Published in final edited form as:

Int J Radiat Biol. 2013 July ; 89(7): 485–492. doi:10.3109/09553002.2013.770579.

Radioprotective efficacy and toxicity of a new family of aminothiols

Richard R. Copp¹, Daniel D. Peebles¹, Cheryl M. Soref¹, and William E. Fahl^{1,2}

¹ProCetus BioPharm, Inc., Madison, WI

²McArdle Laboratory for Cancer Research, University of Wisconsin Carbone Cancer Center, Madison, WI, USA

Abstract

Purpose—A family of 17 new nucleophilic-polyamine and aminothiol structures was designed and synthesized to identify new topical or systemic radioprotectors with acceptable mammalian toxicity profiles. Design elements included: (i) Length and charge of the DNA-interacting, alkylamine backbone, (ii) nucleophilicity of the reactive oxygen species (ROS)-scavenging group, and (iii) non-toxic drug concentration achievable in animal tissues.

Materials and methods—Mouse maximum tolerated doses (MTD) were determined by increasing intraperitoneal (IP) doses. To assess radioprotective efficacy, mice received IP 0.5 MTD doses prior to an LD₉₅ radiation dose (8.63 Gy), and survival was monitored. Topically applied aminothiol was also scored for prevention of radiation-induced dermatitis (17.3 Gy to skin).

Results—The most radioprotective aminothiols had 4–6 carbons and 1–2 amines, and unlike amifostine and its analogs, displayed a terminal thiol from an alkyl side chain that projected the thiol away from the DNA major groove into the environment surrounding the DNA. The five carbon, single thiol, alkylamine, PrC-210, conferred 100% survival to an otherwise 100% lethal dose of whole-body radiation and achieved 100% prevention of Grade 2–3 radiation dermatitis. By mass spectrometry analysis, the one aminothiol that was tested formed mixed disulfides with cysteine and glutathione.

Conclusions—Multiple, highly radioprotective, aminothiol structures, with acceptable systemic toxicities, were identified.

Keywords

Radiation dermatitis; ROS-scavenger; glutathione; cysteine; amifostine

© 2013 Informa UK, Ltd.

Correspondence: Dr William E. Fahl, McArdle, Laboratory for Cancer Research, University of Wisconsin Carbone Cancer Center 1400 University Avenue Madison, Wisconsin 53706 USA. Tel: +1 608 262 1275. Fax: +1 608 262 2824. fahl@oncology.wisc.edu.

Declaration of interest

Daniel Peebles, Cheryl Soref and Richard Copp were employees of ProCetus, Inc., and William Fahl is a paid consultant to ProCetus, Inc.

The authors report no other conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Supplementary material available online

Procedures for new compounds

Introduction

Feuerstein and colleagues originally presented a molecular model illustrating how the naturally occurring polyamine, spermine, bound ionically within the major groove of helical B-DNA, and by doing so, distorted and disrupted base pairing to produce single-stranded bubbles at the site (Feuerstein et al. 1990, 1991). For many electrophilic drugs, alkylation of DNA occurs in two steps: The first step requires electrophilic drug intercalation between adjacent base pairs in helical B-DNA, and a rapid second step involves alkylation of the adjacent base. By condensing and altering helical DNA form, pharmacologic levels of polyamine and polyamine analogs would be expected to significantly reduce alkylation of cellular DNA by electrophilic drugs like cisplatin. As shown in the work of Spothem-Maurizot et al. (1995), the in vitro condensation of DNA by polyamine binding also dramatically reduced the number of single-strand breaks seen when DNA was irradiated. A related protective effect associated with tight-binding of diamines or polyamines to DNA is the p53-dependent, induced expression of waf-1/p21, and the G1/S cell-cycle block that results from induced p21 expression (Marton and Pegg 1995, Kramer et al. 1997, Alm et al. 2000). Additionally, the observation that systemic administration of a free thiol confers radioprotection to rodents was first reported in 1949 (Patt et al. 1949).

