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PHOTODYNAMIC ASPECTS OF SOME METAL COMPLEXES

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Abstract. The potential phototoxic capacity of some metal compounds of clinical significance in dermatology has been investigated by means of the photohemolysis technique. No photosensitized hemolysis of erythrocytes was observed with the chromium, nickel, copper and cobalt compounds studied. On the contrary, nickel and cobalt compounds proved to be efficient in quenching singlet oxygen mediated photo-oxidative membrane damage of red blood cells. Cadmium compounds showed a pronounced photohemolytic activity and the reaction was oxygen dependent. The deuterium test for singlet oxygen showed a significant increase in photohemolytic efficiency. The addition of histidine, a known quencher of singlet oxygen, resulted in a significant inhibition of the photohemolysis. Pronounced photo-oxidative damage to plasma membranes was also observed in vitro cultivated cells by means of scanning electron microscopy.

Key words: Metal compounds; Cadmium sulphide; Photo-oxidative damage; Photohemolysis; Electron microscopy scanning

Several metal compounds which are capable of inducing allergic contact dermatitis in humans also interfere with ultraviolet light irradiation in different ways. Some of these compounds probably act as photosensitizing agents, but others seem to have an inhibitory effect on ultraviolet light induced reactions.

Photodynamic action may be characterized as the oxygen-dependent, sensitized photo-oxidation of a biological substance. Two major primary mechanisms are found to occur. Type I, a direct reaction between sensitizer triplets and the oxidizable substrate, followed eventually by reaction with molecular oxygen. Type II, a reaction which involves the transfer of excitation energy from the triplet sensitizer to molecular oxygen, with the formation of singlet excited oxygen which then may react with the substrate (5, 10, 19).

It is known that several biological photosensitizers act by the way of singlet oxygen which is considered to be an important intermediary in photo-oxidative reactions. Examples of such endogenously occurring photosensitizers are bilirubin (2, 16), the tryptophan metabolite kynurenic acid (15, 21) and probably protoporphyrin (11, 13, 15).

In clinical dermatology, it has been suggested that light sensitivity occurs in association with the following metals: cadmium and various chromium salts. Other metals of clinical importance are not known to possess photosensitizing capacity.

Cadmium in the form of cadmium sulphide, when used as a yellow pigment in tattoos, regularly induces erythema and oedema on exposure to sunlight (1, 7). The reaction is seen on the first exposure and is considered to be phototoxic. Longwave ultraviolet light is responsible for the reaction (1). Little is known about the way in which this photosensitive reaction is mediated.

Patients with chromium dermatitis have been said to exhibit light sensitivity which may thus be one factor in the explanation of the seasonal variations observed (4, 8, 22, 23). In photochemistry, various chromium complexes are known to interact with singlet oxygen mechanisms in a rather complex manner (3, 18).

The purpose of the present study was to investigate the potential phototoxic capacity of some metal compounds of clinical significance regarding damage to biological membranes, as registered by the use of the photohemolysis technique (9, 17, 20), but photo-oxidative damage induced by cadmium sulphide has also been studied by means of in vitro cultivated cells and scanning electron microscopy, using a method reported recently (24).

Furthermore, the biological relevance of the singlet oxygen quenching capacity of nickel and cobalt compounds was studied in reactions induced by the endogenously occurring metabolite, kynurenic acid.

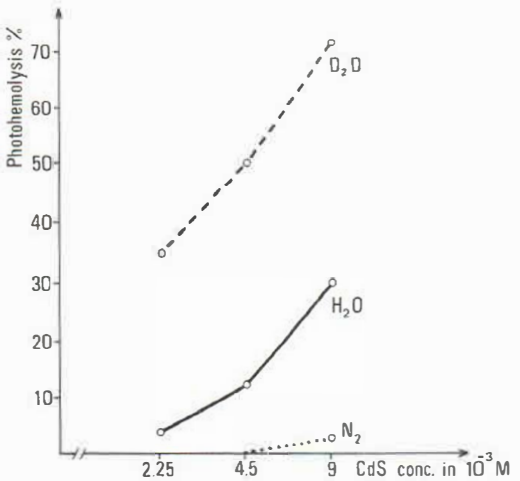


Fig. 1. The degree of photohemolysis as a function of concentration of cadmium sulphide. The experiments were performed with D_2O and H_2O as solvents and in deoxygenated blood (N_2).

