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### UV LIGHT-INDUCED CHANGES IN WASHED PIG PLATELETS\*

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The effect of ultraviolet radiation (UV-A, 360 nm) on the thrombin-induced aggregation of washed pig platelets as well as on the release of adenine nucleotides and proteins was studied. The level in platelets of adenine nucleotides, mainly ADP and ATP, decreased rapidly following the exposure of platelets to a high dose of UV-A (0.5 W/cm<sup>2</sup>, 30 min). Though thrombin-induced aggregation of irradiated platelets was inhibited, the release reaction occurred. The amount of the released adenine nucleotides and proteins was, however, dependent on the dose of UV light. These findings suggest that UV-A light can stimulate the platelet release reaction.

Blood platelets contribute to blood coagulation by forming a haemostatic plug at the site of blood vessel injury. Their physiological and pathological functions are related to their ability to adhere, aggregate and release their granule content.

It has previously been demonstrated (Dickson *et al.*, 1971; Doery *et al.*, 1973) that UV light induced aggregation of mammalian platelets in the absence of exogenous ADP and/or other aggregating agents. This effect was observed at 200-300 nm (with a maximal response at 250 nm), but not at 360 nm.

Our preliminary studies have shown that the 360 nm UV-A light inhibits the ADP-induced aggregation of platelets in pig platelet-rich plasma (Krajewski & Wachowicz, 1982). A similar effect was observed by Roshchupkin *et al.* (1983). Since UV-A alone does not induce aggregation of

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\* This work was supported by the Ministry of Science, Higher Education and Technology within the project R.III.13.4.7.

platelets (Doery *et al.*, 1973) we studied the effect of the 360 nm UV light (UV-A) on the behaviour of thrombin-treated pig platelets as well as the release of adenine nucleotides and proteins.

#### MATERIALS AND METHODS

*Aggregation test.* Fresh blood collected from pig into citric acid/citrate/dextrose (ACD) solution (5:1, v/v) was centrifuged (20 min at 120 g). Platelet-rich plasma was then centrifuged for 20 min at 1 100 g. The platelet pellet was gently suspended and washed twice in modified Tyrode's buffer (140 mM-NaCl, 5 mM-KCl, 15 mM-Tris/HCl) pH 7.4. Washed platelets were exposed to UV irradiation for 10 - 60 min. Thrombin-induced aggregation of control and irradiated platelets was registered in an aggregometer using 2 NIH units of thrombin per  $3 \times 10^8$  platelets.

*The release reaction measurements.* Platelet suspensions were prepared according to Wachowicz & Krajewski (1979). Blood (5 litres) was collected from pigs into 1% disodium EDTA in 155 mM-NaCl, 10 mM-Tris/HCl, pH 7.4 (9:1, v/v). Platelet-rich plasma was obtained by spinning the blood (350 g, 20 min). The platelet pellet obtained by centrifugation of the platelet-rich plasma for 20 min at 2200 g was gently suspended and washed twice in 155 mM-NaCl, 1 mM-EDTA, 10 mM-Tris/HCl buffer, pH 7.4. The final suspensions of platelets were prepared with the same solution without EDTA, at the concentration of 10 - 20 mg of platelet proteins per 1 ml.

*Irradiation of pig platelets.* Samples (5 ml) of platelet suspension were irradiated in open plastic beakers with varying doses of UV-A radiation ( $0.5 \text{ W/cm}^2$ , 15 - 60 min, the lamp L6/58, 220 V, 50 Hz, 1.7 A, 180 W, filter UG 1,  $\lambda$  360 nm: Famed Poland). The depth of the irradiated layer was 2 cm. The samples were stirred during irradiation and thermostated at 37°C. Control samples were kept in the dark at room temperature. In the supernatants obtained from control and irradiated platelets (2200 g, 10 min) the adenine nucleotides and proteins were determined.

*Spectrophotometric determination of extracellular adenine nucleotides.* Samples of supernatants obtained as described above were treated with 1.2 M-HClO<sub>4</sub> (1:1, v/v) and the protein precipitate was removed by centrifugation (2200 g, 10 min). Absorbance at 260 nm was measured and the amount of adenine nucleotides was calculated from the absorbance of an ATP standard in 0.6 M-HClO<sub>4</sub> (Murer, 1968).

