

S-Nitrosoglutathione Photolysis as a Novel Therapy for Antifibrosis in Filtration Surgery

Marie Tannous,¹ Cindy M. L. Hutnik,² David P. Tingey,² and Bulent Mutus¹

PURPOSE. To determine whether a novel peroxynitrite-based photosensitizer S-nitrosoglutathione (GSNO) can produce specific in vitro light-induced cell death of both standard animal lung and human Tenon's capsule (TC) fibroblasts and to compare this effect with that produced by the established photodynamic porphyrin precursor 5-aminolevulinic acid (ALA).

METHODS. V79-4 Chinese hamster lung and human TC fibroblasts were established in tissue culture. GSNO, together with its radioactive tritiated and fluorescent dansylated derivatives, were synthesized. The labeled molecules were prepared to determine the time course of uptake into the fibroblasts. Uptake was monitored by scintillation counting for the tritiated GSNO and confocal fluorescence microscopy for the dansylated GSNO. The uptake of ALA and biosynthesis of its photosensitive product were determined by fluorescence emission spectroscopy of a separate set of fibroblasts. Once uptake was established, both cell lines were incubated with varying concentrations of GSNO or ALA as a function of time (0, 4, or 24 hours) before light exposure (200 msec pulsed visible light, 0.068 W per pulse, for 10 minutes at a distance of 10 cm). After 10 minutes of irradiation, the cells were washed and exposed to fresh tissue culture medium. The effect of the treatment was determined 24 hours later by measuring cell viability.

RESULTS. A 2-minute drug treatment time (0 hours incubation) with GSNO, followed by 10 minutes of irradiation, resulted in approximately 78% of fibroblast cell death at the lowest concentration of GSNO used compared with the control, which was exposed to light, but no GSNO. The higher concentrations of GSNO, or longer drug treatment times before irradiation, did not statistically increase cell death. Maximal cell death was thus obtained using the lowest GSNO concentration (50 mM) and drug treatment time (2 minutes). In contrast, the well-established photosensitizer ALA killed only approximately 4% of cells at the lowest concentration and drug treatment time tested. At drug treatment times of 4 hours and less, increased concentrations of ALA did not produce cell death of more statistical significance. It was not until 24 hours of drug treatment that comparable amounts of cell death were produced by ALA and GSNO. In all experiments similar results were obtained with the animal lung and human TC fibroblasts, suggesting that the source of the fibroblast had no effect on the outcome. The differences in treatment effects between GSNO and ALA were statistically significant under all conditions tested.

CONCLUSIONS. GSNO is able to cause light-specific cell death of human TC fibroblasts at drug treatment times (2 minutes) and irradiation times (10 minutes) that would be compatible with its use in glaucoma filtering surgery. This in vitro performance was superior to that of the well-established photosensitizer ALA, which required treatment times longer than 4 hours to approach the light-specific cell death produced by only 2 minutes of GSNO treatment. (*Invest Ophthalmol Vis Sci.* 2000;41:749-755)

Bleb scarring is the leading cause of failure of glaucoma filtering surgery. Excessive proliferation of subconjunctival fibroblasts, with synthesis and deposition of collagen and glycosaminoglycans, is thought to be the principle

mechanism of failure.¹⁻³ In an attempt to prolong bleb survival, the antimetabolites 5-fluorouracil and mitomycin C have been used to inhibit fibroblast proliferation and therefore to modulate the wound-healing response.^{4,5} Although the use of these antimetabolites has improved the success rate of filtering surgery, the risk of vision-threatening complications has also increased. Mitomycin C in particular has been associated with cystic bleb formation, hypotony maculopathy, and bleb-related endophthalmitis.⁶⁻⁸ The rather imprecise delivery system and lack of identification of standardized treatment parameters⁹ have been recognized as further limitations in the use of these antimetabolites.

Photodynamic therapy (PDT) is a relatively selective method of treating actively proliferating and malignant tissues by the localized application of light. Pretreatment of the tissues with photosensitizing agents results in the photochemical gen-

From the ¹Department of Chemistry and Biochemistry, University of Windsor, Ontario; and the ²London Health Sciences Centre, Department of Ophthalmology, Victoria Campus, London, Ontario, Canada.

