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Restorative Effect of MDP-Lys (L18) on Leukopenia of Cancer Patient Treated with Radiotherapy

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癌患者における放射線療法後白血球減少に対するMDP-Lys（L18）の回復効果

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各種癌患者に対する放射線治療中にみられた白血球減少（≦3,000/mm³）症例に対してmuramyl-dipeptideであるMDP-Lys(L18)；DJ-7041を2週にわたり200μg連日10回（I群）あるいは400μg隔日6回（II群）投与し，その白血球数回復効果を検討した．全体では投与開始前（Day 0）白血球数は平均2,409/mm³であり，投与終了時（Day 14）は平均3,394/mm³であった．主治医有効率は両群で14/23（60.9%）であった．また，投与群間で経時的平均白血球数に差を認めず，有効率も差はなかった．

主な副作用は発熱であり，I群で41.2%，II群で63.6%に認められた．その他注射部位に疼痛を主体とした局所反応および関節痛が観察された．発熱と白血球数の回復促進作用との関係では，発熱が認められた症例で回復が早く，特に桿状核球が発熱の認められなかった症例と比較し増加していた．

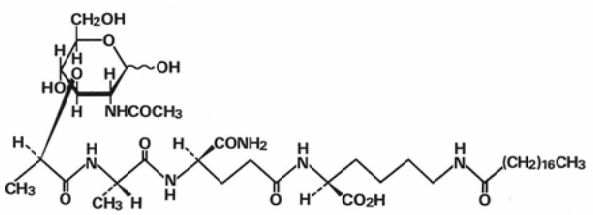
またリンパ球サブセットについては両群ともT4/8比がやや増加する傾向にあった．

以上のことよりMDP-Lys（L18）は放射線照射による白血球減少に対し有効であり，有効性および安全性の評価から200μg連日投与は400μg隔日連日投与に比べより有用であった．

Introduction

In radiotherapy, cancer patients are damaged in normal tissues as well as tumor cells. One of the most serious problems is leukopenia, which may lead to serious infection[1)2)].

MDP-Lys (L18); DJ-7041 is a derivative of muramyl-dipeptides, invented by Daiichi Seiyaku Co.Ltd. The chemical structure is shown in Fig. 1. This compound has activities to augment the host defense mechanisms of normal and immunocompromised animals against infections by *E. coli, P. aeruginosa, S. aureus, C. albicans, S. enteritidis, Corynebacterium kutscheri,* Sendai virus, vaccinia virus, HSV-2[3)~9)]. Mechanisms of the potentiation are suggested to stimulate the function of polymorphonuclear leukocytes[10)], interleukin 1 production[11)] and complement production[12)]. The increase of neutrophil via colony-stimulating factor (CSF) was also shown in leukopenia mice induced with cyclophosphamide and



N^2-[(*N*-acetylmuramoyl)-L-alanyl-D-isoglutaminyl]-N^6-stearoyl-L-lysine

Fig. 1　Chemical structure of MDP-Lys (L18); DJ-7041.

X-ray irradiation[13)14)].

Furthermore, restorative effect of the agent was confirmed in patients receiving anticancer chemotherapies. Recovery of leukocyte count in leukopenia has been shown in a dose-dependent manner[15)].

In this study, we confirmed that MDP-Lys (L18) restored leukocyte count in patients with leukopenia during irradiation treatment of cancer.

Materials and Methods

Cancer patients who developed leukopenia ($\leqslant 3000/mm^3$) during radiation therapy received MDP-Lys (L18). Safety and beneficial effect of MDP-Lys (L18) on leukocyte count were studied under the continued radiation therapy. Patients with age younger than 15 and older than 80, seriously reduced liver function, history of allergy, DIC, endotoxemia, active tuberculosis and apparent bacterial infection were excluded. Pregnant women and women of child-bearing potential were also excluded. Prior to the study, informed consent was obtained from all the patients or their legal guardians. The trial was performed June, 1986 to March, 1987.

Drug: 200 μg or 400 μg MDP-Lys (L18) was dissolved in 1 ml and 2 ml of water for injection, respectively. Patients were allocated to two groups. Patients of Group I were given 200 μg subcutaneously for 5 consecutive days and Group II 400 μg on 3 alternate days over a week. Patients receiving irradiation therapy were injected with MDP-Lys (L18) within 30 minutes after daily irradiation.

