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Preventing Radiation-Induced Injury by Topical Application of an Amifostine Metabolite-Loaded Thermogel

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Title

Preventing radiation-induced injury by topical application of an amifostine metabolite-loaded thermogel

Running title

CPh-1014 mitigates radio-induced injuries

Keywords

irradiation, mucositis, dermatitis, amifostine, topical application

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Abstract

Purpose: Despite the development of high-precision radiotherapy, ionizing radiation inevitably damages healthy tissues. Radiodermatitis and radio-induced oral mucositis are frequent and significant side effects among patients with breast and head and neck cancer, respectively. These radiation-related injuries negatively impact patient quality of life and can lead to unplanned therapeutic breaks, compromising treatment outcomes. Currently, no preventive or mitigating agent has emerged to address these issues. Although amifostine, a well-known free radical scavenger, has proven efficacy against specific radio- and chemo-induced toxicities, severe adverse side effects (reversible hypotension, nausea, emesis, etc.) combined with logistical hurdles are associated with its recommended intravenous route of administration, limiting its use.

Methods and Materials: We developed a thermogel containing the active thiol metabolite of amifostine (CPh-1014) that polymerizes at body temperature and serves as a matrix for topical application onto the skin or mucosa.

Results: Applied before irradiation, CPh-1014 greatly reduced the severity of oral mucositis and dermatitis induced by either a single dose or fractionated irradiation regimens in *in vivo* mouse models. The cytoprotective effect of CPh-1014 was confirmed by the decrease in DNA double-strand breaks in the irradiated epithelium. Noticeably, CPh-1014 did not affect radiotherapy efficacy against tumors grafted at submucosal and subcutaneous sites. In contrast to the intravenous administration of amifostine, CPh-1014 oral application did not induce hypotension in dogs.

Conclusions: CPh-1014 confers radioprotective effects in healthy tissues with reduced systemic side effects without compromising radiotherapy efficacy. We propose CPh-1014 as an easy-to-implement therapeutic approach to alleviate radiotherapy toxicity in breast and head and neck cancer patients.

Introduction

In past decades, the efficacy of radiotherapy has been largely improved due to dose escalation and novel irradiation techniques. However, radiation-related toxicities remain common. Radiation-induced oral mucositis (OM) is the most common debilitating and dose-limiting side effect of irradiation in patients with head and neck cancer (HNC). High-grade mucositis develops in the majority of patients undergoing radiation treatment for HNC (1). This condition sometimes requires parenteral nutrition and hospitalization and can limit dose escalation and lead to unplanned therapeutic breaks, thus negatively impacting efficacy outcomes (2,3). To date, the management of OM has solely relied on improvements in oral hygiene and pain mitigation (4,5). Radiation therapy also induces radiodermatitis in more than 95% of patients with breast cancer, and approximately 30% of women receiving postmastectomy radiotherapy will experience grade 3 skin toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) (6). In severe cases, radiodermatitis can compromise local control because of treatment interruptions. Similar to OM, no agent has emerged as a standard to prevent or treat radiodermatitis (7,8).

Radiotherapy induces extensive DNA damage in cells mainly through the generation of reactive oxygen species (ROS). Amifostine (WR-2721) is a ROS scavenger that has proven efficacy for the prevention of radiation-related toxicities (9-11). Intravenous (IV) injection of amifostine was approved by the FDA in 1999 to manage radiation-induced xerostomia in HNC when salivary glands are exposed. Amifostine is a phosphorylated prodrug that is converted into the free active metabolite amifostine thiol (WR-1065) under the activity of alkaline phosphatase. (9,12). Although amifostine is promising for the management of various radio-induced injuries, severe adverse side effects, such as transient hypotension, nausea and vomiting, are associated with its recommended route of administration, the IV route (13). Slow infusion and continuous monitoring by alert medical staff are recommended for IV administration of amifostine to patients. Adverse side effects,

increased workload, logistical constraints, and patient discomfort dramatically limit clinical use of amifostine and thus encourage research for a better, safer administration route (4,14).

