Journal of Radiation Research, Vol. 61, No. 5, 2020, pp. 791–798
doi: 10.1093/jrr/rraa042
Advance Access Publication: 13 July 2020

Ramipril reduces incidence and prolongates latency time of radiation-induced rat myelopathy after photon and carbon ion irradiation

Maria Saager[1,5,6], Eric W. Hahn[2], Peter Peschke[1,6], Stephan Brons[4,6], Peter E. Huber[3,5,6], Jürgen Debus[3,5,6] and Christian P. Karger[1,6,*]

[1]Department of Medical Physics in Radiation Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[2]Preclinical Imaging Section, Department of Radiology, The University of Texas, Southwestern Medical Center, Dallas, Texas, USA
[3]Clinical Cooperation Unit Molecular Radiooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[4]Heidelberg Ion Beam Therapy Center (HIT), Heidelberg, Germany
[5]Department of Radiation Oncology, University Hospital of Heidelberg, Heidelberg, Germany
[6]National Center for Radiation Research in Oncology (NCRO), Heidelberg Institute for Radiation Oncology (HIRO), Heidelberg, Germany
*Corresponding author. German Cancer Research Center (DKFZ), Dept. of Medical Physics in Radiation Oncology (E040), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany, Phone: +49-6221-42-2575; Email: c.karger@dkfz.de
(Received 30 July 2019; revised 26 April 2020; editorial decision 30 May 2020)

ABSTRACT

To test the hypothesis that the use of an angiotensin-converting enzyme inhibitor (ACEi) during radiotherapy may be ameliorative for treatment-related normal tissue damage, a pilot study was conducted with the clinically approved (ACE) inhibitor ramipril on the outcome of radiation-induced myelopathy in the rat cervical spinal cord model. Female Sprague Dawley rats were irradiated with single doses of either carbon ions (LET 45 keV/μm) at the center of a 6 cm spread-out Bragg peak (SOBP) or 6 MeV photons. The rats were randomly distributed into 4 experimental arms: (i) photons; (ii) photons + ramipril; (iii) carbon ions and (iv) carbon ions + ramipril. Ramipril administration (2 mg/kg/day) started directly after irradiation and was maintained during the entire follow-up. Complete dose-response curves were generated for the biological endpoint radiation-induced myelopathy (paresis grade II) within an observation time of 300 days. Administration of ramipril reduced the rate of paralysis at high dose levels for photons and for the first time a similar finding for high-LET particles was demonstrated, which indicates that the effect of ramipril is independent from radiation quality. The reduced rate of myelopathy is accompanied by a general prolongation of latency time for photons and for carbon ions. Although the already clinical approved drug ramipril can be considered as a mitigator of radiation-induced normal tissue toxicity in the central nervous system, further examinations of the underlying pathological mechanisms leading to radiation-induced myelopathy are necessary to increase and sustain its mitigative effectiveness.

Keywords: Photons; Carbon Ions; Rat Spinal Cord; Myelopathy; Ramipril; Radioprotection

INTRODUCTION

The delivery of the required dose to tumors located in or adjacent to the central nervous system (CNS) is often not possible due to the risk of severe side effects in the surrounding normal tissue. In the spinal cord, radiation-induced damage may result in a breakdown of the blood-spinal cord barrier associated with edema or myelopathy. To minimize the risk of these side effects, the irradiated tissue volume is reduced by tailoring the dose distribution to the tumor by means of conformal irradiation techniques like intensity-modulated radiotherapy (IMRT) [1] or particle therapy [2]. For the latter, protons and carbon ions are currently being used. While both provide an excellent degree of conformity, carbon ions exhibit an increased relative biological effectiveness (RBE) relative to photons, which rises with increasing depth [2]. In spite of these high precision radiotherapy techniques, it is often not possible to completely exclude tissues at risk from the treatment field, as tumors are often located close to normal neuronal structures or even surround these structures.

Various attempts have been made to prevent, mitigate or reduce radiation-induced side effects by administering specific substances e.g. sulfhydryl compounds [3], vitamins [4] or angiotensin-converting

enzyme (ACE) inhibitors [5]. Depending on the time of administration in relation to the radiotherapy course, different strategies exist. While radioprotectors are administered prior to irradiation and aim to reduce or ideally prevent side effects, mitigators are given after radiotherapy. Finally, compounds given to medicate existing radiation-induced effects are defined as treatments [6, 7].

ACE inhibitors (ACEi) have shown to mitigate the radiation tolerance after photon irradiation with late reacting organs like kidney [8–12] or lung [13–16]. Side effects in the kidney could even be treated with ACEi [12, 16]. The extent of radiation-induced damage in the brain [17, 18] and optical nerve [19, 20] was also influenced by ramipril administration. In contrast to other ACE inhibitors [21], ramipril, or rather its active form ramiprilat, is able to cross the blood-brain barrier (BBB) [22]. In the context of these findings, it has to be noted that some of the radiotherapy patients take regularly ACEi for anti-hypertensive treatment.

