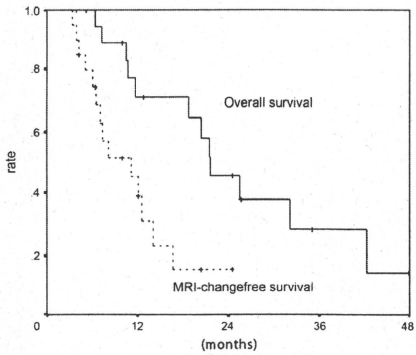

Fig. 3. Kaplan-Meier estimates of overall survival rate and progression-free survival rate for all 20 patients.

yielding a median survival period of 17 months. Compared to the standard schedule x-ray radiotherapy, with a median survival of <15 months (9, 10) or recent IMRT treatments with or without boost and hypofractionation (22, 23), those particle studies, including our present study, may indicate that dose escalation with particle radiotherapy has a potential to improve survival; nevertheless, prominent improvement has not yet been achieved.

It has been reported that age, extent of tumor resection, performance status, tumor size, and tumor location are prognostic factors for survival of patients with GBM (7, 24–26). In addition, Mirimanoff *et al.* (27) determined whether RPA retains its overall prognostic value and what the benefit of concurrent temozolomide is in each RPA class. The overall survival was statistically different among RPA classes III and IV, with median survival times of 17 and 15 months, respectively. Although there are selection biases, *e.g.*, tumors treated were <4 cm in diameter and did not involve risky regions, our data showed better survival, with a median survival period of 21.6 months, than that for the patients of RPA class III, despite the fact that 17 of 20 patients were classified as RPA class IV (Tables 1 and 3).

There has been no prospective large-scale phase I dose escalation study, until the recent report of Tsien *et al.* (28), who reported the results of a multiinstitutional clinical study in which the dose was escalated from 66 to 84 Gy, using a uniform three-dimensional conformal x-ray radiotherapy protocol (33). That study's results indicated that doses up to 84 Gy were well tolerated; however, a definitive survival advantage was not demonstrated, except for 22 cases in which PTV2 (gross tumor volume plus 0.3 cm) were treated with <75 ml and 84 Gy. The median survival of that cohort was 19.4 months, which might be due to not only smaller tumor size with a high dose delivery but also to a high rate of gross total resection (64%).

The most common failure pattern has been reported as local failure in GBM (17, 18). In our study, the occurrence of MRI changes was almost equal in the in-field, border, and extra-field areas (Fig. 3). Fitzek *et al.* (15) reported that all patients developed new areas of gadolinium enhancement during the follow-up period and that enhancements first occurred within the high-dose target volume. However, only 1 of 23 patients had recurrent tumor in the area that received 90 GyE consequently, and the most common failure was in the area that received 60 to 70 GyE or less. Although we agree that tumor control in the border area is a significant subject, prevention of border failure by radiotherapy alone may not be achievable at this time because of uncertainty as to the feasibility of delivering 90 GyE or more to larger PTV, even by using particle radiotherapy.

Cutoff values of tumor size were mentioned in some clinical trials with conformal radiotherapy before this protocol was started. Shaw *et al.* selected solitary recurrent tumors of ≤40 mm in maximum diameter for single- fraction radiosurgery for previously irradiated (60 Gy) recurrent primary brain tumors (29). They reported that the maximum tolerance dose of single-fraction radiosurgery depends on the size of the tumor, and it was 15 Gy for tumors of 31 to 40 mm. Also, Cho *et al.* treated recurrent high-grade gliomas with single- or fractionated stereotactic radiotherapy (30). Volumes of tumors treated in that study were 1 to 54 ml in SRS and 4 to 115 ml in stereotactic radiotherapy. Values of 54 ml and 115 ml are approximately equal to 4.6-cm- and 6.0-cm-diameter spheres, respectively. Finally, Fitzek *et al.* (15) selected patients whose tumor size was less than 60 ml (approximately equal to the largest postoperative diameter of 5 cm). Their clinical target volume (V1) for 90 GyE was the signal-intense rim of the tumor or the remaining cavity plus any residual enhancement on MRI. However, there are no comments on the safety margin in planning V1. Based on these parameters, we selected 4 cm as a cutoff diameter in this study because we added a 5-mm safety margin (1-cm margin in diameter) for clinical target volume, and our dose prescription to the core CTV was 6.6 GyE higher than theirs.

One patient who was treated with this protocol but excluded from analysis had a grade 4 skin injury, and this patient's overall survival period was 16.3 months, indicating that eventual benefit was not remarkable in this case. The CTV1 of this patient was 344.3 cc after encompassing the entire surgical cavity. Combined with the occurrence of leukoencephalopathy in case 11, whose CTV1 was 152.2 cc, we consider that delivering a high dose to a large CTV1 has a high risk; in addition, it might be difficult with this protocol to cover tumor invasion volume by CTV2 or CTV3 if CTV1 is more than 200 cc. Other acute toxicities were within the controllable range, indicating that doses of up to 96.6 GyE are tolerable if the target size is within a range indicated in this study.

As for late toxicity in high-dose radiotherapy for glioblastoma, Tanaka *et al.* (14) reported that 2 of 13 patients who received 90 Gy demonstrated radiation necrosis, diagnosed by biopsy or proton emission tomography images. Also, Fitzek *et al.* (15) mention that 7 of 23 cases who underwent

or immunotherapy as shown in Fig. 2. One patient (case 1) refused any additional therapy. The pathology of 6 patients who underwent reoperation demonstrated mainly a mixture of necrosis, interstitial edema, and degenerated residual GBM cells.

Survival

The overall survival rates after 1 and 2 years were 71.1% and 45.3%, respectively. The median survival periods were 21.6 months (range, 3.9–47.9 months; 95% CI, 15.5–27.7 months). Figure 3 shows the overall survival and MRI change-free survival rates. At the time of analysis, 8 patients were alive and 12 patients were dead; the median follow-up period for survivors was 24.6 months. Ten of 12 patients died of tumor recurrence, and the other 2 patients died of diseases that were not related to tumor recurrence. As mentioned in *Toxicity* (above), one patient (case 11) died of respiratory insufficiency and a disturbance of consciousness 20.4 months after the initial surgery, although there was no evidence of tumor recurrence on the last MRI. The patient's KPS before radiotherapy was 70 %. The other patient (case 15) died of sepsis following cholelithiasis with cholecystitis and panperitonitis 6.4 months after the initial surgery. This patient had moderate diabetes, and the KPS before radiotherapy was 80 %. Table 3 shows the list of outcomes of the patients in this protocol.

It was difficult to distinguish treatment effect from tumor progression at the occurrence of MRI change. Although the eventual diagnosis of "recurrence" can be made based on progression in clinical symptoms and follow-up imaging studies, the precise point of recurrence is frequently difficult to determine because treatment effect and tumor progression might be continuous or mixed. Most patients in this study underwent nuclear medical studies using thallium-201 single-photon emission tomography at some point during the course of follow-up. However, they demonstrated moderately "hot" uptake in the area corresponding to enhancement on MRI in every case, indicating that the diagnostic role was not conclusive.

DISCUSSION

Radiotherapy is the standard treatment for high-grade gliomas after surgical resection because extensive tumor cells remain in the adjacent normal brain tissue. At present, the standard radiotherapy schedule used for patients with GBM is 60 Gy in 30 fractions (9–13). Owing to recent technological advancements in x-ray radiotherapy (*e.g.*, three-dimensional conformal radiation therapy and stereotactic radiosurgery/intensity-modulated radiation therapy), better dose localization to the tumor volume can be achieved. Several prospective studies using these techniques have shown that dose escalation with or without hypofractionation is feasible and that survival is not inferior to standard schedules (15–18, 21–23).