Here, we developed a new thermogel formulation loaded with amifostine thiol, called CPh-1014, and evaluated its efficacy for the prevention of radiation-related toxicities. We used the active thiol metabolite of amifostine to bypass prodrug hydrolysis by alkaline phosphatase. The thermogel polymerizes at body temperature and is suitable for topical application. Its formulation and adhesive properties enabled easy direct application of amifostine thiol to mucocutaneous areas. CPh-1014 application reduced radio-induced DNA damage in mucocutaneous tissues and reduced the severity of mucositis and dermatitis in *in vivo* mouse models without shielding tumors from the efficacy of radiotherapy. Hypotension is one of the most frequent adverse effects of amifostine; we demonstrated that in contrast to the IV injection of amifostine, the application of this amifostine thiol thermogel did not induce orthostatic hypotension in dogs. This study provides a robust and extended rationale to guide the future development of CPh-1014 as a radioprotector in the clinic.

Methods and Materials

***In vivo* mouse experiments**

Animal procedures were performed according to protocols approved by the Ethics Committee XXX (project n° XXX). Female C57BL/6 mice were purchased from Janvier (France) and housed in the XXX animal facility. Irradiation was performed with an X-ray XRAD320 tube.

Thermogels

The thermogel developed by XXX (XXX, XXX) contains the following poloxamers: Poloxamer 407 (Kolliphor® P407 with an average molecular weight/mass of 9840-14600 g/mol); and Poloxamer 188

(Kolliphor® P188 with an average molecular weight/mass of 7680-9510 g/mol) (BASF). Amifostine or amifostine thiol was embedded in the thermogel prior to topical application. Amifostine solution (20 mg/ml) was administered by slow tail vein injection. For better comparisons, the quantities of amifostine in the experiments are expressed as the amifostine thiol equivalent unless otherwise indicated.

Oral mucositis model

To induce OM, we locally irradiated mouse snouts (15,16). Gels were applied to the mouth (5 μ l of gel was spread on each internal cheek and 40 μ l on lips) under chemical anesthesia.

Radiodermatitis model

The dorsal skin of mice was covered with 100 or 200 μ l of gel 30 minutes before irradiation. Anesthetized mice were placed in a lateral decubitus position in restraint devices, and the depilated dorsal skin was taped inside the irradiation field. Gels were removed with water just before irradiation.

Head and neck tumor model

Luciferase-expressing TC1-luc cells were provided by T.C. Wu (Johns Hopkins Medicine, Baltimore, MD, USA). TC1-luc cells were injected at a submucosal site in the right inner lip (500,000 cells in 50 μ l) (17). Tumor growth was monitored using bioluminescence imaging (Xenogen In Vivo Imaging System 50, Caliper Life Sciences, Hopkinton, MA, USA). A few days after tumor cell injection, the mice were randomized according to the bioluminescence imaging signal, allocated to different treatment groups and treated with thermogels (day 0). Thermogels were directly applied onto the tumors (50 μ l) 30 minutes before tumor-localized irradiation. The ethical endpoints for survival were loss of more than 20% of the initial weight for more than 24 h or severe clinical symptoms.

Subcutaneous tumor model

Luciferase-expressing Lewis Lung Carcinoma LL2-luc tumor cells (Caliper Life Sciences) were injected subcutaneously (600,000 cells in 50 μ l). When the tumor volume reached ~ 125 mm³, the mice were randomized according to the tumor size and allocated to different treatment groups (day 0). The tumors were covered with thermogel before irradiation. The relative tumor volume is the tumor volume at a given day ($\text{volume} = \text{length} \times \text{width}^2 / 2$) divided by the tumor volume at day 0.

Immunohistochemical analysis

Tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were processed for gamma-H2AX (Millipore, 05-636) staining. Quantification of gamma-H2AX foci was performed manually on slide scans (digital microscope slide scanning in batches, Hamamatsu, x20).

Tilt test in dogs

Experiments were conducted by Biotrial (Rennes, France) in healthy dogs (nonnaïve adult male beagle dogs, Marshall) trained on the tilt test and previously equipped with a telemetry implant. For the tilt procedure, dogs stood quietly on their four limbs and were then subjected to a rapid postural change comprising a 90 degree tilt in a standing position. The tilt position was maintained for 1 minute. An initial baseline tilt procedure was performed. Then, treatment was administered, and two tilt procedures were conducted after 30 minutes and 1 h.

Statistical analyses

Statistical analyses were performed using GraphPad Prism software (ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; and ****, $p < 0.0001$).