Based on these preclinical results with ACEi in late reacting normal tissues and encouraged by the growing clinical interest to block the renin-angiotensin system to prevent or lessen the severity of radiation-induced normal tissue injuries [23, 24], the present study investigates, whether the administration of ramipril influences the outcome of radiation-induced myelopathy after irradiation of the rat cervical spinal cord. So far, all available normal tissue studies have been performed with photon radiation and just recently, a report was published [25] which describe positive effects of ramipril on the onset and delay of paralysis at high photon radiation doses. As no data for high-LET ion beams with its distinctly different intracellular damage pattern is available, we extend the state of research by using single therapeutic doses with two different beam qualities: carbon ions and photons.

METHODS AND MATERIALS

Animals

A total of 92 eight to ten weeks old adult female Sprague Dawley rats (Charles River, Sulzfeld, Germany) with an average weight of 215 ± 8 g were used in 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. The study was approved by the governmental review committee on animal care (ref. no.: 35–9185.81/G-34/13), and animals were kept under standard conditions at the DKFZ animal laboratory facility.

Experimental setup

The rat cervical spinal cord was irradiated with single doses of either carbon ions or photons. For both irradiation modalities, the field size was 10x15 mm^2 including the cervical segments C1-C6. Rats were positioned vertically in a dedicated mounting device and irradiation was performed from the ventral direction using a horizontal beam. Photons were delivered by a 6 MeV linear accelerator (Artiste, Siemens, Erlangen, Germany) and the field was defined by opening a single leaf pair of the multi-leaf collimator as described previously. Carbon ion irradiation was performed at the Heidelberg Ion-Beam Therapy Center (HIT, Germany) using the active raster scanning technique. For this, the spinal cord was positioned at the center of a 6 cm spread-out Bragg peak (SOBP) ranging from 70 to 130 mm water-equivalent depth corresponding to a dose-averaged linear energy transfer (LET) of 45 keV/μm. As in previous studies, the range of the ions was adapted using a polymethylmethacrylate (PMMA)-bolus of an appropriate thickness in front of the animals [26, 27].

Rats were randomized into 4 experimental arms: (i) photons ($n = 20$), (ii) photons + ramipril ($n = 24$), (iii) carbon ions ($n = 20$), (iv) carbon ions + ramipril ($n = 20$). Eight non-irradiated but otherwise sham treated animals served as controls for the experimental arms with ($n = 4$) and without ($n = 4$) ramipril. Within each experimental arm, different doses were applied using 4 animals per dose level (Table 1). Doses were selected to cover 0–100% response rates for the biological endpoint.

Biological endpoint and ramipril administration

For the experiment, the irreversible biological endpoint radiation-induced myelopathy (paresis grade II) was used. Paresis grade II is defined as neurological symptoms when the animals show regular dragging of the foot with palmar flexion or dragging of extended forelegs [28]. When these definite signs of paralysis were observed within the observation time of 300 days, the endpoint of the experiment was reached and the animals were scored as responder. Rats received ramipril for 300 days in their drinking water available *ad libitum,* with an uptake dose of 2 mg/kg/day starting directly after irradiation. Initiation of ramipril administration as well as the concentration were based on published studies [20] which reported that a minimum dose of 1.5 mg/kg/day is necessary to achieve a biological effect. Rats were checked for weight and general condition once a week. Rats exhibiting paresis grade II were sacrificed according to governmental regulations.

Data analysis

As a reference, dose-response curves were calculated for the experimental arms without ramipril in the same way as in our previous studies [26, 27]. For this, the logistic dose-response model was fitted using actuarial response rates [29]. From the dose-response curves, the TD_{50}-values (dose at 50% probability of paresis grade II) were determined.

Statistics

Dose-response curves were fitted using the maximum likelihood procedure of STATISTICA [30]. Incomplete follow up of animals was considered in the ML-fit using the method of effective sample sizes [29] that corrects the number of treated and responding animals to match actuarial response rates and their variances. As less than 2 response rates differed from 0% and 100%, the standard error could not be estimated reliably by the fitting procedure. The SE was therefore estimated as 25% of the dose difference between the neighbouring 0% and 100% dose levels, which matches previous experience that TD_{50} is separated approximately by 2 SE from the neigbouring 0% and 100% dose levels.

The dependencies of mean and minimum latency time on dose were interpolated by linear regression. The significance of the respective slopes as well as the differences in weight and latency time were analyzed with student's t-test using a significance level of $p = 0.05$.

Table 1. Dose levels and number of animals used for the experiments. Each dose level contained 4 animals. In total 8 controls were included for the experimental arms with and without ramipril, respectively

Study	Dose [Gy]	Total number of animals
Photons		
With ramipril	20, 24, 26, 28, 30, 34	24
Without ramipril	18, 22*, 24, 26, 30	20
Carbon ions		
With ramipril	13*, 15, 17#, 19, 21‡	20
Without ramipril	13, 15, 17, 19◊, 21	20
Controls		
With ramipril	0	4
Without ramipril	0	4

*One animal had to be excluded due to development of mammary carcinoma (239 d and 232 d, respectively).
#Alterations on the spine caused compression of the spinal cord (225 d).
‡One animal died due to unknown reasons (14 d).
◊One animal died during irradiation.