*Protein measurement.* Protein content in the supernatants of control and treated platelets as well as the total platelet proteins were estimated by the microbiuret method (Itzhaki & Gill, 1964).

*Chromatographic separation of adenine nucleotides on DEAE-Sephacel.* Platelet suspensions (control and irradiated) containing 40 - 60 mg of platelet proteins in 5 ml were centrifuged for 15 min at 2200 g and the platelet pellet

was extracted with 0.6 M-HClO<sub>4</sub>. Extracts were pooled, neutralized with 1.2 M-KOH and applied to the DEAE-Sephacel column. Separation of adenine nucleotides was performed as described previously (Kostka & Wachowicz, 1983).

*Thrombin-induced platelet release reaction.* Platelet suspensions (control and irradiated) were incubated for 10 min at 37°C with thrombin (10 NIH units per 1 mg of platelet protein). Incubation was stopped by cooling in an ice-bath and centrifugation for 15 min at 2200 g. In both supernatants, adenine nucleotides and proteins were estimated. Polyacrylamide-gel electrophoresis of thrombin- and UV-released proteins was performed in 5% gel with 1% sodium dodecyl sulphate (SDS) according to Weber & Osborn (1969).

*Platelet lysis estimation.* The extent of platelet lysis following UV irradiation was determined by measuring the loss of lactate dehydrogenase from platelets (Wróblewski & La Due, 1955).

#### RESULTS AND DISCUSSION

The biological activity of blood platelets exposed to UV-A irradiation was in many respects altered. At low doses of UV light (0.5 W/cm<sup>2</sup>, 10 min) the thrombin-induced aggregation of washed pig platelets was inhibited, the inhibition progressing with the increase of the irradiation time (Fig. 1). The exposure of platelets for 10 min to UV-A alone (low dose) was accompanied by the appearance of adenine nucleotides but not proteins in the extracellular medium (Fig. 2). On longer irradiation (30 and 60 min) the adenine nucleotides release amounted to 40% of their total content in the platelets, while only about 2% of the total lactate dehydrogenase activity was found in the medium (Fig. 2). The separation on DEAE-Sephacel column of total adenine nucleotides extracted from control and irradiated platelets revealed in either case the presence of three peaks corresponding to AMP, ADP and ATP, respectively (Fig. 3). Irradiation caused a significant decrease in the amount of both ADP and ATP, especially so after longer irradiation (Table 1, Fig. 3) but the ATP/ADP ratios were unaltered. The patterns of polyacrylamide gel electrophoresis of the supernatants obtained after irradiation with high doses of UV-A light were similar to, but not identical with, those of the thrombin-released material showing that there were some differences between the proteins released by the two treatments applied.

There is no agreement as far as the behaviour of platelets exposed to UV irradiation is concerned. Dickson *et al.* (1971), Doery *et al.* (1973) and Lowler *et al.* (1979) have found that ultraviolet light (UV-C, max. 250 nm) induces aggregation of blood platelets, mainly in the presence of