Results

Thermogel containing amifostine thiol mitigates the severity of oral mucositis induced by a single high-dose irradiation.

To investigate the efficacy of thermogels containing amifostine or amifostine thiol against radiation-induced mucosal reactions, we used a mouse model of OM (15). A lip epidermal mucosal reaction was induced by high-dose irradiation of the mouse snout. The acute reaction was scored macroscopically using the Parkins scoring system (Table 1) (16). The thermogels were applied on the internal oral mucosa (Fig. 1A) and lips (Fig. 1B) to cover an extended part of the oral mucosa 30 minutes before irradiation. After a single 20 Gy dose of irradiation (day 0), the control mice developed a severe mucosal reaction with a mean Parkins score of 5.25 at day 12 (Fig. 1C) and had to be sacrificed in the following days due to severe body weight loss (Figs. 1D and 1E). As expected, intravenous injection of amifostine (2.5 mg/mouse) resulted in a weaker and shorter mucosal reaction (mean score of 2.25 at day 12) with no body weight loss (Figs. 1C and 1D). By contrast, the same dose of amifostine applied topically did not protect against severe mucosal reaction (Fig. 1C). As the conversion rate of amifostine to amifostine thiol at the interface of the gel is unknown, we tested the amifostine thiol gel (CPh-1014). Mice treated with CPh-1014 developed a mucosal reaction (mean score of 4.42 at day 12) but could be kept alive until the end of the experiment (Figs. 1C and 1E) with manageable body weight loss (Fig. 1D). After a single 18 Gy dose irradiation, the control mice developed a slightly less severe mucosal reaction (maximum score of 4.16) with no major body weight loss, while mice treated with topical application of CPh-1014 showed a mucosal reaction of reduced intensity compared with control mice (maximum score of 2.75) (Fig. E1). Thus, CPh-1014 is effective for preventing radio-induced mucosal reactions.

CPh-1014 alleviates the severity of oral mucositis induced by fractionated irradiation by decreasing radio-induced DNA damage.

As fractionation is standard in most radiation therapy curative schedules, we examined the impact of CPh-1014 on OM induced by fractionated irradiation. The thermogel was administered before each fraction of irradiation. The dose of amifostine thiol was reduced to 1.25 mg amifostine thiol per mouse per day to assure tolerability for repeated administration. The snouts of mice were irradiated with four consecutive fractions of 8 Gy, which is biologically equivalent to one fraction of 18 Gy (same biologically effective dose). In the control group, the mucosal reaction peaked at day 15 (score of 5.92), but the body weight loss was manageable (Fig. 2A; Fig. E2A). Amifostine IV 30 minutes before each fraction of irradiation greatly reduced the intensity and duration of mucositis (maximum score of 2.08, and score returned to 0 at day 51 *versus* day 31 for the control and amifostine IV groups, respectively) (Fig. 2A). Interestingly, the topical application of amifostine thiol thermogel was as effective as the IV administration of amifostine (maximum score 2.17, and returned to 0 at day 31) (Fig. 2A). Representative images of swollen red snouts in the control and placebo gel groups are depicted in Fig. E2B. To monitor DNA damage induced by IR, we assessed gamma-H2AX foci formation, a marker of DNA double-strand breaks (DSBs), in the epithelium of the oral mucosa. The number of radio-induced gamma-H2AX foci was dramatically decreased in the IR plus amifostine thiol gel and the IR plus amifostine IV groups (mean of 2.7 and 3.1 foci per epithelial cell, respectively) compared with the IR and the IR plus placebo gel groups (mean of 4.5 and 4.4 foci per cell, respectively) (Figs. 2B and 2C). This result shows that the epithelium is subjected to less radio-induced DNA damage in these two groups, in agreement with the known radioprotective ROS scavenging activity of the amifostine thiol molecule.

Topical application of the amifostine thiol thermogel does not alter the efficacy of IR against head and neck tumors.