Table 2. Comparison of the body weight (g) after 150 and 300 days (d). n represents the number of animals at the specified time point. The student's t-test was utilized to test the significance of the differences in weight using a significance level of $P = 0.05$

Study	150 d	300 d
Photons		
With ramipril	317 ± 23 g (n = 24)	340 ± 36 g (n = 6)
Without ramipril	371 ± 40 g (n = 20)	439 ± 62 g (n = 10)
	$P = 2{\cdot}10^{-6}$	$P = 0.003$
Carbon ions		
With ramipril	329 ± 33 g (n = 19)	344 ± 30 g (n = 8)
Without ramipril	370 ± 41 g (n = 19)	442 ± 91 g (n = 8)
	$p = 0.002$	$p = 0.01$

RESULTS

The irradiation procedure as well as the uptake of ramipril was well tolerated by the animals. Only one animal died during irradiation for unknown reason (Table 1). Four out of 92 rats had to be excluded either due to spontaneous development of mammary carcinoma, death of unknown reason or morphological alterations at the spine leading to paralysis (Table 1).

In the course of follow up, a significant influence of ramipril on body weight was observed at 150 days (Table 2). This average difference increased until the end of the observation time (99 g vs. 54 g for photons and 98 g vs. 41 g for carbon ions).

Dose-response curves and TD_{50}-values

Figure 1 shows the dose-response curves of the irradiated control groups with TD_{50}-values of 24.2 ± 1.0 Gy for photons and 16.0 ± 0.5 Gy for carbon ions. In addition, the response rates of the ramipril treated groups are shown for both radiation modalities. Administration of ramipril decreased the incidence rate for paresis after photon doses of 26, 30 and 34 Gy, which strictly lead to 100% complication probability after photon treatment alone. A similar effect was observed after a carbon ion dose of 19 Gy.

Latency time

Mean and minimum latency time for paresis grade II was generally shorter after carbon ion as compared to photon irradiation with as well as without ramipril (Fig. 2), however the average differences were non-significant ($p > 0.08$). For both irradiation modalities, a prolongation of the minimum as well as the mean latency time was found after ramipril administration as compared to the untreated control arms (Fig. 2). However, also these average differences showed to be non-significant for both radiation modalities ($P > 0.11$). Although there was a general trend of decreasing latency time with increasing dose, the slopes of the linear regression lines for the mean and minimum latency time for carbon ion and photon irradiation with or without ramipril administration were not significantly different from zero ($P > 0.3$).

DISCUSSION

Substantial technological advancements in conformal radiation treatments and imaging technologies together with a better understanding of tumor biology have improved the treatment efficacy of radiotherapy leading to an increase of the number of long-term survivors [31]. Heavy ion radiotherapy with carbon ions is such a high precision technique which holds great potential for patients of various kinds of cancer and is currently investigated in numerous clinical trials [32]. In spite of its