Combining the ability of amines and polyamines to tightly bind to DNA with the reactive oxygen species (ROS)-scavenging capability of thiols into a single molecule was the focus of the U.S. Antiradiation Drug Development Program between 1959 and 1988. This and similar Soviet programs created more than 4000 new molecules for testing in standardized mouse radioprotection assays (Weiss 1997). The best known molecule to emerge from this program, amifostine, is a linear five-carbon alkylamine with two amines and a single, terminal thiol that is phosphate capped (Grdina et al. 1995). Its ability to radioprotect requires enzymatic cleavage of the phosphate to expose the ROS-scavenging thiol.

In this report we describe the design and synthesis of a family of 17 nucleophilic polyamine and aminothiol analogs. In an effort to design and build new pharmacologically active radioprotectors, three design elements were systematically varied: (i) The length and collective positive charge of amine groups and thus of the DNA-interacting alkylamine backbone, (ii) the nucleophilicity of the ROS-scavenging group, and (iii) the non-toxic concentration of drug achievable in animal tissues to confer both DNA-binding and ROS-scavenging efficacy. Testing of the new molecules showed that small aminiothiols with 4–6 total carbons and a single, displayed thiol were pharmacologically the most effective radioprotectors, particularly when compared to larger, thiol-displaying polyamines. The results of this report, consistent with the original design concepts, show that the highly radioprotective PrC-210, PrC-211 and PrC-252 aminiothiols identified in this report actually do contain both a flexible, alkylamine backbone to interact with DNA and a short alkyl side chain with a terminal thiol that is *projected away from the backbone* for ROS or electrophile scavenging in the DNA environs.

Materials and methods

Chemicals

PrC-210, PrC-211 and PrC-240 were synthesized as previously described (Fahl et al. 2007, Copp et al. 2011). Reduced forms of cysteine and glutathione were purchased from Sigma Chemical (St Louis, MO, USA). Amifostine was purchased at the University of Wisconsin Hospitals Pharmacy (Madison, WI, USA). PrC-301, PrC-303, PrC-304, and PrC-307 were synthesized as described in the Supplementary Information, available online at <http://informahealthcare.com/abs/doi/10.3109/09553002.2013.770579>.

DNA precipitation and cell growth inhibition

Sonicated calf thymus DNA (40 µg/ml in 10 mM cacodylate buffer, pH 7.4) was vortexed and incubated at room temperature for 20 min with increasing concentrations of polyamine or aminothiols in wells of a 96-well plate, and following 15 min centrifugation of plates at full speed in an IEC benchtop centrifuge, aliquots of supernate were transferred to a parallel 96-well quartz plate and the A260 of each supernate was recorded as described by Saminathan et al. (1999). Diploid human fibroblasts (Stevens et al. 1988) were plated in Dulbecco's Modified MEM containing 20% fetal bovine serum on 96-well plates, and increasing concentrations of polyamine or aminothiols were added to medium. After 3–4 days growth, cells were harvested and counted to assess growth inhibition.

Animals

Sprague-Dawley rats (female, 35–40 gm b.w.) for radiation dermatitis studies were from Harlan (Indianapolis, IN, USA). Rats were maintained on 12-h light/dark cycle and provided *ad libitum* water and Harlan 8604 lab chow. Prior to irradiation, rats were anesthetized with isoflurane and their backs were clipped using an Oster clipper. Shortly before irradiation, in order to stably position rats on the lead plate within the irradiator, rats received IP injections of sodium pentobarbital (30 µg/gm b.w.). Following a single irradiation to rats' backs, rats were returned to the animal facility. Thirteen days post-irradiation, rats were anesthetized using isoflurane and photographed to score severity of radiation dermatitis in the irradiated area.

ICR mice (female, 20–25 g) were used for maximum tolerated dose (MTD) systemic toxicity (% survival) studies of each aminothiol analog (Harlan). The number of mice per drug treatment dose group was as low as five mice for an aminothiol dose expected to be 100% lethal, or as high as 16 mice for an aminothiol dose expected to be only 10–20% lethal. Mice were maintained on 12-h light/dark cycle and provided *ad libitum* water and Harlan 5305 lab chow. Animal procedures were approved by the University of Wisconsin (Protocol # M0476).

