood mononuclear cell

INTRODUCTION

Psoriasis is a recurrent chronic skin disease, mediated especially by lymphocyte T cell.[1] The role of Lymphocyte T cell is activated an epidermal abnormality that specific for psoriasis.[2] It was proved by expression of cutaneous lymphocyte-associated antigen (CLA) in lymphocyte cell which is homing in the skin starting skin lesion in psoriasis.[3]

The management of psoriasis still becomes a challenge because there was no therapeutic modality which is effective to prevent reccurency.[4] Climatotherapy is a therapeutic modality that combined bathing in sea water therapy with sun bathing.[5] Until now, Climatotherapy is an effective and safe alternative therapy for psoriasis.[6,7]

Balneophototherapy is an artificial climatotherapy, a combination of bathing in high concentrate salt water with an artificial UV light like narrowband ultraviolet B (NB-UVB).[8] Balneophototherapy has been reported as an effective therapy for psoriasis vulgaris with minimal side effect.[9,10] The success of this therapy model based on the effect of ultraviolet light irradiation that induced lymphocyte, macrophage and skin dendritic cell depletion.[11] The effect of bathing in salt water before phototherapy is effect of immune-

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modulated, anti inflammation and anti proliferative because of the highly mineral concentration, releasing of inflammation mediator like leukocyte elastase and cytokine, water that penetrated in to stratum conium will cause increases transmition of UVB.[12]

The culture of lymphocyte T which is homing in the skin lesion of psoriasis showing increases of lymphocyte T with comparison of CD_4^+ to CD_8^+ was 0.85.[13] In the periphery blood of psoriasis patient there was an increased expression of CLA because of lymphocyte T cell migration from circulation into skin. The increased of lymphocyte T CLA^+ CD_4^+ and CD_8^+ connected positively with the severity of the disease.[3] The exposure of narrowband UVB could decreased the viability of periphery blood mononuclear cell by activating an apoptosis mechanism.[14] In the unresponsive patient with climatotherapy proofed to have higher level of serum Mn and Li compare to responsive patient. This because serum Mn reflect a manganese superoxide dismutase activity in psoriasis skin lesion, an enzyme which playing role in the cell differentiation and proliferation. This enzyme suspected played a role in increases of periphery blood mononuclear cell, particularly T lymphocyte. Thus although exposed with UVB, the mechanism of activation cell apoptosis did not happened[15]. While Li play a role in the early stage and in psoriasis exacerbation.

MATERIALS AND METHODS

This research is an *in vitro* study, using simple experimental research method. Subject inclusion criteria were men or women with moderate to severe grade of psoriasis, willing to follow the research and sign for informed consent. Subject exclusion criteria were an erythrodermic psoriasis patient, generalized pustular psoriasis and psoriatic arthritis having systemic therapy and phototherapy unless willing to stop the therapy for 2 weeks before research, pregnant and lactated.

Venous peripheral blood as much as 20 mL was taken from the research subjects. Then the PBMCs were isolated using Histopaque. The layers which were formed after centrifugation, from bottom to top, were erythrocyte, Histopaque, lymphocyte and plasma. Lymphocyte layer which formed cloudy ring then taken by pipette and separated from sterile tube.

The lymphocyte cells that deposited at the bottom of the tube were added with 1 mL complete RPMI media (10% FBS add with 100 units/mL penicillin, streptomycin 2%, PHA 50 µg/mL and fungizone 0.5%) then mixed by pipette until become a suspension of lymphocyte cell.

Next step was to put 100 µL of lymphocyte cell suspension at each wheel, thus each wheel contain of 2 x 10^5 cells. Then, Mn and Li were added with concentration as follows Mn1 0.02 µmol/L; Mn2 0.05 µmol/L; and Mn3 0,08 ìmol/L; Li1 0.08 µmol/L; Li2 0.09 µmol/L; and Li3 0.1 µmol/L also combination of Mn1+Li1, Mn2+Li2, and Mn3+Li3 according to research design.

Then the specimen was exposed with many dose of sub MED narrow band UVB by TL-01 (311-312 nm) lamp. The doses were as followed 150, 200, 250, 500, and 1000 mJ/cm^2. Finally the viability of PBMC was counted by MTT assay and read by ELISA reader 550 nm.

RESULTS AND DISCUSSION

The subject were 5 psoriasis patient with moderate to severe degree of severity, consisted of 4 women (80%) and 1 man (20%). Their average age were 43 ± 9 years old, the youngest was 29 years old while the oldest was 60 years old. Peripheral blood mononuclear cells from those five subjects was doubled at 96 well plates, therefore the case and control group's sample became 12 for each treatment.

The result showed that there were no significant differences in the viability of PBMC that was added with Mn, Li and Mn plus Li in all concentration compare with control ($p>0.05$) after exposure of 150, 200, 250 and 500 mJ/cm^2 NB-UVB. The differences became significant in the treated of Mn3 and Li1 at 1000 mJ/cm^2 NB-UVB (TABLE 1).