Fitzek *et al.* (15) reported that accelerated fractionated proton/photon irradiation to 90 GyE for GBM achieved better survival, with a median survival period of 20 months. More recently, Mizoe *et al.* (16) reported the results of a phase I/II trial involving combination therapy of carbon ion and x-ray for GBM, with doses ranging from 66.8 to 74.8 GyE,

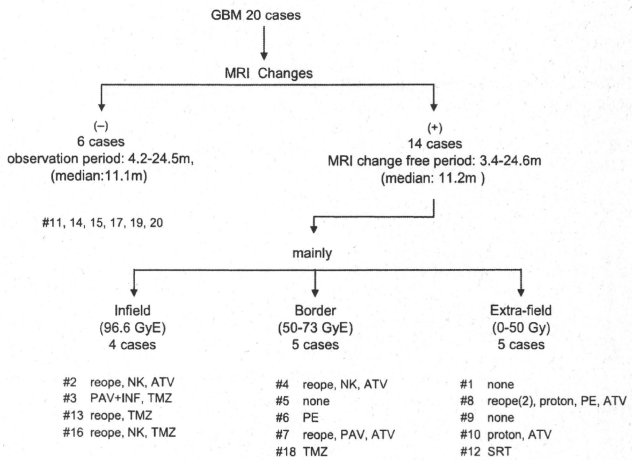

Fig. 2. Patterns of MRI changes and treatment methods after the occurrence of MRI changes. m = month(s); # = number of patients in Table 3; reope = reoperation; PAV = procarvasin plus ACNU plus vincristine; INF = interferon-gamma; TMZ = temozolomide; PE = cisplatin plus etoposide; SRT = stereotactic radiotherapy; NK = natural killer cell therapy; ATV = autologous tumor vaccine.

90 GyE of proton/photon irradiation demonstrated only necrosis, and the remaining 16 patients are thought to have had a mixture of radiation necrosis and recurrent tumor. Tsien *et al.* (28) mention that 8 cases demonstrated grade 3 or 4 radiation necrosis among 209 patients enrolled in their study of dose escalation from 66 to 84 Gy; however, its occurrence was not dose and volume related. In addition, pathological materials taken at the second surgery in 10 cases in their series demonstrated both recurrent GBM, as well as changes consistent with radiotherapy effects. Therefore, MRI changes after radiation doses of 96.6 GyE in our series probably demonstrate a mixture of radiation necrosis and tumor with or without recurrence in the field along the course of observation. Recently, several reports have shown the concept of radiation-induced pseudo-progression (31-33). Pseudo-progression is defined as the increase in size of contrast-enhancing lesions, or new areas with contrast enhancement, immediately after radiotherapy, with subsequent improvement without any further treatment. In our study, compared to a median overall survival period of 21.6 months, a median time to occurrence of MRI change of 11.2 months seemed relatively short. This may indicate that the MRI change in our series included pseudo-progression as well as radiation necrosis. Although detailed histopathological analyses concerning these phenomena are expected, it is beyond the scope of this paper. The other possible reason for prolonged survival period from the occurrence of MRI change might be various combinations of additional or salvage therapy which included reoperation, proton or x-ray irradiation, chemotherapy, or tumor immunotherapy. Although the authors believe that each therapy, including experimental salvage immunotherapy, contributed to some extent to the prolonged survival period, there was no dramatic tumor volume reduction resulting from these additional therapies.

After the results of a randomized phase III trial were published by Stupp *et al.* (10), the use of temozolomide therapy for patients with newly diagnosed GBM was approved in many countries in 2005, and it was approved in 2006 in Japan. Although temozolomide is now accepted as the standard chemotherapy for GBM, postoperative radiotherapy concurrent with ACNU was considered the standard treatment for GBM in Japan in 2001 when this protocol was started (34). This is the reason why we used ACNU in this protocol. Therefore, further clinical trials may be desirable to pursue safety and efficacy of concurrent use of temozolomide with our proton radiotherapy regimen.

CONCLUSIONS

In conclusion, hyperfractionated concomitant boost proton radiotherapy of 96.6 GyE in 56 fractions for supratentorial GBM is tolerable if the target size is well considered. The median survival time was extended to 21.6 months, which is one of the most favorable results reported to date. Further studies are warranted to reveal the effectiveness of dose escalation by proton beams and to pursue the possibility of concurrent therapy with other modalities to control the tumor border region.

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Strahlentherapie
und Onkologie

Original Article

Technical Considerations for Noncoplanar Proton-Beam Therapy of Patients with Tumors Proximal to the Optic Nerve

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Purpose: To investigate technical feasibilities of noncoplanar proton-beam therapy (PBT) on dose reduction to critical organs.
Material and Methods: The degree of mechanical precision, rotational limitations of the gantry and the treatment couch were evaluated, and dose-volume histograms were compared for noncoplanar and coplanar PBT. Following these studies, three patients with tumors proximal to the optic nerve underwent noncoplanar PBT.
Results: Noncoplanar PBT offered advantage in dose reduction to the optic nerve when compared to coplanar therapy. This advantage was more significant if the tumor reduced in size during treatment. None experienced radiation injury to the optic nerve during a short follow-up time of 7–12 months.
Conclusion: Noncoplanar PBT appears to reduce doses to organs at risk.

Key Words: Proton-beam therapy · Irradiation · Noncoplanar · Coplanar · Technical consideration

Strahlenther Onkol 2010;186:36–9
DOI 10.1007/s00066-009-2019-3

Technische Aspekte der nichtkoplanaren Protonenstrahlentherapie bei Patienten mit proximal des Sehnervs gelegenen Tumoren

Ziel: Untersuchung der technischen Möglichkeiten der nichtkoplanaren Protonenstrahlentherapie (PST) im Hinblick auf die Reduktion der Dosisbelastung von kritischen Organen.
Material und Methodik: Evaluiert wurden der Grad der mechanischen Präzision, die Rotationsbeschränkungen der Gantry und des Behandlungstischs, und es wurden Dosis-Volumen-Histogramme für die nichtkoplanare und koplanare PST verglichen. Im Anschluss daran wurden drei Patienten mit proximal des Sehnervs gelegenen Tumoren einer nichtkoplanaren PST unterzogen.
Ergebnisse: Die nichtkoplanare PST war im Hinblick auf die Reduktion der Dosisbelastung des Sehnervs im Vergleich zur koplanaren Therapie von Vorteil. Dieser Vorteil war in den Fällen, in denen die Tumorgröße während der Behandlung abnahm, signifikanter. Bei keinem der Patienten traten während eines kurzen Nachuntersuchungszeitraums von 7–12 Monaten Strahlenschäden am Sehnerv auf.
Schlussfolgerung: Die nichtkoplanare PST scheint die Dosisbelastung für Risikoorgane zu reduzieren.

Schlüsselwörter: Protonenstrahlentherapie · Bestrahlung · Nichtkoplanar · Koplanar · Technische Aspekte

Introduction

Proton-beam therapy (PBT) has been proven to offer the best physical dose distributions when compared to other therapeutic techniques such as three-dimensional conformal, stereotactic, or intensity-modulated radiotherapy [1, 2, 4–7, 11, 12, 15–17, 21–25, 27–30]. Differences in noncoplanar and coplanar PBT lie in the beam direction, that is, in the former the beam enters the target through any spherical angles, whereas in the latter the beam plane is perpendicular to the corporal axis. In this communication, attempts were made to reduce the dose to the optic nerve by selecting beam directions on noncoplanar basis.