MATERIAL AND METHODS

Photohemolysis

The experiments were performed with the photohemolysis technique described by Peterka et al. (17), modified by Kahn & Fleischaker (9) and earlier used in our laboratories (15, 20, 21).

Investigated substances were dissolved in 0.02 M phosphate buffer, pH 7.4, containing 0.14 M NaCl based either on H_2O or D_2O (99.8%). AB Atomenergi, Studsvik, Sweden).

The metal compounds investigated $K_2Cr_2O_7$, Na_2CrO_4, $CrCl_3$, $NiSO_4$, $CoCl_2$, $CuSO_4$, $CuCl_2$, $CdCl_2$, CdS, were tested in the following concentrations, 2, 4, 8, 16, 32 and 64 mg per 100 ml. Molar concentrations of CdS were 2.25×10^{-3}, 4.5×10^{-3}, 9×10^{-3} M corresponding to 32, 64 and 132 mg per 100 ml. These higher concentrations of CdS were used in the experiments with longwave ultraviolet light (UVA) under experimental conditions described below. In quenching experiments with kynurenic acid in a concentration of 5.3×10^{-3} M, $NiSO_4$ and $CoCl_2$ were added in the molar concentrations of 0.4×10^{-3}, 0.75×10^{-3}, 1.5×10^{-3}, 3×10^{-3}, 6×10^{-3} and 12×10^{-3} M.

Packed human red blood cells were washed three times in physiological saline and 0.1 ml was then added to 10 ml of the buffered solutions mentioned above, and poured into 2 mm quartz cuvettes. Controls were incubated in the dark at 37°C. Test suspensions were exposed to ultraviolet light after about 10 min, which was considered sufficient for equilibration of the two types of water. Two types of ultraviolet sources were used. For screening purposes an Osram high pressure xenon arc lamp, XBO 150 W, in a Zeiss microscope lamp house with a quartz collector was used. Irradiations were performed at a distance of 40 cm from the lamp aperture to the test cuvettes. The lamp was equipped with a Schott WG 295 filter to give a sun-spectrum-like radiation. The intensity of the beam under these conditions was 48 mW cm^{-2} and exposure time 0.3×10^3 sec. For selected studies on cadmium compounds and in the quenching experiments with kynurenic acid, nickel and cobalt salts, the investigations were supplemented with irradiation from a Black-Ray B-100 A lamp (Ultra Violet Products Inc.) giving a longwave ultraviolet radiation (UVA) ranging from 350 to 380 nm, with its intensity maximum at 366 nm. In these experiments irradiations were performed at a distance of 12 cm. The intensity of the lamp at this distance was 6.5 mW cm^{-2}. Measurements were performed with a Hewlett & Packard Radiant Flux meter.

After irradiation the test suspensions were centrifuged at 2000 rpm and the optical density of the supernatant fluid determined at 540 nm on a Beckman DB Spectrophotometer after an incubation time of 2 h at 37°C. Results were compared with the dark control and with a total hemolysis control (9, 20), and expressed in percent relative to the 100% hemolysed specimen.

In order to study the oxygen dependence of the photohemolytic reaction induced by cadmium sulphide, the blood was deoxygenated by bubbling nitrogen through the sample for 2 min before the irradiation. The deuterium effect for identification of singlet oxygen (12, 13, 14, 15, 21) in photohemolysis induced by cadmium sulphide was studied by replacing the solvent H_2O by D_2O. The H_2O content of red cell suspensions with D_2O as a solvent, was calculated to be less than 1%. In the dark, no hemolysis was observed after 45 min, either in H_2O or in D_2O. Furthermore, inhibition studies were performed by adding the singlet oxygen quencher histidine (14) in a concentration of 3.2×10^{-4} M.

Cell lines and culture conditions

Experiments with in vitro cultivated cells were performed on normal diploid human glia cells by using the method established and reported in a previous investigation (24). The cells were grown in Eagle's minimal essential medium supplemented with 10% calf serum and antibiotics (100 U/ml of penicillin, 50 μg/ml of streptomycin and 1.25 μg/ml of streptomycin and 1.25 μg/ml of amphotericin B) on 12 mm round glass coverslips no. 0, placed on the bottom of plastic Petri dishes. The cells were harvested 3 days after subcultivation when they were actively dividing, though not yet forming a confluent monolayer.