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Corresponding author: Bulent Mutus, Department of Chemistry and Biochemistry, University of Windsor, 401 Sunset, Windsor, Ontario N9B 3P4, Canada. mutusb@uwindsor.ca

eration of highly reactive oxygen-related intermediates that produce cytotoxicity. Phase III trials are in progress for the treatment of bladder, bronchial, and esophageal carcinomas.¹⁰ The use of PDT in the treatment of various dermatologic disorders has also been well described.¹¹ Considerable attention has recently been drawn to the potential use of PDT to treat choroidal melanomas,¹² choroidal neovascular membranes,¹³ and age-related macular degeneration.¹⁴

Despite the common demonstration of a distinct antifibrotic effect of PDT,^{10,15-17} few reports have examined the effects on ocular fibroblasts. Smyth et al.¹⁸ demonstrated that although the hematoporphyrin derivative photosensitizing agent was absorbed by human subconjunctival fibroblasts and produced a certain dark toxicity, a specific phototoxic effect could not be demonstrated. Hill et al.¹⁹ reported that intravenously administered tin ethyl etiopurpurin failed to prolong bleb survival in a pilot study using rabbits. However, subconjunctival delivery of the photosensitizer 3 hours before filtering surgery appeared to prolong bleb survival in four of six rabbits tested.

To further explore the use of PDT as a potential modulator of antifibrosis in glaucoma filtering surgery, we compared the novel application of *S*-nitrosoglutathione (GSNO) photolysis with the well-characterized porphyrin precursor 5-aminolevulinic acid (ALA) in achieving *in vitro* cell death of both standard and human Tenon's capsule (TC) fibroblasts.

MATERIALS AND METHODS

Cell Culture

The V79-4 Chinese Hamster lung fibroblast cell line was purchased from the American Type Culture Collection (ATCC CCL-93; Rockville, MD). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Burlington, Ontario, Canada) containing 10% heat-inactivated fetal calf serum and 100 units penicillin G, 0.01% streptomycin, and 0.25 μ g amphotericin B in a 5% carbon dioxide incubator with atmospheric air at 37°C. Unless otherwise specified, all chemicals listed in this section were purchased from Sigma-Aldrich (Oakville, Ontario, Canada).

TC fibroblasts were prepared by digestion of a primary explant of a surgical specimen taken at the time of cataract surgery. The patient did not have glaucoma and had no previous history of topical medication use or chronic ocular disease. The microdissected specimen was digested in a mixture of 1 mg/ml collagenase type II, 4 mg/ml trypsin inhibitor and 0.1 mg/ml DNAase, all prepared in balanced salt solution. The digestion mixture was incubated at 37°C for 1 hour and then centrifuged at 800 rpm for 10 minutes. The pellet was resuspended in DMEM and plated onto fibronectin-coated plates. After isolation, the cells were resuspended in a mixture of 90% fetal calf serum and 10% dimethylsulfoxide at a concentration of 2×10^6 cells/ml and frozen in liquid nitrogen until cultures were established as described. When cell cultures became confluent, they were detached from the initial flask using trypsin 0.25% and EDTA 0.02% and replated onto either 60-mm tissue culture dishes (Becton Dickinson Labware, Lincoln Park, NJ) or 22-mm² sterile coverslips (VWR Scientific, Media, PA).

Uptake Studies of ALA

Both V79-4 and TC fibroblasts were treated in an identical manner. The cells were grown to confluence. They were in-

cubated for 24 hours at three different concentrations of ALA (0.05 mM, 0.1 mM, and 1.0 mM) prepared in phosphate-buffered saline (PBS; pH 7.4), both in the absence and presence of 1.0 mM deferoxamine mesylate (DEF; Ciba Pharmaceuticals, Mississauga, Ontario, Canada). After the 24-hour incubation, the cells were removed from the dish by trypsinization and resuspended in 3.0 ml PBS. The fluorescence emission spectrum was recorded on a spectrofluorometer (model F-3010; Hitachi, Tokyo, Japan) at an excitation wavelength of 408 nm.

Uptake Studies of GSNO

Both V79-4 and TC fibroblasts were treated in an identical manner. The cells were grown to confluence on 22-mm sterile coverslips inside 60-mm tissue culture dishes. Uptake was assessed by two methods. The first involved preparation and imaging of the fluorescent dansylated derivative of GSNO (DNS-GSNO) and the second method involved measuring the radioactivity of tritiated GSNO.