Irradiation and aminothiol treatment

Rats and mice were irradiated in a J. L. Shepherd 137Cs irradiator. To induce radiation dermatitis, two anesthetized rats were positioned on a lead plate such that two 1.5 × 3.0 cm windows in the plate allowed irradiation of the same areas on the rats' backs (Peebles et al. 2012). The standard radiation exposure of 17.3 Gy was calibrated with thermoluminescent

dosimeters and administered at a rate of 3.37 Gy/min. For topical aminothiols treatments, aminothiol analog was dissolved in a delivery vehicle of ethanol:propylene glycol:water (ranging from 50:30:20 to 0:50:50, vol:vol:vol) and aliquots of 30 μ l, 25 μ l, 25 μ l and 25 μ l were applied and uniformly spread over the 1.5×3.0 cm area to be irradiated (marked with ink dots) at -2 h, -1 h, -30 min and -10 min. Rats were irradiated at 0 min. As an alternative to topical application, some rats received IP injections of PrC-210 dissolved in water (pH 5.5) 30 min before irradiation. Severity of radiation dermatitis in rats was scored using the same criteria used to score human radiation dermatitis (i.e., http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae3.pdf).

For mouse radiation survival assays, unanesthetized mice were placed in small plastic containers centered in the irradiator and received 8.63 (LD95)–8.75 (LD100) Gy of whole-body radiation at a dose rate of 2.1 Gy/min. Radiation dose was calibrated using thermoluminescent dosimeters. Mice received either IP water or IP aminothiol analog dissolved in water 30 min prior to irradiation. Mice were observed for 30–50 days post irradiation. There were 16–24 mice in each aminothiol treatment group. Chi square analysis with the Gehan-Breslow-Wilcoxon Test (Graphpad software) was used to determine significance of differences between mouse groups.

Results

A family of nucleophilic polyamine and aminothiol radioprotectors

In preparing a new family of nucleophilic polyamines and smaller aminothiols, we expanded upon existing examples in the polyamine and aminothiol literature. The radioprotector molecules described here incorporated both the cell growth inhibition seen with polyamines and shorter alkylamines and the ROS/electrophile scavenging function seen with a range of nucleophiles (Grdina et al. 1995, Kramer et al. 1997). Three parameters in the design matrix and examples of synthesized molecules are shown in Figure 1. These parameters included: (i) The number of positive charges per molecule ‘backbone’, (ii) scavenger nucleophile strength, and (iii) anticipated concentration of nucleophile in at-risk tissues. Representative nucleophilic polyamine and aminothiol structures from different points within the three-dimensional design matrix are shown in Figure 1. Three molecules were also synthesized to test the effect of capping the thiol with either a methyl group (PrC-301) or with an acetyl group (PrC-303, PrC-304). The 17 nucleophilic polyamine and aminothiol analogs that have been synthesized, several of which have been tested in radioprotection assays, are shown in Figure 2.

To begin characterization of the newly synthesized nucleophilic polyamines we titrated them into cell culture wells containing normal human fibroblasts and found that the longer the molecule the more potent the growth inhibition (Figure 3A). We assumed the growth inhibition at very low concentrations of PrC-113 was a function of very tight interaction of the 10 ionizable amine groups in the polyamine arms with DNA. In the data shown in Figure 3B, we saw that as we titrated these same polyamines into 96 wells containing only dissolved calf thymus DNA in buffer, the polyamine:DNA complexes would precipitate when saturated, and saturation was achieved at much lower concentrations for the longer arm polyamines. We expected that these large molecules would be growth inhibitory when added

to cells, and would thus not be useful vehicles for delivering significant thiol scavenger to at-risk cells in animals. PrC-252 and PrC-113 or PrC-117, at opposite corners of the design matrix box in Figure 1, illustrate this point. This realization is the reason we chose to generate smaller aminothiols, with PrC-252 representing the smallest molecule we synthesized.

Aminothiol toxicity spectrum

Each aminothiol analog was initially tested for systemic toxicity by intraperitoneal (IP) injection into mice to establish its Maximum Tolerated Dose (MTD, Table I). Based upon earlier toxicity studies in the field, and to minimize the number of mice used for systemic toxicity assessments, the MTD was defined as the dose that was lethal to 10% of the mice, or LD10 dose (Weiss 1997). Though this seems counterintuitive, statistically, it would take about 10 times as many mice to accurately quantify the LD1 dose than to accurately quantify the LD10 dose. Thus, using the LD10 as the MTD has become common and drug doses for animals are commonly expressed as a fraction of the MTD, e.g., 0.5 MTD.