To exclude the possibility that tumor cells could be protected from IR cytotoxicity by CPh-1014, we used a mouse HNC model (17). Luciferase-expressing TC1-luc cells were injected into the inner lip of

mice. A few days after engraftment, mice were irradiated locally with 4 consecutive fractions of 4 Gy to achieve a moderate antitumor effect of radiotherapy. To mimic the worst situation, thermogels were applied directly onto tumors, as illustrated in Fig. 2D. Tumor growth was monitored using bioluminescence imaging. Irradiation delayed tumor growth (Fig. 2E; two-way ANOVA, Tukey post hoc test, $p < 0.001$) and improved mouse survival (Fig. 2F; median survival of 8 and 23 days after irradiation for the control group and the irradiated without gel group, respectively, log-rank test, $p < 0.001$). Repeated IV injection of amifostine or application of the placebo or amifostine thiol gel did not significantly alter the tumor response to radiotherapy (Figs. 2E and 2F). Whereas nonirradiated tumors presented some DSBs (mean foci per cell of 1.6) as expected for cancer cells, IR alone increased the number of gamma-H2AX foci in epithelial cells (mean, 4.9). The application of the placebo, amifostine thiol gel or the IV injection of amifostine did not significantly alter the radio-induced induction of gamma-H2AX foci (mean, 4.8, 4.5 and 4.6 foci per cell, respectively) (Figs. 2G and 2H). We confirmed the absence of impact of CPh-1014 application on tumor growth after irradiation with a single fraction (Fig. E3). Thus, the application of CPh-1014 directly onto the mucosa reduces DNA damage in epithelial cells but not in tumor cells and protects the mucosa from IR without reducing the antitumor efficacy of radiotherapy irrespective of the irradiation scheme.

In contrast to the intravenous injection of amifostine, the topical application of amifostine thiol thermogel does not induce orthostatic hypotension in dogs.

To study the orthostatic response, blood pressure variations were recorded in conscious dogs subjected to a rapid postural change (upright tilt test) before and 30 minutes and 1 h after the administration of the compounds. Tilting caused an increase in arterial blood pressure (Fig. 2I). Orthostatic hypotension was evoked by intravenous injection of 100 mg/kg amifostine. In contrast, oral application of CPh-1014 at the same equivalent dose did not induce a reduction in systolic blood

pressure, suggesting that topical application of amifostine thiol may show fewer side effects than the systemic administration of amifostine.

Amifostine thiol thermogel reduces the severity of dermatitis induced by fractionated irradiation without protecting subcutaneous tumors from IR.

To evaluate the possible benefit of CPh-1014 against skin toxicities, we developed a murine model of cutaneous radiation injury induced by fractionated irradiation. Briefly, 4 consecutive fractions of 12 Gy were delivered to the dorsal skin of anesthetized mice. Skin reactions were assessed according to a macroscopic scoring system based on the CTCAE grading criteria presented in Table 2. The skin reaction began approximately 15 days after the first day of irradiation, peaked at day 20 and ranged from dry/inelastic skin and desquamation to nonhealing ulceration (Fig. 3A). A larger volume of gel could be applied onto the dorsal skin than onto the snout (Fig. 3B), which permitted us to test higher doses of amifostine thiol. CPh-1014 application decreased the severity score of skin lesions in a dose-dependent manner (Fig. 3A). The IV administration of amifostine at the maximum tolerated dose (2.5 mg/mouse) also showed great efficacy against radio-induced dermatitis. Body weight was unaffected by the development of radiodermatitis or treatment with amifostine thiol gel (Fig. E4A). CPh-1014 application and IV injection of amifostine decreased the number of radio-induced DNA DSBs in keratinocytes (Figs. 3C and 3D). We confirmed the absence of interference of CPh-1014 with the antitumor efficacy of radiotherapy in carcinoma grafted under the skin. Neither the application of amifostine thiol gel nor IV amifostine protected subcutaneous tumors from the antitumor effect of fractionated irradiation (Fig. 3E). Consistent with these observations, gamma-H2AX foci formation in tumors was not altered by these treatments (Figs. 3F and 3G). The application of a larger amount of thermogel dramatically reduced the skin reaction induced by 4 fractions of 12 Gy (Fig. 4A), with only one of 5 mice developing high-grade dermatitis (Fig. E4B). Skin injuries were completely healed by day 24 in 4 out of 5 mice as illustrated in Fig. 4B.