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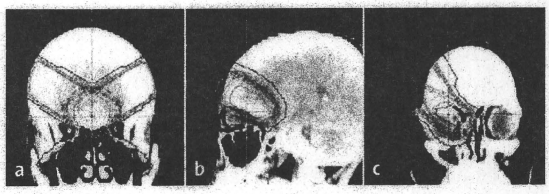
Received: March 16, 2009; accepted: July 24, 2009
Published Online: December 28, 2009

Material and Methods

The PBT system used consists of an isocentrically rotational gantry equipped with an X-ray imager, a rotational treatment couch, a treatment-planning system, a treatment-planning computed tomography (CT) scanner, and an X-ray simulator without any modification of the system [26]. Proton beams ranging from 155 to 250 MeV, generated through a linear accelerator and synchrotron (Probeat, Hitachi, Tokyo, Japan), were spread out and shaped with a ridge filter, double-scattering sheets, multileaf collimators, and a custom-made bolus, to confirm the beams to the treatment-planning data. Since the beam enters the target through unconventional routes in noncoplanar therapy, system precision studies were carried out to ensure that there was no deviation of the isocenter while rotating the gantry and the treatment couch loaded with a 60-kg phantom. This treatment-planning system was used for noncoplanar PBT planning after feeding treatment-planning CT data taken at 2-mm intervals to the system. During each treatment session, the position was precisely adjusted through anterior and lateral orthogonal fluoroscopy unit attached to the treatment unit.



Figures 1a to 1c. Dose distributions of noncoplanar planning for patients who underwent proton-beam therapy. Cases 1 (a), 2 (b), and 3 (c).

Abbildungen 1a bis 1c. Dosisverteilungen der nichtkoplanaren Planung für die einer Protonenstrahlentherapie unterzogenen Patienten. Fälle 1 (a), 2 (b) und 3 (c).

To validate dose distributions obtained by the treatment planning for noncoplanar therapy, CT images of a 10-cm spherical phantom were taken at 2-mm and 5-mm intervals after matching the center of the phantom with that of the treatment-planning CT at various treatment couch angles, including 45° and 90°. Dose distributions obtained were then compared for noncoplanar and coplanar approaches. Range differences were found in noncoplanar beams according to the treatment couch angles and CT image intervals, and maximal difference was within half of CT slice thickness.

Following these phantom studies, dose-volume histograms (DVHs) were obtained for three patients whose tumors were located adjacent to the optic nerve. Both noncoplanar and coplanar DVHs were obtained for each patient and under a condition in which the treatment reduced the tumor size by 1 cm on the beam direction. The maximal depth was measured to adjust the treatment planning.

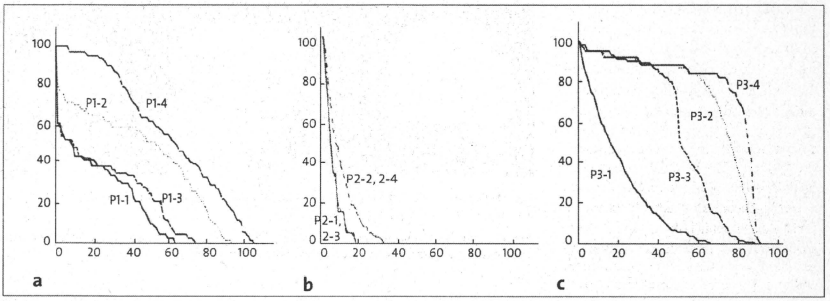
Following these preliminary studies, three patients with tumors close to the optic nerve and chiasm underwent noncoplanar therapy: Case 1 was a 52-year-old woman with olfactory groove meningioma [8, 19]. Her sole complaint was headache with a performance status of 0. She underwent noncoplanar therapy receiving 55.4 GyE in 28 fractions over 45 days to the tumor with an attempt to reduce the optic nerve dose (Figure 1a). Case 2 was a 63-year-old woman with glioblastoma multiforme in the left frontal cortex. After the tumor had been removed macroscopically, she received twice-daily two-modality radiotherapy with X-rays during mornings to a total dose of 50.4 Gy in 28 fractions and PBT at least 6 h after X-ray therapy to a total dose of 46.2 GyE in 28 fractions [3, 14, 20]. Noncoplanar therapy was used to minimize the retinal dose (Figure 1b). Case 3 was a 34-year-old woman having pleomorphic adenoma of the right superior eyelid that recurred after repeated excisions. She underwent PBT because surgery failed to control the tumor [9, 18], receiving 45.5 GyE in 23 fractions to the entire right orbit by coplanar therapy followed by noncoplanar therapy delivering 21.8 GyE in eleven fractions (Figure 1c).

These patients were followed for 7–12 months (median 10 months). Overall treatment time was defined as the period from the first therapy session day to completion of the entire course of treatment. Acute and late treatment-related toxicities were recorded according to the National Cancer Institute's Common Toxicity Criteria version 3.0. The relative biological effectiveness (RBE) of the PBT was assumed to be 1.1 in this study [13].

Results

The isocenter position remained within a 1-mm range when the treatment couch was moved and rotated with a phantom loading. The gantry rotation was not restricted in the coplanar setup, but restricted in the noncoplanar setup: from 270° to 30° when the treatment couch rotated counterclockwise, and from 330° to 90° when the treatment couch rotated clockwise.

Dose distributions and distribution lines for a spherical phantom were the same for both noncoplanar and coplanar setup. Range differences in noncoplanar beams were from 1 to 2.5 mm, which were nearly half of the CT slice thickness, when the ranges of coplanar beams were controlled. In case 1, dose distributions expressed as percent volume versus percent dose revealed that noncoplanar plans appeared desirable (Figure 2a, P1-1) when compared to coplanar plans (Figure 2a, P1-2). Further, the optic nerve dose was reduced when the tumor size was reduced by 1 cm on the beam direction as shown in Figure 2a (P1-3 vs. P1-4). In case 2, DVHs remained unchanged for noncoplanar plans (Figure 2b, P2-1) even if there was a 1-cm reduction in tumor size during therapy (Figure 2b, P2-3).



Figures 2a to 2c. DVHs of the optic nerve doses for cases 1 (a), 2 (b), and 3 (c), respectively. Solid lines of P1-1, P2-1 and P3-1 represent DVHs of noncoplanar planning; dotted lines of P1-2, P2-2 and P3-2 represent DVHs of coplanar planning. When the proton beams reach 1 cm downstream due to tumor shrinkage, the DVHs (P1-3, P2-3 and P3-3 for noncoplanar planning, and P1-4, P2-4 and P3-4 for coplanar planning) suggest superiority of noncoplanar plans in these selected patients.

Abbildungen 2a bis 2c. DVHs der Sehnervdosen für Fälle 1 (a), 2 (b) und 3 (c). Durchgehende Linien von P1-1, P2-1 und P3-1 stellen DVHs der nichtkoplanaren Planung dar; gepunktete Linien von P1-2, P2-2 und P3-2 stellen DVHs der koplanaren Planung dar. Wenn die Protonenstrahlen aufgrund einer Tumorschrumpfung 1 cm hinter den Tumor reichen, deuten die DVHs (P1-3, P2-3 und P3-3 für die nichtkoplanare Planung und P1-4, P2-4 und P3-4 für die koplanare Planung) bei drei der ausgewählten Patienten auf eine Überlegenheit nichtkoplanarer Pläne hin.

The DVHs for coplanar plans (Figure 2b, P2-2) were the same when the tumor reduction during treatment was taken into account (Figure 2b, P2-4), suggesting a higher dose to the optic nerve. In case 3, there was a significant advantage in noncoplanar plans over coplanar plans as shown in Figure 2c. These results suggest that noncoplanar plans offered a considerable advantage in dose reduction to the optic nerve in all three cases, and that this effect was more significant if reduction in tumor size occurred on the beam direction.

