ACTA ONCOLOGICA
2019, VOL. 58, NO. 10, 1337–1339
https://doi.org/10.1080/0284186X.2019.1669819

COMMENTARY

Commentary: RBE in proton therapy – where is the experimental *in vivo* data?

Brita Singers Sørensen

Department of Experimental Clinical Oncology and Danish Center for Particle Therapy, DCPT, Aarhus University Hospital, Aarhus, Denmark

The clinical treatment with protons rely on the relative biological effectiveness (RBE) in order to convert from a physical dose to a biological equivalent dose. Currently, in proton therapy a constant RBE of 1.1 is generically used, as recommended by the International Commission on Radiation Units [1], meaning that a given proton dose is expected to be equivalent to a 10% higher X-ray dose for all tumors, tissues and doses. However, whether this is an adequate solution is under debate [2–4]. There is currently no doubt that an RBE of 1.1 is an oversimplification of the actual biological response to proton irradiation, as it was illustratively demonstrated by Paganetti and coworkers in 2002, where experimental determinations of the RBE *in vitro* and *in vivo* was examined [5].

The RBE is a complex figure, which is influenced by a number of factors. Tissue type, dose and fractionation, and LET are some of the factors known to affect the RBE [6,7]. The LET increases moderately through the Spread Out Bragg Peak, SOBP, with a substantial increase in the very distal edge of the SOBP. This has shown to translate into an increased distal edge RBE *in vitro* [8–11]. This is a critical issue, as the distal edge of the SOBP may be situated in the surrounding normal tissue.

A number of reviews and reports on the possible clinical effects of the RBE have been published in the last few years [2,12–18], and an international expert workshop was held in Dresden with the aim of discussing these issues [19]. Most of the reviews include the conclusion that there is a need for more experimental data in order to shed light on the RBE issue.

The question is which data is needed, and how these are experimentally obtained. It is an open discussion if the *in vitro* effects on the increased RBE in the distal edge can be expected to translate into a clinical impact, and what the magnitude of this increased RBE is in different tissues.

The overall rationale for using proton therapy is to minimize radiation induced morbidity in the normal tissue, but it cannot be avoided that normal tissue receive any radiation, and the RBE of this may be the Achilles heel of the regime. It is possible to extract important information from *in vitro* experiments, as the majority of experimental data within proton radiobiology is based on. However, there is a lack of *in vivo* experiments, which in general are considered a necessary translation from the more hypothesis generating *in vitro* experiments into patient treatment, as *in vivo* studies enable simulation of clinical treatments, and the much more complex biology is taken into account. A substantial part of the *in vivo* data previously obtained for proton irradiation is based on acute endpoints, such as crypt regeneration, acute skin reactions and acute lung pneumonitis, as reviewed in [5]. The data on late endpoints, which is the dose-limiting factor in radiotherapy, are still to a large extent missing.

What is currently needed to shed light on the questions on proton RBE is a thorough experimental evaluation of the radiation induced effects to the normal tissue effects *in vivo*. This needs to be done in a panel of relevant normal tissues models, including functional and tissue endpoints representing both acute and late radiation induced reactions. It should simulate the clinical situation, including normal tissue in the distal edge of the SOBP by examining the effect in different positions in the beam. This has previously been done in the studies on the spinal cord, with radiation-induced myelopathy as endpoint, demonstrating an increased RBE towards the distal edge of the SOBP, although not to the same scale as has previously been observed *in vitro* [20]. Also in order to simulate the clinical situation, dose per fraction needs to be taken into account. Previously obtained *in vivo* data has, beside the spinal cord data from Heidelberg [20], almost exclusively been single fraction studies, using very high doses. The RBE is however influenced by dose and fractionation [21], and it is of importance to perform studies with clinically relevant fractionation schemes in order to obtain relevant data, as single fraction studies may very well underestimate the RBE [22].

The lack of the RBE *in vivo* data has been discussed for years, so why is the appropriate data still not available? *In vivo* experiments are expensive and time consuming, especially when looking at late endpoints, where follow up may be year long. To do the appropriate comparisons, single dose experiments are not covering, instead full dose response curves needs to be obtained, which requires a high number of animals. Furthermore, fractionated animal studies are difficult to do, as they require even more animals and are logistically very demanding. Add to this, that experimental beam time for animal experiments have so far been quite hard to obtain. Luckily, at least the situation with experimental beam time is resolving, with more clinical facilities including experimental rooms [23], and initiatives like KVI-CART, an experimental facility in Groningen with transnational access, and INSPIRE (https://protonsinspire.eu/), a Horizon 2020

CONTACT Brita Singers Sørensen bsin@oncology.au.dk Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark

supported European Research project on infrastructure in Proton International Research, is facilitating an increase in beam time for *in vivo* experiments.

