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Effects of Whole Body UV-Irradiation on Oxygen Delivery from the Erythrocyte*

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Summary. In 16 healthy caucasian volunteers (mean age: 22.2 years) the influence of whole body UV-irradiation on the oxygen transport properties of erythrocytes was investigated. Four hours after irradiation with UV (using the minimal erythema dose, MED) no variation of haemoglobin concentration, hematocrit, mean corpuscular haemoglobin concentration, pH or standard bicarbonate could be found, whereas inorganic plasma phosphate (P_i), calcium, the intraeryhtrocytic 2,3-diphosphoglycerate (2,3-DPG), the activity of erythrocytic phosphofructokinase (PFK) and pyruvatekinase (PK) increased significantly. The half saturation tension of oxygen (P_{50}-value) tended to increase.

The increase of P_i causes – via a stimulation of the glycolytic pathway – an increase in 2,3-DPG concentration and thus results in a shift of the oxygen dissociation curve. It is therefore possible to enhance tissue oxygenation by whole body UV-irradiation.

Key words: UV-irradiation – Oxygen affinity of haemoglobin – 2,3-Diphosphoglycerate – Plasma phosphate – Altitude

Introduction

Vitamin D_3 (Cholecalciferol) is formed in the skin by conversion of 7-dehydrocholesterol by UV-radiation. Further hydroxylation gives 1,25 dihydroxycholecalciferol [1,25 $(OH)_2$ D_3], the active agent which enhances intestinal calcium and phosphate absorption. Furthermore, there are indications that the active vitamin D_3 has an antiphosphaturic effect (Lang 1980).

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An elevation of plasma calcium – caused by elevated intestinal calcium absorption – inhibits the release of parathyrin, which also leads to an increase in renal phosphate reabsorption. Increased exposure to ultraviolet radiation therefore causes an increase in the inorganic plasma phosphate concentration (P_i). It is well known that the level of P_i is of importance in stimulating intraerythrocytic glycolysis (Jahrmärker 1969), which in turn leads to an increase in the concentration of intraerythrocytic 2,3-diphosphoglycerate (2,3-DPG).

It is possible to elevate 2,3-DPG levels in vivo and in vitro by administration of phosphate, either alone or in combination with other substances (Brain and Card 1972; Deuticke et al. 1971; Kerr et al. 1979; Moore et al. 1977). 2,3-DPG is known to be an important allosteric modifier of the affinity for oxygen of human haemoglobin (Benesch and Benesch 1967; Chanutin and Curnish 1967). Binding of 2,3-DPG between the β-chains of human deoxyhaemoglobin stabilizes the low binding affinity (Arnone 1972).

It is therefore conceivable that the oxygen transport properties of the erythrocyte can be influenced by UV-irradiation via the vitamin D_3 pathway. In order to elucidate the sequence of events the following study was performed.

Subjects and Methods

Sixteen healthy, non-smoking, caucasian volunteers between 21 and 26 years of age (mean 22.2) participated in this study. The project was explained to all subjects and informed consent was obtained. Prior to and 4 h after irradiation blood samples were secured by puncture of the cubital vein. The same time course without irradiation was used for control experiments 1 day before. The UV induced effects were calculated from the differences between the parameters measured before and after irradiation (ΔUV) and those in the control experiments (Δcontrols). The changes were given as percent of the initial values.

This kind of calculation was chosen to exclude diurnal variations of the parameters (e.g., P_i) (Böning et al. 1974).

Radiation Sources

Irradiations were performed with 28 Sylvania fluorescent lamps F 75/85W/UV6 mounted in an UV-1000 stand-up irradiation unit (Waldmann Comp., Schwenningen, FRG). These lamps emit at a continuous spectrum between 280 and 350 nm (peak at 313 nm, maximum output $6{,}8 \times 10^{-5}$W/cm^2 at 297 nm). The irradiation was measured with an International Light, Inc., Model IL 700 radiometer system adjusted for maximum transmission at 297 nm.

Irradiation Procedure

The minimal erythema dose (MED) was determined for each subject 1 day prior to the experiments by irradiating template test fields measuring 2 cm in diameter with increasing doses of UV the rest of the body being covered. The MED is defined as the least dose used which elicits a barely visible erythema reaction 24 h after exposure. The individual MED was then used in each subject for total body irradiation. The UV-dose required varied, and ranged from 12.2 to 24.5 (19.2 ± 5.1 ($\bar{x} \pm s$) mJ/cm^2. Despite the fact that the MED is not a physical dose measurement, this approach was chosen because penetration of UV into skin depends on several individual parameters and thus may vary considerably. A constant UV-dose would therefore not permit comparison of the photobiologic effects in different subjects.

