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Antithrombotic activity of F 16618, a new PAR1 antagonist evaluated in extracorporeal arterio-venous shunt in the rat

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ABSTRACT

The purpose of the present work was the evaluation of the antithrombotic activity of a new PAR1 antagonist, F 16618 in arterio-venous shunt in the rat. Arterial thrombosis was induced by insertion of a silk thread (thrombogenic substrate) into an extracorporeal shunt. F 16618 was administered either by intravenous route (0.63–2.5 mg/kg) or by oral route (20–80 mg/kg). Oral activity of F 16618 was compared to that of aspirin (20–80 mg/kg) and clopidogrel (0.63–10 mg/kg). Finally, F 16618 was associated to aspirin and/or clopidogrel to test for possible antithrombotic activity and its effects on bleeding time. SFLLR-induced human platelet aggregation was evaluated in the presence of F 16618, demonstrating the anti-aggregant activity of this compound. F 16618 (1.25 mg/kg) significantly delayed the time leading to occlusion by 52 ± 17%, without affecting bleeding time and in absence of hemodynamic effects. F 16618 given orally dose-dependently increased the time to occlusion. The maximal effect was observed at 40 mg/kg (984 ± 95 s versus 644 ± 17 s in vehicle group). Aspirin and clopidogrel also dose-dependently lengthened time to occlusion, but this effect was associated with an increase of bleeding time. F 16618 (20 mg/kg) orally associated with either aspirin (40 mg/kg) or with clopidogrel (1.25 mg/kg) potentiated the antithrombotic effects of both compounds without further increasing of bleeding time. In conclusion, F 16618 exerted a potent antithrombotic activity by intravenous and oral routes, without affecting bleeding time. Furthermore, the antithrombotic activity was potentiated when combined with aspirin or clopidogrel.

1. Introduction

The role of anti-platelet therapy in the treatment of many cardiovascular disorders is now accepted as antithrombotic strategy. Thus, platelet-active agents such as aspirin, glycoprotein IIb/IIIa antagonists and the thienopyridine derivatives become standard therapeutic agents in both the prevention and the treatment of pathophysiological conditions such as myocardial infarction, stroke and unstable angina [1]. However, these antithrombotic therapies suffer from drawbacks mainly associated with hemorrhagic side effects and lack of desired levels of efficacy [2]. As there is an important interest in the development of safer and more effective oral antithrombotic agents, new approaches of anti-platelet therapy are under investigation. Among these, a promising strategy is targeting thrombin receptors expressed on the platelet membrane using receptor antagonist compounds.

Thrombin mediates its platelet effects primarily through protease-activated receptors (PARs). PARs are G protein-coupled receptors that possess a unique mechanism of activation [3,4]. Thus, thrombin specifically cleaves the extracellular N-terminal region of this receptor to create a new receptor amino terminus which acts as a tethered ligand [5]. PAR can equally be activated in the absence of proteolytic activity, using synthetic peptides called PAR-activating peptides that mimic the tethered ligand sequence.

At present, four distinct subtypes of PARs have been characterized and designated PAR1, PAR2, PAR3 and PAR4, in chronological order of their discovery [5–10]. Among these, PAR1, PAR3, and PAR4 are activated by thrombin [5,11]. Current evidences suggest, however, that PAR1 and PAR4 are functionally the major human platelet thrombin receptors. Indeed, activation of PAR1 and PAR4 is sufficient to trigger platelet aggregation and secretion [5,12]. Based on the observation that the PAR1-activating peptides, SFLLR and TFLLR were unable to induce rodent platelet aggregation *in vitro* or *ex vivo* [13], it has been suggested that PAR1 is not the functional receptor for thrombin in rat platelets. However, we recently demonstrated that selective PAR1 antagonists exert antithrombotic activity in an extracorporeal arterio-venous shunt model in the rat [14]. Three theories have been proposed to explain such a result in the rat. The first would be the existence of a PAR1-like receptor expressed at the platelet membrane, which can be activated by

