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The Effect of Intraspinal Cytosine Arabinoside on the Re-irradiation Tolerance of the Cervical Spinal Cord of Young and Adult Rats

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Intrathecal treatment with cytosine arabinoside (ara-C) in combination with radiation has been used as prophylactic treatment in children with acute lymphatic leukaemia. Animal experiments have shown that ara-C enhances the effect of radiation on the spinal cord when administered shortly before irradiation, and that the long-term recovery after a combined treatment may be impaired. In the present experiments immature, 3-week-old rats, were treated with ara-C and radiation on the cervical spinal cord, and the long-term recovery was examined by reirradiation after different intervals. The endpoint of the study was paresis due to radiation myelopathy. The results showed a clear enhancement of the radiation effect with a dose-modifying factor of 1.2, when ara-C was administered before irradiation. However, no indications for impaired long-term recovery were observed. Additional experiments in adult rats with ara-C treatments during a 6-month interval between two radiation doses also did not suggest any interference between ara-C treatment and long-term recovery of radiation induced injury. It is concluded that for both the adult and immature nervous tissue, only when ara-C is administered intraspinally shortly before irradiation, interaction between ara-C and radiation results in a significant reduction of the isoeffective radiation dose by a factor of 1.2 (1.13–1.37, 95% confidence interval).
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INTRODUCTION

INTRATHECAL TREATMENT with cytosine arabinoside (ara-C) or methotrexate in combination with cranial irradiation, followed by 30 months of intrathecal maintenance therapy, has been used as prophylactic treatment of the central nervous system in asymptomatic children with acute lymphatic leukaemia [1, 2]. However, these patients exhibited prominent abnormalities on computed tomography scans [1], and a significant diminution of the cognitive abilities of these children was observed [2]. It was not possible in these studies to identify the contribution of both treatment modalities; however, also after separate treatment with any of these modalities central nervous system toxicity has been reported [3–5].

Previous animal experiments indicated that intrathecal as well as high-dose intraperitoneal ara-C treatments reduce the isoeffective doses for induction of paresis after spinal cord irradiation by a factor of maximally 1.3 in adult rats [6, 7]. In addition, in the adult rats the long term recovery after irradiation combined with ara-C seemed to be reduced in comparison to radiation alone [6].

In the present experiments the rat cervical spinal cord was used as an experimental model to examine further the temporal interactions of ara-C and irradiation. The influence of age on the interaction between ara-C and radiation was studied in 3-week-old, developing rats. Furthermore we investigated whether ara-C interferes with long term recovery of radiation damage when applied not in combination with the irradiation treatment but during the recovery period in adult rats. For that purpose, ara-C was administered using an "intensive" treatment schedule, mimicking a sandwich treatment with radiotherapy and chemotherapy, and an "extended" schedule mimicking chemotherapy maintenance treatment during a recovery period of 6 months between two irradiations in adult animals.

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METHODS AND MATERIALS

Female Wistar rats (CPB/WU) were used in this study at an age of 22–26 days (weanling rats) and at an age of 15 weeks (adult rats). Prior to irradiation, the animals were anaesthetised with Ethrane inhalation [8]. Positioning was facilitated using a lucite fixation set-up, enabling us to irradiate six animals simultaneously.

At the age of 3 weeks irradiation was performed with 250 kV X-rays at a focus-spinal cord distance of 50 cm. The total neck was irradiated, with the head and the rest of the body shielded with 3 mm lead. Exposure of a 12 mm segment of the cervical and upper thoracic spinal cord (C1 through T1-T2) was carried out with 250 kV X-rays, filtered with 1 mm Cu, at a dose rate of 0.6 Gy/min.

Reirradiations after 1–3 months and all irradiations of adult rats were performed on a linear accelerator at a focus-spinal cord distance of 100 cm, with the head and body shielded with 70 mm lead blocks close to the skin. Exposure of a 18 mm segment, also including cervical and upper thoracic spinal cord, was carried out with 4 MeV photons at a dose-rate of 2.2 Gy/min. Doses of X-rays were corrected with a factor of 1.1 for the difference in relative biological effectiveness (RBE) between X-rays and 4 MeV photons (ED_{50} 4 MeV/ED_{50} 250 kV = 1.1 [9]).

