Nephron 33: 253–256 (1983)

0028-2766/83/0334-0253$2.75/0

Influence of Inhibitor of Glucose Utilization on the Blood Platelet Function

P. Tisoň[a], *P. Kubisz*[b], *P. Černáček*[a], *R. Dzúrik*[c]

[a]3rd Medical Clinic, Comenius University Medical School, Bratislava, Czechoslovakia;
[b]Hemostasis Laboratory, Department Hospital, Čadca, Czechoslovakia; [c]Bionics Research Institute, Bratislava, Czechoslovakia

Key Words. Inhibitor of glucose utilization · Blood platelets · Function of platelets

Abstract. The inhibition of glycolysis by an inhibitor of glucose utilization isolated from urine of the uremic subjects reflects in: (1) decreased platelet aggregation induced by adenosine diphosphate, adrenaline, or collagen, respectively; (2) decreased platelet factor 4 release induced by the same inductors; (3) decreased availability of platelet factor 3, and (4) inhibition of retraction of reptilase clot. It is concluded that the inhibition of glycolysis by 'inhibitor of glucose utilization' contributes to the functional changes of platelets and thus to the alteration of hemostasis in uremic patients.

Introduction

It is generally accepted that a functional platelet disturbance contributes to the bleeding defect of patients with chronic renal failure. Disturbance of the activation of platelet factor 3 [10] and of the aggregation [7] and adhesion [6] of platelets has been described by several groups and has been attributed by some to uremic toxins, such as phenolic compounds or guanidinosuccinate [9].

Adenosine triphosphate plays an important role in platelet participation in hemostasis, and of course, glucose is the main substrate for adenosine triphosphate synthesis. Thus, inhibition of glycolysis in various tissues [3] including the platelets [13] by phenolic acids, guanidinosuccinic acid, and the middle molecular 'inhibitor of glucose utilization' (IGU) could explain the functional platelet defect of uremia.

IGU is a peptide with a molecular weight of just less than 1,000 daltons, and it was the first middle molecule to be isolated from uremic serum and urine [4]. In muscle it interferes with glycolysis at the level of phosphofructokinase [1, 2]. No exact method for IGU quantitation has yet been developed, but on the basis of bioassay studies it has become clear that it accumulates in the sera of uremic subjects and that it is excreted in the urine of patients with chronic renal failure to a greater extent than in normal subjects.

In this paper the effect of IGU on basic platelet functions has been studied and the results are presented. IGU was chosen for further study because it interferes with glycolysis in the platelets [13], and the effects of a purified middle molecule on platelet function have not yet received attention.

Materials and Methods

Isolation of Platelets

9 parts of venous blood obtained from healthy blood donors were mixed with 1 part of 0.1 mmol/l sodium citrate. Platelet-rich plasma (PRP) was obtained by centrifuging (280 *g*) the blood sample for 20 min at room temperature. The platelet count was adjusted to 300,000 ± 20,000/μl, and the samples were used within a period of 3 h. Platelet-poor plasma (PPP) was prepared by PRP centrifugation (20,000 *g*) for 20 min at 6 °C.

Incubation of Platelets

8 parts of PRP and 1 part of 0.9% NaCl containing 1.1 μg nitrogen per milliliter IGU, which was in freeze-dried form, were preincubated at 37 °C for 5 and 30 min, respectively. Afterwards the incubation was started by adding the individual inductors as shown in the 'Results' section.

Platelet Aggregation

It was measured on a Corning EEL 169 aggregameter equipped with a recorder.

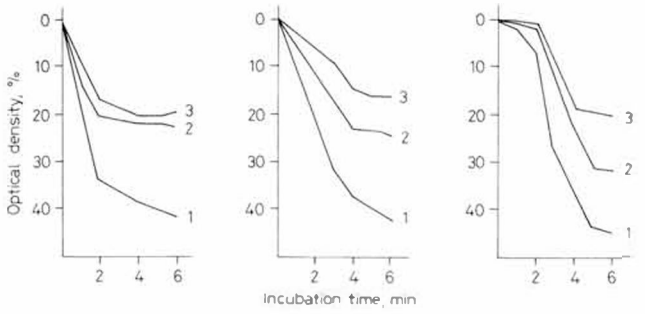

Fig. 1. a Effect of IGU on aggregation of platelets induced by $4.5 \cdot 10^{-6}$ mol/l of ADP. 1 = PRP-buffer, incubation 5–30 min (37 °C); 2 = PRP-IGU, incubation 5 min (37 °C); 3 = PRP-IGU, incubation 30 min (37 °C). b Effect of IGU on the platelet aggregation induced by $6 \cdot 10^{-6}$ mol/l of adrenaline. Same meaning of curves as in a. c Effect of IGU on the platelet aggregation induced by $20 \cdot 10^{-6}$ mol/l of collagen. Same meaning of curves as in a.