Blood Parameter Estimation

Haemoglobin concentration (Hb) was measured spectrophotometrically after conversion to cyanmethaemoglobin. Hematocrit (Hct) was determined in capillary tubes centrifuged with a microhematocrit centrifuge at 16,000 rpm for 4 min. Mean corpuscular haemoglobin concentration (MCHC) was calculated by dividing the haemoglobin concentration by the hematocrit.

The acid-base-status was measured with the blood microsystem of Radiometer (BMS II), whereby standard bicarbonate was determined using the Siggaard-Andersen curve nomogram. Oxygen affinity, expressed as the half saturation tension of oxygen (P_{50}-value), was determined by the microequilibration technique of Astrup et al. (1965). The blood was exposed to humidified gas mixtures of known composition in temperature regulated microtonometers (37° C). The gas mixture, which was composed of pure nitrogen, carbon dioxide and oxygen (Wösthoff OHG, Bochum, FRG), contained 6% CO_2, 4.7% or 3.76% O_2 and made up to 100% with N_2. The fractional O_2-concentration used for equilibration corresponds to P_{O_2}-values below and above the expected P_{50}-values. After equilibration for 15 min the oxygen saturation of haemoglobin was determined with an OSM II (Radiometer, Denmark). The P_{O_2}-value of the equilibrating gas mixture was corrected to pH 7.4 using a Bohr coefficient of −0.48 (Dill et al. 1940). The value of log $S_{O_2}/100 - S_{O_2}$ (S_{O_2} : oxygen saturation) was plotted against that of log P_{O_2} and P_{50} was then determined graphically. The concentrations of P_i and intraeryhtrocytic ATP were measured using Boehringer kits. The intraeryhtrocytic 2,3-DPG concentration was measured by the method published by Rose and Liebowitz (1970) supplied by Sigma Chemical Corporation and calculated as μmol/g haemoglobin. Plasma calcium concentration (Ca) was measured using a Boehringer kit.

The activities of the main enzymes of the intraerythrocytic glycolysis, namely phosphofructokinase (PFK) and pyruvatekinase (PK), were determined according to Schröter (1974), in a Zeiss photometer at a wavelength of 340 nm (37° C, pH 7.5) using Boehringer reagents.

Statistical analysis was performed employing standard parametric and non-parametric tests (Student's *t*-test and Wilcoxon test) (Sachs 1968).

Results

The initial values (mean ± SEM) of all subject measured are listed in Table 1.

Hb, Hct, MCHC, pH, and the concentration of standard bicarbonate were in the normal range and no significant variation could be found 4 h after UV-irradiation in comparison to controls. Figure 1 illustrates the effect of irradiation shown as the difference between ΔUV and Δcontrol. Four hours after irradiation with UV a highly significant ($p < 0.001$) increase of P_i, Ca, 2,3-DPG, PFK, and PK occurs. The P_{50}-value tended to increase, indicating a decrease in oxygen affinity, but the changes were not significant ($p < 0.08$).

Table 1. Initial values of plasma calcium (Ca), inorganic phosphate (P_i), 2,3-diphosphoglycerate (2,3-DPG), half saturation tension (P_{50}) and the erythrocytic enzyme-activities of phosphofructokinase (PFK) and pyruvate kinase (PK) (means ± SEM)

Ca (mmol/l)	2.45	0.03
Pi (mmol/l)	1.28	0.19
2,3-DPG (μmol/g Hb)	13.95	0.5
P_{50} korr. (mm Hg)	25.5	0.9
PK (U/g Hb)	9.25	0.82
HK (U/g Hb)	0.59	0.57

