

(4) .ベータグルカンの放射線防護効果 Radioprotection Effect of β -D-glucan

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INTRODUCTION: Recently, radioprotective agent without a side effect is sought in cancer therapy. We reviewed antioxidation, immunisation activity using mice so that radioprotection effect of β -glucan of conclusive evidence by immunological enhancement.

MATERIALS AND METHODS: Radiation protection from death and stimulating leukocyte recovery by oral administrations consecutively of β -D-glucan (*Enterococcus Faecalis* 2001), 200 mg/kg and 400mg/kg b.w., once a day, before whole-body x-rays irradiation was confirmed by tests with C3H mice, meanwhile, its radioprotective actions compared to immunological enhancement. Based on the studies of survival, behavior of hematograms, and numbers of lymphocytes, whole body following irradiation, it was demonstrated that β -D-glucan was an effective radioprotector.

RESULTS: The survival of irradiated mice protected by β -D-glucan was significantly increased and statistically higher than that of mice pre-treated with oral administration. After administration of β -D-glucan, enhanced CD4 and CD8 cells, numbers of NK cells and CD4 and CD8 cells in mice were found and lymphocytes numbers was higher than in irradiated control group. Stimulated recovery of leukocyte, lymphocytes, and NK cells counts were observed in mice pre-treated with EF 2001. All above-mentioned results were similar to those in mice protected by β -D-glucan, but the protecting actions of β -D-glucan on promoting recovery of nucleated cells and leukocyte counts were significantly higher than those of β -D-glucan.

CONCLUSION: It could be deduced that the uncertainly radioprotective action against death is induced by a possible process of enhanced regeneration of the leukocyte stem cells due to not only strengthened radioresistance and increased numbers of remained leukocyte cells, but also enhanced post-irradiation repair or promoted proliferation of the leukocyte stem cells. This effect of β -D-glucan may have some therapeutic implications for radiation-induced injuries. We can analyze a result of this study than this thing as follows. We think that CD 4 and CD 8 did immunological enhancement of β -D-glucan than helper T cells and suppressor T cell activation from their having been a rise. In addition, we think that indicating the activation of cell-mediated immune

(5) X線照射による筋管形成阻害に対するIGF-1の効果 Effect of IGF-1 on Inhibition of myotube formation by X-ray radiation :

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INTRODUCTION : Skeletal muscle is relatively stable tissue composed of differentiated muscle fibers. However, growth and repair of skeletal muscle are carried out in response to damage or stretch. Skeletal muscle is relatively resistant to X-ray radiation, but the formation of multinucleated myotubes is delayed or suppressed by X-ray irradiation of myoblasts^{1), 2)}.

Expression of insulin-like growth factor-1 (IGF-1) increases during generation and regeneration of skeletal muscle³⁾. The overexpression of IGF-1 result in myofiber hypertrophy⁴⁾.

PURPOSE : In this study, we investigated whether IGF-1 improve the delay or suppression of myotube formation induced by X-ray irradiation.

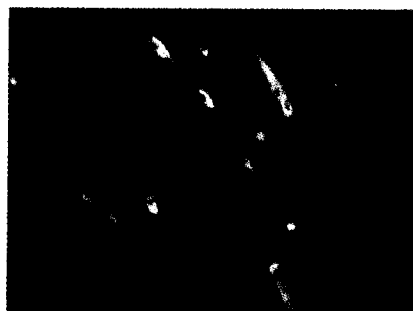
MATERIALS AND METHODS : Mouse derived myoblast C2C12 were seeded at a density of 4×10^4 cells/cm² on a 24-well cell desk plate (Sumitomo Bakelite). After an overnight culture in DMEM medium supplemented with 10% fetal bovine serum (FBS), X-ray irradiation at 2 or 4 Gy using an X-ray generator (MBR-1520R; Hitachi Medical Corporation, at 150 kV and 20 mA with an aluminum filter of 0.5 mm and a copper filter of 0.1 mm (90 cGy/min)) was conducted, and then the medium was replaced with DMEM medium with 2% FBS and the culture was continued for 6 days to induce differentiation. Differentiation of cells into myocytes and myotubes was identified by fluorescence immunostaining with an anti-myosin (skeletal muscle) monoclonal antibody

(Histofine).

