Dose-volume effects in rat spinal cord irradiated with protons

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CHAPTER 4

Influence of adjacent low dose fields on tolerance to high doses of protons in rat cervical spinal cord

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Abstract

Purpose: The dose-response relationship for a relatively short length (4 mm) of rat spinal cord has been shown to be significantly modified by adjacent low dose fields. In an additional series of experiments, we have now established the dose-volume dependence of this effect.

Methods and Materials: Wistar rats were irradiated on the cervical spinal cord with single doses of unmodulated protons (150 MeV) to obtain sharp lateral penumbras, using the shoot through technique which employs the plateau of the depth-dose profile rather than the Bragg peak. Three types of inhomogeneous dose-distributions were administered:

Twenty millimeters of cervical spinal cord were irradiated with variable subthreshold (= bath) doses (4 and 18 Gy). At the center of the 20-mm segment, a short segment of 2 mm or 8 mm (= shower) was irradiated with variable single doses. These inhomogeneous dose-distributions are referred to as symmetrical bath-and-shower experiments. An asymmetrical dose-distribution was arranged by irradiating 12 mm (= bath) of spinal cord with a dose of 4 Gy. The caudal 2 mm (= shower) of the 12 mm bath was additionally irradiated with variable single doses. This arrangement of inhomogeneous dose-distribution is referred to as asymmetrical bath-and-shower experiment. The endpoint for estimating the dose-response relationships was paralysis of the fore and/or hind limbs, and confirmed by histology.

Results: The 2 mm bath and shower experiments with a 4 Gy bath dose, showed a large shift of the dose-response curves compared with the 2 mm single field giving lower ED$_{50}$ values of 61.2 Gy and 68.6 Gy for the symmetrical and asymmetrical arrangement, respectively, compared with an ED$_{50}$ of 87.8 Gy after irradiation of a 2 mm field only. By increasing the bath dose to 18 Gy, the ED$_{50}$ value decreased further to 30.9 Gy.

For an 8 mm field, adding of a 4 Gy bath dose did not modify the ED$_{50}$ obtained for an 8 mm field only (23.2 and 23.1 Gy).

Conclusions: The spinal cord tolerance of relatively small volumes (shower) is strongly affected by a low dose irradiation (= bath) of adjacent tissue. The results of all “bath-and-shower” experiments show the effect of a low bath dose to be highest for a field of 2 mm, less for 4-mm and absent for 8 mm.

Adding a 4 Gy bath to only one side of a 2 mm field still showed a large effect. Since glial progenitor cells are known to migrate over at least 2-3 mm, this observation indicates that interference with stem cell migration is not the most likely mechanism of a bath effect.
Influence of Adjacent Low Dose Fields on Tolerance Dose of Spinal Cord

**Introduction**

In contrast to the extensive knowledge of dose-fractionation effects on spinal cord tolerance, only limited information is available for dose-volume relationships. For rat cervical spinal cord, dose-volume effects after homogeneous irradiation are observed for irradiated lengths shorter than 8 mm (1,2,3). Irradiation of 1 cm up to the full length of rat cervical and thoracic cord (6 cm) does not show significant differences in iso-effective doses for paralysis due to white matter necrosis (4).

In a recent publication applying inhomogeneous dose-distributions, we reported that the high iso-effective dose (ED$_{50}$) for induction of white matter necrosis in a small region (= shower) of 4 mm decreased significantly when the adjacent tissue was irradiated with a subthreshold dose (= bath) (5). The dose-response curve for the 4 mm single field showed an unexpectedly large downward shift by applying a bath dose of only 4 Gy, showing an iso-effective dose (ED$_{50}$) of 39 Gy compared with 53.7 Gy for the 4 mm field only. This effect on the dose-response curve for white matter necrosis is referred to as bath-effect. It is not clear what mechanisms account for this substantial decrease of the spinal cord tolerance by a dose as low as 4 Gy. A possible explanation for the bath-effect is a reduced migration of oligodendrocyte progenitor cells (OPC) from the low dose (bath) to the high dose (shower) segment (5,6). Since the migration distance of OPC’s is limited to approximately 2 mm (7) a question was whether the bath-effect would still exist for shower volumes < 4 mm. To obtain a complete picture of the relative importance of the bath-and-shower effect over a range of cord lengths, an additional series of experiments were performed for shower lengths of 2 and 8 mm.
Materials and methods