These patients completed noncoplanar therapy without interruptions. In case 1, the patient suffered from grade 1 acute dermatitis during therapy, but 1 year after PBT, the primary tumor was under control with no late adverse event. In case 2, local failure occurred 6 months after PBT requiring palliative care. In case 3, the patient experienced grade 2 acute dermatitis but no late toxicity 10 months after PBT.

Discussion

Since the dose distribution depends on the proton stopping power that is influenced by a CT Hounsfield value, there is uncertainty in dose distribution within nonhomogeneous tissue. Considering the fact that the RBE value increases at the end of the Bragg peak, these two factors may result in differences in prescribed and actual doses. Therefore, minimizing the radiation dose to the organs at risk should be made by cutting the beam with a collimator, or selecting a proper port rather than by means of manipulating the proton stopping power. A noncoplanar technique calls for an increase in port selection for the tumor when attempting to exclude the organs at risk. In the three cases treated via noncoplanar ports, the use of a collimator made it possible to avoid the optic nerve, apparently a better approach when compared to coplanar ports. Safe treatment is required particularly in the case of benign tumors. Small range differences of 1–2 mm make a big difference in doses given, and these differences become bigger when the biological factor of RBE is in consideration. In addition, fraction doses may be associated with optic nerve toxicities [10]. Therefore, considerable caution is needed for selecting treatment planning when proton beams directly go to optic nerve.

Range uncertainty occurs in each setup, and in addition, the tumor may be reduced in size as therapy proceeds requiring in-range changes. In our series, no reduction in tumor size occurred during treatment, however, this possibility should be taken into account. When the range was lengthened by 1 cm in terms of dose distributions, dose distributions were much better for noncoplanar than those of coplanar ports (Figure 2a).

Currently, the isocenter position was confirmed with an X-ray fluoroscopy system before each therapy and by daily confirmation of its position three-dimensionally, noncoplanar therapy was given after relocating the patient to a specified position, omitting final adjustments. However, mechanical errors incurred during these procedures were found to be of < 1 mm. Currently, treatment-planning CT for noncoplanar therapy is taken at 2-mm intervals, resulting in an error of ≤ 1 mm. These two errors, possible isocentric error after rotating table and range uncertainty by CT slice interval, were in addition to those incurred by coplanar PBT. Thus, noncoplanar therapy is preferred to coplanar therapy if the sum of these errors is

covered by better dose distributions provided by the former. Noncoplanar PBT has advantages in selected patients. In our series, only three patients underwent noncoplanar PBT out of 190 patients with head or head-and-neck tumors.

Conclusion

For selected cases, noncoplanar PBT may reduce doses to critical organs even when the tumor shrinks during treatment. Further improvements in PBT system are necessary to broaden the application of this modality.

Acknowledgments

The study was supported in part by a Grant-in-Aid for Cancer Research (15-9) from the Ministry of Health, Labor, and Welfare of the Japanese Government.

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Cancer Letters 293 (2010) 52–57

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Edaravone, a known free radical scavenger, enhances X-ray-induced apoptosis at low concentrations

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ARTICLE INFO

Article history:
Received 13 October 2009
Received in revised form 19 December 2009
Accepted 23 December 2009

Keywords:
Edaravone
Radiosensitizer
Apoptosis
p53

ABSTRACT

Edaravone has been reported to have a radioprotective effect at high concentrations. We now report that a lower dose of edaravone enhanced X-ray-induced apoptosis of some cell lines harboring p53 wild-type status, such as MOLT-4, Nalm-6, and HepG2. The knock-down of p53 using siRNA in MOLT-4 cells abolished the radiosensitizing effect of edaravone. Enhanced phosphorylations of p53 at Ser 15 and Ser 20 and up-regulation of PUMA, a p53 target protein, were observed after X-irradiation in the presence of edaravone. We conclude that the low dose of edaravone sensitized cells to X-irradiation by promoting the p53-dependent apoptotic signaling pathway.

1. Introduction

Edaravone (MCI-186; 3-methyl-1-phenyl-2-pyrazolin-5-one) is a drug widely used clinically for the treatment of acute cerebral infarction [1] and is known to scavenge free radicals as an electron donor [2]. We previously reported that in the human T-cell leukemia cell line, MOLT-4, 3 mg/ml of edaravone suppressed X-ray-induced apoptosis by inhibiting both reactive oxygen species (ROS) and p53 [3]. The MOLT-4 cell line is highly sensitive to X-rays and undergoes p53- and caspase-dependent apoptosis, showing nuclear condensation and DNA fragmentation [4]. p53 is a well-studied transcription factor associated with the determination of the cells to undergo apoptosis or other fates after DNA damage. After DNA damage, p53 stability is increased by phosphorylation, and the accumulated p53 induces the transcription of its target genes [5], including the p53-upregulated modulator of apoptosis (PUMA). PUMA is a BH3-only protein belonging to the Bcl-2 family that regulates apoptosis and plays a key functional role in the process of p53-mediated apoptosis [6–9]. Overexpressing a dominant-negative form of p53 in MOLT-4 cells results in resistance to radiation-induced apoptosis [10].

Various compounds have been reported to be effective as radiosensitizers, such as wortmannin. Wortmannin is an extensively studied inhibitor of the phosphatidylinositol 3-kinase family, ataxia telangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PK). Wortmannin has also been reported to enhance X-ray-induced apoptosis through the inhibition of DNA repair [11,12]. Tomita et al. [13] also demonstrated that wortmannin enhances

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0304-3835/$ - see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2009.12.020

X-ray-induced apoptosis possibly through the JNK/SAPK pathway in MOLT-4 cells.

Because the clinical concentration of edaravone in human blood is estimated to be approximately 1000-fold lower than that used in the previous study [14], we sought to determine the effect of lower concentrations of edaravone on X-ray-induced apoptosis. The presumption was that lower concentrations of edaravone would show a milder radioprotective effect. However, contrary to our expectations, we found that even a lower dose of edaravone enhanced the X-ray-induced apoptosis through the p53 pathway.

2. Materials and methods

2.1. Cell culture

Human T-cell leukemia MOLT-4 cells, MOLT-4 stable transfectants overexpressing short hairpin (sh)-type p53 small interfering RNA (siRNA) (p53 knock-down MOLT-4), human pre-B-cell leukemia Nalm-6 cells, and human hepatocellular carcinoma (HepG2) cells were cultured in Dulbecco's Modified Eagle Medium (Sigma) containing 5% fetal bovine serum (Hyclone) and antibiotics (100 units/ml of penicillin/streptomycin), and incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. To generate p53 knock-down MOLT-4 cells, MOLT-4 cells were transfected by electroporation (Gene Pulsar II, Bio-Rad; 0.25 kV, 950 microfarads) with the ApaLI-linearized vectors (GeneSuppressor System, p53 siRNA plasmid and the negative control shRNA plasmid, IMGENEX), and selected on 0.16% soft agar culture containing 0.8 mg/ml G418 for 3 weeks.

2.2. Chemicals

Edaravone was kindly provided by the Mitsubishi Tanabe Pharma Corporation (Tokyo, Japan). Edaravone (52.5 mg) was dissolved in 192.5 μl of 2 M NaOH and 1.05 ml of DDW, and then adjusted to pH 8.8 with 2 M HCl. Finally, physiological saline was added to adjust the final concentration of edaravone to 30 mg/ml. Edaravone was added to the cells 5 min before X-irradiation.

2.3. X-irradiation

X-irradiation was performed with an X-ray generator (Pantak HF 350, Shimadzu) at 200 kVp and 20 mA, with a filter of 0.5 mm Cu and 1 mm Al, and at a dose rate of 1.35–1.40 Gy/min.

