tenosynovial giant cell tumor/pigmented villonodular synovitis. Cancer 2012;118:1649–55.
2. Reynders K, Illidge T, Siva S, et al. The abscopal effect of local radiotherapy: using immunotherapy to make a rare event clinically relevant. Cancer Treat Rev 2015;41:503–10.
3. Formenti SC, Demaria S. Systemic effects of local radiotherapy. Lancet Oncol 2009;10:718–26.
4. Levy A, Chargari C, Marabelle A, et al. Can immunostimulatory agents enhance the abscopal effect of radiotherapy? Eur J Cancer 2016;62:36–45.
5. Grimaldi AM, Simeone E, Giannarelli D, et al. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. Oncoimmunology 2014;3:e28780.
6. Chandra RA, Wilhite TJ, Balboni TA, et al. A systematic evaluation of abscopal responses following radiotherapy in patients with metastatic melanoma treated with ipilimumab. Oncoimmunology 2015;4:e1046028.
7. Golden EB, Chhabra A, Chachoua A, et al. Local radiotherapy and granulocyte-macrophage colony-stimulating factor to generate abscopal responses in patients with metastatic solid tumors: a proof-of-principle trial. Lancet Oncol 2015;16:795–803.
8. Staals EL, Ferrari S, Donati DM, et al. Diffuse-type tenosynovial giant cell tumour: Current treatment concepts and future perspectives. Eur J Cancer 2016;63:34–40.

LETTER TO THE EDITOR

The relative biological effectiveness of carbon ion irradiations of the rat spinal cord increases linearly with LET up to 99 keV/μm

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To the Editor,

Based on preliminary work at the Lawrence Berkley Laboratory [1], carbon ion beams developed to a mature technology in radiotherapy, which has now been applied successfully in patients for two decades [2–4]. As in proton radiotherapy, the depth-dose profile (Bragg peak) of carbon ions allows for highly conformal irradiations of the tumor. However, the rise of the relative biological effectiveness (RBE) with penetration depth towards the distal end of a spread-out Bragg peak (SOBP) is much more pronounced for carbon ions than for protons [5]. Quantitatively, the RBE is given by the ratio of a photon and an isoeffective carbon ion dose at a defined endpoint. However, the RBE is a complex quantity depending on particle type, linear energy transfer (LET), dose, and also on biological properties of the irradiated tissue. To consider the increased effectiveness of carbon ions, the RBE is calculated by bio-mathematical models. In Europe, clinical treatment planning is done using the local effect model (LEM), which is presently available in two versions (LEM I [6] and IV [7]). Although LEM I has been used for all patients so far, LEM IV was extended by including information on the spatial DNA double stand break density. It is, however, an open question, which version is more accurate.

To validate the LEM in vivo, the RBE for radiation-induced myelopathy in the rat spinal cord has been determined in several studies [8–11] and comparisons with LEM predictions were made. In these studies RBEs were calculated for the tolerance doses at 50% complication probability (TD_{50}) determined from dose-response experiments. Although initial studies [8,9] demonstrated a strong dose-dependence of the RBE, the LET-dependence was not investigated in detail.

This was the starting point of a second series of experiments, where the rat spinal cord was irradiated with single and split doses at six different positions of a 6 cm SOBP covering a LET range from 16 to 99 keV/μm [10,11]. These data showed a linear increase of RBE with LET, where the slope of the curve increased with decreasing fractional dose [11]. For the single fraction experiment at 99 keV/μm, however, an inconsistently low RBE combined with a shallow dose-response curve was found. As this was only observed in the single but not in the split dose experiments, we repeated the 99 keV/μm single dose experiment and report here on the results.

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Material and methods

Animals

Thirty young adult female (196.5 ± 7.5 g) Sprague Dawley rats (Charles River, Sulzfeld, Germany) were used for this study. Irradiations were performed under general gaseous anesthesia with a mixture of 4% Sevoflurane (Abbott, Wiesbaden, Germany) and oxygen at 2 l/min, using a 50 ml disposable syringe as a mask. All experiments were approved by the governmental review committee on animal care (35-9185.81/G62-08), and animals were kept under standard conditions at the DKFZ animal laboratory facility.

Experimental setup

The experimental setup is identical to the single dose study [10]. Briefly, the spinal cord was located at 127 mm (dose-averaged LET 99 keV/μm) of a 6 cm SOBP ranging from 70 to 130 mm water-equivalent depth (beam energy 187–260 MeV/u), which was calculated with the treatment planning system TRiP [12]. The field size was $10 \times 15\,\mathrm{mm}^2$ including the cervical segments C1-C6 [8–11]. The position of the spinal cord within the SOBP was adjusted using an appropriate polymethyl-methacrylate (PMMA)-bolus.

