Dose-volume effects in rat spinal cord irradiated with protons

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CHAPTER 2

Dose-volume effects in the rat cervical spinal cord after proton irradiation

Hendrik P. Bijl, Peter van Luijk, Rob P. Coppes, Jacobus M. Schippers, Antonius W.T. Konings and Albert J. van der Kogel

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Abstract

Purpose: To estimate dose-volume effects in the rat cervical spinal cord with protons.
Methods and Materials: Wistar rats were irradiated on the cervical spinal cord with a single fraction of unmodulated protons (150-190 MeV) using the shoot through method, which employs the plateau of the depth-dose profile rather than the Bragg peak. Four different length of the spinal cord (2, 4, 8 and 20 mm) were irradiated with variable doses. The endpoint for estimating dose-volume effects was paralysis of fore and/or hind limbs.
Results: The results obtained with a high-precision proton beam showed a marginal increase of ED\textsubscript{50} when decreasing the irradiated cord length from 20 mm (ED\textsubscript{50} = 20.4 Gy) to 8 mm (ED\textsubscript{50} = 24.9 Gy), but a steep increase in ED\textsubscript{50} when further decreasing the length to 4 mm (ED\textsubscript{50} = 53.7 Gy) and 2 mm (ED\textsubscript{50} = 87.8 Gy). These results generally confirm data obtained previously in a limited series with 4-6 MV photons, and for the first time it was possible to construct complete dose-response curves down to lengths of 2 mm. At higher ED\textsubscript{50} values and shorter lengths irradiated, the latent period to paralysis decreased from 125 to 60 days.
Conclusions: Irradiation of variable lengths of rat cervical spinal cord with protons showed steeply increasing ED\textsubscript{50} values for lengths of less than 8 mm. These results suggest the presence of a critical migration distance of 2-3 mm for cells involved in regeneration processes.
Dose-Volume Effects in Irradiated Rat Spinal Cord

Introduction

The spinal cord is a critical organ in radiotherapy and a typical late reacting tissue in response to radiation (1-4). The incidence of radiation myelopathy is less than 5% using doses of 57-61 Gy (5). Many clinicians assume that the probability of myelopathy increases by increasing length of irradiated spinal cord although there is no clear clinical evidence (6).

Knowledge of dose-volume effects is important for uniform and non-uniform dose delivery in critical, late reacting organs like the spinal cord. Limited data are available from photon experiments with small (1,2,4,7,8) and large animal (9-12). These data show that within a certain range of volumes, less damage on organ functioning takes place when the field length is reduced.

It is important, especially for small field lengths, that the edges of the radiation field are sharply demarcated. Most of the data on dose-volume effects have been obtained after irradiation with photons. Because proton beams may deliver sharp demarcations with good spatial accuracy, these particles are very suited to study dose-volume effects in small animals with millimeter precision. More knowledge on the biological effects after proton irradiation is desirable because the clinical application of these beams is increasing. The relative biological effectiveness (RBE) of protons depends among others on the position of the target tissue in the beam.

Here we report on an investigation of dose-volume effects in single-volume, single-dose experiments with unmodulated plateau protons, avoiding dose deposition by protons from the Bragg peak. It is expected that the RBE of these irradiations will be close to unity (13). Rat spinal cord (C1-T2 region) has been irradiated and the dose-effect relationships for paralysis was determined for different irradiated lengths. For the first time, a complete dose-response curve for a 2 mm field was established. The results are compared with previous results obtained after irradiation with photons in limited series (1,2,6,8). The high-precision irradiation technique as presented in this study is currently used to study...
dose-response relationships of inhomogeneous dose distributions in rat cervical spinal cord.

Methods and materials

Animals

Male Wistar rats weighing 200-250 grams were used in this study. After irradiation, the rats were housed two per cage and provided with food and water ad libitum. All experiments were carried out in agreement with the Netherlands Experiments on Animal Act (1977) and the European Convention for the Protection of Vertebrates Used for Experimental Purpose (Strasbourg, March 18, 1986).

The rats were checked for development of paralysis of fore and/or hind limbs at regular intervals of 1 week. Rats were scored as responders when they showed paralysis of the fore or hind limbs, or both. The time from irradiation to the time of definitive neurological symptoms (paralysis) is referred to as the latent period.

When definitive neurological symptoms had developed, the rats were killed and the spinal columns were decalcified and processed for histology. The cut-off time to establish the early delayed endpoint of paralysis due to white matter necrosis was set at 210 days in all dose groups. This is based on a rat spinal cord model characterized by two distinct pathologies, selective white matter necrosis and late vasculopathies (2). In this model, selective white matter necroses resulted in paralysis well within 210 days whereas late vascular lesions in the white and gray matter were observed after a varying latent period of at least 8 but generally 12 months or more.