For mice given a lethal dose of any of the PrC-aminothiols in Figure 2, the death mechanism appeared to be the same (generalized muscle spasm that if not subsided, resulted in death in a few minutes). High dose amifostine toxicity and death looked similar though the nature of the muscle spasm was somewhat different. A previous publication showed that the PrC-210 aminothiol, while as good or better a radioprotector as amifostine, had neither of the limiting toxicities of amifostine (Soref et al. 2012). As shown in Table I, MTD doses for the PrC-210 aminothiol were also established in mice and rats by subcutaneous and oral delivery routes. Some PrC-210 efficacy data for radiodermatitis prevention and survival were previously published (Peebles et al. 2012) and are provided here for comparison to the other aminothiol analogs described here for the first time.

Aminothiol-conferred radioprotection

We optimized two dosing parameters before we tested the ability of a single dose of each aminothiol analog to confer systemic protection against a single dose of whole body radiation. Figure 4 shows that a single IP dose of PrC-210 (252 $\mu\text{g/gm}$ b.w.) administered between 7.5 and 60 min before whole-body radiation conferred highly significant radioprotection (P values of < 0.0001 – 0.001) versus the sham-treated 0 min control (5% survival). Figure 5 shows that a 0.5 MTD IP dose (252 $\mu\text{g/gm}$ b.w.) of PrC-210 conferred 100% survival against the 8.63 Gy dose of whole-body radiation, and that there was a clear dose-dependent loss of radioprotection with decreasing doses of PrC-210. As shown in Figure 5, there was a 5% survival in mice that received an IP vehicle injection followed 30 min later by an 8.63 Gy radiation dose. With the Figure 5 data, we went back and scored the number of days post-irradiation to the day at which half of the mice that would eventually die in each of the treatment groups had died. In the 0 $\mu\text{g/gm}$ group, half of the mice died by day 12; in the 211 $\mu\text{g/gm}$ group, half (one mouse) died on day 16; the other groups were between 12 and 16 days. From these data we decided for screening purposes to compare radioprotection conferred by 0.5 MTD doses of each aminothiol analog, and to administer the IP doses 30 min before irradiation.

Table I, columns 6 and 7, provide a summary of the *systemic* radioprotection conferred by each of the seven tested aminothiols. The notable results included: (i) PrC-210 was the most potent radioprotector conferring 100% protection at the 0.5 MTD dose, (ii) amifostine was nearly equal in its radioprotective efficacy, (iii) PrC-211, which is the PrC-210 molecule with exposed, terminal amines, was about 50% as potent as PrC-210, (iv) oral doses of PrC-210 were 100% radioprotective in both mouse and rat radiation assays, (v) the oxidized, disulfide form of PrC-210 was ineffective as a radioprotector, and (vi) both methyl (PrC-301) and acetyl (PrC-304) capping of the terminal sulfhydryl group for possible activation of the prodrug forms by either a methylase or acetylase, respectively, abolished all radioprotection.

Table II, column 3, provides additional results showing that when PrC-210, PrC-252 and PrC-240 were administered *systemically* by IP injection, they were also 100% effective in preventing radiation dermatitis in rats that received 17.3 Gy of radiation to a 1.5×3.0 cm patch of skin on their backs. PrC-210 and PrC-211 could also provide 100% prevention of Grade 2–3 radiation dermatitis when applied *topically* prior to skin irradiation (Figure 6). Interestingly, the small aminothiol, PrC-252, that completely prevented radiation dermatitis when given IP, was only 68% effective in preventing radiation dermatitis when applied *topically* to skin prior to skin irradiation. In order to test a smaller version of PrC-210 for efficacy, PrC-211 was synthesized without terminal methyl groups on the two backbone amines (Figure 2). Though radioprotective, it was half as potent as PrC-210 (Figure 6).