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thrombin but not by the PAR1-activating peptide SFLLRN (or SFFLRN in the case of the rodent thrombin receptor's N-terminal tethered ligand [15]) and which could be recognized by the PAR1 antagonists. The second hypothesis would be an effect on monocyte–platelet interactions. Monocytes possess PAR1 [16] known to play a major role in thrombosis. Platelet interaction with the monocytes in the bloodstream induces activation of monocyte, which subsequently become more adhesive, more migratory and more procoagulant. Additionally, monocytes and platelets contribute to an inflammatory phenotype of the endothelium. This effect ultimately leads to an increase of monocyte and platelet adhesion and to activation of these cells [17]. As a final possible explanation, the lack of selectivity of these PAR1 antagonists towards other receptors distinct from the PAR family cannot be excluded [14].

In an extracorporeal arterio-venous shunt model of low-shear rate, simple and reproducible hemodynamic and rheological conditions of the experiments are important and should be controlled [18,19]. In this model, a thrombus is induced that consists of platelets, erythrocytes and fibrin [14,20]. We have shown that a single administration of PAR1 antagonist, namely ER-121958 or SCH-203099 exerted an antithrombotic effect, which was comparable to that observed with conventional agents [14]. Moreover, a contribution of PAR4 could be reasonably excluded in this context. We used this model to further characterize the properties of the recently induced new non-peptidic small molecule PAR1 antagonist termed F 16618 [21,22]. The aims of this study were firstly to describe the antithrombotic activity of F 16618 administered either intravenously or orally and secondly to evaluate whether pharmacological activity of F 16618 could be additive to those of other therapeutic classes of antithrombotic agents.

2. Materials and methods

2.1. Human platelet aggregation

Apheresis platelet concentrates were collected from healthy donors from the French Blood Transfusion Agency. The platelets were incubated with or without F 16618 (10 or 100 μM) for 30 min without stirring before adding different concentration of SFLLR (0.1–100 μM). Aggregation was measured and expressed as a percent change in light transmission. Results are expressed as a percentage of control area under curve (AUC) ± SEM.

2.2. Preparation of animals

Animals were housed and tested in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility in strict compliance with all applicable regulations and the protocol was carried out in compliance with French regulations and with local Ethical Committee guidelines for animal research.

Two hundred nineteen male Crl: OFA (SD) rats weighing 280–300 g at the date of experiment were purchased from Charles River (Laboratories France, Iffa-Credo, L'Arbresles, France). They were housed in the Centre de Recherche Pierre Fabre animal facilities for at least two weeks before use. Throughout this period, they had free access to food and drinking water. The animal house was maintained on a 12-h light/dark cycle (lights on at 7 a.m.) at an ambient temperature of 20 ± 2 °C.

The caudal vein was cannulated for anesthesia (60 mg/kg pentobarbital sodium, Sanofi-Aventis, France) and for intravenous administration of compounds. The animals underwent a tracheotomy and were mechanically ventilated at 60 respirations/min (2.5 ml/respiration, Ventilator model 683, Harvard Apparatus, Holliston, MA, USA) but respiratory rate and tidal volume could be adjusted for maintaining blood gases within a physiological range (ABL 700, Radiometer). Animal temperature was maintained at 38 °C ± 0.5 °C with a heating pad (Homeothermic blanket control unit, Harvard Apparatus). Polyethylene catheters were inserted into both femoral arteries for sampling blood and continuously measuring arterial pressure via a DTX™ PLUS transducer (Becton Dickinson, Le Pont de Chaix, France), connected to a Gould amplifier (Gould Instruments, France).

