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Original Contribution

Anti-cancer effect of pharmacologic ascorbate and its interaction with supplementary parenteral glutathione in preclinical cancer models

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ABSTRACT

Two popular complementary, alternative, and integrative medicine therapies, high-dose intravenous ascorbic acid (AA) and intravenous glutathione (GSH), are often coadministered to cancer patients with unclear efficacy and drug–drug interaction. In this study we provide the first survey evidence for clinical use of iv GSH with iv AA. To address questions of efficacy and drug–drug interaction, we tested 10 cancer cell lines with AA, GSH, and their combination. The results showed that pharmacologic AA induced cytotoxicity in all tested cancer cells, with IC_{50} less than 4 mM, a concentration easily achievable in humans. GSH reduced cytotoxicity by 10–95% by attenuating AA-induced H_2O_2 production. Treatment in mouse pancreatic cancer xenografts showed that intraperitoneal AA at 4 g/kg daily reduced tumor volume by 42%. Addition of intraperitoneal GSH inhibited the AA-induced tumor volume reduction. Although all treatments (AA, GSH, and AA + GSH) improved survival rate, AA + GSH inhibited the cytotoxic effect of AA alone and failed to provide further survival benefit. These data confirm the pro-oxidative anti-cancer mechanism of pharmacologic AA and suggest that AA and GSH administered together provide no additional benefit compared with AA alone. There is an antagonism between ascorbate and glutathione in treating cancer, and therefore iv AA and iv GSH should not be coadministered to cancer patients on the same day.

Intravenous (iv) vitamin C or ascorbic acid (AA) has been used in cancer treatment for many years [1–8]. Until recently the extent of iv AA use in complementary, alternative, and integrative medicine (CAIM) practices was unknown [9]. From the reported survey data, it is now known that iv AA is in wide use and doses up to 200 g per infusion are used to treat a variety of conditions, including cancer. Intravenous AA infusions are remarkably safe, with very few adverse events reported in approximately 20,000 patients in 2 years' time.

Intravenous AA has a very different mechanism of action compared to its oral use [10]. Mechanistic studies show that intravenous, but not oral, AA produces pharmacologic plasma concentrations that result in the production of hydrogen peroxide (H_2O_2) in the extracellular space [11–13] and then result in neoplastic cytotoxicity [12–15]. These plasma concentrations of ascorbate are attainable in human clinical trials [12,16]. Taken as a whole, iv AA is a prodrug for H_2O_2 production in the extracellular space, resulting in targeted neoplastic cell death, and as such is a pro-oxidative therapy.

Intravenous glutathione (GSH) is another CAIM therapy known anecdotally to be in wide use. Unfortunately the extent and pattern of iv GSH use by survey, unlike iv AA, have never been determined. There are animal studies, case reports, and small clinical trials using supraphysiologic doses of parenteral GSH in a variety of disorders, including cancer, but none addressed the actual numbers of infusions of GSH given to patients each year [17–24].

GSH is a tripeptide of glutamate, cysteine, and glycine that actively scavenges reactive oxygen species such as superoxide, hydroxyl radical, and notably, H_2O_2[25–29]. GSH has been shown to work synergistically with AA to remove and quench reactive oxygen species at physiologic concentrations of AA (<200 μM) that are often achieved with oral supplementation [30–32]. However, at supraphysiologic concentrations obtained by iv administration, at which AA acts as a pro-oxidant inducing the production of H_2O_2, the synergistic relationship may not hold true. In fact if GSH's role is to clear H_2O_2, the therapeutic benefit of iv AA may be nullified by coadministration of iv GSH.

Anecdotal evidence shows that CAIM practitioners are concurrently administering iv AA with iv GSH during the same clinic visit, but the extent and frequency are not known. To assess the actual use of iv GSH and the concomitant use of iv GSH and iv AA in patients, we

Abbreviations: AA or AscH, ascorbic acid or ascorbate; $Asc^{\bullet}$, ascorbate radical; CAIM, complementary, alternative, and integrative medicine; DHA, dehydroascorbic acid; GSH, glutathione; IC_{50}, 50% inhibition concentration; ip, intraperitoneal; iv, intravenous; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; $O_2^{\bullet-}$, superoxide radical; ROS, reactive oxygen species.

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conducted a survey among CAIM practitioners. As the plausibility of AA as a cancer treatment increases and mounting research ties this to AA's pro-oxidant activity, it is imperative to study whether the combination of GSH as an antioxidant benefits or antagonizes the AA effect. Here we investigate the effect in cancer cell lines and in an animal model when combining the two treatments. We hypothesize that there may be interference in the antineoplastic effect by increased clearance of H_2O_2 when iv AA is administered concurrent with iv GSH.