Animals

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

As described in previous papers (1,8) the irradiated rats were checked for the development of paralysis of the fore and/or hind limbs. Only animals with paralysis within 210 days after irradiation were recorded as responders. The cut-off time to establish the endpoint for paralysis due to white matter necrosis was set at 7 months. This cut-off point was chosen since the incidence of paralysis due to white matter necrosis occurs within a latent period of 5-6 months. An extra month was added to the follow-up period to account for the very few relatively late responders (9).

The rat cervical spinal cord was irradiated with single doses of unmodulated 150 MeV proton beams from AGOR (Accelerateur Groningen ORsay) cyclotron of the Kernfysisch Versneller Instituut in Groningen (1,8). The spinal cord was located at a total water equivalent depth of 3 cm. It should be emphasized that in the employed “shoot through” technique, the Bragg peak in the depth-dose distribution was not used since this would be at much longer depth. The transversal beam size, defined by a collimator position of 15 cm in front of the rat, determined the length of the irradiated length of the cervical spinal cord. The dose rate was in the range of 15-20 Gy/min. The administered doses were monitored during the experiments.

Dose-distribution arrangements

Symmetrical bath and shower: A 20 mm segment (= bath) of the cervical spinal cord was irradiated with variable single subthreshold doses. A 2 mm or 8 mm segment (= shower) was irradiated with variable single doses and
superimposed in the center of the 20 mm bath segment at the fourth cervical vertebra (Fig. 1a and 1b). Since the bath was located on both sides adjacent to the shower, the experiment is referred to as the symmetrical bath and shower arrangement. The variable doses used in the 2 mm and 8 mm symmetrical bath and shower experiments are listed in Table 1.

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**Figure 1.** Schematic view of the setup for the three experiments. The corresponding dose distributions are shown: *symmetrical* 2 mm bath and shower (a), *symmetrical* 8 mm bath and shower (b) and *asymmetrical* 2 mm bath and shower (c). The used combination of irradiation fields are depicted on the left of the corresponding graph. (A.U. = Arbitrary Units).
Asymmetrical bath and shower (Fig. 1c): A 12 mm segment (= bath) was irradiated with a single dose of 4 Gy. The shower was located in the caudal 2 mm of the bath and was irradiated with various single doses (Table 1).

Table 1. Overview of the experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Bath Dose (Gy)</th>
<th>Total Doses (Gy) in 2 and 8 mm segment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Homogeneous irradiation:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mm single field:</td>
<td>-</td>
<td>67-112</td>
</tr>
<tr>
<td>8 mm single field:</td>
<td>-</td>
<td>20-28</td>
</tr>
<tr>
<td><strong>Inhomogeneous irradiation:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mm symmetrical bath &amp; shower:</td>
<td>4</td>
<td>40-80</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>24-46</td>
</tr>
<tr>
<td>8 mm symmetrical bath &amp; shower:</td>
<td>4</td>
<td>18-26</td>
</tr>
<tr>
<td>2 mm asymmetrical bath &amp; shower:</td>
<td>4</td>
<td>55-95</td>
</tr>
</tbody>
</table>

Irradiation protocol

The cervical spinal cord was irradiated with an unmodulated 150 MeV proton beam from the AGOR cyclotron of the Kernfysisch Versneller Institute in Groningen.