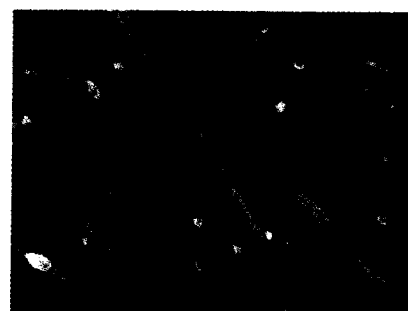
RESULTS : The results of immunostaining are shown below. Decreased myotube formation was observed after X-ray radiation of 2 Gy and this change was reversed by adding 5 ng/ml IGF-1.



0 Gy-IGF-0 ng/ml



2 Gy-IGF-0 ng/ml



2 Gy-IGF-5 ng/ml

CONCLUSION : The decrease in the number of differentiated cells and reduction in myotube formation induced by X-ray radiation of 2 Gy were inhibited by IGF-1. The X-ray irradiation at 2 Gy is used in multi-fractionated irradiation in radiotherapy, and therefore IGF-1 may be useful for reduction of radiation damage.

References

- 1) Santini MT et al., *Int J. Radiat Biol* 58: 463-474 (1990).
- 2) Ikeda S et al., *Int J Radiat Biol* 76: 403-411 (2000).
- 3) Florini JR et al., *Endocrine Rev* 17: 481-517 (1996).

β -glucanに対する放射線防護効果と免疫賦活作用
Radioprotection Effect and Immune Activity for β -D-glucan

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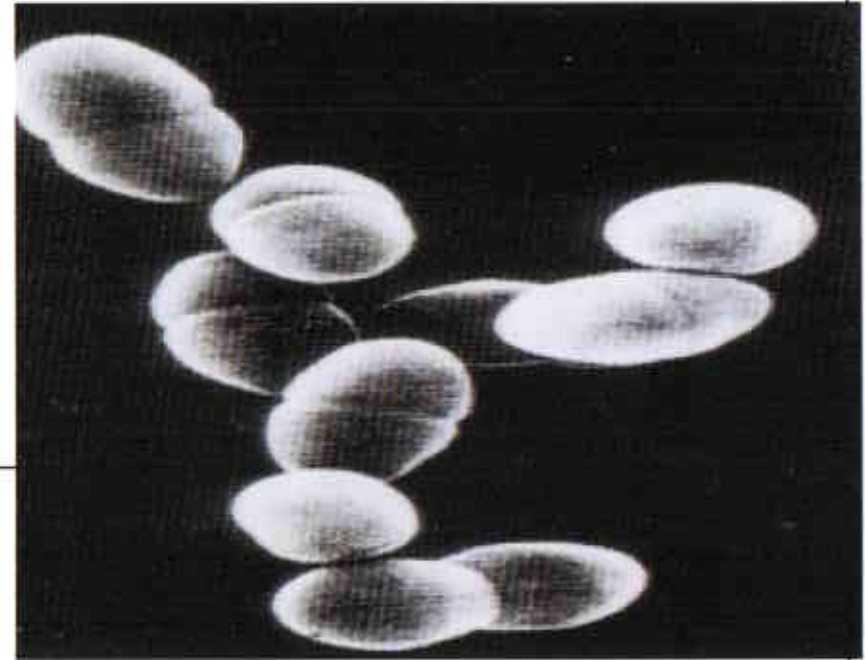
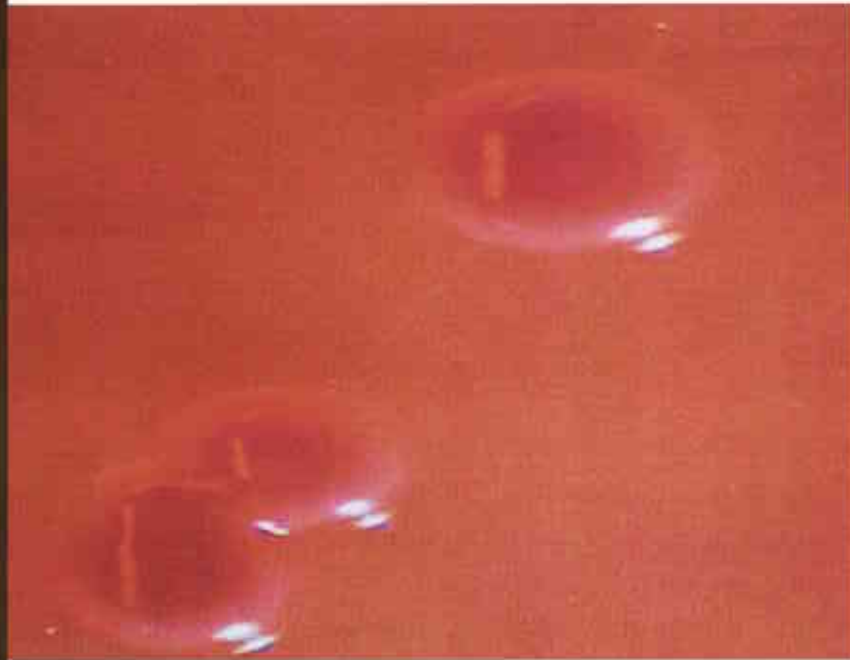
Purpose