Methods

Survey methods

The study was reviewed by the Human Subjects Committee/Institutional Review Board at the University of Kansas Medical Center and categorized as an exempt study. Survey forms were distributed to practitioners attending an annual CAIM conference in April 2011. Surveys were distributed and collected on 3 successive days. Participants were asked whether they administer high-dose iv vitamin C or iv GSH and, if they use both, whether they administer the two agents concurrently in the same patient. Participants were also asked to detail the use of iv GSH by answering specific questions about dosing amounts and frequency of dosing (see Survey S1 for the survey form used).

Cells and reagents

Human lung cancer cell line A549, breast cancer cell line MCF-7, and mouse melanoma cell line B16 were purchased from the American Type Culture Collection (Manassas, VA, USA). Human pancreatic cancer cell line HPAF-II was kindly donated by Dr. R. Puri, FDA/CBER (Bethesda, MD, USA), MIA PaCa-2 by Dr. J. Cullen, University of Iowa (Iowa City, IA, USA), PANC-1 by Dr. M. Brownstein, J. Craig Venter Institute (Rockville, MD, USA), murine pancreatic cancer line PAN-02 by Dr. A. Sandler, Children's Hospital Medical Center (Washington, DC, USA), human ovarian cancer cell line OvCar5 by Dr. P. Eck, NIDDK/NIH (Bethesda, MD, USA), and prostate cancer cell line PC-3 by Dr. B. Li, University of Kansas Medical Center (Kansas City, KS, USA). A human cervical cancer cell line transfected with the fluorescent hydrogen peroxide sensor HyPer (HeLa-HyPer-Cyto) was donated by Dr. M. Levine, NIDDK/NIH, and cells were maintained in DMEM supplemented with 10% fetal bovine serum and 100 μg/ml G418. Cell lines were independently authenticated where applicable (RADIL, Columbia, MO, USA). Glutathione injectables were purchased from Wellness Pharmacy (Birmingham, AL, USA) and stored at 4 °C. Ascorbate (Sigma, St. Louis, MO, USA) was prepared as 1 M stock solutions in sterile water, with sodium hydroxide added drop-wise to adjust the pH to 7.0. Aliquots stored frozen at −80 °C were thawed for single use.

Cell viability assay

Cells (1×10^4) in exponential growth phase were cultured at 37 °C in 5% CO_2/95% air in the recommended growth medium containing 10% fetal bovine serum and exposed to serial dilutions of AA and/or GSH for 48 h. Because of oxidation and ascorbate disappearance over the incubation time, the actual exposure time is relevant to clinical iv AA use, in which pharmacological AA concentrations typically persist for 6–8 h [9,12,16]. Before being assessed for relative proliferation, cells were washed with PBS and changed into fresh medium. Relative viability was detected by colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, based on the ability of living, but not dead, cells to reduce MTT to formazan [33,34]. Half-inhibitory concentration (IC_{50}) was defined as the concentration of drug that inhibited cell growth by 50% relative to untreated control. Cells did not reach plateau phase during the 48-h experiment period.

Assessment of H_2O_2 in HeLa-HyPer-Cyto cells

HyPer is the first fully genetically encoded fluorescent sensor capable of specifically detecting intracellular H_2O_2[35,36]. HeLa-HyPer-Cyto cells express HyPer protein in the cell cytoplasm. Cells at 80% confluence were exposed to AA (10 mM), GSH (640 μM), or the combination of AA and GSH. At 2 h of exposure, imaging for green fluorescence (500/528 nm) was performed with an Olympus IX71 fluorescence microscope and DP71 camera (Olympus, Center Valley, PA, USA). Fluorescence intensity was detected with a Synergy 2 fluorescence and luminescence plate reader (Biotek, Winooski, VT, USA) at excitation of 420 ± 9 or 500 ± 9 nm and emission of 500 ± 20 nm. The changes in HyPer fluorescence intensity at 500/520 relative to 420/520 were used to indicate changes in H_2O_2 concentrations in the cells [35].

Mouse tumor model and treatment procedures

The syngeneic murine pancreatic carcinoma PAN-02 cells (1×10^6) suspended in normal saline solution were injected subcutaneously into the flank of female athymic mice (Ncr-nu/nu ages 5–8 weeks). When tumor volume reached ~30 to 40 mm^3, the mice were randomly assigned to treatment groups: control group received daily saline solution osmotically equivalent to AA; ascorbate group received 4 g ascorbate/kg body wt daily; GSH group received 800 mg GSH/kg body wt daily. For combinations, AA was administered first, followed immediately by GSH. All drugs were administered by intraperitoneal injection. Longitudinal tumor volume was calculated from caliper measurements using volume $=(\text{length})\times(\text{width})^2\times0.5$. Body weight was measured on a digital pan balance. Treatment stopped at day 18, and the mice continued to be monitored until they reached preset criteria for euthanasia as described in a protocol approved by the Institutional Animal Care and Use Committee. Survival rate was recorded until day 30, when all mice were euthanized.

