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THE ROLE OF OXYGEN IN THE PHOTODAMAGING EFFECT ON ERYTHROCYTES OF SOME PHOTOALLERGIC AND PHOTOTOXIC COMPOUNDS

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Abstract. Red blood cells irradiated with longwave ultraviolet light in the presence of a number of photoallergic or phototoxic compounds showed a marked loss of K^+ ions. This process was strongly restricted by anaerobic conditions. During irradiation, oxygen was consumed. Since pre-irradiated photosensitizers were not toxic to erythrocytes, it is concluded that during irradiation, oxidative processes take place in the erythrocyte. The concentrations of the investigated photoallergic compounds required to induce photohaemolysis, were several times higher than those required for the phototoxic compound protoporphyrin. These findings indicate that photoallergic compounds, like the photodynamic compounds, also have photo-oxidative capacities, but to a much lower degree.

Key words: Photosensitizers; Photohaemolysis; Photo-oxidation

Dermatology knows two groups of chemicals capable of causing photosensitivity reactions in the skin, i.e. phototoxic and photoallergic compounds (3, 5), which are called photosensitizers. Clinically, one of the main differences between a phototoxic and a photoallergic reaction is that the former develops after the first exposure and is dose-dependent, whereas the latter is a delayed reaction and is not strictly dose-dependent.

Photoallergic compounds are used mainly as drugs and disinfectants. Their clinical effects on the skin have been described by Harber (5). The mechanism underlying the action of these compounds differs from that of phototoxic reactions. The work of Sulzer (21) has shown that binding of a photoallergic compound to a protein during exposure to light leads to the formation of what is called a photohapten. As a secondary reaction, the body produces antibodies directed against the photohapten, and this process can eventually result in an immunological reaction and inflammation.

Both photoallergic and phototoxic compounds have been investigated in in vitro experiments, particularly as regards their action on erythrocytes and yeast cells. The aim of these studies was to analyse their action mechanism, to test new drugs and disinfectants with respect to photosensitizing properties, and to search for inhibitors of the sensitizing reactions (6, 10, 11, 12, 18, 19, 20) or to find evidence of the existence of an endogenous sensitizer (23). The biochemical basis of the reaction caused by photoallergens in vitro is still unknown. In the type of experiment undertaken in this study an immunological reaction can be avoided. Possible mechanisms include a photo-oxidative process, damage inflicted on the erythrocyte at binding of the photoallergic compound to the cell membrane, and the formation of toxic compounds.

An example of an oxidative process is the photohaemolysis caused by protoporphyrin, which has been studied extensively (6, 18, 19, 20). In this case certain amino acids in the erythrocyte membrane are oxidized (20). According to Goldstein (4), oxidation of lipids also plays a role in this photohaemolytic process.

Binding of a photosensitizer to the erythrocyte membrane might disturb the functional and structural integrity of the cell membrane. Such binding to erythrocytes is known in the case of the organic mercurials parachlorobenzoate (PCMB) (1) and parachloromercuribenzene sulfonate (PCMBS) (22).

A third possibility is that erythrocytes might be poisoned by a photochemically altered photo-

Table 1. *Loss of potassium ions by erythrocytes (0.5% suspension) in the presence of photosensitizer irradiated for 30 min under aerobic and anaerobic conditions*

Photosensitizer	Concentration (mmol)	% K^+ efflux	
		Under aerobic conditions	Under anaerobic conditions
Bithionol	0.007	35	20
Tribromsalicylanilide	0.05	55	40
Promethazine	0.15	40	20
Protoporphyrin	0.0035	60	20
Anthracene	0.017	60	15
Demethylchlortetracycline	0.4	70	40
Sulfanilamide	3.2	45	15
Chlorpromazine	0.065	50	25

sensitizer, primarily resulting, for example, in a disturbance of the enzyme metabolism of the cell.

The present study was performed in order to gather more information about the biochemical basis of the haemolysis induced by various photosensitizers. Photohaemolysis experiments were performed in vitro in the presence and absence of oxygen, and the oxygen consumption was determined in relation to the injurious effects on the red blood cells. In addition, the loss of potassium ions (K^+ efflux) by erythrocytes was measured.

