BRAIN
RESEARCH

Research report

Fractionated radiation facilitates repair and functional motor recovery after spinal cord transection in rat

Nurit Kalderon[a,*], Su Xu[b,c], Jason A. Koutcher[b,c], Zvi Fuks[d]

[a]*Sloan-Kettering Institute for Cancer Research, New York, NY 10021, USA*
[b]*Department of Medical Physics, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA*
[c]*Department of Radiology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA*
[d]*Department of Radiation Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA*

Abstract

Previous studies suggest that motor recovery does not occur after spinal cord injury because reactive glia abort the natural repair processes. A permanent wound gap is left in the cord and the brain-cord circuitry consequently remains broken. Single-dose x-irradiation destroys reactive glia at the damage site in transected adult rat spinal cord. The wound then heals naturally, and a partially functional brain-cord circuitry is reconstructed. Timing is crucial; cell ablation is beneficial only within the third week after injury. Data presented here point to the possibility of translating these observations into a clinical therapy for preventing the paralysis following spinal cord injury in the human. The lesion site (at low thoracic level) in severed adult rat spinal cord was treated daily, over the third week postinjury, with protocols of fractionated radiation similar to those for treating human spinal cord tumors. This resulted, as with the single-dose protocol, in wound healing and restoration of some hindquarter motor function; in addition, the beneficial outcome was augmented. Of the restored hindlimb motor functions, weight-support and posture in stance was the only obvious one. Recovery of this motor function was partial to substantial and its incidence was 100% instead of about 50% obtained with the single-dose treatment. None of the hindlimbs, however, regained frequent stepping or any weight-bearing locomotion. These data indicate that the therapeutic outcome may be further augmented by tuning the radiation parameters within the critical time-window after injury. These data also indicate that dose-fractionation is an effective strategy and better than the single-dose treatment for targeting of reactive cells that abort the natural repair, suggesting that radiation therapy could be developed into a therapeutic procedure for repairing injured spinal cord. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

Natural repair mechanisms are activated in the adult spinal cord in response to injury. These repair mechanisms commence healing the wound and reconstructing the cord tissue, including reforming the damaged blood vessels and regrowing the severed axons [15,17,23,29,37]. However, by the end of the third week after the injury, the repair is aborted and decay processes take over, yielding a perma-

nent wound gap and muscle paralysis beyond the lesion (Fig. 1A). Clinically, complications in wound healing are due to the failure/abnormality of the basic repair processes, e.g., ulceration of the wound in human tissues is due to inadequate vascularization during healing [10]. In the spinal cord, the blood vessels fail to reinstate a normal blood supply at the damaged area (e.g., Refs. [3,4,28]); accordingly, as in other tissues [10], the injury inevitably results in progressive decay at the lesion site [4,17,35,38]. This chronic tissue decay has a pivotal role in the devastating consequences of spinal cord injury: the severed nerve fibers fail to cross the widening wound gap, leaving the cord beyond the injury site permanently disconnected
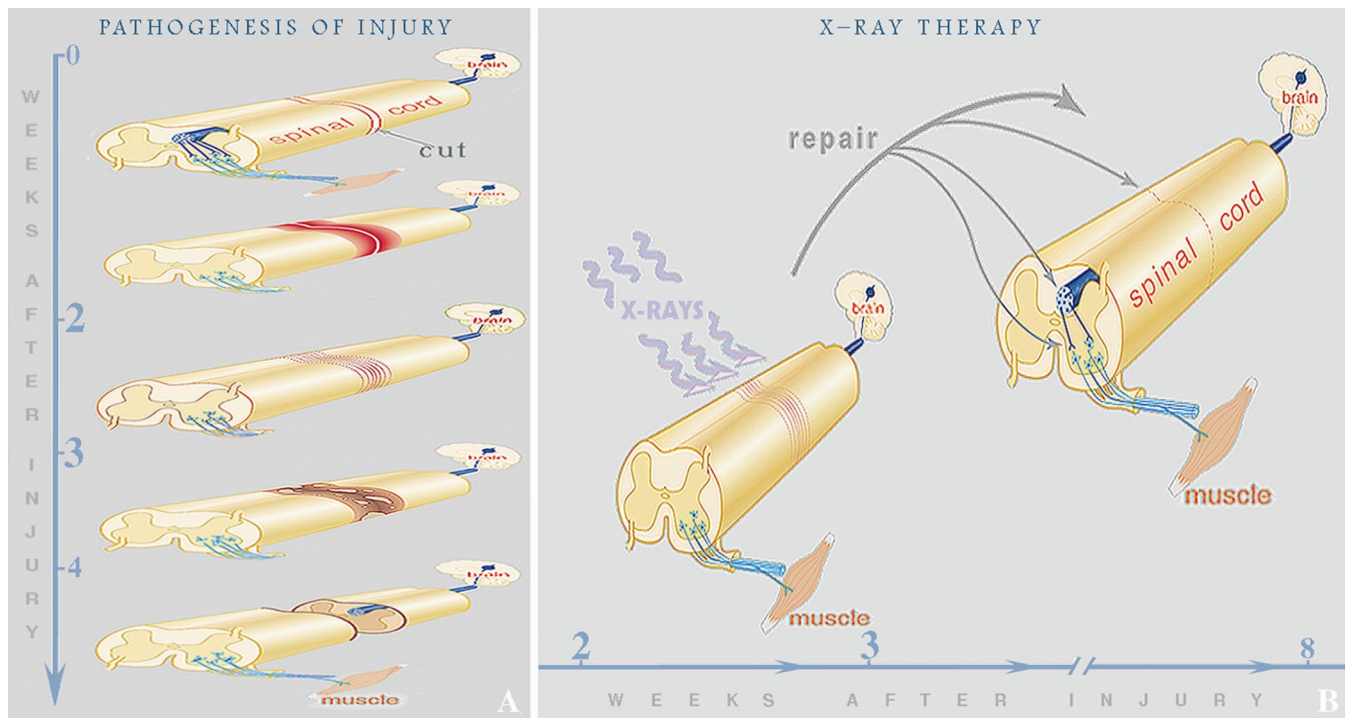
Fig. 1. The natural course of pathogenesis in spinal cord injury and its possible prevention by a timed X-ray intervention – a schematic illustration based on previous observations [23,24]. (A) Consequences of injury; (based on Fig. 2 in Ref. [23]). A normal course of wound healing events proceeds through the third week postinjury: at the first few days postinjury the two cord stumps are separated by a gap, whereas during the third week the cord seems to be almost fused with very little signs of the initial cut. The destructive processes seem to take over at the fourth week postinjury, being noticeable as discrete sites of disintegration; these lead eventually to a permanent wound gap. (B) Localized, single-dose (17.5–20 Gy) x-irradiation of the lesion site, delivered within the third week postinjury, prevents much of the destructive outcome [23,24]: tissue decay is averted and structural continuity is restored; consequently, the severed corticospinal fibers re-grow across the lesion site into the distal stump [23], re-instate some of the disrupted circuitry with cord neurons and regain control over hindlimb muscle function [24].

