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APPLICABILITY OF THE LINEAR-QUADRATIC MODEL TO HYPOFRACTIONATED RADIOTHERAPY

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Abstract:

Background: The linear-quadratic (LQ) model is used for evaluating fractionation schedules in radiation oncology. It has been suggested that the biologically effective dose based on the LQ model cannot be applied to hypofractionated radiotherapy.

Methods: Glioblastoma cells were transplanted into nude mice. Four equivalent dose fractionation schedules (12 Gy/1 fraction (fr), 15.06 Gy/2 fr, 18.16 Gy/4 fr, and 20.96 Gy/8 fr) were calculated based on the LQ model. The tumor size was measured to evaluate the number of days required for the relative tumor volume to double and become five times the initial value (TGD_2 and TGD_5, respectively). A histopathological study was performed after the evaluation of growth delay.

Result: The mean TGD_2 of the unirradiated control group and 12 Gy/1 fr, 15.06 Gy/2 fr, 18.16/4 fr, and 20.96 Gy/8 fr groups was 5.69, 22.64, 37.26, 36.81, and 28.96 days, respectively. The mean TGD_5 was 17.83, 48.03, 60.30, 60.40, and 64.94 days, respectively. All TGDs of the irradiated groups were longer than those of the control group. However, no significant differences were observed among the four irradiated groups. Histologically, irregular giant cells were found, but there were no significant differences among the four groups. There was no significant difference in the Ki-67 labeling index and the CD133 and CD44 expression among the groups.

Conclusion: The four dose fractionation schedules equivalent to 12 Gy/1 fr yielded comparable responses, suggesting that the LQ model may be applicable to hypofractionated radiotherapy, with a high dose per fraction of up to 12 Gy.

Key Words: Linear-Quadratic (LQ) Model, Biologically Effective Dose (BED), Stereotactic Irradiation (STI), Tumor Growth Delay (TGD)

Ⅰ. Introduction

Radiation therapy has progressed dramatically over the last few decades due to the advances in radiation physics and medical engineering. These advances have significantly improved radiation dose distribution in radiotherapies such as stereotactic irradiation (STI), intensity-modulated radiation therapy, and volumetric modulated arc therapy[1]. It has often been reported that these techniques provide better control of brain tumors and reduce neurotoxicity. However, it has been suggested that further studies are needed to compare the efficacy of the different radiation therapy techniques and to investigate the optimal radiation dose fractionation[1]. Several studies have focused on the radiobiological aspects of single high-dose stereotactic radiosurgery (SRS) and hypofractionated stereotactic radiation therapy (SRT) at a high dose per fraction[2, 3].

In recent years, linear quadratic (LQ) models and the biologically effective dose (BED) based on the LQ models, as follows, have been used frequently[3, 4]:

$E = n(\alpha d + \beta d^2) = \alpha (nd)(1 + d / \alpha/\beta) = \alpha$ (total dose) (relative effectiveness)

BED = (total dose) (relative effectiveness) $= E / \alpha = nd(1 + d / \alpha/\beta)$

(E: biological effect, BED: biologically effective dose, n: number of fractions, d: single dose, α/β: α/β ratio)

However, the validity of the LQ model for various fractionation schedules in hypofractionated radiotherapy is controversial. It remains unclear whether the model is appropriate for STI therapies, such as SRS and SRT[5-9].

In the present study, the relationships among the different fractionation schedules of hypofractionated radiotherapy for brain tumors were evaluated in vivo to assess the applicability of the LQ model to STI therapies, such as SRS and SRT.

Ⅱ. Materials and methods

Tumors and animals: Glioblastoma cells[10] of human origin and male nude mice that were 6 to 8 weeks old (BALB/cAJcl-nu/nu, CLEA Japan, Inc., Tokyo) were utilized in the present study. The tumors were transplanted subcutaneously into the right thigh of the mice. Each group consisted of 8 mice. However, tumors that showed evident ulceration with necrotic changes were excluded from the study because it was not possible to evaluate the tumor size precisely.

