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Prevention of Toxicities by Cytoprotection: The Role of Amifostine in the Supportive Care of Cancer for Patients with Head and Neck Cancer

Jens Büntzel[1]*, Henno Welgemoed[2] and Rene-Jean Bensadoun[3]

[1]Department of ORL, Head Neck Surgery, Nordhausen, Germany

[2]Clinigen Group, Burton-upon-Trent, United Kingdom

[3]Centre de Haute Energie, Nice, France

***Corresponding author:** Jens Büntzel, Department of ORL, Head Neck Surgery, Nordhausen, Germany, Email: Jens.Buentzel@shk-ndh.de

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ABSTRACT

Free radicals are responsible for the effects and toxicities associated with irradiation. For this reason, head and neck cancer patients often use trace elements or vitamins to act as scavengers of free radicals in order to prevent the side effects that occur during and after the treatment phase. Amifostine is the only clinically approved free radical scavenger that effectively prevents irradiation toxicities in the head and neck region. The primary benefit of amifostine is that the therapeutic only acts in normal cells and not in tumour tissue. As a result, the prophylactic use of amifostine leads to reductions in dry mouth as well as stomatitis. The following chapter aims to summarise the important pharmacological and clinical studies that have been conducted with amifostine. Additionally, this chapter will include information on the impact of modern radiotherapy techniques such as intensity-modulated radiation therapy (IMRT) on the acute and late toxicities that occur. Specific subgroups of patients derive the most benefit from the combination of modern radiotherapy concepts with selective cytoprotection by amifostine. Further amifostine research should be therefore focused on these subgroups.

Keywords: Amifostine; Dry mouth; Mucositis; Radiochemotherapy; IMRT; Selective cytoprotection

PART 1 - CYTOPROTECTION WITH AMIFOSTINE - A SHORT INTRODUCTION

SUMMARY

Most cytotoxic regimens share the common characteristic of a narrow therapeutic index, namely the relative inability to differentiate between normal and target tissues. This lack of selectivity could increase patient morbidity and hinder the therapeutic efficacy of some treatment modalities. Therefore, a broad-spectrum cytoprotective agent would be a valuable option to improve patients' quality-of-life **(QoL)** and permit the delivery of higher cumulative chemotherapy and/or radiation doses.

DISCOVERY

Amifostine (also known as WR-2721) was originally developed by the Walter Reed Institute of Research to protect against the toxic effects of nuclear radiation [1-3]. Preclinical experimentation suggested that WR-2721 was able to protect animals from lethal doses of irradiation [3].

The dose reduction factor **(DRF)** has been used to measure the potency of the radioprotective effect with amifostine. This parameter is used to define the highest radiation dose that can be administered with amifostine to reach a predetermined toxicity in comparison with the use of radiation alone. For example, a DRF of two allows the administration of double the radiation dose and the radioprotective agent should therefore be able to provide a highly protective effect. Table 1 shows the protective activity of amifostine against radiation-induced damage to a variety of normal murine tissues in terms of the DRF results. The normal tissues that had DRFs above two were parotid gland, spermatogonia, and bone marrow [4-6].

Table 1: Summary of DRFs for amifostine against radiation-induced damage to different normal murine tissues.

Tissue	Amifostine (mg/kg)	Assay system	DRF	Reference
Parotid gland	400	Salivary flow rate	2.9	Menard, et al. [7]
Bone marrow	200 200	Survival of colony-forming units LD50	2.3 1.8	Travis, et al. [8]
Lung	400 400 400	LD50 (pneumonitis) Damage to Type II cells Damage to endothelial cells	1.37 1.24 1.20	Travis, et al. [9]
Kidney	400	Tissue damage or kidney weight	1.33-1.34	Williams and Denekamp [10]
Oesophagus	400 400	LD50 (acute damage) LD50 (chronic damage)	1.6 1.49-1.54	Ito, et al. [11]
Spermatogonia	400 400	Genetic damage Survival of testicular stem cells	2.4 1.31-1.37	Benova [12] Meistrich, et al. [4]
Skin	200-500	Tissue damage	1.24-1.64	Stewart and Rojas [5]

DRF, dose reduction factor; LD50, median lethal dose.

ORGANIC THIOPHOSPHATE

Amifostine's polyamine-like structure alongside its sulphydryl group contributes to the ability of amifostine to provide protection against the toxicities arising from therapeutic radiation as well as numerous cytotoxic drugs [13, 14]. Amifostine is a prodrug that is hydrolysed, to a free-thiol active metabolite (WR-1065) and a symmetric disulphide (WR-33278), following enzymatic cleavage of a terminal phosphate group by membrane-bound alkaline phosphatase [2] (Figure 1).