Rats dying without neurological symptoms were recorded as intercurrent deaths. Only rats with clear neurological symptoms due to radiation-induced injury of the cervical spinal cord were recorded as responders. In general, neurological symptoms developed well before the cutoff time of 210 days (2). In four rats developing neurological impairment at later times, the definitive score
was determined by histological examination, but not included in the data of Table 1. These histological data will be published in a separate report.

**Irradiation Protocol**

Six rats at a time were placed vertically at equal distance in a perspex frame with the head fixed, after being anesthetized by a mixture of fentanyl citrate/fluanisone, water, and midazolam (1:2:1), at a dosage of 3.4 mL/kg (i.p). Rats were irradiated on the cervical region (region C1-T2) of the spinal cord. The center of the proton beam coincided with an alignment laser, used to position the spinal cord. For all field lengths, the fourth cervical vertebra was set as the center of the field. After the full dose to one animal, the frame was shifted by remote control until the next rat was in position.

The rats were irradiated with a single dose of unmodulated 150 or 190 MeV proton beams from the AGOR (Accelerateur Groningen-Orsay Netherlands) cyclotron of the Kernfysisch Versneller Instituut in Groningen. With this technique a homogeneous dose is deposited along the beam direction, with a penumbra (80-20%) of approximately 1 millimeter. It should be emphasized that the Bragg peak in the depth-dose distribution was not used. Instead the target field was located at a total depth of 3 centimeters. This irradiation method is usually referred to as ‘shoot through technique’. The transversal beam size, defined by a collimator positioned at 15 cm in front of the rat, determined the length (i.e. volume) of the irradiated part of the cervical spinal cord. The dose rate was in the range of 10-15 Gy/min.

The field sizes varied between 2x10 mm and 20 x 10 mm, where the 10 mm was in lateral direction so that the cervical spinal cord was irradiated homogeneously over its full cross section. Variable lengths of 2-20 mm of the cervical spinal cord were irradiated with a range of doses (Table 1).

**Dosimetry**

During irradiation the dose was monitored by a parallel plate ionization chamber mounted in front of the collimator. The relation between the number of
monitor units (MU) and the dose in the target was calibrated with a standard reference ionization chamber (Farmer type, PTW30001). For this calibration a sufficiently large ($\varnothing = 7$ cm) irradiation field was used to ensure the prescribed homogeneous irradiation of the Farmer chamber. The Farmer chamber was surrounded by a polyethylene phantom to account for the tissue and frame material around the spinal cord. The dose profiles of the 2, 4, 8 and 20 mm fields are shown in Figure 1.

The small collimator apertures used in this study induced distortions in the dose distribution, resulting in a reduction of the dose per MU up to 20%. Therefore, for these small fields the dose distribution in the two transversal dimensions have been measured by means of a fluorescent screen, which is positioned at the beam exit-side of a 3 cm sheet of polyethylene simulating the depth in tissue. It has been shown that the light output of this screen is a very accurate measure of the dose (14). The spatial resolution is sufficient to be able to correctly measure the smallest dose distribution (0.22 mm standard deviation) (15). The light output of the screen was calibrated against the Farmer chamber in the $\varnothing 7$ cm field.

Figure 1. Dose profiles of the 2, 4, 8 and 20 mm fields. The profiles were measured at the same number of monitor units and normalised to the response in the 7 cm field. In the 7 cm field the dose per MU was measured using a Farmer type ion chamber. The dose per MU can be seen to decrease with decreasing field size. All doses reported in this study are corrected for this effect.
Data analysis

Each of the dose response curves was constructed by probit-analysis. The significance of differences was tested with the $t$ test. For the statistical analysis we used the SPSS 9.0 software (SPSS Inc.) for Windows (Microsoft Corporation).

Table 1. Summary of the spinal cord lengths, the single doses, number of rats irradiated and the number of responders in each volume group.

<table>
<thead>
<tr>
<th>Volume (mm)</th>
<th>Dose (Gy)</th>
<th>Irradiated</th>
<th>Responding</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>67</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>78.5</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>112</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>4 (group I)</td>
<td>33</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>36</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>6</td>
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<td>4</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>4 (group II)</td>
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<td>6</td>
<td>0</td>
</tr>
<tr>
<td>44</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
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<td>7</td>
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<td>29</td>
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<tr>
<td>24</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
Results

Single-dose tolerance

The dose-response curves for the incidence of the endpoint paralysis after single dose irradiation of the cervical spinal cord are shown in Figure 2. The data used to construct these curves are listed in Table 1. As can be seen in Figure 2, a marked shift in dose-response relationships was seen for irradiated field lengths of 8 mm or less. These results do not suggest a difference in slope of dose-response curves with decreasing volumes. The ED50 values for paralysis in the field lengths less than 8 mm show a steep increase (Table 2).