Each experiment comprised 5–6 dose levels with 5–6 animals per dose group. Examinations for signs of neurological impairment were performed 3 times a week. Animals were scored as

responders when they showed regular dragging of their feet with palmar flexion, dragging of extended forelegs, or inability to walk on their forelegs when lifted by the tail. When these definite signs of paresis were seen, rats were killed and the irradiated part of the spinal column was dissected out for histological examination. Rats for which the neurological damage could be attributed to spinal cord compression by a tumour, or damage to vertebrae resulting in spinal cord compression, were not recorded as responders but as intercurrent deaths.

Dose–response curves were constructed by probit-analysis. Response rates were corrected with the life-table method when intercurrent deaths occurred [10, 11]. For legitimate probit analysis at least 2 points between 0% and 100% are needed. Because of the steep dose–response curves not all experiments fulfilled this requirement. When less than two groups with response between 0% and 100% were observed, the dose–response curves were fitted by eye, and the highest dose giving no response and the lowest dose giving 100% response were considered to represent the range of ED_{50} values. Significance of differences in response were tested using the Fisher's "exact probability test" [12].

Ara-C (Cytosar®, Upjohn) was administered intraspinally, 20 mg/ml dissolved in physiological saline. Rats were lightly anaesthetised with ether. A small incision in the skin was made at the level of L6 to L7, and a 0.6 mm × 25 mm needle was placed between vertebrae L6 and L7. Induced movement of the tail or hind legs upon positioning of the needle, or after slight movement of the needle, was regarded as a positive indication of the right positioning of the needle tip in the spinal canal. The injected volume was 0.06 ml in young rats (40–60 mg/kg), and 0.12 ml in adult rats (30 mg/kg). After ara-C administration the incision was closed with a surgical clip. In the young rats administration was performed 15–30 min before the first irradiation, unless stated differently. In the adult rats ara-C was administered either 6 times in 2 weeks or 8 times in 8 weeks during the long-term recovery period. A schematic representation of the experimental set-up is given in Fig. 1.

RESULTS

Ara-C and radiation interaction and long-term recovery in 3-week-old rats

Cervical spinal cord irradiation of the 3-week-old animals with doses above tolerance resulted in paresis of the front legs after a latent period of 2–3 months. The histology of the spinal cord of the paralysed animals showed a generalised diffuse demyelination, with sometimes necrotic areas and small haemorrhages in the white matter. The histopathological basis of these lesions has been described in detail elsewhere [6, 13, 14].

The dose–response curves, for paresis after single dose irradiation treatments of 3-week-old rats with and without ara-C administration 15–30 min before or 5–15 min after irradiation, are shown in Fig. 2. Control single dose irradiation yielded an ED_{50} of 21.3 Gy (20.6–21.8 Gy, 95% CI) in 3-week-old rats; after intraspinal injection with 0.06 ml physiological saline the ED_{50} was 22.0 Gy (19.6–24.6 Gy), indicating no sensitising effect from the injection alone. It can be seen in Fig. 2 that both the ara-C administration 15–30 min before as well as 5–15 min after irradiation shifted the dose–response curve to lower doses compared to X-rays alone. Comparison of the dose–response data showed a significant ($P < 0.05$) modification by a factor of 1.2 (1.13–1.37, 95% CI) with ara-C treatment before irradiation. Ara-C treatment within 15 min after irradiation showed a lesser reduction of the ED_{50} to 20.2 Gy (18.6–21.8 Gy); however the difference in response between sham-treatment and the com-