Uptake Studies of DNS-GSNO

Preparation of DNS-GSNO. Oxidized glutathione (100 mg in 20 ml 0.1 M Tris buffer; pH 8.0) was shaken at room temperature for 24 hours with 0.01 M dansyl chloride in 1.0 ml absolute ethanol. The mixture was passed over a G-25 Sephadex 2.5×10 -cm gel filtration column. The first fluorescent fraction was collected and lyophilized. This material was reduced with 10 mM β -mercaptoethanol. Gel-exclusion chromatography was repeated, and the first fluorescent fraction was collected and lyophilized. Transnitrosylation was performed by incubating this material (in Dulbecco's PBS [DPBS; pH 7.4]), with 100-fold molar excess (over free thiol) of GSNO for 1 hour at room temperature. GSNO was prepared as previously reported.¹ The DNS-GSNO was protected from ambient light by aluminum foil.

Treatment of Cells. The cells were removed from the incubator, the tissue culture medium removed, and the cells rinsed twice with Hanks' balanced salt solution. The cells were then incubated at room temperature with a 1.0 mM solution of DNS-GSNO in DPBS for 1, 2, 5, and 10 minutes. The DNS-GSNO was aspirated, and the coverslips were inverted onto microscope slides using mounting medium (Vectashield Fluorescence Medium; Vector, Burlingame, CA) as an interface.

Confocal Microscopy. The cells were imaged with a confocal laser scanning microscope (model 1024 MRC; Bio-Rad, Hercules, CA) with a $\times 20$ to $\times 60$ objective (Eclipse E800 IMT2; Nikon, Garden City, NY). An argon blue laser excitation source was passed through a combination of a T1 triple dichroic and T2A 560 DRLP filter system.

Uptake Studies of ³H-GSNO

Preparation of ³H-GSNO. Twenty microcuries ³H-glutathione (³H-GSH; Mandel Scientific, Guelph, Ontario, Canada) was added to 1 mM GSNO (10 ml), which was then divided and stored at -20°C .

Treatment of Cells and Measurement of Radioactive Uptake. One-milliliter fractions of ³H-GSNO were diluted 20-fold in Earle's balanced salt solution (EBSS) to achieve a concentration of 50 μ M ³H-GSNO (0.1 μ Ci/ml). Cells were pelleted by centrifugation after trypsinization and washing in EBSS. One milliliter ³H-GSNO was added to the cell pellet and

incubated for 1-, 2-, and 5-minute intervals. The reaction was stopped by suction filtration through a 0.45- μ m nitrocellulose filter followed by washing with EBSS. The filter paper was placed in 10 ml scintillation fluid (Cytoscint; ICN, Costa Mesa, CA) and shaken at room temperature overnight. The radioactivity was measured after 18 hours on a multipurpose scintillation counter (model LS 6500; Beckman, Mississauga, Ontario, Canada). Nonspecific binding was determined by adding 1.0 ml of 50 M 3 H-GSNO to the filter paper followed by treatment identical with that of the cell-containing specimens. The nonspecific binding was subtracted from the radioactivity measured in the presence of cells.

Photodynamic Treatment. Cells were grown to confluence. After removal of the culture medium, the cells were treated with three different concentrations of either ALA or GSNO (0.05 mM, 0.1 mM, and 1.0 mM). The ALA experiments were performed both in the absence and presence of 1.0 mM DEF added along with the ALA.

Drug-treated cells at each concentration were further divided into three groups. The first group of cells was irradiated immediately after drug addition, which, in practical terms, corresponded to a drug treatment time of approximately 2 minutes before irradiation. Because the cells were not placed in the CO₂ incubator before light application, this treatment time corresponded to 0 hours of incubation. The other two groups of cells were incubated for an additional 4 or 24 hours at 37°C in 5% CO₂ after drug addition but before light treatment. These incubation times thus correspond to drug treatment times of 4 and 24 hours, respectively.

The cells were radiated with a fiber optic spectrometer (model S2000; Ocean Optics, Dunedin, FL), which delivered 200-msec pulses of visible light through a 3-mm aperture held at a distance of 10 cm after passing through a cupric sulfate filter (1 mM). A power meter (Spectra Physics, Mountain View, CA) recorded an energy of 0.068 W per pulse. The radiation time was arbitrarily 10 minutes.

After irradiation, the incubation solutions were removed and replaced with fresh tissue culture medium. The cells were incubated for another 24 hours before measurement of cell viability. Twenty-four hours was thought to be a reasonable period in which cells affected by the treatment would be dead and those unaffected would continue to proliferate and remain viable.

In these photodynamic experiments, control samples were composed of cells that received the same volume of medium as the treated cells, but without drug. Control cells were incubated and irradiated in a manner identical with the drug-treated cells.