To a large extent, the experimental design for proton radiobiology can be built upon the knowledge gained from years of photon radiobiology experience. However, there are some significant differences. Importantly, proton research requires a very high degree of precision in positioning the target. To be able to resolve the differential effects in the very distal edge of the SOBP, precision is a crucial subject. It also raises considerations about the used models. As the LET is increasing through the SOBP, looking at the effect in larger targets will be an average RBE of a range of LET values. This was the issue in the studies of crypt regeneration in whole body irradiated mice [24], where the obtained RBE values do not reflect the spatial distribution of the RBE. To do this it will be necessary to determine independent RBE values at different depth in the SOBP, by using an endpoint that can be quantified in small volumes, for example, by imaging, or by using targets that are only a few millimeters, as this will give the spatial resolution through the SOBP. New solutions to this spatial issue is currently emerging, as Parodi et als setup for high precision image-guided proton irradiation of small animals [25], which enables studies of differential effects in different positions in animals.

In DCPT, the Danish Center for Particle Therapy, we are using a setup used for many years for photon research in Aarhus for our proton RBE *in vivo* experiments. This is based on irradiating mouse legs, with endpoints of both acute skin damage [26], and radiation induced fibrosis, a late damage, estimated with the leg contracture assay [27,28]. We have fine-tuned this setup to be able to move the target in 4 mm steps in through the SOBP, and determined an increased effect towards the distal edge using acute damage as endpoint [29].

A large amount of valuable radiobiological photon data have been obtained using orthovoltage X-ray irradiation, which has been the most common experimental platform. Also today, small animal irradiators are commonly low energy [30]. However, a differential radiobiological effect between orthovoltage and megavoltage irradiation has been known for many years, and doing comparison studies, it is necessary to be aware what the data are comparing to. As for the proton response, the differential normal tissue response between orhtovoltage and megavoltage X-ray are also tissue dependent. It is hence recommended to use high-energy reference radiation, with 6 MV photons being the clinically most relevant radiation source [19].

The final evaluation on the impact of variable proton RBE lies within the clinic and in clinical data, as in the study by Peeler and colleges demonstrating a correlation between MRI changes and LET in pediatric patients treated for ependymoma [18], and in the publication on MRI changes in glioma patients after proton therapy [31]. Nevertheless, important information should be obtained from experimental *in vivo* data. Current RBE models are to a considerable degree based on *in vitro* data on clonogenic survival [13,32], which is most likely not a very good surrogate for normal tissue damage. For this, high quality, clinical relevant *in vivo* data are needed. As the request for *in vivo* data on proton RBE has been a topic of interest for several years, the experimental platforms and knowledge has been on the way, and the next years will hopefully show a plethora on experimental normal tissue data on proton RBE.