Carbon ion irradiations were performed at the Heidelberg Ion-Beam Therapy Center (HIT) using the raster scanning method [13]. Thirty animals were irradiated in groups of five animals using single doses of 10.5, 11.5, 12.5, 13.5, 14.5 or 15.5 Gy. Five additional animals served as sham treated controls. Prescribed doses refer to measurements with a pinpoint ionization chamber (TM31009, PTW Freiburg, Germany) as described previously [10].

Follow-up and biological endpoint

After irradiation, rats were checked for weight and general condition once a week. The biological endpoint of this study was radiation-induced myelopathy (paresis grade II) meaning that both forelimbs show signs of paralysis within 300 days [8]. Rats exhibiting this endpoint were sacrificed and scored as responders.

Data analysis

The logistic dose-response model

$$P(D) = \frac{e^{b_0+b_1D}}{1+e^{b_0+b_1D}}$$

was adjusted using actuarial response rates. Censored animals were included using the method of effective sample sizes [14]. In Equation 1a, D is the total dose and b_0 and b_1 are the fit parameters from which the TD_{50} can be calculated:

$$TD_{50} = -\frac{b_0}{b_1}.$$

Using the previously published dose-response curves for photons [9], the RBE was calculated as the ratio of the TD_{50}-values for photons and carbon ions.

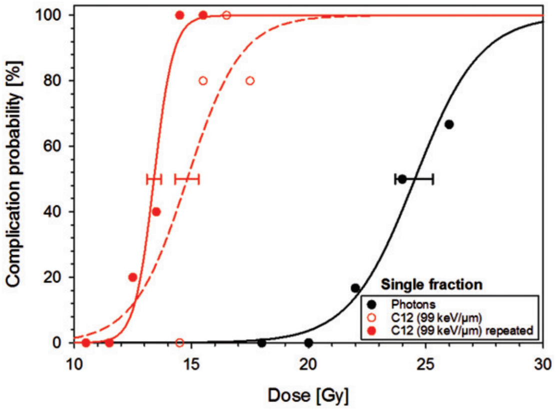

Figure 1. Single fraction dose-response curves for the original [10] and repeated carbon ion experiment at 99 keV/μm together with the reference curve for 15 MV photons [9]. The curve of the present study shows an increased slope and a lower TD_{50}-value. Error bars indicate the uncertainty (1 SE) of TD_{50}.

RBE calculation

For comparison with measurements, the RBE was calculated at the position of the spinal cord within the SOBP and for the measured TD_{50}-value using LEM I and IV together with the standard parameters of the two versions [11].

Statistics

The logistic model was adjusted using the maximum likelihood fitting procedure of STATISTICA [15]. Standard errors (SE) of TD_{50} and RBE were calculated by error propagation considering the correlation of b_0 and b_1. Ninety percent confidence limits (90% CL) were additionally calculated using Fieller's Theorem [16].

Results

No weight reduction was observed during follow-up and none of the animals died from acute radiation toxicity. Within three weeks after treatment, a slight or complete transient hair loss as well as moist desquamation restricted to the irradiation field was observed on the ventral side of the rat's throat. Two rats (10.5 and 12.5 Gy) had to be censored due to spontaneous development of mammary carcinomas at 220 days and 290 days, respectively. Minimum and mean latency time of the onset of paresis grade II were 136 days and 181 days, respectively (range 136–275 days).

Figure 1 displays the original and the repeated single fraction dose-response curves for carbon ions at 99 keV/μm together with the reference curve for 15 MV photons. Compared to the original measurement the slope of the dose-response curve is much steeper and the TD_{50}-value is reduced from 14.8 ± 0.5 Gy (13.9–15.8) to 13.4 ± 0.3 Gy (12.8–14.0) (±SE, 90% CL). Using the photon TD_{50}-value of 24.5 ± 0.8 Gy (23.3–26.7), an increase of the RBE from 1.66 ± 0.08 (1.53–1.79) to 1.83 ± 0.07 (1.72–1.95) (±SE, 90% CL) was determined. As a consequence, the RBE now continues the linear dependence on LET (Figure 2). At 99 keV/μm the measured RBE agrees well with the prediction of LEM IV whereas LEM I shows large deviations.

