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(54) ANTI-AGGREGANTS

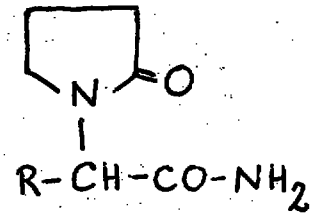
(71) We, UCB, of 4, Chaussée de Charleroi, Saint-Gilles-lez-Bruxelles, Belgium, a Body Corporate organised under the laws of Belgium, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—.

The present invention is concerned with pharmaceutical compositions which possess anti-aggregation and deaggregation properties with regard to blood platelets (thrombocytes) and with a process for the treatment of vascular diseases in non-human mammals.

The compositions according to the present invention are useful for the treatment of all diseases and especially of vascular diseases, in which it is necessary to inhibit platelet aggregation or to eliminate platelet aggregates already formed. They are also useful for the prophylaxis of vascular diseases.

The active compounds used in these compositions are known and their activity on the nervous system, especially the so-called nootropic activity, is also already known.

Thus, according to the present invention there is provided a pharmaceutical composition comprising at least one active compound of the general formula:—



(I)

wherein R is a hydrogen atom or a methyl or ethyl radical, said composition being in the form of a dosage unit containing 500 mg. of active compound, or in the form of a solution for intramuscular or intraperitoneal administration containing 1 g. of active material in 5 ml. of double distilled water or in the form of a potable solution containing 100 mg. of active compound per ml. of water + propylene glycol.

Thus, the following three 2 - (2 - oxo - pyrrolidino) - alkylamides are covered by this general formula:

compound A: 2 - oxo - 1 - pyrrolidineacetamide (piracetam) (R=hydrogen)
compound B: 2 - (2 - oxo - pyrrolidino) - propionamide (R=methyl)
compound C: 2 - (2 - oxo - pyrrolidino) - butyramide (R=ethyl).

Compound A and the preparation thereof are described and claimed in British Patent Specification No. 1,039,113 and compounds B and C and the preparation thereof, are described and claimed in British Patent Specification No. 1,309,692, which is a Patent of Addition to the first-mentioned British Patent Specification.

The present invention provides a process for the treatment of vascular diseases in non-human mammals, in which it is necessary to inhibit platelet aggregation or to eliminate already formed platelet aggregates by the use of an anti-aggregation agent, which comprises administering to a non-human mammal, as an anti-aggregation agent, a therapeutically-effective dose of a compound of the above-given general formula (I).

In the course of clinical investigations carried out with these compounds in order to determine their influence on a therapy in association with indirect anti-coagulants (for example phenprocoumon), there was observed a remarkable decrease of platelet aggregation in the plasma of patients suffering from vascular diseases, without any influence, either direct or indirect, being exerted upon the anti-coagulant action of the anti-coagulants used concurrently.

These clinical observations led to an attempt to confirm this activity on the platelets by conventional methods of investigation used in this field. Indeed, compounds having such an activity are particularly desirable but hitherto it has not been possible to find compounds which are satisfactory from all points of view. In the first place, the role of such compounds is to act at the very first appearance of the symptoms of thrombosis in such a manner as to avoid the appearance thereof at the level of the damaged vascular endothelium. It is imperative that, in contradistinction to anti-coagulants, the anti-aggregants do not modify the aptitude to coagulation (in particular the speed of formation of fibrin) so as to be able to administer concurrently an anti-aggregant and an anti-coagulant in the prophylactic treatment of thrombo-embolisms.

The tendency of platelets to aggregate in the human plasma increases with age, as well as in subjects suffering from vascular diseases, including chronic arterial obstructions and venous obstructions. Similarly, in the case of subjects who do not suffer from vascular diseases, the tendency for platelets to aggregate increases after infections. The anti-coagulant compounds (for example phenprocoumon or heparin) have, as a rule, an influence on the fibrin formation phase, without remedying the increase of the tendency of platelets to aggregate. For this reason, intensive research has recently been carried out in order to discover medicaments which do not have any action on the mechanism of haemostasis but which diminish or suppress platelet aggregation. For example, acetylsalicylic acid has been found to have an important action not only on platelet aggregation but, unfortunately, also on haemostasis, which limits its therapeutic utility in this field.

The same applies to other anti-aggregant compounds, such as indomethacin and phenylbutazone, which have an undesirable influence on the effective dose of indirect anti-coagulants, for example phenprocoumon. This excludes an attempt to carry out a therapy combining these two types of drugs. An ideal anti-aggregant compound must reduce, or even eliminate, the tendency to aggregation without having any secondary effects, especially upon haemostasis. In other words, at the concentration at which these compounds inhibit platelet aggregation, they must not have any other influence on the other platelet factors responsible for the role of platelets in haemostasis. In particular, it is eminently desirable that these drugs do not have any influence on the mechanism of coagulation and, in particular, upon the formation of thrombin. Hitherto, such medicaments have been extremely rare (see, for example, E. Wenzel *et al.*, Med. Welt. *25*/1976).