2.3. Extracorporeal arteriovenous shunt

Animals were prepared according to the method described by Freund et al. [23]. Briefly, the right carotid artery and the left jugular vein were exposed and carefully isolated from surrounding tissues. The shunt (30 cm) was constructed with polyethylene catheters. A silk thread placed in the central part of the shunt was used to act as the thrombogenic substrate. The polyethylene tubing used was coated with silicon. The shunt was filled with 0.2 ml/kg heparin solution (10 IU/ml; Choay Laboratories, France). A thermal microprobe (type IT-23, Physitemp Instruments Inc., Clifton, NJ) was secured onto the central part of the shunt and was connected to a thermocouple (Gould Instruments). Blood flow was then established through the shunt by unclamping, thereby rapidly raising the shunt temperature to values slightly lower than the rectal temperature of the rat. Shunt temperature reached a plateau phase and then fell rapidly, coinciding with increased thrombotic obstruction of blood flow across the shunt. Time leading to occlusion of the shunt (reported as time to occlusion) was defined as the elapsed time between the start of carotid blood flow and the time at which the shunt temperature was 1 °C higher than baseline temperature and corresponded to the formation of an occlusive thrombus with complete interruption of carotid blood flow.

2.4. Experimental protocol

Three protocols were used for this study:

A/evaluation of F 16618 alone by i.v. route.
B/evaluation of F 16618 and other antithrombotic drugs alone by oral route.
C/evaluation of F 16618 associated to other antithrombotic drugs by oral route.

2.4.1. Protocol A

Following stabilisation of hemodynamic and blood gases, treatment (drug/vehicle) was intravenously administered (1 ml/kg) over 5 min, 10 min before shunt opening. Two groups were performed for this protocol:

- 1/vehicle (10% dimethylsulfoxide in sterile saline, $n = 19$).
- 2/F 16618 (0.63 mg/kg, $n = 8$; 1.25 mg/kg, $n = 9$; 2.5 mg/kg, $n = 10$) dissolved in 10% dimethylsulfoxide in sterile saline.

2.4.2. Protocol B

All drugs (vehicle, F 16618, aspirin, and clopidogrel) were administered in a 1% aqueous solution of methylcellulose (10 ml/kg) *via* a rigid gastric tube. F 16618 was administered 15 min, aspirin 90 min and clopidogrel 210 min before anesthesia.

- 1/vehicle ($n = 21$).
- 2/F 16618 (10 mg/kg, $n = 8$; 20 mg/kg, $n = 8$; 40 mg/kg, $n = 7$ and 80 mg/kg, $n = 8$).
- 3/aspirin (20 mg/kg, $n = 8$; 40 mg/kg, $n = 8$ and 80 mg/kg, $n = 8$).
- 4/clopidogrel (0.63 mg/kg, $n = 8$; 1.25 mg/kg, $n = 8$; 2.5 mg/kg, $n = 8$; 5 mg/kg, $n = 8$ and 10 mg/kg, $n = 6$).

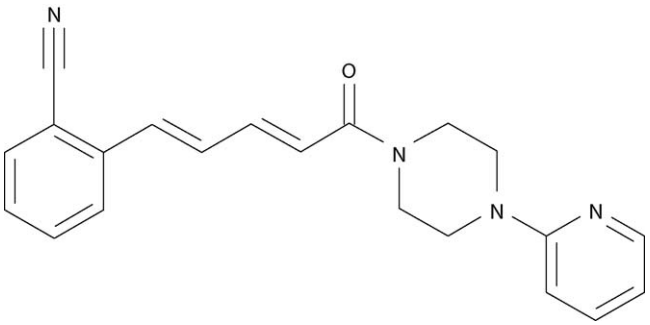

Fig. 1. The chemical structure of F 16618.