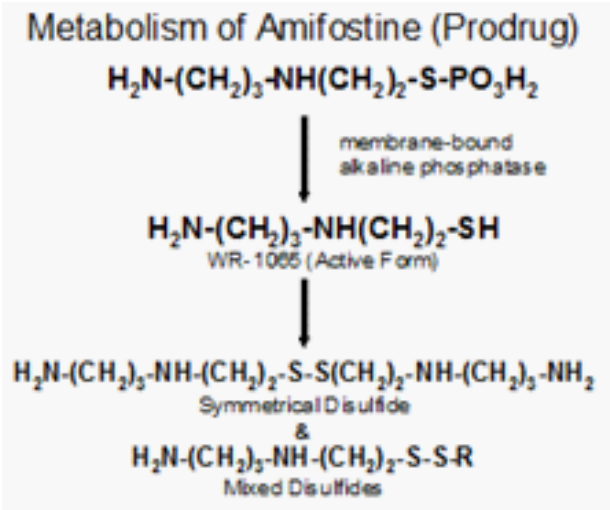

Figure 1: Amifostine metabolism

MECHANISM OF ACTION

WR-1065 is readily taken up into normal cells and it acts by binding with and detoxifying the damaging molecules that are produced by ionising radiation and some chemotherapeutic agents. This mechanism of action effectively induces a temporary state of acquired resistance to radiation- and chemotherapeutic-related toxicities. Several cytoprotective mechanisms have been identified (Table 2), including the scavenging of oxygen free radicals formed by ionising radiation, the donation of hydrogen from the free thiol to repair damaged target molecules, the binding of active species to alkylating and platinum agents, the partial removal of preformed platinum-deoxyribonucleic acid **(DNA)** adducts, and WR-1065 acting as an alternate target to DNA or ribonucleic acid **(RNA)**. Preclinical investigations have identified a number of other amifostine-induced effects that could affect cellular response to cytotoxic agents such as inhibition of apoptosis, alteration of gene expression, and modification of enzyme activity [1,15-21].

The symmetrical disulphide, WR-33278, also exerts cytoprotective effects. WR-33278 is similar in structure to the polyamine spermine. Polyamines are known to participate in a number of cellular processes, including chromatin stabilisation, DNA synthesis, gene expression,

and protein conformation, which suggests that WR-33278 may also exhibit similar activities. Preclinical investigations have shown that WR-33278 binds to DNA in a dose-dependent manner and enhances the relaxation (unwinding) of DNA supercoils mediated by topoisomerase-1. In addition, WR-33278 is also capable of protecting Chinese hamster ovarian (CHO) cells from radiation induced mutagenesis [1, 15, 22, 23].

Table 2: Cytoprotective mechanisms of amifostine.

• Scavenging of free radicals, such as those produced by ionising radiation
• Donation of hydrogen to damaged molecules
• Binding to and detoxifying active species of alkylating agents or platinums
• Partial removal of preformed platinum-DNA adducts

DNA, deoxyribonucleic acid.

RADIOPROTECTION

In preclinical investigations, amifostine protected a variety of normal murine tissues from radiation-induced toxicities. These tissues included the parotid gland, spermatogonia, bone marrow, lung, kidney, oesophagus, intestine, and skin. Subsequent studies showed that amifostine affords an elevated degree of cytoprotection against high-dose radioiodine therapy-induced salivary gland damage and radiation-induced mucositis. The hypothesis that amifostine may protect the salivary gland from ionising radiation was suggested following autoradiographic analysis in which relatively high concentrations of radiolabelled amifostine were found in mouse salivary glands compared with tumour tissue. Based on these findings, the rat parotid gland was used as a model in subsequent studies to evaluate the radioprotective effects of amifostine. Similarly to the human parotid gland, the rat parotid gland is a pure serous gland which is highly sensitive to ionising radiation [4-12,24-30].

CYTOPROTECTION

Amifostine afforded protection against bone marrow toxicity induced by a range of antineoplastic agents including cisplatin, carboplatin, cyclophosphamide, nitrogen mustard, melphalan, mitomycin-C, carmustine, 5 fluorouracil, paclitaxel, daunorubicin, doxorubicin, mitoxantrone, and diaziquone in both normal and tumour-bearing animals. Preclinical studies also demonstrated that amifostine was capable of protecting against radiation-induced immunologic toxicity, intestinal crypt cell toxicity, cisplatin-induced nephrotoxicity and neurotoxicity, kanamycin-induced ototoxicity, and cyclophosphamide and bleomycin-induced pulmonary toxicity. Similarly, pre-treatment with amifostine afforded protection against chemotherapy- and radiotherapy-induced mutagenesis and carcinogenesis. In addition, amifostine exhibited a stimulatory effect on normal bone marrow cells in the absence of neoplastic exposure [31-60].

SELECTIVE CYTOPROTECTION

Differences in the microenvironment between normal tissue and tumour tissue promotes the selective uptake of amifostine and its metabolites. This selective uptake results in higher intracellular concentrations in normal, healthy cells and organs compared with tumour cells (Figure 2).