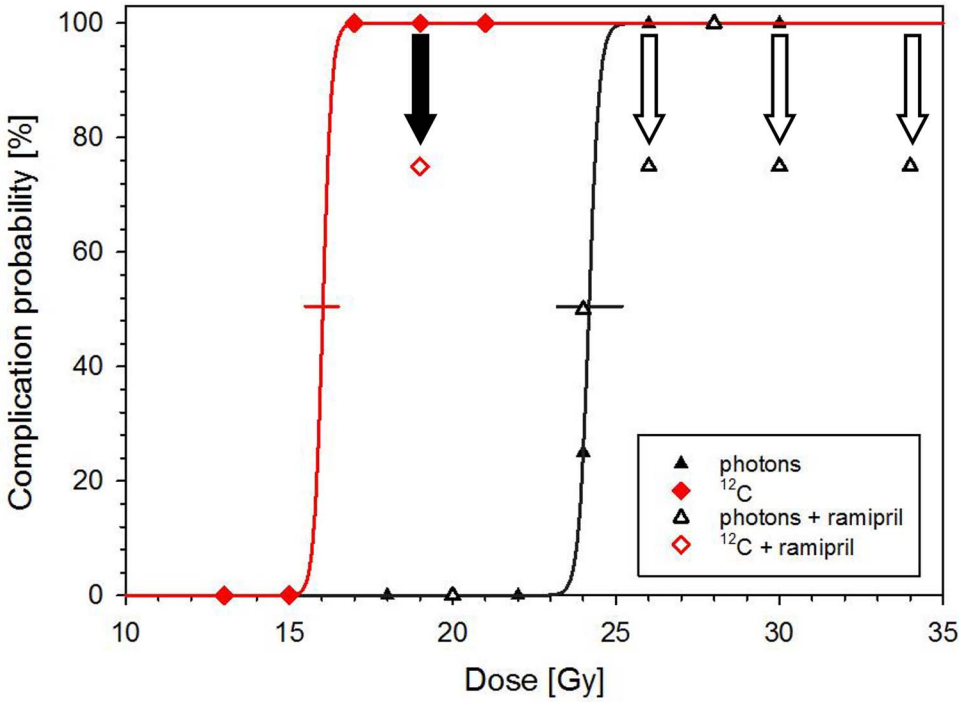

Fig. 1. (a) Dose-response curves for carbon ion (^{12}C) (diamonds) and photon irradiation (triangles) fitted to the response data without ramipril (closed symbols) at 300 d after irradiation. Ramipril administration decreases the response rates at high doses (100% effect level) for 1 out of 3 dose levels after carbon ion (closed arrow, open diamonds) and for 3 out of 4 dose levels after photon irradiation (open arrows, open triangles).

improved tumor conformity, radiation-induced side effects are still a matter of concern, especially because of the higher relative biological effectiveness (RBE) of particles. Radiation-induced myelopathy is a long-term sequela of head and neck and thoracic cancer radiotherapy occurring months or even years post treatment [33]. Consequently, there is worldwide an ongoing search for appropriate radiomitigators or radioprotectors, which could help to increase the normal tissue resistance to irradiation and hence improve tumor control rates by allowing higher doses applied to the tumor.

Normal tissue model and ramipril administration

In the past, the rat spinal cord model was used to quantitatively examine fractionation effects, dose-volume relationships and the biological effectiveness of protons and heavier ions [26, 34, 35]. Most of these preclinical evaluations were performed in female rats because they are easy to handle and can be readily kept for the long follow-up times, necessary to quantify the biological endpoint of radiation myelopathy.

The use of ramipril in the present study was based on promising results of several studies which investigated the potential of this drug to mitigate late side effects in the CNS [17–20, 36]. Ramipril was given via drinking water with an uptake dose of 2 mg/kg/day starting with the application directly after radiotherapy. As previously shown, the effect of ramipril is not influenced by water [37] and the prodrug is rapidly resorbed by the body and converted in the liver to its active form ramiprilat. The resorption is also not influenced by food intake [38]. The selection of the starting point of ramipril administration as well as the concentration were based on published studies. Ryu *et al.* [20] showed that a ramipril uptake dose of 1.5 mg/kg/day starting with the administration two weeks after low-LET irradiation led to a better preservation of the functional integrity and morphology of the optic nerve as compared to the irradiated controls. In contrast, a dose of 0.5 mg/kg/day had no effect. No mitigative effect was obtained when the drug was applied 4 weeks after irradiation.

During the first three weeks after irradiation, a slower gain in body weight was observed in irradiated groups receiving ramipril as compared to the irradiated controls. Within this period, the animals drank 40–65 mL ramipril solution and the intake was two- to three-fold higher as in the controls (20 mL ramipril solution per day). Based on these observations, a mean intake of 30 mL/day was used for the calculation of the ramipril concentration in the drinking water. After an initial adaption phase, the ramipril receiving animals started gaining weight. A decrease of the age-related gain in body weight due to ACEi intake (perindopril) was also described by Weisinger *et al.* [39] and is not a result of a reduced food intake, but rather due to the enhanced metabolic turnover. As a result, the animals exhibit a reduced fraction of adipose tissue [39] which explains the lower body weight throughout the experiments as compared to the irradiated controls (Table 2).

Evidence of mitigative effects

In the general case, dose response curves are described by several parameters: the efficacy (location of curve along the dose axis), the