Irradiation: X-rays (MBR-1520R, Hitachi Power Solutions Co., Hitachi, Japan) were used for the irradiation process under the following conditions:

150 kVp, 20 mA, 0.5 mm Al-filter, 0.1 mm Cu-filter

Dose fractionation schedules: In this study, the LQ model was utilized, with the assumption that the α/β ratio of the tumors was 10 Gy. The equivalent dose for each fractionation schedule was calculated. A summary of the dose fractionation schedules, BED, and equivalent dose in 2 Gy fractions (EQD_2: dose delivered in 2 Gy fractions that are biologically equivalent to a total dose) is shown in Table 1. Mice were irradiated with total doses of 12 Gy/1 fraction (fr), 15.06 Gy/2 fr, 18.16 Gy/4 fr, or 20.96 Gy/8 fr depending on their group.

Table 1. Summary of the dose fractionation schedules, with BED and EQD2

Fraction number	Fraction size	Total dose	BED_{10}	EQD_2 (α/β:10)
1	12.00 Gy	12.00 Gy	22.00 Gy	26.40 Gy
2	7.53 Gy	15.06 Gy	22.00 Gy	26.40 Gy
4	4.54 Gy	18.16 Gy	22.00 Gy	26.40 Gy
8	2.62 Gy	20.96 Gy	22.04 Gy	26.45 Gy

BED, biologically effective dose; EQD_2, equivalent dose in 2 Gy fractions

Tumor volume evaluation and irradiation timing: The shorter diameter (a) and longer diameter (b) of each tumor were measured every 3 days using a caliper. The tumor volume (V) was calculated using the following formula[11]: $V = a^2b/2$. Irradiation of the tumor in the right thigh was started when the relative tumor volume exceeded 200 mm^3. The other part of the body was shielded with lead during the irradiation. In the 2 fr, 4 fr, and 8 fr groups, irradiation was carried out twice daily at intervals of more than 10 hours in order to allow for enough time for sublethal damage repair and to reduce the effect of different treatment periods.

Tumor diameters were measured every 3 days following irradiation, and the time (days) required for the relative tumor volume to double and become five times the initial value (tumor growth delay 2 and 5, TGD_2 and TGD_5, respectively) was evaluated[11]. The Kruskal-Wallis test was used to compare the growth delay. Tumors were fixed in formalin and embedded in paraffin for histopathological examination after the evaluation of growth delay. Hematoxylin and eosin staining was performed to evaluate morphological changes. Additionally, immunohistochemistry was performed using anti-Ki-67 mouse monoclonal antibody (clone 7B11, Life Technologies, CA, USA), anti-CD133 rabbit polyclonal antibody (AbFrontier, Seoul, Korea), anti-CD44 mouse monoclonal antibody (clone DF1485, Leica Biosystems, Newcastle, UK), and anti-Nestin mouse monoclonal antibody (clone 10C2, MBL, Nagoya, Japan).

This animal experiment was approved by the Nara Medical University Animal Care and Use Committee.

Ⅲ. Results

Changes in the relative tumor volume and the mean relative volume (± standard error) are shown in Figs. 1 and 2, respectively. The mean TGD (± standard deviation) in each group is shown in Table 2. The mean TDG_2 of the control group and 12 Gy/1 fr, 15.06 Gy/2 fr, 18.16 Gy/4 fr, and 20.96 Gy/8 fr groups was 5.69, 22.64, 37.26, 36.81 and 28.96 days, respectively. The mean TGD_5 was 17.83, 48.03, 60.30, 60.40, and 64.94 days, respectively. The TGD_2 and TGD_5 of all four irradiated groups were much longer than those of the control group, and three of them (15.06 Gy/2 fr, 18.16 Gy/4 fr, and 20.96 Gy/8 fr) showed significant differences when compared with the control group ($p<0.05$). However, there was no significant difference in the TGD_2 and TGD_5 among the four irradiated groups.