Statistics

Mixed linear regression was used to compare group-specific tumor progress over time. Survival-rate comparison and single-point comparison between groups used paired *t* test. All statistic analysis was two-sided and assessed for significance at the 5% level.

Results

Usage of iv GSH and the concurrent usage of iv ascorbate by CAIM practitioners

As the usage of iv AA in CAIM has been published in detail [9], our focus for this survey was iv GSH use. One hundred sixty-nine survey forms were distributed to attendees at the CAIM conference in April 2011. Ninety-four forms were returned, a response rate of 55.6%. Of the respondents, 75.5% (71/94) had a MD degree, and others were ND,

Table 1
Number of survey respondents (CAIM practitioners) who use iv glutathione and concurrently use iv ascorbate.

Survey forms distributed	Respondents	Use iv AA	Use iv GSH	Use iv AA and iv GSH		
				Same patient	Same day	Same bag
169	94 (55.6% response rate)	89 (85.4% concurrently use Iv GSH)	85 (89.4% concurrently use iv AA)	76	65	13

Survey forms were distributed to the attendees of a CAIM conference in April 2011. GSH, glutathione; AA, ascorbate; iv, intravenous.

Table 2
Dose and frequency of iv glutathione used by survey respondents.

	Mean	Median	Range
Doses of GSH (mg/treatment)	1567	1750	300–4000
Frequency (doses/week/respondent)	9.4	15.5	1–30

DO, RN, MN, PA, and nurse practitioners. Eighty-nine respondents used iv AA, 85 used iv GSH. Of the 85 who used iv GSH, 76 (89.4%) coadministered iv AA and iv GSH to the same patient; 65 of them (76.5%) administered both drugs on the same day, and 13 of them (15.3%) mixed the two drugs into the same infusion bag (Table 1). The overall concurrent use of both iv ascorbate and iv GSH was 80.9% (76/94) in all respondents (Table 1). Furthermore, 85.4% (76/89) of the respondents who used iv AA coadministered iv GSH to the same patient (Table 1). With the large scale of iv AA usage by CAIM practitioners (exceeding 10,000 patients per year [9]), there is a compelling need to investigate potential drug–drug interactions between iv AA and iv GSH.

The total number of iv infusions of GSH given was estimated at 808 infusions per week by the survey respondents, with a wide range of 1–30 infusions per practitioner per week (Table 2). The dose of iv GSH per infusion ranged from 300 to >4000 mg, averaging 1567 mg/infusion (Table 2). Intravenous GSH was used for a variety of conditions, labeled as detoxification (82 respondents, 24.55% of total usage), neurologic disorders (74 respondents, 22.16%), neuropathy (64 respondents, 19.16%), cancer (43 respondents, 12.87%), infections (36 respondents, 10.78%), and other conditions (35 respondents, 10.48%) (Fig. 1).

Sensitivity of various cancer cells to pharmacologic concentrations of ascorbate

Studies have shown that AA in pharmacologic concentrations achievable with iv infusion can induce H_2O_2-dependent cell death in cancer cells [12–15,37–40]. We tested the cytotoxic effect of pharmacologic AA in 10 cancer cell lines. Tested cells were incubated with 0–20 mM AA, which is relevant to clinical concentrations achieved with iv AA. All tested cells were sensitive to AA treatment, although there were variations in their sensitivity (Fig. 2). AA concentrations causing a 50% decrease in cell survival (IC_{50} values) ranged from 0.5 mM for MCF-7 (breast cancer) to 3 mM for B16 (melanoma), concentrations easily achievable from iv infusion [10].