MATERIALS AND METHODS

Eight photosensitizers were tested in this study, i.e., three photoallergic compounds: bithionol (obtained from K. & K. Laboratories), tribromsalicylanilide (from the same source), and promethazine (Specia); three phototoxic compounds: protoporphyrin methylester (Fluka), anthracene (from the same source), and demethylchlortetracycline (Lederle Laboratories Division) and also two compounds which, according to Harber (5), are capable of inducing both types of photosensitizing reactions: sulfanilamide (Fluka) and chlorpromazine (Specia).

Blood was obtained from healthy volunteers and heparinized. After centrifugation, the plasma and the buffy coat were removed. The erythrocytes were washed twice in an isotonic buffered NaCl solution (pH 7.4) (17).

Erythrocyte suspensions of 0.5 and 2.5% in buffered saline solution containing 0.05 mM glucose were used. Stock solutions of the photosensitizers were made in methanol or acetone, and 0.2 to 0.6 ml of this stock solution was added to 10 ml erythrocyte suspensions.

The suspension was then irradiated in a Pyrex glass cuvette of a Gilson oxygraph provided with a micro-Clark electrode (15). During irradiation the suspension was agitated with a magnetic stirrer. For irradiation of the samples containing protoporphyrin, a normal super high pressure mercury lamp (Philips HPL 125 W) was used. The other photosensitizers were irradiated with the same lamp, but surrounded by a Pyrex glass balloon. The distance between lamp and cuvette was always 5 cm; the output of the HPL lamp, measured with a thermopile (Kipp) provided with a Schott KGl filter, was 5 mWatt/cm² and with the Pyrex balloon 8 mWatt/cm².

Anaerobic conditions were provided by adding 3 I.U. glucose oxidase per ml suspension, after which the cuvette was closed and all the dissolved oxygen was consumed by oxidation of the glucose. The moment at which oxygen exhaustion occurred could be read on the recorder of the oxygraph, after which irradiation was started.

The temperature was regulated by a thermostat (Haake FK 10). The K^+ efflux was measured by a flame photometer (Kipp). All experiments were carried out several times at various concentrations of sensitizer and erythrocytes.

RESULTS

Four series of experiments were performed in this study:

1. *Determination of the influence of anaerobic conditions on the K^+ efflux from erythrocytes in the presence of various photosensitizers and light*

A well-known parameter for the extent of photochemical damage to erythrocytes is the determination of the loss of K^+ ions from these cells (14, 16). Table I shows that under aerobic conditions roughly 50% K^+ efflux was found after 30 minutes of irradiation. Distinctly lower values were obtained under anaerobic conditions, which points to a marked inhibitory factor. The general inhibitory effect did not alter significantly when the photosensitizer concentration and the irradiation time were decreased or increased.

Sulfanilamide and demethylchlortetracycline induced a definite K^+ loss only when relatively high concentrations were used.

Control experiments performed with glucose oxidase showed that this enzyme, which was added in order to obtain anaerobic conditions, did not alter the sensitivity of the erythrocytes to photochemical reactions. The small amount of organic solvent also had no influence on the erythrocytes.

2. *Determination of the oxygen consumption by the erythrocytes–photosensitizer mixture in the presence of light*

The results of these experiments are given in Table II. A 2.5% erythrocyte suspension was used and

Table II. *Consumption of oxygen by erythrocytes (2.5 % solution) in the presence of photosensitizer irradiated for 90 min at 4°C in air-saturated buffer solution*

Photosensitizer	Applied concentration (mMol)	% K^+ efflux	Consumed nM O_2 in 90 min	Consumed nM O_2 in 90 min/0.1 mM sensitizer (calculated)
Bithionol	0.015	60	100	670
Tribromsalicylanilide	0.1	50	210	210
Promethazine	0.6	60	215	360
Protoporphyrin	0.007	45	290	140
Anthracene	0.05	50	125	250
Demethylchlortetracycline	0.4	40	600	150
Sulfanilamide	13.6	35	680	5
Chlorpromazine	0.13	75	130	100

concentrations of the photosensitizers were taken such that the oxygen consumption could be measured and a marked K^+ loss would be obtained during an irradiation period of 90 minutes.