Scanning electron microscopy (SEM)

Alterations induced by longwave ultraviolet light (UVA) from the Black-Ray lamp and cadmium sulphide in the concentrations of 4.5×10^{-3} and 9×10^{-3} M were studied. The period of incubation prior to irradiation was 1 hour. Exposure time was 1.2×10^3 sec.

Subsequent to fixation for 60 min at 37°C in 2% glutaraldehyde in 0.1 M Na-cacodylate HCl buffer with 0.1 M sucrose (pH 7.2; total osmolality=510 mOsmol; vehicle osmolality=300 mOsmol), the cells were post-fixed in 2% OsO_4 in *s*-collidine buffer (pH 7.2) for 90 min at 22°C. A brief rinse in 0.15 M cac buffer (pH 7.2) at 22°C was interposed between the two fixations. Care was taken not to dry the cells during the replacement of medium with the aforementioned glutaraldehyde fixative, warmed to 37°C. After post-fixation, dehydration was performed in a

graded series (50%, 70%, 75%, 80%, 85%, 90%, 95%, 100%) of ethanol. The cells were then brought to acetone and critical-point dried from CO_2 in a Polaron E 3000 apparatus. After mounting on stubs with silver conductive paint, the specimens were coated with a 300 Å thick layer of gold in a Polaron sputter apparatus. The specimens studied in a Jeol JSM-SL microscope at 10 kV (24).

RESULTS

Photohemolysis

No significant photosensitized hemolysis of red blood cells was observed with the chromium compounds investigated under the experimental conditions used, when compared with controls. Similar negative results were obtained with the nickel, copper and cobalt compounds studied.

By contrast, when cadmium chloride was tested for sensitizing action in this system using the xenon arc lamp, a significant photohemolysis was observed, augmented with increasing concentrations of cadmium and reaching a maximum of 30% hemolysis. Cadmium sulphide was also photohemolytically active with a roughly similar percentage of hemolysis, despite the insolubility of the substance, which had to be used as a suspension not completely dissolved in the buffer. When irradiations with exclusively UVA were performed, a similar degree of hemolysis was seen as in the experiments with the xenon arc lamp.

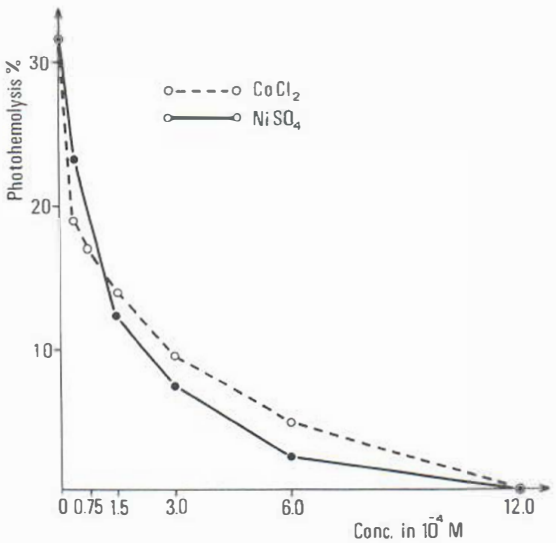


Fig. 2. Inhibition of photohemolysis induced by kynurenic acid in a concentration of 5.3×10^{-3} M, by the addition of $NiSO_4$ (solid line) and $CoCl_2$ (dotted line) in increasing concentrations.