from the brain, and the related muscles remain paralyzed (Fig. 1A).

Several therapeutic strategies have been developed that result in successful regrowth of severed brain-cord fiber tracts beyond the lesion site [6,9,11,14,18,26,30,31,33,36]. These strategies are aimed not at preventing but at correcting the deficiencies caused by the injury; they bridge or bypass the wound gap thereby providing a favorable terrain for the re-growing fiber tracts, e.g., bridging with peripheral nerve grafts [9,11].

We have developed a therapeutic strategy which is aimed at preventing the deficiencies caused by the injury; by eliminating reactive cells that interfere with natural repair processes in transected adult rat spinal cord we prevented tissue decay [23,24] (Fig. 1B). We have identified one cellular component, the reactive astrocyte, that appears to play an important role in aborting the natural repair, since elimination of this cell with x-irradiation, as used to remove proliferating cells [16,32], enables the continuation of the natural intrinsic repair [22,23]. Timing is critical. It was possible to reduce the reactive gliosis provided the radiation was delivered within the period of 2–3 weeks after injury [22]; optimal elimination was achieved at 15–18 days postinjury. Along with the prevention of gliosis, it was possible to prevent much of the

destructive outcome of transection injury in adult rat spinal cord [23,24]; both tissue decay and paralysis were prevented by single-dose (17.5–20 Gy) irradiation on day 17 or 18 postinjury (Fig. 1B). The incidence and the degree of recovery were not maximal, e.g., the incidence of functional motor recovery was about 50% [24].

Thus far, we have employed irradiation conditions (single-dose protocols) that were useful in clarifying the pathogeny of spinal cord injury; they are not however in clinical use. Fractionated radiation therapy, as used clinically in cancer treatment, involves repetitive daily doses of irradiation at the range of 2–4 Gy given usually over a course of several weeks. Safe radiation protocols for the treatment of human tumors have been developed, employing total doses that eradicate tumors without exceeding the normal tissue tolerance. For example, the clinical tolerance dose values for the human spinal cord are 45–50 Gy and 33 Gy when delivered in daily fractions of 2 Gy and 3 Gy, respectively [25,27,34].

Here, we question whether the strategy of eliminating inhibitory cells could be developed into a clinical therapeutic procedure for repairing injured spinal cord. To answer the question, we determine the capacity of fractionated radiation therapy in facilitating repair in severed rat spinal cord when applied over the third week after injury.

2. Materials and methods

2.1. Injury

Adult Sprague–Dawley female rats (Charles River Breeding Laboratories), 3–6 months old were used. The surgical procedure and spinal cord injury are the same as described previously [24]. Briefly, the rat is anesthetized with 7% chloral hydrate injected i.p. (0.6 ml per 100 g of body weight), and with 0.2% Stadol (butorphanol) injected s.c. (0.01 ml per 100 g of body weight). First, the exposed spinal cord is completely severed (cordotomized) at T10–T11 by transecting it from top-to-bottom with microscissors but avoiding the posterior blood vessels. Next, to assure a complete severance, a loop is made around the entire cord tissue which remained intact with a surgical monofilament nylon suture #8-0, and the loop-enclosed tissue is cut. Upon completion of the injury, strips of Gelfoam (Upjohn) are placed in between the muscles and the bony structures around the cord: one leaning on top of the spinous processes of vertebra T10 and T12 and two along each of the sides between the vertebra and the side muscles. The overlying back muscles are sutured, the skin is closed with surgical wound clips, and the rat is given an s.c. injection of long-acting penicillin (300,000 units). Bladders were expressed manually 3–4 times a day until automatic bladder function has been resumed (within 1–2 weeks postsurgery), and 1–2 times per day thereafter. The animal care was in accordance with the Institutional Animal Care and Use Committee guidelines.

