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Prevention of Radiation-induced Central Nervous System Toxicity: A Role for Amifostine?

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Abstract. *Purpose: To review the role of amifostine (WR-2721) in ameliorating radiation-induced central nervous system (CNS) toxicity. Materials and Methods: Literature review and presentation of preliminary animal experiments designed to test the efficacy of both intrathecal and subcutaneous application of amifostine. Results: Despite its inability to cross the blood-brain barrier, amifostine appears promising because it protects blood vessels against radiation-induced damage. Vascular damage is one of the most important components in the development of CNS toxicity after radiotherapy. Furthermore, the increased permeability of the blood-brain barrier during fractionated radiotherapy might allow penetration of amifostine. Three animal studies with systemic administration found positive results after brain irradiation with different fractionation schedules, total doses and amifostine doses. One study where amifostine was given after radiotherapy showed no protection, suggesting that the timing of the drug application is crucial. Further data suggest that either intrathecal or systemic administration might protect the spinal cord as well. In our experience with spinal cord irradiation, systemic administration was more effective than intrathecal. Regarding CNS protection, the optimum dose of amifostine has yet to be determined. Conclusion: Several independent experiments provided preliminary evidence that modulation of the radiation response of the CNS in vivo by systemic administration of amifostine is possible and feasible. Additional studies are warranted to investigate the protective effect with differing regimens of administration, more clinically relevant fractionation regimens and longer follow-up.*

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As the central nervous system (CNS) is a major dose-limiting organ for the radiotherapy of various cancers, particularly for those arising within the CNS, a large body of experimental studies have investigated its radiation response. They have led to important insights into long-term recovery, fractionation sensitivity and the kinetics of sublethal repair, as well as tolerance to re-irradiation (1-6). This knowledge has subsequently been translated into the clinic to define clinical tolerance doses and to evaluate altered fractionation schedules. In addition, technological advances, such as stereotactic and intensity-modulated radiotherapy, help to better shape the dose distribution to the target volume and thus spare more of the normal tissues.

The development of strategies of response modification has remained unsatisfactory, at least in terms of clinical impact. If innovative approaches could be developed, many patients would be able to receive more effective treatment. To address this notion, we reviewed the literature about the clinically available radioprotective compound amifostine (WR-2721). The drug is a member of a sulfhydryl-containing class of agents, which act through scavenging of radiation-induced free radicals. Amifostine needs to be converted into its free thiol form, WR-1065 (*via* hydrolysis by alkaline phosphatase). Reportedly, amifostine can also affect cells directly, for example by inducing a G1 arrest and the expression of p21 in certain cell types (7). Several studies suggested clinical benefits such as protecting thoracic organs as well as salivary glands against the side-effects of cancer radiotherapy (8-12). Such studies also proposed that, besides intravenous infusion, subcutaneous administration is feasible and effective (13). Amifostine does not cross the blood-brain barrier. To circumvent this problem, some groups initially evaluated intrathecal administration. Meanwhile, systemic application has been studied as well. Because clinical data on CNS radioprotection by amifostine are not available yet, this review focuses on experimental results. In addition, we summarize our group's experience with amifostine in a rodent myelopathy model, induced by cervical spinal cord irradiation. An overview of underlying principles and models

Table I. *Overview of experimental studies of central nervous system (CNS) radioprotection.*

Reference	Animals	CNS region	RT schedule	AF schedule	Follow-up	Results
Guelman *et al.*	Neonatal Wistar rats	Cephalic end	1 x 5 Gy	Subcutaneously 100 mg/kg	30 days (90 days for 1 endpoint)	Sign. protection
Alaoui *et al.*	Young Sprague-Dawley rats	Whole body (brain)	1 x 2.5 Gy	Intraperitoneal 75 mg/kg	6 hours	No sign. protection
Lamproglou *et al.*	Young Wistar rats	Whole brain	10 x 3 Gy	Intraperitoneal 37.5, 75 and 150 mg/kg	7.5 months	37.5 mg/kg not effective; 150 mg/kg caused 34% mortality; 75 mg/kg reduced memory dysfunction
Plotnikova *et al.*	Adult Wistar rats	Whole brain	1 x 25 Gy (earlier study with 40 or 60 Gy)	Intraperitoneal 300 mg/kg	18 months	Protection against vascular damage, necrosis and death after 25 Gy only
Spence *et al.*	Adult F-344 rats	Spinal cord	1 x 20-38 Gy	Intrathecal 0.33 mg	36 weeks	Protection with DMF 1.3
Nieder *et al.*	Adult F344 rats	Spinal cord	2 fractions, high dose	Intrathecal 0.3 mg	12 months	No sign. protection
Nieder *et al.*	Adult F344 rats	Spinal cord	2 fractions, high dose	Subcutaneous 200 mg/kg	12 months	Protection at 36 Gy-level