For completeness, another set of experiments was performed to estimate the dark toxicity of GSNO in the absence of light and the nonspecific drug-free phototoxicity of 350 nm light. In one experiment, cells shielded from light were divided into two groups. One group received various concentrations of GSNO, and the other group received drug-free aliquots of equal volume. After 24 hours of incubation in the dark, a dark toxicity of approximately 25% was observed in the dark drug-treated samples compared with the dark control samples.

In another experiment, cells shielded from light were divided into two groups. One group received light treatment using parameters identical with those outlined in the photodynamic experiments, whereas the other group was kept in the dark. After 24 hours of incubation in the dark, a drug-free

phototoxicity of approximately 30% was noted. This rate of drug-free phototoxicity was automatically accounted for in the photodynamic experiments because, as stated, both the controls and the drug-treated cells were irradiated in an identical manner. The percentage of cell viability therefore represents the fraction of cells surviving in the presence of drug plus light over those surviving after light treatment alone.

Cell Viability Studies

Cell viability was determined using the trypan blue assay (Gibco) and a hemocytometer (Bright-Line; Reichert-Jung, Hauser Scientific, Horsham, PA).

Statistical Analysis

The variables of interest were drug (GSNO, ALA-DEF, and controls), concentration (0.05 mM, 0.1 mM, and 1.0 mM), and incubation interval (0, 4, and 24 hours). The dependent variable in all cases was the percentage of cell viability. The total sample size was 54, divided between the two cell populations (human TC fibroblasts and V79-4 Chinese hamster lung fibroblasts). Analyses were performed with commercial software (SuperANOVA; SAS Institute, Cary, NC).

Analyses were performed to examine the main effects of drug, concentration, and incubation interval and any interactions between them. Because the control cell samples did not receive different concentrations of any drug, they were excluded from any analysis examining the effect of concentration, in which case in some analyses there were two levels of drug (GSNO and ALA-DEF). Analyses were performed separately for the two different fibroblast groups, and then the data were combined to see whether group (human TC fibroblasts and V79-4 Chinese hamster lung fibroblasts) was significant.

RESULTS

Uptake Studies of ALA

Fibroblastic uptake of ALA and its subsequent biosynthesis into the active fluorescent photosensitizer protoporphyrin IX (PpIX) was demonstrated by recording a characteristic fluorescence emission spectrum (Fig. 1). Excitation of a cell suspension at 408 nm produced an emission maximum between 631 and 635 nm for both the animal lung and TC fibroblasts. The presence of DEF enhanced the emission intensity produced by the ALA product by more than threefold. On the basis of this

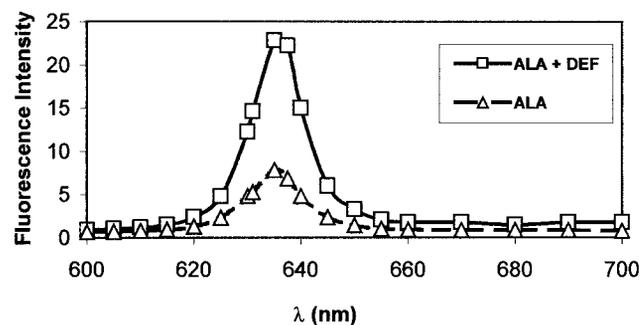


FIGURE 1. Steady state fluorescence emission spectrum of 1.0×10^6 cells/ml of V79-4 fibroblasts after 24 hours of incubation with 1-mM concentrations of ALA alone or 1 mM ALA plus 1 mM DEF. Excitation wavelength was 408 nm.

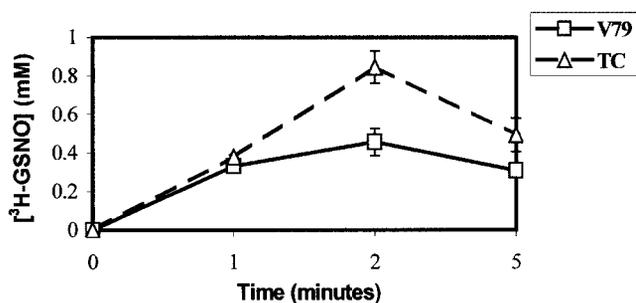


FIGURE 2. Concentration of ³H-GSNO measured after treating 1.4×10^6 cells/ml of both V79-4 and TC fibroblasts with the tritiated compound for various times.

result, DEF was included in all the photodynamic experiments with ALA.