From this point of view, the action of the compounds of general formula (I), namely, piracetam and its two higher homologues, is particularly interesting. These compounds do not modify the distribution of the thrombocytes (platelets) according to their volume, nor the morphological parameters of the thrombocytes observed under the microscope, even at high concentrations of the order of 5×10^{-2} mol. The three compounds very effectively inhibit aggregation induced by adenosine diphosphate (ADP) up to concentrations of 10^{-5} mol. Furthermore, it is their specificity which is most interesting because, besides inhibiting the aggregation induced by ADP, they do not affect the other platelet functions. Although this effect has been observed for the first time clinically with piracetam *in vivo*, it appears that this compound, at the same concentration but *in vitro*, does not seem to be *a priori* as interesting, especially as compared with compounds B and C. This is bound up with fact that *in vitro* tests give an orientation or an idea of the activity of the test compound, without it being possible *a priori* to transpose these conclusions integrally *in vivo*.

The following tests serve to demonstrate these conclusions:

A. Presentation of the various methods used.

1. *Thromboelastographic measurements on citrate plasma rich in platelets.*

In a TEG apparatus (Hartert's apparatus), a human citrate plasma rich in platelets obtained from healthy subjects is recalcified. In this plasma, the number of thrombocytes and the concentration of fibrinogen are known and constant. Recalcifying is carried out by the use of a 0.1 mol solution of calcium chloride. The role of the citrate is to prevent any coagulation before starting the experiments. Indeed, the citrate ions behave as a chelating agent towards the calcium ions present in the plasma, which

are the origin of the coagulation process. The coagulation process may thus be started at any moment by a simple recalcification by adding calcium chloride. It is to be recalled that this thromboelastographic technique makes use of the increase in viscosity of a blood preparation in the course of coagulation. The principle of this technique consists in subjecting to an oscillating angular movement a cup containing the preparation being investigated and of studying the effects of the coagulation on a cylinder suspended at the end of a torsion wire and immersed in the preparation. At the beginning of coagulation, the plasma contained in the cup entrains the cylinder in its oscillating movements. Coagulation time $t=0$ is the moment at which the activation of the factors responsible for the coagulation is initiated by the addition of calcium ions. Further, r=the latent period which precedes the effective beginning of coagulation, am=the maximum amplitude of the oscillations of the cylinder, k=the time interval from the moment when the cylinder starts oscillating to the moment when the amplitude of movement becomes equal to the maximum amplitude obtained under the same conditions with a plasma without platelets and having a normal fibrinogen content.

The total coagulation time of a preparation is constituted by the sum $r + k$. The kinetics of the coagulation is expressed by the parameters r and $r + k$ and the intensity of the coagulation by the maximum amplitude am of the oscillations of the cylinder.

The equation

$$\frac{am}{r+k}$$

expresses the speed of solidification of the coagulum (clot).

In the same manner, there is also measured the maximum elasticity of the thrombus (in the following Tables max.E), which is an indirect measure of the shrinkage of the fibrin coagulum induced by the thrombocytes. The determination of the maximum

$$\frac{1}{2}\text{-lysis}$$

time is used in order to show that there is no activation of the fibrinolytic system.

2. *Measure of the coagulation.*

The partial recalcification time of a plasma treated with kaolin (PTT test) is used as a measure of the capacity of the formation of intrinsic prothrombinase (see H. Vinazzer, Gerinnungsstörungen in der Praxis, pub. Fischer Verlag, Stuttgart, 1972). For the speed of formation of extrinsic thrombin, there is used the prothrombin time measurement test (PTZ test) and the hepatoquick test (see Vinazzer, *loc. cit.*).

The phase of polymerisation of the fibrin is studied by the time of coagulation induced by thrombin, reptilase and thrombin°coagulase (see E. Wenzel *et al.*, Dtsch.med. Wsch., *15*, 746—756/1974 and P. E. Gregoire, Biochemie pathologique, pub. Presses Acad, Europeennes, Paris, Maloine, 1972, Chapter 39, p.831 (Les Maladies Hemorragiques).

3. *Morphological studies of the thrombocytes and analysis of the distribution of platelets according to their volume.*

The morphology of the thrombocytes, smeared on a slide of an interference microscope (enlargement × 400), is used as a very sensitive test for evaluating the functional alteration of the thrombocytes. This test essentially gives non-quantitative indications and, in particular, enables alterations in the energy metabolisms of the thrombocytes to be detected.