2.4. Dye exclusion tests

One hundred microliters of cell suspension (approximately 5×10^5 cells/ml) was mixed with 25 μl of 1% erythrosin B in PBS. The numbers of stained (dead) cells and unstained (live) cells were counted and the viability (%) was calculated as follows:

Viability(%)
= (number of unstained cells/total cell number) × 100

2.5. Annexin V binding assay

The extent of apoptosis was determined by Annexin V-FITC and propidium iodine (PI) staining, using the MEBCYTO Apoptosis Kit (MBL). Flow cytometric analysis was carried out with an EPICS flow cytometer (XL System II, Beckman Coulter), using a single laser emitting excitation light at 488 nm. In the FITC/PI diparametric plot, cells in quadrant four (upper FITC/ lower PI) were considered to be in the early stage of apoptosis. More than 5000 cells were subjected to the analysis.

2.6. Quantification of intracellular ROS

The amount of intracellular ROS production was measured by chloromethyl-2′, 7′-dichlorodihydro-fluorescein diacetate (CM-H_2-DCFDA, Molecular Probes). MOLT-4 cells were incubated in the dark with approximately 5 μg/ml of probe CM-H_2-DCFDA for an hour, and the fluorescence intensity was analyzed by an EPICS flow cytometer (XL System II, Beckman Coulter) using a laser excitation and emission wavelength of 492–495 nm and 517–527 nm, respectively.

2.7. Western blotting

Cells were lysed in a SDS sample buffer (1% SDS, 3% β-mercaptoethanol, 5% glycerol, 62.5 mM Tris–HCl, pH 6.8). Proteins were separated by 10% or 15% SDS–PAGE and were transferred onto polyvinylidene difluoride membranes (Immobilon, Millipore). After blocking for 30 min in 5% skim milk in TBS (20 mM Tris–HCl, pH 7.5, 150 mM NaCl) supplemented with 0.05% Tween-20 (TBS-T), the membranes were incubated overnight at 4 °C in TBS-T containing 5% skim milk and primary antibodies. The primary antibodies were anti-p53 (clone DO-1, Santa Cruz Biotechnology), anti-phospho p53 at Ser 15 (Calbiochem), Ser 6, Ser 9, Ser 20, and Ser 392 (cell signaling), anti-cleaved caspase-3 (cell signaling), anti-caspase-7 (MBL), anti-PUMA (Calbiochem), and anti-β-actin (Sigma, AC-15). After rinsing three times with TBS-T, the membranes were incubated for 2 h at room temperature in TBS-T containing 5% skim milk and secondary antibodies conjugated with horseradish peroxidase (DAKO). The membranes were then washed three times with TBS-T, once with TBS, and developed using an ECL-plus kit (GE Healthcare). The signals were obtained by exposure to X-ray films (Hyperfilm MP, GE Healthcare).

2.8. Statistical analysis

All experiments were repeated at least three times. The results are expressed as the mean ±standard deviation (SD) of the mean. All laboratory data were evaluated according to standard statistical methods, using commercially available computer programs such as Microsoft Excel 2000. Statistical differences were determined using the Student's *t*-test. In all tests, *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Edaravone significantly enhances X-ray-induced cell death at low concentrations

First, the cytotoxicity of edaravone was determined by using the dye exclusion test [3]. Cell viability was examined in cultures treated with 0.15, 0.75, 1.5, 3, and 6 mg/ml edaravone. With concentrations of edaravone up to 3 mg/ml, the cell viabilities were more than 60%, which was considered acceptable (Fig. 1A). On the other hand, when treated with 6 mg/ml edaravone, cell viability was less than 30% and this was considered overly cytotoxic (Fig. 1A). These results are consistent with the previous report [3].

To determine the effect of edaravone on X-ray-induced cell death, MOLT-4 cells were treated with various concentrations of edaravone, and then subjected to 2 Gy X-irradiation 5 min later. Cell viabilities were determined 20 h after the treatment. When MOLT-4 cells were X-irradiated without edaravone, the cell viability was 36.2 ± 6.3% (Fig. 1A). When MOLT-4 cells were X-irradiated in the presence of edaravone at concentrations of 0.15, 0.75, and 1.5 mg/ml, the cell viabilities were 9.7 ± 2.1%, 5.7 ± 3.5%, and 7.2 ± 6.3%, respectively (Fig. 1A). These results were significantly lower than that in the absence of edaravone ($P < 0.05$). The enhancement of X-ray-induced cell death was observed in a time- and dose-dependent manner ($P < 0.05$) (Fig. 1B and C). These results indicate that low doses of edaravone enhanced X-ray-induced cell death. Considering the cytotoxicity of edaravone (Fig. 1A), the combined effect of these concentrations of edaravone and X-irradiation on MOLT-4 cell viability was considered supra-additive. On the other hand, when MOLT-4 cells were X-irradiated in combination with 2.7 or 3 mg/ml of edaravone, the cell viability increased significantly ($P < 0.05$) (Fig. 1A), which was compatible with the previous report [3]. Because 0.75 mg/ml of edaravone appeared to enhance X-ray-induced MOLT-4 cell death most effectively, this dose was used for all subsequent experiments in this study.

Although not as remarkable as noted in MOLT-4 cells, the radiosensitizing effect of edaravone was also observed in human pre-B-cell leukemia Nalm-6 and hepatocellular carcinoma HepG2 cells ($P < 0.05$) (Fig. 2A and B). Thus, the radiosensitizing effect of a low dose of edaravone is not limited to MOLT-4 cells, but is observed in cells with p53 wild-type status.

To determine whether DMSO (dimethylsulfoxide), another free radical scavenger, has a similar radiosensitizing effect in low doses, a dye exclusion test was performed on MOLT-4 cells treated with 0.2% or 1% DMSO, and then subjected to X-irradiation. The viability of the cells treated with 1% DMSO before X-irradiation was significantly greater than that of the cells treated only with X-irradiation ($P < 0.05$), indicating that 1% DMSO had a radioprotective effect. On the other hand, the viability of the cells treated with 0.2% DMSO before X-irradiation was almost the same as that of the cells treated only with X-irradiation (Fig. 2C). These results indicate that low doses of free radical scavengers do not always show the radiosensitizing effect.

3.2. Low dose of edaravone (0.75 mg/ml) enhances X-ray-induced MOLT-4 cell death by promoting apoptosis

To determine whether the radiosensitizing effect of edaravone is mediated by apoptosis, the effect of edaravone on the induction of apoptosis was examined by using flow cytometric studies with Annexin V-PI staining. When MOLT-4 cells were treated with either edaravone or 2 Gy X-ray, the percentages of Annexin V+/PI− cells in the early stage of apoptosis were 2.04 ± 0.21% and 12.43 ± 1.96%, respectively (Fig. 3A and C). When MOLT-4 cells were subjected to 2 Gy X-irradiation in addition to edaravone, the result was 39.57 ± 4.48% (Fig. 3B and C). This indicated that adding 0.75 mg/ml edaravone significantly increased the frequency of X-ray-induced apoptosis ($P < 0.05$), and that the combined effect of edaravone and X-irradiation was supra-additive. The next investigation was on the effect of edaravone on the activation of caspase-3 and -7, which are known as apoptotic effectors and play crucial roles in the execution of apoptosis [15]. Treatment with X-irradiation combined with edaravone resulted in an earlier induction of the active forms of caspase-3 and -7. With X-irradiation alone however, the same results were not obtained until 8 h after the treatment (Fig. 3D). These data indicate that the radiosensitizing effect of a low dose of edaravone is due to the enhancement of apoptosis.