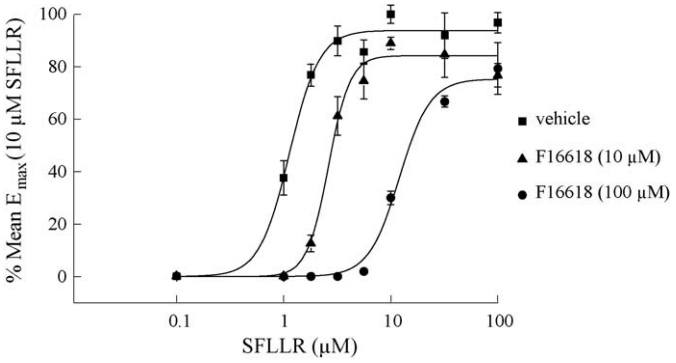

Fig. 2. Effects of F 16618 on SFLLR-induced human platelet aggregation. Apheresis platelet concentrates were collected from healthy donors. Data are means ± SEM.

2.4.3. Protocol C

Drugs were administered as for protocol B. Four further groups were performed:

- 1/aspirin (40 mg/kg) + clopidogrel (1.25 mg/kg), $n = 8$.
- 2/aspirin (40 mg/kg) + F 16618 (20 mg/kg), $n = 8$.
- 3/clopidogrel (1.25 mg/kg) + F 16618 (20 mg/kg), $n = 8$.
- 4/aspirin (40 mg/kg) + clopidogrel (1.25 mg/kg) + F 16618 (20 mg/kg), $n = 8$.

2.5. Measured parameters

The analogic arterial pressure signal was digitised at 500 Hz and simultaneously recorded by means of data acquisition software (Notocord-hem Evolution, Notocord Systems, Croissy sur Seine, France). Mean arterial pressure (MAP) in mmHg was calculated as MAP = (systolic arterial pressure + 2 diastolic arterial pressure)/3. Heart rate (HR) in bpm was derived from arterial pressure. Rectal temperature and the temperature in the shunt were also recorded throughout the experiment. These parameters are the average of all successive determinations taken during a 30 s recording period.

2.6. Drugs

For intravenous administration, F 16618, 2-[5-Oxo-5-(4-pyridin-2-yl-piperazin-1-yl)-penta-1,3-dienyl]-benzonitrile hydrochloride (Fig. 1), synthetized in the Centre de Recherche Pierre Fabre by the Division of Medicinal Chemistry was dissolved in 10% dimethylsulfoxyde. For oral administration, aspirin (Aspegic, Sanofi-Aventis, Paris, France), clopidogrel (Plavix, Sanofi-Aventis, Paris, France) and F 16618 were suspended in a 1% aqueous solution of methylcellulose. The suspension was continuously stirred until administered to animals. The doses of drugs employed are in terms of their corresponding bases.

2.7. Tail bleeding time

The tail bleeding time test was essentially performed as described by De Clerck et al. [24] and Wollny et al. [25] and was done at the end of the shunt occlusion experiment. The tail was cut at 2 mm from the tip, after warming the tail at 37 °C for 3 min. The bleeding time was measured from the moment the tail was cut until bleeding had stopped completely for 30 s. In the treated-groups, a bleeding time that exceeded four times the average value obtained in the vehicle group was considered as non-occluded and was noted as being a 300% increase in bleeding time.

F 16618, aspirin and clopidogrel were tested in this procedure.

2.8. Statistical analysis

All data were expressed as mean ± SEM. Analysis of variance, with or without repeated measures, followed by Dunnett's test was used to assess significance among and between groups, respectively (Sigma Stat version 2.03, Jandel, Germany). A $P < 0.05$ level was chosen for significance.

3. Results

3.1. Human platelet aggregation

The PAR1 agonist peptide SFLLR induced a concentration-dependent human platelet aggregation (EC_{50}: 1.13 μM, 95% confidence limits: 0.94–1.32 μM). From 10 μM, F 16618 significantly inhibited SFLLR-induced platelet aggregation in a competitive manner, EC_{50}: 2.62 μM (2.25–3.05 μM) and 12.0 μM (9.6–18.5 μM) in the presence of 10 and 100 μM F 16618, respectively (Fig. 2).