- ・ EF2001 由来の β -glucanの免疫増強作用の検討
- ・ 血球細胞への影響
- ・ フローサイトメリーによるCD4,CD8,CD16への影響の検討
- ・ 腫瘍成長抑制との検討
- ・ 放射線治療時の放射線照射による副作用の低減の検討



放射線防護効果および免疫効果への検討

Enterococcus Faecalis



Enterococcus Faecalis under microscope (left) $\times 20$, (right) $\times 12900$

Materials and Methods



血球細胞への影響

- ・実験動物:ICRマウス(5週齢、♂、1群10匹)
- ・使用機器:X線照射装置(フィリップス社製、MG226/4.5)
:全自動血球計測器(日本光電celltac- α)
- ・投与方法: β -glucan 200, 400mg, 800mg/kg でo.p.にて毎日投与
- ・照射条件:2Gy照射(0.355Gy/min)
- ・統計処理:t- test, Dunntts-test

フローサイトメトリーによるTリンパ球の解析

- ・実験動物: C57BL (5週齢、♂、1群10匹)
- ・解析装置: フローサイトメーター (BD社製)
- ・抗体: マウスCD3/CD4/CD8/CD16および
陰性コントロール
- ・投与方法: β -glucan; 200mg/kgをo.p.にて毎日投与
- ・解析: 2Gy全身照射後、7日後



・実験群(血球細胞、CD4,CD8,CD16、がん)

- ① Control群(蒸留水)
- ② 200mg/kg投与群
- ③ 400mg投与群
- ④ 800mg/kg投与群
- ⑤ 2Gy照射群
- ⑥ 200mg/kg + 2Gy照射群

腫瘍成長抑制

- ・実験動物: ICRマウス(5週齢、♂、1群10匹)
- ・担癌方法: Cancer cells: SCC-7 (2×10^6)を右大腿部の皮下に接種
- ・投与方法: β -glucan 200mg, 400mg, 800mg/kgをo.p.にて毎日投与

・腫瘍サイズの測定:腫瘍接種後7日後から腫瘍摘出

まで1日おきにノギスで測定

・腫瘍体積の算出方法:

$$\text{腫瘍体積}[\text{mm}^3] = \frac{1}{2} \times (\text{長径}) \times (\text{短径})^2$$