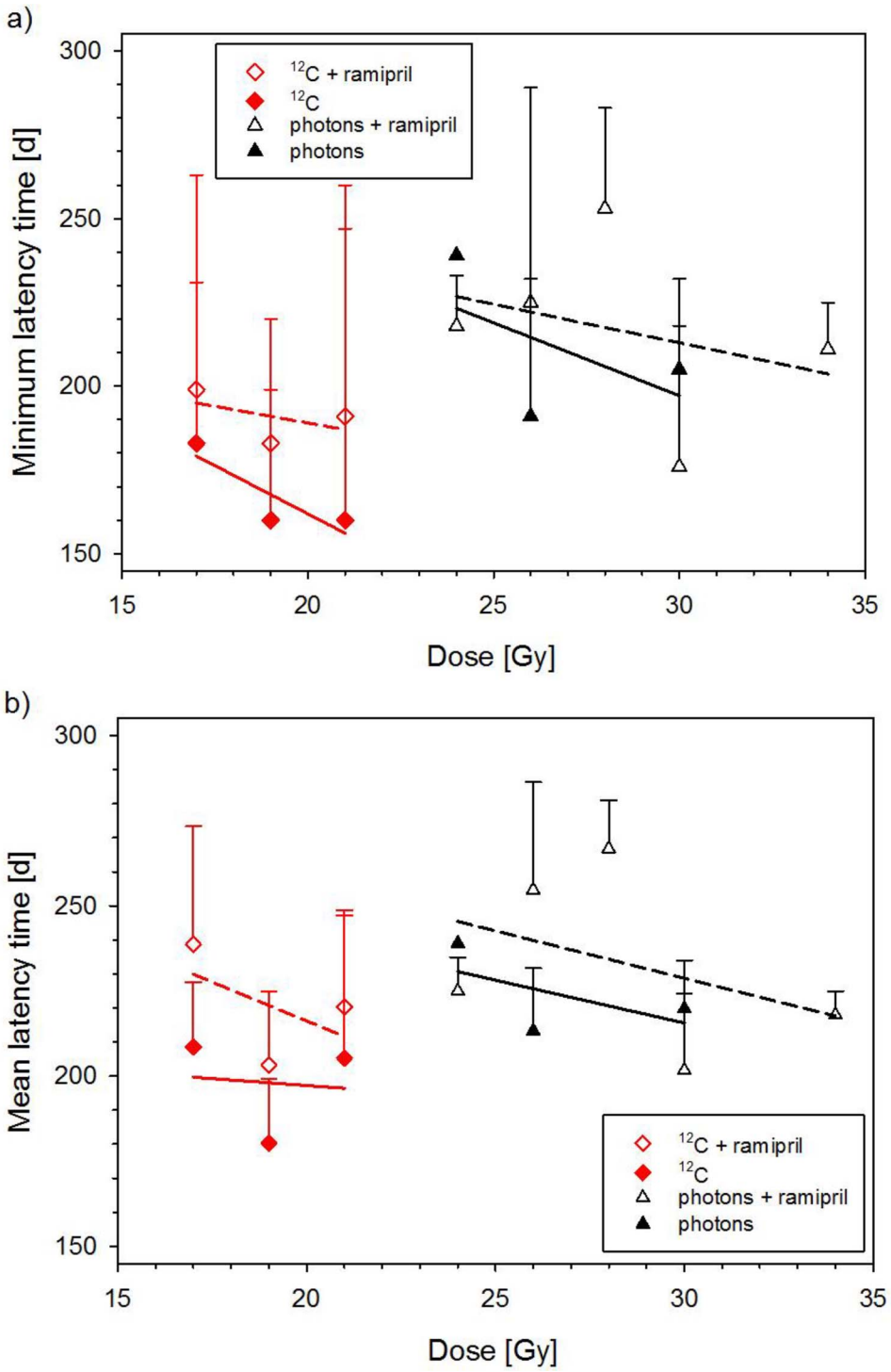

Fig. 2. Minimum (a) and mean (b) latency time for the onset of paresis grade II after carbon ion (^{12}C) (diamonds) and photon (triangles) irradiation, respectively, with (open symbols, dotted line) and without (closed symbols, solid line) ramipril administration displayed together with linear regression lines. The error bars represent the range (minimum latency time, (a)) and single standard deviation (mean latency time, (b)), respectively, of the latency time within one dose group. Note: Mean and minimum latency times include all animals showing paresis grade II within 300 days.

slope (change in response per unit dose) as well as minimal dose (highest dose leading to no response) and the so-called ceiling effects (greatest attainable response). Radiation treatments without drugs, usually assume sigmoid curves with a ceiling effect of 100% and a minimal dose that is symmetrical to TD_{50}. Carbon ion treatment without ramipril (Fig. 1) exhibits a RBE of 1.51 ± 0.08, which is in excellent agreement with a previous experiment [27]. High-LET radiotherapy employs a high effectiveness, reflected in an increased percentage of clustered DNA damage along the particle tracks. Both the proportion and the degree of complexity of this clustered DNA damage increase with LET and compromise accurate DNA repair [40]. In the spinal cord, the increased LET accelerates molecular and cellular alterations such as cytokine upregulation and inactivation of radiosensitive cells leading to enhanced demyelination, a larger extent of blood vessel perforation, tissue hypoxia and functionally to a faster appearance of myelopathy [41, 42].

No difference in efficacy, slope and at the lower barrier ("non responders") for photons and carbon ions with and without drug was observed. A striking difference, however, was found for the ceiling effect, showing that the probability to achieve the selected biological endpoint is influenced by the drug (Fig. 1).

Taking the dose-response curves of the experimental arms without ramipril as a reference, the reduced incident rates of 75% (3 out of 4 ramipril treated animals responded) were found in several high dose groups at 100% effect level for both carbon ion and photon irradiations, (Fig. 1). The 95%-confidence interval of these reduced incident rates ranges from 19.4% to 99.4% and the probability predicted by the dose-response curves of the experimental arms without ramipril coincides with the upper boundary of this interval for incidences at several dose levels. As this incidence pattern is highly unlikely, it has to be attributed to the administration of ramipril. The significance of this observation as well as the reproducibility of the spinal cord model and the resulting dose-response curves are very well supported by previous studies using the same experimental setup. In these experiments, the tolerance doses TD_{50} differed only by 0.1 Gy for carbon ions- and by 0.3 Gy for photon irradiations [26].