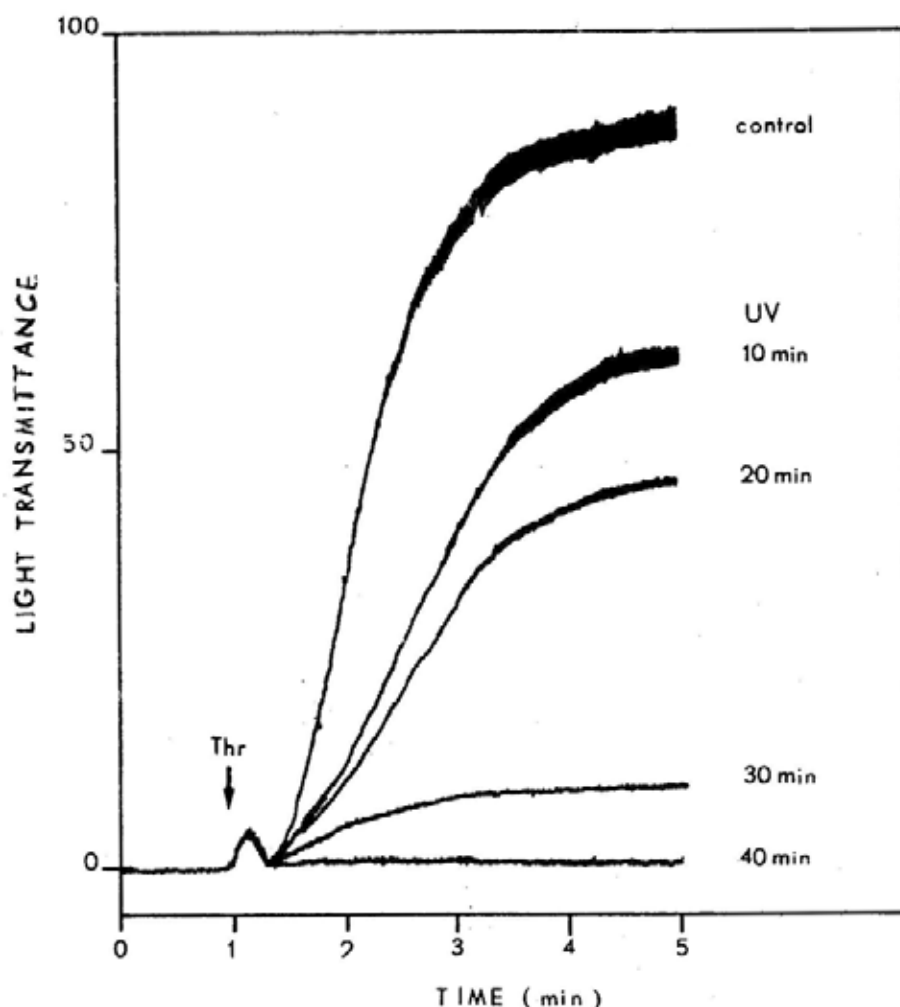


Fig. 1. Effect of UV-A radiation ( $0.5 \text{ W/cm}^2$ , 360 nm) on the thrombin (Thr)-induced aggregation of washed pig platelets.

fibrinogen. However, this reaction was accompanied by the release of only very small amounts of adenine nucleotides and serotonin.

On the other hand, thrombin-induced aggregation of UV-C (250 nm) irradiated platelets was inhibited both in the presence and absence of fibrinogen (Lawler *et al.*, 1979). A similar observation was made by Roshchupkin *et al.* (1983) who demonstrated that UV-B and UV-A irradiation of platelet-rich plasma resulted in inhibition of ADP-induced platelet aggregation. These findings are in agreement with our observation (Krajewski & Wachowicz, 1982) that UV-A light inhibits ADP-induced aggregation of platelets in platelet-rich plasma as well as thrombin-induced release of