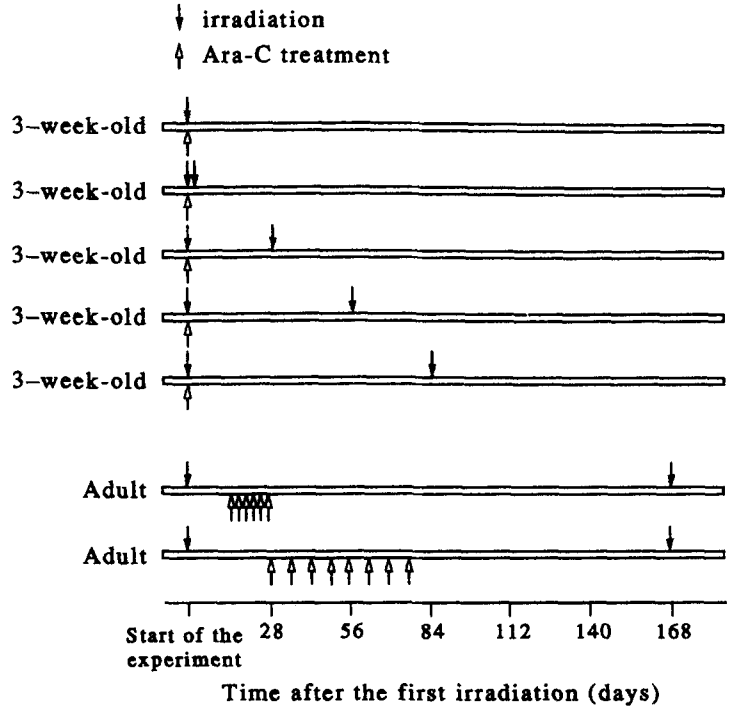

Fig. 1. Schematic representation of the timing of ara-C and radiation treatment of 3-week-old (young) and adult rats. In the 3-week-old rats the ara-C was administered 15–30 min before the first irradiation dose, with reirradiation treatments after intervals from 1 day to 84 days. In the adult rats ara-C treatments were given either 3 times a week at week 3 and 4 after the first irradiation, or once a week at week 5–12 after the first irradiation. Reirradiation of the adult rats was always 6 months after the first irradiation.

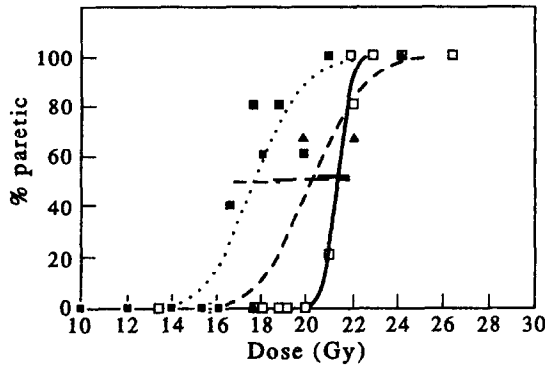

Fig. 2. Dose–response curves for paresis after single dose irradiation of the cervical spinal cord of 3-week-old rats. —□— control without ara-C treatment; ....■.... ara-C treatment 15–30 min before irradiation; ---▲--- ara-C treatment 5–15 min after irradiation.

bined treatment with irradiation followed by ara-C application is not significant at the 95% significance level. When ara-C was injected 4 h after irradiation no modification of the radiation effect could be observed (estimated ED_{50} = 21.1 Gy).

The effect of ara-C on long-term recovery of 3-week-old rats is shown in Fig. 3. Dose–response curves are shown for animals which received an initial treatment with ara-C followed by 12 Gy within 30 min, followed by reirradiation after different intervals. In Table 1 the reirradiation ED_{50} values derived from these probit curves are summarised, together with previously derived ED_{50} values [9] for retreatment after an initial irradiation with 12 Gy or 14.9 Gy without ara-C.

One day after an initial dose of 12 Gy preceded by ara-C treatment, the reirradiation ED_{50} was found to be 13.8 Gy. Significant recovery ($P < 0.05$) took place during the first month after the initial treatment, with an ED_{50} of 16.3 Gy after 28 days. The intervals of 56 and 84 days between treatments showed a slight but not significant further increase in ED_{50}. The results of long-term recovery after 14.9 Gy X-rays alone and 12 Gy preceded by ara-C treatment are graphically presented in Fig. 4. As can be seen, recovery after 84 days is not complete for ara-C treated as well as control irradiated animals; The preirradiated rats are significantly ($P < 0.05$) more sensitive than control rats, and the reirradiation ED_{50} values are still