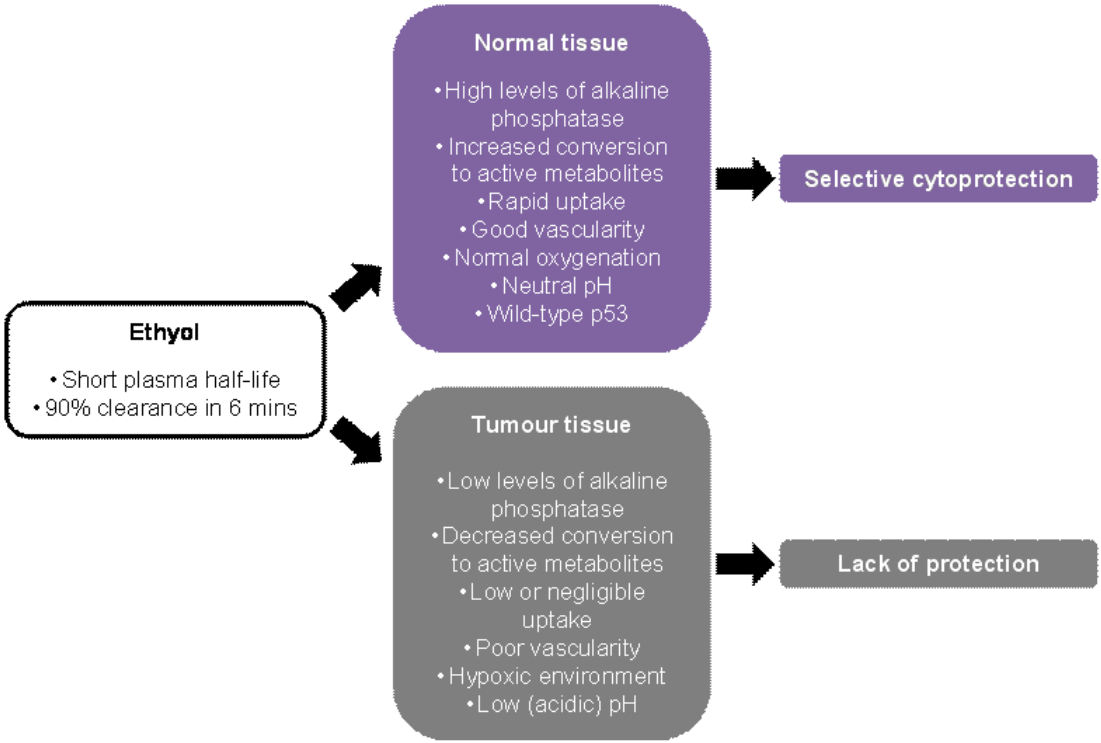

Figure 2: Mechanisms of selective cytoprotection by amifostine.

Early studies using radiolabelled drugs showed that the concentrations of amifostine and its metabolites were much higher in normal tissues than in tumours following an intraperitoneal injection of amifostine to rodent models. In a further study, rats with implanted mammary carcinomas were injected with amifostine either intravenously or subcutaneously 5 days a week for 3 weeks. The concentrations of WR-1065 were subsequently measured in normal tissues and tumours using high-performance liquid chromatography **(HPLC)**. This study showed that the concentrations of WR-1065 were higher in normal tissues, such as the kidneys and parotid glands, than in tumour tissue (Figure 3). The results from this study also demonstrated that WR-1065 does not accumulate in normal tissue after repeated dosing [61,62].

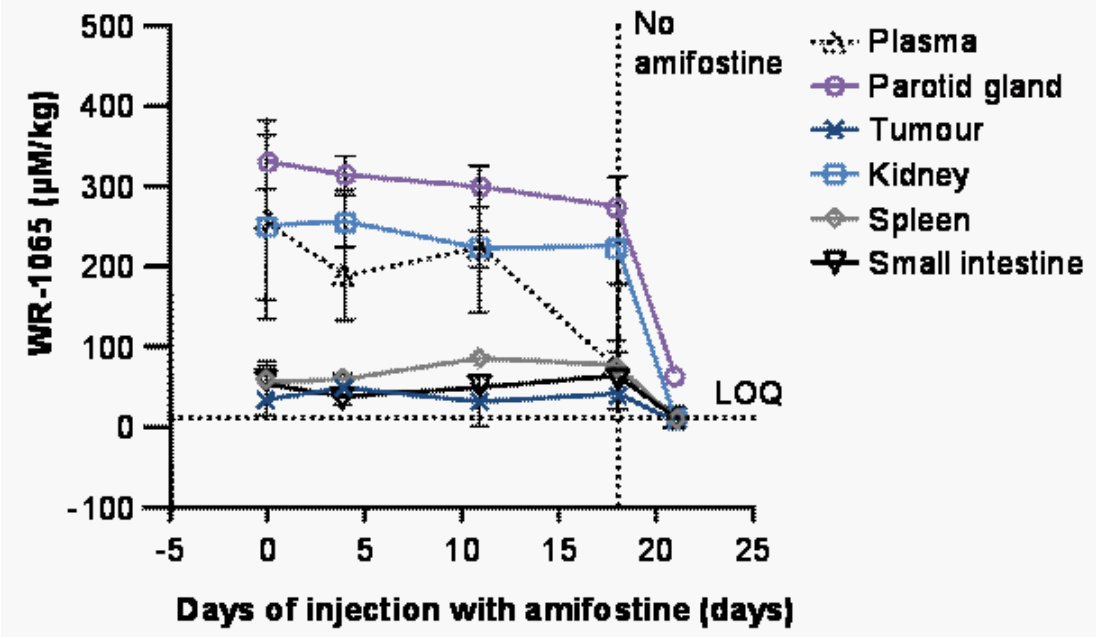

Figure 3: Concentrations of WR-1065 in normal and tumour tissues of rats bearing mammary carcinomas following daily administration of amifostine.

LOQ, limit of quantification.

Tissue-specific expression of alkaline phosphatase is an important factor that determines amifostine selectivity. In vitro studies have shown that the specific activity of alkaline phosphatase can be as much as 275-fold higher in normal lung cells compared with human non-small cell lung cancer **(NSCLC)** cells. A detailed analysis of the expression of intestinal-type alkaline phosphatase in a variety of normal tissues (breast, lung, colon, head, and neck) and their malignant counterparts has been previously performed using immunohistochemical techniques. A strong expression of nuclear and cytoplasmic alkaline phosphatase was observed in all of the cellular constituents (epithelium, stroma [fibroblasts], and vessels [endothelium]) of normal tissues. In contrast, 60% of tumours had low levels of alkaline phosphatase expression in both epithelial cells and stroma. Furthermore, only 10% to 15% of tumours had alkaline phosphatase reactivity in both the nucleus and cytoplasm and this activity was restricted to the epithelial cells. Preclinical investigations have also shown that just 6% to 17% of tumour blood vessels have detectable levels of alkaline phosphatase expression [63,64].

