

IRRADIATION OF EPIDERMAL CELL SUSPENSIONS IN THE PRESENCE AND ABSENCE OF PROTOPORPHYRIN

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Abstract. It is not known with certainty whether the epidermis of patients with erythropoietic protoporphyria (EPP) reacts primarily to irradiation or secondarily to lesions in the dermis. In this investigation suspensions of epidermal cells obtained from suction blisters were irradiated with violet light in the presence and absence of protoporphyrin. Differences between the controls and protoporphyrin-treated cells were not observed with any of the methods used. Furthermore, it was found that the epidermis of EPP patients does not contain measurable amounts of protoporphyrin. It is therefore suggested that the epidermal lesions in EPP patients are secondary to dermal lesions.

Erythropoietic protoporphyria (EPP) is a dominantly inherited disorder characterized by sensitivity to light with a wavelength of about 400 nm (5). The patients have an elevated protoporphyrin level in erythrocytes, faeces, and plasma. Erythrocytes of EPP patients haemolyse when irradiated with light with a wavelength of about 400 nm, in the presence of oxygen. Haemolysis also occurs when normal erythrocytes to which protoporphyrin has been added are irradiated under the same conditions (6).

It is not yet known exactly what happens in the skin of EPP patients under irradiation. Do the epidermis cells react primarily to irradiation in the presence of protoporphyrin or are the epidermal changes secondary to lesions in the underlying dermis?

The present investigation was done to determine the effect of irradiation with light with a wavelength of about 400 nm on a suspension of epidermal cells, with and without added protoporphyrin, in the presence of oxygen.

METHODS AND RESULTS

Epidermis was obtained by making suction blisters according to Kiistala (4). The cup carried an adapter-plate containing nineteen holes ($\varnothing$ 6 mm), and the

apparatus was placed on the shaved abdominal skin of adult guinea pigs (weight about 1 kg) for 2 to 3 hours at a pressure of -220 to -230 mmHg. The animals were anaesthetized with 0.4 ml Nembutal (60 mg/ml, Abbott). After removal of the apparatus the skin was disinfected with 70% alcohol and the blister roofs were removed. The fragments of epidermis were placed in TC Medium 199 w/o phenol red (Difco) and kept at 37°C for about 1 hour before being trypsinized with 0.25% trypsin (British Drug Houses) in a solution containing 7% sucrose and 0.1% glucose in phosphate buffer (pH 7.2 to 7.4), to obtain a cell suspension.

Because the cells proved to be very vulnerable shortly after trypsinization, which removed the cell coat, they were incubated overnight in a culture medium containing 70% medium 199, 20% human serum, and 10% embryonic extract, to permit them to recover.

The cell suspension was then divided into 4 roller tubes. Protoporphyrin (in quantities of between 0.14 and 14 μ g/ml) was added to two of these tubes, and immediately afterward one drop of suspension was taken from each tube and stained with trypan-blue. As a rule, at this moment between 20 and 25% of the cells took up the dye (in 20 experiments), indicating that those cells had not recovered. Two tubes, one with and one without added protoporphyrin, were irradiated with violet light (high-pressure Hg bulb, Philips 57236E/74 HPL-N 125 W; distance 10-20 cm)¹ shortly after the addition of the protoporphyrin. The remaining two tubes were kept in the dark throughout. During irradiation, one drop of cell suspension was taken from the tubes at 30-

¹ This is the same light source as has been used in the photohaemolysis experiments (6); in these experiments haemolysis did not take place after irradiation of normal red cells.

min intervals and stained with trypan-blue. Up to 60 minutes no increase of trypan-blue stainable cells was seen. After 90 min, the percentage of cells taking up the dye increased in the irradiated suspensions to 30-40, but there was no difference between the cell suspensions with any of the added concentrations and those without protoporphyrin. After 2 hours of irradiation the percentage of cells taking up trypan-blue was 50 to 70, irrespective of whether protoporphyrin had been added or not, and the total number of cells had decreased sharply due to disintegration. In the control tubes, both with and without protoporphyrin, even after 2 hours no increase in the percentage of trypan-blue stainable cells was seen.