The volume analysis of the particles with a channel analyser (Coulter Counter Channelyzer), which is electronic and automatic, enables quantitative but less sensitive data to be obtained for the more pronounced alterations of the thrombocytes. Thus, by this method, the typical morphological alterations of the thrombocytes or the process of fragmentation or also the aggregation phenomena may be precisely determined quantitatively. Smaller modifications are, however, not detected by this test (see H. Holzhüter *et al.*, Thromb et diath. Suppl., in the press, 1976; see also H. Holzhüter *et al.*, Verh.Dtsch.Ges.f.Inn.Med., 1974).

4. *Turbidimetric measurement of the aggregation process.*

In an aggregometer modified according to Born (ELVI model) or in an Eppendorff universal aggregometer, there is placed, with continuous agitation, a plasma rich in

platelets (PRP) at 37°C. After a constant preliminary incubation period of 30 minutes, to the PRP is added ADP (10^{-4} to 10^{-6} mol) or collagen (20 μg to 4 μg/ml. PRP) and the process of aggregation is directly recorded in the form of curves of decrease of extinction or of increase of transmission. The maximum change of extinction per minute, or the time which, on the extinction curve, separates the first point of inflection from the lowermost point on this curve (the so-called reaction time) are taken as measurement parameters. These measurements are completed by an analysis of the distribution of the non-aggregated thrombocytes according to their volume.

B. Results of the tests.

Unless otherwise stated, the concentrations indicated for the compounds A, B and C are the final concentrations in the test samples in moles per litre.

1. *Influence of compounds A, B and C on the functional aptitudes of human thrombocytes.*

To a PRP obtained from the plasma of 10 healthy persons, there are added increasing amounts of compounds A, B and C, whereafter the samples are incubated for 30 minutes at ambient temperature, after which there are carried out:

an analysis of the volume distribution of the particles (Coulter counter);
thromboelastographic tests in a recalcified PRP;
morphological examination of the thrombocytes smeared on a microscope slide.

Results and conclusions.

Even high concentrations (1 mol) of compounds A and C only have a very minimum influence on the morphology of the spread thrombocytes (slight increase of the unspread thrombocyte forms). At concentrations of 10^{-2} mol or less, this effect is no longer detectable for any of the compounds A, B or C. The results obtained are given in the following Table I (the normal value for unspread thrombocytes lies between 35 and 45%).

TABLE I

Compound (mol)	Evaluation	% unspread thrombocytes
blank I (NaCl)	slight inhibition	39
blank II (NaCl)	slight inhibition	41
A 1	slight increase of inhibition	43
A 10^{-1}	as blank	38
A 10^{-2}	as blank	40
A 10^{-3}	as blank	37
B 1	as blank	39
B 10^{-1}	as blank	40
B 10^{-2}	as blank	37
B 10^{-3}	as blank	40
C 1	slight increase of inhibition	41
C 10^{-1}	as blank	39
C 10^{-2}	as blank	40
C 10^{-3}	as blank	39

The distribution of the thrombocytes according to their volume is not modified, even by high concentrations of the three compounds tested.

The following Table II indicates the number of thrombocytes at increasing concentrations of compounds A, B and C.

TABLE I I

Compound (mol)	Number of thrombocytes
Blank	95,000
A 10^{-1}	107,000
10^{-2}	98,000
10^{-3}	97,000
B 10^{-1}	97,000
10^{-2}	98,000
10^{-3}	97,000
C 10^{-1}	95,000
10^{-2}	94,000
10^{-3}	89,000

Furthermore, the functional thromboelastographic parameters of the platelets and, in particular, the solidity of the thrombus (which is an indirect measure of the contraction of the coagulum under the effect of the thrombocytes) is not significantly influenced even by high concentrations of compounds A, B or C. In the following Table III, there are given the results of the thromboelastogram, measured on solutions containing 9 parts of PRP and 1 part of test compound.

TABLE III

Compound (mol)	r	k	r + k	am	$\frac{am}{r+k}$	max. E
blank PRP	1.6	0.5	2.1	4.9	2.33	96.1
blank NaCl	2.0	0.8	2.8	5.4	1.93	117.4
A 10^{-3}	1.5	0.5	2.0	4.7	2.35	88.7
10^{-4}	1.7	1.1	2.8	3.3	1.18	49.3
10^{-5}	1.5	1.1	2.6	3.0	1.15	42.9
blank PRP	1.6	0.5	2.1	4.9	2.33	96.1
blank NaCl	2.0	0.8	2.8	5.4	1.93	117.4
B 10^{-3}	1.9	0.8	2.7	6.0	2.22	150.0
10^{-4}	2.3	1.2	3.5	4.9	1.4	96.1
10^{-5}	1.4	0.4	1.8	6.0	3.3	150.0
blank PRP	6.4	3.0	9.4	4.7	0.5	88.7
blank NaCl	6.5	2.4	8.9	5.6	0.63	127.3
C 10^{-3}	5.8	2.2	8.0	4.6	0.58	85.2
10^{-4}	5.0	3.7	8.7	3.9	0.45	63.9
10^{-5}	4.1	1.4	5.5	4.5	0.82	82.2

Table III shows the influence of compounds A, B and C on the maximum solidity of the thrombus and on the time of formation of the coagulum by the thrombo-elastographic tests on a human PRP before and after the addition of the test compounds. Final concentrations of 10^{-2} to 10^{-5} mol of these compounds influence the maximum solidity of the thrombus and the time of formation of the coagulum only to a very minor degree.