3.2. Intravenous administration of F 16618 (protocol A)

3.2.1. Hemodynamic effects

The hemodynamic effects of intravenous administration of F 16618 are summarized in Table 1. Mean arterial pressure and heart rate values did not significantly differ between groups of rats under baseline conditions. F 16618, even at 2.5 mg/kg, was devoid of notable changes on mean arterial pressure and heart rate compared to vehicle (7.7 ± 1.4% versus 4.2 ± 2.9%, P = NS, and 1.6 ± 0.2% versus 0.8 ± 1.2%, P = NS, respectively, Table 1).

Table 1
Effects of F 16618 (0.63–2.5 mg/kg) and its vehicle administered by intravenous route on mean arterial pressure and heart rate.

Dose F 16618 (mg/kg)	n	Mean arterial pressure			P	Heart rate			P
		Baseline (mm Hg)	Post administration (mm Hg)	Variations (%)		Baseline (bpm)	Post administration (bpm)	Variations (%)	
Vehicle	19	121 ± 5	125 ± 4	4.2 ± 2.9	NS	406 ± 8	409 ± 8	0.8 ± 1.2	NS
0.63	8	121 ± 5	126 ± 5	4.5 ± 1.7	NS	399 ± 11	408 ± 9	2.4 ± 1.0	NS
1.25	9	132 ± 7	137 ± 6	4.7 ± 2.1	NS	428 ± 10	428 ± 1	0.2 ± 0.7	NS
2.5	10	118 ± 5	127 ± 6	7.7 ± 1.4	NS	402 ± 10	409 ± 111	1.6 ± 0.2	NS

Data are means ± SEM.

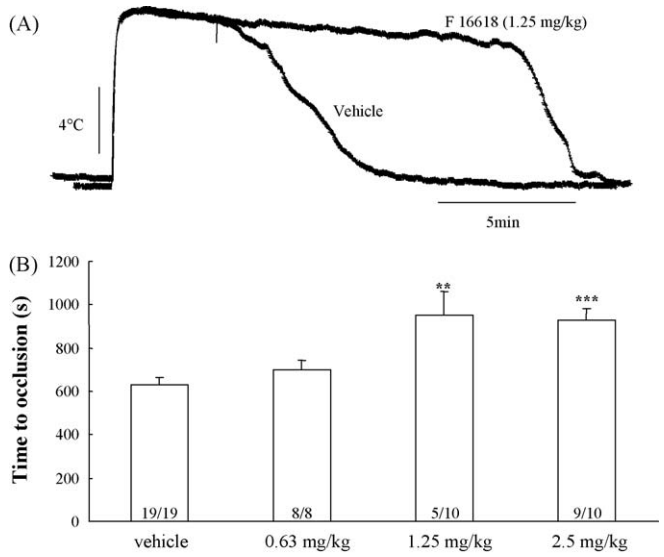

Fig. 3. Effects of F 16618 (by i.v. route) on time leading to occlusion during an arterio-venous shunt in the rat. Typical recording of temperature into the shunt in presence of vehicle or F 16618 (A). Dose–responses of F 16618 (0.63–2.5 mg/kg, B). Numbers inside columns represent the ratio of shunts closed after 20 min. Data are means ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle.

3.2.2. Effects of F 16618 on the time leading to occlusion

The average time leading to occlusion of vehicle group was 628 ± 39 s. At 0.63 mg/kg, F 16618 failed to affect the time to occlusion (699 ± 46 s, P = NS). On the other hand, F 16618 from 1.25 mg/kg significantly increased the time leading to occlusion (Fig. 3B). Fig. 3A shows a representative recording in the presence of F 16618. Time to return to baseline temperature in the shunt was almost twice as long when compared to vehicle.

3.2.3. Effects of F 16618 on bleeding time

In the vehicle group, the average bleeding time was 393 ± 29 s. Table 2 summarizes bleeding time with different doses of F 16618. No notable changes of this parameter, even at 2.5 mg/kg, were recorded.