RT: radiotherapy; AF: amifostine

has already been published (14-16). Briefly, radiation myelopathy (RM) is thought to result from complex dynamic interactions between parenchymal and endothelial cells, their progenitors and the microenvironment within the spinal cord (14, 16, 17). Early intervention with growth factors appears to prevent development of RM (15, 16). Thus, investigation of other agents with radioprotective properties is warranted.

Amifostine and radiation treatment of the brain

Guelman *et al.* (18) investigated cerebellar morphological damage and motor gait impairment induced by neonatal radiation treatment with a single fraction of 5 Gy in rats. Amifostine 100 mg/kg subcutaneously *(s.c.)* 30 minutes prior to exposure partially prevented the development of this type of neurotoxicity. In contrast, the radiation-induced cerebellar noradrenaline concentration change 30 and 90 days after irradiation was not prevented. Interestingly, in this particular model, *s.c.* administration might lead to higher CNS concentration of the radioprotector than in adult animals because the blood-brain barrier permeability is higher. The disadvantages of the study are the limited clinical relevance of the fractionation scheme, especially in very young individuals, and the short follow-up of 30 days for motor gait evaluation and cerebellar histological changes. In contrast to these encouraging results, Alaoui *et al.* (19) found no protection of the brain when amifostine 75 mg/kg was injected intraperitoneally (*i.p.*) in conjunction with 2.5 Gy irradiation in 15-day-old rats. However, the injection was performed 20 minutes after irradiation, which might be too late. In addition, the study focused on the very early endpoints of neuronal damage and symptoms such as somnolence, gait disturbance and hypolocomotion 6 hours after irradiation. Interestingly, such side-effects were ameliorated by administration of two blockers of glutamate-mediated neurotransmission. An overview of the most relevant data from each study is provided in Table I. Lamproglou *et al.* (20) treated 45-day-old rats with whole-brain radiotherapy with saline or different doses of amifostine. Ten fractions of 3 Gy were administered. They evaluated learning and memory tasks (avoidance tests) for up to 7.5 months after irradiation. Compared to saline, doses of 75 or 150 mg/kg administered 1 hour before irradiation resulted in statistically significant differences, suggesting prevention of these transitory side-effects. A dose of 37.5 mg/kg was less effective. At the highest dose level, 34% of amifostine-treated rats died. The last study available for

Table II. *Overview of animal groups treated with saline or amifostine.*

RT schedule	Treatment	No. of animals irradiated	No. of response	Latency (days after RT)	No. censored	Days after RT	% paresis (actuarial)
16+17 Gy	Amifostine *i.t.*	9	5	159, 168, 168, 187, 198	1	69	62.5
16+20 Gy	Amifostine *i.t.*	9	6	147, 155, 159, 159, 159, 162	3	94, 121, 300*	86
16+17 Gy	Amifostine *s.c.*	8	2	211, 278	4	1, 1, 1, 308*	40
16+20 Gy	Amifostine *s.c.*	8	4	113, 161, 179, 351	0		50
16+17 Gy	Saline *s.c.*	8	1	158	4	120, 133, 195, 228	16
16+20 Gy	Saline *s.c.*	6	6	147, 150, 150, 151, 158, 163	0		100
16+20 Gy	Saline *i.t.*	6	4	142, 160, 163, 163	2	124, 129	100

RT: radiation therapy, *s.c.*: subcutaneous, *i.t.*: intrathecal
* rats censored because of tumor development

review was performed with a significantly higher radiation dose and longer follow-up (21). Protection against microvascular damage, which in this particular whole-brain irradiation model started to appear 12 months after single-fraction treatment with 25 Gy in adult rats, as well as reduced incidence of brain necrosis and subsequent death were achieved by *i.p.* injection of gammaphos (S-2-(3-amino-propylamino) ethylphosphorothioat or WR-2721) 3 minutes before irradiation. Earlier experiments with higher doses of 40 and 60 Gy were unsuccessful (22). A major limitation of this work is the restriction to just one relevant radiation dose level, *i.e.* 25 Gy, which makes it impossible to obtain the dose-modification factor.