Plasma alkaline phosphatase does not appear to hydrolyse amifostine. Thus, the endothelial cells of blood vessels within tissues may be the first site that circulating amifostine comes into contact with active alkaline phosphatase. The high concentrations of alkaline phosphatase, present in the endothelial cells of capillaries and arterioles from normal tissues, will increase the conversion of amifostine to free thiol, which is required for rapid local uptake. In contrast, as

tumours typically have poor vascularisation with lower concentrations of endothelial cell alkaline phosphatase, drug delivery may be impaired in these tissues. Amifostine may also diffuse into the interstitial spaces between vessel walls and undergo hydrolysis via stromal alkaline phosphatase. The lower stromal concentrations of alkaline phosphatase observed in tumours may therefore also be responsible for the reduced conversion of amifostine to its active metabolites in the tumour environment [15,64,65].

Tumour hypovascularisation also results in a low (acidic) interstitial pH relative to normal tissues. Although acid phosphatase is active at low pH, it does not dephosphorylate amifostine. In contrast, alkaline phosphatase is more enzymatically active at the more neutral pH found in normal tissues. Thus, the rate of free thiol **(WR-1065)** generation increases as the pH increases from acidic to neutral pH. This pH effect is supported by in vitro data which demonstrated that the rate constant for the uptake of WR-1065 across cell membranes is markedly accelerated with small differences in pH. The highest level of uptake was observed with a pH of 7.4, which is typically found in normal tissues compared with the relative acidity of some tumours. Poor vascularisation of tumours may also cause increased hypoxia relative to normal tissues. The radioprotective efficacy of amifostine is known to correlate with tissue oxygen supply because both WR-1065 and WR-33278 compete with oxygen for free radicals. In vivo, the greatest level of amifostine-mediated cytoprotection was observed with the intermediate oxygen levels found in normal tissue and lower levels of cytoprotection were observed in the hypoxic areas of tumours [1,2,66,67].

Preclinical data suggest that the metabolites of amifostine may mediate selective cytoprotection through their effects on the cell cycle and DNA repair apparatus. For example, WR-1065 has been shown to bind to and activate several cellular DNA transcription factors, including nuclear factor kappa B **(NF-κB)**, activator protein-1 **(AP-1)**, and p53. In non-transformed cells, WR-1065 protected the cells from paclitaxel toxicity in a p53-dependant manner. However, malignant cells were not protected from the effects of paclitaxel by WR-1065. These results suggest that p53-mediated cellular growth arrest is an important mechanism of selective cytoprotection by amifostine in normal cells. Amifostine has also been shown to induce changes in the cell cycle status of mouse bone marrow cells in the presence of chemotherapeutic drugs or whole-body gamma irradiation [68-71].

AMIFOSTINE DOES NOT IMPAIR THE ANTI-TUMOUR EFFICACY OF CYTOTOXIC THERAPY

The protection that amifostine provides is not apparent in the tumour environment. Several clinical and preclinical studies demonstrated that tumour protection was not evident in a wide range of cancer cell lines and xenograft models including human NSCLC cells, human neuroblastoma cells, human ovarian cancer cells, acute lymphoblastic leukaemia **(ALL)** cell lines, and a non-seminomatous germ cell tumour. Furthermore, in human tumours, human cell lines,

tumour xenografts in nude mice, and tumours taken directly from patients, amifostine did not appear to affect the antitumor efficacy of a broad range of chemotherapeutic agents [35,63,72-78].

TIMING

The timing of amifostine administration is critically important. Effective cytoprotection was observed in several animal models when amifostine was administered prior to the cytotoxic agent, whereas administration of amifostine after the cytotoxic agent afforded no protection. For example, cytoprotection was observed when amifostine was administered prior to cisplatin (Figure 4), although no protection was reported when amifostine was administered after the cytotoxic agent. In addition, cytoprotection was also observed when WR-1065 was administered concomitantly with radiation (Figure 5) [30,37].

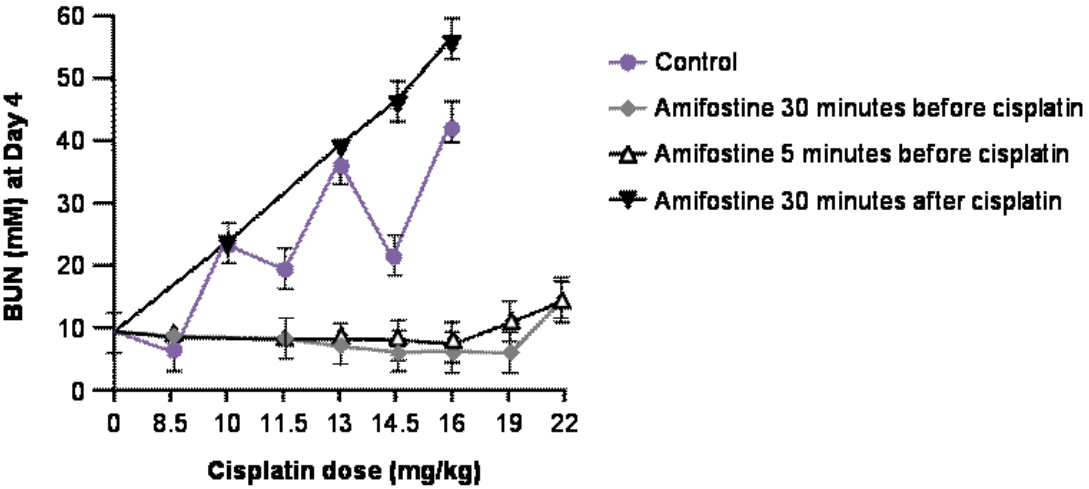

Figure 4: The effect of amifostine (200 mg/kg IV) on cisplatin-induced nephrotoxicity in BALB/C mice (n=8) when administered 30 minutes before, 5 minutes before, or 30 minutes after cisplatin as measured by BUN levels on Day 4.

BUN, blood urea nitrogen; IV, intravenous.