3.3. Effects of edaravone on the production of intracellular ROS

CM-H_2-DCFDA, a fluorescence-based probe recently developed to detect the intracellular production of ROS, was used in a flow cytometry sys-

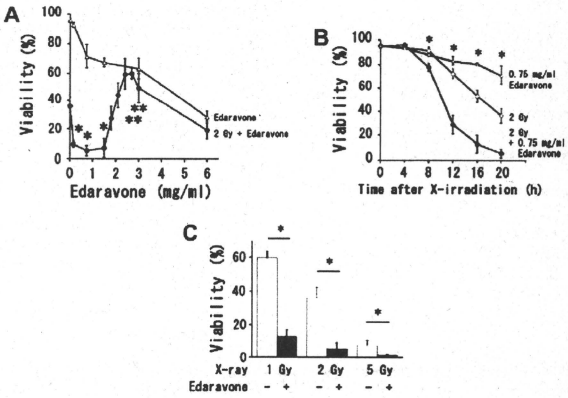

Fig. 1. Low concentrations of edaravone enhanced X-ray-induced cell death in MOLT-4 cells. (A) Cytotoxicity of edaravone and effects of various concentrations of edaravone on 2 Gy X-ray-induced cell death are shown. Cell viabilities were determined 20 h after treatment with the indicated concentrations of edaravone with or without X-irradiation. *$P < 0.05$, meaning significantly lower than that of the X-irradiated cells in the absence of edaravone. **$P < 0.05$, meaning significantly higher than that of the X-irradiated cells in the absence of edaravone. (B) The time-course cell viability after treatment with 2 Gy X-irradiation and/or 0.75 mg/ml edaravone. *$P < 0.05$, meaning that the viability of the X-irradiated cells in the absence of edaravone is significantly higher than that of the X-irradiated cells in the presence of 0.75 mg/ml edaravone. (C) X-ray-dose response of cell death in the absence or presence of 0.75 mg/ml edaravone. *$P < 0.05$.

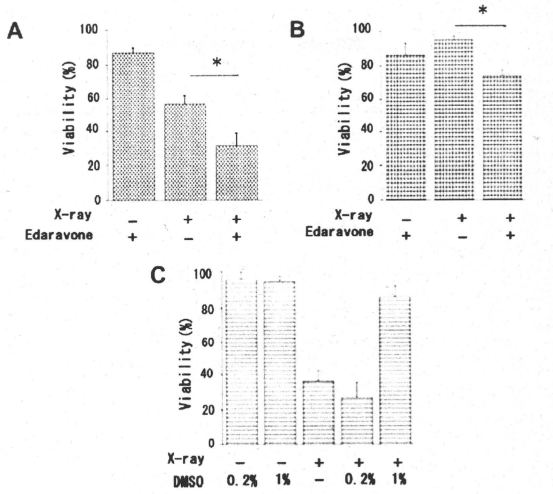

Fig. 2. Low concentrations of edaravone also enhanced X-ray-induced cell death in Nalm-6 (A) and HepG2 (B) cells. In Nalm-6 and HepG2 cells, cell viabilities were determined by dye exclusion test with erythrosine B 20 h after the treatment with 0.75 mg/ml edaravone and/or 2 Gy X-irradiation. $^{*}P < 0.05$. (C) Another free radical scavenger, DMSO, did not show the radiosensitizing effect at a low dose. Cell viability of MOLT-4 was determined 20 h after X-irradiation in the absence or presence of DMSO.

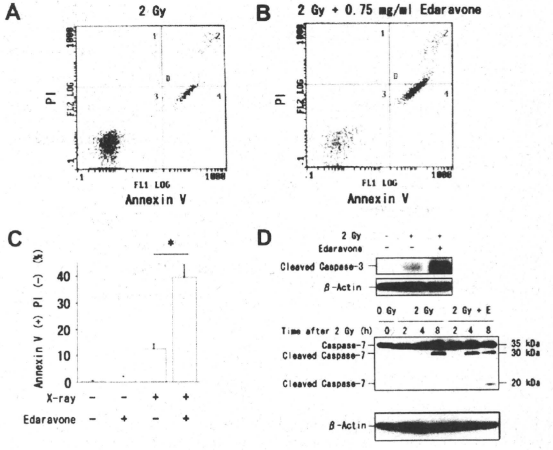

Fig. 3. A low dose of edaravone enhanced X-ray-induced apoptosis. (A–C) Apoptosis was quantified by Annexin V-PI staining. Cells were harvested 8 h after each treatment (2 Gy X-irradiation and/or 0.75 mg/ml edaravone). (A) Two Gy X-irradiation only. (B) Two Gy X-irradiation and 0.75 mg/ml edaravone. (C) The percentage of the Annexin V+/PI− cells is indicated. $^{*}P < 0.05$. (D) Cells were X-irradiated in the absence or presence of 0.75 mg/ml edaravone (E), then harvested at the indicated times. Western blot analysis was performed using anti-cleaved caspase-3 and anti-caspase-7. β-Actin as a loading control is shown.

tem to study the effect of edaravone on the X-ray-induced production of intracellular ROS [16]. CM-H_2-DCFDA is passively diffused into and trapped within the cells, and is deacetylated by intracellular esterases. It is subsequently oxidized to a fluorescent product in the presence of intracellular ROS. The oxidation of CM-H_2-DCFDA can be monitored as a convenient determinant of the level of intracellular oxidative stress. X-irradiation at 20 Gy induced an approximately 11-fold increase in basal CM-H_2-DCFDA fluorescence, which was significantly suppressed by adding 0.75 mg/ml edaravone 5 min before X-irradiation ($P < 0.05$) (Fig. 4A). Moreover, the suppressive effect was even greater when 3 mg/ml edaravone was added ($P < 0.05$). This result indicates that the radiosensitizing effect of edaravone is not mediated by promoting ROS generation.

3.4. p53 is involved in the radiosensitizing effect of a low dose (0.75 mg/ml) of edaravone

To determine whether p53 is involved in the radiosensitizing effect of edaravone, we used one of two stable p53 knock-down transformants, which were generated using a vector that overexpressed p53 siRNA [17]. A clone was used which showed a better suppression of p53. It was reconfirmed that this clone showed significantly lower levels of p53 expression than the wild-type MOLT-4 cells even after X-irradiation with or without edaravone (Fig. 4B). This clone was X-irradiated at 2 Gy with or without edaravone and the cells were subjected to the dye exclusion test. Differences of cell viabilities at 20 h after X-irradiation for the cells untreated or treated with edaravone were not significant, i.e., 88.63 ± 2.02% and 86.13 ± 4.75%, respectively (Fig. 4C). The suppression of p53 by siRNA abolished the radiosensitizing effect of edaravone. Therefore, these results suggest that the p53 pathway is involved in the radiosensitizing effect of edaravone. Next, the effects of edaravone were analyzed on the accumulation or phosphorylation of p53 by Western blot analysis with total or phospho-specific antibodies. The results showed that edaravone enhanced the phosphorylation of p53 at Ser 15 and Ser 20 (Fig. 4D). Since phosphorylation of p53 at Ser 15 and 20 has been reported to play an important role in apoptosis induced by DNA damage [18], our results suggest that edaravone specifically stimulates DNA damage-induced apoptosis signaling. On the other hand, the accumulation of p53 and its phosphorylation at Ser 6, Ser 9, and Ser 392 induced by X-irradiation did not increase significantly in the presence of edaravone (Fig. 4D).

Since edaravone enhances DNA damage-induced apoptosis, we next investigated the expression of p53 target genes, especially an apoptosis-related protein PUMA, which is a pro-apoptotic Bcl-2 family protein and induced by DNA damage [8,19]. The expression of PUMA was apparent 4 h after 2 Gy X-irradiation, and it increased significantly when a low dose of edaravone was added before X-irradiation (Fig. 4E). On the other hand, the expression of PUMA induced by X-irradiation was suppressed in the presence of 3 mg/ml edaravone (Fig. 4E), which is compatible with its radioprotective effect shown previously for that dose [3]. The expression of PUMA in the p53 knock-down transformants was not as apparent as that in wild-type MOLT-4 cells after X-irradiation in the presence or absence of edaravone (Fig. 4B). The results indicate that the low dose of edaravone enhanced the expression of the p53 target gene PUMA, and suggest that the enhanced expression contributed to the promotion of apoptosis.