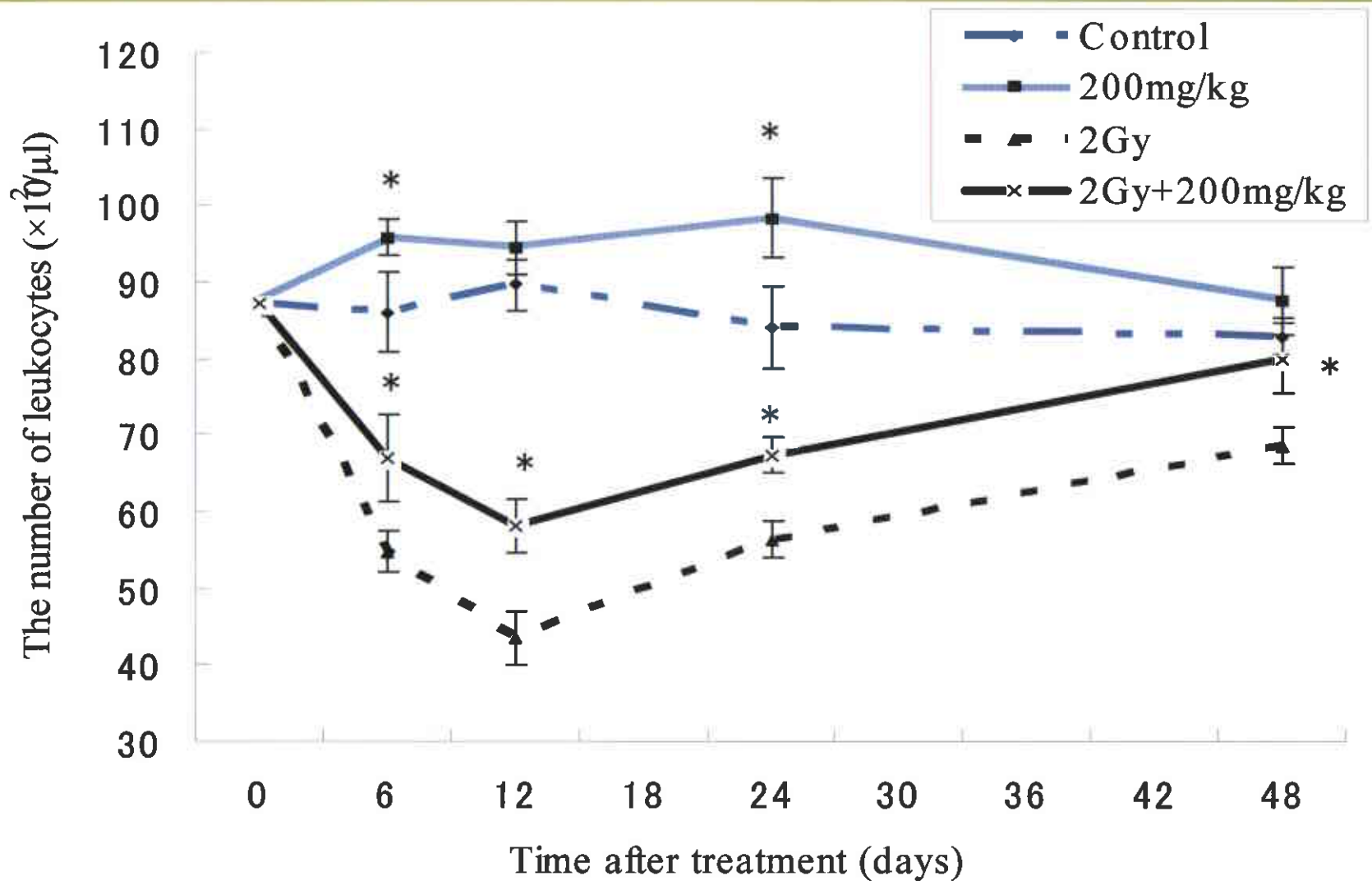
・腫瘍成長抑制率の算出方法:

$$\text{腫瘍抑制率} = (C_w - T_w) / C_w \times 100(\%)$$

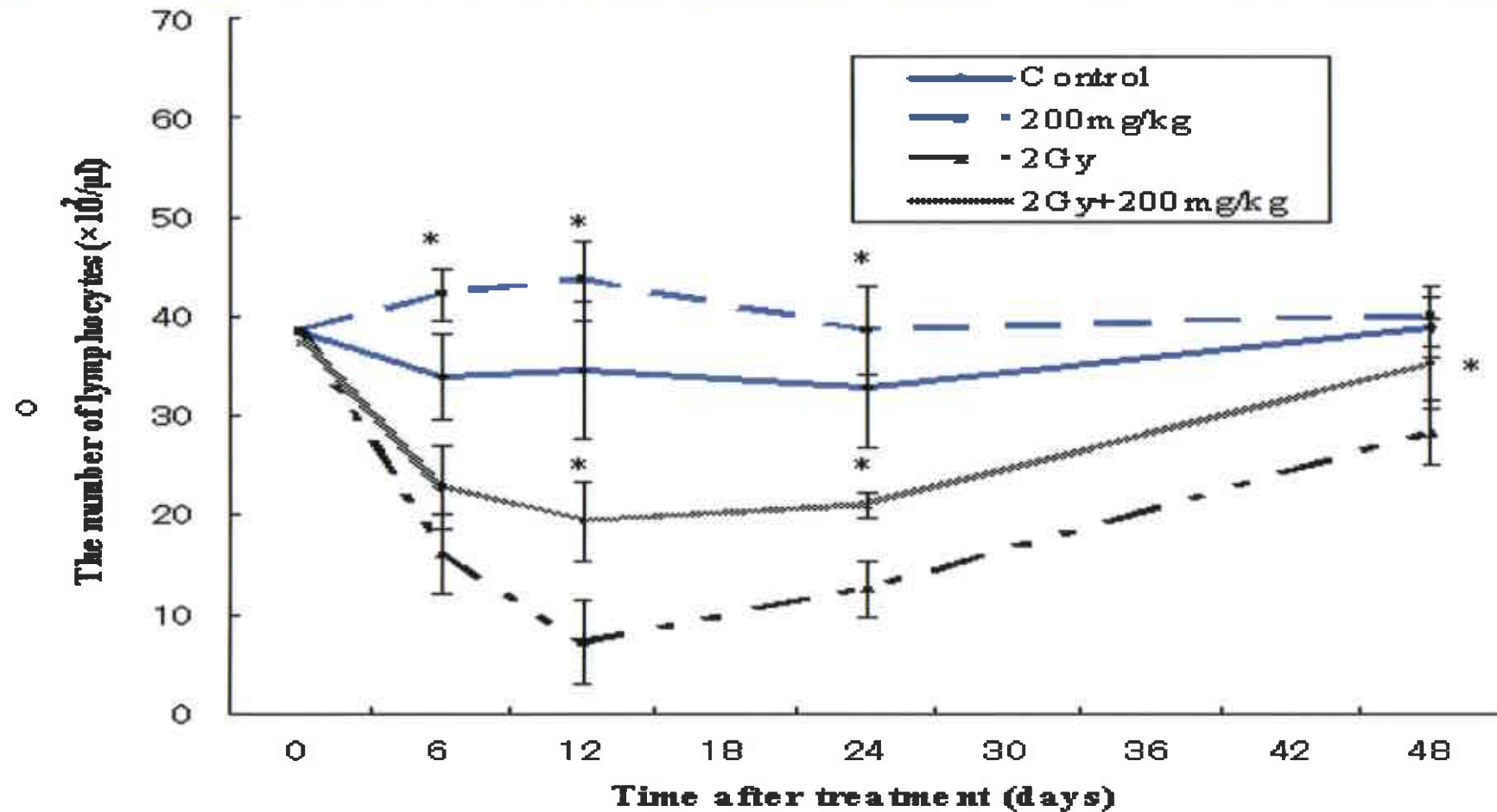
C_w :対照群の腫瘍重量の平均

T_w :検体群の腫瘍重量の平均

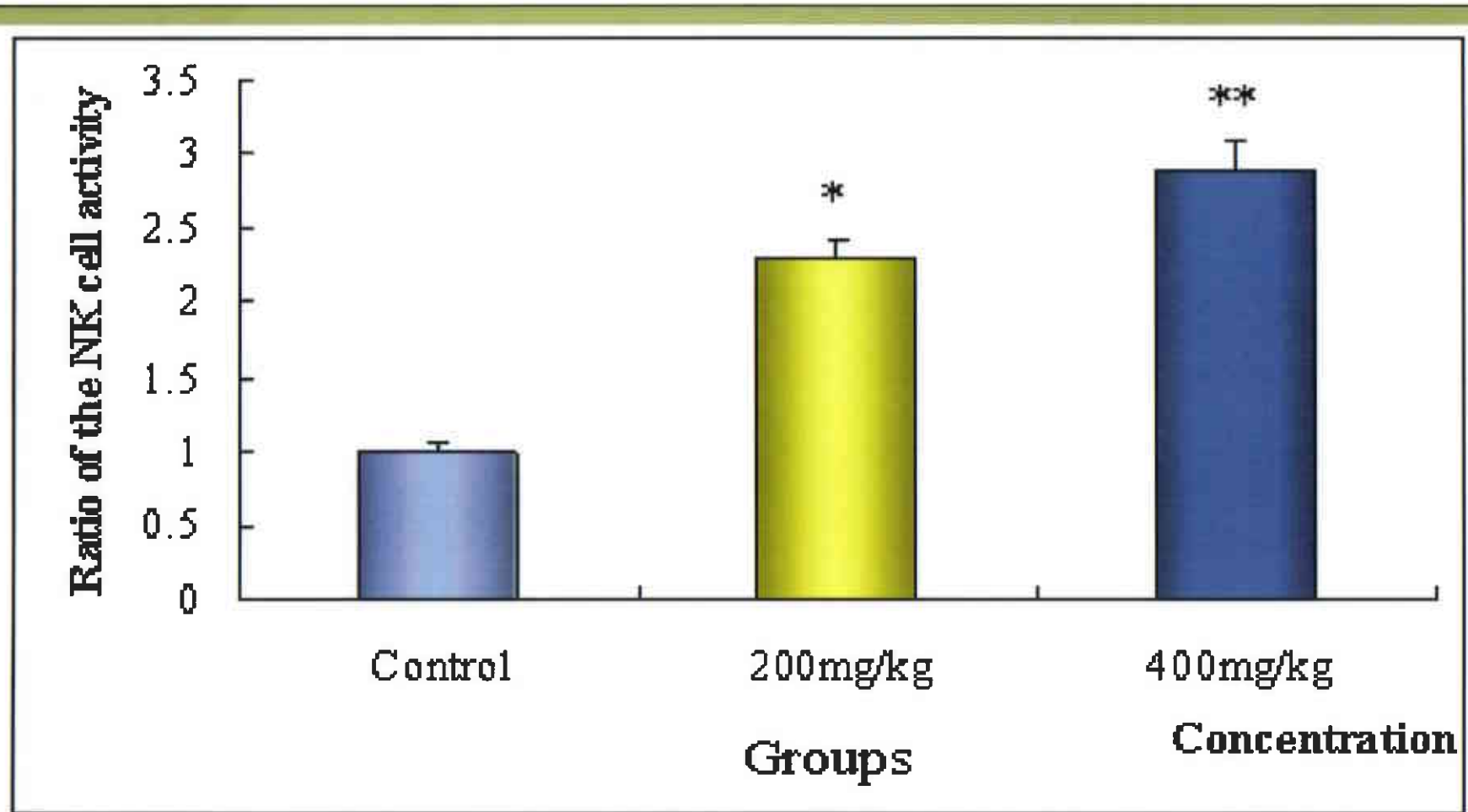
・統計処理: t-test, Dunntts-test



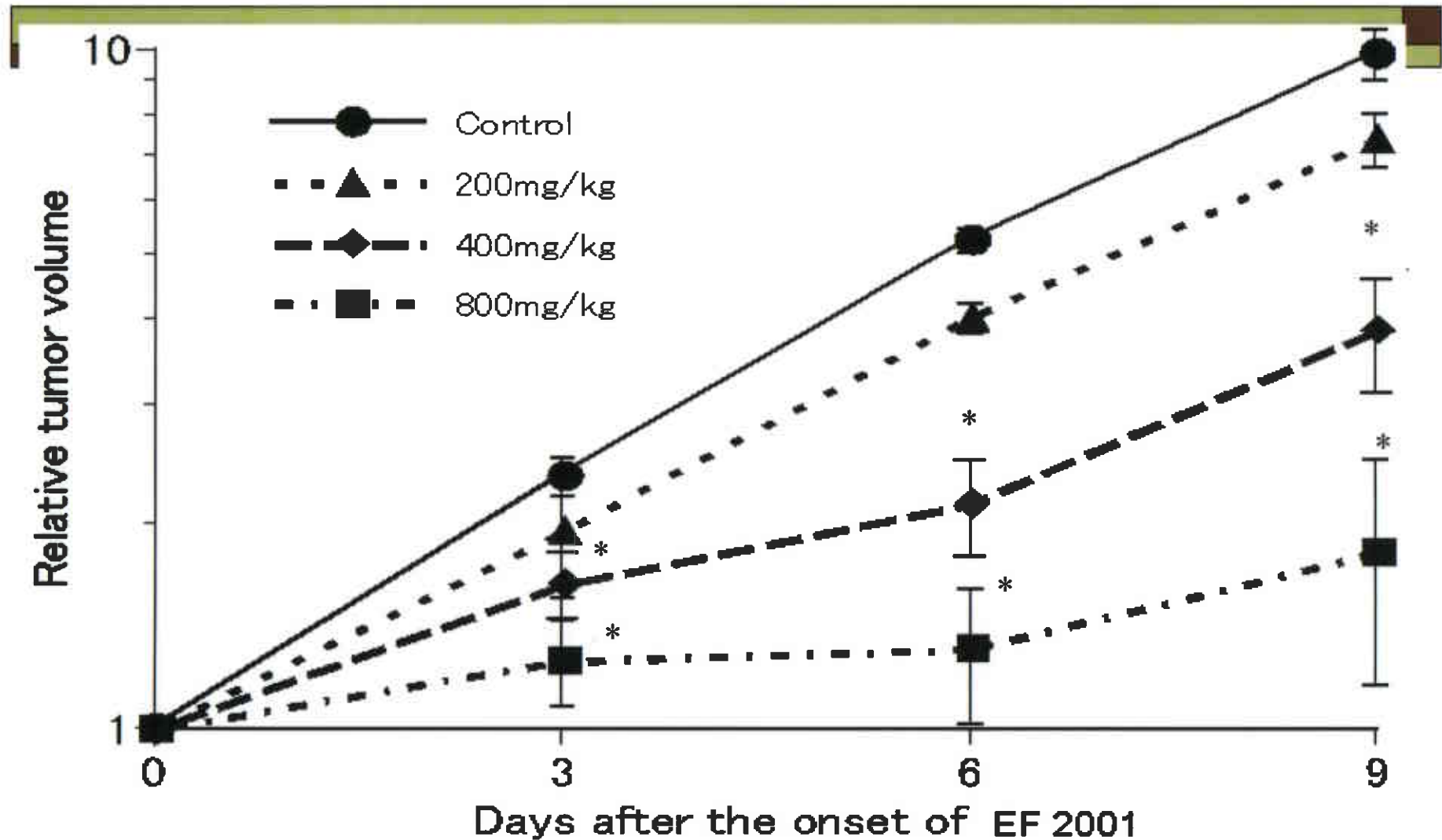