Uptake Studies of GSNO

Both V79-4 and TC fibroblasts demonstrated maximum uptake of ³H-GSNO after 2 minutes of incubation (Fig. 2). By 5 minutes, the recovered amount of radioactivity had declined. Fluorescence microscopy of the DANS-GSNO treated cells confirmed that, by 2 minutes, the cytoplasm of the fibroblasts was

qualitatively as fluorescent as those treated for longer periods (Fig. 3).

Assessment of Phototoxicity

Treatment of the V79-4 animal lung fibroblasts with three concentrations of either ALA-DEF or GSNO (0.05 mM, 0.1 mM, and 1.0 mM) at 2 minutes (0 hours incubation), 4 hours, or 24 hours of drug treatment time showed that GSNO produced a greater amount of light-specific cell death at the two shortest treatment intervals (Fig. 4). This difference was noted even at the smallest concentration (0.05 mM). After 2 minutes of drug treatment, less than 22% of the cells were viable after treatment with GSNO compared with 96% still viable with ALA-DEF. At the longest incubation interval (24 hours), the effect of ALA-DEF on cell viability was comparable to that of GSNO at all three concentrations (Fig. 4).

In the analysis of the V79-4 animal lung fibroblasts, the main effect, drug, was significant ($F(2,24) = 79.823$, $P = 0.0001$) and the Tukey-Kramer post hoc procedure showed that all pairwise differences between the means were significant ($P < 0.01$). Examination of the means in Figure 4, when collapsed against all concentrations, showed that GSNO killed the most cells, with only 16% of cells remaining viable, whereas just over 45% of cells treated with ALA-DEF and all the cells in the control sample remained viable.

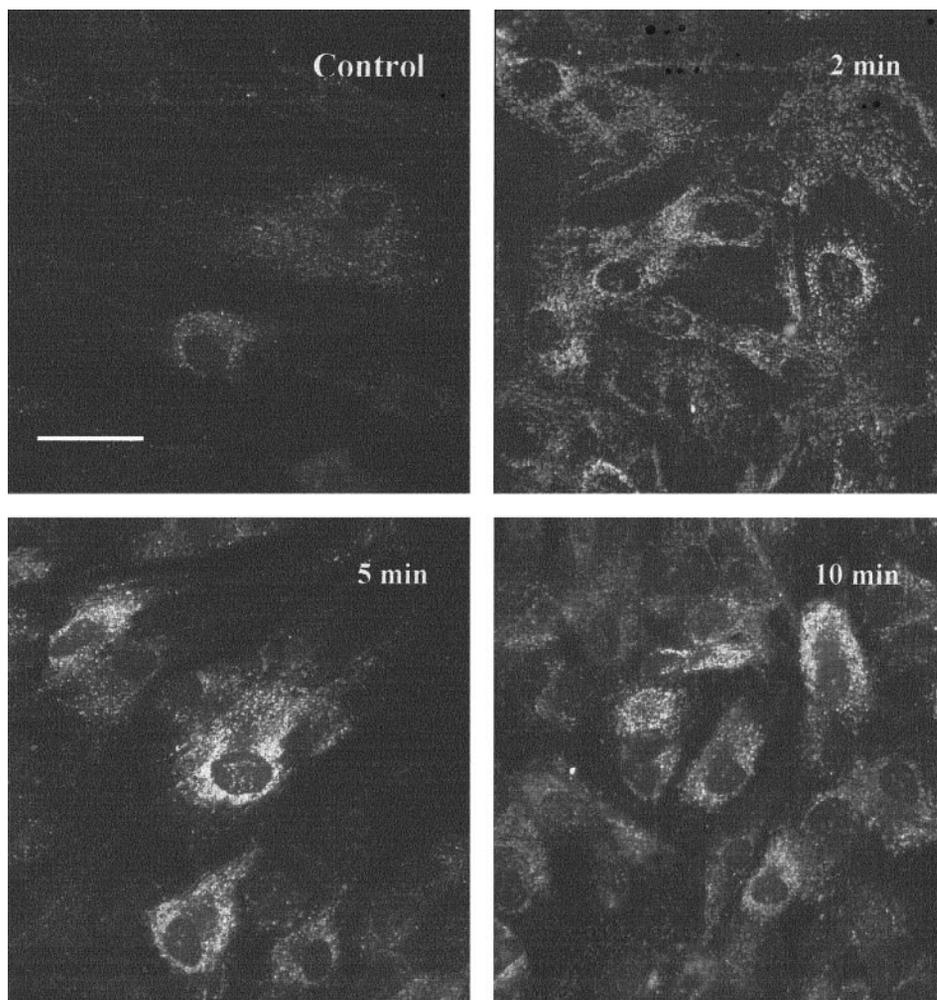


FIGURE 3. Fluorescence microscopic images of TC fibroblasts after incubation of the cells with DANS-GSNO for various times.