The data in Figure 6 also demonstrate the profound impact that inclusion of ethanol has upon efficient topical delivery of these aminothiols to at-risk cells within the 12 epidermis. Though 100% efficacy for PrC-210 is seen in a delivery vehicle without ethanol (0:50:50; ethanol:propylene glycol:water) the potency of PrC-210 increases 3.8-fold ($1400 \div 370$ mM) when ethanol assumes a large percentage (50:30:20) of the topical delivery vehicle. These small aminothiols retain solubility within a 50% ethanol vehicle well above 1 M concentration. A preliminary version of this data has also been previously published (Peebles et al. 2012). To better understand why four topical applications of PrC-210 to rat skin were required prior to irradiation to fully prevent radiation dermatitis, we considered possible ways that an aminothiol might be consumed or sequestered when administered by any route to an animal. Table III illustrates that in a test tube, the PrC-210 aminothiol is fully capable of reacting with the two principal physiologic thiols, cysteine and glutathione to form mixed disulfides that are very likely to be inactive radioprotectors because the PrC-210 disulfide itself was found to be an ineffective radioprotector (Figure 2, Table I).

Discussion

By chemically combining flexible, alkylamine, DNA-interacting domains with ROS-scavenging thiols that were projected away from the alkylamine backbone by an alkyl side chain, a new, small family of nucleophilic polyamines and aminothiols was created. One family member, PrC-210, an aminothiol with a molecular weight of 148, was shown to be a highly effective radioprotector when administered topically to prevent radiation dermatitis or systemically to confer 100% survival to an otherwise 100% lethal dose of whole-body radiation. In the library of 17 new molecules, smaller 4–6 carbon molecules with 1–2 amine

groups and a single thiol were far more effective radioprotectors, particularly when compared to larger, thiolated polyamines. Mass spectrometry studies showed the formation of mixed disulfides when PrC-210 was incubated with the physiologic thiols cysteine or glutathione. The need to first saturate the tissue 'sinks' could account for the need to administer multiple applications of the PrC-210 aminothiols to the skin prior to irradiation in order to achieve 100% prevention of radiation dermatitis. Previous studies of spermine and spermidine catabolism and design of synthetic polyamine analogs like DENSPM to defeat or modify catabolism of the drug molecule showed that terminal amines on alkylamine backbones were targets for acetylation by the cellular enzyme spermidine/spermine-N(1)-acetyltransferase (SSAT) (Bergeron et al. 2001, Yarlett et al. 2007, Pegg 2008). This literature provides the most likely explanation of the greater potency of PrC-210 with methylated terminal amines versus PrC-211 with exposed terminal amines (Figure 2).

Replacing the two remaining amine protons in PrC-210 with methyl groups to yield PrC-307 resulted in a much more hydrophobic and toxic molecule with a 2.5-fold lower IP MTD (166 $\mu\text{g/gm}$ vs. 422 $\mu\text{g/gm}$) and as a result, almost no radioprotective efficacy. Only very low molar concentrations of PrC-307, and hence thiol (i.e., $\text{MTD} = 166 \mu\text{g/gm} \times 0.5 = 83 \mu\text{g/gm}$) could be used without toxicity to the mouse.

During the preliminary rounds of planning and synthesizing thiolated or nucleophilic polyamines with a weaker N-ethyl nucleophile (PrC-117 vs. PrC-113) we found that the long polyamines provided potent (nM range) growth suppression of normal, diploid human fibroblasts (Figure 3). When we saw this, we speculated that the long polyamines, with 24–32 carbons and 8–10 amines total (e.g., PrC-112 and PrC-113), had very tight ionic interaction with DNA. This speculation was supported by the results of the DNA precipitation assay (Figure 3B).

From the PrC-113 result in Figure 3, it was apparent that only 100–200 nM concentrations of PrC-113 could be introduced into growing human cells without growth suppression. If the side chain amine nucleophile of PrC-113 was exchanged for a side chain thiol, to now yield the PrC-117 molecule (Figure 2), the 32 carbon backbone and growth suppression characteristic would be unchanged. When we tested this point, by increasing the PrC-117 concentration to low mM concentrations in order to achieve a molar excess of thiol compared to the ROS that would be generated when tissue or cells were irradiated, we saw massive drug-induced growth arrest long before we could achieve target concentrations of thiol. This design problem was corrected in a next generation of aminothiol molecules, whereby shortening the alkylamine backbone and diminishing its DNA affinity, we were then able to use mM concentrations of the DNA-interacting backbones with now mM concentrations of attached thiol. This provided a large molar excess of thiol over radiation-induced ROS with little associated growth suppression and cell toxicity.