Leukocyte counts on different days after irradiation in mice of different groups. The number of leukocyte was calculated from the pre-irradiation values taken as 100%. The bars represent standard deviation. * Statistically significant ($P < 0.05$) from the control group.



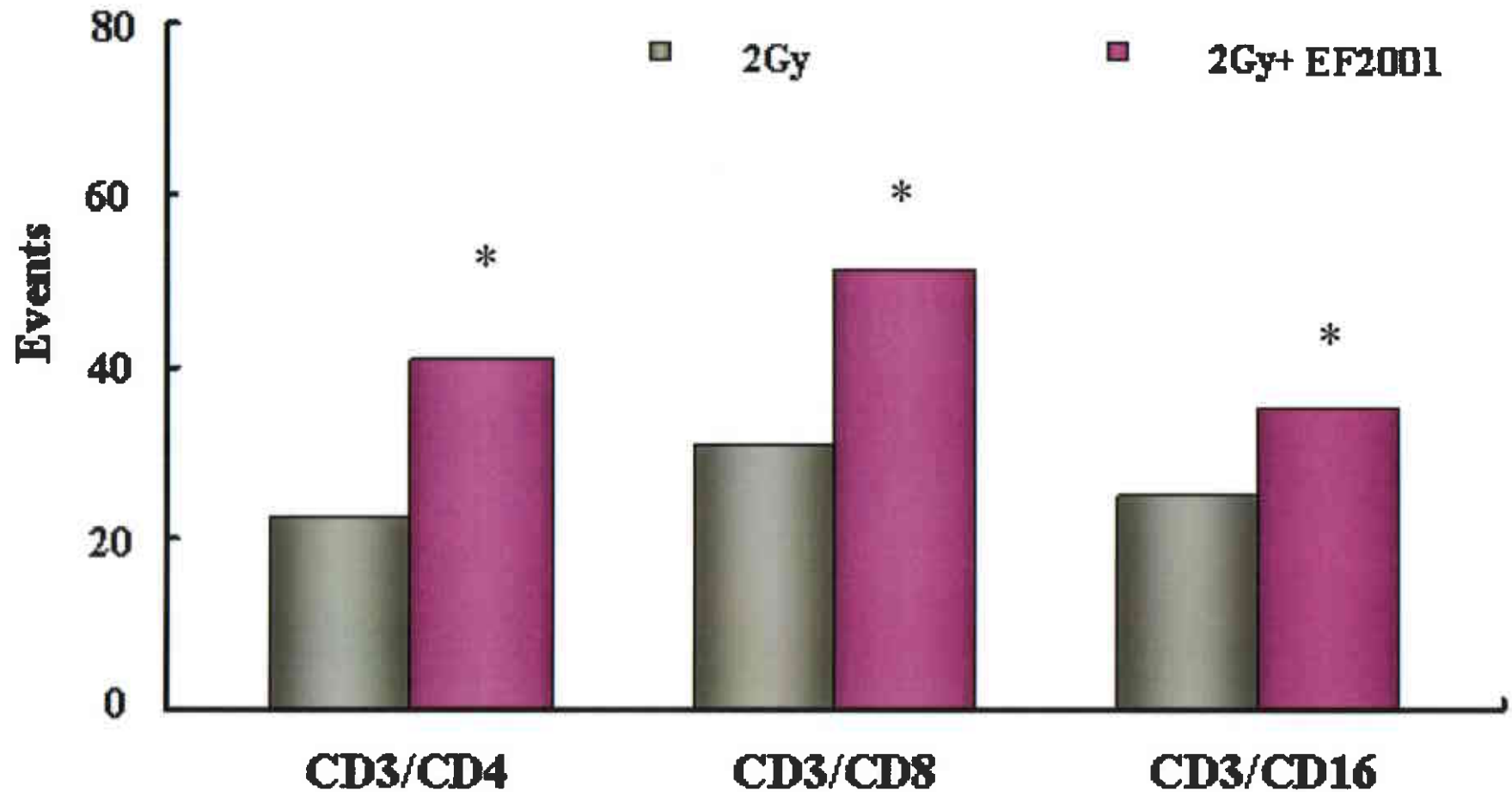
Lymphocyte counts on different days after irradiation in mice of different groups. The number of lymphocyte was calculated from the pre-irradiation values taken as 100%. The bars represent standard deviation. * Statistically significant ($P < 0.05$) from the control group.



