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THE EFFECT OF UV-LIGHT ON FREE SULFHYDRYL GROUPS IN WASHED PIG PLATELETS

In this work the effect of ultraviolet radiation (UV-A, 360 nm) on the behaviour of fast reacting sulfhydryl groups in washed pig platelets was evaluated. The level of platelet free -SH groups increased rapidly following the exposure of platelets to UV-A light (0.5 W/cm^2: 15, 30 and 60 min.). This increase seems to be only partially dependent on the -SH groups connected with the released material.

INTRODUCTION

Blood platelets lose their haemostatic function under a variety of conditions - irradiation or storage [5, 7]. These changes in platelet function are correlated with structural and metabolic alterations [7]. Our recent studies demonstrated that ultraviolet irradiation caused the inhibition of platelet aggregation accompanied by the changes in the level of platelet adenine nucleotides and proteins [6, 7]. Reactive free sulfhydryl groups play an important role in the function of blood platelets, too [1-3]. The purpose of this work was to examine the behaviour of free sulfhydryl groups of platelet membranes following UV-A irradiation and the possible relationship of these changes to the alterations in blood platelet function.

MATERIALS AND METHODS

Preparation of washed pig platelets: blood was collected from pig into plastic container with 1% EDTA in buffered saline (9 : : 1, v/v). Platelet rich plasma (PRP) was obtained from whole blood by centrifugation at 350 x g, 15 min. Red and white cells contamination was reduced by 1 min. centrifugation at 1500 x g. Platelets were then separated by spinning the PRP at 1200 x g, 20 min. The platelet pellet was washed twice with buffer: 155 mM NaCl, 10 mM Tris-HCl, pH 7.4 containing 1 mM EDTA. Finally, the platelet pellet was suspended in the same buffer.

Irradiation of pig blood platelets: The samples (5 ml) of platelet suspensions in buffer (10 mg of platelet protein in ml) were irradiated in open plastic beaker with different doses of ultraviolet radiation: 15, 30 and 60 min. (0.5 W/cm^2, the lamp EMITA VP 60, Famed, Poland, 220 V, 50 Hz, 1.8 A, 180 W, filter UG 1, 360 nm) in dark, with stirring. The control samples were kept at room temperature for the same period of time.

Thrombin-induced release reaction: Platelet suspensions were incubated with thrombin (10 NIH units/mg of platelet protein) at $37^{o}C$ for 5 min. and then incubation was stopped by cooling the samples in an ice bath. The released material was separated from the cells by centrifuging for 15 min. at 2200 x g.

Spectrophotometric determination of free -SH groups on the platelets with 5,5´-dithiobis (2,-nitrobenzoic acid) (DTNB): 1.5 ml buffer containing 155 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 7.4 and 3.5 ml of DTNB always prepared before use (1 ml of 10 mM DTNB in 0.1 M phosphate buffer and 50 ml of 0.1 M phosphate buffer, pH 8.0) were added do 0.5 ml suspension of platelets (control, irradiated and thrombin-treated) or to 0.5 ml of supernatant. The concentration of coloured thiol anions (3-carboxylato--4-nitrothiophenolate) released into the reaction mixture were estimated by reading the absorbance at 412 nm against the blanks consisting of reagent mixtures without blood platelets. For the calculation of sulfhydryl group concentration the calibration curve was prepared with cysteine-HCl as a standard (10-50 nmol). Free sulfhydryl groups fast reacting with DTNB were measured:

1) in fresh intact platelets (3-4 hours after the collection of blood), 2) in UV-A irradiated platelets (different doses) directly after irradiation, 3) in thrombin-stimulated platelets, 4) in the material released from platelets into extracellular medium after thrombin or UV-A irradiation.

RESULTS

In fresh pig platelets the level of free thiols, rapidly reacting with DTNB was estimated to be about 5 nmol/mg of platelet protein (Table 1). The UV-A irradiation (0.5 W/cm^2, 15, 30, 60 min.) of these platelets resulted in a significant ($p < 0.001$) increase of the thiols (Table 1). Thrombin treatment of platelets also caused some changes in the level of -SH groups (Table 2) that are less significant than in case of platelets after 60 min. UV-A action (Table 3). In corresponding supernatants (thrombin and UV-A released material) the amount of sulfhydryl groups was found to be almost the same (Tables 2, 3).