Dosimetry

The dose-profiles were measured in 2D using the CCD/scintillator system developed by Boon et al (10), which has a spatial resolution of $\sigma = 0.22$ mm (11). These dose profiles were calibrated before the first irradiation using 0.6 cc cylindrical ion chamber PTW-30001 (Farmer chamber, PTW Freiburg) at 3-cm depth in a $\varnothing$ 70 mm field. The output factors of all fields with respect to the $\varnothing$ 70 mm field were obtained by comparing screen measurements, taken at an equal number of monitor units (12). The reported dose values are the 100% values.
Endpoints

The animals were checked for development of paralysis of fore and hind limbs at least twice weekly. The cut-off time to establish the endpoint for paralysis due to white matter necrosis was set at 7 months (9). Paralysis due to white matter necrosis occurs within 5-6 months after irradiation (9). An extra month of follow-up was added to account for the very low incidence of relatively late responders. Animals were scored as responders when they showed paralysis of the fore and/or hind limbs. The non-responders were kept in follow-up for 20 months to rule out a possibly developing second wave of paralysis due to late vascular damage (2,13). The time from irradiation to the time of paralysis is referred to as the latent period.

Statistical analysis

The dose-response curves were constructed by probit analysis. SPSS 9.0 (SPSS Inc.) was used for statistical analysis. Graphs were constructed with KaleidaGraph (Synergy software).

Results

Dose-response relationship

To complete the investigations of the effect of a low dose volume adjacent to variable shower-lengths, two sets of experiments were performed with a shower field of 2 or 8 mm.

Fig. 2 shows the dose-response curves for the set of experiments with a 2 mm shower. The large influence of the symmetrical bath dose of 4 Gy on the dose-response curve for the 2 mm field is expressed in a shift of the dose-response curves by 27 Gy. Increasing the bath dose to 18 Gy results in a further shift of the dose-response curve by 57 Gy. The ED$_{50}$ value for the homogeneously irradiated 2 mm segment decreases from 87.8 Gy to 61.2 Gy or 30.9 Gy for bath doses of 4 and 18 Gy, respectively. In agreement with previous results from 4 mm shower lengths, the ED$_{50}$ values are significantly different from the ED$_{50}$ for the homogeneously irradiated 2 mm (Table 2).
Figure 2. Dose-response curves for the 2 mm experiments after symmetrical and asymmetrical bath and shower irradiation compared with homogeneously irradiated 2 mm single fields (1). The graphs show the impact of an asymmetrical 4 Gy (diamonds), symmetrical 4 Gy (squares) and symmetrical 18 Gy (triangles) bath dose on the dose-response curve for the 2 mm single field (circles). Error bars: 95% C.I.

After applying an asymmetrical bath dose adjacent to the 2 mm field, a bath-effect is still present. The asymmetrical bath irradiation showed a smaller shift of the dose-response curve compared to the symmetrical bath irradiations (Fig. 2). The ED$_{50}$ value for the asymmetrical bath-and-shower irradiation (ED$_{50} = 68.6$ Gy) is higher than the ED$_{50}$ for the symmetrical bath-and-shower (ED$_{50} = 61.2$ Gy). This difference between the ED$_{50}$ values is borderline significant with a small overlap of 95% CI (Table 2).
Table 2. ED$_{50}$ values for symmetrical and asymmetrical bath and shower experiments compared with ED$_{50}$ values for homogeneously irradiated 2 and 8 mm single fields.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Bath Dose (Gy)</th>
<th>ED$_{50}$ (Gy)</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous irradiation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mm single field (1):</td>
<td>-</td>
<td>87.8</td>
<td>80-96</td>
</tr>
<tr>
<td>4-mm single field (1):</td>
<td>-</td>
<td>53.7</td>
<td>49-62</td>
</tr>
<tr>
<td>8 mm single field (1):</td>
<td>-</td>
<td>24.9</td>
<td>22-29</td>
</tr>
<tr>
<td>20-mm single field (1):</td>
<td>-</td>
<td>20.4</td>
<td>-</td>
</tr>
<tr>
<td>Present 8 mm single field:</td>
<td>-</td>
<td>23.2</td>
<td>22-24</td>
</tr>
<tr>
<td>Inhomogeneous irradiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mm symmetrical bath &amp; shower:</td>
<td>4</td>
<td>61.2</td>
<td>55-68</td>
</tr>
<tr>
<td>8 mm symmetrical bath &amp; shower:</td>
<td>4</td>
<td>23.1</td>
<td>22-24</td>
</tr>
<tr>
<td>2 mm asymmetrical bath &amp; shower:</td>
<td>4</td>
<td>68.6</td>
<td>64-74</td>
</tr>
</tbody>
</table>

After applying an asymmetrical bath dose adjacent to the 2 mm field, a bath-effect is still present. The asymmetrical bath irradiation showed a smaller shift of the dose-response curve compared to the symmetrical bath irradiations (Fig. 2). The ED$_{50}$ value for the asymmetrical bath-and-shower irradiation (ED$_{50} = 68.6$ Gy) is higher than the ED$_{50}$ for the symmetrical bath-and-shower (ED$_{50} = 61.2$ Gy). This difference between the ED$_{50}$ values is borderline significant with a small overlap of 95% CI (Table 2).