For the clinical endpoint used in our study, there are clear detection criteria, however, the applied follow-up time is an implicit parameter of this endpoint. As the follow-up in our experiment was 300 d, it remains ambigous whether the reduced incidence at high doses would have persisted permanently or if these animals would have expressed myelopathy only at a later time point. Analyzing the latency times (Fig. 2), however, clearly shows an increased latency time for all responding animals for all ramipril-treated dose groups of both photons and somewhat more pronounced for carbon ions. These findings suggest the existence of a limited protection of the spinal cord tissue by ramipril which seems not to be affected by radiation quality. Both, the reduced incident rates at high dose levels and the prolonged latency time at intermediate doses leads us to interpret the effect of ramipril on the development of radiation-induced myelopathy as being mitigative.

This study not only confirms positive effects on the onset and delay of radiation induced myelopathy at high photon doses [25] but is concurrently the first finding of a mitigative effect with ramipril for high-LET radiation. Similar effects of ramipril in terms of functionality and morphology have been observed for stereotactic low-LET irradiation of the optic nerve [19]. The cognitive function of rats treated with ramipril was improved compared to the only irradiated control group [18]. A prolongation of latency time for the onset of ataxia in the forelimbs after low-LET irradiation was also observed by Hornsey *et al.* [43] using the vasoactive drug dipyridamol.

The underlying mechanisms are still under debate. ACE is part of the renin-angiotensin-aldosterone-system (RAAS), which regulates blood pressure, fluid balance and electrolyte homeostasis [44, 45]. The enzyme converts inactive angiotensin I to angiotensin II (Ang II), a multifunctional growth factor and immunomodulator that stimulates the production of vasoconstrictors, influences cell growth, tissue fibrosis, differentiation, apoptosis [46] and promotes a variety of inflammatory processes in the vasculature of the kidney, liver, heart and lung [47–49]. Moreover, an organ-specific RAAS was identified in neuronal and glial elements in the brain as well as in the cerebrospinal fluid [50] and the expression of angiotensin receptors in the spinal cord was proven [51]. As RAAS is known to be up-regulated in normal tissues weeks to several months after irradiation [52, 53], there is evidence that inhibition of Ang II by ramipril not only has an indirect impact on blood vessels, which play an important role in the development of radiation-induced myelopathy [54–56] but also down-regulates a variety of Ang II-induced pro-inflammatory events. Radiation-induced VEGF expression and a reduction of microglial infiltration seems to be central effectors which are down-regulated by ramipril [25]. Finally, an important advantage that makes RAAS targeting an interesting strategy for improving the radiotherapeutic ratio is the fact that progression, vascularization and metastasis in cancer cells is also inhibited [57].

CONCLUSION

Successful post-irradiation therapeutic intervention could offer both substantial reductions in late effects and substantial dose escalation and hence will have a major impact on clinical radiotherapy. This study demonstrated that ramipril blocks radiation-induced Ang II-associated pathways effectively and attenuates consequences of ionizing radiation for both photon and high-LET carbon ion irradiation. Yet, as the inhibition of Ang II through ACEi is not sufficient to completely protect from late effects in the rat spinal cord, the drug is considered to be mitigative. Combining ramipril with additional targeting compounds might raise the mitigative efficacy. Moreover, to increase and sustain the mitigative effectiveness, a more precise understanding of the underlying pathological mechanisms leading to radiation-induced myelopathy is necessary. Finally, in view of the use of ACEi as anti-hypertensive drug, radiotherapy treatments combined with relatively high ACEi doses are safe, at least in this animal model.

CONFLICT OF INTEREST NOTIFICATION

The authors have no conflict of interest to report.

PRESENTATION AT A CONFERENCE

Parts of this study were presented at the 61st Annual meeting of the Radiation Research Society, Weston, FL, USA, September 19–22, 2015.

FUNDING

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, KFO 214).

ACKNOWLEDGEMENT

We greatly acknowledge the excellent experimental conditions provided by the Heidelberg Ion-Beam Therapy Center (HIT).