Amifostine and radiation treatment of the spinal cord

Spence *et al.* (23) administered amifostine intrathecally in Fisher F344 rats 45 minutes prior to single-fraction irradiation of the cervical spinal cord. Based on earlier toxicity studies, the dose was 0.33 mg. They found a radiation dose-dependent prolongation of the median latent time to RM. The additional time without RM was 12 weeks in the intermediate dose range (+63% compared to controls) and 2 weeks (+10%) after high radiation doses. The dose-modification factor was 1.3 during a follow-up time of 36 weeks. The short follow-up is the most important limitation of this study, because it is known that rats might develop RM with 2 peaks, *i.e.* after approximately 5-6 and 10-12 months (1, 5, 16). It has subsequently been demonstrated that opening of the blood-brain barrier by injection of hypertonic arabinose into the internal carotid artery permits entry of amifostine into the ipsilateral cerebral hemisphere (24). To our knowledge, this difficult mode of delivery has not yet been evaluated in conjunction with radiation treatment. It should be noticed that radiation treatment also modifies the permeability of the blood-brain barrier. Thus, the actual penetration of systemically administered agents into the CNS might vary during a fractionated course of radiotherapy.

Own amifostine spinal cord experiments: materials and methods. Female adult Fisher F-344 rats (12 weeks old and weighing approximately 180-210 g) were purchased from Sasco Inc., Wilmington, MA, USA. The animals were housed in conventional rodent facilities as previously described (15, 16). Details of anesthesia and radiation technique have already been published (2, 15, 16). In our established rodent myelopathy model, rats were treated in a prone position with ^{60}Co-gamma irradiation to a 1.5-cm segment of the cervical spinal cord using a single anterior field (at a dose rate of approximately 0.5 Gy per minute and source skin distance 70 cm). The dose was prescribed to a depth of 1.3 cm based on lateral radiographs. Radiation was administered in two fractions where the first fraction was 16 Gy and the second fraction either 17 Gy or 20 Gy given 24 hours apart. Previous experiments showed that a total radiation dose of 36 Gy consistently induced a 100% incidence of myelopathy. Animals were anesthetized during irradiation by inhalation of 1.5-2.0% halothane (plus oxygen 0.5 l/min) using a semi-circuit inhalation anesthesia system to immobilize them in the desired position.

For the purpose of intrathecal injection of either saline or amifostine, a stainless steel canula was implanted into the cisterna magna. All implanted material was removed completely after the end of saline or amifostine injection. Groups of 8-9 rats each received intrathecal (*i.t.*) (0.3 mg) or *s.c.* injection (40 mg) of amifostine 30-45 minutes prior to each fraction of radiation. Control groups were treated either with *i.t.* or *s.c.* injection of physiological NaCl solution. Table II summarizes all groups that formed the basis of this experiment. Rats were monitored every other day for at least 12 months (clinical study endpoint) for development of paresis as a sign of spinal cord damage. As soon as neurological signs were unequivocal, rats were sacrificed by CO_2 inhalation and specimens of the cervical spinal cord were harvested and prepared for histopathological examination. Thus, the clinical diagnosis was verified histologically by identifying lesions

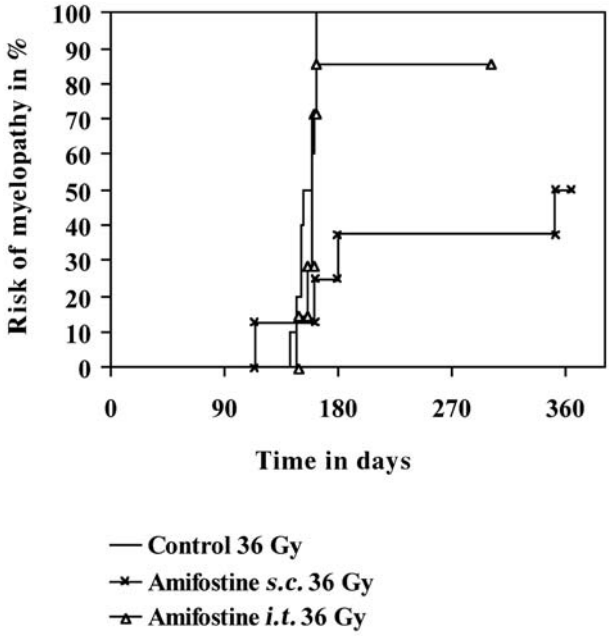

Figure 1. *Kaplan-Meier analysis of time to radiation myelopathy after cervical spinal cord irradiation in Fisher F-344 rats treated with saline vs. amifostine subcutaneously or intrathecally, respectively ($p<0.05$ for saline vs. subcutaneous amifostine, log rank test).*