Platelet Factor 3

0.1-ml (tested) samples were collected at 5 and 30 min and transferred into another test tube containing 0.1 ml Michaelis buffer and 0.1 ml stypven solution (10 μg/ml of Michaelis buffer). 0.1 ml 0.025 mol/l $CaCl_2$ was added immediately to the test material and the coagulation time of the sample measured [11].

Platelet Factor 4

0.4 ml of each preincubated sample were mixed with 0.06 ml heparin (1.4 U/ml) and 0.1 ml of thrombin (22 NIH U/ml) and the coagulation times determined. Total release of platelet factor 4 was measured by incubating 1.8 ml PRP with 0.15 ml Michaelis buffer and 0.05 ml 30% Triton X-100 for 3 min at 37 °C. The results obtained were considered as 100% of platelet factor 4 release. The percentage of platelet factor 4 release in the different samples tested was calculated from a standard curve which had been drawn from the results obtained by the determination of platelet factor 4 activity in serial dilution of tritonized PRP with PPP [11].

Reptilase Clot Retraction Test

0.1 ml of serum obtained during retraction of collagen-reptilase clots was added to 0.3 ml of PRP. The test tubes and the controls containing 0.1 ml of the 0.9% NaCl instead of serum were incubated for exactly 2 min at 37 °C. Then 0.1 ml solution of reptilase was added and clot retraction was evaluated after 30 min incubation at 37 °C [12].

Isolation of IGU

The isolation was performed from urine of patients suffering from chronic renal failure as published previously [4, 5]. There is no exact method for IGU determination. However, the used IGU concentration (1.1 μg N/ml medium) exerts 30% inhibition of glucose utilization in rat hemidiaphragm which is the usual inhibition found in the sera of the uremic subjects [2]. Thus, it is supposed that the IGU concentration used in these studies resembles to those found in plasma of the uremic subjects.

The statistical validity of the measurements was evaluated by the Student's t test.

Results

Aggregation of Platelets

The effect of IGU on the platelet aggregation induced by adenosine diphosphate (ADP; fig. 1a), adrenaline (fig. 1b), or by collagen (fig. 1c) has shown that IGU inhibited platelet aggregation of both, but predominantly the second aggregation wave. Moreover, it was found that 30 min preincubation with IGU reflected in a more pronounced effect.

Release of Platelet Factor 4

IGU inhibited the release of platelet factor 4 (table I) in the presence of any of the inductors, but especially in the presence of adrenaline or ADP.

Table I. Effect of IGU on release of platelet factor 4 (mean ± SEM)

PRP	Time, min	Platelet factor 4, %			
		ADP	adrenaline	collagen	buffer
Control	0	4.8 ± 0.5 (5)	4.9 ± 0.7 (5)	4.4 ± 0.4 (5)	4.5 ± 0.5 (5)
	5	36.0 ± 2.2 (5)	39.5 ± 2.3 (5)	49.3 ± 2.9 (5)	4.4 ± 0.3 (5)
	30	52.0 ± 1.7 (5)	51.5 ± 2.1 (5)	62.6 ± 1.4 (5)	4.6 ± 0.5 (5)
IGU	0	4.6 ± 0.9 (5)	4.7 ± 1.0 (5)	4.2 ± 0.6 (5)	4.2 ± 0.4 (5)
	5	13.1 ± 2.7* (5)	10.8 ± 2.1* (5)	30.4 ± 1.7* (5)	4.3 ± 0.4 (5)
	30	19.5 ± 2.6* (5)	20.2 ± 2.6* (5)	40.8 ± 2.1* (5)	4.2 ± 0.3 (5)

Concentrations of inductors are the same as in figure 1.
Numbers in parentheses refer to the number of samples.
* $p < 0.001$.

Table II. Effect of IGU on availability of platelet factor 3 (mean ± SEM).

PRP	Time, min	Stypven time, s			
		ADP	adrenaline	collagen	buffer
Control	0	32.5 ± 1.4 (5)	33.6 ± 2.0 (5)	33.9 ± 1.2 (5)	34.8 ± 1.4 (5)
	5	20.0 ± 1.3 (5)	21.0 ± 1.4 (5)	32.1 ± 1.2 (5)	35.8 ± 1.2 (5)
	30	17.0 ± 0.9 (5)	17.3 ± 1.2 (5)	17.4 ± 1.4 (5)	32.1 ± 0.8 (5)
IGU	0	35.5 ± 1.9 (5)	34.3 ± 1.3 (5)	35.0 ± 0.7 (5)	36.6 ± 0.9 (5)
	5	25.5 ± 1.6* (5)	27.2 ± 1.5* (5)	28.1 ± 1.6** (5)	34.1 ± 0.8 (5)
	30	22.2 ± 1.7* (5)	22.1 ± 1.8* (5)	22.4 ± 1.9* (5)	33.7 ± 0.9 (5)

Concentrations of inductors are the same as in figure 1.
Numbers in parentheses refer to the number of samples.
* $p < 0.05$; ** $p < 0.02$.