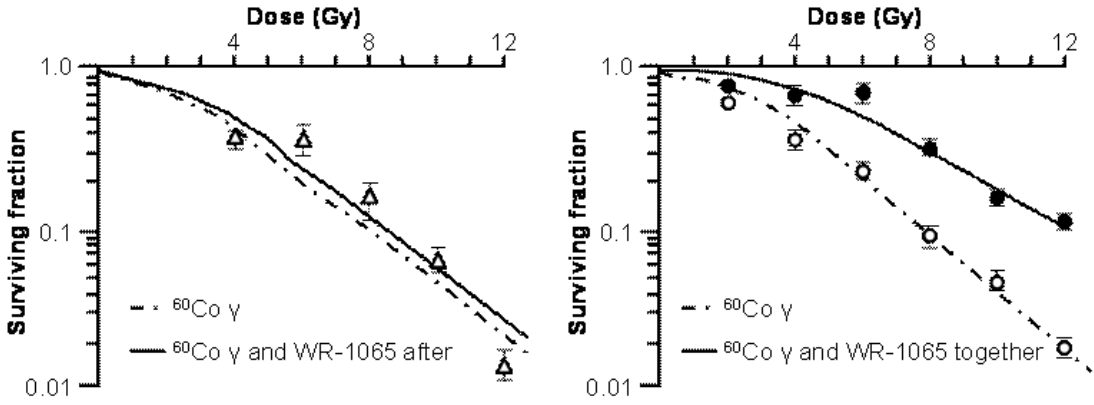

Figure 5: Cell survival of V79 cells exposed to 60Co γ-rays in the absence or presence of 4 mM WR-1065, the active metabolite of amifostine

SUBCUTANEOUS (SC) ADMINISTRATION

The pharmacology of amifostine following intravenous **(IV)** and SC administration was investigated using a rat mucositis model. Protection against radiation-induced mucositis was observed in animals pre-treated with IV amifostine up to 4 hours prior to irradiation (Figure 6) and with SC amifostine up to 8 hours prior to irradiation (Figure 7). Following multiple doses of amifostine and irradiation, both routes of amifostine administration were equally protective against radiation-induced mucositis in this animal model. Moreover, the data suggested that complete protection was dependent on daily dosing with amifostine prior to each fraction of irradiation. These data were supported by pharmacokinetic studies in rats and monkeys showing comparable levels of WR-1065 in the parotid glands and kidneys following SC or IV administration of amifostine. The findings from these investigations provided the impetus for clinical studies evaluating the effectiveness and safety of amifostine following SC administration [29].

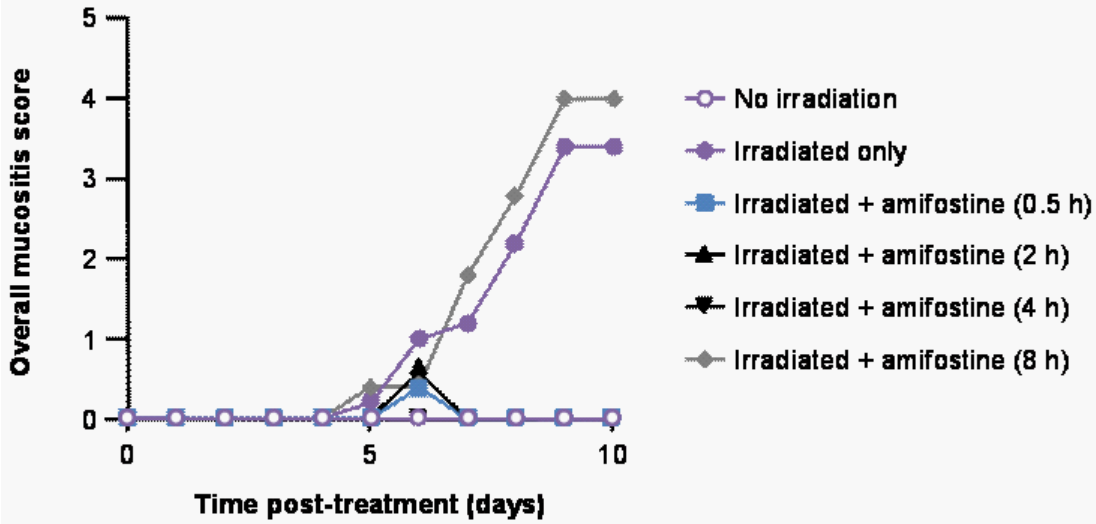

Figure 6: Amifostine administered intravenously protects against radiation-induced mucositis in a rat model.

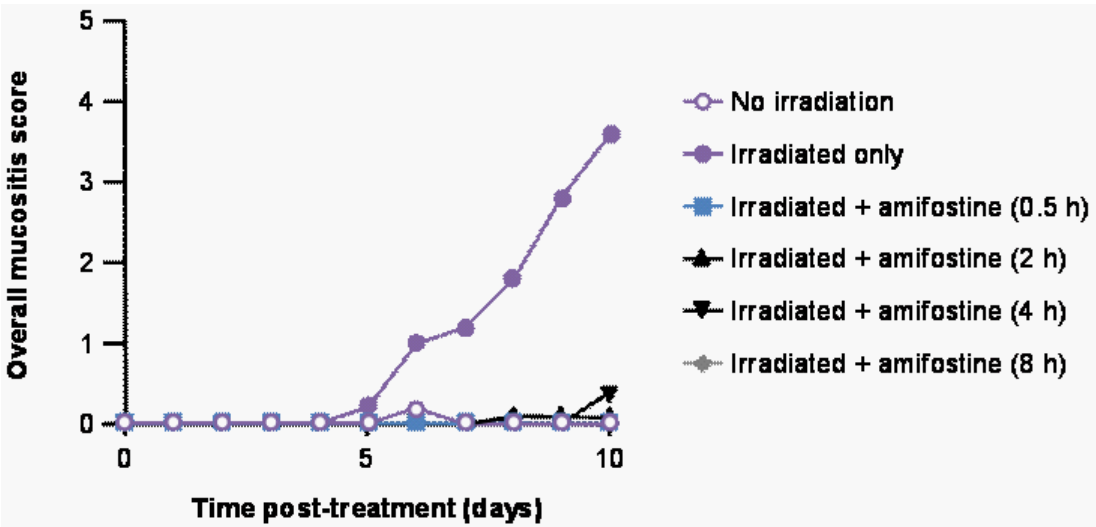

Figure 7: Amifostine administered subcutaneously protects against radiation-induced mucositis in a rat model.

