Influence of Dicoumarol Derivatives on Plasma Fibrinolytic System

From the Department of Physiopathology, Institute of Haematology, Warsaw, Poland

Marta Niewiarowska and Zenon Wegrzynowicz

Dicoumarol derivatives inhibit synthesis of prothrombin, Factor VII, IX, X, XI (3, 5, 9, 10, 14, 18). The purpose of this work was to examine the mode of action of these anticoagulants on both the plasma fibrinolytic system and interrelationship between coagulation and activation of fibrinolysis.

Materials and Methods

In this study the following preparations were used:

1. The extract from rabbit brain (antirabbies vaccin, mfd. by Warszawska Wytwórnia Surowic i Szczepionek — W. W. S. S., Warsaw) was used as a source of thromboplastin.
2. Streptokinase (Distreptaza mfd by W. W. S. S.).
3. Bovine fibrinogen prepared according to Astrup and Müllertz (2).
4. Bovine thrombin prepared by the method of Alexander and Landwehr (1), slightly modified.
5. Spontaneously active human and guinea-pig plasmin obtained by the method of Niewiarowski and Latallo (15).
6. Sintrom (3-[α-[4'-nitrophenyl]-β-acetyl-aethyl]-4-oxycoumarin, mfd Geigy).
7. Marcoumar (3-[1'-phenyl-propyl]-4-oxycoumarin, mfd Hoffman-La Roche).
8. Pelentan (Tromexan), mfd SPOFA, Czechoslovakia.

The above mentioned anticoagulants were given orally during long-term therapy to 104 patients with coronary disease. The patients were treated in the Outpatient Department for Thrombotic Diseases in the Institute of Haematology, Warsaw, by Dr. M. Wirecki and Dr. J. Sawulis.

The same anticoagulants were also administered to the rabbits by the aid of a gastric tube in one large single dose: sintrom — 8 mg per kg of weight, marcoumar — 9 mg per kg of weight, pelentan — 300 mg per kg of weight.

The analytical technics, used in this work, were as follows: 1. Determination of prothrombin time (16); 2. Determination of fibrinogen level (16); 3. Determination of fibrinolysis in whole plasma (7, 11); 4. Determination of fibrinolysis time after recalcification of euglobulin fraction of plasma (4, 11, 13); 5. Determination of plasminogen activated by streptokinase (12);

6. Determination of antiplasmin activity according to the authors own method. The test was performed in the system:

0,1 ml of plasma diluted 1 : 10 with saline
0,1 ml of plasmin
Incubation 3 min. at 37° C
0,1 ml of thrombin
0,2 ml 0,2% fibrinogen

The results of the examination of the patient's plasma were compared with the results of the examination of healthy donor's plasma. These results were expressed as antiplasmin index. It is the quotient of the values:

$$\text{Index} = \frac{\textit{clot lysis time of sample with patient's plasma}}{\text{clot lysis time of sample with donor's plasma.}}$$

In every set of determinations the lysis time of the blank sample containing 0.9% NaCl instead of plasma was determined. The activity of plasmin expressed in clot lysis time varied from 2 min. 30 sec. to 4 min. 30 sec.

Results

I. The plasma fibrinolytic system in patients during long-term anticoagulant therapy.

We have examined 104 patients. According to prothrombin time the patients were divided into 3 groups: 1) with prothrombin time between 15—20 sec.; 2) with prothrombin time between 20—30 sec.; 3) with prothrombin time longer than 30 sec. (Normal prothrombin time was 15—16 sec.).