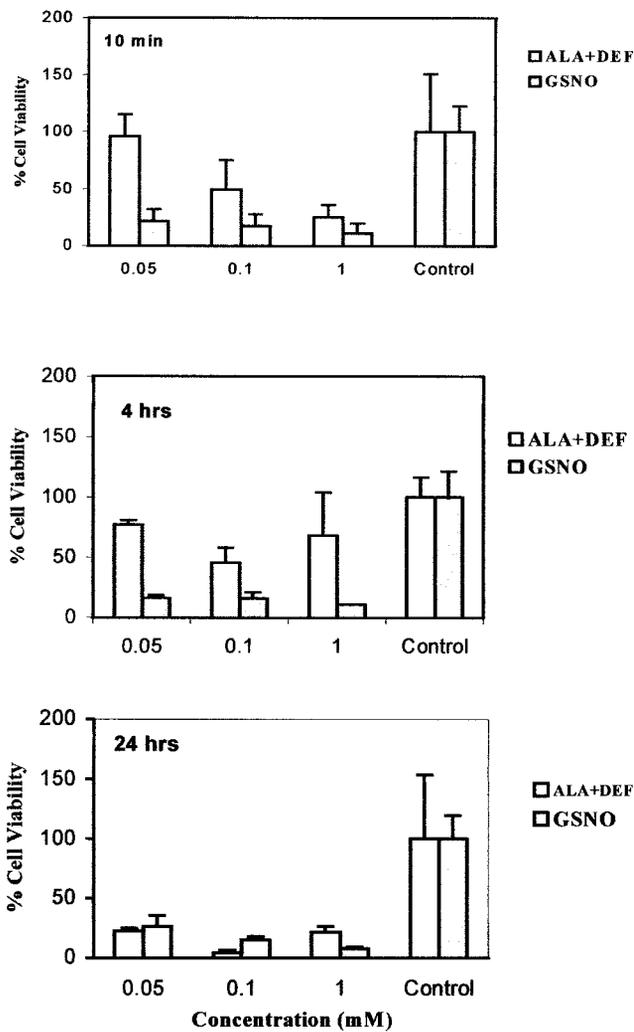


FIGURE 4. Comparison of the effect of different concentrations and incubation times on the cell viability of V79-4 fibroblasts in the presence of ALA-DEF versus GSNO. Data are means.

The effect of incubation interval on the V79-4 animal lung fibroblasts did not quite reach the level of significance ($P < 0.08$), but the interaction between drug and incubation interval was significant ($F(2,18) = 3.273, P < 0.05$). Treatment of the V79-4 animal lung fibroblasts at 2 minutes (0 hours incubation), 4 hours, or 24 hours of drug treatment time, showed that GSNO produced a greater amount of light-specific cell death at the two shortest treatment intervals (Fig. 4). This difference was noted even at the smallest concentration (0.05 mM). Only at the longest incubation interval (24 hours) was the effect of ALA-DEF on cell viability comparable to that of GSNO (Fig. 4).

The level of drug concentration was not a significant effect in the V79-4 animal lung fibroblasts, nor were there any significant interactions involving concentration. This demonstrated that the lowest concentrations tested were just as effective as the higher concentrations.

As in the V79-4 animal lung fibroblasts, treatment of TC fibroblasts at the same incubation periods produced similar results (Fig. 5). Drug was significant ($F(2,24) = 695.922; P = 0.0001$) and the Tukey-Kramer post hoc procedure showed that all pairwise differences between the means were significant ($P < 0.01$). Again, when collapsed against all concentra-

tions, GSNO killed the most cells in this cell population, with less than 10% of cells remaining viable, compared with just over 42% of cells treated with ALA-DEF and all the cells in the control sample remaining viable.

The effect of incubation interval was significant in this group ($F(2,24) = 20.302, P = 0.0001$) and the Tukey-Kramer post hoc procedure revealed that all pairwise differences between the means were significant ($P < 0.01$). The interaction between drug and incubation interval was also significant ($F(2,18) = 14.135; P = 0.0001$). At both 0 and 4 hours of incubation, GSNO resulted in more cell death than ALA-DEF.