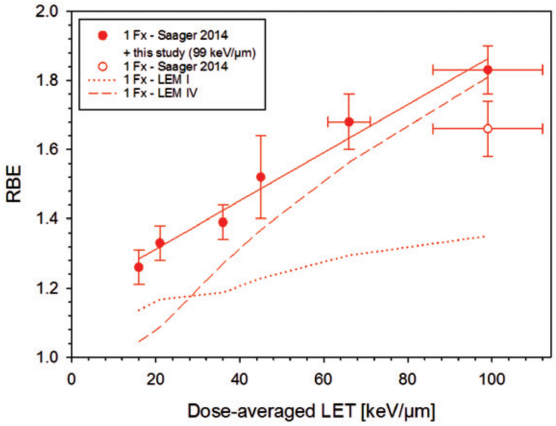

Figure 2. Measured dependence of RBE on LET: Compared to our previous study [10], a higher RBE was measured at 99 keV/µm including previous data [10] the LET-dependence is well described by a linear regression line. At 99 keV/µm, the RBE prediction of LEM IV agrees well with the measured data, whereas LEM I shows large deviations. Towards very low LET values, LEM I agrees better than LEM IV. Vertical error bars represent 1 SE while horizontal error bars represent a ± 2 mm positioning uncertainty of the spinal cord within the SOBP.

Discussion

Model-based RBE-calculations are an intrinsic feature in carbon ion treatment planning. Accurate RBE-modeling is required as the RBE directly affects the prescribed carbon ion dose, which is related to the dose of an isoeffective photon treatment by the so-called clinical RBE, and it is also needed to achieve a homogeneous RBE-weighted dose within the target volume. Although the assumption of an inaccurate clinical RBE may be practically compensated by a clinical dose finding process, errors in the functional dependence of the RBE on LET and dose may still lead to over- or under-dosages within the normal tissue or tumor, and thus may compromise therapeutic outcome.

The rat spinal cord is a well established and reliable model for studying late reactions in normal tissue [17–23]. With this model, we initiated a large study to evaluate the LET-dependence of the RBE at two different dose levels and to compare the results with predictions of LEM I and IV [10,11]. As expected from the experimental design, we could show that the RBE increases linearly with LET and that the slope increases with decreasing fractional dose. However, for the single dose, but not for the split dose experiment at 99 keV/µm there was an exception as the RBE decreased relative to that at 66 keV/µm. In addition, the slope of the dose-response curve, which increased continuously with LET up to 66 keV/µm, decreased again [10]. Both findings originated from an increased heterogeneity in the observed incidences at the different dose levels (Figure 1). Although a decrease of the RBE beyond a certain LET-value is expected by the so-called *overkill effect*, in vitro data suggest that the RBE increase up to 200 keV/µm [24]. Therefore, the results of this experiment appeared inconsistent with the available database.

The experimental procedure was retrospectively analyzed. All 12 experimental arms [10,11] were treated under identical conditions using the same equipment and no suspicious observations were made in the single fraction experiment at 99 keV/µm in the animals during follow-up. Moreover, all animals showed hair loss at the expected location ruling out a misalignment of the animals. In the previous experiment at 99 keV/µm, however, the dose groups were irradiated in two cohorts (11.5, 13.5, 15.5, 16.5 Gy vs. 12.5, 14.5 and 17.5 Gy) for logistic reasons. For the second cohort, inconsistently low incidences were found at 14.5 and 17.5 Gy (Figure 1). This result could be explained by a slight over-range of the ions in the second cohort, which would lower the LET and hence the biological effectiveness. Analysis of the robustness of the irradiation conditions revealed that even small range errors will be critical in this respect.

We therefore repeated this experiment with a single cohort of animals using the identical setup and taking special quality assurance measures to guarantee high mechanical precision (±1 mm in all directions). As a result we obtained a steep sigmoid dose-response curve and an RBE-value, which fits well to a continuous linear increase with LET. The increased steepness of the new dose-response curve indicates a reduced response heterogeneity, which could be explained by a higher setup reproducibility of the repeated experiment. These findings and the consistency with the results of the split dose experiment [11] suggest that the RBE of the present study is more reliable than that of our previous study [10]. It has to be noted, however, that the exact slope and shape of the RBE versus LET curve is expected to depend on the detailed composition of the radiation field as the dose-averaged LET does not fully reflect the microscopic features of the energy deposition of the ions.

As a conclusion, we have now established a large and consistent RBE database for late reactions of the rat spinal cord and we used these data to test the functional dependence of the LEM predictions on LET and dose. In general, a much better agreement of the data with the LEM IV predictions is observed in the SOBP as compared to the predictions of LEM I; only at very low LET values LEM IV shows larger deviations than LEM I.

Transferring these results to patients, the actual RBE-weighted dose in the normal tissue at the distal edge of the SOBP would be significantly higher than predicted in the LEM I-based treatment plans. Accordingly, increased rates of late side effects would have been expected which, however, have not been observed up to now. It has to be noted, however, that the clinically applied dose per fraction is much lower than in our single dose study. Moreover, potential inaccuracies of LEM I may have been compensated by prescribing a safe and effective dose. Further fractionated studies as well as analysis of clinical side effects are therefore required to answer the question whether LEM I or LEM IV is more accurate in patients. With this respect, the data of the present study contributes to this increasing data base.