PART 2 - INTENSITY-MODULATED RADIATION THERAPY: A NEW ERA OF IRRADIATION FOR HEAD AND NECK CANCER PATIENTS

The advent of intensity-modulated radiation therapy **(IMRT)**, a three-dimensional conformal radiotherapy **(3DCRT)** technique, has excited the profession of radiation oncology more than any other new invention since the introduction of the linear accelerator. Although only a handful of institutions pioneered IMRT for head and neck cancer during the mid-1990s, this technique has become commonplace during the last decade. The aim of IMRT is to provide enhanced conformal dose distribution compared with standard 3DCRT and this in turn results in improved sparing of normal structures (i.e. parotid glands). This sparing potentially translates into fewer late side effects **(xerostomia)** and improved QoL for the patients [79]. Through the use of IMRT, the physician can identify the target volumes(s) and the organs at risk **(OARs)** for each particular clinical condition.

IMRT has emerged as an effective technique for delivering the full radiation dose to the tumour and regions at risk while reducing the exposure to surrounding healthy tissues. One of the most significant complications of radiotherapy to the head and neck region is hyposalivation, and its related complaint of xerostomia (subjective oral dryness). The results of a study that pooled all head and neck radiation regimens found that the weighted prevalence of xerostomia was 6% before treatment and 93% during irradiation [80]. This study also showed that a slightly lower prevalence was observed 1 month after therapy compared with 2 years post-treatment. Saliva plays an important role in maintaining mucosal integrity, promoting oral wound healing, taste perception, formation of food bolus, initiation of food ingestion, swallowing, and speech [81]. Alterations in the oral microbiome, reduced oral clearance, changes in saliva composition (e.g. decreased buffer capacity, pH, immunoglobulin concentrations, and defensins), and dietary changes may increase the risk of mucosal infections and rapidly progressing dental demineralisation and caries [82]. A substantial decrease in salivary function therefore has a significant impact on QoL and results in the additional burden of long-term dental care [83,84].

Both dysphagia and aspiration risk structures **(DARS)** are susceptible to damage during IMRT [85]. In particular, alterations to the tongue base, pharyngeal constrictors, the larynx, and the autonomic neural plexus were found to be crucial in the development of post-radiotherapy dysphagia. Clinical studies have confirmed that reducing the radiation dose to DARS decreases dysphagia risk [86–88], although dysphagia continues to be a significant clinical problem [89].

Irradiation of the salivary glands results in loss of gland function, which begins early during the course of radiotherapy [90] and has been shown to induce apoptosis in parotid glands in a dose-dependent manner [91]. A modest improvement in xerostomia has been observed a few months after radiotherapy which suggests the occurrence of either functional recovery or adaptive or compensatory functioning by the non-irradiated salivary glands. However, most patients have

persisting oral dryness for the rest of their life following the use of 3DCRT. With IMRT preserving more of the major salivary glands, long-term oral dryness is reduced. However, a significant proportion of patients still experience xerostomia, especially during the 2 years following cancer treatment and when concomitant chemotherapy, or targeted agents like cetuximab are used [92].

It is noteworthy that the maximum prescribed dose has empirically evolved as the highest tolerated dose that takes into account the surrounding normal structures. A 'definitive' treatment typically includes two to three different dose levels administered to the same patient. Using IMRT, a different dose can be delivered to various targets through the use of sequential plans **(**as for **3DCRT)** or with a simultaneous integrated boost **(SIB)**. It has been shown that the latter approach provides better dose conformity compared to several consecutive plans [93]. When a single plan is prescribed, the main clinical target volume **(CTV1)** receives both a higher total dose and a higher dose/fraction **(d./f.)** compared with the other CTVs. This results in a higher biologically equivalent dose **(BED)** being delivered to the CTV1. However, since all dose levels are delivered through the same number of fractions, the target tissue, as well as healthy tissues, must receive different fractionations.

Nowadays, IMRT is unequivocally the radiotherapy reference technique for head and neck cancer. Chemoradiation (concomitant use of external radiotherapy and systemic chemotherapy or targeted therapy) is mostly used for advanced cases, with a high level of local-regional control following the use of optimal full-dose treatment. However, late side effects, especially xerostomia, remain important when combined modalities are used, leaving a potentially important role for amifostine cytoprotection. Hence, in our opinion, high-dose combined modality protocols should involve the use of amifostine to optimise therapeutic regimens and QoL in this type of patients [94].

PART 3 - CYTOPROTECTION FOR HEAD AND NECK CANCER PATIENTS: A CLINICAL STUDY REVIEW

There are two extended reviews that investigate the impact of amifostine on the course of head and neck cancer; Bourhis et al. (2011) and Gu et al. (2014) [95,96]. Both reviews are central to understanding the key points of the role of amifostine as a supportive care treatment in patients with head and neck cancer.