Concomitant medication: Anticancer drugs except the irradiation, drug influencing leukocyte count and blood transfusion were prohibited. But prophylactic use of antipyretics or antibiotics was permitted.

Observation: White blood cell (WBC) count and differential count were measured on Days 4, 7, 14 and 21 (after the commencement of MDP-Lys (L18) administration). Hematology, blood chemistry including renal and liver functions were carried out prior to treatment, and on Days 7, 14 and 21. In order to study the effect of MDP-Lys (L18) on immune system, lymphoblast transformation by PHA as a mitogen and lymphocyte subpopulation ($T3^+$, 4^+, 8^+, and IgG-FcR^+ T, $Ia1^+$) were examined prior to treatment and on Days 7, 14 and 21.

Evaluation: Restorative effect of MDP-Lys (L18) on leukopenia was evaluated in four grades; extremely increased (excellent), moderately increased (good), unchanged (fair) and decreased (poor). Safety evaluation was made in four grades; no side effect, side effect without treatment, side effect with treatment and side effect which caused withdrawal from the study. Usefulness was also judged in four categories; extremely useful, useful, fairly useful and not useful based on restorative effect of leukocyte and safety evaluation. These evaluations were made by the doctors in charge.

Results

Twenty-eight cancer patients, 7 males and 21 females, were enrolled in this study as shown in Table 1. The age ranged from 35 to 78 years old. Nine had uterine cancer, 5 malignant lymphoma and 6 breast cancer. They received radiotherapy with X ray and/or γ ray for cancer therapy, which lead to myelosuppression. The fraction dose was 1.8—2 Gy (180—200 rad)/day and 4—5 times/week. Total dose of radiation ranged from 8 to 40 Gy (800 to 4000 rad) at the enrollment.

Twenty-eight patients were included in the analysis of safety. After excluding 3 patients (each for no measurement of leukocyte count, DIC and diarrhea due to radiation), 25 patients were evaluated for usefulness. Total 23 patients were analyzed for effectiveness after excluding 2 patients who discontinued medication due to adverse reaction.

As shown in Fig. 2, the recovery of white blood cell (WBC) was confirmed in MDP-Lys (L18) administration during 2 weeks. In differential counts of WBC, increase of neutrophil was prominent.

Table 1　Patient characteristics

	Items	Group I 200μg consecutive-day dosing	Group II 400μg alternate-day dosing
Sex	male	7	
	female	10	11
Age	—39	2	3
	40—49	3	1
	50—59	4	2
	60—69	2	3
	70—	6	2
	mean±SE	58.9±3.6	54.5±4.4
	range	36—78	35—78
Cancer of	uterine (cervix)	3	6
	lung	1	
	mediastinum	1	
	malignant lymphoma	5	
	breast	3	3
	testis	1	
	ovary	1	
	oral cavity		1
	thyroid		1
	larynx	1	
	rectum	1	

Table 2　Effectiveness of MDP-Lys(L18) judged by physicians in charge

Items	excellent	good	fair	poor	statistical evaluation*
Group I		7	4	2	W : NS
(200μg consecutive-day dosing)	(53.8%)				
Group II		7	1	2	F : NS
(400μg alternate-day dosing)	(70.0%)				

*W ; Wilcoxon test, F ; Fisher exact test　NS ; not significant

Monocyte and basophil also increased. Although Group II (400 μg alternate day dosing) showed greater value of WBC on Day 7 than Group I (200 μg consecutive day dosing), no statistical significance was observed. WBC of both groups recovered in the same manner.

Clinical effectiveness evaluated by doctors in charge was shown in Table 2. Effective rates were 53.8% in Group I and 70.0% in Group II.

Fever was observed dose-dependently in 41.2% (7/17) of Group I and 63.6% (7/11) of Group II (Table 3). The other adverse effects were local reaction at injection site mainly with pain, tenderness and redness; pain in joint; burning sensation; headache; urticaria; eruption; nausea; and pain in anterior chest wall. Increase of eosinophil was observed in a patient with eruption. Increase of C-reactive protein and erythrocyte sedimentation rate were also observed. Safety evaluation by doctors is shown in Table 4. The incidence of side effect of Group II was 81.8% (9/11), including 3 cases in which MDP-Lys (L18) was discontinued.