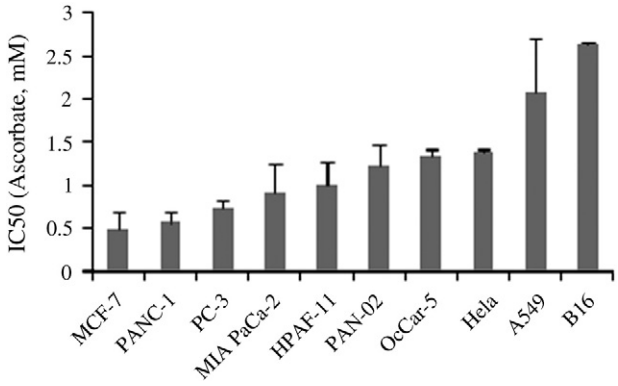

Fig. 2. Sensitivity of cancer cells to pharmacological concentrations of ascorbate. Human pancreatic cancer (MIA PaCa-2, HPAF-II, and PANC-1), lung cancer (A549), ovarian cancer (OvCar5), breast cancer (MCF-7), prostate cancer (PC-3), and cervical cancer (HeLa) cell lines, a mouse melanoma (B16) and a mouse pancreatic cancer cell line (Pan-02) were exposed to serial dilutions (0–20 mM) of ascorbate. Cells were washed with PBS and viability was detected using MTT assay at 48 h of treatment. IC_{50} was determined for the concentrations that reduced viability by 50% compared with matched untreated cells. All data represent two to four individual experiments, each done in triplicate (±SD).

Glutathione reduces the cytotoxicity of ascorbate toward cancer cells

Having established the dose response of ascorbate-induced cytotoxicity for each cell line, we added GSH cotreatment to the AA treatment. To determine the GSH dose, cells were incubated with a series of dilutions of GSH for 48 h (data not shown). A concentration relevant to clinical GSH use (640 μM) [26] provided 0–25% decrease in

Other uses described by respondents	Number of respondents
Autism	7
Chronic Fatigue syndrome	6
Fibromyalgia	3
Presbycusis	1
Dental amalgam removal	1
Autoimmune disease	1
Methylation/glucoronidahorl, sulfation issues	1
Malabsorption	1
Asthma	1
Chemical sensitivity	2
Cold	1
Surgey,Emergent situation	1
Vasculitis	1
Gut healing	1
Encephalopathy	1
Pain	1
Depression	1
Headache	1
Itching	1
Learning disability	1
Thyroid disorder	1

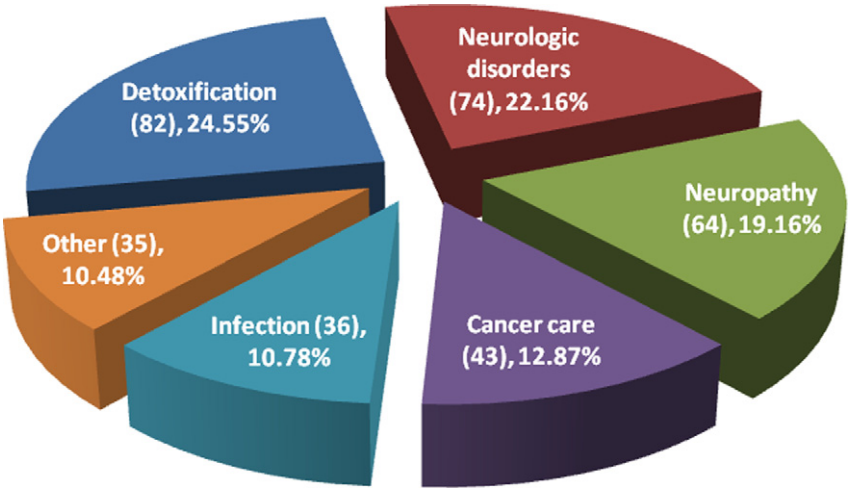

Fig. 1. Number of survey respondents (CAIM practitioners) who use intravenous glutathione for various conditions. The number in the parentheses is the number of practitioners who use iv GSH for the indicated condition. The percentage denotes the portion of usage for the indicated condition in all usages.