Discussion

There is a need to identify ways to protect against and/or mitigate the acute side effects of IR to spare quality of life and optimize treatment efficacy by avoiding any treatment break due to severe acute toxicity in normal tissues. Amifostine has well-known cytoprotective properties and reduces the incidence of radio-induced acute and late xerostomia when the salivary glands are exposed (18). Amifostine has been considered promising for managing OM in patients with HNC; however, studies have reported conflicting results and weak evidence of efficacy (4,19). The difference may be due to the increased uptake of amifostine by the salivary glands (20,21).

As toxic side effects were associated with the intravenous route of administration of amifostine, alternative routes were investigated. The subcutaneous route of administration showed no clear benefit in terms of patient compliance, efficacy or toxicity (22). The application of cotton pads soaked with amifostine solution in the oral cavity was shown to reduce the mucosal reaction induced by a single 30 Gy dose irradiation in guinea pigs (23). In these conditions, the uptake of amifostine was 25-fold higher in the oral tissues than in the plasma (24). However, amifostine is likely poorly metabolized in the mouth due to the decreased level of alkaline phosphatase (25), and the concentration of amifostine thiol in the oral mucosal tissues was found to be only approximately one-tenth that of the prodrug (24). In line with a poor conversion of amifostine into amifostine thiol in the mouth, it was demonstrated that compared with other organs, the oral mucosa is only marginally protected by amifostine (26). Stokman and colleagues showed that the oral topical administration of amifostine thiol, given as a mouth rinse for 1 minute, to non-small-cell lung cancer patients treated with gemcitabine and epirubicin failed to significantly reduce epirubicin-induced OM, likely due to weak tissue absorption of amifostine thiol (27). These data suggest that mucosal

and epidermal tissues may be challenging and that efficient exposition to amifostine thiol may require high doses and extended duration contact.

We thus aimed to develop and investigate a topical way to administer amifostine that could result in a sustained local delivery of amifostine thiol to the mucosa or skin, with fewer side effects compared with systemic administration. We used a novel thermogel that has mucocutaneous adhesive properties (28,29). As the thermogel is a viscous solution at room temperature, it can easily be applied to the mucosa or skin; subsequent polymerization creates sustained contact between the resulting gel and the epithelium. The application of a thermogel containing amifostine did not show any efficacy against radio-induced mucositis (Fig. 1C), in contrast to the results obtained in guinea pigs. However, in that study, the amifostine doses were more than ten times higher than in our study (23). In contrast, the application of a dose of amifostine thiol as low as 2.5 mg/mouse showed great efficacy against radio-induced injury induced by either a single high dose (20 or 18 Gy) or fractionated irradiation. Interestingly, in the case of fractionated irradiation, which is more clinically relevant, the application of the amifostine thiol thermogel efficiently mitigated both OM and dermatitis, with an efficacy similar to that observed for IV amifostine.

A striking correlation between gamma-HA2X foci induction and radioprotection was observed in our study. The analysis of gamma-H2AX foci was focused on the epithelial cell layer because damage to basal cells is responsible for the first changes in skin/mucosa integrity (30,31). Our results suggest that CPh-1014 indirectly reduces the severity of inflammation by diminishing primary damage. As we observed a reduction in gamma-H2AX foci mainly in the epithelium and to a lesser extent in underlying tissues (tumors and derma), we hypothesized that the penetration and absorption of the compound might be limited to the first layer of cells, which could account for the absence of impact of CPh-1014 on tumor cells. Unfortunately, we could not properly quantify gamma-H2AX foci in deep tissues due to sampling conditions, and further studies are needed to validate this hypothesis.

Amifostine is a selective cytoprotector of normal tissues (14,32). Indeed, there is a greater accumulation of the active metabolite of amifostine in healthy tissues than in tumors following the IV injection of amifostine (14). In our study, we directly administered the active metabolite of amifostine. To exclude whether such treatment could affect radiotherapy antitumor efficacy, we used an orthotopic HNC model and a subcutaneous tumor model. Gels were directly applied to the tumors to mimic the worst conditions, even though the gel would not generally be applied in such close proximity to the tumors in the clinic. The radio-induced tumor growth delay and mouse survival extension were not altered by the application of CPh-1014 in both tumor models.

