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Research Report

Radiation-induced endothelial cell loss and reduction of the relative magnitude of the blood flow in the rat spinal cord

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ABSTRACT

The main purpose of the present study was to examine the time-dependent alterations in the endothelial cell density that occur in the first 180 days after irradiation of the spinal cord and the functional role of these alterations in the spinal cord blood flow. An irradiated cervical spinal cord rat model (C2-T2 segment) was generated using a ^{60}Co irradiator to deliver 30 Gy. A significant loss of forelimb motor function was observed 180 days post-irradiation. The number of neurons in the anterior horn of the spinal cord began to decrease significantly 3 days post-irradiation compared with normal controls, reaching the lowest number at 90 days post-irradiation. A significant reduction in the endothelial cell density was observed from 14 days post-irradiation in the white matter and from 3 days post-irradiation in the gray matter. The lowest endothelial cell density was reached at 30 days post-irradiation in the white matter and at 60 days post-irradiation in the gray matter. A significant reduction in the microvessel density was observed from 3 days post-irradiation in both the white matter and the gray matter. The lowest microvessel density was reached at 90 days post-irradiation in both the white matter and the gray matter. A significant reduction in the relative magnitude of spinal cord blood flow was observed from 21 days post-irradiation. The lowest relative magnitude of spinal cord blood flow was reached at 90 days post-irradiation. We did not find any evidence of demyelination. The results revealed that a single 30-Gy irradiation dose resulted in impaired forelimb motor

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function, a decreased number of neurons, and reduced endothelial cell density, microvessel density and relative magnitude of spinal cord blood flow. However, a 30-Gy single-dose irradiation was not sufficient to induce demyelination in the rat spinal cord.

1. Introduction

Approximately 40% of all cancer patients receive radiation therapy during their illness (Wong and Van der Kogel, 2004). The efficacy of radiation therapy is largely restricted by the limitations on the radiation doses allowed for normal tissues. Radiation-induced spinal cord injury (RI-SCI, radiation myelopathy clinically) is a major toxic side effect of radiation therapy for tumors located within or near the spinal cord and is a key impediment to delivering curative radiation doses (Nordal and Wong, 2004). RI-SCI causes deficits that involve motor, sensory, and autonomic neural functions. Histopathological changes, predominantly vascular damage and demyelination, normally occur after a latent period of several months to years (Atkinson et al., 2003; Chiang et al., 1992; Rezvani et al., 2001; Hubbard and Hopewall, 1978; Van der Kogel, 1979, 1980, 1991). Hence, the vascular endothelial cell has been proposed as one of most important targets of the late effects of RI-SCI (Rezvani et al., 2001; Li et al., 2003, 2004; Coderre et al., 2006; Myers et al., 1986; Ruifrok et al., 1994).

A previous report has demonstrated that white-matter necrosis occurs after a latent paralysis period of approximately 6 months following 30–40 Gy single-dose irradiation and that vascular damage occurs after a latent paralysis period of longer than 1 year following single-dose irradiation of less than 20 Gy in the rat radiation myelopathy model (Chiang et al., 1992). After the administration of the ED100 dose of 22 Gy, which causes forelimb paralysis in the cervical spinal cord (C2-T2) model, an early dose-dependent endothelial cell response and acute blood–brain barrier disruption have been observed within 180 days secondary to white-matter necrosis (Nordal and Wong, 2004; Li et al., 2003, 2004). However, there is no evidence about the time course of endothelial cell responses and their relationships to spinal cord blood flow within 180 days after irradiation in the cervical spinal cord (C2-T2) model.

The aim of the present study was to examine time-dependent alterations in endothelial cell density within 180 days post-irradiation and the functional effects of these alterations. We irradiated the rat cervical spinal cord with a single dose of 30 Gy, determined the time course of the endothelial cell response in the spinal cord, and specifically examined whether the endothelial cell response might play a causative role in the changes in the spinal cord blood flow and the behavioral dysfunction.

2. Results

2.1. Radiation damaged motor function

To determine the effect of a 30-Gy single-dose irradiation of the spinal cord, behavioral tests were performed using the forelimb paralysis scoring system. As shown in Fig. 1, the irradiated rats exhibited significant forelimb motor function impairment compared with the normal controls at 180 days post-irradiation. However, we did not observe total paralysis at 180 days post-irradiation. These results suggested that a 30-Gy single-dose irradiation was not sufficient to induce total paralysis in the RI-SCI model.

2.2. Radiation induced neuronal damage and loss in the rat spinal cord

Next, we asked whether irradiation could result in histological damage to the spinal cord. The histology of spinal cord sections was examined using H&E and Nissl staining. The staining revealed that a 30-Gy single-dose irradiation primarily injured the neurons in the spinal cord. A markedly thickened cytoplasm, significant swelling and distension of the soma, and hazy Nissl bodies were observed in the neurons between 30 days and 90 days post-irradiation. After 90 days post-irradiation, this neuronal damage was reversed to some degree. Activated microglial cells were observed in the white matter (Fig. 2A).

We also counted the number of neurons by Nissl staining in the anterior horn of the spinal cord following irradiation. The number of neurons began to significantly decrease at 3 days post-irradiation (14.50 ± 2.06) compared with the normal controls (17.70±1.81). The number of neurons reached its lowest (5.70±0.97) point at 90 days post-irradiation and was partially restored (11.50±1.78) at 180 days post-irradiation (Fig. 2B).

2.3. Radiation induced alterations in endothelial cell density and microvessel density and reduced the spinal cord blood flow

Previous reports have described endothelial cell loss at up to 14 days during the early phase of response to irradiation

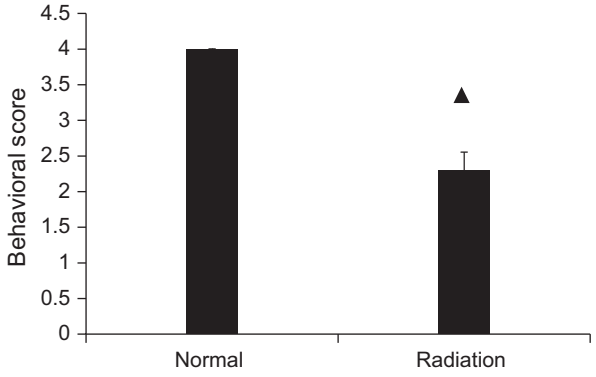

Fig. 1 – Partial forelimb paralysis was observed at 180 days post-irradiation ($n=5$, $^{*}P<0.01$). Error bars represent S.E.M.