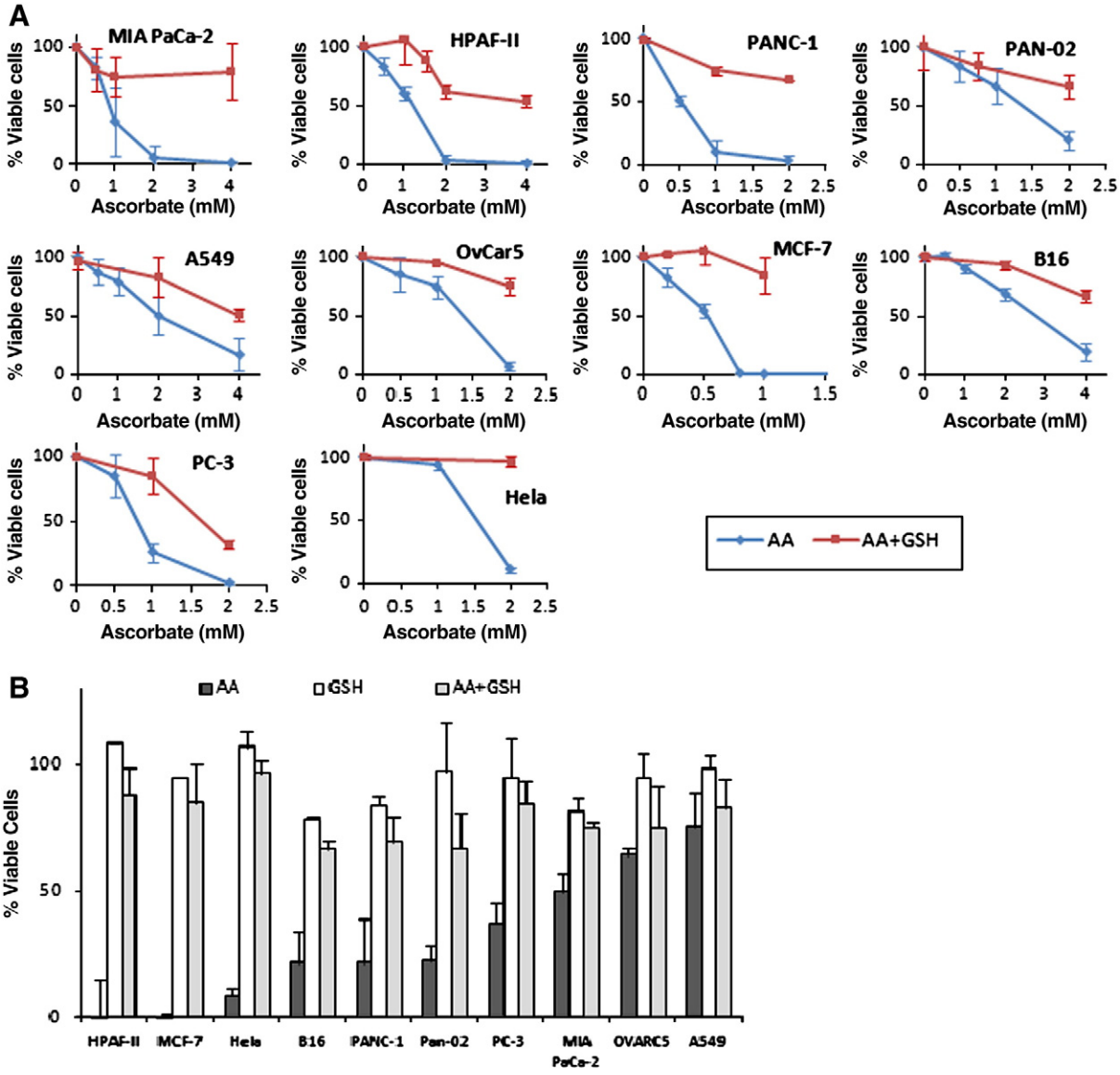

Fig. 3. Glutathione reduces the cytotoxicity of ascorbate toward cancer cells. Human pancreatic cancer (MIA PaCa-2, HPAF-II, and PANC-1), lung cancer (A549), ovarian cancer (OvCar5), breast cancer (MCF-7), prostate cancer (PC-3), cervical cancer (HeLa), mouse melanoma (B16) and mouse pancreatic cancer (PAN-02) cell lines were treated with ascorbate (AA) at various concentrations, with or without glutathione (GSH; 640 μM). Cell viability was examined using MTT assay at 48 h of treatment. (A) Dose response of cancer cell viabilities to the treatments. Blue lines show AA treatment alone, red lines show AA + GSH. (B) Percentage of cell viability in treated cancer cells relative to untreated cells. The concentrations of AA were 1 mM for MCF-7, PC-3, and MIA PaCa-2; 1.5 mM for HPAF-II and OVCAR5; 2 mM for PANC-1, Pan-02, A549, and HeLa; and 4 mM for B16. GSH was 640 μM for all cell lines. All data represent two to four individual experiments each done in triplicate (±SD).

cell viability, depending on the cell line tested (Fig. 3B). This clinically relevant GSH concentration was chosen to combine with AA. Cells were incubated with AA or with AA + GSH for 48 h and viability was assessed. Across the tested concentration range of AA, addition of GSH dramatically reduced AA-induced cytotoxicity compared with AA treatment alone (Figs. 3A and B). The dose–response curves of each tested cell line shifted toward much less or no response to AA treatment (Fig. 3A). When GSH was combined, an impairment in the sensitivity to pharmacologic ascorbate treatment occurred in all the cancer cells tested, regardless of cell type or sensitivity of the cells to either AA or GSH alone.