2.2. Radiation

Irradiation was delivered by an X-ray generator, a hybrid orthovoltage unit operating at 320 kVp, 10 mA with 0.5 mm Cu filtration, at a dose rate 146.5 cGy/min, at a distance of 50 cm from the skin. Treatment was delivered through a posterior approach while the rat was sedated with acepromazine maleate injected i.p. (0.5 mg per 100 g of body weight) and shielded with lead except for the exposed treatment field which was centered at the site of lesion and measured 25 mm×20 mm (long×wide). Two dose fractionation protocols were used; these protocols, which were delivered over 6 sequential days, consisted: either of 6 fractions of 3.4 Gy delivered once a day or of 11 fractions of 2.2 Gy delivered twice a day with an interval of 10–14 h in between the dose fractions.

2.3. Evaluation of motor recovery

Motor function of each of the rats, the irradiated and the unirradiated controls, was recorded periodically (once every few weeks), starting on day 14 postinjury, by videotaping the animals' behavior when they were placed on a flat metallic surface for about 5 to 10 min per session. Occasionally, the animals were also videotaped when placed on an uneven (providing traction) flat surface, on a thin-layered sponge. Hindquarter motor function of each of the rats was evaluated and scored from the related videotape records.

2.4. Tissue harvest and histology

Harvest of cord tissue samples containing the lesion site and histology were performed as previously described [23]. Briefly, anesthetized rats were perfused with a phosphate-buffered saline solution and then with a solution of 6% formaldehyde in phosphate-buffered saline, and after removal from the vertebrae cord tissue samples were further fixed and frozen [23]. Frozen cord samples were cryostat-sectioned (20 μm thick) in a horizontal plane; the serially collected sections were stained for routine histology with thionin and examined by light microscopy.

2.5. Magnetic resonance imaging (MRI) in vivo

MRI scans of the lesion site of the spinal cord were obtained on a 4.7 T/33 cm bore CSI Omega imaging spectrometer (Bruker, Fremont, CA, USA) equipped with shielded gradients (70 mT/m) and a linear 'birdcage' coil (15 cm diameter). The anesthetized rat was placed in the linear 'birdcage' coil which was placed in the magnet with the lesion site at the center of the magnet. Images were acquired using a spin-echo pulse sequence. To localize the cord lesion site a sagittal scout image was obtained with the following parameters: repetition time=300 ms, echo time=16 ms, with field of view=60 mm, four excitations, 128×128 imaging matrix, 2 mm thick slice, and eight adjacent slices with 0.5 mm gap in between slices. Subsequently, T_1- and T_2-weighted sagittal images were obtained with the following parameters: T_1-weighted (repetition time=500 ms, echo time=16 ms, 8 excitations) and T_2-weighted (repetition time=3500 ms, echo time=40 ms, 4 excitations) [8] with field of view=60 mm, 128×128 imaging matrix and 1 mm thick slice, collecting 8 adjacent slices with 0.5 mm gap.

3. Results

3.1. Motor recovery by fractionated radiation

The effectiveness of fractionated-radiation protocols in facilitating repair was studied in a completely transected spinal cord; an injury which permits an unequivocal determination of functional motor recovery below the lesion site due to regeneration of severed descending fiber tracts. The injury was at the T10–T11 level, which results in a complete loss of function and control of the hindlimbs (Figs. 2B, 3A and 4A) as compared with a normal rat with intact spinal cord (Fig. 2A). Our previous data suggested that recovery of motor function is dependent on and
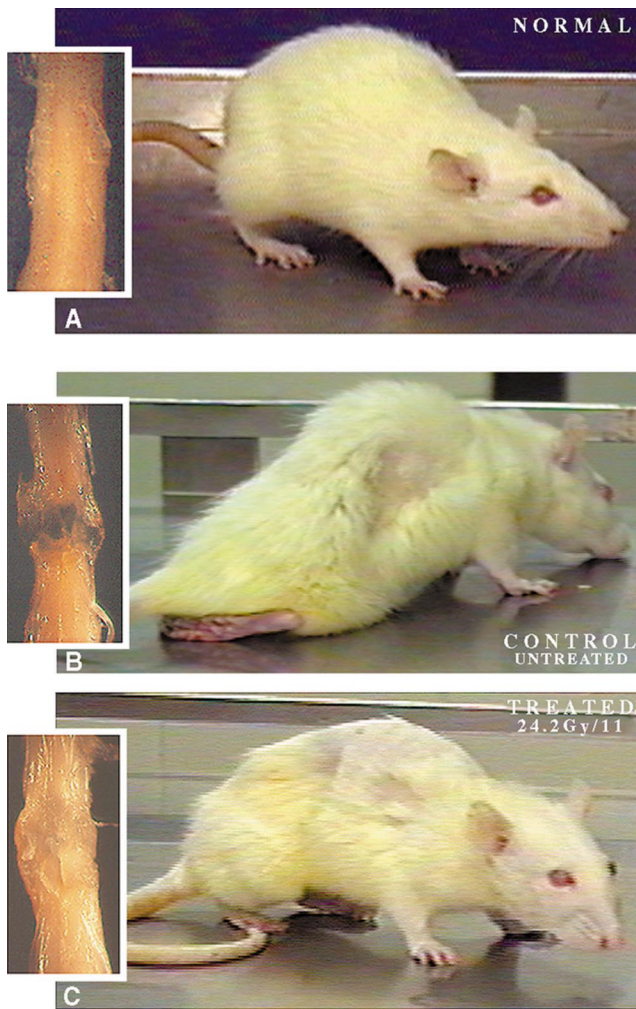
Fig. 2. Wound repair and hindlimbs' motor recovery – weight support – by fractionated radiation therapy. A composite of still images taken from video recordings of three rats (A–C) (one normal and two differently treated cordotomized rats, 6 months postinjury) combined with photographs of a segment of their spinal cord, at the level of the injury, placed as inset at their respective left side. (A) Normal rat in stance and its intact spinal cord. (B) Control untreated cordotomized rat and its cord, 10 months postinjury. (C) Treated (11 fractions of 2.2 Gy) cordotomized rat and its cord, 10 months postinjury. Comparison of the three shows that the untreated rat is paralyzed in its posterior body supporting its posture and weight with the knees and the tail's base, and its cord has an extensive cavitation 2–3 mm in size. In contrast, the irradiated rat looks almost like the normal rat, its hindlimbs support a complete elevation of its hindquarters, the tail's base is off the ground, and its cord shows a considerable repair of the wound.