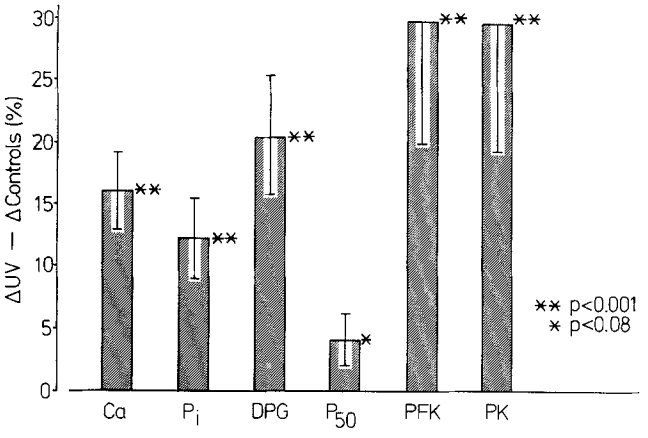

Fig. 1. Effect of UV-irradiation on the concentration of calcium (Ca), inorganic plasma phosphate (P_i), 2,3-diphosphoglycerate (2,3-DPG), half saturation tension (P_{50}), phosphofructokinase (PFK) and pyruvatekinase (PK) 4 h after UV-irradiation using the minimal erythema dose

Discussion

The data obtained from these experiments show a significant increase in the concentration of plasma phosphate and calcium following whole body-irradiation with UV. It is well known that inorganic plasma phosphate concentration influences the intraeryhtrocytic 2,3-DPG concentration and oxygen affinity via the glycolytic pathway (Brain and Card 1972; Deuticke et al. 1971; Jahrmärker 1969; Kerr et al. 1979; Moore et al. 1977). An increase in oxygen affinity has been correlated with low levels of 2,3-DPG in hypophosphatemic states (Lichtman et al. 1971), conversely a decrease in affinity has been observed with high levels of 2,3-DPG in association with the hyperphosphatemic state in renal disease (Lichtman and Miller 1970).

The metabolism of the red cell is influenced by relatively small variations in P_i concentration, and these changes occur within a short period of time. This has been shown by Brain and Card (1972), who reported that oral and intravenous administration of isotonic phosphate to normal volunteers leads to a 10–20% increase in 2,3-DPG levels over a 5-h period.

In our study, a significant increase in inorganic plasma phosphate could be detected 4 h after irradiation. This causes a stimulation of the glycolytic pathway which in turn leads to an increase in the 2,3-DPG concentration and thus results in a shift to the right of the oxygen dissociation curve. Indications for stimulated glycolysis after irradiation are given by increased erythrocytic enzyme activities (Fig. 1).

In earlier studies performed at moderate altitude (1,500–3,000 m) a continuous increase in plasma phosphate was detected in individuals while staying at this altitude (Humpeler and Skrabal 1978; Humpeler et al. 1979; Humpeler and Mairbäurl 1981). This could be explained by the fact that the intensity of UV-irradiation increases with increasing altitude. During all studies

at moderate altitudes a simultaneous increase in 2,3-DPG with a concomitant decrease in oxygen affinity was observed. An increase in pH due to respiratory alkalosis and a decrease in oxygen saturation of haemoglobin is presently under discussion as the main factors responsible for this increase of 2,3-DPG. However, at moderate altitudes the decrease in arterial oxygen saturation – if seen at all – is very small and there is also no marked alkalosis. Thus, these mechanisms cannot explain the rise in 2,3-DPG concentration sufficiently. Another possible explanation for such an increase in 2,3-DPG could be the rise in plasma phosphate concentration caused by an increased UV-irradiation. Indirect evidence for our assumption that the oxygen transport property of haemoglobin might be influenced by UV-radiation via the vitamin D_3 and phosphate metabolism can be deduced from the findings of Lund and Sorensen (1979). In 596 Danish subjects these authors found a relationship between the duration of sun exposure and serum 25-OHD_3. In 20 subjects, after a 3-week vacation in the sun, Cech et al. (1979) found a 39,3% increase of 25-hydroxy-vitamin D_3. Offermann and Biehle (1978) were able to demonstrate in a group of old people that the 25-hydroxy-vitamin D_3 concentration depends on the average duration of sun exposure. Furthermore, old people who regularly attend a senior citizen's group had a higher plasma phosphate concentration than people of similar age who were hospitalized or were in an old people's home.

Sommer-Tsilenis et al. (1980) observed an increase in plasma 25-hydroxy-cholecalciferol and a significant increase in plasma phosphate concentration in a group of psoriasis patients after a 3-weeks irradiation with UVA/UVB. Davie and Lawson (1980) showed that this plasma 25 (OH) D_3 responses to vitamin D_3 synthesized by ultraviolet irradiation of skin is brisk.

From the results of this study we conclude that it is possible to influence eryhtrocyte glycolysis by whole body UVB-irradiation via calcium and phosphate metabolism. This, in turn, leads to an increase in the intraerythrocytic 2,3-DPG concentration, causing an improved oxygen delivery from the erythrocytes.

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