REFERENCES

1. Bortfeld T. IMRT: A review and preview. *Phys Med Biol* 2006;51:R363–79.
2. 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.
3. Singh VK, Fatanmi OO, Wise SY et al. The potentiation of the Radioprotective efficacy of two medical countermeasures, gamma-Tocotrienol and Amifostine, by a combination prophylactic modality. *Radiat Prot Dosimetry* 2016;172:302–10.
4. Sridharan V, Tripathi P, Sharma S et al. Effects of late administration of pentoxifylline and tocotrienols in an image-guided rat model of localized heart irradiation. *PLoS One* 2013;8:e68762.
5. van der Veen SJ, Ghobadi G, de Boer RA et al. ACE inhibition attenuates radiation-induced cardiopulmonary damage. *Radiother Oncol* 2015;114:96–103.
6. Johnke RM, Sattler JA, Allison RR. Radioprotective agents for radiation therapy: Future trends. *Future Oncol* 2014;10:2345–57.
7. Prasanna PG, Stone HB, Wong RS et al. Normal tissue protection for improving radiotherapy: Where are the gaps? *Transl Cancer Res* 2012;1:35–48.
8. Geraci JP, Sun MC, Mariano MS. Amelioration of radiation nephropathy in rats by postirradiation treatment with dexamethasone and/or captopril. *Radiat Res* 1995;143:58–68.
9. Molteni A, Moulder JE, Cohen EP et al. Prevention of radiation-induced nephropathy and fibrosis in a model of bone marrow transplant by an angiotensin II receptor blocker. *Exp Biol Med (Maywood)* 2001;226:1016–23.
10. Moulder JE, Fish BL, Cohen EP. Treatment of radiation nephropathy with ACE inhibitors. *Int J Radiat Oncol Biol Phys* 1993;27:93–9.
11. Moulder JE, Fish BL. Angiotensin converting enzyme inhibitor captopril does not prevent acute gastrointestinal radiation damage in the rat. *Radiat Oncol Investig* 1997;5:50–3.
12. Moulder JE, Fish BL, Cohen EP. Treatment of radiation nephropathy with ACE inhibitors and AII type-1 and type-2 receptor antagonists. *Curr Pharm Des* 2007;13:1317–25.
13. Ward WF, Molteni A, Ts'ao CH et al. Radiation pneumotoxicity in rats: Modification by inhibitors of angiotensin converting enzyme. *Int J Radiat Oncol Biol Phys* 1992;22:623–5.
14. Molteni A, Moulder JE, Cohen EF et al. Control of radiation-induced pneumopathy and lung fibrosis by angiotensin-converting enzyme inhibitors and an angiotensin II type 1 receptor blocker. *Int J Radiat Biol* 2000;76:523–32.
15. Molthen RC, Wu Q, Fish BL et al. Mitigation of radiation induced pulmonary vascular injury by delayed treatment with captopril. *Respirology* 2012;17:1261–8.
16. Moulder JE, Fish BL, Cohen EP. ACE inhibitors and AII receptor antagonists in the treatment and prevention of bone marrow transplant nephropathy. *Curr Pharm Des* 2003;9:737–49.
17. Jenrow KA, Brown SL, Liu J et al. Ramipril mitigates radiation-induced impairment of neurogenesis in the rat dentate gyrus. *Radiat Oncol* 2010;5:6.
18. Lee TC, Greene-Schloesser D, Payne V et al. Chronic administration of the angiotensin-converting enzyme inhibitor, ramipril, prevents fractionated whole-brain irradiation-induced perirhinal cortex-dependent cognitive impairment. *Radiat Res* 2012;178:46–56.
19. Kim JH, Brown SL, Kolozsvary A et al. Modification of radiation injury by ramipril, inhibitor of angiotensin-converting enzyme, on optic neuropathy in the rat. *Radiat Res* 2004;161:137–42.
20. Ryu S, Kolozsvary A, Jenrow KA et al. Mitigation of radiation-induced optic neuropathy in rats by ACE inhibitor ramipril: Importance of ramipril dose and treatment time. *J Neurooncol* 2007;82:119–24.
21. Robbins ME, Zhao W, Garcia-Espinosa MA et al. Renin-angiotensin system blockers and modulation of radiation-induced brain injury. *Curr Drug Targets* 2010;11:1413–22.
22. Nordstrom M, Abrahamsson T, Ervik M et al. Central nervous and systemic kinetics of ramipril and ramiprilat in the conscious dog. *J Pharmacol Exp Ther* 1993;266:147–52.
23. Harder EM, Park HS, Nath SK et al. Angiotensin-converting enzyme inhibitors decrease the risk of radiation pneumonitis after stereotactic body radiation therapy. *Pract Radiat Oncol* 2015;5:e643–9.
24. Sio TT, Atherton PJ, Pederson LD et al. Daily Lisinopril vs placebo for prevention of Chemoradiation-induced pulmonary distress in patients with lung cancer (alliance MC1221): A pilot double-blind randomized trial. *Int J Radiat Oncol Biol Phys* 2019;103:686–96.
25. Clausi MG, Stessin AM, Tsirka SE et al. Mitigation of radiation myelopathy and reduction of microglial infiltration by Ramipril, ACE inhibitor. *Spinal Cord* 2018;56:733–40.
26. 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.
27. 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.
28. Ruifrok AC, Stephens LC, van der Kogel AJ. Radiation response of the rat cervical spinal cord after irradiation at different ages: Tolerance, latency and pathology. *Int J Radiat Oncol Biol Phys* 1994;29:73–9.
29. Walker AM, Suit HD. Assessment of local tumor control using censored tumor response data. *Int J Radiat Oncol Biol Phys* 1983;9:383–6.
30. StatSoft, Inc. *STATISTICA for Windows (Software-System for data analysis)*, Version 9.1. www.statsoft.com. 2010.
31. Baumann M, Krause M, Overgaard J et al. Radiation oncology in the era of precision medicine. *Nat Rev Cancer* 2016;16:234–49.

