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THE INHIBITORY EFFECT OF UV-LIGHT ON THROMBIN-INDUCED PIG PLATELET AGGREGATION

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Krajewski, T., Pietrucha, T. and *Wachowicz, B.*: The inhibitory effect of UV-light on thrombin-induced pig platelet aggregation. Acta physiol. pol., 1989, 40 (4): 413—417. The effect of ultraviolet radiation on the thrombin-stimulated aggregation of washed pig platelets was studied. It was observed that UV radiation (UV-A, 0.5 W/cm^2) inhibited aggregation of pig platelets induced by thrombin. Plasma may have a protective role against the damaging effect of UV light on platelet aggregation.

Key words: *blood platelet; aggregation; ultraviolet radiation*

Ultraviolet irradiation is responsible for many important biological phenomena, e.g., virus inactivation, vitamin D production, initiation of carcinogenic process, etc.

The biological activity of blood platelets exposed to UV-light is also changed. Dickson et al. (1971) observed that UV irradiation at the wavelength of 253 nm induced aggregation of human platelets in the presence of extracellular fibrinogen, but Lawler et al. (1979) showed a decreased thrombin-stimulated aggregation of irradiated platelets. Our earlier experiments demonstrated that UV-A radiation (360 nm) is responsible for the inhibition of both ADP-induced aggregation [Krajewski and Wachowicz, 1982] and a thrombin-stimulated release reaction [Krajewski et al., 1985].

The aim of the present work was to study the effect of UV-A irradiation (occurring in the spectrum sunlight, wavelength about 366 nm) on the thrombin-induced pig platelet aggregation and the protective role of plasma in this process.

MATERIAL AND METHODS

Blood (1 l) was collected from the pig aorta (slaughterhouse) into a plastic container with anticoagulant ACD (85 mM sodium citrate, 65 mM citric acid and 110 mM dextrose). Care was taken to discard the initial 50—100 ml of blood in order to avoid contamination of the sample with tissue thromboplastin. To one volume of ACD five volumes of blood were added. The blood was centrifuged for 20 min at 350$\times$g (10°C), and then the platelet rich plasma (PRP) was centrifuged again for 20 min at 2000 $\times$ g. The platelet pellet obtained after centrifugation of PRP was gently suspended in 10 to 15 volumes of Ca^{2+} free — Tyrode's buffer containing 140 mM NaCl, 5 mM KCl, 15 mM Tris-HCl, 5.5 mM glucose, pH 7.4. The washing medium was then removed by centrifugation (20 min, 2000 $\times$ g). The procedure was repeated and finally the platelet pellet was suspended in the same buffer.

Samples (5 ml) of platelet suspension (in open plastic beakers) were exposed in dark to varying doses (20—120 min) of UV irradiation (0.5 W/cm^2, the lamp VP-60, Famed, Poland, filter UG1), the dominating wavelength being 366 nm. The depth of the irradiated layer was 2 cm. The samples were carefully stirred during irradiation by a magnetic stirrer and thermostated at 37°C. Control samples were kept in the dark under the same conditions.

Platelet aggregation was done by the turbidimetric method of Born (1962). Thrombin-induced aggregations of the control and irradiated platelets were registered in a dual channel aggregometer (made in Poland, Kraków) using 2 NIH units of thrombin (Biomed Serum and Vaccine Manufacturers in Lublin) per 3 $\times$ 10^8 platelets. The platelet count was adjusted to 3 $\times$ 10^8 platelets by microscopic counting using a Bürker chamber. The thrombin solution was prepared freshly each day (200 u NIH/ml) and kept on ice during the procedure.

All the surfaces coming in contact with the blood and blood platelets were made of plastic or siliconized.

RESULTS

Thrombin at a concentration of 2 NIH units/3$\times$10^8 platelets caused a distinct, irreversible aggregation of pig platelets registered in aggregometer (Figs. 1—3, controls). Our results indicated that ultraviolet irradiation (366 nm) inhibited this thrombin-induced aggregation of a washed pig platelet suspension. This inhibitory effect increased with an extension of the time of irradiation. After 2 h irradiation the aggregation of platelets was reduced to 15.4 $\pm$ 3.2% (Fig. 1., Tab. 1). The exposure of platelet rich plasma to UV-A irradiation under the same conditions had almost no effect on the thrombin-induced aggregation of the irradiated platelets (Fig. 2).

In the case of 2 h irradiation of blood platelets suspended in buffered saline diluted plasma (3 : 1, v/v) a slight inhibitory effect was observed (70.3 $\pm$ 11.5%) in comparison with thrombin-induced aggregation of control platelets (Fig. 3).