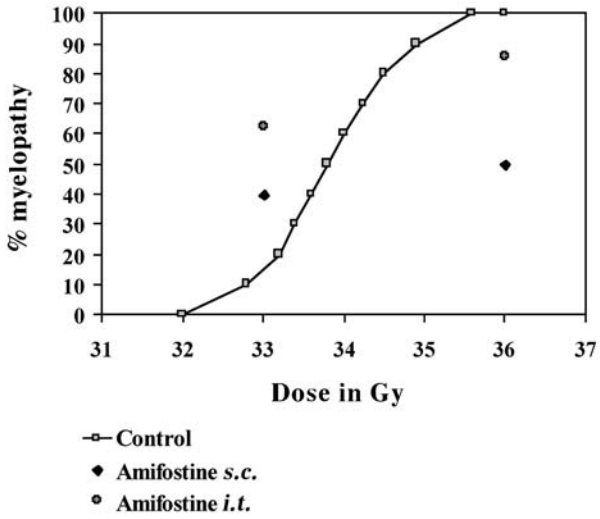

Figure 2. *Dose-effect curve for radiation myelopathy after cervical spinal cord irradiation in Fisher F-344 rats treated with saline and results of intrathecal or subcutaneous amifostine injection.*

consistent with myelopathy. The incidence of myelopathy was calculated using Kaplan-Meier estimates and compared for a significant difference using the log rank test.

Own amifostine spinal cord experiments: results. Animals censored before day 135 had to be sacrificed for progressive dyspnoea and weight loss due to chronic inflammation and obstruction of the upper aerodigestive tract, or died of the aforementioned side-effects. Animals censored beyond day 300 had to be sacrificed for the development of radiation-induced tumors. Those tumors were developing in the irradiated areas extra- and/or intraspinally, sometimes encroaching the spinal cord or the nerve roots and causing symptoms similar to paresis caused by myelopathy. This was confirmed histologically. As shown in Table II, no significant spinal cord protection from *i.t.* amifostine was seen. Figure 1 illustrates that *s.c.* amifostine reduced the incidence of myelopathy after 36 Gy. However, no such reduction was seen after 33 Gy (graph not shown). For comparison, a complete dose-response curve for radiation alone from a parallel experiment (16) is shown in Figure 2.

Amifostine and protection of blood vessels

It has long been recognized that radiation treatment damages blood vessels, particularly the microvasculature. Recently, an increasing number of reports described the occurrence of carotid stenosis or ischemic stroke after external beam radiotherapy for head and neck tumors (25-27). In the study by Cheng *et al.* (26), 95 irradiated patients with stenosis of 15-49% in duplex ultrasound were compared with 74 patients with a matched degree of carotid artery stenosis who had not received radiation therapy. Both groups were prospectively evaluated for a mean follow-up of 36 months. It was found that stenosis in previously irradiated patients progressed more rapidly, yet there was no difference in development of new symptoms or mortality. The authors adjusted for other covariates such as sex, age, smoking, diabetes and hypertension. The studies by Lam *et al.* (27), Dubec *et al.* (28) and Carmody *et al.* (29) also suggest an increased risk of carotid artery stenosis several years after radiotherapy. Vascular damage, including malformations and stenosis, has been described after radiotherapy to the brain in pediatric patients (30).

Several recent publications provide increasing evidence that amifostine or its active metabolite might be able to protect blood vessels. Grdina *et al.* (31) reported that WR-1065 induced activation of nuclear transcription factor kappaB and enhanced MnSOD gene expression in human vascular endothelial cells *in vitro*. A different *in vitro* experiment suggested that amifostine induces endothelial cell proliferation both under irradiated and non-irradiated conditions (32). Comparable results were reported in the non-irradiated area vasculosa of fertilised eggs, leading to

subsequent neovascularisation (33). Giannopoulou *et al.* (34) used the chicken embryo chorioallantoic membrane as model and showed a reduction in radiation-induced apoptosis of cells and in decrease of blood vessels. Kruse *et al.* (35) showed in a rat model of single-fraction heart irradiation that amifostine 160 mg/kg *i.p.* protected against reduced coronary and aortic flow. A small reduction in perivascular fibrosis and interstitial fibrosis was also apparent, as well as protection of the incidentally radiated lung tissue within the portal. These data support the evaluation of systemically administered amifostine with the aim of preventing one of the major components of CNS toxicity, *i.e.* vascular damage. Furthermore, they suggest that collection of long-term follow-up data on carotid stenosis and stroke from head and neck tumor patients irradiated as participants of the randomised amifostine trials would be a worthwhile effort. If different incidence rates were detected, prospective clinical evaluation of this endpoint should be considered. If amifostine protects blood vessels, the question arises as to whether this is a general phenomenon, *i.e.* does it also apply to tumor blood vessels?