correlates with restoration of structural continuity [23,24]. Once tissue decay was prevented and structural continuity was established it was found (by antero- and retrograde tracing, by electrophysiologic recording and by visual observations) that some of the severed descending fibers re-grew across the lesion site into the distal stump [23], re-instated some of the disrupted circuitry and regained control of hindlimb muscle function [24] (Fig. 1B).

Therefore, we examined here only two events, the beginning and the end of the repair cascade, i.e., restoration of structure and recovery of function, respectively. Structural examination of the lesion site [23] and behavioral evaluation of hindlimb motor function [24] were the assays used here to determine the effectiveness of fractionated radiation in eliciting repair.

Two dose fractionation protocols were examined: a 20.4 Gy ($n=7$) or a 24.2 Gy dose ($n=8$), delivered in fractions of 3.4 Gy once a day or 2.2 Gy twice a day, respectively, starting on day 15 postinjury. In all studied rats, treated ($n=15$) and untreated ($n=6$), there was a recovery within a day postinjury of the distal spinal reflex responses elicited, for example, by pinching of the tail. Selection for irradiation was random and motor function in the hindquarters of treated and untreated rats was monitored and periodically recorded up to 15 months postinjury.

In all rats that were treated with the dose-fractionated protocols some of the paralysis in their hindquarters was prevented as compared with the control untreated rats (Figs. 2, 3 and 4). Complete transection of the cord results in complete loss of function and control of the hindlimbs. The posterior body, distal to the cut, is paralyzed and lies flat on the surface (Figs. 2B, 3A, and 4A); the feet are splayed or tucked under the lower abdomen, and the rat supports its hindquarters' posture and weight with the knees and the base of the tail. In addition, with time after the injury, as locomotion is carried out by the forelimbs and the paralyzed hindquarters' muscles atrophy the torso becomes larger in size in comparison with the posterior body and the rat develops a hump (Figs. 2B, 3A and 4A).

In all irradiated rats some of the hindlimb motor functions were restored; the most obvious of these was weight-support and posture in stance, the only restored hindlimb motor function which can be objectively characterized (Fig. 4). Some very important behavioral functions, e.g., feeding and grooming, are carried out for lengthy periods of time (several minutes) in stance only, i.e., when stationary. Weight support and posture are the motor functions that participate in these vital behaviors. A normal rat holds the food or grooms its face with the two 'hands' while its 'legs' are bent in a sitting position (with the rump held up or on the ground). A rat cordotomized at the waist, after the injury, stops using both 'hands' for holding food or grooming; instead it uses them one at a time when the other 'hand' supports its anterior body weight and posture while the posterior body lies flat on the ground. The irradiated rats regained the ability, partial to substantial, to support the posterior body weight (Fig. 4B–E) and standing posture with the hindlimbs (Figs. 2C and 3xA). One of these irradiated rats can be seen supporting its posterior body weight and posture with the 'legs' and is simultaneously performing face grooming with its 'hands' (Fig. 3xA, inset); it was involved in this behavior for several minutes. The posture of the posterior body with the bent 'legs' in a sitting position looks almost
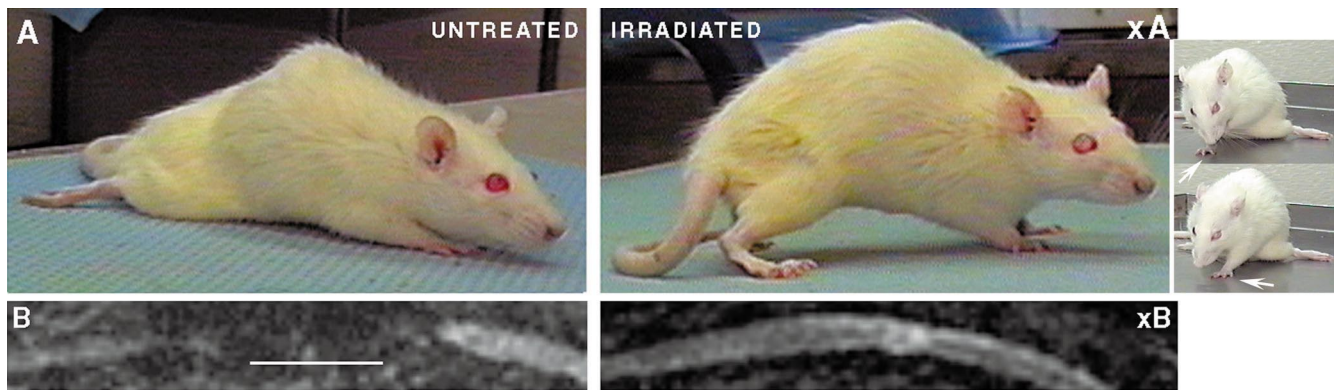
Fig. 3. In vivo views of the structural and functional repair. A composite of still images taken from video recordings of two differently treated cordotomized rats (A & xA) combined with MRI scans of the corresponding lesion site at their cords (B & xB). (A & xA). The untreated control rat, 15 months postinjury (A), its posterior body is paralyzed, whereas the irradiated (6 fractions of 3.4 Gy) rat, 13 months postinjury (xA), shows a considerable motor recovery; its hindlimbs regained the capacity of complete elevation of its hindquarters including the base of the tail off the ground. The treated rat, in two additional images (xA inset) is seen: holding its posterior body off the ground when the bent 'legs' are positioned wide open leaning on the tilted paws, and simultaneously grooming its face with one 'hand' at time (top with the left and bottom with the right) while the other 'hand' (arrow) is on the ground. (B & xB). Spin-echo T_2-weighted MRI sagittal scans of the lesion site. The control untreated cord (B), 20 months postinjury, has extensive tissue decay and cavitation. In contrast, the irradiated cord (xB), 17 months postinjury, shows considerable wound repair; the cord is for the most part continuous, with some fluid accumulation (high signal, white) around the lesion site. (Bar=10 mm).