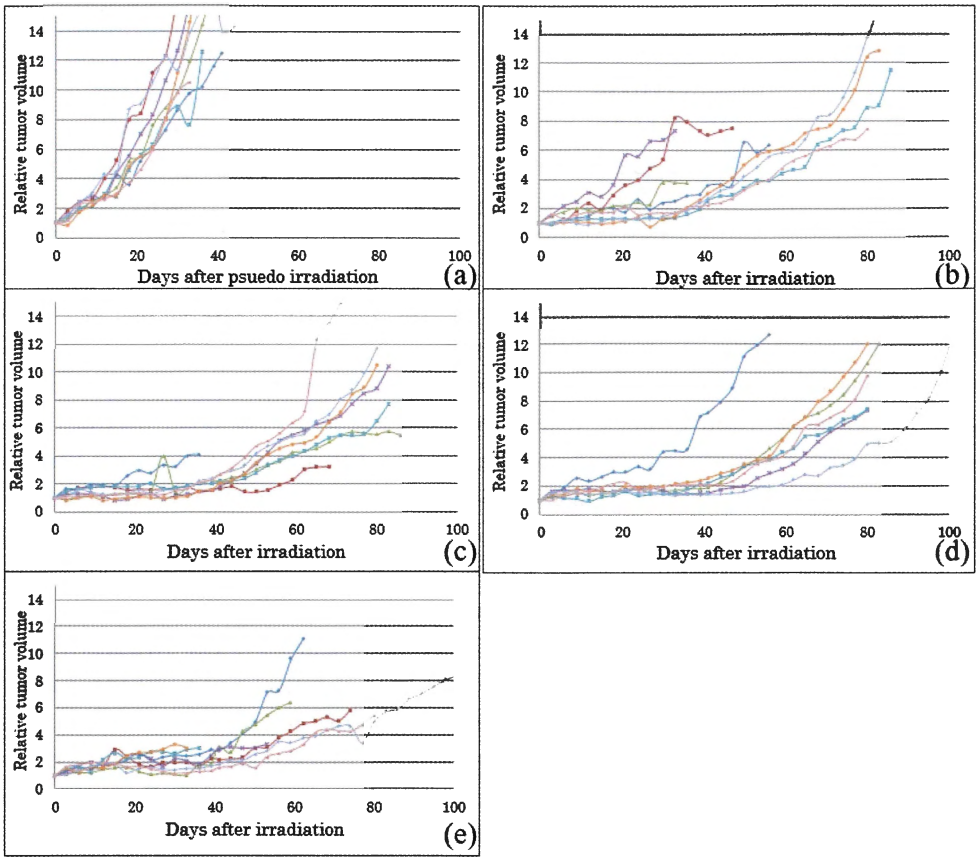


Fig. 1. Relative tumor volume after irradiation. (a) Control, (b) 12 Gy/1 fr, (c) 15.06 Gy/2 fr, (d) 18.16 Gy/4 fr, (e) 20.96 Gy/8 fr.

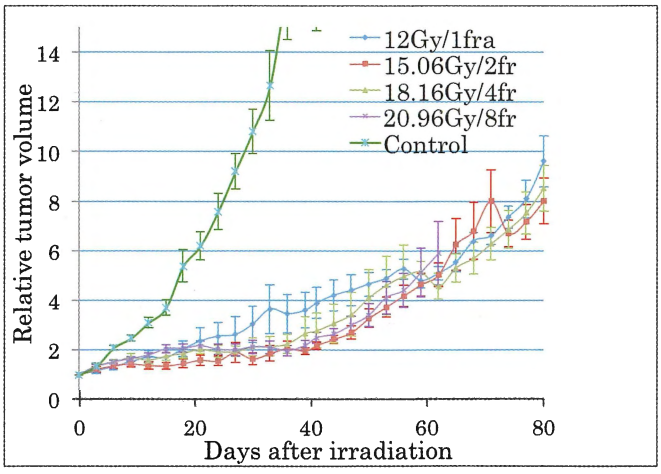
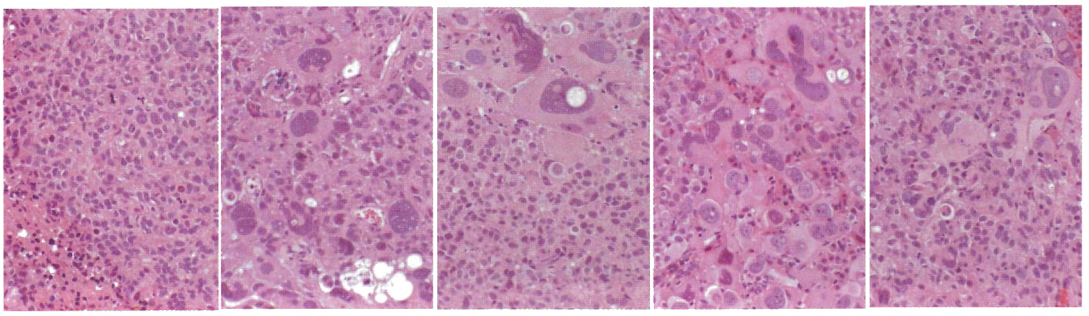