As previously noted, this new thermogel could be useful for preventing and/or reducing dermatitis and mucositis in patients with breast cancer and HNC, respectively. For HNCs, the current formulation can easily be applied in the oral cavity, further optimization should be made to treat less accessible sites (sites damaged by irradiation of oropharyngeal, hypopharyngeal, or laryngeal cancers for example). Interestingly, as patients with HNC undergoing radiotherapy can experience acute mucositis and acute dermatitis simultaneously (33,34), CPh-1014 could even be applied both to the mucous membranes in the mouth and the skin in the neck region. CPh-1014 might also be beneficial in other indications, for example, in cases of pelvic irradiation (30). CPh-1014 could be studied in radiotherapy with concomitant chemotherapy for further preclinical evaluations. In HNCs, this treatment regimen induces more severe mucositis (cisplatin-based radiochemotherapy) or slightly different forms of mucositis (cetuximab-based radiochemotherapy). Additionally, studying the impact of CPh-1014 on cutaneous reactions (rash) induced by chemotherapy and checking that CPh-1014 does not induce rashes, unlike the subcutaneous injection of amifostine (22), could be interesting.

In 2004, Palifermin, a recombinant human keratinocyte growth factor, became the only drug approved for the treatment of severe OM. However, the use of Palifermin remains restricted to patients with hematologic cancers undergoing high-dose chemotherapy, followed by bone marrow

transplantation in the United States. However, in randomized trials in locally advanced HNC, Palifermin had no effect on narcotic use, patient-reported pain, and chemoradiotherapy compliance, thus compromising its role in the management of HNCs (35,36). Photobiomodulation or low-level laser therapy has emerged as promising supportive care to prevent OM as well as radiodermatitis (37). However, this treatment requires new equipment, trained medical staff and treatment scheduling. Superoxide dismutases, whose activity constitutes an important antioxidant defense in organisms, have been shown to exert significant radioprotective effects (1,38). The small molecule superoxide dismutase mimetic GC4419 has recently entered a phase 3 clinical trial (NCT03689712) after the completion of a positive phase 1b/2a trial. An exploratory analysis showed a trend toward a reduction of chemoradiotherapy-induced oral mucositis in head and neck cancer patients (39,40). At this stage, reported toxicities for GC4419 include nausea/vomiting and facial paresthesia. No published *in vivo* preclinical or clinical study has clearly established the impact of GC4419 on tumor growth. One limitation of GC4419 might result from the inconvenience of the administration route, as a 60-minute intravenous infusion before each fraction of irradiation is currently used to administer GC4419.

Here, we propose an easy-to-use thermogel that patients can apply without the assistance of medical staff. Our study demonstrates that CPh-1014 exerts therapeutic effects against acute mucosal and epidermal lesions induced by IR, probably by decreasing the primary damage to the epithelium. Moreover, this gel has no impact on the antitumor effect of radiotherapy and may have fewer side effects than IV-administered amifostine, as demonstrated in a canine model. Additional preclinical, pharmacodynamic, pharmacokinetic and tolerance studies are now required before the launch of a clinical proof-of-concept trial.

In conclusion, CPh-1014 represents a novel easy-to-implement and affordable strategy to manage radio-induced injuries for patients with HNC and breast cancer that warrants further investigation in clinical settings.

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Figure Captions

Figure 1

Topical application of thermogel containing amifostine thiol mitigates the severity of oral mucositis induced by a single fraction of high-dose irradiation.

A-B, Images illustrating the method of thermogel application: on the internal cheeks (A) and onto the lips (B). C-E, To induce oral mucositis, we irradiated the snouts of C57BL/6 mice at 20 Gy at day 0. Thirty minutes before irradiation, a thermogel containing amifostine (Amif.) or amifostine thiol (Amif. thiol) or a placebo gel was applied (final volume, 50 μ l); alternatively, amifostine was injected intravenously (Amif. IV). An equivalent of 2.5 mg of amifostine thiol per mouse was administered for each condition. Macroscopic Parkins score (C) and body weight (D) were recorded throughout the experiment (mean \pm SEM; n=6). Mice were humanely euthanized upon the presentation of defined ethical endpoints (loss of >20% of the initial weight for two consecutive days, clinical signs of prostration, weakness and suffering). Survival analysis for the different treatment groups (Kaplan-Meier curves, (E)).

Figure 2

Topical application of CPh-1014 reduces DNA damage in the mucosa and the severity of oral mucositis induced by fractionated irradiation (A-C), without protecting tumors (D-H) and has fewer side effects than does IV amifostine (I).