Effects of amifostine on tumors

In vitro, human U87 and U251 glioma cells exposed to WR-1065 activated the nuclear transcription factor kappaB and enhanced the MnSOD gene expression, as seen in microvascular endothelial cells (31). Eventually, the compound protected 4 different glioma cell lines *in vitro* (36). Rat glioma treated with cisplatin with or without amifostine enlarged to a significantly different extent (37). However, there was no significant difference in cisplatin-induced DNA adduct formation evaluated by immunohistochemistry. Whether the larger volume of amifostine-exposed tumors resulted from the compound itself or from artefacts induced by the toxicity of cisplatin in the other group of animals can not be judged definitively on the basis of the data presented by the authors. This dilemma is also reflected in the controversial discussion of a large number of experiments published earlier. Their interpretation is not easy from today's point of view. This has led to different conclusions, as recently summarized by Koukourakis (38) and by Brizel and Overgaard (39). In general, there is no clear indication that amifostine protects tumor cells and normal tissues to the same extent. Numerous clinical studies in different tumor entities also support this statement, although they were not designed to investigate this particular question (8, 9, 11-13, 40, 41). Given the large number of patients so far treated with amifostine, it appears unlikely that worse tumor control or survival would not have been detected. Despite the limited statistical power, which was comparable to that of most amifostine studies, unexpected negative effects on outcome were identified in a head and neck cancer radiotherapy trial for granulocyte colony-stimulating factor (42). The potentially selective mode of action might result from reduced expression of an alkaline phosphatase isoenzyme, which is involved in the hydrolysis of amifostine to its active metabolite in tumors and their blood vessels (43).

Discussion

Despite recent advances in conformal and intensity-modulated radiotherapy, the CNS still represents a major dose-limiting organ. Radiation-induced necrosis and other sequelae are serious, multifactorial conditions that usually develop in a time- and dose-dependent manner after a threshold dose has been exceeded. Currently, both fractionation and advanced treatment planning and delivery can reduce radiation-induced CNS toxicity. Driven by advances in our understanding of tissue responses to radiation, several groups evaluated potential modifiers of radiation responses. The general aim of this study was to review the role of amifostine in this context. Despite its inability to cross the blood-brain barrier, the drug appears interesting because it protects blood vessels against radiation-induced damage. Vascular damage is one of the most important components in the development of CNS toxicity after radiotherapy. Furthermore, the increased permeability of the blood-brain barrier during a fractionated course of radiotherapy might allow penetration of amifostine.

Regarding experiments of brain irradiation, one study revealed negative results. However, this lack of radioprotection can be explained by delayed administration of the drug. Three other studies with systemic administration found positive results after different fractionation schedules, total doses and amifostine doses. However, they can be criticized for their limited follow-up and leave us with open questions regarding the treatment schedule. Thus, more systematic approaches should be made to better define the role of amifostine. We propose two different experimental models, *i.e.* fractionated whole-brain irradiation and single fraction treatment as used for radiosurgery. In both models, different doses of amifostine, starting with 75 mg/kg, should be examined and the follow-up should extend to at least 12 months. Furthermore, it is necessary to study a range of radiation doses to obtain the dose-modification factor, *i.e.* the shift of the dose-response curve to higher doses in amifostine-treated animals. The effects of whole-brain irradiation as well as fractionated partial brain irradiation should also be studied in young animals, because radioprotection of the relatively sensitive developing CNS would have enormous clinical relevance, for example when treating pediatric brain tumors.