Positive correlation between increase in fever and WBC was suggested as shown in Fig. 4. But, WBC count increased by more than 800/mm^3 as well in the patients who did not develop fever. The patients with

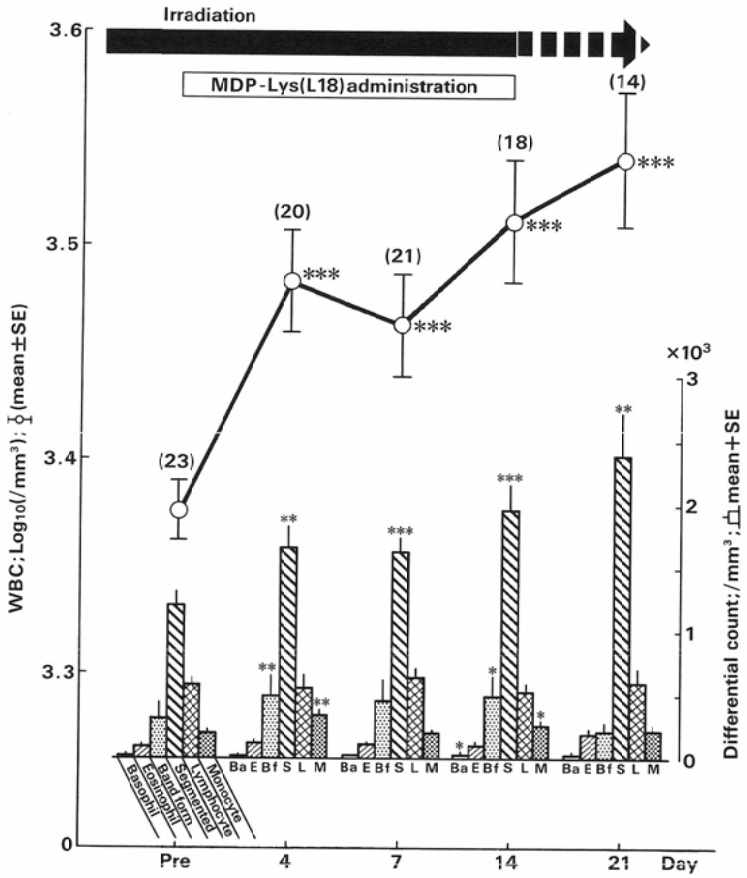


Fig. 2 Restoration of WBC and differential count by MDP-Lys (L18).
*; $p<0.05$, **; $p<0.01$, ***; $p<0.001$.

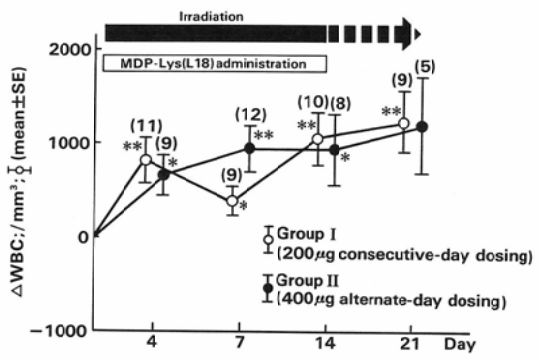


Fig. 3 Increases of WBC classified by dosing groups.
*; $p<0.05$, **; $p<0.01$.

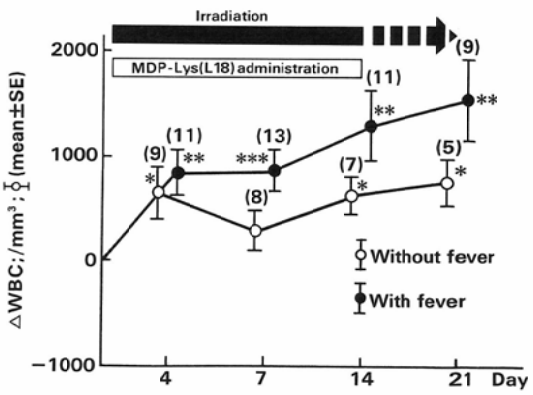


Fig. 4 Increases of WBC with or without fever.
*; $p<0.05$, **; $p<0.01$, ***; $p<0.001$.