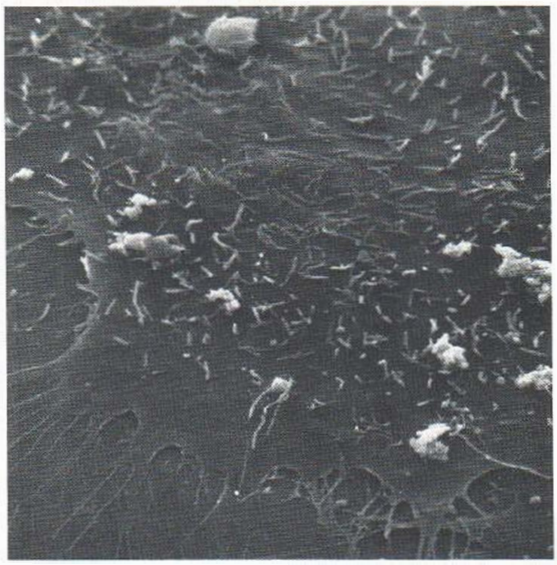
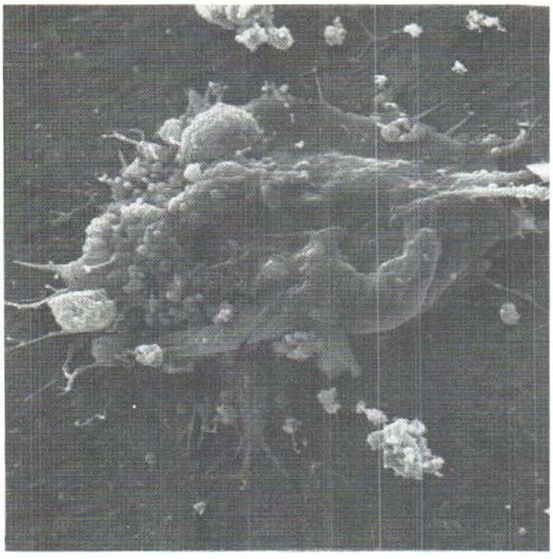


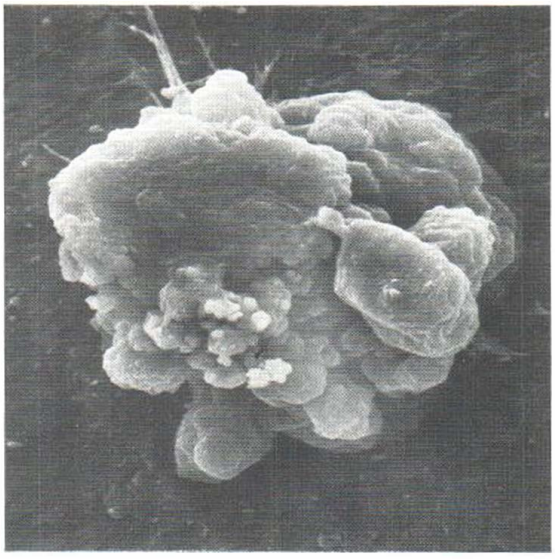
Fig. 3. Scanning electron microscope picture of a control glia cell treated with cadmium sulphide only (9×10^{-3} M) and no UVA irradiation. The cell show a quite normal appearance (×3400).

The oxygen dependence of the photochemical reaction induced by cadmium sulphide and UVA was studied by bubbling nitrogen through the sample in order to deoxygenate the blood. The degree of photohemolysis was 30% in the control sample, while only 2.5% was found in the deoxygenated blood (Fig. 1). The deuterium test for singlet oxygen showed a significant increase in the photohemolytic efficiency in the perdenterated solvent which at the lower sensitizer level amounts to a factor of 10, though the increase is smaller at the higher sensitizer concentrations, with 73% photohemolysis in D_2O when compared with 30% in H_2O for the highest concentration of cadmium sulphide used (Fig. 1). The addition of histidine in a concentration of 3.2×10^{-4} M to cadmium sulphide (9×10^{-3} M) in H_2O caused a slight though significant inhibition of the photohemolysis, from 30% to 20%.

Singlet oxygen quenching capacity of nickel and cobalt compounds

When the singlet oxygen quenching capacity of nickel and cobalt compounds was studied on photohemolysis induced by kynurenic acid and UVA, a considerable inhibition was observed and the photohemolysis was reduced from 32% to 0% with about the same efficiency in both metals (Fig. 2).





Figs. 4–5. Surface changes of glia cells treated with cadmium sulphide (9.0×10^{-3} M) and UVA (1.2×10^{3} sec) as shown in the scanning electron microscope. Advanced cellular damage with swelling and blebbing of the cells is noted (Fig. 4, ×1 800; Fig. 5, ×2 500).