like the posture of a normal rat. Early signs of motor recovery, related to weight support and posture, were noticed already during the second month postinjury; these gradually improved with time stabilizing at about 4–6 months postinjury.

In addition, all treated rats maintained rounded body shape (Figs. 2C, 3xA and 4B–E) and did not develop the rectangular hump at the lesion level that is typical of the untreated rats. This rounded body shape indicates that most of the hindquarter muscles regained some motor function and therefore did not atrophy. In some of the irradiated rats (n=5) there was also recovery of occasional hindlimb stepping; this was noticeable at about 5 months and became more pronounced later up to 9 months postinjury. None of the hindlimbs, however, regained frequent stepping or any weight-bearing locomotion. Finally, no significant difference was observed in the hindquarter's motor function when placed on smooth (Fig. 2) or uneven surface (Fig. 3).

3.2. Degree and incidence of functional recovery by fractionated radiation

The degree of functional recovery in the irradiated rats was variable, e.g., Fig. 4B–E. At the present no meaningful neurologic tests exist for evaluating the degree of functional recovery below the lesion in an animal whose spinal cord was completely transected. Several neurologic tests are available for evaluating the deficits/residual motor function in the rat's hindlimb following spinal cord contusion/crush injury. However, these tests have very limited use in our study as they evaluate the activity of the spared fiber tracts not the activity of the regenerated tracts; further, they mostly focus on rating locomotion. For
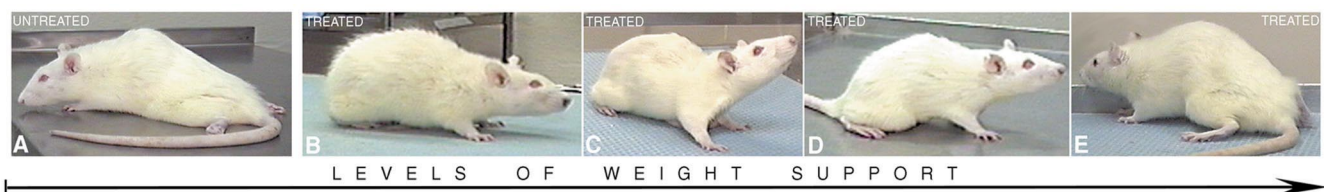

Fig. 4. Levels of restored weight support and posture. A composite of still images taken from video recordings of 5 different rats, a control untreated (A) and 4 treated (B–E) cordotomized rats, 6–9 months postinjury. (A) Untreated paralyzed rat, its hindlimbs cannot support its posterior body weight. (B–E) Irradiated rats which show different levels of hindlimb motor recovery related to weight support and posture as determined by the distance of the posterior body from the ground and by the positioning of the feet away from the body. Seen are three levels of weight support: a low level, score of 2 (B); a medium level, scores of 3–4 (C–D); and a high level, score of 5 (E); only one of the four rats (E) regained some capacity of elevating of the base of the tail off the ground.

example, the BBB locomotor rating scale [1] rates the residual level of normal motor function of the rat hind-limbs on a scale of 1 to 21, when the scores 10–21 are devoted to locomotion capacity. However, this scale does not include any scoring for the different levels of restored weight support observed in the individual rats or any rating for the restored motor function unrelated to the hindlimb. Using this rating scale: the restored hindlimb motor function related to weight-support observed in each of the irradiated cordotomized rats is equivalent to locomotor score 9 [1]; whereas, the hindlimb motor function in cordotomized untreated rats equals a locomotor score of about 2 (this, due to local reflex activity at the distal cord) [2]. Thus, according this rating scale, the degree of functional recovery in all our treated rats is identical, equivalent to seven locomotor scores of the BBB rating scale [1].

Here, to assess and gain an insight into the range of degree of hindlimb motor recovery related to weight-support when in stance we have used an arbitrary cumulative 7-point scoring scale: three points were assigned to three muscle groups (hip, thigh, and shank) involved in each hindlimb weight support function, and one point was assigned to the muscles involved in elevation of the base of the tail off the ground (Fig. 5). Using this scoring 'scale', the lowest degree of restored weight support in the irradiated rats was a score of 2 (Fig. 4B) and the highest was a score of 7 (Figs. 2C and 3xA); other intermediate levels can be seen in Fig. 4C–E. The degree of hindlimb weight-support of the individual rats at about 4–5 months postinjury was scored and summarized (Fig. 5) by examining the videotaped behavior records of each of the rats. According to this assessment, about half (7/15) of the

irradiated rats regained the capacity to support their hindquarters' weight with hip and thigh muscles, while only about one quarter (4/15) of them regained weight support with hip, thigh, and shank muscles.