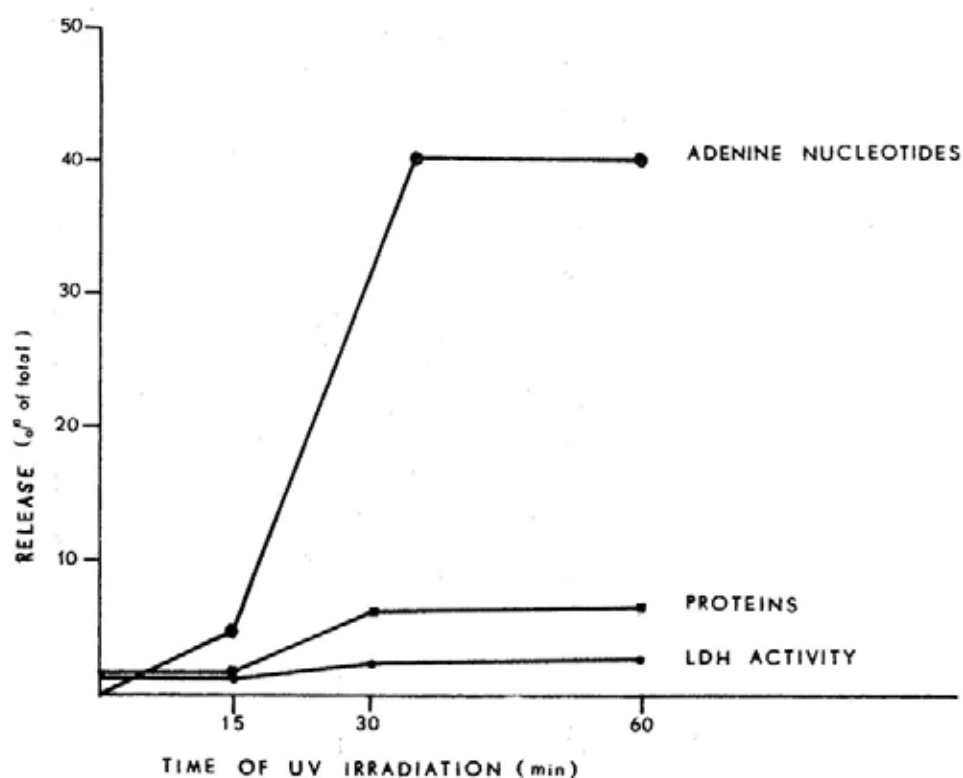


Fig. 2. Effect of UV-A on the release of platelet adenine nucleotides, proteins and lactate dehydrogenase (LDH) activity. The amounts of the released compounds are expressed as percentage of their total amount in the cell.

Table 1

The effect of UV-A ( $0.5 \text{ W/cm}^2$ , 15 and 30 min) on adenine nucleotide content in washed pig platelets.

Values are expressed as nanomoles/mg of platelet proteins.

	total	AMP	ADP	ATP	ATP/ADP	other
Control	77.5	14.8	26.4	30.7	1.16	5.6
S.D.	13.3	2.3	4.4	6.9	0.04	1.1
Irradiated for 15 min	74.8	14.5	22.8	27.8	1.20	9.7
S.D.	9.1	2.7	2.4	2.5	0.02	2.0
<i>p</i>	< 0.01	> 0.05	< 0.01	< 0.001		
Irradiated for 30 min	44.5	8.1	12.6	13.6	1.07	10.2
S.D.	8.8	2.8	2.8	5.1	0.03	3.2
<i>p</i>	< 0.001	< 0.001	< 0.001	< 0.001		

Student's *t* test for paired data was used.

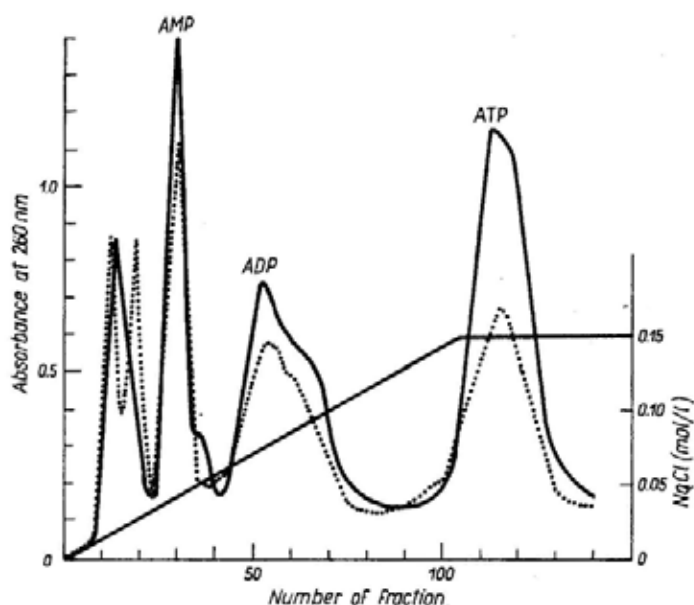


Fig. 3. DEAE-Sephacel column chromatography of adenine nucleotides extracted from washed pig platelets: control (—) and irradiated for 30 min (○). The column (1.2 × 18 cm) was equilibrated with 0.1 M-Tris/HCl buffer, pH 7.6, and the nucleotides were eluted with a linear NaCl concentration gradient up to 0.154 M in the same buffer. Fractions of 2.3 ml were collected at a flow rate of 0.5 ml/min.