Table 3　Side effects and abnormal findings

Items	Group I 200μg consecutive-day dosing	Group II 400μg alternate-day dosing
Fever	7/17(41.2%)	7/11(63.6%)
Local reaction at injection site	2/17(11.8%)	3/11(27.3%)
Pain in joint	1/17(5.9%)	2/11(18.2%)
Burning sensation		1/11(9.1%)
Headache	1/17(5.9%)	2/11(18.2%)
Urticaria		1/11(9.1%)
Eruption	1/17(5.9%)	
Nausea		1/11(9.1%)
Pain in anterior chest wall		1/11(9.1%)
Increase of eosinophil		1/11(9.1%)
Increase of C-reactive protein	5/11(45.5%)	2/4 (50.0%)
Increase of erythrocyte sedimentation rate	2/12(16.7%)	1/4 (25.0%)

Table 4　Safety evaluation of MDP-Lys(L18) judged by physicians in charge

Items	no side effect	side effect			statistical evaluation*1
		continue without treat.	continue with treat.	withdrawal for	
Group I	10	2	4	1*2	W : P=0.0444
(200μg consecutive-day dosing)			(41.2%)		
Group II	2	3	3	3*3	χ^2 : P=0.0797
(400μg alternate-day dosing)			(81.8%)		

*1 : W ; Wilcoxon test, χ^2 ; Chi square test

*2 : The reason of withdrawal was fever with pain in joint, headache and eruption which disappeared within 3 days

*3 : The reasons of withdrawal were fever, fever with headache and headache, nausea, urticaria, pain in joint and pain in anterior chest wall. These symptoms disappeared within 4 days

Table 5　Usefulness of MDP-Lys(L18) judged by physicians in charge

Items	extremely useful	useful	fairly useful	not useful	statistical evaluation*
Group I		7	5	2	W : NS
(200μg consecutive-day dosing)	(50.0%)				
Group II		5	4	2	χ^2 : NS
(400μg alternate-day dosing)	(45.5%)				

*W ; Wilcoxon test, χ^2 ; Chi square test, NS ; not significant

fever, however, had prominent increase of band form leukocyte compared with those without fever.

Lymphocyte count was not changed during the observation period. But T4/8 ratio increased slightly during MDP-Lys (L18) administration. There was no significant change of other parameters, $T3^+$, $T4^+$, $T8^+$, IgG-FcR^+T, $Ia1^+$ and lymphoblast transformation by PHA (Fig. 5).

As shown in Table 5, usefulness of MDP-Lys (L18) was slightly better in Group I (50.0%, 7/14) than in Group II (45.5%, 5/11).