Table III. Effect of IGU on retraction of reptilase clot (mean ± SEM)

PRP	Retraction of reptilase clot, %
Control	
ADP	72 ± 4.7 (5)
Adrenaline	70 ± 5.0 (5)
Collagen	78 ± 4.2 (5)
Buffer	4 ± 0.9 (5)
IGU	
ADP	11 ± 2.3* (5)
Adrenaline	6 ± 0.9* (5)
Collagen	7 ± 1.7* (5)
Buffer	3 ± 1.8 (5)

Concentrations of inductors: ADP 10^{-4} mol/l; adrenaline 10^{-4} mol/l; collagen 50 μg/ml.
Number in parentheses refer to the number of samples.
* $p < 0.001$.

Availability of Platelet Factor 3

IGU inhibited the availability of platelet factor 3 after preincubation with any of the three inductors (table II). However, if the inhibitions of the release of platelet factor 4 and the availability of platelet factor 3 were compared, it was found that IGU inhibited the release of platelet factor 4 much more intensively.

Retraction of Reptilase Clot

As can be seen from table III, IGU interfered with the retraction of reptilase clot markedly.

Discussion

Inhibition of glycolysis by IGU [13] probably accounts for the marked inhibition that has been shown in all the tests of platelet function, for IGU has not been shown to alter cellular oxidative processes [2]. IGU interferes with glycolysis at the level of phosphofructokinase [1], and it has been shown in erythrocytes that this inhibition is non-competitive [8].

The defect of platelet function in chronic renal failure is probably not caused by a single inhibitor, but could be the consequence of the combined action of uremic toxic metabolites and a middle molecular weight regulator such as IGU [13].

References

1 Dzúrik, R.; Krajči-Lazáry, B.: The effect of uremic serum on carbohydrate metabolism in rat diaphragm. Experientia *23:* 798–799 (1967).
2 Dzúrik, R.; Valovičová, E.: Glucose utilization in muscle during uremia: in vitro study. Clinica chim. Acta *30:* 137–142 (1970).
3 Dzúrik, R.; Božek, P.; Řezníček, J.; Oborniková, A.: Blood level of middle molecular substances during uremia and hemodialysis. Proc. Eur. Dial. Transplant Ass. *10:* 263–269 (1973).
4 Dzúrik, R.; Hupková, V.; Černáček, P.; Valovičová, E.; Niederland, T.R.: The isolation of an inhibitor of glucose utilization from the serum of uraemic subjects. Clinica chim. Acta *46:* 77–83 (1973).
5 Dzúrik, R.: Pathophysiology of the carbohydrate metabolism in renal insufficiency. Rev. Czech. Med. *20:* 179–188 (1974).
6 Eknoyan, G.; Wacksman, S.J.; Glueck, H.I.; Will, J.J.: Platelet function in renal failure. New Engl. J. Med. *280:* 677–681 (1969).
7 Evans, E.P.; Branch, R.A.; Bloom, A.L.: A clinical and experi-

mental study of platelet function in chronic renal failure. J. clin. Path. *25:* 745–753 (1972).

8 Gajdoš, M.; Dzúrik, R.: Erythrocyte glycolysis in uremia: dynamic balance caused by the opposite action of various factors. Int. Urol. Nephrol. *5:* 331–336 (1973).

9 Horowitz, H.I.: Uremic toxins and platelet function. Archs intern. Med. *126:* 823–826 (1970).

10 Horowitz, H.I.; Stein, I.M.; Cohen, B.D.: Further studies on the platelet-inhibiting effect of guanidinosuccinic acid and its role in uremic bleeding. Am. J. Med. *49:* 336–345 (1970).

11 Kubisz, P.; Suranová, J.: The effect of alpha and beta receptor blocking agents on collagen induced platelet release reaction. Thromb. Diath. haemorrh. *27:* 278–291 (1972).

12 Kubisz, P.; Suranová, J.: Reptilase clot retraction test. Pathol. Biol. *23:* 269–275 (1975).

13 Tisoň, P.; Černáček, P.; Silvánová, E.; Dzúrik, R.: Uremic 'toxins' and blood platelet carbohydrate metabolism. Nephron *28:* 192–195 (1981).

Accepted: October 13, 1982

Dr. Paul Tisoň, NsP akad. L. Dérera,
III. interná klinika LFUK,
Limbová 5,
809 46 Bratislava (Czechoslovakia)