To investigate the bath-effect at relatively large shower lengths, a final series was performed with a shower length of 8 mm. In Fig. 3, the dose-response curves for an 8 mm field only and symmetrical bath-and-shower arrangement are shown. After applying the 4 Gy bath dose on both sides of the 8 mm field, the dose-response curve for the 8 mm field is not shifted and coincides with that for the 8 mm field only. This is in strong contrast with the results obtained for 2 mm and 4 mm (8) bath-and-shower experiments. The similar ED$_{50}$ values of 23.2 and 23.1 Gy for the 8 mm single field and 8 mm bath-and-shower irradiation, respectively
(Table 2), express the lack of a bath-effect. Thus a bath-effect is highest for the smallest fields of 2 mm, intermediate at 4 mm and disappears at 8 mm.

![Figure 3. Dose-response curves for the 8 mm experiments. The symmetrical 4 Gy (squares) bath and shower dose-response curve is not different from the 8 mm single field (circles) curve. Error bars: 95% C.I.](image)

**Latent period**

The mean latent periods (MLP) for the first signs of paralysis after irradiation are shown in Fig. 4a. For the 2 mm as well as for the 8 mm experiments, the MLP are dose dependent with shorter latencies after increasing doses in the 2 and 8 mm segments. This trend is in agreement with previously published data (1,14). Our experiments clearly show the existence of a threshold dose (a highest dose at which no paralysis develops) which varies with irradiated length from approximately 78 Gy (2 mm) to 20 Gy (20 mm) (1,9). Applying low doses around the 2 and 4 mm fields induce a response after lower shower doses, but after longer latent times (Fig. 4b and 4c). As can be seen in Fig. 4c, the MLP increases with decreasing
maximum doses in the 4-mm single field only, as well as for the bath-and-shower experiments. Adding the 4 Gy bath results in shorter latencies at equal maximum doses. In contrast with the 2 mm experiments, the mean latent periods for the 8 mm experiments are not different (Fig. 4d). Obviously, the 4 Gy bath does not show a noticeable effect on the latent periods in the 8 mm experiments.

Figure 4. Latent periods for the single field experiments (a), 2 mm bath-and-shower (b), 4 mm bath-and-shower (c) and 8 mm bath-and-shower (d). For simplicity, the latencies for specific dose range per single field experiment are depicted in gray rectangles. Error bars: standard error of mean.
Discussion

In a previous paper on inhomogeneous dose-distributions, we observed a large decrease of the iso-effective dose (ED$_{50}$) for a 4 mm segment (= shower) when surrounded by a dose as low as 4 Gy (bath-effect). In this study, we further investigated the extent of this effect for shorter and longer lengths of cervical cord. The present experiments show a larger bath-effect for a shower of 2 mm even after asymmetric (one sided) bath doses. However, when increasing the shower length to 8 mm, the bath-effect disappears (Table 2).

It is hypothesized that: (1) the adjacent low dose (bath-dose) has a negative impact on the regenerative capacity in the high dose field (shower) and/or (2) the bath-dose induces indirect effects that prevent the repair of tissue, leading to the development of white matter necrosis at lower doses.

