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# Deferoxamine photosensitizes cancer cells in vitro ☆

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**Abstract**

Effect of the iron chelator deferoxamine (DF) on the production of endogenous porphyrins was studied in adenocarcinoma WiDr cells and erythroid K562 cells in vitro. Porphyrin fluorescence was observed in the cells in vitro incubated with DF. The fluorescence spectra recorded in the cells were similar to that of protoporphyrin IX (PpIX). The amount of PpIX generated by DF was around 5% of the ALA effect. Around 90% of the WiDr cells incubated in vitro with DF (0.5 mM, 24 h) and then exposed to light (400–460 nm, 20 min) were photodynamically inactivated. In conclusion, the present study describes a novel approach of using iron chelating agents without 5-aminolevulinic acid (ALA) to photosensitize cancer cells.


*Keywords:* Endogenous porphyrins; Ferrochelatase; Heme biosynthesis; Phototoxicity

Photodynamic therapy (PDT) is a new treatment modality of cancer based on administration of photosensitizing agents (photosensitizers) that together with light kill cancer cells [1,2]. The administration of 5-aminolevulinic acid (ALA), a natural precursor of heme, induces accumulation of the photosensitizer protoporphyrin IX (PpIX) [3,4].

Ferrochelatase converts PpIX into the heme by inserting ferrous iron into PpIX. Thus, the presence of iron chelators may promote the formation of PpIX from ALA [5]. Ethylenediaminetetraacetic acid (EDTA) and deferoxamine (DF) are the most common and clinically used metal chelators [6,7]. The iron chelator DF with high affinity binds iron with a large preference over other metal ions [8,9]. It has been found that combined incubation of ALA and iron chelators in cells in vitro enhances the accumulation of PpIX [10,11]. The effect of an iron chelator alone on the endogenous porphyrins has not been demonstrated so far. In the present study, we report that the iron chelator deferoxamine generates PpIX, and in combination with light photokills cancer cells in vitro.

## Materials and methods

*Chemicals.* Deferoxamine mesylate (DF), 5-aminolevulinic acid hydrochloride (ALA), RPMI-1640 medium, penicillin/streptomycin solution, L-glutamine, trypsin–EDTA, phosphate-buffered saline (PBS), and other chemicals were obtained from Sigma–Aldrich Norway AS (Oslo, Norway). Foetal calf serum was obtained from Gibco-BRL (Life Technologies, Roskilde, Denmark). A 10 mM stock solution of ALA in RPMI-1640 medium was prepared ex tempera before every experiment and then was diluted to a final concentration of 0.2 mM.

*Cell cultivation.* Human WiDr cell line is derived from a primary adenocarcinoma of the rectosigmoid colon [12]. Erythroid K562 cells originate from human chronic myeloid leukaemia [13]. The cells were grown in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% foetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, and incubated in 25 $cm^2$ cell culture flasks (Nunc AS, Roskilde,

☆ *Abbreviations:* ALA, 5-aminolevulinic acid; DF, deferoxamine (desferrioxamine); EDTA, ethylenediaminetetraacetic acid; PDT, photodynamic therapy; PpIX, protoporphyrin IX; rel. u., relative units.

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Denmark) or plastic 12-well dishes (Corning, NY) at 37 °C in a humidified 5% $CO_2$ atmosphere. WiDr cells attach to the bottom of a flask, while K562 cells float as single cells in suspension. Both cell lines were subcultured twice a week. The WiDr cells were subcultured using 0.01% trypsin in 0.02% EDTA. The cell lines were periodically tested for mycoplasma and no contamination was found.

For the experiments, WiDr cells were seeded out into 12-well plates (around $7.5 \times 10^4$ cells, 2 ml medium per well) or 25 $cm^2$ cell culture flasks (around $5 \times 10^5$ cells, 5 ml medium per flask) and incubated for 2 days at 37 °C for proper attachment to the substratum. K562 cells were transferred into 25 $cm^2$ cell culture flasks (around $5 \times 10^5$ cells, 5 ml medium per flask) immediately prior to incubation with a drug. The cells were then exposed to different concentrations of DF, to 0.2 mM DF or to 1 mM ALA, whichever was necessary, in culture medium without serum. The cell viability was not reduced under incubation for 24 h without serum.

*Determination of cell viability.* The survival of the WiDr cells was measured using the colorimetric thiazolyl blue tetrazolium bromide (MTT). The MTT test developed by Mosmann [14] is based on the ability of mitochondrial enzymes to reduce tetrazolium salt (MTT) to purple formazan crystals. The absorbance values therefore allow comparing cell viability. The MTT assay has been reported to be reliable for evaluation of PDT-induced cytotoxicity [15]. MTT was dissolved in phosphate-buffered saline (PBS; 0.01 M; pH 7.4) at 2 mg/ml, filtered to be sterile, and stored at 4 °C. At 24 h after drug incubation, stock solution was added to each well of an assay (50 μl/1 ml medium), and plates were incubated at 37 °C for 4 h. The medium was removed and the cells were washed with ice-cold PBS solution. The formazan crystals were dissolved by adding 200 μl isopropanol per well. The dishes were shaken for 5 min and then 25 μl of the obtained supernatants was transferred from each well into a 96-well microplate and diluted with 175 μl isopropanol per well. The optical density was read at 570 nm on a Multiskan MS (type 352, Labsystems, Helsinki, Finland) plate reader. Untreated control cells were compared with treated cells. Cell viability (cell survival) was expressed as a percentage of viable treated cells relative to untreated control cells.