Tab. 1: Euglobulin fibrinolysis and plasma prothrombin time

Prothrombin time sec.	Numb. of determinat.	Mean time of euglobulin fibrinolysis in min.	Standard error	t	p
$\leqslant 20$	18	231	11.9	$t_1 = 4.10$	< 0.001
20—30	39	312	18	$t_2 = 3.14$	$0.01 > p > 0.001$
> 30	44	486	53.8	$t_3 = 4.59$	$p < 0.001$

t_1 = calculated by the comparison of group 1 a. 2
t_2 = calculated by the comparison of group 3 a. 2
t_3 = calculated by the comparison of group 1 a. 3

Table 1 represents interrelationship between euglobulin fibrinolysis time and prothrombin time in patients plasma. It can be seen that in cases with prolonged prothrombin time the fibrinolysis time was also lengthened attaining its maximal values in the third group. The differences between the values of

fibrinolysis time are statistically significant. The correlation coefficient between euglobulin fibrinolysis and prothrombin times of examined plasma is 0,53.

Tab. 2: Plasminogen activity and plasma prothrombin time

Prothrombin time sec.	Numb. of determinat.	Mean time of plasminogen activity in sec.	Standard error	t	p
$\leqslant 20$	24	1127	57.7	$t_1 = 1.66$	$p_1 = < 0.1$
20—30	36	1007	43.65	$t_2 = 1.33$	$0.2 > p_2 > 0.1$
> 30	38	1086	40.01	$t_3 = 0.58$	$0.6 > p_3 > 0.5$

t_1 = calculated by the comparison of group 1 a. 2
t_2 = calculated by the comparison of group 3 a. 2
t_3 = calculated by the comparison of group 1 a. 3

It can be seen from table 2 that no correlation exists between plasminogen level and prothrombin time of plasma. Similarly we could not find any relationship between the fibrinogen level and the prothrombin time in patient's plasma. Table 3 shows that the increase of antiplasmin activity is parallel with the prolongation of the prothrombin time. The statistical significance of this findings was also shown, but correlation coefficient was 0,155.

Tab. 3: Antiplasmin activity and plasma prothrombin time

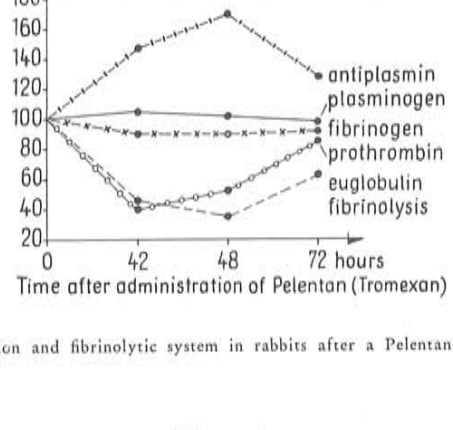
Prothrombin time sec.	Numb. of determinat.	Mean of antiplasmin activity index	Standard error	t	p
< 20	19	1.09	10.33	$t_1 = 2.96$	< 0.01
20—30	40	1.72	18.28	$t_2 = 0.44$	< 0.7
$\geqslant 30$	44	1.84	19.92	$t_3 = 3.19$	$0.01 > p > 0.001$

t_1 = calculated by the comparison of group 1 a. 2
t_2 = calculated by the comparison of group 3 a. 2
t_3 = calculated by the comparison of group 1 a. 3

II. The plasma fibrinolytic system after a single dose of anticoagulants.

The findings in human patients as described above were confirmed by the results in 33 rabbits treated with pelentan. Prolongation of prothrombin time, increase of antiplasmin activity and lengthening of euglobulin fibrinolysis time were noted (fig. 1). Both fibrinogen and plasminogen level did not show any

significant changes. Similar results were obtained when 21 rabbits were treated with sintrom or marcoumar.



The coagulation and fibrinolytic system in rabbits after a Pelentan administration.

Discussion

Changes in plasma fibrinolytic system after the administration of anticoagulants (dicoumarol derivative) are mentioned only in a few publications. Witte (21) found that the plasminogen level is influenced by these anticoagulants. No activation of fibrinolysis in the whole plasma was observed (7, 11). Opinions concerning the changes of fibrinogen level in the course of dicoumarol derivatives therapy are rather contradictory (8, 19).