White matter necrosis

The temporal sequence of the processes involved in the pathogenesis of white matter necrosis is not fully understood. After single field and single dose irradiation endothelial cells and oligodendrocytes undergo dose-dependent apoptosis within 24 hours after irradiation (15,16,17,18,19). This is followed by a dose-dependent disruption of the blood-spinal cord barrier (BSCB) at 24 hours after irradiation (19,20) and a decline of oligodendrocyte cell density (18,21). After the early apoptosis of endothelial cells (EC), there is a dose-dependent reduction in EC density within 24 hours (17,19). The endothelial cell density increases after 7 to 14 days with a recovery of the BSCB function at 1 week after irradiation (17). The decline of the oligodendrocyte population is not restored to normal levels and the process of demyelination is reflected in a significant decrease of the proteolipid protein (PLP) gene expression after 4 weeks followed by some recovery and again a decline 2-3 weeks before paralysis (18). At a lower dose, the demyelination is less pronounced. After 93 days to 120 days a late phase of increased vascular permeability is observed (20,22,23). We hypothesize that these early events occur both in the 2 and 8 mm segments and that other processes have to be involved in
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the course of radiation-induced white matter necrosis to explain the difference in ED$_{50}$ values for bath-and-shower effect.

**Oligodendrocyte progenitor cells and migration**

In a previous paper on inhomogeneous dose-distributions (8) we suggested that the bath-dose impaired the migration of oligodendrocyte progenitor cells (OPCs) from the bath region into the high dose shower region. Chari and Blakemore (24) showed that 7 mm of the thoracic spinal cord repopulated completely by OPCs from adjacent unirradiated tissue within 6 weeks after a single dose of 40 Gy. This repopulation occurred at the rate of approximately 0.5 mm/week in the first month. The repopulation process was capable of restoring the density of progenitors to that of normal tissue and was not associated with a secondary progenitor loss in tissue from which progenitor cells were generated. Irradiation of the region (= bath) adjacent to the 2 and 8 mm shower results in a local reduction of the OPCs and may reduce and delay migration. This delay of the interaction of OPCs with demyelinating lesions may have a deleterious effect on remyelination (25) and is reflected in the 2 mm and previous 4 mm bath-and-shower experiments by a shift to lower doses of the dose-response curves (8). The present asymmetrical 2 mm bath-and-shower experiment shows a shift to lower doses of the dose-response curve compared with the 2 mm field only (Table 2). One would expect no effect of the one-sided bath dose given that the migration of OPCs from the unirradiated other side of the high dose segment is not affected by irradiation. Thus, contribution from migrated progenitor cells to the repair of radiation-induced damage in the high dose segment may only partly explain the bath effect.

**Involvement of cytokines, growth factors and vasoactive mediators**

Parallel to the radiation effects on target cells, other mechanisms and/or effects such as the release of cytokines (CK), growth factors (GF) and vasoactive mediators may affect locoregional physiological processes. Astrocytes and microglia are the mean sources of CK and GF that regulate and modulate
oligodendroglia proliferation, differentiation, migration, survival, permeability of the blood-brain barrier and the function of neurons (23,26,27,28). Little is known about the time course of up and down regulation of these substances during the latent period preceding white matter necrosis, although observed increases of vasoactive compounds (27) and GF (23) are associated with phases of increased vascular permeability. Modulating of radiation-induced damage with growth factors was as observed after irradiating the mouse spinal cord (29) and rat spinal cord (30,31,32). Furthermore, it has also been shown that CK’s and GF’s exert an influence on latent periods (31,32,33). In the current bath-and-shower experiments for 2 and 4-mm lengths, the induction and release of cytokines in the bath region may affect the physiological processes in the shower region which is reflected in a decrease of the ED50 values. Since glial progenitors are known to migrate over at least 2-3 mm, the diffusion of CK and GF may inhibit this migration and proliferation of progenitor cells in the unirradiated tissue adjacent to the shower in the asymmetrical bath-and-shower experiment.

Conclusions

The spinal cord tolerance of relatively small volumes (= shower) is strongly affected by a low dose irradiation (= bath) of adjacent tissue. The results of all “bath-and-shower” experiments show the effect of a low bath dose to be highest for a field of 2 mm, less for 4-mm and absent for 8 mm.

Adding a 4 Gy bath to only one side of a 2 mm field still showed a large effect. Since glial progenitor cells are known to migrate over at least 2-3 mm, this observation indicates that interference with stem cell migration is not the most likely mechanism of a bath effect.
References