A significant aim in preparing this library of molecules was to identify molecules that could be applied topically to skin and mucosa before radiotherapy exposure to prevent radiation dermatitis or mucositis. By applying the aminothiol drug topically, one essentially removes any concerns about systemic, aminothiol-conferred protection of tumor cells during radiotherapy. A second aim in preparing and testing this library of molecules was to identify

candidates that could be administered systemically without eliciting either the nausea/emesis or hypotension/fainting toxicities that are commonly encountered when amifostine is administered to human subjects (Rose 1996). In a separate, initial report we showed that the PrC-210 aminothiols elicited neither the emesis nor hypotension effects in preclinical animal models, both of which perfectly re-created the emesis and hypotension responses when amifostine was administered at the ferret and rat equivalents to the 0.5 MTD amifostine dose that is commonly used in humans (Soref et al. 2012). Screening of the systemically active PrC-252 aminothiol in these same nausea and fainting toxicity models should be done.

In the studies so far, the PrC-210 aminothiol: (i) is shown to be the most effective radioprotector in this library, and is as effective as amifostine with the same Dose Reduction Factor (DRF) of 1.6 (Peebles et al. 2012), (ii) lacks the nausea/emesis and hypotension/fainting side-effects of amifostine in appropriate preclinical models, and (iii) has 100% efficacy in preventing radiation dermatitis when applied topically to skin. Based on these results we believe it is a logical candidate for clinical development as a topical and systemic radioprotector in healthy humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grant #CA-22484 from NCI as well as research funds from ProCetus BioPharm, Inc.

References

- Alm K, Berntsson PS, Kramer DL, Porter CW, Oredsson SM. Treatment of cells with the polyamine analog N, N11-diethylnorspermine retards S phase progression within one cell cycle. *European Journal of Biochemistry*. 2000; 267:4157–4164. [PubMed: 10866819]
- Bergeron RJ, Wiegand J, McManis JS, Weimar JR, Smith RE, Algee SE, Fannin TL, Slusher MA, Snyder PS. Polyamine analogue antidiarrheals: A structure-activity study. *Journal of Medicinal Chemistry*. 2001; 44:232–244. [PubMed: 11170633]
- Copp RR, Peebles DD, Fahl WE. Synthesis and growth regulatory activity of a prototype member of a new family of aminothiol radioprotectors. *Bioorganic and Medicinal Chemistry Letters*. 2011; 21:7426–7430. [PubMed: 22041059]
- Fahl, WE.; Peebles, DD.; Copp, RR. Amino thiol compounds and compositions for use in conjunction with cancer therapy. U.S. patent 7,314,959. 2007. See <http://patft.uspto.gov>
- Feuerstein BG, Pattabiraman N, Marton LJ. Molecular mechanics of the interactions of spermine with DNA:DNA bending as a result of ligand binding. *Nucleic Acids Research*. 1990; 18:1271–1282. [PubMed: 2320418]
- Feuerstein BG, Williams LD, Basu HS, Marton LJ. Implications and concepts of polyamine-nucleic acid interactions. *Journal of Cellular Biochemistry*. 1991; 46:37–47. [PubMed: 1874798]
- Grdina DJ, Shigematsu N, Dale P, Newton GL, Aguilera AJ, Fahey RC. Thiol and disulfide metabolites of the radiation protector and potential chemopreventive agent WR-2721 are linked to both its anti-cytotoxic and anti-mutagenic mechanisms of action. *Carcinogenesis*. 1995; 16:767–774. [PubMed: 7728953]
- Kramer DL, Fogel-Petrovic M, Diegelman P, Cooley JM, Bernacki RJ, McManis JS, Bergeron RJ, Porter CW. Effects of novel spermine analogues on cell cycle progression and apoptosis in MALME-3M human melanoma cells. *Cancer Research*. 1997; 57:5521–5527. [PubMed: 9407962]