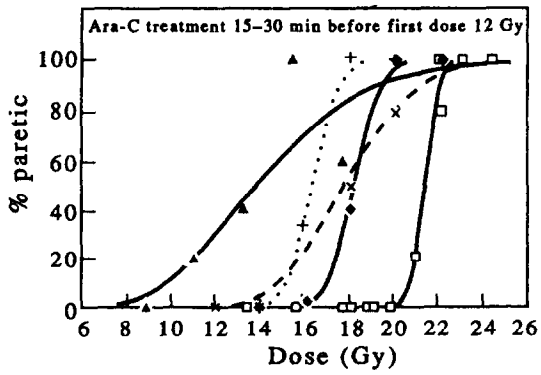

Fig. 3. Dose–response curves for paresis after reirradiation of the cervical spinal cord at different time intervals after an initial treatment with ara-C followed by 12 Gy X-rays at the age of 3 weeks. --▲-- 1 day interval; ....+.... 1 month interval; ---×--- 2 months interval; --◆-- 3 months interval; —□— single dose control (without ara-C treatment).

lower than the ED_{50} of the previously unirradiated spinal cord (21.3 Gy). The most important conclusion to be derived from Fig. 4, is that the present data suggest similar recovery for ara-C treated animals and control animals, when matched for equal effect of the initial treatment.

In Table 2 the latency times for development of paresis, after retreatment with doses representing ED_{80}–ED_{99}, for the different recovery intervals, of animals which received a first treatment at 3 weeks, are presented. The mean latency after the second treatment ranged from 66 to 137 days after the second treatment. The latent times show a general trend to increase after increasing treatment intervals, however, these differences are not significant. The scheduling of the radiation and ara-C treatment had no significant influence on the latency after the second treatment.

Long-term recovery in adult rats

In adult animals, the ED_{50} after single dose irradiation is 21 Gy with a latency of 247 ± 18 days (mean ± S.D.) for paresis development, which is significantly longer than the latency after irradiation of 3-week-old rats [9]. Without long term recovery (1 day interval), the ED_{50} after a first dose of 15 Gy is 16.2 Gy (15.5–17.3 Gy, 95% CI), indicating that the first dose of 15 Gy represents about 45% of the biological effect of the ED_{50} for paresis in a split dose treatment, assuming a fractionation response according to the LQ model with an α/β value of 2 Gy [13, 14]. When 6 months of additional recovery time is allowed, the ED_{50} for reirradiation increases to 18.3 Gy (17.4–19.4 Gy, 95% CI). In Fig. 5 the results of repeated ara-C treatments during the 6 months recovery period, after a first dose of 15 Gy is shown. As can be seen, no difference was observed between iso-effect doses without ara-C treatment (reirradiation ED_{50} = 18.3 Gy), with 'intensive' treatment with six injections in 2 weeks (reirradiation ED_{50} = 18.2 Gy; 16.6–19.9 Gy, 95% CI), or with a 'protracted' treatment with eight injections in 2 months (reirradiation ED_{50} = 18.6 Gy;16.9–21.2 Gy 95% CI). Also the latent times of paresis development were similar in the three treatment groups; the latent times were 160 ± 21 days, 158 ± 17 days, and 147 ± 7 days (mean ± S.D.) after the second treatment for, respectively, control, 'intensive' ara-C treatment or 'protracted' ara-C treatment, combined with reirradiation after 6 months.

DISCUSSION

Because of the frequent use of ara-C in combination with radiation treatment, and the possibility of radiation retreatment in these patients, quantitative information about the interaction between ara-C and radiation during and between irradiations is of interest. Previous experiments indicated that intraspinally injected ara-C reduced the isoeffect doses (or tolerance) for the white matter necrosis syndrome in the adult rat spinal cord by a factor of 1.2–1.3 when ara-C was administered 30 min before irradiation [6]. Also, intraperitoneal treatment of adult rats with high doses (9 g/kg) given 2 h before irradiation resulted in a reduction of the spinal cord radiation tolerance by a factor of approximately 1.2 [7]. The present results confirm this effect of ara-C on the radiation sensitivity for immature 3-week-old rats; when administered within 30 min before irradiation, a reduction in isoeffective dose of 1.2 (1.13–1.37, 95% CI) was seen.