On the basis of these results, it should be concluded that irradiation with a mercury lamp is rather toxic for epidermal cells, and that the addition of different amounts of protoporphyrin does not alter the reaction pattern. However, the question arose as to whether the cell suspensions contained sufficient amounts of protoporphyrin, which is known to be a very labile compound that is quickly broken down by light. Consequently, during one of the experiments a 1-ml sample of the cell suspension containing added protoporphyrin was taken before and 30 min after irradiation. The protoporphyrin determinations, performed as described elsewhere (3), showed that less than 10% of the added amount of protoporphyrin (14 $\mu\text{g/ml}$) remained in the irradiated sample. No decrease was observed in the non-irradiated samples.

Because this approach did not make it possible to demonstrate differences between cells irradiated in the presence and absence of added protoporphyrin, another method was sought to demonstrate any changes not detectable with trypan-blue staining. It was decided to start by looking at the cells in the ordinary light microscope, but it turned out to be very difficult to make good slides of the cell suspensions for light microscopical examination. The cells did not stick to the glass when fixed in the wet state, and drying before fixation altered their appearance. To solve this problem, phase-contrast microscopy was used, which enabled us to study the structure of living cells. For this purpose Falcon culture discs (3010, 60 $\times$ 15 mm, with absorbent ring) were used. The centre compartment was provided with a grid before being filled with culture medium (70% medium 199 and 30% serum) to the level of the grid, on which a piece of atoxic cellophane (washed with distilled water and sterilized) was placed. The cell suspension

was then placed on the cellophane. Nutrients and waste products could diffuse through the cellophane and oxygen could be taken up from the air. The uptake of oxygen was thought to be better in this way than in roller tubes, because of the absence of a thick liquid layer between the cells and the air.

After overnight incubation, a wet slide was made with the cells on the cellophane for phase-contrast microscopy before and after irradiation. The time after which the cells began to change and the kind of changes were established as criteria. Again, there were no differences in ten experiments between cells irradiated in the presence and absence of protoporphyrin (in the same amounts as mentioned above). Overnight incubation in a medium containing protoporphyrin (50 $\mu\text{g}/100\text{ ml}$, as can occur in the blister fluid of EPP patients) (3) also gave no differences with respect to the controls in four experiments. After 30 min of irradiation all of the cells showed changes, however. The cell membrane gave rise to a large bubble and in the nucleus a Brownian movement became visible, indicating decreased viscosity due to the breakdown of large molecular compounds. Longer irradiation led to complete destruction of the cells.

DISCUSSION

Since no specific changes could be demonstrated in epidermal cells after irradiation in the presence of protoporphyrin, the question was raised of whether the epidermis of EPP patients contains protoporphyrin. A method had meanwhile been developed to determine very small amounts of protoporphyrin (3). This method had been applied to epidermis of EPP patients obtained from suction blisters. These experiments showed that the epidermis of EPP patients did not contain a measurable amount of protoporphyrin. This observation is in contrast with the findings in the blister fluid, which clearly contained protoporphyrin in all of the cases and in some even more than was found in the plasma.

The literature indicates that the most important pathological changes in the skin lesions of EPP patients as visible with the light microscope occur in the upper level of the dermis, where amorphous material is found in and around the capillary walls (5). Electron microscopically, too, pathological changes are only found in the dermis (1). Only in biopsy samples of skin taken at the site of swelling provoked by irradiation, have lipid droplets also

been seen in the basal layers of the epidermis (2), but this may be secondary to injuries of the dermis.

CONCLUSION

Because of the great sensitivity of epidermal cells to light and the rapid breakdown of protoporphyrin by light, an increased sensitivity in vitro of epidermal cells to light in the presence of protoporphyrin could not be demonstrated by the methods described here. In addition, no protoporphyrin could be demonstrated in the epidermis of EPP patients. It is therefore thought to be more likely that the epidermis is affected secondarily, which is also suggested by the results of previous histological and electron-microscopical investigations that have been reported in the literature.

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