Repeated dose effect of β -glucan on the NK activity in mice. Groups of ten mice each were subjected to each treatment. Results represent means \pm S.D. * Statistically significant ($P < 0.05$) from the control group.



Effect of β -glucan on the tumor growth in mice inoculated with SSC-7 carcinoma cells. Groups of ten mice each were subjected to each treatment. Results represent means \pm S.D. * Statistically significant ($P < 0.05$) from the control group.



The increased percentage of CD4+, CD8+ and CD16+ T-lymphocytes in PBLs compared to the experimental data baselines of the groups. The unit is in percentage (%). Significantly different from * $P < 0.05$ Control group vs. β -glucan groups by Dunnett test.

Conclusion

▲ 血球細胞への影響

照射群に比べて β -D-グルカン投与群のほうが白血球数、リンパ球数の増加、Tリンパ球数のサブセットにおいて β -D-グルカン投与によりヘルパーT細胞、キラーT細胞の増加がみられたことから、免疫増強作用が認められた。また、照射による血球数の減少が抑制され早期回復が認められたことから放射線防護効果が示唆された。



β -1,3-D-グルカン、 β -1,6-D-グルカンが免疫増強作用に関与。主として β -D-グルカンがラジカルスカベンジャーとして働き、酸化による血球の細胞膜破壊を防いで放射線防護効果と免疫作用により、PPSCからの造血が活性化されたと推測する。

▲ 抗腫瘍効果

- ▲ Control群に比べて β -D-グルカン投与群により、腫瘍成長抑制が認められた。



β -D-グルカンによりマクロファージが活性化され、マクロファージはNK、LAK細胞を活性化する。TNF α が直接腫瘍を壊死に至らしめたと考えられる。

β -グルカン

免疫増強活性 \longrightarrow β (1-3)・(1-6)D-Glucan

β (1-3)D-Glucan \longrightarrow 腸管吸収困難性から腸内壁のリンパ球の活性化
腸内善玉菌が分解(腸内細菌叢との関連)

β (1-6)D-Glucan \longrightarrow マクロファージの活性化 \Rightarrow
T-リンパ球、B-リンパ球活性

ガン治療の三大療法

外科療法、放射線療法、化学療法



いずれも人体に負担となり免疫機能を弱らせる。



副作用のない
放射線防護剤

免疫療法

Internal mechanism