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Disclosure statement

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

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References

1. Castro JR, Linstadt DE, Bahary JP, et al. Experience in charged particle irradiation of tumors of the skull base: 1977-1992. Int J Radiat Oncol Biol Phys. 1994;29:647–55.
2. Combs SE, Debus J. Treatment with heavy charged particles: systematic review of clinical data and current clinical (comparative) trials. Acta Oncol. 2013;52:1272–86.
3. Kamada T. Clinical evidence of particle beam therapy (carbon). Int J Clin Oncol. 2012;17:85–8.
4. Kamada T, Tsujii H, Blakely EA, et al. Carbon ion radiotherapy in Japan: an assessment of 20 years of clinical experience. Lancet Oncol. 2015;16:93–100.
5. Suit H, DeLaney T, Goldberg S, et al. Proton vs carbon ion beams in the definitive radiation treatment of cancer patients. Radiother Oncol. 2010;95:3–22.
6. Scholz M, Kellerer AM, Kraft-Weyrather W, et al. Computation of cell survival in heavy ion beams for therapy. The model and its approximation. Radiat Environ Biophys. 1997;36:59–66.
7. Elsasser T, Weyrather WK, Friedrich T, et al. Quantification of the relative biological effectiveness for ion beam radiotherapy: direct experimental comparison of proton and carbon ion beams and a novel approach for treatment planning. Int J Radiat Oncol Biol Phys. 2010;78:1177–83.
8. Debus J, Scholz M, Haberer T, et al. Radiation tolerance of the rat spinal cord after single and split doses of photons and carbon ions. Radiat Res. 2003;160:536–42.
9. Karger CP, Peschke P, Sanchez-Brandelik R, et al. Radiation tolerance of the rat spinal cord after 6 and 18 fractions of photons and carbon ions: experimental results and clinical implications. Int J Radiat Oncol Biol Phys. 2006;66:1488–97.
10. Saager M, Glowa C, Peschke P, et al. Carbon ion irradiation of the rat spinal cord: dependence of the relative biological effectiveness on linear energy transfer. Int J Radiat Oncol Biol Phys. 2014;90:63–70.
11. Saager M, Glowa C, Peschke P, et al. Split dose carbon ion irradiation of the rat spinal cord: Dependence of the relative biological effectiveness on dose and linear energy transfer. Radiother Oncol. 2015;117:358–63.
12. Jäkel O, Kramer M, Karger CP, et al. Treatment planning for heavy ion radiotherapy: clinical implementation and application. Phys Med Biol. 2001;46:1101–16.
13. Haberer T, Becher W, Schardt D, et al. Magnetic scanning system for heavy ion therapy. Nucl Instrument Methods Phys Res Sec A. 1993;330:296–305.
14. Walker AM, Suit HD. Assessment of local tumor control using censored tumor response data. Int J Radiat Oncol Biol Phys. 1983;9:383–6.
15. StatSoft I. STATISTICA für Windows [Software-System für Datenanalyse]. Version 6; 2004. Available from: www.statsoft.com.
16. Finney DJ. Statistical method in biological assay. 3rd ed London: Charles Griffin; 1978.
17. van der Kogel AJ, Barendsen GW. Late effects of spinal cord irradiation with 300 kV X rays and 15 MeV neutrons. Br J Radiol. 1974;47:393–8.
18. Bijl HP, van Luijk P, Coppes RP, et al. Unexpected changes of rat cervical spinal cord tolerance caused by inhomogeneous dose distributions. Int J Radiat Oncol Biol Phys. 2003;57:274–81.
19. Leith JT, McDonald M, Powers-Risius P, et al. Response of rat spinal cord to single and fractionated doses of accelerated heavy ions. Radiat Res. 1982;89:176–93.
20. Okada S, Okeda R, Matsushita S, et al. Histopathological and morphometric study of the late effects of heavy-ion irradiation on the spinal cord of the rat. Radiat Res. 1998;150:304–15.
21. Bijl HP, van Luijk P, Coppes RP, et al. Dose-volume effects in the rat cervical spinal cord after proton irradiation. Int J Radiat Oncol Biol Phys. 2002;52:205–11.
22. Bijl HP, van Luijk P, Coppes RP, et al. Regional differences in radiosensitivity across the rat cervical spinal cord. Int J Radiat Oncol Biol Phys. 2005;61:543–51.
23. Bijl HP, van Luijk P, Coppes RP, et al. Influence of adjacent low-dose fields on tolerance to high doses of protons in rat cervical spinal cord. Int J Radiat Oncol Biol Phys. 2006;64:1204–10.
24. Weyrather WK, Ritter S, Scholz M, et al. RBE for carbon track-segment irradiation in cell lines of differing repair capacity. Int J Radiat Biol. 1999;75:1357–64.