2. *Influence of high concentrations of compounds A, B and C on the formation of intrinsic and extrinsic thrombin and on the rate of polymerisation of the fibrin.*

To a plasma obtained from healthy donors, there are added increasing concentrations of the test comouunds, whereafter the samples are incubated at ambient temperature for 30 minutes. As a blank, there are used, the same plasmas to which there is added the same volume of the solvent which is used for the test compounds (9 parts of isotonic sodium chloride solution per one part of citrate). The pH values of the solutions thus obtained vary between 7.2 and 7.6.

Results and conclusions.

Even high concentrations of the three test compounds (up to 10^{-2} mol) do not have any influence on the formation of the intrinsic thrombin (PTT test) and of the extrinsic thrombin (quick-test).

Concentrations higher than 10^{-2} mol of the three test compounds have a very distinct inhibiting action on the polymerisation of fibrin. However, the thrombin time is only very slightly influenced.

For concentrations equal to or below 10^{-2} mol (for example 10^{-2} and 10^{-3} mol), neither the polymerisation of fibrin nor the thrombin formation phase are influenced to any notable extent.

The results of these tests are given in the following Table IV.

TABLE IV

Compound (mol)	thrombin time	PTT	Quick	fibrinogen	reptilase	thrombin coagulase
blank I (NaCl)	17.8	35.5	100	168	16.8	20.3
A 10^{-1}	22.9	36.1	90	144	34.1	54.0
10^{-2}	19.0	34.6	89	132	18.4	21.4
10^{-3}	20.2*	32.0*	100*	210*	15.8*	24.8*
blank II (NaCl)	20.8*	31.8*	100*	240*	15.0*	26.5*
blank I (NaCl)	17.8	35.5	100	168	16.8	20.3
B 10^{-1}	24.9	38.5	85	144	37.3	42.8
10^{-2}	18.3	34.5	89	140	17.6	22.6
10^{-3}	21.1*	31.8*	100*	240*	16.5*	25.6*
blank II (NaCl)	20.8*	31.8*	100*	240*	15.0*	26.5*
blank I (NaCl)	17.8	35.5	100	168	16.8	20.3
C 10^{-1}	22.9	36.1	90	144	34.1	54.0
10^{-2}	19.0	34.6	89	132	18.4	21.4
10^{-3}	20.2*	32.0*	100*	210*	15.8*	24.8*
blank II (NaCl)	20.8*	31.8*	100*	240*	15.0*	26.5*

* The asterisk indicates that blank II corresponds to the tests carried out at the concentration of 10^{-3} mol. These results are expressed in seconds, except for fibrinogen, where they are expressed in mg/100 ml.

3. *Influence of compounds A, B and C on platelet aggregation induced by collagen or ADP.*

The influence of increasing concentrations of compounds A, B and C on platelet aggregation induced by high and low concentrations of ADP or collagen is studied quantitatively. PRP is incubated for 30 minutes with the treatment substances up to concentrations of the order of 10^{-5} mol. As a blank, there is used PRP with a constant number of thrombocytes, either as such or with the addition of a buffer. Each test lasts about 4 hours, bearing in mind the alteration of the platelets which takes place in any case when human thrombocytes are left at ambient temperature (they have a spontaneous tendency to aggregate). Consequently, all the blanks are repeated after each series of tests.

In the first preliminary tests, there is used the turbidimetric method modified according to Born's principle (ELVI turbidimeter). As a measure of the aggregation (or deaggregation), there is used the extinction change measured by graphic extrapolation of the recorded extinction curve. In addition to this, there is also taken into consideration the slope of the curve and the maximum transmission change during the course of the reaction.

The quantitative measurement of the aggregation and deaggregation phenomena is evaluated by the decrease in the absolute number of platelets (decrease in the number of non-aggregated thrombocytes in the plasma after the beginning of the aggregation process). On the other hand, there is recorded the volumetric distribution of the free platelets, which are not aggregated, during the aggregation process. There is thus obtained a direct measurement of the aggregation kinetics and of the deaggregation process.

For the aggregation experiments induced by ADP or collagen, there are used plasmas from healthy subjects which contain, on average, between 80 and 120,000 platelets/mm^3. All the experiments were repeated several times.

Results:

Compounds A, B and C inhibit platelet aggregation induced by ADP or by collagen, without having an effect on the distribution of the thrombocytes according to their volume. Indeed, on the contrary, the influence of ADP and of collagen on this latter is suppressed (this influence manifests itself by a decrease of the bigger thrombocytes which are particularly functionally active and by a relative increase of the number of smaller thrombocytes, which are relatively less active, always speaking functionally).