4. Discussion

We found that a low dose of edaravone enhanced X-ray-induced cell death (Fig. 1A–C). This radiosensitizing effect was observed in multiple p53 wild-type cell lines (Figs.

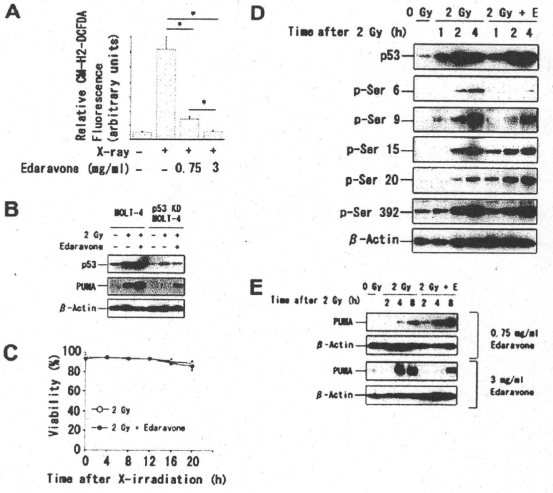

Fig. 4. p53 is involved in the radiosensitizing effect of low dose of edaravone. (A) Intracellular ROS determined by the CM-H_2-DCFDA flow cytometry system. The amount of intracellular ROS after treatment (20 Gy X-irradiation with or without 0.75 or 3 mg/ml edaravone) is shown. Edaravone was added 5 min before X-irradiation. The ROS production of each sample was quantified as described in Materials and methods. Data shown are means ± SD from at least three independent experiments. *$P < 0.05$. (B) MOLT-4 cells and its stable transformants (p53 knock-down (KD) MOLT-4 cells) were X-irradiated with or without 0.75 mg/ml edaravone, then subjected to Western blot analysis with p53 or PUMA antibody. β-Actin is used as a loading control. (C) MOLT-4 and p53 knock-down MOLT-4 cells were X-irradiated with 0.75 mg/ml edaravone, and harvested for the indicated times. (D) Effects of the low dose of edaravone (E) on X-ray-induced accumulation or phosphorylations of p53 at Ser 6, Ser 9, Ser 15, Ser 20, or Ser 392. β-Actin was used as a loading control. (E) Effects of edaravone on X-ray-induced induction of p53 targets, PUMA. MOLT-4 cells were X-irradiated in the absence or presence of the indicated concentrations of edaravone, and harvested at the indicated times. β-Actin was used as a loading control.

1A–C, 2A and B), but not in p53 knock-down MOLT-4 (Fig. 4C). The radiosensitization was mainly caused by enhancing apoptosis (Fig. 3A–D), although ROS was partially suppressed (Fig. 4A), indicating that the radiosensitizing effect was not due to enhancement of ROS generation. We next investigated whether DNA damage signaling pathways were up-regulated, especially the p53-dependent pathway. Although p53 accumulation did not change after X-irradiation in the absence or presence of edaravone, the phosphorylation of p53 at Ser 15 and Ser 20 was enhanced by adding edaravone before X-irradiation (Fig. 4D). Moreover, up-regulation of the p53 target gene, PUMA, was observed with the addition of a low dose of edaravone (Fig. 4E).

PUMA has been reported to play a causal role in p53-dependent apoptosis. Villunger et al. [8] demonstrated that DNA damage-induced apoptosis decreased in PUMA-disrupted mouse fibroblasts and loss of PUMA protected lymphocytes from cell death. The expression of PUMA, which is induced by p53, might contribute to the radiosensitizing effect of edaravone. Our results suggest that the enhancement of phosphorylations of p53 alter the expression pattern of the p53 target genes, causing the promotion of apoptosis.

A question which remains to be answered is why a low dose of edaravone enhances X-ray-induced apoptosis even though it partially eliminates the intracellular ROS generated by X-irradiation (Fig. 4A). In the previous study, a higher dose (3 mg/ml) of edaravone completely eliminated intracellular ROS generated by X-irradiation, causing the suppression of p53 accumulation and phosphorylation at Ser15 [3]. In the present study, p53 phosphorylation at Ser 15 and Ser 20 were enhanced in the presence of edaravone after X-irradiation. The phosphorylation of p53 at Ser 15 or Ser 20 has been shown to be mediated by the ATM, leading to p53 activation [20]. A low dose of edaravone might effect on the activity of ATM or the related protein kinases, resulting in the enhanced phosphorylation of p53, at least at Ser 15 and Ser 20.

We conclude that a low dose of edaravone (0.75 mg/ml) used in this study enhanced X-ray-induced apoptosis by modifying p53 transcriptional activity. The results of the current study may have important clinical implications for radiation therapy.

5. Conflicts of Interest

None declared.

Acknowledgements

We thank all the members of our laboratory for their help and encouragement. We also thank Dr. T. Kondo (University of Toyama) for various suggestions and instructions. This study was supported in part by the Mitsubishi Tanabe Pharma Corporation (Tokyo, Japan). This study was also supported in part by the Radiation Effects Association.

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SECTION 2. RADIOTHERAPY

CQ38 **Should radiotherapy be recommended for intrahepatic tumors?**

RECOMMENDATION

Three-dimensional conformal radiotherapy can be considered for conditions to which no other therapies are applicable; but, as yet, sufficient scientific evidence is not available. **(grade C1)**

There is no recommendation based on scientific evidence for the fractionation or total dose of radiotherapy.

SCIENTIFIC STATEMENT

Because there are no reports of studies on the indications for radiotherapy, we examined the selection of subjects in articles describing studies evaluating 3-D conformal radiotherapy for hepatocellular carcinoma. The eligibility criteria for subjects in these reports were roughly classified into two groups. One was reports on studies involving patients with portal vein tumor thrombosis. The other was reports on studies that focused on patients with unresectable tumors. For some of the patients in the latter, portal vein tumor thrombosis was included as a reason for the tumors being unresectable. Eligibility criteria for subjects such as the stage of hepatocellular carcinoma, liver function and combinations of other treatments varied among the reports. Furthermore, the fractionation and total dose of radiotherapy also differed among the reports. In four reports on prospective studies, the treatment results in patients with portal vein tumor thrombosis (LF10584[1] level 4, LF10824[2] level 4, LF11100[3] level 4, LF11708[4] level 4) were reported to be response rates of 50–80.5% and 1-year survival rates of 25–40.6%. Some of these reports concluded that the survival rate improved significantly in responders as compared with non-responders (LF10824[2] level 4, LF11402[5] level 4, LF11707[6] level 4). There were reports that the scheduled treatment could not be completed due to deterioration of general condition, and many patients dropped out of the studies (LF11707[6] level 4, LF11100[3] level 4). Tumor enlargement and worsening of general condition were documented as reasons for dropping out, but the possibility of adverse events associated with radiotherapy could not be ruled out. However, it was concluded in all of these reports that radiotherapy could be performed safely.

There are also reports on stereotactic radiotherapy and particle radiotherapy (proton therapy/heavy particle radiotherapy) (LF11470[7] level 4, LF11086[8] level 4, LF11279[9] level 4, LF10646[10] level 4, LF11353[11] level 4). Adequate long-term results have not yet been reported, but the local control rate is reportedly 81–100% for 2–5 years. In a report by Romero *et al.* (LF11086[8] level 4), one of eight patients died of hepatic failure after stereotactic radiotherapy for primary liver cancer. In a report by Kawashima *et al.* (LF10646[10] level 4), hepatic failure was noted in eight of 30 patients who underwent proton therapy because their tumors were unresectable and ablation treatment was not applicable. Four of these patients were reported to have died without recurrence 6–9 months after treatment. All reports pointed out that the risk of adverse events was high for patients with decreased liver function but concluded that the therapy could be safely given if appropriate patients were selected.