Finally, the data from the two populations were combined to see whether there was any difference between the two types of fibroblasts. The difference was not significant, although at all incubation times the TC fibroblasts seemed slightly more sensitive to ALA-DEF and GSNO than the V79-4 fibroblasts, in that fewer cells remained viable after photodynamic treatment.

As with the V79-4 animal lung fibroblasts, the level of drug concentration was not a significant effect, nor were there any significant interactions involving concentration.

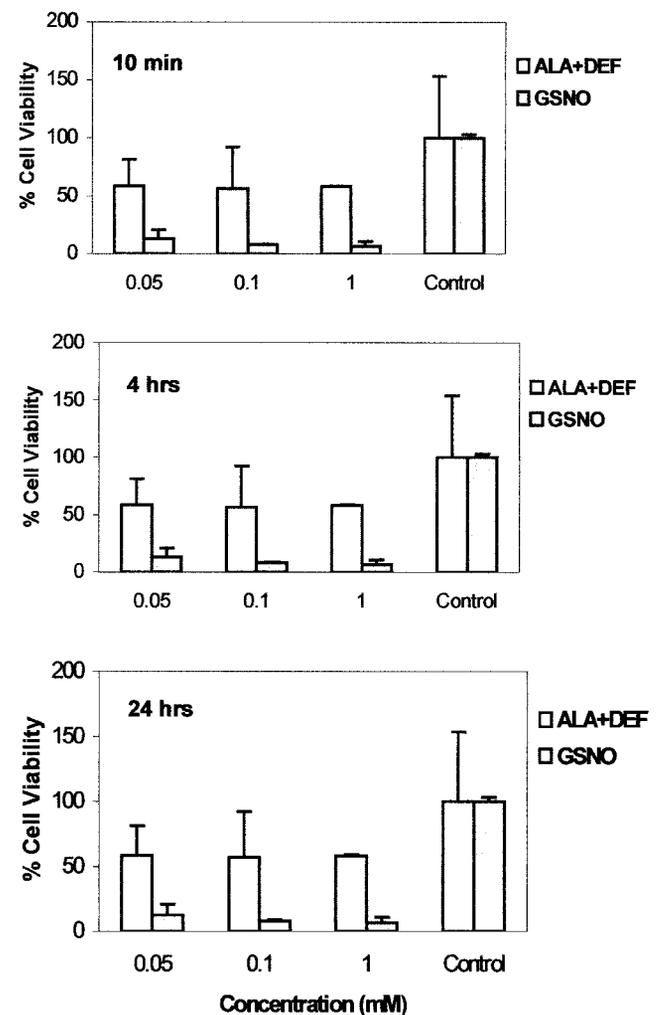


FIGURE 5. Comparison of the effect of different concentrations and incubation times on the cell viability of TC fibroblasts in the presence of ALA-DEF versus GSNO. Data are means.

DISCUSSION

Although the concept of mitomycin C has revolutionized glaucoma filtering surgery, the potential for associated vision-threatening complications remains a concern. As such, a constant search for a novel, less toxic, more controlled antifibrotic therapy still exists.

PDT has been recognized as an effective and controlled method of achieving antiproliferation of a variety of cell types since the late 1980s, yet it has been met with the same skepticism that all new therapeutic modalities face when introduced into the clinical forum.²¹ With increasing reports of its success in treating hyperproliferating tissues throughout the body, interest in its clinical potential has recently heightened.

The selectivity of PDT is partly due to the observation that, in some systems, both endogenous and exogenous photosensitizers tend to accumulate preferentially in actively proliferating tissue and, after a localized application of light energy, the reactive cytotoxic species that are formed tend to be transient.²² PDT is also selective because light energy can be controlled in its location, area, duration, wavelength, and radiant flux. A large number of photosensitizing agents have been reported,¹¹ but none of these current agents seem to have ideal therapeutic properties.

An ideal photosensitizer would selectively accumulate in the proliferating target tissue if administered parenterally, thus minimizing systemic toxicity. If the principal treatment effect were cell death, rather than cell cycle-specific antiproliferation, then the least invasive route of topical application would be preferred, together with minimal treatment time (duration and frequency) and drug dose. The photosensitizer would demonstrate a high rate of activation by localized application of light, with minimal dark reaction, thus minimizing damage to adjacent tissues. Photosensitizing concentrations of the active species would rapidly fall to nontoxic levels once treatment was completed. In this way, the treatment would be highly controlled and titratable and would have minimal effect when exposed to ambient sunlight.