No significant difference could be detected in between the functional recovery outcome of the two fractionated-radiation protocols as determined by statistical analysis, the Mann–Whitney test, and by comparing the distribution profiles of degree of recovery in the two-treatment groups. The distribution of degree of recovery into a low (1–2.9), medium (3–4.9), and high (5–7) score in the 20.4 Gy- and 24.2 Gy-treated groups was 29%: 57%: 14% and 25%: 50%: 25%, respectively.

An additional observation of the study is that by using dose-fractionation vs. single-dose protocols the incidence of functional recovery was increased from 55 to 100% (Fig. 5). All rats ($n=15$) that were treated with the dose-fractionated protocols regained some hindlimb motor recovery related to weight-support (Fig. 5). In contrast, as reported previously [24], only 6 of the 11 cordotomized rats that were treated with the single-dose protocols regained some hindlimb motor recovery related to weight-support (Fig. 5). Statistical analysis, Mann–Whitney test, shows that these fractionated radiation protocols are significantly ($0.005>P>0.001$) better than the single-dose protocol in promoting functional recovery.

3.3. Wound healing by fractionated radiation

The lesion sites were examined at the end of the experiment in the fixed cord tissues macroscopically (Fig. 2) and microscopically in the tissue sections. A few of the lesion sites were also examined in vivo by MRI, in rats
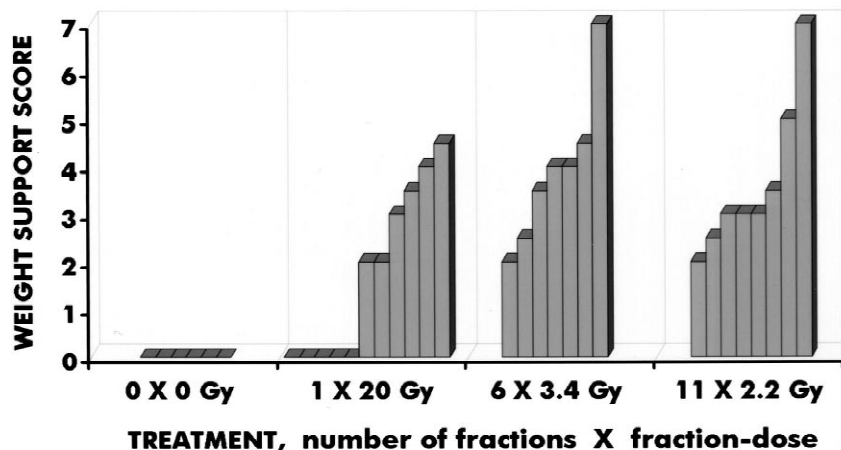

Fig. 5. Degree and incidence of restored weight support in the different treatment groups. Plotted are: individual scores of the hindquarters' weight support in each of the cordotomized and differently treated rats (bars) which were assessed by examining the rats' individual videotaped behavior records. An unirradiated group ($n=6$), and three irradiated groups receiving: fractionated-doses, 20.4 Gy/6 ($n=7$) or 24.2 Gy/11 ($n=8$); or a single-dose, 20 Gy ($n=11$) (the data for the single dose were obtained previously [24]). The incidence of recovery was 100% in the two fractionated-dose groups and 55% in the single-dose group. Scoring: a cumulative 7-point scoring was used to quantify the degree of hindlimb motor recovery related to weight-support/ elevation of the hindquarters in stance. In this scoring, 3 points are assigned to each hindlimb for weight-support by 3 muscle groups: 1 – hip-thigh muscles, plantar foot placement; 1 – thigh-shank muscles, positioning of knee; 1 – shank-heel muscles, positioning of ankle; and 1 point is assigned for elevation of the base of the tail off the ground. A partial weight-support in any of the last three muscle groups was scored as 1/2 a point.

that were maintained more than a year after the injury (Fig. 3B and xB). Analysis of the lesion site at the cord both ex vivo and in vivo showed that in the control unirradiated rats a continuous large cavity was formed (Figs. 2B and 3B). The size of the wound gap appears to vary with time postinjury; for example, the wound gap in two fixed cords that were harvested at different times after injury reached a size of about 3 mm by 10 months postinjury and a size of about 8 mm by 21 months postinjury (in cords seen in Fig. 2B and Fig. 3B, respectively). MRI scans in vivo of the lesion site suggest that tissue decay is much more extensive than observed macroscopically in the fixed tissue. In the same untreated severed cord, the cavitation measured in vivo about 14 mm (Fig. 3B) and in the fixed tissue about 8 mm. Similar extent of pervasiveness of degenerative processes at the lesion site was noted in other studies (e.g., Refs. [12,13]). For example, in a study which examined the wound area 4 months after complete transection in rat spinal cord the cavity was about 2–3 mm in the fixed tissue whereas the abnormal area in the cord as seen in vivo in the MRI scans measured about 10–12 mm [13].

In contrast to the untreated rats, in all the irradiated rats tissue decay was prevented (Figs. 2C and 3xB) and structural continuity was restored (Fig. 3xB) to varying degrees; macroscopically, the lesion site showed partial to substantial wound healing. Histological analysis of serial tissue sections showed that none of the treated cords regained a complete structural continuity throughout the entire volume of the lesion site, as obtained previously in 11.5% of the partially (~75%) severed spinal cords [23,24]. MRI analysis of the lesion site in the irradiated cords, 17 months postinjury, suggests that the degenerative processes and tissue decay were prevented for the most part by the radiation therapy. However, the localized high signal at the lesion site (Fig. 3xB) which could be indicative of chronic inflammation suggests a deficiency/ complication in the wound healing process which either was not corrected by the radiation therapy or was secondarily caused by tethering and compression of the cord by adjacent tissues.