32. 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:e93–e100.
33. Schultheiss TE, Higgins EM, El-Mahdi AM. The latent period in clinical radiation myelopathy. *Int J Radiat Oncol Biol Phys* 1984;10:1109–15.
34. Kirkpatrick JP, van der Kogel AJ, Schultheiss TE. Radiation dose-volume effects in the spinal cord. *Int J Radiat Oncol Biol Phys* 2010;76:S42–9.
35. 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;128:115–20.
36. Kim JS, Yun I, Choi YB et al. Ramipril protects from free radical induced white matter damage in chronic hypoperfusion in the rat. *J Clin Neurosci* 2008;15:174–8.
37. Allen LV Jr, Stiles ML, Prince SJ et al. Stability of ramipril in water, apple juice, and applesauce. *Am J Health Syst Pharm* 1995;52:2433–6.
38. Kohl RR, Kolozsvary A, Brown SL et al. Differential radiation effect in tumor and normal tissue after treatment with ramipril, an angiotensin-converting enzyme inhibitor. *Radiat Res* 2007;168:440–5.
39. Weisinger HS, Begg DP, Egan GF et al. Angiotensin converting enzyme inhibition from birth reduces body weight and body fat in Sprague-Dawley rats. *Physiol Behav* 2008;93:820–5.
40. Goodhead DT. Mechanisms for the biological effectiveness of high-LET radiations. *J Radiat Res* 1999;40:1–13.
41. Okada S, Okeda R. Pathology of radiation myelopathy. *Neuropathology* 2001;21:247–65.
42. Saager M, Peschke P, Welzel T et al. Late normal tissue response in the rat spinal cord after carbon ion irradiation. *Radiat Oncol* 2018;13:5.
43. Hornsey S, Myers R, Jenkinson T. The reduction of radiation damage to the spinal cord by post-irradiation administration of vasoactive drugs. *Int J Radiat Oncol Biol Phys* 1990;18:1437–42.
44. Garg M, Angus PW, Burrell LM et al. Review article: The pathophysiological roles of the renin-angiotensin system in the gastrointestinal tract. *Aliment Pharmacol Ther* 2012;35:414–28.
45. Speth RC, Giese MJ. Update on the renin-angiotensin system. *J Pharmacol Clin Toxicol* 2013;1:1004.
46. Mehta PK, Griendling KK. Angiotensin II cell signaling: Physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol* 2007;292:C82–97.
47. Candido R, Allen TJ, Lassila M et al. Irbesartan but not amlodipine suppresses diabetes-associated atherosclerosis. *Circulation* 2004;109:1536–42.
48. Phillips MI, Kagiyama S. Angiotensin II as a pro-inflammatory mediator. *Curr Opin Investig Drugs* 2002;3:569–77.
49. Okamura A, Rakugi H, Ohishi M et al. Upregulation of renin-angiotensin system during differentiation of monocytes to macrophages. *J Hypertens* 1999;17:537–45.
50. Young CN, Davisson RL. Angiotensin-II, the brain, and hypertension: An update. *Hypertension* 2015;66:920–6.
51. McKinley MJ, Albiston AL, Allen AM et al. The brain renin-angiotensin system: Location and physiological roles. *Int J Biochem Cell Biol* 2003;35:901–18.
52. Cohen EP, Fish BL, Moulder JE. Successful brief captopril treatment in experimental radiation nephropathy. *J Lab Clin Med* 1997;129:536–47.
53. Cao S, Wu R. Expression of angiotensin II and aldosterone in radiation-induced lung injury. *Cancer Biol Med* 2012;9:254–60.
54. Coderre JA, Morris GM, Micca PL et al. Late effects of radiation on the central nervous system: Role of vascular endothelial damage and glial stem cell survival. *Radiat Res* 2006;166:495–503.
55. Morris GM, Coderre JA, Bywaters A et al. Boron neutron capture irradiation of the rat spinal cord: Histopathological evidence of a vascular-mediated pathogenesis. *Radiat Res* 1996;146:313–20.
56. Morris GM, Coderre JA, Whitehouse EM et al. Boron neutron capture therapy: A guide to the understanding of the pathogenesis of late radiation damage to the rat spinal cord. *Int J Radiat Oncol Biol Phys* 1994;28:1107–12.
57. Hosseinimehr SJ. The use of angiotensin II receptor antagonists to increase the efficacy of radiotherapy in cancer treatment. *Future Oncol* 2014;10:2381–90.