The earliest data on spinal cord radiotherapy plus amifostine rely on intrathecal application (23). They were encouraging with a dose-modification factor of 1.3. Unfortunately, our own experience was less favorable. With longer follow-up than in the experiments by Spence *et al.* (23), we could not repeat their findings. Our rat model of spinal cord irradiation has been used previously to study various

other aspects, such as fractionation (1, 15, 16). The results of the control groups were consistent with our previous experience in term of the latency to RM and effective doses. Intrathecal manipulation did not alter the sensitivity of the spinal cord, as demonstrated from the comparison of intrathecal with subcutaneous saline administration. Also, rates of toxicity to the aerodigestive tract and tumor induction did not differ significantly between the control and the amifostine groups. Thus, the results can not be explained by any difficulties with the model. However, we also investigated systemic administration, *i.e.* subcutaneous injection of amifostine. The latter mode of delivery resulted in better protection, although a complete dose-response curve is not available yet. Taking the clinical treatment regimes for spinal cord into account, we feel that conventionally fractionated radiotherapy will provide a more relevant model for further experimental studies. It is also clear that intrathecal drug administration is hardly feasible in a clinical setting.

In general, the hypothesis that modulation of early radiation-induced CNS reactions by pharmacological treatment is able to prevent late toxicity appears to be valid. In the past, several pragmatic neuroprotective approaches have been undertaken. Fike *et al.* (44) showed that a-difluoromethylornithine (DFMO), a polyamine-synthesis inhibitor given 2 days before to 14 days after ^{125}I brachytherapy, reduced the volume of radionecrosis and the contrast-enhancement in dog brain. Kondziolka *et al.* (45) implanted C6 glioma into rat brain and performed a single fraction gamma-knife treatment, with or without *i.v.* administration of U-747389G (50-60 minutes before radiosurgery), a 21-aminosteroid that is largely selective to endothelium. The drug prevented development of perifocal edema and radiation-induced vessel damage in the healthy brain region within steep dose-gradients just outside the target volume. This effect might be caused by antioxidative and membrane-stabilizing properties, leading to reduced secretion of arachidonic acid from damaged cell membranes. Hornsey *et al.* (46) showed that the vasoactive drug dipyridamol (starting 17 weeks after single dose irradiation) reduced the incidence of myelopathy in rats. The ED_{50} increased by 2-3 Gy. Rezvani *et al.* (47) used neural stem cell transplantation to protect rats against RM. Their results were encouraging, however, follow-up was shorter than 12 months. Furthermore, they conducted the study in younger rats whose immature CNS might react differently.

Radiobiological strategies ideally aim at increasing the radiation tolerance of normal tissue without protecting the tumor at the same time. Whether this criterion is fulfilled by amifostine is currently unknown and a matter of debate (38, 39). Accumulating clinical evidence suggests that differential protection can be achieved. Yet, most of the studies were underpowered and not primarily designed to answer the question definitively. None of them included patients with CNS tumors.

Conclusion

Several independent experiments provided preliminary evidence that modulation of the radiation response of the CNS *in vivo* by systemic administration of amifostine is possible and feasible. Additional studies are warranted to investigate the protective effect with differing regimens of administration, more clinically relevant fractionation regimens and longer follow-up.

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References

1 Ang KK, van der Kogel AJ, Van Dam J *et al*: The kinetics of repair of sublethal damage in the rat cervical spinal cord during fractionated irradiations. Radiother Oncol *1*: 247-253, 1984.

2 van der Schueren E, Landuyt W and Ang KK: From 2 Gy to 1 Gy per fraction: sparing effect in rat spinal cord? Int J Radiat Oncol Biol Phys *14*: 297-300, 1988.

3 Ruifrok AC, Kleiboer BJ and van der Kogel AJ: Fractionation sensitivity of the rat cervical spinal cord during radiation retreatment. Radiother Oncol *25*: 295-300, 1992.

4 Wong CS, Minkin S and Hill RP: Re-irradiation tolerance of rat spinal cord to fractionated X-ray doses. Radiother Oncol *28*: 197-202, 1993.

5 Wong CS and Hao Y: Long-term recovery kinetics of radiation damage in rat spinal cord. Int J Radiat Oncol Biol Phys *37*: 171-179, 1997.

6 Ang KK, Jiang GL, Feng Y *et al*: Extent and kinetics of recovery of occult spinal cord injury. Int J Radiat Oncol Biol Phys *50*: 1013-1020, 2001.

7 Lee EJ, Gerhold M, Palmer MW *et al*: p53 protein regulates the effects of amifostine on apoptosis, cell cycle progression, and cytoprotection. Br J Cancer *88*: 754-759, 2003.