Glutathione inhibits pharmacologic ascorbate-induced H_2O_2 production

The mechanism of action of AA-induced cancer cell death is through H_2O_2 formation as AA is oxidized (catalyzed by transition metals), with $Asc^{\bullet}$ serving as intermediary [12–15,37,38]: AscH + $O_2 \rightarrow Asc^{\bullet} + O_2^{\bullet -} \rightarrow DHA + H_2O_2$. GSH is able to prevent ascorbate oxidation [30], or reduce H_2O_2 directly [25–29], and thus decrease H_2O_2 concentration. The decrease in H_2O_2 concentration could then result in attenuation of the ascorbate-induced cytotoxicity [13,37]. To assess H_2O_2 production by pharmacologic AA with and without addition of GSH, we used a human cervical cancer cell line, HeLa-HyPer-Cyto, that expresses a transfected H_2O_2 fluorescent sensor—HyPer protein—in its cytoplasm. HyPer has one fluorescence-emission peak at ~516 nm and two excitation peaks at ~420 and ~500 nm [35]. Upon H_2O_2 exposure, HyPer changes fluorescence with a decease at ~420/516 nm proportional to the increase at ~500/516 nm [35]. As shown in Fig. 4A, treatment with 2 mM H_2O_2 enhanced the fluorescence of HyPer at 500/520 nm, which serves as a positive control. Glutathione alone decreased the endogenous fluorescence showing as relative fluorescence intensity in Figs. 4A and 3B, consistent with its role as a H_2O_2 reducer. At 2 h of AA treatment, the green fluorescence of HyPer at 500/520 nm was greatly enhanced compared with untreated control

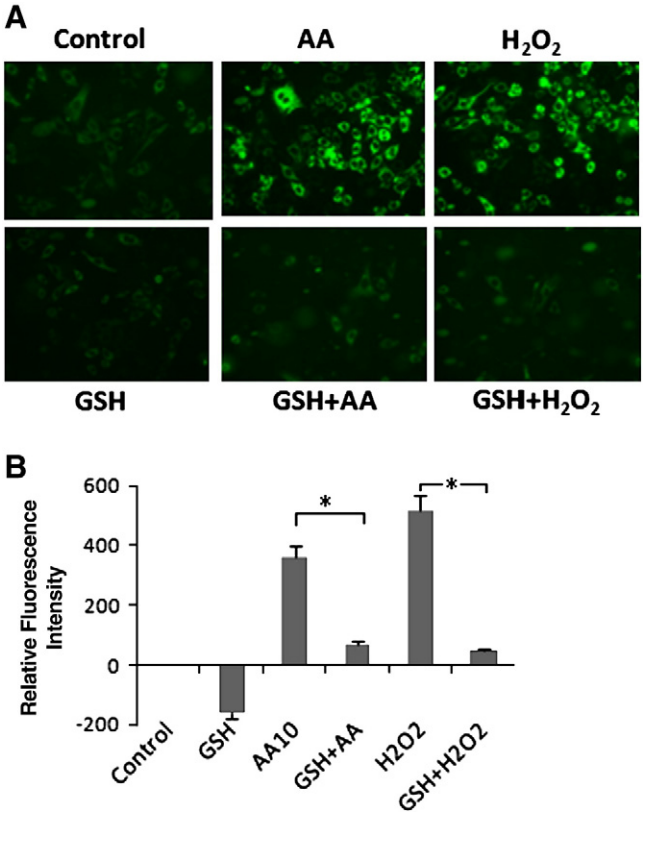

Fig. 4. Glutathione reduces ascorbate-induced H_2O_2 production. HeLa-HyPer-Cyto cells at 80% confluence were treated with AA (10 mM), GSH (640 μM), or AA + GSH for 2 h. H_2O_2 (2 mM) was added as a positive control. (A) Imaging of HyPer green fluorescence. (B) Relative fluorescence intensity indicates intracellular H_2O_2 concentrations and was determined by the changes in HyPer fluorescence at excitation/emission of 500/520 nm relative to 420/520 nm. Data were normalized to untreated control cells and represent two individual experiments, each done in six repeats (±SD). $^*P<0.05$.

