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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Key Words: Central nervous system, brain, spinal cord, radiation therapy, 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. 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 caused 34% mortality; 75 mg/kg reduced memory dysfunction by 25 Gy only.

RT: radiotherapy; AF: amifostine
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) ethylphosphorothioate 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.

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

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

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

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 CO2 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
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
arises as to whether this is a general phenomenon, prospective clinical evaluation of this endpoint should be participants of the randomised amifostine trials would be a and stroke from head and neck tumor patients irradiated as collection of long-term follow-up data on carotid stenosis 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 125I 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 dipyriramol (starting 17 weeks after single dose irradiation) reduced the incidence of myelopathy in rats. The ED50 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 regimes and longer follow-up.

**Acknowledgements**

Supported by the Dr. Mildred Scheel Foundation for Cancer Research, Bonn, Germany, and the Bavarian State Ministry of the Environment, Public Health and Consumer Protection, Munich, Germany.

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Accepted May 3, 2004
Accepted October 11, 2004