For compound A, this is demonstrated, in the first place, by photometric methods.

In the following Table V, there are given the results of extinction curves (but not the curves themselves). Aggregation is induced by ADP at different concentrations (10^{-4}, 10^{-5} and 10^{-6} mol). Compound A (0.14 mol) is incubated with the PRP for 30 minutes.

In the following Table, the symbols have the following meanings:

Tr=the time which, on the extinction curve, separates the first point of inflection from the lowermost point on this curve;

Lz=the angle giving a measure of the maximum speed of aggregation;

Max E=the absolute extinction difference in the plasma, before and after aggregation.

TABLE V

Final concentration of ADP	Compound (mol)	Tr	Lz	Max. E	Number of thrombocytes	
					before aggregation	after aggregation
10^{-4} mol	NaCl (blank)	0.5	70	610	112,000	20,000
	A 0.14	1.0	60	340	109,000	78,000
10^{-5} mol	NaCl (blank)	0.9	71	470	107,000	59,000
	A 0.14	1.3	31	115	101,000	79,000
10^{-6} mol	NaCl (blank)	1.5	33	220	112,000	54,000
	A 0.14	–	0	95	105,000	83,000

Table VI gives the results of the extinction curves but this time the concentration of compound A is varied. The operating conditions are the same as above. The ADP is used at a concentration of 10^{-6} mol. The non-diluted solution of compound A is 1.41 mol.

TABLE VI

Test solutions	Final concentration of A (mol)	Tr	Lz	Max. E	No. of thrombocytes before aggregation
9 parts PRP + 1 part NaCl	–	0.9	62	370	152,000
9 parts PRP + 1 part A (1 : 0)	0.14	–	180	140	152,000
9 parts PRP + 1 part A (1 : 5)	0.028	1.0	45	270	152,000
9 parts PRP + 1 part A (1 : 10)	0.014	1.2	59	380	160,000
9 parts PRP + 1 part A (1:100)	0.0014	1.0	57	350	160,000
9 parts PRP + 1 part A (1:1000)	0.00014	1.0	58	370	160,000

In the accompanying drawings, there is also given an example of platelets volume distribution curves obtained with compound A under the following conditions:
0.9 ml. PRP, incubated for 30 minutes with compound A (0.14 mol);
0.1 ml. of a 10^{-4} mol solution of ADP.

Figure I represents the platelet volume distribution curve when using compound A before aggregation, while Fig. II represents the corresponding curves obtained with a blank (sodium chloride).

The abscissae of the curves give the channels of 0 to 100 (according to the principle of the channel analyser) and the ordinates the number of thrombocytes corresponding to a given channel.

Table VII below gives the results obtained on the photometric curves and on the platelet volume distribution curves for compounds A, B and C. The operating conditions are as follows:
aggregation induced by 0.1 ml of 10^{-6} mol solution of ADP;
0.9 ml. PRP, incubated for 30 minutes with the test compound.

TABLE VII

Product final molar concentration	Tr	Lz	Max E		Number of thrombocytes		Peak	
			1st summit	2nd summit	before aggr.	after aggr.	before aggr.	after aggr.
blank NaCl	0.9	66°	420	730	95,000	58,000	19	14
A 10^{-1}	–	0°	220	200	107,000	76,000	19	17
A 10^{-2}	0.9	62°	290	340	98,000	37,000	17	15
A 10^{-3}	0.9	62°	270	300	97,000	27,000	18	13
B 10^{-1}	–	0°	80	70	97,000	78,000	17	16
B 10^{-2}	0.9	64°	380	260	98,000	50,000	15–20	15
B 10^{-3}	0.8	66°	450	500	97,000	26,000	16	5 15
C 10^{-1}	–	0°	80	80	95,000	84,000	17	17
C 10^{-2}	1.1	66°	390	290	94,000	55,000	17	15
C 10^{-3}	1.4	70°	420	400	89,000	30,000	16	1 16
blank NaCl	0.9	65°	500	760	98,000	11,000	19	5

The same experiments were carried out for aggregations induced by different concentrations of collagen. In Table VIII (concentration of collagen 4 μg/ml) and Table IX (concentration of collagen 20 μg/ml) given hereinafter, there are given the results obtained on the photometric curves and on the platelet volume distribution curves using compounds A, B and C. The operating conditions are as follows:
aggregation induced by collagen at the rate of 20 and 4 μg of collagen (in suspension) per ml. of PRP;
0.9 ml. of PRP incubated for 30 minutes with the test compound.