- Marton LJ, Pegg AE. Polyamines as targets for therapeutic intervention. *Annual Review of Pharmacology and Toxicology*. 1995; 35:55–91.
- Patt HM, Tyree EB, Straube RL, Smith DE. Cysteine protection against X irradiation. *Science*. 1949; 110:213–214. [PubMed: 17811258]
- Peebles DD, Soref CM, Copp RR, Fahl WE. ROS-scavenger and radioprotective efficacy of the new PrC-210 aminothiols. *Radiation Research*. 2012; 178:57–68. [PubMed: 22702647]
- Pegg AE. Spermidine/spermine-N(1)-acetyltransferase: A key metabolic regulator. *American Journal of Physiology, Endocrinology and Metabolism*. 2008; 294:995–1010.
- Rose PG. Amifostine cytoprotection with chemotherapy for advanced ovarian carcinoma. *Seminars in Oncology*. 1996; 23(4 Suppl.):83–89. [PubMed: 8783673]
- Saminathan M, Antony T, Shirahata A, Sigal LH, Thomas T, Thomas TJ. Ionic and structural specificity effects of natural and synthetic polyamines on the aggregation and resolubilization of single-, double-, and triple-stranded DNA. *Biochemistry*. 1999; 38:3821–3830. [PubMed: 10090772]
- Soref CM, Hacker TA, Fahl WE. A new aminothiol radioprotector free of nausea and hypotension side effects at high radioprotective doses. *International Journal of Radiation Oncology, Biology and Physics*. 2012; 82:701–707.
- Spotheim-Maurizot M, Ruiz S, Sabattier R, Charlier M. Radioprotection of DNA by polyamines. *International Journal of Radiation Biology*. 1995; 68:571–577. [PubMed: 7490507]
- Stevens CW, Brondyk WH, Burgess JA, Manoharan TH, Haene BG, Fahl WE. Partially transformed, anchorage-independent human diploid fibroblasts result from overexpression of the c-sis oncogene: Mitogenic activity of an apparent monomeric Platelet-Derived Growth Factor 2 species. *Molecular and Cellular Biology*. 1988; 85:2089–2096. [PubMed: 3290648]
- Weiss JF. Pharmacologic approaches to protection against radiation-induced lethality and other damage. *Envir Hlth Perspect*. 1997; 105:1473–1478.
- Yarlett N, Wu G, Waters WR, Harp JA, Wannemuehler MJ, Morada M, Athanasopoulos D, Martinez MP, Upton SJ, Marton LJ, Frydman BJ. *Cryptosporidium parvum* spermidine/spermine N1-acetyltransferase exhibits different characteristics from the host enzyme. *Molecular Biochemistry and Parasitology*. 2007; 152:170–180.

Figure 1.

The matrix of three parameters that were used to design each of the 17 nucleophilic polyamine and aminothiols molecules synthesized. Examples of two nucleophilic polyamines and two subsequently synthesized aminothiols are shown. All synthesized structures are shown in Figure 2. This Figure is reproduced in color in the online version of *International Journal of Radiation Biology*.

Figure 2.
Structures of synthesized aminothiols and nucleophilic polyamines.

Figure 3.

(A) Inhibited growth of normal human fibroblasts in culture by nucleophilic polyamines. Molecules were added at indicated concentrations to log phase, growing cultures of diploid human fibroblasts in 96-well plates, and cell numbers were measured four days later. (B) Polyamines were added at the indicated concentrations to 96-well plates containing buffer with a standard amount of dissolved, high molecular weight, calf thymus DNA (40 $\mu\text{g}/\text{ml}$ in 10 mM cacodylate buffer, pH 7.4). After incubation at room temp for 10 min, plates were spun for 20 min to pellet any precipitated material. The OD260 for aliquots of the well supernatants was then measured.

Figure 4.

Survival in groups of mice given single IP injections of PrC-210 (252 $\mu\text{g/gm b.w.}$) at the indicated times prior to a single dose of whole-body radiation (8.63 Gy). There were 24 mice in each treatment group. Chi square analysis indicated that all treatment group % Survival scores were significantly different than the 0 min control group with P values ranging between <0.0001 and 0.01 (7.5 min).

Figure 5.

Survival in groups of mice given single IP injections of PrC-210 at the indicated doses 30 min prior to a single dose of whole-body radiation (8.63 Gy). There were 24 mice in each treatment group. Chi square analysis gave *P* values of <0.0001 for doses of 150 µg/gm or greater.

Figure 6.