A, Macroscopic Parkins score (mean \pm SEM; n=4-6); mouse snouts were irradiated with 4 consecutive daily fractions of 8 Gy. A thermogel containing amifostine thiol (Amif. thiol) or a placebo gel (placebo) was applied or amifostine was injected intravenously (Amif. IV) before each fraction of irradiation. An

equivalent of 1.25 mg of amifostine thiol per mouse per day was administered for each condition. B-C, The experiment was the same as that in A except that mice were sacrificed 5 h after the first fraction of irradiation. Representative images of immunohistochemical gamma-H2AX staining of mucosal sections; the epithelium is distinguished by a dotted line (scale=50 μm) (B); quantification of gamma-H2AX foci in epithelial cells (mean \pm SD; one-way ANOVA, Tukey post hoc test) (C). D-H, Luciferase-expressing TC1-luc tumor cells were injected into the right inner lip of mice. Four days later, mice were treated with or without topical application of amifostine thiol gel onto the tumor (representative image, (D)) or treated with intravenous injection of amifostine before each fraction of irradiation (4 consecutive daily fractions of 4 Gy). One group was left untreated and nonirradiated (control without gel). Tumor growth was followed by bioluminescence imaging (mean \pm SEM) (E), and survival was analyzed (Kaplan-Meier, (F)) (n=4-6). Analysis of immunohistochemical staining of gamma-H2AX in tumor sections (tumors were excised 5 h after the first fraction of irradiation): representative images (scale=50 μm) (G) and quantification of gamma-H2AX foci in tumor cells (mean \pm SD; ns, not significant) (H). I, Tilt test in dogs. Dogs were tilted to a 90° upright position for one minute before (Tilt baseline), 30 minutes after (Tilt 30 minutes) or 1 h after (Tilt 1 h) intravenous injection of amifostine (10, 30 or 100 mg amifostine/kg) or topical application (oral) of amifostine thiol (equivalent to 100 mg amifostine/kg) or placebo gels. Variations in systolic arterial blood pressure are depicted (mean \pm SEM; two-way ANOVA, Tukey post hoc test).

Figure 3

Topical application of CPh-1014 reduces the severity of radiodermatitis and radio-induced DNA damage without altering the efficacy of IR against tumors.

A, Macroscopic scores (mean \pm SEM; n=5-7); the dorsal skin of mice was irradiated with 4 consecutive daily fractions of 12 Gy to induce a skin reaction. Before each fraction of irradiation, thermogels were applied (100 μl) or amifostine was injected intravenously. The indicated dose corresponds to the

dose per mouse per day expressed as the amifostine thiol equivalent. B, Thermogels were applied onto the dorsal depilated skin of mice, as illustrated in the image. C-D, Immunohistochemical gamma-H2AX staining of dorsal skin harvested 5 h after the first fraction of irradiation: representative pictures of skin sections, the epidermis is indicated by a dotted line (Amif. Thiol 5 mg; Amif. IV 2.5 mg; scale 50 μ m) (C); quantification of gamma-H2AX foci in keratinocytes (mean \pm SD; one-way ANOVA, Tukey post hoc test) (D). E, Mice were injected subcutaneously with Lewis Lung Carcinoma tumor cells. At day 0, mice were treated with or without topical application of amifostine thiol gel onto the tumor or intravenous injection of amifostine 30 minutes before each fraction of irradiation (localized irradiation with 4 fractions of 5.5 Gy). Relative tumor growth (mean \pm SEM; n=6-7). F-G, Immunohistochemical gamma-H2AX staining of tumors harvested 5 h after the first fraction of irradiation: representative images of tumor sections (Amif. Thiol 5 mg; Amif. IV, 2.5 mg) (F); quantification of gamma-H2AX foci in tumor cells (mean \pm SD; ns, not significant) (G).

Figure 4

High-dose amifostine thiol thermogel shows remarkable efficacy against radiodermatitis.

A, Radiodermatitis macroscopic score (mean \pm SEM; n=5-6); a thermogel containing amifostine thiol (10 mg per mouse per day) or a placebo gel was applied (200 μ l) thirty minutes before irradiation of the dorsal skin of mice (4 fractions of 12 Gy from day 0 to day 3). B, Representative images of skin reactions for each group at day 24. The white depilated area corresponds to the irradiated zone.