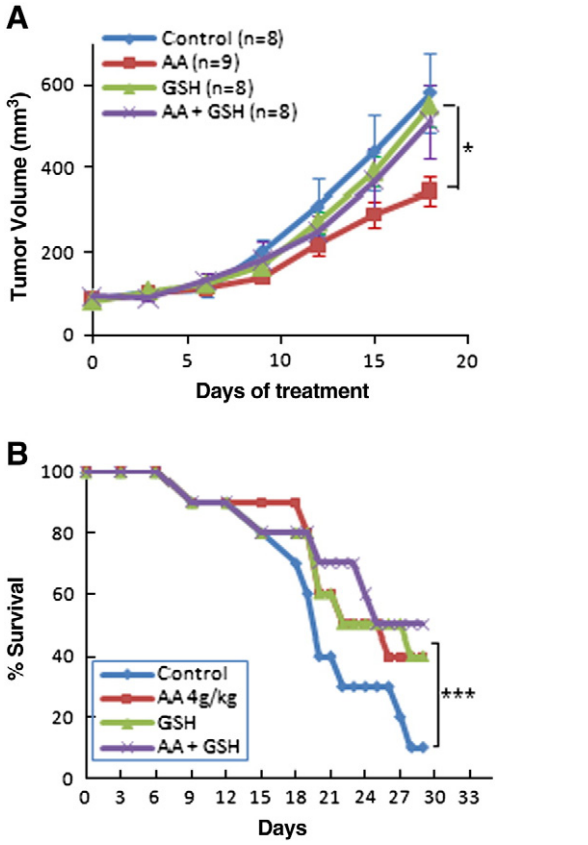

Fig. 5. Glutathione reduces the tumor-inhibitory effect of ascorbate in a mouse pancreatic cancer model. Pan02 subcutaneous xenografts were treated with ip doses of ascorbate (AA) 4 g/kg/day, glutathione (GSH) 800 mg/kg/day, or the combination of AA and GSH (AA + GSH). Control mice were treated with saline osmotically equivalent to AA. Tumor volume was calculated from caliper measurements using the equation volume = (length × width × width)/2. Treatment stopped at day 18, and the mice were monitored until they reached the preset criteria for euthanasia. (A) Longitudinal tumor growth under each treatment. (B) Survival of mice with each treatment. $^*P<0.05$, $^{***}P<0.005$. Standard errors are shown.

(Figs. 4A and B), indicating an increase in H_2O_2 concentration in the cells. By adding 640 μM glutathione to the ascorbate treatment, the fluorescence intensity was lowered and showed no difference compared to untreated control cells or cells treated with GSH alone (Figs. 4A and B). These experiments demonstrated that glutathione inhibited the expected AA-induced H_2O_2 production.

Glutathione reduces the tumor-inhibitory effect of ascorbate in a mouse pancreatic cancer model

To validate the in vitro attenuation of cytotoxicity by GSH in AA-treated cancer cells, we tested the combination of GSH and AA treatment in vivo. A mouse syngeneic pancreatic cancer cell line, PAN-02, was used to form subcutaneous xenografts in nude mice. Treatment was given by daily ip injections of saline as a control, 4 g/kg AA, 800 mg/kg GSH, or the combination of AA and GSH. AA treatment alone inhibited the growth of this aggressive tumor by 42% compared with saline-treated control (Fig. 5A), consistent with published studies [12]. AA treatment also improved survival rate (Fig. 5B). GSH treatment alone did not exhibit any increase or decrease in tumor growth (Fig. 5A). The addition of GSH to AA treatment attenuated the effect of AA, resulting in identical tumor growth compared to saline-treated control (Fig. 5A). Interestingly, GSH treatment alone showed a benefit in improved survival rate, but the combination of GSH with AA did not provide further improvement in survival rate compared with either AA or GSH alone (Fig. 5B). Taken together, adding GSH treatment simultaneously to high-dose AA treatment offers no benefit over AA treatment alone, and it appears that GSH antagonizes ascorbate-induced tumor growth inhibition when these two treatments are used together.

Discussion

High-dose iv and intramuscular vitamin C or AA has endured as a therapy for infectious disease and cancer since its introduction in the first half of the 20th century [1–8,41,42]. Because of negative reports regarding its utility in chronic disease [43–45], high-dose iv AA has largely been abandoned by conventional medicine and has become a therapy primarily practiced by CAIM practitioners [9,46]. However, recent in vitro and in vivo research findings support the use of high-dose iv AA in cancer treatment and provide a mechanism for neoplastic cytotoxicity [12–15,37–40]. Parenteral administration of high doses of AA generates ascorbate radical in the extracellular space, which in turn reacts with a transition metal to produce H_2O_2. It is H_2O_2 that acts as the cytotoxic agent and, as such, high-dose AA is a prodrug for H_2O_2 with apparent targeted neoplastic cytotoxicity. Our data in this study confirm the pro-oxidant anti-cancer effect of ascorbate using clinically relevant conditions, as the AA-induced cancer cell death was attenuated by concurrent administration of the anti-oxidant GSH.

Because the administration of iv AA has been out of the purview of conventional medical practice but in use in CAIM practices, it has only recently been found what the extent of use is [9]. Our survey data in this paper showed that iv GSH is often used concurrent with iv AA

and administered to cancer patients on the same day and even within the same infusion (Tables 1 and 2 and Fig. 1). However, there is no scientific evidence to support the combination and no information about efficacy or drug–drug interactions. This preliminary report indicates that there is antagonism between ascorbate and glutathione in treating cancer, and therefore iv AA and iv GSH should not be coadministered on the same day to cancer patients.