8 Antonadou D, Petridis A, Synodinou M *et al*: Amifostine reduces radiochemotherapy-induced toxicities in patients with locally advanced non-small cell lung cancer. Semin Oncol *(Suppl 18)*: 2-9, 2003.

9 Brizel DM, Wasserman TH, Henke M *et al*: Phase III randomized trial of amifostine as a radioprotector in head and neck cancer. J Clin Oncol *18*: 3339-45, 2000.

10 Bourhis J, De Crevoisier R, Abdulkarim B *et al*: A randomized study of very accelerated radiotherapy with and without amifostine in head and neck squamous cell carcinoma. Int J Radiat Oncol Biol Phys *46*: 1105-8, 2000.

11 Komaki R, Lee JS, Milas L *et al*: Effects of amifostine on acute toxicity from concurrent chemotherapy and radiotherapy for inoperable non-small-cell lung cancer: report of a randomized comparative trial. Int J Radiat Oncol Biol Phys *58*: 1369-1377, 2004.

12 Vacha P, Fehlauer F, Mahlmann B *et al*: Randomized phase III trial of postoperative radiochemotherapy +/- amifostine in head and neck cancer. Is there evidence for radioprotection? Strahlenther Onkol *179*: 385-389, 2003.

13 Koukourakis MI, Kyrias G, Kakolyris S *et al*: Subcutaneous administration of amifostine during fractionated radiotherapy: a randomized phase II study. J Clin Oncol *19*: 2226-2233, 2000.
14 Nieder C, Andratschke N, Price RE *et al*: Innovative prevention strategies for radiation necrosis of the central nervous system. Anticancer Res *22*: 1017-1023, 2002.
15 Nieder C, Price RE, Rivera B *et al*: Experimental data on administration of insulin-like growth factor-1 and basic fibroblast growth factor for prevention of radiation myelopathy (German). Strahlenther Onkol *178*: 147-152, 2002.
16 Andratschke NH, Nieder C, Price RE *et al*: Modulation of rodent spinal cord radiation tolerance by administration of platelet-derived growth factor. Int J Radiat Oncol Biol Phys *60*: 1257-1263, 2004
17 Tofilon PJ and Fike JR: The radioresponse of the central nervous system: a dynamic process. Radiat Res *153*: 357-370, 2000.
18 Guelman LR, Zorrilla Zubilete MA, Rios H *et al*: WR-2721 (amifostine, ethyol) prevents motor and morphological changes induced by neonatal X-irradiation. Neurochem Int *42*: 385-391, 2003.
19 Alaoui F, Pratt J, Trocherie S *et al*: Acute effects of irradiation on the rat brain: protection by glutamate blockade. Eur J Pharmacol *276*: 55-60, 1995.
20 Lamproglou I, Djazouli K, Boisserie G *et al*: Radiation-induced cognitive dysfunction: the protective effect of ethyol in young rats. Int J Radiat Oncol Biol Phys *57*: 1109-1115, 2003.
21 Plotnikova ED, Levitman MK, Shaposhnikova VV *et al*: Protection of microvasculature in rat brain against late radiation injury by gammaphos. Int J Radiat Oncol Biol Phys *15*: 1197-1201, 1988.
22 Plotnikova, ED, Levitman MK, Shaposhnikova VV *et al*: Protection of microcirculation in rat brain against late radiation injury by gammaphos. Int J Radiat Oncol Biol Phys *10*: 365-368, 1984.
23 Spence AM, Krohn KA, Edmondson SW *et al*: Radioprotection in rat spinal cord with WR-2721 following cerebral lateral intraventricular injection. Int J Radiat Oncol Biol Phys *12*: 1479-1482, 1986.
24 Lamperti A, Conger AD, Jenkins O *et al*: WR-2721 entry into the brain across a modified blood-brain barrier. Radiat Res *115*: 303-313, 1988.
25 Dorresteijn LD, Kappelle AC, Boogerd W *et al*: Increased risk of ischemic stroke after radiotherapy on the neck in patients younger than 60 years. J Clin Oncol *20*: 282-8, 2002.
26 Cheng SW, Ting AC, Ho P *et al*: Accelerated progression of carotid stenosis in patients with previous external neck irradiation. J Vasc Surg *39*: 409-415, 2004.
27 Lam WW, Leung SF, So NM *et al*: Incidence of carotid stenosis in nasopharyngeal carcinoma patients after radiotherapy. Cancer *92*: 2357-2363, 2001.