Table 1

The effect of UV-A irradiation on the level of thiol groups (nmol/mg of platelet protein) in washed pig platelets

Pig	Control platelets suspension	UV-A irradiated platelets		
		15 min.	30 min.	60 min.
		suspension		
1	3.2	11.6	7.3	13.5
2	3.8	12.6	12.6	29.6
3	7.5	13.1	11.7	12.6
4	7.4	8.2	8.2	11.3
5	3.9	18.4	20.9	22.3
6	4.4	12.3	7.9	10.5
7	8.6	15.6	24.2	26.6
$\bar{x}$	5.5	13.1*	13.3*	18.0*
SD	2.2	3.2	6.7	7.9

* $p < 0.001$; Student's t test for paired data was used.

Table 2

The effect of thrombin on the level of -SH groups in platelets (nmol/mg of platelet protein)

Pig	Control platelets		Thrombin-treated platelets	
	suspension	supernatant	suspension	supernatant
1	4.3	0.1	8.0	4.3
2	3.2	0.3	7.1	3.9
3	5.1	0.0	6.9	2.5
4	3.7	0.3	6.8	2.9
5	4.6	0.1	6.0	1.9
6	4.6	0.1	6.9	2.3
$\bar{x}$	4.2*	0.15*	7.0*	2.9*
SD	0.7	0.1	0.5	0.9

Corrected for -SH of added thrombin.

* $p < 0.001$; Student's t test for paired data was used.

Table 3

The amount of -SH groups (nmol/mg of platelet protein) in platelet suspension and in the material released from platelets after UV-irradiation (60 min.)

Pig	Control platelets suspension	Irradiated platelets	
		suspension	supernatant
1	4.3	7.4	2.9
2	5.2	21.7	3.7
3	5.1	6.8	2.1
4	3.7	7.0	1.9
5	4.6	8.5	4.0
6	4.5	10.3	2.9
$\bar{x}$	4.5*	10.3*	2.9
SD	0.5	5.7	1.1

* $p < 0.01$ Student's t test for paired was used.

DISCUSSION

Reactive free sulfhydryl groups play an essential role in the function of blood platelets [1-3]. A n d o and S t e i n e r [1, 2] demonstrated the presence of at least two classes of thiols in the platelet membrane. Approximately one half of the total thiols was found to be masked in native membrane. Ultraviolet irradiation of platelets resulted in the exposure of additional thiols (Tables 1, 3) to the reacting with DTNB, -SH reagent with a very limited membrane permeability [4]. This high amount of thiols in irradiated platelets, corrected for -SH groups of released proteins (supernatant) indicates that the increase of sulfhydryl groups reacting with DTNB is not totally dependent on the released material (Table 3). This phenomenon seems to be associated with the changes of molecular structure of platelet membrane proteins and the exposure of additional thiols masked in native platelets.

On the other hand, ultraviolet light irradiation can lead to the cleavage of disulfide bonds in the cell membrane proteins forming together with lipids a target for ultraviolet radiation [8, 9].

The mechanism of UV action on blood platelets leading to the changes of platelet function seems to be connected with some conformational changes of platelet proteins as a result of cleavage and the exchange of sulfhydryl-disulfide relation in the platelet membrane proteins.

REFERENCES

[1] A n d o Y., S t e i n e r M. (1973), Biochim. Biophys. Acta, 311, 38-46.

[2] A n d o Y., S t e i n e r M. (1973), Biochim. Biophys. Acta, 311, 26-38.

[3] A n d o Y., S t e i n e r M., B a l d i n i M. G. (1974), Blood., 43, 121-129.

[4] E l l m a n G. L. (1959), Arch. Biochem. Biophys., 82, 70-77.

[5] Jamieson G. A., Ordinas A. (1981), Vox Sanguinic, 40, 18-21.

[6] Krajewski T., Wachowicz B. (1982), IRCS Med. Sci., 10, 1015-1016.

[7] Krajewski T., Wachowicz B., Bończak M., Pietrucha T. (1985), Acta Biochim. Pol., 32, 155-162.

[8] Murphy T. M. (1983), Physiol. Plant., 58, 381-385.

[9] Roshupkin D. J., Anosov A. K., Potapenko A. Y., (1983), Photobiochem. Photobiophys., 5, 307-313.

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WPŁYW UV NA POZIOM WOLNYCH GRUP SULFHYDRYLOWYCH W PŁYTKACH KRWI ŚWINI

Badano wpływ promieniowania ultrafioletowego (UV-A, 360 nm) na zachowanie się w płytkach krwi wolnych grup sulfhydrylowych szybko reagujących z DTNB. Wykazano, że poziom grup -SH gwałtownie wzrasta w napromienionych płytkach (15, 30, 60 min, UV-A, 0,5 W/cm^2). Wzrost ten jest tylko w nieznacznym stopniu zależny od grup -SH związanych z materiałem uwalnianym z płytek.