Table 2. ED50 values for different lengths of the rat cervical spinal cord. The 4 mm (combined) is the combination of the data of group I and II.

<table>
<thead>
<tr>
<th>Volume (mm)</th>
<th>ED50 (Gy)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>87.8</td>
<td>80.4-96.4</td>
</tr>
<tr>
<td>4 (group I)</td>
<td>51.8</td>
<td>47.7-57.1</td>
</tr>
<tr>
<td>4 (group II)</td>
<td>55.6</td>
<td>52.4-58.6</td>
</tr>
<tr>
<td>4 (combined)</td>
<td>53.7</td>
<td>49.2-61.9</td>
</tr>
<tr>
<td>8</td>
<td>24.9</td>
<td>21.6-28.6</td>
</tr>
<tr>
<td>20</td>
<td>20.4</td>
<td>-</td>
</tr>
</tbody>
</table>

The results of the two repeated 4 mm experiments (group I and II) are consistent, and have been evaluated as one group. The 8 mm and 20 mm irradiated lengths show no significant difference in the ED50 values. As can be seen in Table 2, there is a significant difference between the ED50 values of the 8 mm, 4 mm and 2 mm experiments, 24.9 Gy, 53.7 Gy and 87.8 Gy, respectively.

Latency to paralysis

The mean latent period per dose group for each separate field length experiment is shown in Table 3. When combining the results of all experiments, a continuous decrease in latent time was seen with increasing dose in smaller field
lengths. A remarkable observation is the almost complete lack of overlap between the different volume groups (Fig. 3).

Figure 2. Dose-response curves for paralysis in fore and/or hind limbs after single dose irradiation of variable lengths of the rat cervical spinal cord. The curves are fitted by probit analysis. Irradiated lengths: (□) 20 mm; (◊) 8 mm; (+) 4 mm (I); (○) 4 mm (II) and (△) 2 mm.

Figure 3. Mean latency period in days (± standard error of the mean) per dose group. (□) 20 mm; (◊) 8 mm; (+) 4 mm (I); (○) 4 mm (II); (△) 2mm. The dotted vertical lines indicate the highest dose in each volume group without responders.
Discussion

The present study confirms the existence of a large field length effect in the rat cervical spinal cord in irradiated field lengths smaller than 8 mm. This result is consistent with and extends previous photon experiments and clearly shows the steeply increasing ED$_{50}$ with decreasing field length to 2 mm (1,2,6,8). The high precision proton beam allowed an accurate determination of the dose-effect relationships of the rat spinal cord to as short a length as 2 mm. Dose-response curves were very steep and derived ED$_{50}$ values were as high as 87.8 Gy as a single dose to a length of 2 mm.

The ED$_{50}$ values obtained for the 20 mm, 8 mm and 4 mm field lengths show only small differences compared with previously obtained photon data (6, 8) and clearly show the consistency of the three data sets (Fig. 4). A study of the photon beam profiles used in the previous experiments (6) showed that the dose profiles had a long tail of 2 mm which resulted in a dose of about 10% of D$_{\text{max}}$ at 1 mm from the field edges. This had no impact in the response of 8 mm and 20 mm irradiated field lengths since there is no significant dose-volume effect in irradiated lengths $\geq$ 8 mm. For the smaller lengths of cord the irradiated field length is more critical and the longer tail of the beam profile could be the explanation for the reduced ED$_{50}$ value (45 Gy) in the 4 mm photon experiment. Since the ED$_{50}$ values from the present study are generally similar to the ED$_{50}$ values of previous photon experiments, we can conclude that there is no evidence that the RBE value of the used plateau protons is significantly different from the RBE value for photons, hence the RBE is not significantly different from unity. This is somewhat different from a conclusion in a recently published review on RBE values in proton therapy, stating that the RBE value of entrance plateau protons is approximately 1.1 for various in vivo and in vitro studies. However, this value is depending upon the irradiated tissue and the dose per fraction (13).
Figure 4. ED$_{50}$ values of the 20 mm, 8 mm, 4 mm and 2 mm field lengths from the present study compared with ED$_{50}$ values from previous photon experiments. The error bars indicate the 95% CI.

As shown in Figure 3, the latent period decreases with increasing dose especially in smaller irradiated field lengths, in agreement with earlier studies. A striking observation is the almost complete lack of overlap between the different field length groups. In the 8 mm field length experiment there is a 100% response at the highest dose level, however at that dose level there is no response in the 4 mm experiment. This observation is also present at the highest dose level in the 4 mm experiment compared with that dose level in the 2 mm experiment. In the 2 mm experiment there is a minimal latent period of about 60 days at doses of 101-112 Gy and a maximum of 83 days at 78.5 Gy. The minimal value for the 4 mm and 8 mm groups are 99 days at 58 Gy and 154 days at 26 Gy. In previous photon experiments (4 mm to 20 mm) the latent period varied from 120-210 days (1,2).
This variation in the latency could be a reflection of radiation-induced damage to different targets (glial cells and blood vessels) in the pathophysiology of white matter necrosis.