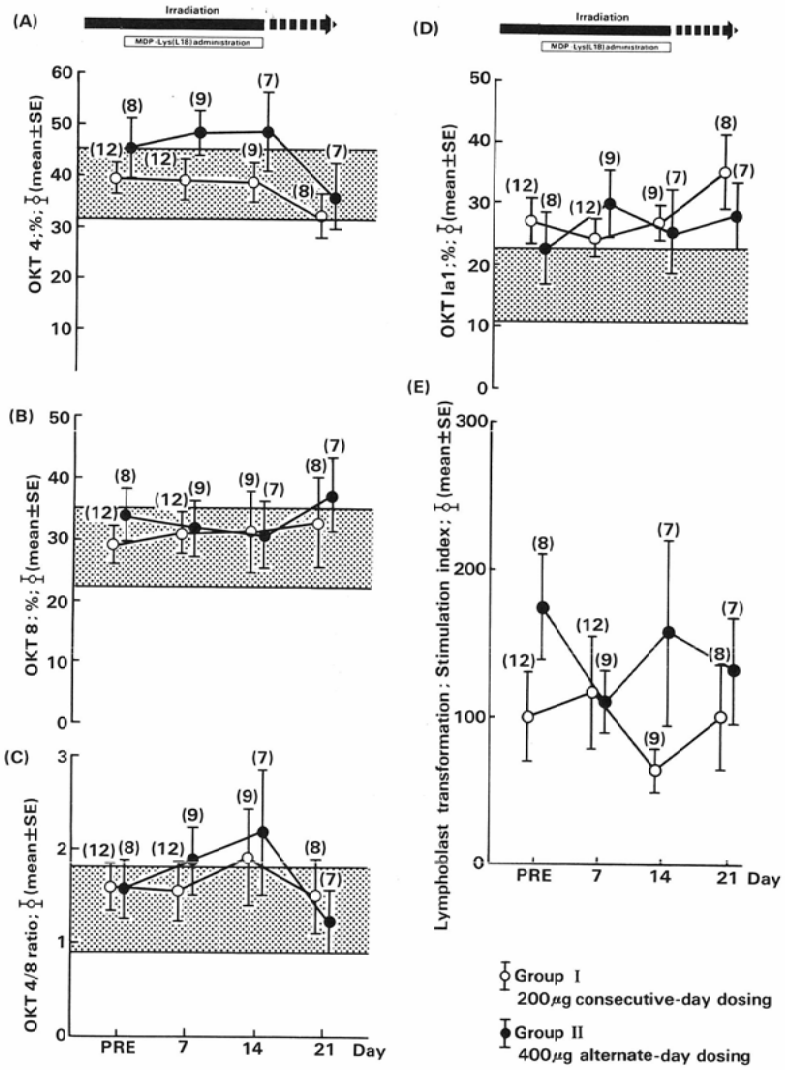


Fig. 5 Immunological parameters.
A; $T4^+$, B; $T8^+$, C; T4/8 ratio, D; $Ia1^+$, E; Lymphoblast transformation by PHA.

Discussion

Radiotherapy is known to suppress the function of phagocytes and the immunity to micro-organisms[16)17)]. It is reported that the function of lymphocytes was also suppressed with irradiation[18)19)]. Leukopenia is one of the parameters for immunosuppression by radiotherapy.

If irradiation is not stopped, WBC which reduced the number during radiotherapy cannot increase the number without treatment[20)21)]μ. For clinical successful radiotherapy, to prevent WBC decrease is critical. This study was conducted to confirm the therapeutic effect of MDP-Lys (L18) on WBC restoration in patients with leukopenia ($\leqslant$3000/mm^3).

Previous dose study suggested that 200 μg or 400 μg of MDP-Lys (L18) were potent to recover from WBC nadir, the same doses were selected in this study to confirm the potency.

Results of this study confirmed the restorative effect on WBC, especially on neutrophils as reported in the previous report[15]. Mechanism of leukocyte increase by MDP-Lys (L18) has been reported as the potentiation of colony stimulating factor (CSF) production by macrophages. MDP is known to stimulate the production of CSF in vitro[22)~24)]. On the other hand, it has been reported that the in vitro potentiation by MDP-Lys (L18) of CSF from macrophages is 10 times greater than its parent compound, MDP[11]. Although macrophages/monocytes produces G-CSF, we have not yet clarified what kind of CSF is produced after injection of MDP-Lys (L18) in vitro and in vivo. Increase of neutrophils, monocytes and basophils in this study suggested a strong possibility of potentiation of the other type of CSF as well as G-CSF.

Another possibility of this restorative effect in peripheral blood is mobilization of mature WBC from the marginal pool. This mobilization of WBC could be also stimulated by interleukin 1 (IL-1). It has been reported that MDP-Lys (L18) stimulates the production of IL-1 from macrophages in vitro, which action was stronger than MDP[11]. Furthermore, 41.2% and 63.6% of the patients developed dose-dependent fever in Group I and Group II, respectively. The patients with fever exhibited more increase of WBC count than those without fever. These facts suggest that the primary reason for the increase in WBC count and for the development of fever lies in IL-1 which acts as an endogenous pyrogen.

Concerning the side effect, fever was seen most frequently as long as local reaction at injection. This result is similar to previous reports[15]. Fever disappeared within 24 hours, and local reaction at injection site within a few days. On the other hand, joint pain was observed in 1 and 2 patients of Group I and Group II, respectively. Joint pains were present with fever in 2 patients and disappeared within 24 hours after withdrawal. MDP derivatives are known to cause the adjuvant arthritis in rat[25)26)], but it is unknown that the joint pain observed in this study was associated with arthritis.

It is known that therapeutic radiation leads to the immunosuppression. It also decreases the number of lymphocytes, and their subpopulations[18], when it is given at a large dose. But these changes were not found in this study. This might relate to differences of irradiation method or MDP-Lys (L18) administration.

Although efficacy rate in Group II (400 μg 3 alternate days) was slightly better than that of Group I (200 μg 5 consecutive days), the latter group has less adverse reactions. These results suggest that 200 μg regimen may be recommended for further study.

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