Bourhis et al. focused on the question of survival during radiotherapy in patients taking amifostine [95]. The results of this study showed that amifostine had no negative impact on survival. This is an important finding as radiotherapy uses free radicals to attack the cancer, whilst amifostine is acting as a scavenger of free radicals. Amifostine was found to be ineffective at protecting tumours, demonstrating the selectivity of its mechanism of action. The survival curves produced showed the epidemiological equivalency of this pharmacological model. The authors included all Phase II and Phase III studies and analysed the quality of the data and follow-up observations. Furthermore, they were able to extend the follow-up time for each included patient by collecting individual survival data at the time of preparing the review. It should be noted that only 50% of all treated patients were included in the study because of a lack of original data sources. In total, 1,119 patients from 12 clinical trials were included.

Gu et al. analysed the clinical effects of amifostine when it was used as a supportive therapy for patients with head and neck cancer in randomised trials [96]. In patients treated with amifostine, significant reductions in acute mucositis (Grade 3/4), xerostomia (Grade 2-4), late xerostomia, and dysphagia (Grade 3/4) were observed. Amifostine improved oral comfort during radiotherapy and helped to avoid malnutrition caused by irradiation. However, the included data were not strong enough to demonstrate these effects in the subgroup of patients receiving radiochemotherapy regimens. This study also found that the occurrence of haematological side effects was not influenced by amifostine. The authors included 17 trials involving a total of 1,167 patients. The authors ranked the included studies regarding their quality and four high-quality trials are discussed in detail below.

The pivotal study examining the use of amifostine was published by Wasserman et al. in 2000 [97]. In total, 301 patients took part in this study which aimed to compare the efficacy of radiotherapy alone and radiotherapy combined with a daily dose of amifostine. The study found that patients experienced a systematic and significant reduction in acute and late xerostomia if the patients received daily IV doses of 200 mg/m^2 amifostine 15–30 minutes before irradiation. However, amifostine had no impact on the grade of mucositis which was observed. The patients in this study were not administered any pre-medication before the trials, as this was deemed to be unnecessary. The study is the largest trial involving amifostine in patients receiving radiotherapy. Critical points from this study were:

- An open-label design was used and therefore there was no placebo control or any other form of blinding for comparative purposes.
- The dosage of radiotherapy varied between 50 and 70 Gy and the study included patients receiving both adjunctive and primary radiotherapy.
- The authors of the study experienced difficulties categorising mucositis and dry mouth in accordance with Radiation Therapy Oncology Group **(RTOG)** recommendations.

Büntzel et al. conducted an open-label randomised Phase II trial involving 39 patients receiving a radiochemotherapy protocol [98]. In this trial, irradiation doses of between 60 and 70 Gy were used. Carboplatin was administered as radiosensitiser on Days 1-5 and 21-25 before each radiotherapy course. The authors administered 500 mg amifostine intravenously before each chemotherapy cycle, alongside antiemetic pre-medication with 8 mg ondansetron and 12 mg dexamethasone. The methodology of the study ensured that the time interval between starting the course of amifostine and the end of radiotherapy administration did not exceed 45 minutes. Results from 28 patients (14 per arm) showed that amifostine significantly reduced Grade 3-4 mucositis, Grade 2 xerostomia, Grade 2 ageusia, and Grade 3-4 dysphagia. As no haematological side effects were observed following amifostine treatment, the authors added a further 11 patients to the amifostine arm and the same clinical effects were observed. Critical points from this study include:

- The randomised Phase II protocol design was not able to investigate statistical differences between the groups
- The primary endpoint was not well defined. Dysphagia, xerostomia, mucositis, and ageusia are all qualitative toxicities
- The resulting statistical power was too low to obtain realistic statistical results
- Despite the potential drawbacks, this Phase II trial demonstrated clinically relevant indications for the use of amifostine in head and neck cancer patients.

A single Phase III trial was the only placebo-controlled, double-blind amifostine study involving patients with head and neck cancer [99]. The study involved 132 patients who each received 300 mg/m^2 amifostine or placebo on the same days that carboplatin was administered (Days 1-5 and 21-25) and 200 mg/m^2 amifostine or placebo on the days of radiotherapy alone (Days 6-20 and 26-35). Anti-emetic pre-medication and glucocorticoids were only given on the days that chemotherapy was administered. The primary endpoint of the study was the rate of Grade 3-4 mucositis, which was not reduced following amifostine treatment. Acute and late xerostomia were similar in both groups, although the toxicity and treatment interruption frequencies were significantly higher in the amifostine group. Several issues were identified with the protocol:

- Anti-emetic pre-medication was only given on the days when amifostine was administered to combat the side effects of nausea and vomiting. However, this pre-medication was not given on the days where radiotherapy was administered alone. Patients receiving chemotherapy may experience a higher incidence of nausea and vomiting compared with patients who receive radiotherapy, as indicated by Bourhis et al [95].
- Nausea increases the rate of mucositis which decreases patient compliance with the supportive strategy
- As more treatment interruptions were observed in the amifostine arm, the potential impact on xerostomia could have been reduced
- In summary, this Phase III trial demonstrated the potential pitfalls involved in amifostine usage and highlighted the problems of a combined modality approach.

In 2002, Antonadou et al. published a study involving the use of carboplatin-including chemoradiotherapy to treat patients with head and neck cancer [100]. The dosage of amifostine administered was 300 mg/m^2 IV on each treatment day. In total, the results for 22 amifostine-pre-treated patients were compared with the findings from 23 patients who received radiochemotherapy alone. The primary study endpoint was Grade 3/4 mucositis/dysphagia and the incidence of late xerostomia. From the second week of combined therapy, symptoms of both mucositis and dysphagia were reduced in the amifostine arm. A reduced rate of late xerostomia was observed during the first 12 months of follow-up, although the authors did not note any differences in haematological toxicities. Critical discussions are awaited regarding this Phase II protocol which enroled only a small number of patients per group and used the statistical methodology described above.