Fig. 2. Comparison of the mean relative tumor volume (± standard error)

Table 2. Mean tumor growth delay (± standard deviation): days required for the relative tumor volume to double (TGD_2) and become five times the initial value (TGD_5).

Dose/fraction	TGD_2	TGD_5
Control	5.69± 1.40	17.83±2.63
12.00 Gy/1fr	22.64±12.91	48.03±17.18
15.06 Gy/2fr	37.26±11.75	60.30± 6.46
18.16 Gy/4fr	36.81±15.81	60.40±14.10
20.96 Gy/8fr	28.96±14.93	64.94±14.23

Histologically, most tumor cells of the control group were small with round or irregular nuclei and were relatively monotonous compared with those of the irradiated groups. In the four irradiated groups, tumor cells were more pleomorphic than in the unirradiated control, and in addition to small atypical cells, irregular mono- or multi-nucleated giant cells were frequently seen (Fig. 3). However, no significant differences were found among the irradiated groups. Ki-67 immunohistochemistry and the Ki-67 labeling index (LI) are shown in Figs. 4 and 5, respectively. The Ki-67 LI of the control group and 12 Gy/1 fr, 15.06 Gy/2 fr, 18.16 Gy/4 fr, and 20.96 Gy/8 fr groups was 31.6%, 21.1%, 22.6%, 21.5%, and 16.1%, respectively. There were no significant differences among the four irradiated groups ($p>0.05$). Most of the tumor cells were CD133-positive, CD-44 positive, and weakly Nestin-positive. However, no significant differences were found among the groups (Fig. 6).



(a) (b) (c) (d) (e)

Fig. 3. Hematoxylin and eosin staining. (a) Control, (b) 12 Gy/1 fr, (c) 15.06 Gy/2 fr, (d) 18.16 Gy/4 fr, (e) 20.96 Gy/8 fr.

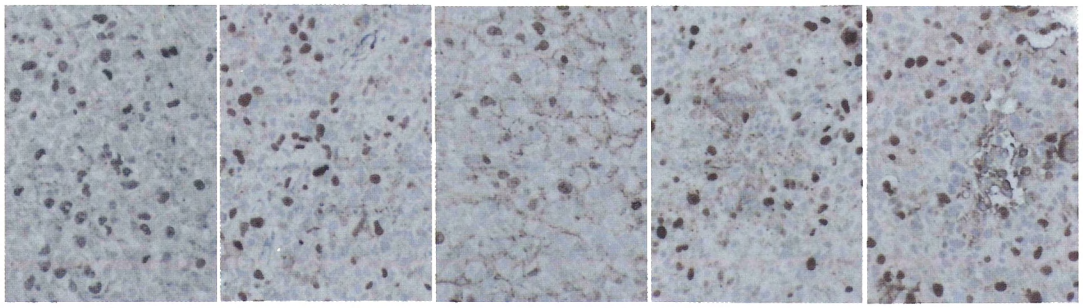


Fig. 4. Ki-67 immunostaining. (a) Control, (b) 12 Gy/1 fr, (c) 15.06 Gy/2 fr, (d) 18.16 Gy/4 fr, (e) 20.96 Gy/8 fr.

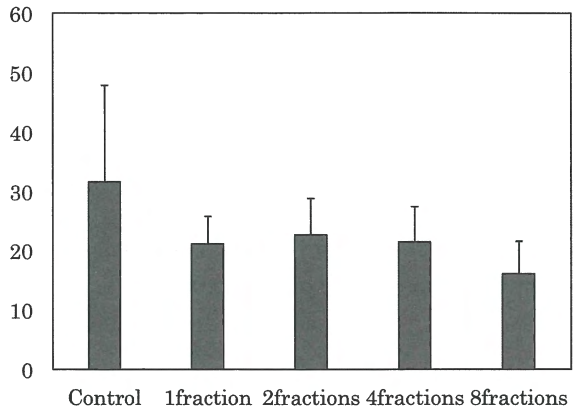


Fig. 5. Ki-67 labeling index (+standard deviation) (%).