TABLE 1. The average of PBMC viability and p value after the addition of PBMC with Mn, Li, Mn + Li in different concentration and exposure of 150, 200, 250, 500, and 1000 mJ/cm^2 NB-UVB

	The average of PBMC viability (p)				
Doses of NB- UVB (mJ/cm^2)	150	200	250	500	1000
Mn1	0,741 (0,977)	0,694 (0,817)	0,678 (0,908)	0,231 (0,127)	0,249 (0,275)
Mn2	0,805 (0,544)	0,687 (0,773)	0,678 (0,817)	0,193 (0,050)	0,182 (0,050)
Mn3	0,735 (0,564)	0,651 (0,236)	0,632 (0,453)	0,244 (0,827)	0,184 (**0,045**)
Li1	0,779 (0,665)	0,670 (0,371)	0,638 (0,386)	0,170 (0,050)	0,184 (**0,046**)
Li2	0,776 (0,908)	0,644 (0,214)	0,647 (0,773)	0,179 (0,050)	0,206 (0,127)
Li3	0,772 (0,817)	0,665 (0,686)	0,647 (0,564)	0,179 (0,050)	0,189 (0,050)
MnLi1	0,762 (1,000)	0,674 (0,544)	0,660 (0,603)	0,206 (0,050)	0,206 (0,050)
MnLi2	0,789 (0,773)	0,709 (0,862)	0,675 (0,885)	0,195 (0,127)	0,174 (0,050)
MnLi3	0,809 (0,817)	0,660 (0,371)	0,652 (0,603)	0,135 (0,050)	0,154 (0,050)
Control	0,777	0,696	0,679	0,267	0,265

Because of there were no significant differences of the average at the exposure of 100, 150, 200, 250 mJ/cm^2 NB-UVB, then the doses were increased to 500 mJ/cm^2 and 1000 mJ/cm^2. Peripheral blood mononuclear from a subject were doubled with 96 well plates, therefore became 3 sample in case and control groups. The control group without exposure of 500 mJ/cm^2 and 1000 mJ/cm^2 NB - UVB were compared with control group with exposure. The result showed a decreased in the PBMC viability in the NB-UVB exposed group, even not significantly different (FIGURE 1).

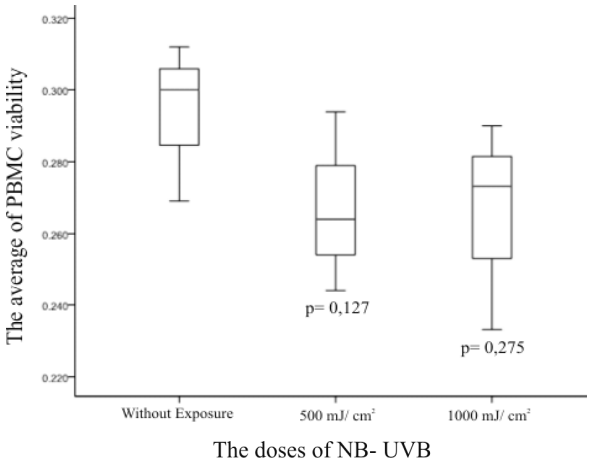

FIGURE 1. Graphic of the average of PBMC viability without exposure of 500 and 1000 mJ/cm2 doses of NB –UVB

The result showed that there were a decrease in the viability of PBMC which was added with the different concentration of Mn, Li, Mn + Li compared to control group, after exposure of 500 and 1000 mJ/cm^2 NB – UVB, but not significantly different unless in the group adding with Mn3 (0.08 µL concentration) and Li1 (0.08 µL concentration) and exposure of 1000mJ/cm^2 NB-UVB (FIGURE 2 and 3).

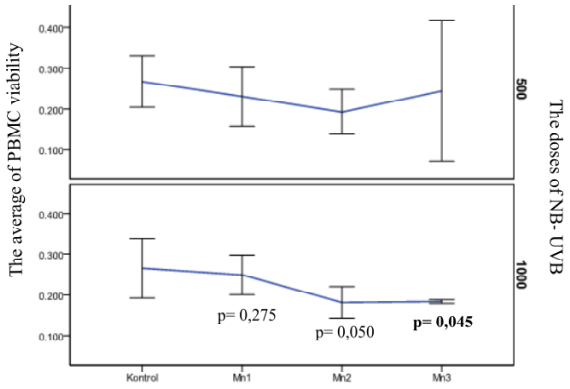

FIGURE 2. Graphic of the average of viability PBMC which add with Mn in different concentration and exposure of 500 and 1000 mJ/cm2 NB –UVB

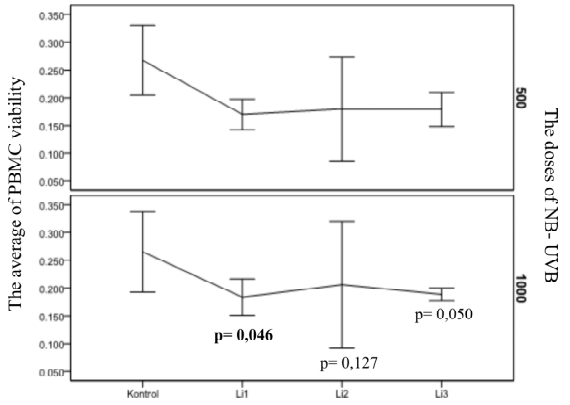

FIGURE 3. Graphic of the average of viability PBMC which add with Li in different concentration and exposure of 500 and 1000 mJ/cm2 doses of NB –UVB