The survival of the K562 cells was determined by the trypan blue dye exclusion assay [16]. Trypan blue stains dead or dying cells, whereas viable cells are able to repel the dye and do not stain. The K562 cells were incubated with trypan blue for 5 min. An aliquot of 10 μl was pipetted into a chamber of a Glasstic slide (Hycor Biomedical, Garden Grove, CA) and the number of dead cells was counted by a hemocytometer.

*Intracellular formation of PpIX.* The WiDr and K562 cells were incubated with the drugs for 24 h as described in Cell cultivation. After addition of fresh RPMI medium without serum (5 ml), the WiDr cells were removed from the bottom of the flasks by means of a soft cell scraper (Kebo Lab., Norway). The cells were centrifuged and the supernatants were removed. Two millilitres of solvent consisting of 1% sodium dodecyl sulphate (SDS) in 1 N perchloric acid and methanol (1:1 vol/vol) was added into the pellets [17]. After 5 min, the cells were centrifuged and for further measurements the supernatants were used. The production of PpIX was measured spectroscopically using a luminescence spectrometer (Perkin-Elmer LS50B, Norwalk, CT) equipped with a R3896 photomultiplier tube (Hamamatsu, Japan). The fluorescence of porphyrins, mainly PpIX, was excited at 405 nm and the fluorescence emission was measured at 605 nm, corresponding to the maxima of PpIX in the solvent used to digest the cells. A long-pass cut-off filter (530 nm) was used on the emission side of the luminescence spectrometer to block scattered excitation light.

*Phototoxicity studies of PpIX induced by DF in cells in vitro.* For the light exposure experiment, WiDr cells were seeded in plastic 12-well dishes (Corning, NY) at 37 °C in a humidified 5% $CO_2$ atmosphere. The cells were incubated for 24 h with 0, 5, 50, and 500 μM DF, respectively. They were then exposed to light (400–460 nm, maximum at 420 nm, 10 $mW/cm^2$) from four fluorescent tubes (Philips TLK 40 W/03, Eindhoven, The Netherlands) for 0, 2.5, 5, 10, 15, and 20 min. Immediately after light exposure the medium was removed and the cells were washed with ice-cold PBS. The cell viability was determined by the MTT assay as described above. The data represent averages of three parallels in each group. The experiments were repeated two times.

## Results

### *Porphyrin induction by DF*

At concentrations up to 0.5 mM DF was not toxic to the erythroid cells and was only moderately toxic to the adenocarcinoma cells (Fig. 1). Incubation of both cell lines with DF induced formation of porphyrins (Fig. 2). Spectral analysis revealed that mainly PpIX was formed after incubation of the cells with DF (Fig. 3). At a concentration of 1 mM DF induced around 4.5%

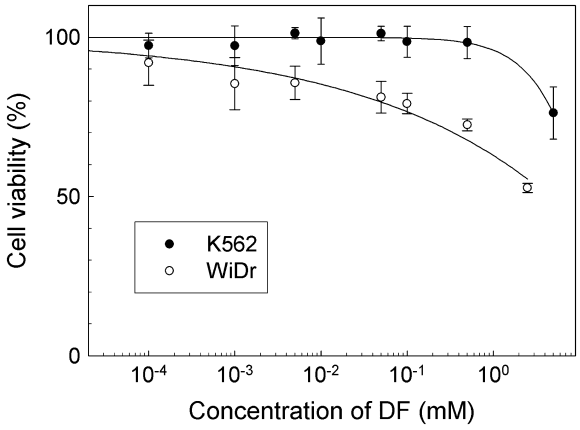


Fig. 1. Cytotoxicity of DF. The cell survival was assayed using MTT (WiDr cells) or trypan blue (K562). The cells were incubated with DF for 24 h. The data are averages of three samples. The error bars represent standard deviation.

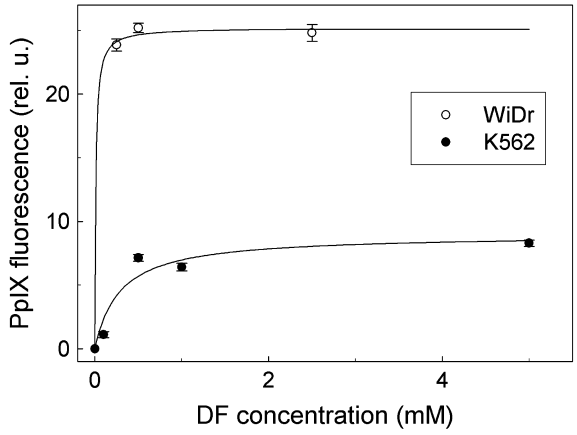


Fig. 2. Accumulation of PpIX in the cells incubated with DF for 24 h. The data are averages of three samples. The error bars represent standard deviation.