One of the most highly characterized photosensitizing agents is PpIX, which is biosynthesized when cells are treated with ALA.^{15-17,23} It has been used to treat a variety of malignancies and skin disorders.¹¹ The properties of rapid photobleaching and clearance from skin within 24 hours of application, focused interest away from the prototypical hematoporphyrin derivative that was used in many of the early studies.²³

In the present study, ALA was shown to produce photosensitizing concentrations of PpIX in both the V79-4 Chinese Hamster lung fibroblasts and human TC fibroblasts. In accordance with previous reports, the presence of the iron chelator DEF enhanced the accumulation of PpIX¹⁶ but seemed to have its greatest effect at the shortest incubation times. Unlike Smyth et al.¹⁸ who did not demonstrate a specific photodynamic cytotoxicity in human TC fibroblasts with porfimer (Photofrin; QLT Phototherapeutics, Pearl River, NY), a definite phototoxic effect was observed in both the animal lung and human TC fibroblasts with ALA-DEF. However, incubation periods on the order of hours were required to achieve a substantial effect on cell viability, which would not be ideal for use in a surgical setting.

Implicit in the definition of photodynamic therapy is the understanding that cell death is due to the photochemical

generation of reactive oxygen intermediates that cause direct cytotoxicity or indirect damage by compromise of the local vascular system.²⁴ The relatively recent discovery that nitric oxide may also mediate cytotoxicity, by either necrosis or apoptosis,²⁵ has suggested that a novel approach to photodynamic therapy may be the use of nitric oxide-based photosensitizers.

Initial work from our laboratory demonstrated that nitric oxide could be photolytically released from the *S*-nitrosothiol derivative of glutathione, using visible light and could produce a cytotoxic effect on HL-60 leukemia cells.²⁶ The cytotoxicity was shown to be independent of local oxygen concentration and therefore occurred by mechanisms different from those described in classic photodynamic therapy.²⁰ The potential benefits of this mode of therapy were the specific photolytic liberation of nitric oxide from this natural carrier and the fact that the *in vivo* half-life of nitric oxide is on the order of seconds, thus permitting a great deal of control over treatment duration. Further investigations showed that, after photolytic release, nitric oxide could combine with the superoxide anion under certain conditions, producing peroxynitrite, which had a potent cytotoxic effect on human colon adenocarcinoma cells.²⁷

This present study demonstrated that GSNO was rapidly incorporated into both animal lung and human TC fibroblasts after only 2 minutes of contact. A specific cytotoxic effect was observed after light exposure. Dark toxicity was also observed, resulting in approximately 25% cell death. However, because the proposed treatment route would be topical and because the nitric oxide generated would be so short lived, the potential toxicity to local tissues, or the system toxicity, would be expected to be minimal. Studies using an animal model of glaucoma filtering surgery are currently in progress to examine this issue. At a concentration of 0.05 mM, and after no additional incubation beyond the time required for light treatment (10 minutes), GSNO produced a significant degree of cytotoxicity. This was in contrast to ALA-DEF, which performed best after 24 hours of incubation.

In summary, in both standard animal lung and human TC fibroblasts, GSNO effectively inhibited proliferation at concentrations, incubation times, and irradiation times compatible with its potential use in glaucoma filtering surgery. ALA, the more established photosensitizer, was as effective as GSNO only after 24 hours of preirradiation drug treatment. This, and also because ALA must be coadministered with the metal chelator DEF for maximum effectiveness, makes it less attractive for use in filtration surgery. This *in vitro* study demonstrates that GSNO possesses many characteristics that support its potential use as an antiproliferative agent in glaucoma filtering surgery. Although not specifically examined in this report, it is likely that GSNO is cytotoxic to fibroblasts rather than inhibitory at the cell cycle level. In this way, it is at least more similar to mitomycin C than is 5-fluorouracil, and application may be needed only at the time of surgery. However, unlike mitomycin C, the effects of GSNO are more likely to be titratable because the greatest toxicity is related to light exposure, which can be well controlled. There remains the possibility that, analogous to 5-fluorouracil, it may be possible to inject GSNO subconjunctivally in the postoperative period and irradiate to further effect the wound-healing process. *In vivo* studies are currently under way using an animal model of glaucoma filtering surgery to determine whether this antifibro-

blastic effect will translate into increased bleb survival and to assess the specific tissue effects and potential ocular toxicity of this novel photosensitizer.

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