TABLE VIII

Test compound (mol)	Tr	Lz	Max. E	Number of thrombocytes		Peak	
				before aggr.	after aggr.	before aggr.	after aggr.
blank (NaCl)	7.5	80°	820	106,000	4,000	14	3
A 10^{-2}	6.4	79°	700	106,000	4,000	14	3
B 10^{-3}	6.2	80°	790	106,000	10,000	14	3
10^{-4}	7.3	78°	820	106,000	5,000	14	3
C 10^{-3}	7.0	78°	790	106,000	5,000	14	3

TABLE IX

Test compound (mol)	Tr	Lz	Max. E	Number of thrombocytes		Peak	
				before aggr.	after aggr.	before aggr.	after aggr.
blank (NaCl)	4.0	82°	1060	111,000	5,000	13	3
A 10^{-1}	8.0	74°	790	106,000	18,000	14	11
10^{-2}	6.4	79°	860	106,000	4,000	14	3
B 10^{-1}	7.5	70°	630	106,000	33,000	14	12
10^{-2}	4.3	77°	990	106,000	4,000	14	3
C 10^{-1}	8.0	0°	340	106,000	78,000	14	11
10^{-2}	5.1	81°	900	106,000	4,000	14	3

Table IX shows that the three compounds A, B and C, at a final concentration of 10^{-1} mol, inhibit the aggregation induced by 20 μg. of collagen. The descending order to activity of the compounds is C, B, A, not only in the optical measurements but also in the measurement of the thrombocyte count. At 10^{-2} mol, the activity is not observable. Table VIII shows, on the other hand, insofar as it concerns the aggregation induced by a smaller quantity of collagen (4 μg.), that only compound B is active in a dosage equal to 10^{-3} mol or even 10^{-4} mol.

From what has been stated hereinbefore, it can be seen that, especially in the case of aggregation induced by ADP, the three compounds appear to be equally active. The measurements made with collagen appear to indicate, however, the superiority of B and C over A. The examination of the form of the aggregation curves also shows an increase of the speed of deaggregation.

The general conclusion which can be drawn from these tests is that, leaving concentration considerations aside, the three compounds have a very similar type of action: they diminish, in a particularly effective manner, the aggregation of platelets induced by ADP and increase the speed of deaggregation during the phenomenon of the ADP aggregation. They also protect the platelets against the morphological modifications induced by ADP or by collagen. Thus, the "platelet anti-aggregant" activity of these compounds appears to be very interesting. This is all the more so, considering that this activity is not simultaneously accompanied by an undesirable influence on the mechanism of coagulation and, in particular, upon the formation of thrombin.

A comparison of the activity of compound C with that of a known anti-aggregant, namely lysine acetylsalicylate, sold under the Registered Trade Mark "Aspisol" and designated hereinafter as "compound X", shows the medical interest of the first compound (as well as that of its homologues: compounds B and A).

Compound X has, in fact, superior antiaggregant properties, as can be seen from Table X below giving the results of the aggregation test induced by collagen.

TABLE X

Compound (mg/ml)	Maximum speed of aggregation Lz	Maximum extinction Max. E	Aggregation latency	Interpretation
Blank	51°	320	27	normal
C (2)	61°	390	39	normal type of aggregation,
(4)	51°	400	52	but delayed
X (2)	24°	200	64	slowed down speed, reduced intensity, very marked delay
(4)	0	0	∞	total inhibition

It can be seen that, under the prevailing conditions, compound C slightly delays aggregation in proportion to the concentration used. This delay is interpreted as a slight inhibition. Compound X inhibits aggregation partially at 2 mg/ml., but completely at 4 mg/ml.

However, unlike compound C, compound X has two secondary effects, which might be harmful:

1. Table XI, giving the comparative results of thromboelastography, shows that compound X slows down the formation of the coagulum because of its anti-coagulant properties, this effect being proportional to the concentration used. However, compound C does not show this.

TABLE XI

Compound (mg/ml)	Time for the formation of the coagulum r+k	Interpretation
C (2)	2.8	normal
(4)	2.3	normal
X (2)	4.6	marked slowing down of the speed of coagulation
(4)	5.2	

2. Tables XII and XIII, which give the distribution of the platelets according to their volume before and after the addition of collagen show that, whereas compound C leaves this distribution practically unchanged, compound X produces an increase of the percentages of the smaller platelets, which are functionally less active (smaller contracted platelets and cellular fragments).