We have shown above that the increase in antiplasmin activity and the inhibition of fibrinolysis in the euglobulin occurs in humans in the course of long-term anticoagulant therapy as well as in rabbits after administration of a large single dose of these anticoagulants. We suggest, that the inhibition of fibrinolysis is mainly due to increase of plasmin inhibitor because significant no changes in plasminogen and fibrinogen were observed. We found similar correlation between fibrinolysis and antiplasmin studying activation of fibrinolysis in physical hypothermia (14a). Elsner (6) described also the activation of fibrinolysis correlated with the decrease of plasmin inhibitor in his study on fibrinolysis in pregnancy and in certain obstetrical conditions.

The question of the inhibition of fibrinolysis may be important for biological and clinical evaluation of anticoagulant drugs. The increase in antiplasmin activity may render the intravascular clots more resistant to the dissolution processes. In this context we should like to quote Wessler's (20) results on

the formation and dissolution of clots in isolated segments of dog vessels. Wessler observed some advantages of heparin as compared with dicoumarol.

In some experiments clot formation in the isolated vessels of dicoumarolized dogs occured at prothrombin times as low as 10%. In the conditions of Wessler's experiments heparin prevented thrombus formation more effectively than dicoumarol. It is quite possible that Wessler's results may be partially explained by our findings as well as by Buluk and Januszko's (4) work. The latter found that heparin inactivates antiplasmin in the euglobulin fraction of plasma.

The activation of fibrinolysis during blood clotting may be due to a decrease of plasmin inhibitor. (Rechnic et al. [17]). Treatment with dicoumarol derivatives causing hypoprothrombinemia increases at the same time inhibition of fibrinolysis. The phenomenon of inhibition of fibrinolysis which we have described may be a part of the system of dynamic equilibrium in vivo between blood clotting factors and fibrinolytic enzymes.

Summary

The influence of some coumarine derivatives (anticoagulant drugs: pelentan [tromexan], sintrom, marcoumar) on plasma fibrinolytic activity has been investigated. The cases studied included a number of patients with coronary thrombosis treated in long-term anticoagulant therapy and rabbits treated with a single dose of the drug.

The anticoagulants have no significant effect either on fibrinogen or plasminogen level. All of the above mentioned drugs cause a prolongation of the euglobulin fibrinolysis time and an increase of the antiplasmin level. These two changes may be correlated to the prolongation of the prothrombin time.

The authors suggest that the described phenomena may be useful for biological and clinical evaluation of anticoagulant drugs.

Résumé

Nous avons examiné l'influence des derivées de dicoumarol — les anticoagulants: pelentan (tromexan), sintrom, marcoumar — sur le systeme fibrinolytique du plasma. Les cas etudies etaient les malades souffrants de la maladie coronaire qui etaient traité par les anticoagulants a longue distance. Nous avons etudié egalement les lapins qui ont recue une seule et grande dose des anticoagulants.

Les anticoagulants sont sans effet sur le niveau du plasminogéne et fibrinogene. Cependent nous avons constaté un allongement de la fibrinolyse dans des euglobulines et une augmentation du taux de l'antiplasmine plasmatique au cours du traitement par les anticoagulants examinés. Ces changements sont liées a un allongement du temps de prothrombine.

Nous croyons que les phenomenes que nous avons decrit soient importants pour une meilleurs evaluation clinique et biologique des anticoagulants.

Zusammenfassung

Der Einfluß einiger Coumarinderivate — Pelentan (Tromexan), Sintrom, Marcoumar — auf die fibrinolytische Aktivität von Plasma wurde untersucht. Das Versuchsmaterial bestand aus Patienten, die langdauernder Antikoagulantientherapie unterworfen waren. Außerdem wurde die Wirkung bei Kaninchen untersucht, die eine einmalige Dosis des Antikoagulans erhielten.

Die Behandlung mit Antikoagulantien beeinflußte den Fibrinogen- und Plasminogenspiegel nicht. Hingegen verursachen alle untersuchten Antikoagulantien eine Verlängerung der Euglobulin-Fibrinolyse und einen Anstieg des Antiplasminspiegels. Diese Veränderungen können zu der Verlängerung der Prothrombinzeit in Beziehung gesetzt werden.