The results of previous experiments suggested that ara-C could impair the capacity of long-term recovery after irradiation treatment in adult rats [6]. However, the present data obtained in 3-week-old rats give no indication of inhibition of long term recovery after a first treatment with combination of ara-C and

Table 1. Reirradiation tolerance at different intervals following a first irradiation at the age of 3 weeks with or without intraspinal ara-C treatment

1st dose (Gy)	Interval to second dose (days)	Reirradiation ED_{50} (Gy) X-rays only ‡§	Reirradiation ED_{50} (Gy) Ara-C + X-rays ‡
Single dose	—	21.3 (20.5–21.7)	
Sham treatment	—	22.0 (19.6–24.7)	17.7 (16.8–18.7)
12.0	1	17.3 (15.6–19.2)	13.8 (11.4–17.4)
12.0	28	18.7†	16.3†
12.0	56	>18*	17.7 (16.0–20.7)
12.0	84	20.0†	18.1†
14.9	1	14.5 (13.2–15.3)	
14.9	28	16.8 (16.2–17.5)	
14.9	56	16.9 (15.8–17.9)	
14.9	84	17.2 (16.3–17.9)	

*Highest dose in series; no response observed. †Derived from dose–response curve fitted by eye. ‡95% confidence limits in parenthesis. §Data from Ref. 9, except sham treatment.

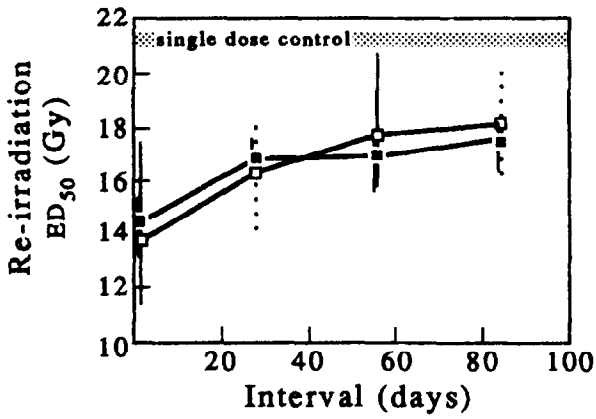

Fig. 4. Reirradiation ED_{50} (Gy) at different time intervals after a first irradiation of the cervical spinal cord at 3 weeks with 14.9 Gy (■) and 12 Gy preceded by ara-C treatment (□). Error bars: 95% confidence interval; dotted error bars 1 and 3 months after 12 Gy preceded by ara-C represent the estimated range of ED_{50} values. The shaded area indicates the ED_{50} for paresis of previously unirradiated control rats.

Table 2. Latency times to paresis development after a second irradiation dose of ED_{80}–ED_{99}, following a first irradiation with or without combination with intraspinal ara-C treatment at the age of 3 weeks

1st dose (Gy)	Interval to second dose (days)	Latency in days after second treatment [mean ± S.D. (median)] X-rays only*	Ara-C + X-rays
Single dose	—	88 ± 30 (85)	
Sham treatment	—	60 ± 7 (58)	69 ± 16 (65)
12.0	1	80 ± 4 (81)	100 ± 4 (100)
12.0	28	103 ± 13 (105)	80 ± 5 (79)
12.0	56	—	106 ± 52 (81)
12.0	84	127 ± 9 (126)	137 ± 16 (135)
14.9	1	66 ± 7 (67)	
14.9	28	83 ± 20 (77)	
14.9	56	102 ± 9 (101)	
14.9	84	115 ± 7 (112)	

*Data from Ref. 9, except sham treatment.

irradiation. Assuming an enhancement factor of 1.2 for the combination of ara-C with irradiation, ara-C preceding a radiation treatment with 12 Gy is comparable with a single dose of 14.4 Gy radiation only. As can be seen in Fig. 4, the recovery pattern after treatment with 14.9 Gy is not significantly different from the recovery after ara-C plus 12 Gy. This suggests that ara-C treatment in combination with irradiation only results in enhancement of the effect of the first irradiation treatment, and does not interfere with the long-term recovery after the initial combined treatment. Also the fact that ara-C treatment 15 min after irradiation results in a minor decrease in ED_{50} (statistically not significant), and that treatment 4 h after irradiation did not result in a changed isoeffect dose, show that interaction between ara-C and radiation only is observed when applied within a limited time.