All of the photosensitizers under study consumed oxygen. The amount of oxygen consumed per mmol photosensitizer was calculated. Protoporphyrin showed the highest consumption per mmol, and sulfanilamide the lowest. In control experiments without photosensitizer no decrease in oxygen pressure was observed. With respect to the K^+ loss from the erythrocytes, bithionol caused a marked K^+ efflux even without irradiation. The other compounds did not lead to this phenomenon.

3. *Oxygen consumption by irradiated photosensitizers in the absence of erythrocytes*

To find out whether photosensitizers are able to take up oxygen during irradiation, the experiments listed in Table II were repeated but without the addition of erythrocytes. Under these conditions, all of the solutions except those containing protoporphyrin (Table III) showed a marked uptake of oxygen. The amount of protoporphyrin used in these experiments was apparently too low to permit the measurement of oxygen consumption.

4. *Determination of K^+ efflux by erythrocytes in the presence of pre-irradiated photosensitizers*

To ascertain whether toxic compounds were formed during irradiation, the sensitizers were irradiated in an oxygen-containing buffer, added to red blood cell suspensions, and then kept in the dark for 90 minutes.

In none of these cases was a higher K^+ loss found than in those in which the non-irradiated photosensitizer had been added.

DISCUSSION

When the results of our experiments are summarized, we arrive at the following findings:

(a) The K^+ loss decreased markedly after exhaustion of the oxygen content.

Table III. *Effect of photosensitizers in 1.8 ml air-saturated buffer solution at 4°C irradiated for 10 min*

Photosensitizer	Concentration (mMol)	Consumed nM O_2 in 10 min	Consumed nM O_2 in 10 min/0.1 mM photosensitizer (calculated)
Bithionol	0.015	35	233.3
Tribromsalicylanilide	0.1	390	390.0
Promethazine	0.6	515	85.8
Protoporphyrin	0.007	–	–
Anthracene	0.05	400	800.0
Demethylchlortetracycline	0.4	30	7.5
Sulfanilamide	13.6	135	1.0
Chlorpromazine	0.13	390	300.0

(*b*) During irradiation of the erythrocyte–photosensitizer solutions the consumption of oxygen could be demonstrated, the highest being found for protoporphyrin.

(*c*) All photosensitizers (except protoporphyrin) consumed oxygen during irradiation.

(*d*) Pre-irradiated sensitizers did not damage red blood cells.

From these findings it may be concluded that the haemolytic activities of the investigated photosensitizers are of the oxidative type.

It does not seem likely that the oxygen is used for the conversion of the sensitizer into a toxic compound that caused the K^+ efflux in our experiments, as the irradiated sensitizers did not have a toxic effect when added to erythrocytes. However, Johnson found that pre-irradiated chlorpromazine caused haemolysis when added to red blood cells (8, 9). This controversial finding can probably be explained by two factors which differed from the conditions prevailing in our experiments: Johnson used stronger light during a short period of irradiation and his use of Petri dishes meant that a large surface area of his solutions was exposed to the air.

The possibility that binding between sensitizer and erythrocyte takes place during irradiation cannot be excluded. From the work of Alani (1) it is known that photoallergic compounds are able to bind to a protein during irradiation. This has been demonstrated for, among others, chlorpromazine (2) and salicylanilides (7). Such binding might also explain why photohaemolysis can only be induced by these compounds when relatively high concentrations are used. Another explanation for the need for these high concentrations may be that the photoallergic compounds in question only have the capacity to oxidize special targets in the cell membrane, for example sulfhydryl groups. The fact that photohaemolysis can be induced in vitro not only by phototoxic compounds but, under certain conditions, also by photoallergic compounds, merits further investigation. Studies to determine which kinds of biochemical compounds can be photo-oxidized by photoallergic and phototoxic compounds are in progress.

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