Disclosure statement

The author report no conflicts of interest

References

[1] ICRU Report 78. Prescribing, recording and reporting proton-beam therapy. J ICRU 2007.
[2] Ödén J, DeLuca PM, Orton CG. The use of a constant RBE = 1.1 for proton radiotherapy is no longer appropriate. Med Phys. 2018;45(2):502–505.
[3] Jones B. Why RBE must be a variable and not a constant in proton therapy. Br J Radiol. 2016;89(1063):20160116.
[4] Buchsbaum JC, McDonald MW, Johnstone PAS, et al. Range modulation in proton therapy planning: a simple method for mitigating effects of increased relative biological effectiveness at the end-of-range of clinical proton beams. Radiat Oncol. 2014;9(1):2.
[5] Paganetti H, Niemierko A, Ancukiewicz M, et al. Relative biological effectiveness (RBE) values for proton beam therapy. Int J Radiat Oncol Biol Phys. 2002;53(2):407–421.
[6] Paganetti H, van Luijk P. Biological considerations when comparing proton therapy with photon therapy. Semin Radiat Oncol. 2013;23(2):77–87.
[7] Tommasino F, Durante M. Proton radiobiology. Cancers (Basel). 2015;7(1):353–381.
[8] Bettega D, Calzolari P, Chauvel P, et al. Radiobiological studies on the 65 MeV therapeutic proton beam at Nice using human tumour cells. Int J Radiat Biol. 2000;76(10):1297–1303.
[9] Sørensen BS, Overgaard J, Bassler N. In vitro RBE-LET dependence for multiple particle types. Acta Oncol. 2011;50(6):757–762.
[10] Britten RA, Nazaryan V, Davis LK, et al. Variations in the RBE for cell killing along the depth-dose profile of a modulated proton therapy beam. Radiat Res. 2013;179(1):21–28.
[11] Wouters B, Skarsgard L, Gerweck L, et al. Radiobiological intercomparison of the 160 MeV and 230 MeV proton therapy beams at the Harvard Cyclotron Laboratory and at Massachusetts General Hospital. Radiat Res. 2015;183(2):174–187.
[12] Willers H, Allen A, Grosshans D, et al. Toward a variable RBE for proton beam therapy. Radiother Oncol. 2018;128(1):68–75.
[13] Paganetti H, Blakely E, Carabe-Fernandez A, et al. Report of the AAPM TG-256 on the relative biological effectiveness of proton beams in radiation therapy. Med Phys. 2019;46(3):e53–e78.
[14] Lühr A, von Neubeck C, Krause M, et al. Relative biological effectiveness in proton beam therapy – current knowledge and future challenges. Clin Transl Radiat Oncol. 2018;9:35–41.
[15] Mohan R, Peeler CR, Guan F, et al. Radiobiological issues in proton therapy. Acta Oncol. 2017;56(11):1367–1373.
[16] Jones B. Clinical radiobiology of proton therapy: modeling of RBE. Acta Oncol. 2017;56(11):1374–1378.
[17] Grassberger C, Paganetti H. Varying relative biological effectiveness in proton therapy: knowledge gaps versus clinical significance versus clinical significance. Acta Oncol. 2017;56(6):761–762.
[18] Peeler CR, Mirkovic D, Titt U, et al. Clinical evidence of variable proton biological effectiveness in pediatric patients treated for ependymoma. Radiother Oncol. 2016;91:395–401.
[19] Lühr A, von Neubeck C, Pawelke J, et al. Radiobiology of proton therapy: results of an international expert workshop. Radiother Oncol. 2018;128(1):56–67.
[20] Saager M, Peschke P, Brons S, et al. Determination of the proton RBE in the rat spinal cord: is there an increase towards the end of the spread-out Bragg peak? Radiother Oncol. 2018;128(1):115–120.

[21] Dasu A, Toma-Dasu I. Impact of variable RBE on proton fractionation. Med Phys. 2013;40(1):011705.

[22] Marshall TI, Chaudhary P, Michaelidesova A, et al. Investigating the implications of a variable RBE on proton dose fractionation across a clinical pencil beam scanned spread-out bragg peak. Int J Radiat Oncol Biol Phys. 2016;95(1):70–77.

[23] Dosanjh M, Jones B, Pawelke J, et al. Overview of research and therapy facilities for radiobiological experimental work in particle therapy. Report from the European Particle Therapy Network radiobiology group. Radiother Oncol. 2018;128(1):14–18.

[24] Gueulette J, Bohm L, De Coster BM, et al. RBE variation as a function of depth in the 200-MeV proton beam produced at the National Accelerator Centre in Faure (South Africa). Radiother Oncol. 1997;42(3):303–309.

[25] Parodi K, Assmann W, Belka C, et al. Towards a novel small animal proton irradiation platform: the SIRMIO project. Acta Oncol. 2019;58:1470–1475.

[26] Von der Maase H. Effect of cancer chemotherapeutic drugs on the radiation-induced skin reactions in mouse feet. Br J Radiol. 1984;57(680):697–707.

[27] Sørensen BS, Horsman MR, Alsner J, et al. Relative biological effectiveness of carbon ions for tumor control, acute skin damage and late radiation-induced fibrosis in a mouse model. Acta Oncol. 2015;54(9):1623–1630.

[28] Stone HB. Leg contracture in mice: an assay of normal tissue response. Int J Radiat Oncol Biol Phys. 1984;10(7):1053–1061.

[29] Sørensen BS, Bassler N, Nielsen S, et al. Relative biological effectiveness (RBE) and distal edge effects of proton radiation on early damage in vivo. Acta Oncol. 2017;56(11):1387–1391.

[30] Verhaegen F, Dubois L, Gianolini S, et al. ESTRO ACROP: technology for precision small animal radiotherapy research: optimal use and challenges. Radiother Oncol. 2018;126(3):471–478.

[31] Eulitz J, Troost EGC, Raschke F, et al. Predicting late magnetic resonance image changes in glioma patients after proton therapy. Acta Oncol. 2019;58:1536–1539.

[32] McNamara AL, Schuemann J, Paganetti H. A phenomenological relative biological effectiveness (RBE) model for proton therapy based on all published in vitro cell survival data. Phys Med Biol. 2015;60(21):8399–8416.