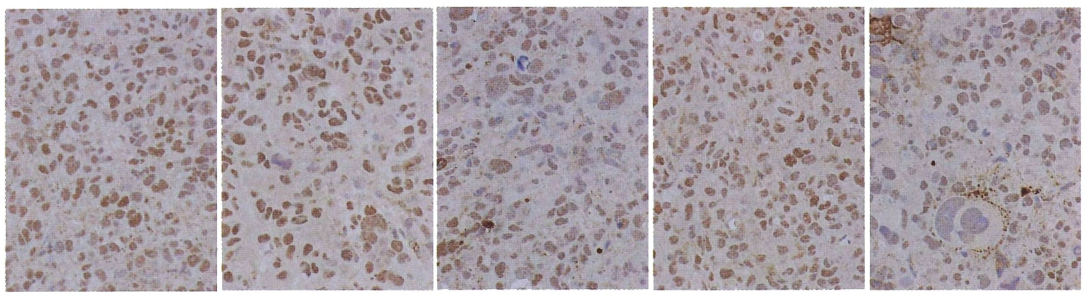


Fig. 6. CD133 immunostaining. (a) Control, (b) 12 Gy/1 fr, (c) 15.06 Gy/2 fr, (d) 18.16 Gy/4 fr, (e) 20.96 Gy/8 fr.

Ⅳ. Discussion

The LQ model is most commonly used for evaluating various dose fractionation schedules in radiation oncology. However, it is contentious whether the model applies to hypofractionated high-dose STI therapies, such as SRS and SRT, because the fraction size of STI is much larger than the conventional fraction size of 2 Gy[5-9]. Therefore, this study was conducted to examine the applicability of the LQ model to hypofractionated radiotherapy.

All four irradiated groups, 12 Gy/1 fr, 15.06 Gy/2 fr, 18.16 Gy/4 fr, and 20.96 Gy/8 fr, which are biologically equivalent according to the LQ model, showed a significant growth delay when compared with the unirradiated control group. However, no significant differences were observed among the four irradiated groups. Furthermore, many irregular mono- or multi-nucleated giant cells, which were rare in the control group, were observed in the histopathological examinations of the four irradiated groups. However, no significant differences among the four irradiated groups were observed. The immunohistochemical examination demonstrated that the Ki-67 LI of the four irradiated groups was lower than that of the control group. However, no significant differences were found among the four irradiated groups. The immunohistochemical examination of CD133, CD44, and Nestin expression did not demonstrate any significant differences among the groups.

These results do not necessarily imply that the radiation effects of the four fractionation schedules were equal. However, the results do suggest that these four fractionation schedules have similar or equivalent biological effects.

When calculating the equivalent BED for tumors, the α/β ratio is usually assumed to be around 10 Gy, because a tumor's α/β ratio is reported to be as large as about 10 Gy[4, 12] for common radiation therapy that uses doses of around 2 Gy. However, if the fraction size of a single irradiation is much larger than 2 Gy, there is a possibility of additional effects resulting from endothelial cell damage, enhanced tumor immunity, and stem cell damage [5, 13]. Furthermore, the α/β ratio of the blood vessels and stroma is usually assumed to be as small as 2 or 3 Gy. The LQ model allows us to predict that the effect of a single high-dose irradiation might be more than that calculated by the LQ model using the α/β ratio of 10 Gy[4].

In the present in vivo study, neither growth delay nor the histopathological results revealed any significant differences in the radiation effects among the four different fractionation schedules. This is in contrast to the above prediction. Morphological changes in tumor cells were similar in the irradiated groups, but vascular changes were not evident. Brenner[7] suggested that the LQ model was reasonably well-validated, both experimentally and theoretically, up to about 10 Gy/fraction, and would be reasonable for use up to about 18 Gy/fraction.