The result showed that there were no effects on the PBMC viability after the addition of different concentration of Mn, Li, Mn + Li in and exposure of 150, 200, and 250 mJ/cm^2 NB – UVB. At dose of NB-UVB reach 1000 mJ/cm^2, there was a decreasing of PBMC viability, but not significantly different. The factors that could influence the result were concentration of Mn, Li, Mn + Li, doses of exposure and other mechanism that might be involved in phototherapy response of psoriasis lesion healing process.

The concentration of Mn and Li that were used in this research were based on the research of climatotherapy at Dead Sea. Serum Mn of psoriasis patients who were responsive to climatotherapy was 0.02 ± 0.02 µmol/L, while in unresponsive patient the concentration was 0.08 ± 0.03 µmol/L. Serum Li of responsive psoriasis patients with climatotherapy was 0.08 ± 0.03 µmol/L, while in unresponsive patient the concentration was 0.1 ± 0.03 µmol/L.[15] Those concentration were used as baseline which were expected as the limit concentration that might influenced the PBMC viability. The concentration of Mn and Li that higher than in the psoriasis patients serum were not used due to no information its effect on PBMC viability.

Peripheral blood mononuclear cell exposed to NB – UVB at 60, 180, and 360 mJ/cm^2 dose, has been proved could induce caspase activity that depend on the dose and duration of the exposure. An exposure of NB-UVB at 180mj/cm^2 could activate an apoptosis of PBMC.[14] This research proved that exposure of NB – UVB at 500 and 1000 mJ/cm^2 could decrease PBMC viability even though not significantly different.

According to the reference, psoriasis patients who are unresponsive to climatotherapy has been proved have hingher serum level of Mn and Li than patient who responsive to climatotherapy.[15] But this research which was specific for PBMC psoriasis patient showed that addition of different concentration of Mn, Li, Mn + Li and exposure of NB – UVB 1000mJ/cm^2 tend to decrease the cell viability.

This research proved that Li did not influence PBMC viability in psoriasis patients. Therefore, it was suspected that there was other role, in early stage and in exacerbation of psoriasis that was through inositol pathway of adenylate cyclase system by decreasing cyclic adenosine monophosphate (cAMP) and inositol then calcium intercellular become decrease.[15] This decreasing made keratinocyte proliferation increase, chemotaktic and leukocyte polimononuclear increase and releasing of cytokine proinflamation.[16] Releasing cytokine activation in psoriasis lesion was very complex; also there was an indication that releasing of cytokine not only in lymphocyte cell; but the cytokine also stimulated the neighbourhood cell like dendrite cell, macrophage and keratinocyte to release more cytokine.[18]

Lithium was proved could influence keratinocyte cell at basal layer to proliferate, increased mitosis cell speed and intercellular oedema.[19] Lithium stimulate neutrophyl cell of psoriasis patient to release inflammatory mediator (lysosomal enzyme) and to increase activity of myelo peroxide enzyme and catalase in psoriasis patient compare to normal individual.[20] In psoriasis the main effect of high Li level might influence inflammation through granulocyte pathway not from lymphocyte. This was proved by the increased proliferation and decreased bactericide capacity of granulocyte but not influence lymphocyte function. This research was performed in PBMC normal individual therefore need to be proof in psoriasis patient.[21]

The serum Mn level of psoriasis patient describes manganese superoxide dismutase activity that play role in cell differentiation and proliferation.[15] This was proved with increase expression of mRNA manganese superoxide dismutase in psoriasis skin lesion.[22] Mitogenesis in lymphocyte T cell could be blockade by adding of 100 µmol/L Mn for 4-6 hours.[23] In this research the smaller concentration of Mn was used which was 0.02-0.08 µmol/L with longer evaluation time which was 3 days.

Based on positive result of climatotherapy in psoriasis patient that physical effect of bathing in Dead Sea salt water will increase desquamation effect and skin hydration, thus could increase penetration of UV into the skin, immunomodulation effect, anti inflammation effect and anti proliferation effect cause by high mineral level, releasing inflammatory mediators like leukocyte elastase and cytokine.[12,14] Therefore, beside *in vitro* effect to PBMC also need to consider the clinical effect of

bathing in Dead Sea salt water for the successful of Balneophototherapy in psoriasis.

CONCLUSION

Mangan and Li in this research were proved not significantly influence the viability of PBMC in low dose (sub MED) and the viability tends to decrease at doses 2 MED of NB-UVB exposure. Therefore, it was suspected that Mn and Li level was not an obstacle projector for psoriasis healing process. Further research is really needed to know the influences of Mn and Li concentration which higher in psoriasis patient serum and influences of Mn and Li to other cell which also play role in psoriasis pathogenesis, like dendritic cell, fibroblast, macrophage and keratinocyte cell.

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