4. Discussion

This study shows that fractionated radiation can facilitate wound healing and motor function recovery following transection injury in adult mammalian spinal cord. Our data also show that the incidence of inherent natural repair, both structural and functional, is maximized when employing radiation protocols similar to those used in a clinical setting. These data imply that dose-fractionation is more effective in eradicating the cells that abort the natural repair than the single-dose radiation, suggesting that the strategy of eliminating inhibitory cells could be developed into a bona fide therapeutic procedure for repairing the injured spinal cord. However, because the degree of

recovery was neither uniform nor maximal, the data also indicate that there is room for improvement, primarily in achieving a complete, normal wound healing.

Our structural analyses both in vivo and in the fixed tissue suggest that the radiation protocols used in this study enabled partial wound healing. The complications in wound healing in the injured spinal cord are linked to the inadequate re-vascularization at the lesion site [4,17,35,38]. The reasons for the failure of the blood vessels to reinstate a normal blood supply in the damaged spinal cord are still unknown. Nevertheless, in some studies, the astrocyte has been implicated to have a detrimental role in the fate of the newly formed blood vessels (e.g., Refs. [19–21]). Thus, we speculate that the strategy of cell elimination enabled the reforming blood vessels to reinstate a normal blood supply in some portions of the damaged cord. Since our previous data show that gliosis can be reduced during the period 2–3 weeks after injury [22,23], it is assumed that distributing the radiation therapy throughout the entire critical time window, e.g., a 10-day period starting on day 12 after injury, might yield a better and uniform degree of wound repair.

In assessing the degree of functional recovery, it needs to be emphasized that the experimental conditions were extremely harsh, thus a priori reducing the extent of structural and functional repair, and that the regenerating axons do not necessarily reach their normal target, thus resulting in abnormal circuitry and some meaningless function. For example, apart from being cut, the cord is also surrounded by and pulled out with a suture to assure its complete severing. Further, the exposed cord in the absence of the protecting bony structure (due to the laminectomy) is subjected, after wound closure, to chronic compression by the adjacent muscles. Because of these experimental conditions it is plausible that some of the descending tracts regenerate better than other tracts leading to preferential recovery of certain motor functions. For example, it is plausible that in the rat the corticospinal tract due to its central location, at the ventral portion of the dorsal funiculus [7], is less subject to physical pressure and regenerates 'better' than other tracts that run at the circumference of the cord. Consequently, the restored circuitry for the most part would be abnormal, which may explain the lack of recovery of any coordinated locomotion.

Radiation therapy is a preventive procedure in that it eliminates a key player in triggering the onset of pathogenesis in spinal cord injury. However, unlike the circumventing/corrective therapeutic strategies [6,9,11,14,18, 26,30,31,33,36], which could be employed for treating chronic spinal cord injuries, this preventive therapeutic strategy can be used only within a very limited window of opportunity after injury. It seems that radiation therapy could be used also in conjunction with abating therapies that reduce the secondary damage, e.g., clinical use of methylprednisolone to reduce inflammation immediately,

within 8 h, after injury [5]. Finally, since radiation therapy is a very powerful and clinically safe procedure for removing the critical pathogenic cells, this therapy holds realistic promise to be developed into a clinical procedure that would eradicate paralysis as the inevitable result in human spinal cord injury.

Acknowledgements

References

[1] D.M. Basso, M.S. Beattie, J.C. Bresnahan, A sensitive and reliable locomotor rating scale for open field testing in rats, J. Neurotrauma 12 (1995) 1–21.

[2] D.M. Basso, M.S. Beattie, J.C. Bresnahan, Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection, Exp. Neurol. 139 (1996) 244–256.

[3] J.L. Beggs, J.D. Waggener, Microvasculature regeneration following spinal cord injury: the growth sequence and permeability properties of new vessels, in: R.A. Thompson, J.R. Green (Eds.), Advances in Neurology: Complications of Nervous System Trauma, Vol. 22, Raven Press, New York, 1979, pp. 191–206.

[4] A.R. Blight, Morphometric analysis of blood vessels in chronic experimental spinal cord injury: hypervascularity and recovery of function, J. Neurol. Sci. 106 (1991) 158–174.

[5] M.B. Bracken et al., A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury, New Engl. J. Med. 322 (1990) 1405–1411.

[6] B.S. Bregman, E. Kunkel-Bagden, L. Schnell, H.N. Dai, D. Gao, M.E. Schwab, Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors, Nature 378 (1995) 498–501.

[7] L.T. Brown, Projections and termination of the corticospinal tract in rodents, Exp. Brain Res. 13 (1971) 432–450.

[8] J.H. Chen, H.E. Avram, L.E. Crooks, M. Arkawa, L. Kaufman, A.C. Brito, In vivo relaxation times and hydrogen density at 0.063–4.85 T in rats with implanted mammary adenocarcinoma, Radiology 184 (1992) 427–434.

[9] H. Cheng, Y. Cao, L. Olson, Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function, Science 273 (1996) 510–513.

[10] R.S. Cotran, V. Kumar, T. Collins, Tissue repair: cellular growth, fibrosis, and wound healing, in: T. Robbins (Ed.), Pathologic Basis of Disease, 6th Edition, W.B. Saunders, Philadelphia, PA, 1999, pp. 89–112.

[11] S. David, A.J. Aguayo, Axonal elongation into peripheral nervous system 'bridges' after central nervous system injury in adult rats, Science 214 (1981) 931–933.