Whereas the cytotoxic effect of high-dose AA is confirmed again in our study, combining GSH with AA significantly reduced the cytotoxic effect of AA. This antagonism occurs by GSH interfering with AA-induced H_2O_2 production (Fig. 4). GSH is a well-known H_2O_2 scavenger that directly reduces H_2O_2 to H_2O [25–29]. In addition, by chelating the transition metal, GSH can prevent *trans*-metal interactions with ascorbate radical and thus reduce H_2O_2 formation [30]. As H_2O_2 formation is an essential mechanism of AA antineoplastic action, the combination of GSH counteracts this important catalysis.

Consistent with published studies [12,15], our in vivo data demonstrate the effects of AA single-drug treatment in inhibiting pancreatic cancer growth (Fig. 5A). Of concern, addition of GSH to AA completely attenuated the tumor-inhibitory effect of AA treatment. GSH also failed to provide any additional survival benefit compared to AA single-drug treatment. It is fair to conclude from these data that adding GSH concurrent with AA treatment has no benefit over AA treatment alone and results in a detrimental effect through drug–drug interaction, with inhibition of the expected H_2O_2 formation by AA oxidation.

GSH plays a pivotal role in drug metabolism through Phase I and Phase II metabolizing systems, through which elimination of drugs and their metabolites is enhanced [47]. This is important for cell and tissue detoxification, but may also inactivate or eliminate effective drug components or drug metabolites. GSH can decrease neurotoxicity associated with platin-based chemotherapy, through removal of platinum from the dorsal root ganglia, and spares nerve damage [17–21]. Of note, no reduction in the chemotherapeutic effect was observed in clinical trials when iv GSH was added before platin-based chemotherapy administration [17–21]. This benefit, however, may not extend to other types of chemotherapy. In fact, many studies suggest that elevated glutathione or glutathione synthesis ability contributes to chemoresistance in cancer cells [31,48–53]. In multidrug-resistant neoplastic cells, the GSH/glutathione *S*-transferase level is elevated, making these cells less likely to generate the reactive oxygen species (ROS) that can tip the cells into apoptosis [53–55]. In this regard, classic alkylating drugs and platin-based chemotherapy are exempt because of their reliance on pathways for cytotoxicity other than ROS-related ones [53]. Therefore, narrow classes of cytotoxic drugs such as platin-based chemotherapy may allow for coadministration of GSH given the beneficial findings in clinical trials [18–20]. However, when ROS are critical for neoplastic cell death, GSH is predicted to interfere and should not be coadministered on the same day.

It may be wise to expand the use of GSH with other chemotherapeutic regimens but perhaps only on days apart from chemotherapy administration, so that the benefit of tissue protection could be achieved while diminishing the risk of potential interference in antineoplastic effects. The pharmacokinetic behavior of iv GSH and the combining drug should be considered when suggesting a reasonable time of administration.

In this report we have demonstrated the reduction of the chemotherapeutic effect of high-dose AA by coadministration of GSH in cultured cells and parenterally in animal models and would predict a similar effect in humans. It would be a wise first step to investigate the pharmacokinetics of AA and GSH coadministration in healthy humans with an assessment of production of ascorbate radical and H_2O_2. In this way drug–drug interactions can be assessed outside of the clinical setting.

Because GSH is important in drug metabolism, antioxidant defense, immunology, nutrient metabolism, and regulation of cellular events [29,56], GSH has a powerful role in cellular protection and is implicated in cancer prevention [31,57,58]. Interestingly, in our animal experiment, GSH treatment alone did extend survival despite having no effect on tumor growth. As such, GSH could provide a tool for clinical practice in settings in which protection from highly oxidative processes is needed or in situations in which drug metabolism may need supporting. However, in clinical settings in which treatments are dependent on ROS and free radicals, such as conventional chemotherapy, GSH effects are counterproductive. Furthermore it may be said that when GSH is used with AA and certain chemotherapeutic drugs, GSH acts as a double-edged sword. There are obvious benefits of GSH use in reducing oxidant damage to normal cells such as nervous tissue, but in the case of pharmacologic AA, there is a reduction in H_2O_2 production when used concurrently, with potential for dampening down signals that trigger neoplastic cell death. At this juncture, it does not appear that high-dose iv GSH acts as a pro-oxidant in the manner that iv AA does, although further research is needed. Based on the reported findings, we advise iv AA and iv GSH should not be coadministered on the same day in the clinical setting of cancer treatment. Simply put, GSH is an antioxidant and pharmacologic iv AA is a pro-oxidant, and when used concurrently effects can be canceled.

Acknowledgments

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