In 2006, a Multinational Association of Supportive Care in Cancer **(MASCC)** working group published a review of the literature regarding the effect of amifostine for the prevention of cancer-therapy induced mucositis [101]. The only potential positive effects of amifostine attested in this review were the reduction of acute oesophagitis in patients treated with chemoradiation for lung cancer, and the reduction of proctitis in patients treated with radiation for rectal cancer (IV, SC, or intra-rectal route).

To reduce the treatment side effects and increase the acceptance of amifostine in patients with head and neck cancer, a stringent course of pre-medication with serotonin **(5-HT3)** antagonists should be given to patients to prevent emesis or nausea. Furthermore, patients who would benefit from amifostine treatment require therapy within a specific timeframe. As the complexity of the therapeutic regimen increases, the frequency of treatments will also increase. In order to combat the potential side effects associated with these treatments, the use of SC amifostine cytoprotection during fractionated radiotherapy has been suggested as a potential administration route [102]. In a Phase II study of 14 patients with head and neck cancer, a reduction in oral mucositis was observed in patients administered a SC dose of 500 mg amifostine 20 minutes before irradiation.

A summary of the principal methodological features from clinical studies involving amifostine is shown in Table 3.

Table 3: Summary of the principal methodological features from clinical studies involving amifostine [96].

	Adequate sequence generation?	Allocation concealment?	Blinding?	Incomplete outcome data addressed?	Free of selective reporting?	Free of other bias?
Amrein 2005						
Antonadou 2002						
Bourhis 2000						
Braaksma 2005						
Brizel 2000						
Buentzel 1998						
Buentzel 2006						
Fan 2001						
Haddad 2009						
He 2004						
Jellema 2006						
Jiang 2009						
Koukourakis 2000						
Peng 2006						
Veerasarn 2006						
Yu 2009						
Zhang 2010						

PART 4 - THE CONCEPT OF CYTOPROTECTION WITH AMIFOSTINE IN THE ERA OF INTENSITY-MODULATED RADIATION THERAPY

The results of an expert consensus meeting held in February 2016 are presented below. The aim of this consensus meeting was to link present IMRT developments with earlier amifostine research data in order to find a potential new role for selective cytoprotection.

The use of IMRT has enhanced individual irradiation planning alongside reducing the side effects of treatment. Patients who require additional supportive care need to be identified, as patients with higher grades of toxicities should ideally receive prophylactic treatment with amifostine. In these patients, amifostine could be administered on a daily basis using a bolus IV administration or a short infusion. A dose of 200 mg/m^2 amifostine should be used for radioprotection and 300 mg/m^2 should be used for radiochemoprotection. The time between IV application and the end of radiotherapy should not exceed 1 hour and anti-emetic pre-medication is recommended. The following technical and individual criteria should be taken into account when amifostine is used:

- Technical criteria:

1. For patients treated with radiotherapy doses of greater than 25 Gy to the salivary glands, each tumour located near the salivary gland could benefit from amifostine treatment.
2. T3 or T4 situation. The larger the primary tumour, the greater the proportion of oropharyngeal mucosa that will be affected by the irradiation. If such patients receive amifostine, a reduction in dysphagia should hopefully be observed.
3. Bilateral neck disease **(N2c or N3)** requires radiotherapy doses of at least 50-60 Gy. Large regions of the oropharyngeal mucosa will be affected and have to be protected by amifostine. If less soft tissue of the neck is included in the irradiation fields, the use of amifostine may not be required.

- Individual Criteria:

1. Patients with clinical manifestations of malnutrition have a poorer prognosis compared with well-nourished individuals [103]. To improve the prognosis of the head and neck cancer patient, their nutritional situation must be stabilised. Each patient with malnutrition should receive amifostine to avoid further negative developments. Signs of poor nutritional situations include weight loss, negative bioimpedance phase angles, low body mass index (BMI), or swallowing pathologies.
2. In cases involving a secondary primary cancer or recurrent disease, the patient will have already received primary radiotherapy and must be re-irradiated. As the patient has already undergone radiotherapy, the mucosa is already disturbed and further damage should be avoided. A small study has showed that amifostine can have a positive impact on potential cumulative toxicities [104,105].

3. Mucositis rates have increased since radiochemotherapy was first used to treat patients with head and neck cancer. Following the use of cetuximab as a simultaneous antibody infusion, some patients have experienced high grade stomatitis despite local mouth cleaning. Simultaneous application of cytotoxics or antibodies should therefore be carefully considered as they may reduce the potential benefit observed with amifostine.

Each of the above criteria should be considered as reasons to discuss the individual usage of amifostine despite the use of IMRT techniques to treat head and neck cancer. It is important to take into account that the daily administration of amifostine will be affected by the need for repeated IV injections and time limitations as well as the side effects of the therapy. SC application should be investigated further to identify potential dosing regimens [102].

In conclusion, the potential for parotid-sparing therapy to cause xerostomia underscores the need for additional measures to protect salivary tissues and preserve salivary function. The use of amifostine to prevent the typical side effects of multimodal therapies is currently recommended by several internationally-respected organisations including MASCC and the American Society of Clinical Oncology **(ASCO)** [106,107]. Cytoprotection with amifostine is therefore a relevant treatment option in the era of IMRT. Usage is recommended in defined subgroups of patients with head and neck cancer in order to improve QoL as well as the effectiveness of the radiation therapy. Fundamentally, amifostine research has set the scientific benchmark for free radical scavengers in the field of head and neck oncology.

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