TABLE XII

(before addition of collagen)

Compound (mg/ml)	Percentages of platelets measured along the following volumes								Interpretation
	0 to 2.16 μ^3	2.16 to 4.56 μ^3	4.56 to 6.9 μ^3	6.9 to 9.36 μ^3	9.36 to 11.76 μ^3	11.76 to 14.16 μ^3	14.16 to 16.56 μ^3	16.56 to 18.96 μ^3	
Blank	2.6	11.04	21.82	21.50	16.19	10.59	6.73	4.42	normal
C (2)	4.35	15.13	24.0	20.7	13.7	8.8	5.6	3.75	normal
(4)	6.14	14.4	23.7	21.0	14.0	8.6	5.3	3.5	normal
X (2)	5.3	15.9	24.3	20.7	13.8	8.3	5.0	3.2	normal
(4)	6.4	16.6	25.65	20.94	13.21	7.6	4.3	2.7	normal

underlined = maximum

TABLE XI II

(after addition of collagen)

Compound (mg/ml)	Percentages of platelets measured among the following volumes										Interpretation
	0 to 2.16 μ^3	2.16 to 4.56 μ^3	4.56 to 6.96 μ^3	6.96 to 9.36 μ^3	9.36 to 11.76 μ^3	11.76 to 14.16 μ^3	14.16 to 16.56 μ^3	16.56 to 18.96 μ^3	18.96 to 21.36 μ^3	21.36 to 23.76 μ^3	
Blank	2.6	11.0	21.8	21.5	16.2	10.6	6.7	4.4	2.9	1.8	(1)
C (2)	4	14.1	23.4	21.1	14.4	9.1	5.9	3.9	2.6	1.6	(2)
(4)	6	15.6	23.8	20.1	13.5	8.3	5.4	2.3	1.53	–	(2)
X (2)	21.7	26.1	22.7	13.2	7.0	3.9	2.4	1.3	1	0.7	(3)
(4)	50.1	22.4	12.9	6.5	3.4	2.15	1.13	0.72	0.45	0.24	(4)

underlined = maximum

(1) normal distribution without collagen = blank

(2) normal

(3) mixed distribution: cellular fragments – platelets

(4) characteristic distribution of cellular fragments. Very few whole platelets.

Consequently, compound C, as well as compounds B and A, can advantageously replace compound X in all cases where the latter might be contraindicated, more particularly, in the following situations:

(a) gastro-duodenal ulcers or persons having had such antecedents;
(b) any surgical operation and, in particular, vascular or cardiac aerterial reconstruction surgery, for instance open heart operations;
(c) thrombosis diseases in general (cardiac, cerebral, members);
(d) general platelet pathology (thromboasthenia, thrombopoenia, thrombocythemia).

C. Posology.

The experiments concerning the metabolism of compounds A, B and C have shown that a dosage of 8.5 to 500 mg/kg administered three times a day at 8 hour intervals enables a blood level to be obtained which is sufficient to inhibit platelet aggregation *in vivo*.

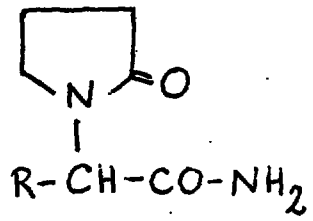
D. Type of administration.

The intensity and speed of resorption of these compounds enables the desired blood levels to be obtained, whatever the route of administration be (oral, intramuscular, intraperitoneal or intravenous).

E. Indications.

Compounds A, B and C can be used for the following indications: myocardial infarct resulting from platelet hyperaggregatability or hyperadhesivity, extracorporeal circulation or the use of valve prostheses, thromboembolic diseases and coronary hyperaggregability.

The present invention also provides a process for the treatment of vascular diseases in non-human mammals in which it is necessary to inhibit platelet aggregation or to eliminate already formed platelet aggregates by the use of an anti-aggregation agent, which comprises administering, as an anti-aggregation agent, a therapeutically effective dose of an active compound of the general formula:—



wherein R is a hydrogen atom or a methyl or ethyl radical.

For carrying out this process, the active compound can be in the form of a composition comprising the active compound in admixture with a solid or liquid pharmaceutically acceptable carrier or diluent.

The compositions used in the process according to the present invention can be all conventional oral and parenteral forms of administration, for example, tablets, capsules, dragees, syrups, solutions, suspensions, drops and suppositories. For this purpose, at least one active material is mixed with a solid or liquid pharmaceutical carrier or diluent and subsequently brought into the desired form. Solid carrier materials include, for example, starch, lactose, mannitol, methyl cellulose, talc, highly dispersed silicic acid, high molecular weight fatty acids (such as stearic acid), gelatine, agar-agar, calcium phosphate, magnesium stearate, animal and vegetable fats and solid high molecular weight polymers (such as polyethylene glycol). Compositions suitable for oral administration can, if desired, contain flavouring and/or sweetening materials. As injection medium, it is preferred to use water which contains conventional additives for injection solutions, such as stabilising agents, solubilising agents and/or buffers. Additives of this type include, for example, acetate and tartrate buffers, ethanol, complex-forming agents (such as ethylenediamine-tetraacetic acid and the non-toxic salts thereof) and high molecular weight polymers (such as polyethylene oxide) for viscosity regulation.