28 Dubec JJ, Munk PL, Tsang V *et al*: Carotid artery stenosis in patients who have undergone radiation therapy for head and neck malignancy. Br J Radiol *71*: 872-875, 1998.
29 Carmody BJ, Arora S, Avena R *et al*: Accelerated carotid artery disease after high-dose head and neck radiotherapy: is there a role for routine carotid duplex surveillance? J Vasc Surg *30*: 1045-1051, 1999.
30 Kortmann RD, Timmermann B, Taylor RE *et al*: Current and future strategies in radiotherapy of childhood low-grade glioma of the brain. Part II: Treatment-related late toxicity. Strahlenther Onkol *179*: 585-97, 2003.
31 Grdina DJ, Murley JS, Kataoka Y *et al*: Differential activation of nuclear transcription factor kappaB, gene expression, and proteins by amifostine's free thiol in human microvascular endothelial and glioma cells. Semin Radiat Oncol 1*2 (Suppl 1)*: 103-111, 2002.
32 Andreopoulos D, Schleicher UM, Cotarelo CL *et al*: Radioprotection of human endothelial cells with amifostine. Strahlenther Onkol *175 (Suppl 4)*: 34-36, 1999.
33 Plasswilm L, Hanjalic A, Hoeper J *et al*: Microvessel density and endothelial cell proliferation after amifostine (Ethyol) administration *in vivo*. Anticancer Res *19*: 4241-4245, 1999.
34 Giannopoulou E, Katsoris P, Parthymou A *et al*: Amifostine protects blood vessels from the effects of ionizing radiation. Anticancer Res *22*: 2821-2826, 2002.
35 Kruse JJ, Strootman EG and Wondergem J: Effects of amifostine on radiation-induced cardiac damage. Acta Oncol *42*: 4-9, 2003.
36 Kataoka Y, Murley JS, Patel R *et al*: Cytoprotection by WR-1065, the active form of amifostine, is independent of p53 status in human malignant glioma cell lines. Int J Radiat Biol *76*: 633-639, 2000.
37 Bergstrom P, Johnsson A, Bergenheim T *et al*: Effects of amifostine on cisplatin induced DNA adduct formation and toxicity in malignant glioma and normal tissues in rat. J Neurooncol *42*: 13-21, 1999.
38 Koukourakis MI: Amifostine: is there evidence of tumor protection? Semin Oncol *30 (Suppl 18)*: 18-30, 2003.
39 Brizel DM and Overgaard J: Does amifostine have a role in chemoradiation treatment? Lancet Oncol *4*: 378-381, 2003.
40 Leong SS, Tan EH, Fong KW *et al*: Randomized double-blind trial of combined modality treatment with or without amifostine in unresectable stage III non-small cell lung cancer. J Clin Oncol *21*: 1767-1774, 2003.
41 Werner-Wasik M, Axelrod RS, Friedland DP *et al*: Preliminary report on reduction of esophagitis by amifostine in patients with non-small-cell lung cancer. Clin Lung Cancer *2*: 284-289, 2001.
42 Staar S, Rudat V, Stuetzer H *et al*: Intensified hyperfractionated accelerated radiotherapy limits the additional benefit of simultaneous chemotherapy--results of a multicentric randomised German trial in advanced head-and-neck cancer. Int J Radiat Oncol Biol Phys *50*: 1161-71, 2001.
43 Giatromanolaki A, Sivridis E, Maltezos E *et al*: Down-regulation of intestinal-type alkaline phosphatase in the tumor vasculature and stroma provides a strong basis for explaining amifostine selectivity. Semin Oncol *29 (Suppl 19)*: 14-21, 2002.
44 Fike JR, Gobbel GT, Marton LJ *et al*: Radiation brain injury is reduced by the polyamine inhibitor alpha-difluoromethylornithine. Radiat Res *138*: 99-106, 1994.
45 Kondziolka D, Mori Y, Martinez AJ *et al*: Beneficial effects of the radioprotectant 21-aminosteroid U-74389G in a radiosurgery rat malignant glioma model. Int J Radiat Oncol Biol Phys *44*: 179-184, 1999.
46 Hornsey S, Myers R and Jenkinson T: The reduction of radiation damage to the spinal cord by post- irradiation administration of vasoactive drugs. Int J Radiat Oncol Biol Phys *18*: 1437-1442, 1990.
47 Rezvani M, Birds DA, Hodges H *et al*: Modification of radiation myelopathy by the transplantation of neural stem cells in the rat. Radiat Res *156*: 408-412, 2001.

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