In the present study a latency of about 60 days (after $\geq 100$ Gy to 2 mm) is the minimal time period from irradiation to failure of functional integrity of the spinal cord. The existence of a plateau in the latent time-dose response may be present, but we have studied limited dose ranges and only a few dose groups at the ED$_{100}$ dose level (101-112 Gy). Geraci and Mariano reported latent periods varying from 9-3 weeks after single dose irradiation (100-150 Gy). It was concluded that the latent period is also dose dependent after single doses of 1.5x ED$_{100}$ or more (16). The latent period after irradiation is generally assumed to be determined by the turnover time of the functional cells and the critical cell number below which the tissue fails to maintain functional integrity (17). In experiments with juvenile rats it was shown that the latent periods increased almost linearly from about 2 weeks after irradiation at the age of 1 week to about 200 days after irradiation at the age of 8 weeks (17). Similar results have been reported by Geyer et al. (18). This increasing latent period with age in juvenile rats is probably a result of increasing lifetime of the functional cells up to the age of 7-8 weeks. The labeling index of glial cells above the age of 2 weeks is almost constant (17). White matter necrosis is the major cause of paralysis occurring within 120-210 days after irradiation with single doses down to approximately 20 Gy (1,2). In the literature, opinions about the pathogenesis of white matter necrosis have often focused on either a primary glial or a primary vascular origin or a combination of both (1,2,4, 7,9). White matter necrosis is characterized by demyelination, loss of axons, focal necrosis and liquefactive necrosis, after single doses of $\geq 20$ Gy (2). At the low end of the dose range, the lesions may remain as scattered foci. After higher doses the individual foci rapidly expand and coalesce into larger necrotic areas. Hopewell et al. (8) showed that the extent of white matter necrosis is less in a large volume (16 mm) than in small volumes (8 and 4 mm) at iso-effective doses for neurological damage. In the 16 mm volume experiment, the white matter necrosis was randomly distributed and no extensive lesions were seen in dorsal, ventral and
lateral white matter columns. The histology of the 4 and 8 mm volume experiments showed extensive white matter necrosis of dorsal, ventral and lateral white matter columns. Gray matter necrosis and nerve root necrosis was only observed in the histology of the small volumes. A first impression of the histology in the present study confirms the observations of Hopewell et al. of the differential distribution of white matter necrosis in large and small volumes, but necrosis of the gray matter and nerve root was not observed. The definitive histological evaluation of the present study will be presented in a separate paper. Vascular edema is usually associated with the development of white matter necrosis and the role of this component of vascular injury at various dose levels is not clear. With increasing dose a hemorrhagic component becomes more conspicuous and this may be involved in the shortening of the latent period.

A clear-cut biological interpretation of the findings in the present dose-volume study is still not possible. The field length effects could partly be related to a limited migration of functional cells or precursors from the edges of small-irradiated field lengths (8,19). Franklin et al. demonstrated the existence of a limited migration distance of approximately 2 mm for recruiting target cell (i.e. remyelinating cells) into areas of demyelination (20). In the 2 mm and 4 mm experiments, the migration of remyelinating cells from the edges is sufficient to restore the eradicated glial cell population. However, with increasing dose the incidence of vascular injuries in gray and white matter is increasing (1-4,7,8) and hemorrhages or extensive vascular leakage are the major cause of white matter necrosis. Direct evidence for vascular changes prior to necrosis has been shown in the irradiation of spinal cord of the rat (21). The vascular changes consisted of endothelial cell nucleus enlargement, blood vessel dilatation, blood vessel wall thickening and astrocyte hypertrophy. The latent periods for the appearance of these factors were clearly dose-related. Recent studies in the rat spinal cord with \textsuperscript{10}Boron capture agents showed evidence for an apparent lack of involvement of glial progenitor cells in the pathogenesis of white matter necrosis. These experiments allowed preferential irradiation of blood vessels and relative sparing of the parenchyma and showed white matter necrosis comparable with that after
irradiation with thermal neutrons alone (22). The present data are therefore not conclusive for unraveling the pathophysiological mechanisms of white matter necrosis. Histopathological studies are in progress and will be published in a separate paper.

Conclusions: Irradiation of variable lengths of 2-20 mm of rat spinal cord with 150-190 MeV protons showed very steep dose-response curves, with ED$_{50}$ values increasing from 20.4 Gy (20 mm) to 87.8 Gy (2 mm).

A continuous decrease in latent time was seen with increasing dose in smaller field lengths, showing almost no overlap of the latent periods between the different field lengths. A minimal latent period of approximately 60 days was observed in the 2 mm experiment after a dose of $\geq$ 100 Gy.
References