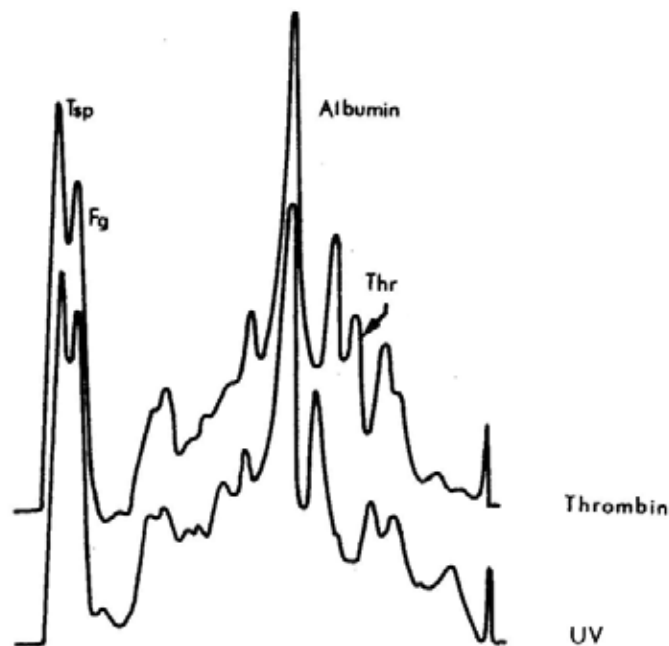


Fig. 4. Densitogram of SDS-polyacrylamide-gel electrophoresis (5% gel) of proteins released from washed pig platelets by UV-A radiation and by thrombin treatment. Tsp, thrombospondin; Fg, fibrinogen; Thr, thrombin.

proteins from platelets in suspension, but not the release of adenine nucleotides, the latter even increasing by about 30% as compared with the control. The release of about 40% of adenine nucleotides (ATP and ADP) in response to UV-A irradiation observed in our present experiments seems to be caused by a specific secretory process (release from granules) rather than by damage to the cells, since even after a high dose of UV-A light only slight lysis was observed, as measured by lactate dehydrogenase activity in the extracellular phase.

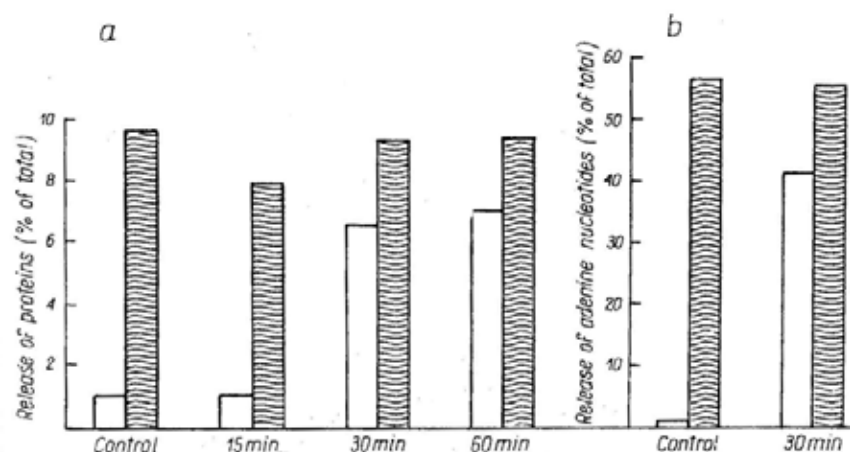


Fig. 5. Release of protein (a) and adenine nucleotides (b) from UV-A irradiated pig platelets, with or without thrombin treatment. Compounds released from platelets: open columns, without thrombin treatment; dashed columns, treated with thrombin following irradiation.

Since membranes of platelets are known to possess specific receptors for thrombin (Tam & Detwiler, 1978), ADP (Lips *et al.*, 1980) and other agents responsible for platelet aggregation, we suggest that UV-A irradiation induces in membranes some changes which influence the behaviour of platelets.

The mechanism of UV light action on platelet behaviour is being studied.

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Received 16 December, 1984:

revised 25 January, 1985