WHAT WE CLAIM IS:—

1. A pharmaceutical composition comprising at least one active compound of the general formula:—

Consequently, compound C, as well as compounds B and A, can advantageously replace compound X in all cases where the latter might be contraindicated, more particularly, in the following situations:

(a) gastro-duodenal ulcers or persons having had such antecedents;
(b) any surgical operation and, in particular, vascular or cardiac aerterial reconstruction surgery, for instance open heart operations;
(c) thrombosis diseases in general (cardiac, cerebral, members);
(d) general platelet pathology (thromboasthenia, thrombopoenia, thrombocythemia).

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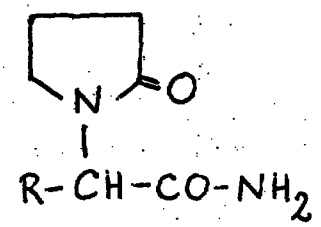
D. Type of administration.

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E. Indications.

Compounds A, B and C can be used for the following indications: myocardial infarct resulting from platelet hyperaggregatability or hyperadhesivity, extracorporeal circulation or the use of valve prostheses, thromboembolic diseases and coronary hyperaggregability.

The present invention also provides a process for the treatment of vascular diseases in non-human mammals in which it is necessary to inhibit platelet aggregation or to eliminate already formed platelet aggregates by the use of an anti-aggregation agent, which comprises administering, as an anti-aggregation agent, a therapeutically effective dose of an active compound of the general formula:—



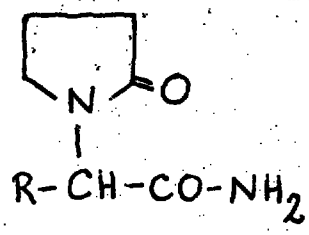
wherein R is a hydrogen atom or a methyl or ethyl radical.

For carrying out this process, the active compound can be in the form of a composition comprising the active compound in admixture with a solid or liquid pharmaceutically acceptable carrier or diluent.

The compositions used in the process according to the present invention can be all conventional oral and parenteral forms of administration, for example, tablets, capsules, dragees, syrups, solutions, suspensions, drops and suppositories. For this purpose, at least one active material is mixed with a solid or liquid pharmaceutical carrier or diluent and subsequently brought into the desired form. Solid carrier materials include, for example, starch, lactose, mannitol, methyl cellulose, talc, highly dispersed silicic acid, high molecular weight fatty acids (such as stearic acid), gelatine, agar-agar, calcium phosphate, magnesium stearate, animal and vegetable fats and solid high molecular weight polymers (such as polyethylene glycol). Compositions suitable for oral administration can, if desired, contain flavouring and/or sweetening materials. As injection medium, it is preferred to use water which contains conventional additives for injection solutions, such as stabilising agents, solubilising agents and/or buffers. Additives of this type include, for example, acetate and tartrate buffers, ethanol, complex-forming agents (such as ethylenediamine-tetraacetic acid and the non-toxic salts thereof) and high molecular weight polymers (such as polyethylene oxide) for viscosity regulation.

WHAT WE CLAIM IS:—

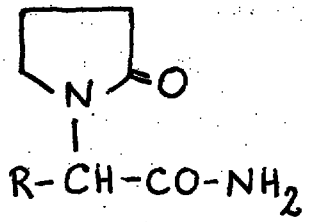
1. A pharmaceutical composition comprising at least one active compound of the general formula:—



wherein R is a hydrogen atom or a methyl or ethyl radical, said composition being in the form of a dosage unit containing 500 mg. of active compound, or in the form of a solution for intramuscular or intraperitoneal administration containing 1 g. of active material in 5 ml. of double distilled water, or in the form of a potable solution containing 100 mg. of active compound per ml. of water + propylene glycol.

2. A pharmaceutical composition according to claim 1, substantially as hereinbefore described.

3. A process for the treatment of vascular diseases in non-human mammals in which it is necessary to inhibit platelet aggregation or to eliminate already formed platelet aggregates by the use of an anti-aggregation agent, which comprises administering, as an anti-aggregation agent, a therapeutically effective dose of an active compound of the general formula:—



wherein R is a hydrogen atom or a methyl or ethyl radical.

4. Process according to claim 3, wherein the active compound is used in admixture with a solid or liquid pharmaceutically acceptable carrier or diluent.

5. A process according to claim 3 or 4, wherein administration is oral, intramuscular, intraperitoneal or intravenous.

6. A process according to any of claims 3 to 5, wherein the therapeutically effective dose is from 8.5 to 500 mg./kg., administered three times a day at 8 hour intervals.

7. A process according to any of claims 3 to 6, wherein the compound used is 2-oxo-1-pyrrolidine-acetamide.

8. A process according to any of claims 3 to 6, wherein the compound used is 2-(2-oxo-pyrrolidino)-propionamide.

9. A process according to any of claims 3 to 6, wherein the compound used is 2-(2-oxo-pyrrolidino)-butyramide.

10. A process according to claim 3 for the treatment of vascular diseases in non-human mammals, substantially as hereinbefore described and exemplified.

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