COMMENTS

There were no reports with a high evidence level. The majority described the results of prospective or retrospective studies equivalent to phase I/II trials without a control group. With the recent progress in radiation concentrating techniques in the field of radiotherapy, irradiation to the liver, which had not previously been performed, has been started. While there is general agreement that irradiation to the liver in patients with severely decreased liver function is dangerous, sufficient data on the eligibility criteria for patients who can safely receive this therapy and a tolerable dose for the liver have not yet been accumulated. Neither have long-term results been adequately pooled as yet. Due to these

considerations, scientific evidence was determined to be inadequate.

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CQ39 Does prognosis improve after radiotherapy?

RECOMMENDATION

There is no scientific evidence suggesting that radiotherapy alone improves prognosis. However, prognosis may improve if transcatheter arterial chemoembolization (TACE) is combined with radiotherapy, but, as yet, sufficient scientific evidence is not available. **(grade C1)**

SCIENTIFIC STATEMENT

No reports on RCT showing the role of radiotherapy were found. However, retrospective studies reported data suggesting the improvement of prognosis with the addition of TACE to radiotherapy. There were five reports on studies comparing survival time between patients who received TACE alone and patients who received TACE plus radiotherapy. In all of these reports, patient background characteristics and the contents of treatment varied, but four of these reports (LF10273[1] level 3, LF10847[2] level 3, LF11020[3] level 3, LF11032[4] level 3) stated that the combination with radiotherapy was a significant prognostic factor. Of these five reports, one by Cheng *et al.* (LF11336[5] level 3) was the sole report documenting no significant difference in survival time between the TACE alone and the TACE plus radiotherapy groups.

In addition, reports on radiotherapy in combination with arterial infusion chemotherapy (LF10649[6] level 4, LF11384[7] level 4), in combination with TACE (LF11178[8] level 4) and radiotherapy alone (LF11101[9] level 4) stated that total radiation dose was a prognostic factor for survival. In reports on radiotherapy alone in patients with portal vein tumor thrombosis or unresectable tumors, prognosis was demonstrated to be dependent on radiation dose (LF11707[10] level 4, LF11354[11] level 4, LF10822[12] level 4). However, both reports on radiotherapy in combination with arterial infusion chemotherapy (LF10649[6] level 4, LF11384[7] level 4) concerned studies involving patients with bile duct cancer and colon cancer with liver metastasis. Because whether or not it was applicable to hepatocellular carcinoma was not mentioned, caution should be taken when interpreting.

Based on the above, although sufficient scientific evidence is not available, prognosis may improve when radiotherapy is combined with TACE versus administrated alone.

COMMENTS

Patient background characteristics and treatment contents also varied among the aforementioned reports. Moreover, the majority of these studies were retrospective and comparative, namely, they do not provide adequate scientific evidence. In order to establish the significance of radiotherapy, RCT are required. Prior to such RCT, however, a consensus must be reached on the disease states and levels of liver function which would be indications for radiotherapy.

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CQ40

Can radiotherapy be indicated for distant metastasis of hepatocellular carcinoma?

RECOMMENDATION

Radiotherapy is generally useful for alleviation of pain associated with bone metastasis; thus, this treatment is recommended. In terms of the dose fractionation schedule, no evident difference in efficacy is noted between single and fractionated irradiation. **(grade B)**

Performing whole-brain irradiation as a standard treatment for brain metastasis can be recommended. **(grade B)**

For solitary brain metastasis, it is advisable to also perform stereotactic irradiation in addition to whole-brain irradiation. When metastatic lesions number two to four, it is preferable to consider adding stereotactic irradiation. **(grade B)**

SCIENTIFIC STATEMENT

No clinical studies in patients with only distant metastasis of hepatocellular carcinoma with a high evidence level have as yet been conducted. Consequently, as the only currently available high evidence level reports, we present herein statements based on study data obtained from a published work search conducted without specifying the organ of origin.

The pain relief rate achieved by radiotherapy for painful bone metastasis was high at 50–90% (LF11732[1] level 1a, LF11721[2] level 1a). Although there is no RCT directly comparing radiotherapy with non-treatment, it is instituted as a standard treatment for pain relief. In a multicenter study comparing it with the fractionated irradiation method performed by the Radiation Therapy Oncology Group (LF11730[3] level 1b), four fractionated regimens were compared: 40.5 Gy/3 weeks and 20 Gy/1 week for isolated metastasis, and 15 Gy/1 week to 30 Gy/2 weeks for multiple metastases. Partial remission rates were 85% and 82% for isolated metastasis, and 78% to 87% for multiple metastases, respectively. There was no significant difference in remission rates according to the differences in fractionated irradiation. Nor was there any significant difference between the two groups in time to remission or duration of remission. Based on these results, short-term low-dose radiotherapy has been considered to be as effective as long-term therapy. A meta-analysis (LF11732[1] level 1a) also corroborated this conclusion, and single irradiation was found to be appropriate for treatment to relieve pain. However, the re-irradiation rate is high in the single irradiation group, such that treatment with fractionated irradiation should be also considered.

For brain metastasis, the results of an RCT on the ECOG reported by Horton *et al.* in 1971 (LF11745[4] level 1b) revealed that whole-brain irradiation prolonged survival and improved the general condition. RCT of whole-brain irradiation comparing the efficacy among several fractionated irradiation methods, such 20 Gy/1 week, 30 Gy/2 weeks and 40 Gy/4 weeks, continue to be reported. Still, none of these reports has yet been able to identify particularly effective fractionated irradiation using survival time, the improvement rate of symptoms or the duration of maintenance of general condition as indexes. The stereotactic irradiation technique has advanced recently and is now being widely used. In brain metastasis patients with two to four lesions measuring no more than 2.5 cm in diameter at maximum, the addition of stereotactic irradiation to whole-brain irradiation, which was a standard treatment, did not significantly improve survival; however, a single-center, RCT performed by Kondziolka *et al.* (LF11746[5] level 1b) showed that such an addition significantly increased the control rate of intracerebral lesions. In addition, Andrews *et al.* (LF11734[6] level 1b) investigated the significance of adding stereotactic irradiation for the treatment of brain metastasis patients with one to three lesions measuring no more than 4 cm in diameter at maximum in a multicenter RCT. Stereotactic irradiation was demonstrated to significantly prolong survival in patients with a single brain metastasis. An RCT by JROSG 99-1 (LF11735 level[7] 1b) investigated the

appropriateness of performing stereotactic irradiation alone by omitting whole-brain irradiation as a treatment alternative for brain metastasis patients with a few lesions. With one to four brain metastases, survival was not shortened due to the omission of whole-brain irradiation, but it was demonstrated that the combination with whole-brain irradiation significantly decreased the intracerebral recurrence rate. Based on these observations, stereotactic irradiation alone can be a treatment alternative for four or fewer lesions, but whole-brain irradiation is still important as a standard treatment at present.

COMMENTS

Critical aspects of the treatment of distant metastasis are alleviation and prevention of symptoms associated with tumors. In particular, tumor control is directly linked to survival in brain metastasis patients; thus, the selection of an appropriate treatment policy is critical. As mentioned above, however, because there are relatively few studies with a large number of hepatocellular carcinoma patients with distant metastasis, whether these statements are applicable to distant metastasis of hepatocellular carcinoma is unknown. When applying the statements in this CQ, attention should be paid to this issue.

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