Concerning the influence of ara-C treatment on latency the present results in 3-week-old rats confirm and extend earlier results after high dose intraperitoneal treatment of adult rats with ara-C [7]; no significant effect of the ara-C treatments on latency time could be detected in the young animals when ara-C was given in combination with irradiation.

The present data from the 3-week-old rats clearly confirm

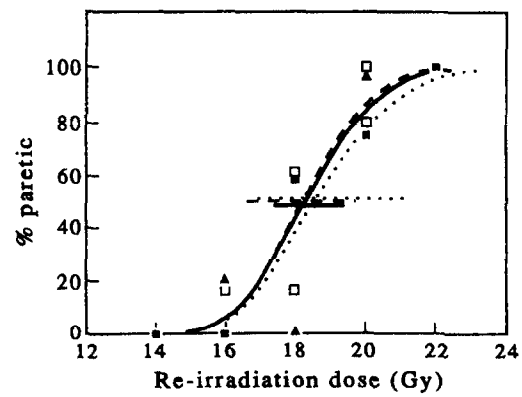

Fig. 5. Dose–response curves for paresis after reirradiation of the cervical spinal cord of adult rats irradiated with 15 Gy 6 months before. —□— control, no treatment during the 6 month interval; ....■.... 'intensive' ara-C treatment, 30 mg/kg 3 times a week in week 3 and 4 after the first irradiation; - - -▲- - - 'protracted' ara-C treatment, 30 mg/kg a week for weeks 5–12 after the first irradiation.

previously published results for adult animals, showing an enhancement of the radiation effect when ara-C was administered immediately before irradiation. One hypothesis about the mechanism of the sensitising effect of ara-C on the radiation response is the inhibition of sublethal damage repair, as demonstrated for human leukaemic blast cells and HeLa cells *in vitro* [15, 16]. An alternative mechanism may be the inhibition of potentially lethal damage (PLD) repair, a general action also observed for halogenated pyrimidines on cells in culture [17]. PLD repair, observed in non-dividing, plateau phase cells, may be of importance for the radiation reaction of the spinal cord, because of the limited cell turn-over in the CNS [18]. Definite conclusions about the inhibitory effect of ara-C on SLD or PLD repair in the cervical spinal cord after irradiation cannot be drawn from the present experiments; however, the lack of significant differences between control irradiation and ara-C treatment within 5–15 min after irradiation do not support the suggestion of a significant contribution of inhibition of SLD or PLD repair to the radiation response after ara-C treatment *in vivo* in the 3-week-old rats. A more likely explanation may be an increased radiosensitivity in the presence of ara-C, as recently shown for halogenated pyrimidines in *in vitro* experiments [19].

The present results in 3-week-old rats do not support an initial observation in adult rats that ara-C may reduce long-term recovery of radiation induced spinal cord damage [6]. The reason for the possible difference between the results presently observed in 3-week-old rats and the previous observations in adult rats is not known. One of the explanations for the difference in results may be a difference in effect of ara-C treatment on recovery kinetics of 3 week old and adult rats. As reported before [9], long term-recovery is faster and starts earlier in 3-week-old rats than in adult rats. Long-term recovery starts immediately in the 3 week old rats, but after a lag period of about 2 months in adult rats [9, 13, 14]. When ara-C prolongs this lag-time, or influences the rate of long-term recovery differently in 3-week-old and adult rats, this may influence the extent of long-recovery, as well as the latent time to damage development after the second treatment as reported for adult rats [6].

In addition to a lack of effect of ara-C on the long term recovery of the immature rats, the present experiments with adult animals show that 'intensive' as well as 'protracted' treatment with ara-C during a recovery period does not result in reduction of the ED_{50} of a reirradiation after 6 months following an initial irradiation with a single dose of 15 Gy (Fig. 5). This indicates that neither an intensive treatment nor maintenance treatment with intrathecal ara-C interacts with long-term recovery, when given between irradiation courses.

Summarising, the results indicate that ara-C reduces the isoeffective irradiation dose by a factor of about 1.2 in young as well as adult animals when administered intraspinally shortly before irradiation. No significant effects of ara-C treatment on long-term recovery or latency to paresis development in 3-week-old as well as adult rats were observed.

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