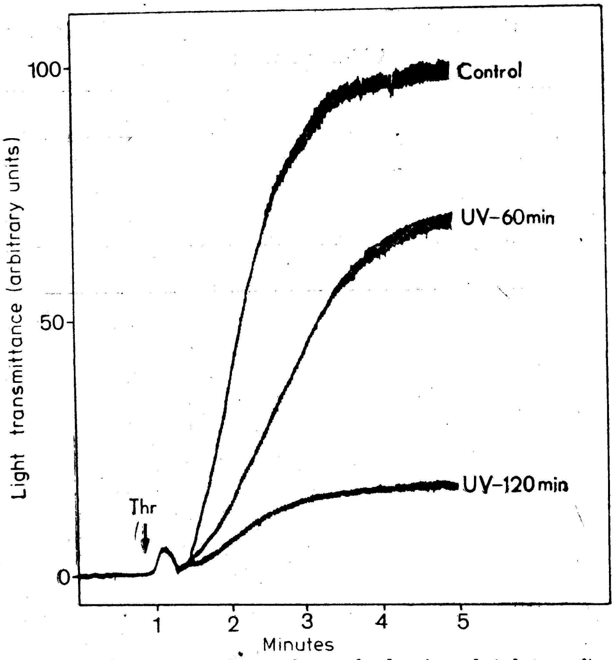

Fig. 1. Thrombin-induced aggregation of washed pig platelets after UV-irradiation (20—120 min).

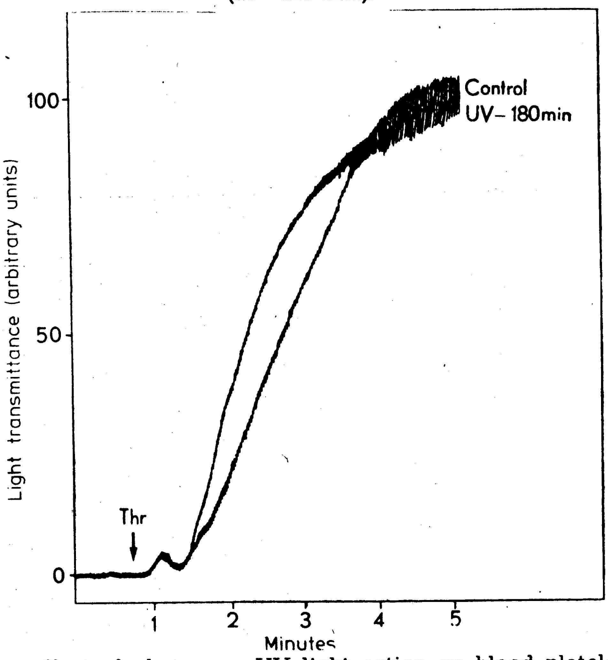

Fig. 2. Protective effect of plasma on UV light action on blood platelets. Platelet rich plasma was exposed to UV-source for 60, 120, 180 min and then isolated platelets were suspended in Tyrode's buffer, pH 7.4. Thrombin-induced aggregation of platelets (2 units per 3×10^8 platelets) was measured.

Table 1. Inhibition of thrombin-induced aggregation of washed pig platelets after UV-irradiation

Time of irradiation (min)	20	40	60	80	100	120
% aggregation	84.8	68.8	60.6	46.0	30.8	15.4
SD	11.2	16.3	10.6	10.9	4.5	3.2

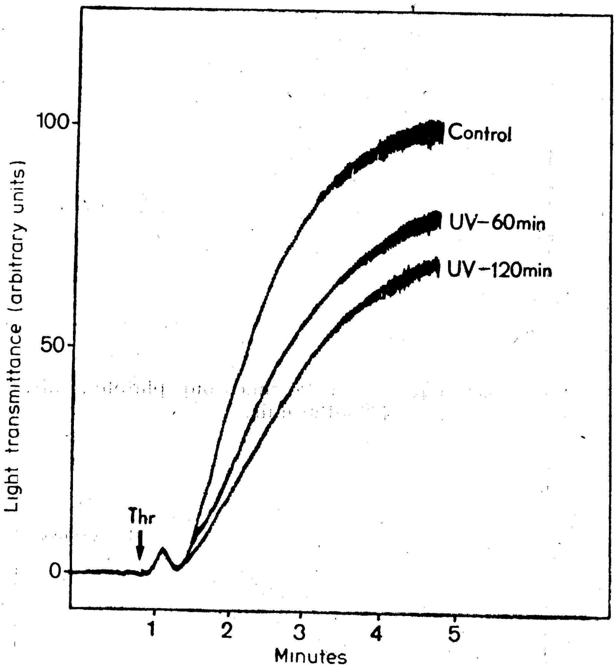

Fig. 3. Aggregation of pig platelets isolated from previously irradiated platelet suspension in treefold diluted PRP.

DISCUSSION

Our results suggest that plasma may have a protective role against the damaging effect of UV-A irradiation on platelet function, e.g. thrombin-induced aggregation. Some part of sunlight irradiation penetrates the human dermis to the capillary blood vessels and may be responsible for the changed function of blood platelets.

The mechanism by which ultraviolet radiation inhibits thrombin-induced aggregation of platelets remains to be elucidated. The radiation damage to the platelet membrane structure, peroxidation of membrane lipids and production of free radicals [Krajewski and Wachowicz, 1982; Roshchupkin et al., 1983] should be taken into account. The protective effect of plasma on the UV-irradiated platelets seems to be connected with the presence of proteins partially absorbing the energy of radiation and the presence of enzymes (ceruloplasmin) with radioprotective and antioxidant effects [Roshchupkin et al., 1983; Gutteridge et al., 1980].

The UV radiation damage to the platelet membrane structure might also affect the availability of a surface receptor for thrombin and in this way interferes with a physiological interaction of blood platelet and thrombin.

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