In addition to its effect on blood vessels, Kirkpatrick et al.[5] suggested that high-dose-per-fraction radiotherapy may affect the radioresistant subpopulations of tumor cells, such as cancer stem cells, differently. Immunohistochemically, the expression of the cancer stem cell-related markers, CD133, CD44, and Nestin, showed no significant differences among the four fractionation groups. However, the various limitations of this study have to be considered.

Otsuka et al.[6] strongly suggested that LQ formalism and BEDs should not be used for *in vivo* tumor responses to high-dose-per-fraction radiotherapies such as STI. In contrast to their con-

clusion, our study showed that the equivalent dose fractionation calculated using BED_{10} resulted in comparable responses, suggesting that its use may be appropriate for such tumors. Nevertheless, further studies are required to evaluate the effect of fraction doses greater than 12 Gy.

V. Conclusion

A comparison of four equivalent dose fractionation schedules calculated using the LQ model showed comparable responses *in vivo*. The results suggest that the BED may be applicable to hypofractionated radiotherapy with a high dose-per-fraction of up to 12 Gy.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content in and writing of the paper.

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References

1) Scaringi C, Agolli L, and Minniti G. : Technical Advances in Radiation Therapy for Brain Tumors. Anticancer Res. **38** : 6041-6045, 2018.
2) Park, H. J., Griffin, R. J., Hui, S., Levitt. S. H., and Song, C. W. : Radiation-induced vascular damage in tumors : Implications of vascular damage in ablative hypofractionated radiotherapy (SBRT and SRS). Radiat. Res. **177** : 311–327, 2012.
3) Joiner, M.C. and Kogel, A.J. : Basic Clinical Radiobiology. 4th ed., Hodder Arnold, London, pp100-134, 2009.
4) Hall, E. J., and Giaccia, A. J. : Radiobiology for the radiologist. 8th ed., Lippincott Williams & Wilkins, Philadelphia, pp429-434, 2019.
5) Kirkpatrick, J. P., Meyer, J. J., and Marks, L. B. : The linear-quadratic model is inappropriate to model high dose per fraction effects in radiosurgery. Semin. Radiat. Oncol. **18** : 240–243, 2008.
6) Otsuka, S., Shibamoto, Y., Iwata, H., Murata, R., Sugie, C., Ito, M., and Ogino, H. : Compatibility of the linear-quadratic formalism and biologically effective dose concept to high-dose-per-fraction irradiation in a murine tumor. Int. J. Radiat. Oncol.Biol.Phys. **81** : 1538-1543, 2011.
7) Brenner, D. J. : The Linear-Quadratic model is an appropriate methodology for determining isoeffective doses at large doses per fraction. Semin. Radiat. Oncol. **18** : 234–239, 2008.

8) Matsuyama, T., Kogo, K., and Oya, N. : Clinical outcomes of biological effective dose-based fractionated stereotactic radiation therapy for metastatic brain tumors from non-small cell lung cancer. Int. J. Radiat. Oncol. Biol. Phys. **85** : 984-990, 2013.

9) Kim, Y. J., Kwan, H. C., Kim, J. Y., Lim, Y. K., Min, H.S., Lee, S. H., Kim, H. J., Gwak, H.S., Yoo, H., and Lee, S. H. : Single-dose versus fractionated stereotactic radiotherapy for brain metastases. Int. J. Radiat. Oncol. Biol. Phys. **81** : 483-489, 2011.

10) Ishiuchi, S., Tsuzuki, K., Yoshida, Y., Yamada, N., Hagimura, N., Okado, H., Miwa, A., Kurihara, H.,Nakazato, Y., Tamura, M., Sasaki, T., and Ozawa, S. : Blockage of Ca(2+)-permeable AMPA receptors suppresses migration and induces apoptosis in human glioblastoma cells. Nat. Med. **8** : 971-978, 2002.

11) Joiner, M.C. and Kogel, A.J. : Basic Clinical Radiobiology. 4th ed., Hodder Arnold, London, pp91-92, 2009.

12) Joiner, M.C. and Kogel, A.J. : Basic Clinical Radiobiology. 4th ed., Hodder Arnold, London, p122, 2009.

13) Brown J.M., Carlson D.J., and Brenner D.J. : The tumor radiobiology of SRS and SBRT : are more than the 5 Rs involved? Int. J. Radiat. Oncol. Biol. Phys. **88** : 254-62, 2014.