Relative potencies of topically applied PrC-210 and PrC-211 aminothiols in preventing radiation dermatitis in rat model. There were four rats in each treatment group. Addition of 50% ethanol to topical delivery vehicle increased PrC-210 topical potency by 3.8-fold, i.e., 1400 mM/370 mM.

Table I

Systemic toxicity and radioprotection against whole-body radiation conferred by new aminothiols.

Molecule name	MW	(1) Mouse IP MTD	(2) Rat IP MTD	(3) Mouse oral MTD (µg/g b.w.)	(4) Rat oral MTD	(5) Mouse SC MTD	(6) Mouse % survival ^e	(7) Rat % survival ^f
PrC-210	148	504 ^a 422 ^b	485	1780	1974	431	100% (at IP 0.5 MTD: 252 µg/g) 98% (at IP 0.5 MTD: 211 µg/g) 100% (at ORAL 0.87 MTD: 1550 µg/g) ^g	100% (at oral 0.5 MTD: 900 µg/g)
PrC-210 Disulfide	294	155	-	-	-	-	37% (at IP 0.9 MTD: 140 µg/g) <5% (at IP 0.5 MTD: 78 µg/g)	-
PrC-211	120	475	-	-	-	-	100% (at IP 0.9 MTD: 427 µg/g) 47% (at IP 0.5 MTD: 238 µg/g) 37% (at IP 0.25 MTD: 118 µg/g)	-
PrC-301	162	625	-	-	-	-	<5% (at IP 0.5 MTD: 312 µg/g)	-
PrC-303	274	1860	-	-	-	-	ND ^h	-
PrC-304	188	1340	-	-	-	-	<5% (at IP 0.5 MTD: 670 µg/g)	-
PrC-307	176	166	-	-	-	-	12% (at IP 0.5 MTD: 83 µg/g)	-
Amifostine	214	800 ^c 750 ^d	-	-	-	-	98% (at IP 0.5 MTD: 400 µg/g)	-

^a Determined using probit analysis of survival data;^b Determined using best fit analysis of survival data;^c Published value;^d Experimentally determined;^e 8.63 Gy whole-body radiation;^f 9.5 Gy whole-body radiation;^g This dose was the only one tested;^h not determined.

Table II

Topical (or IP) aminothiols prevention of radiation-dermatitis.

Molecule name	MW	(1) Drug dose	(2) Drug application route	<i>n</i>	(3) Radiation dermatitis (% clear skin ^a)
Vehicle	-	-	Topical	12	0%
PrC-210	148	370 mM (50:30:20) ^b	Topical	10	100
		1200 mM (0:90:10) ^b		4	100
		200 µg/g b.w.	IP	2	100
PrC-211	120	1400 mM (50:30:20) ^b	Topical	3	55
		2200 mM (0:90:10) ^b			100
PrC-252	105	Expt. 1 300 mM	Topical	3	10
		600 mM		3	45
		900 mM		3	57
		1800 mM		3	68
		Expt. 2 450 mM	Topical	3	70
		180 µg/g b.w.	IP	2	100
PrC-240	188	150 µg/g b.w.	IP	2	100
Amifostine	214	100 mM	Topical	4	0

^aPercentage of irradiated skin that is clear of any scab material 13 days following 17.3 Gy radiation dose to a 1.5 × 3.0 cm rectangle on rat's dorsal back;

^b(ethanol:propylene glycol:water).

Table III

Reactivity of PrC-210 thiol with cysteine-thiol or glutathione-thiol.

Molecules in incubation	Mass spectrometry peaks (pre-reaction) m/z, amu	Mass spectrometry peaks (post-reaction ^a) m/z, amu
PrC-210 thiol	149.1: PrC-210 thiol	149.1: PrC-210 thiol
PrC-210 thiol ⁺	149.1: PrC-210 thiol	149.1
Cysteine thiol	122.0: Cysteine thiol	122.0 268.1 PrC-210-Cysteine disulfide, 1 charge 134.5 PrC-210-Cysteine disulfide, 2 charges
PrC-210 thiol ⁺	149.1: PrC-210 thiol	149.1
GSH ^b thiol	308.1: GSH thiol	346.1:
	346.1: Potassium ⁺ -GSH	456.4: PrC-210-GSH disulfide 615.5: GSH disulfide

^a Reaction was in water, pH 5.5, 24 h at room temperature in the dark;^b Glutathione.