[12] J.C. Falconer, P.A. Narayana, M.B. Bhattacharjee, S.J. Liu, Quantitative MRI of spinal cord injury in a rat model, Magn. Reson. Med. 32 (1994) 484–491.

[13] M. Fraidakis, T. Klason, H. Cheng, L. Olson, C. Spenger, High-resolution MRI of intact and transected rat spinal cord, Exp. Neurol. 153 (1998) 299–312.

[14] R. Grill, K. Murai, A. Blesch, F.H. Gage, M.H. Tuszynski, Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury, J. Neurosci. 17 (1997) 5560–5572.

[15] L. Guth, C.P. Barrett, E.J. Donati, F.D. Anderson, M.V. Smith, M. Lifson, Essentiality of a specific cellular terrain for growth of axons into a spinal cord lesion, Exp. Neurol. 88 (1985) 1–12.

[16] E.J. Hall, Radiobiology for the Radiologist, 3rd Edition, J.B. Lippincott, Philadelphia, PA, 1988.

[17] P.J. Horner, P.G. Popovich, B.B. Mullin, B.T. Stokes, A quantitative spatial analysis of the blood–spinal cord barrier. II. Permeability after intraspinal fetal transplantation, Exp. Neurol. 142 (1996) 226–243.

[18] Y. Iwashita, S. Kawaguchi, M. Murata, Restoration of function by replacement of spinal cord segments in the rat, Nature 367 (1994) 167–170.

[19] C.B. Jaeger, A.R. Blight, Spinal cord compression injury in guinea pigs: Structural changes of endothelium and its perivascular cell associations after blood–brain barrier breakdown and repair, Exp. Neurol. 144 (1997) 381–399.

[20] R.C. Janzer, M.C. Raff, Astrocytes induce blood–brain barrier properties in endothelial cells, Nature 325 (1987) 253–257.

[21] B. Jiang, M.A. Bezhadian, R.B. Caldwell, Astrocytes modulate retinal vasculogenesis: effects on endothelial cell differentiation, Glia 15 (1995) 1–10.

[22] N. Kalderon, A.A. Alfieri, Z. Fuks, Beneficial effects of x-irradiation on recovery of lesioned mammalian central nervous tissue, Proc. Natl. Acad. Sci. USA 87 (1990) 10058–10062.

[23] N. Kalderon, Z. Fuks, Structural recovery in lesioned adult mammalian spinal cord by x-irradiation of the lesion site, Proc. Natl. Acad. Sci. USA 93 (1996) 10079–10084.

[24] N. Kalderon, Z. Fuks, Severed corticospinal axons recover electrophysiologic control of muscle activity after X-ray therapy in lesioned adult spinal cord, Proc. Natl. Acad. Sci. USA 93 (1996) 10085–10090.

[25] D.C. Khan, S. Malhotra, R.E. Stevens, A.D. Steinfield, Radiotherapy for the treatment of giant cell tumor of the spine: a report of six cases and review of the literature, Cancer Invest. 17 (1999) 110–113.

[26] Y. Li, P.M. Field, G. Raisman, Repair of adult corticospinal tract by transplants of olfactory ensheathing cells, Science 277 (1997) 2000–2202.

[27] R.B. Marcus, R.R. Million, The incidence of myelitis after irradiation of the cervical spinal cord, Int. J. Radiat. Oncol. Biol. Phys. 19 (1990) 3–8.

[28] P.G. Popovich, P.J. Horner, B.B. Mullin, B.T. Stokes, A quantitative spatial analysis of the blood–spinal cord barrier. I. Permeability changes after experimental spinal contusion injury, Exp. Neurol. 142 (1996) 258–275.

[29] S. Ramón y Cajal, in: Degeneration and Regeneration of the Nervous System, Vol. 2, Oxford University Press, London, 1928, pp. 482–713, translated by R.M. May.

[30] O. Rapalino et al., Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats, Nat. Med. 4 (1998) 814–821.

[31] P.J. Reier, B.S. Bregman, J.R. Wujek, Intraspinal transplantation of embryonic spinal cord tissue in neonatal and adult rats, J. Comp. Neurol. 247 (1986) 275–296.

[32] P. Rubin, G.W. Casarett, in: Clinical Radiation Pathology, W.B. Saunders, Philadelphia, PA, 1968, pp. 609–661.

[33] L. Schnell, M.E. Schwab, Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors, Nature 343 (1990) 269–272.

[34] T.E. Schultheiss, Spinal cord radiation 'tolerance': doctrine versus data, Int. J. Radiat. Oncol. Biol. Phys. 15 (1990) 219–221.

[35] H. Shingu, I. Kimura, Y. Nasu, A. Shiotani, M. Oh-hama, A. Hijioka, J. Tanaka, Microangiographic study of spinal cord injury and myelopathy, Paraplegia 27 (1989) 182–189.

[36] B.T. Stokes, P.J. Reier, Fetal grafts alter chronic behavioral outcome after contusion damage to the adult rat spinal cord, Exp. Neurol. 116 (1992) 1–12.

[37] W. Tetzlaff, S.W. Alexander, F.D. Miller, M.A. Bisby, Response of facial and rubrospinal neurons to axotomy: changes in mRNA expression for cytoskeletal proteins and GAP-43, J. Neurosci. 11 (1991) 2528–2544.

[38] Z. Zhang, L. Guth, Experimental spinal cord injury: Wallerian degeneration in the dorsal column is followed by revascularization, glial proliferation, and nerve regeneration, Exp. Neurol. 147 (1997) 159–171.