Scanning electron microscopy (SEM)

The alterations caused by cadmium sulphide and UVA on in vitro cultivated cells when studied by scanning electron microscopy (SEM) consisted of advanced cellular damage with enormous swelling and blebbing of the cells and the development of retraction fibrils (Figs. 4, 5). Cells treated with cadmium sulphide only and no UVA irradiation showed a quite normal appearance with no further alterations (Fig. 3).

DISCUSSION

The photosensitizing capacity of various metal compounds of clinical importance in dermatology has been investigated with regard to expected photo-oxidative damage of cellular membranes. The technique of photohemolysis has been used, which is a reproducible and standardizable method suitable for the selective study of photobiological problems. It can be used for screening purposes on substances expected to cause photo-oxidative reactions resulting in cellular damage. However, photosensitizers which do not require molecular oxygen for their action and/or react with nuclear material will be ineffective in this system. Nevertheless, erythrocytes offer a unique material for the study of cell membrane photosensitization without other complicating structures and several important endogenously occurring photoactive substances such as protoporphyrin (11, 17), bilirubin (16) and kynurenic acid (21) are known to induce a pronounced photohemolysis. Similar photo-oxidative membrane damaged is seen with many other commonly used pharmacological photosensitizers, viz. chlorpromazine, demethylchlortetracycline, griseofulvin, etc. (9, 15, 20).

Concerning chromium compounds, earlier findings have indicated a possible photosensitizing capacity in patients with chromium dermatitis (4, 22, 23). Furthermore, it has been shown that the excited state of some chromium complexes can be quenched by molecular oxygen to give singlet oxygen, but the reverse process—singlet oxygen quenching by metal chelates—is also conceivable (3, 18). In the present study no significant hemolysis of red blood cells was observed with the chromium compounds investigated under the prevailing experimental conditions. Further studies on the probable photosensitizing capacity of these substances in other in vitro and in vivo systems will be required and standardized photopatch testing of patients with chromium dermatitis seems necessary. These investigations are now in progress.

The nickel compounds investigated failed to elicit photohemolysis. Nor are clinical photoreactions in patients with manifest nickel dermatitis known to

occur. Interestingly enough, nickel and cobalt compounds proved to be efficient in quenching singlet oxygen mediated photo-oxidative membrane damage, here provoked by the use of kynurenic acid (Fig. 2). The biological implications of this finding are uncertain but may be of importance amongst other quenchers of singlet oxygen in pathological reactions mediated by this mechanism.

Cadmium sulphide is used in the production of photoelectric cells, and thin crystals show absorption from 510 nm through the region of longwave and shortwave ultraviolet light (6).

Phototoxic reactions are known to occur in patients where cadmium sulphide has been used in tattoos and longwave ultraviolet light has been considered responsible for the reaction (1). This concept is confirmed by the present study where photohemolysis could be elicited by UVA irradiation exclusively. Little has been known about how this photosensitized reaction is mediated. The present data reveals that the reaction is strictly oxygen dependent (Fig. 1). In the deuterium test for singlet oxygen reactions the expected increase in lifetime of singlet oxygen would be around tenfold, giving a corresponding increase in photohemolysis. The photohemolytic efficiency of cadmium sulphide is increased in the perdeuterated solvent, as compared with water, which at the lower sensitizer level amounts to a factor of 10 (Fig. 1). The smaller effect observed at the higher sensitizer concentrations may be ascribed to the fact that the extensive photohemolysis observed in D_2O at these levels approaches its maximum, and is no longer proportional to the number of primary chemical events. These findings seem to indicate (at least in part) a singlet oxygen pathway in photoreactions induced by cadmium compounds, which is further corroborated by the fact that the singlet oxygen quencher histidine, when added, gives a significant inhibition of the photohemolysis.

In the present study cadmium sulphide showed a pronounced capacity for photo-oxidative damage to biological membranes, as seen in the experiments performed with red blood cells. Similarly, pronounced damage was also seen in in vitro cultivated cells with conspicuous plasma membrane alterations revealed by scanning electron microscopy (Figs. 4, 5). Whether or not other cellular constituents are damaged by cadmium sulphide and UVA remains to be investigated by means of transmission electron microscopy.

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