Die Autoren sind der Ansicht, daß die beschriebenen Phänomene von Wert für die biologische und klinische Beurteilung von Antikoagulantien sein können.

References

(1) Alexander, B., Landwehr, G.: Evolution of prothrombin conversion accelerator in stored human plasma and prothrombin fractions. Amer. J. Physiol. 159: 332 (1949).
(2) Astrup, T., Müllertz, S.: The fibrin plate method for estimating fibrinolytic activity. Arch. Biochem. Biophys. 40: 346 (1952).
(3) Bachmann, F., Duckert, F., Geiger, H., Baer, P., Koller, F.: Differentiation of factor VII complex. Studies on the Stuart-Prower factor. Thromb. Diath. haem. 1: 169 (1957).
(4) Buluk, K., Januszko, T.: Heparyna a fibrynoliza. Patol. pol. 8: 107 (1957).
(5) Douglas, A. S.: A mode of action of coumarin drugs. Brit. med. Bull. 11: 39 (1955).
(6) Elsner, P.: Fibrinolyse in Schwangerschaft und Geburt. Fortschritte der Geburtshilfe und Gynäkologie, Nr. 6 (1957), (S. Karger, Basel—New York).
(7) Halse, T.: Fibrinolyse. Freiburg (1948), Cantor ed.
(8) Irish, V. V., Jaques, L. B.: The effect of dicoumarol upon plasma fibrinogen. Amer. J. Physiol. 143: 101 (1945).
(9) Koller, F., Loeliger, A., Duckert, F.: Experiments on a new clotting Factor (Factor VII). Acta haemat. (Basel) 6: 1 (1951).
(10) Koller, F.: Le Facteur X. Rev. Hémat. 10: 362 (1952).

(11) Kopec, M., Niewiarowski, S.: Czynnosc fibrynolityczna osocza osob zdrowych. Pol. Arch. Med. wewnet. 26: 1322 (1956).
(12) Kowalski, E., Latallo, Z., Niewiarowski, S.: Untersuchungen über die Aktivierung von Plasminogen und Inaktivierung von Plasmin. Folia haemat. (Lpz.) 75: 225 (1957).
(13) Kowarzyk, H., Buluk, K.: Postepy badan nad krzepnieciem krwi. Postepy Medycyny Doswiadczalnej i Higieny 2: 1 (1950).
(14) McElfresh, A. E., Özge, A.: The effect of coumarin drugs upon Plasma Thromboplastin Component. J. Lab. clin. Med. 49: 753 (1957).
(14a) Niewiarowska, M.: L'influence de l'hypothermie par refroidissement sur la coagulation du sang et sur la fibrinolyse chez le chat. Rev. Hémat. 12: 650 (1957).
(15) Niewiarowski, S., Latallo, Z.: unpublished.
(16) Quick, A. J.: Hemorrhagic Diseases. Philadelphia 1957, Lea and Febiger.
(17) Rechnic, M., Siwinska, M., Czerwinska, B.: Antyfibrynolizyna krwi a thrombinogeneza. Arch. Immunol. Ter. Dosw. 5: 347 (1957).
(18) Sise, H. S., Kumball, D. M., Adams, D.: Plasma Thromboplastin Component Deficiency produced by prolonged administration of prothrombopenic anticoagulants. Proc. Soc. exp. Biol. (N. Y.) 89: 91 (1955).
(19) Smith, W. B., Rosenfeld, R., Shinovara, G. Y.: Human plasma fibrinogen levels in various pathological states. Fed. Proc. 10: 371 (1951).
(20) Wessler, S.: Studies on intravascular coagulation. II. A comparison of the effect of dicoumarol and heparin on clot formation in isolated venous segments. J. clin. Invest. 32: 652 (1953).
(21) Witte, S.: Profibrinolysin, Thrombin-Inhibitor und Antithrombin bei Thrombose und während einer antikoagulierender Therapie. Intern. Tagung über Thrombose und Embolie, Basel (1954).