3.3. Oral administration of F 16618 (protocol B)

Table 3 presents baseline mean arterial pressure and heart rate values obtained for different groups of rats. Overall, the baseline mean arterial pressure and heart rate values did not differ between groups, apart from some exceptions described in Table 3.

When vehicle was administered by oral route, the time to occlusion was very close to that obtained after intravenous administration (636 ± 25 s, n = 21, versus 628 ± 39 s, n = 19, respectively). F 16618 given orally dose-dependently increased the time leading to occlusion. The maximal effect was observed at 40 mg/kg (984 ± 95 s versus 644 ± 17 s in the vehicle group, $P < 0.001$). As shown in Fig. 4, aspirin and clopidogrel dose-dependently also lengthened the time to occlusion. Under aspirin, the maximal effect was measured at 80 mg/kg (837 ± 57 s, $P < 0.001$). Clopidogrel showed a greater antithrombotic efficacy than aspirin. Indeed, clopidogrel at 1.25 mg/kg presented a significant antithrombotic activity, whereas aspirin at 20 mg/kg failed to affect the time to occlusion. Furthermore, clopidogrel at 10 mg/kg totally prevented the shunt closing since no arterio-venous shunt was closed at the end of the experiment, whereas all shunts (n = 21) were closed in the vehicle group.

Table 2
Effects of F 16618 and its vehicle, intravenously administered on bleeding time.

Treatment i.v.	Dose (mg/kg)	Bleeding time (s)	Variations (%)	P
Vehicle		393 ± 29		
F 16618	0.63	316 ± 53	−20 ± 13	NS
	1.25	366 ± 32	−7 ± 8	NS
	2.5	358 ± 26	−9 ± 9	NS

Data are means ± SEM.

Table 3
Effects of F 16618, aspirin, clopidogrel and their vehicle, orally administered, on mean arterial pressure and heart rate.

Treatment	Dose (mg/kg)	n	MAP P (mmHg)	HR P (bpm)
Vehicle		21	126 ± 4	404 ± 7
F 16618	10	8	126 ± 7	436 ± 16*
	20	8	124 ± 4	422 ± 11
	40	7	147 ± 5**	415 ± 14
	80	8	124 ± 6	423 ± 15
Aspirin	20	8	140 ± 4	431 ± 16
	40	8	116 ± 5	395 ± 9
	80	8	107 ± 6*	365 ± 11**
Clopidogrel	0.63	8	133 ± 4	411 ± 7
	1.25	8	136 ± 6	427 ± 9*
	2.5	8	131 ± 5	401 ± 15
	5	8	124 ± 5	375 ± 6*
	10	6	134 ± 6	409 ± 17

Data are means ± SEM.
* $P < 0.05$.
** $P < 0.01$ versus vehicle.

3.4. Effects of F 16618 associated to other antithrombotic agents (protocol C)

Fig. 5 shows different associations studied in the present procedure. F 16618 (20 mg/kg) and aspirin (40 mg/kg) shared the same threshold of antithrombotic activity, but their association markedly increased the time leading to occlusion by 45 ± 11% (versus vehicle). Similarly, the combination of F 16618 (20 mg/kg) and clopidogrel (1.25 mg/kg) potentiated the antithrombotic effect by 61 ± 13%. On the other hand, the association of the three compounds failed to further increase the time to occlusion indeed, aspirin (40 mg/kg) associated to clopidogrel (1.25 mg/kg) lengthened the time to occlusion by 66 ± 9%.

Clopidogrel (1.25 mg/kg) or aspirin (40 mg/kg) significantly increased the bleeding time, to 751 ± 166 and 1066 ± 165 s, respectively, versus 490 ± 54 s in the vehicle group. Fig. 6 clearly shows that the addition of F 16618 (20 mg/kg) did not prolong the bleeding time when it was administered with aspirin or clopidogrel. As expected, the association of aspirin and clopidogrel dramatically increased the bleeding time (>1400 s, Fig. 6).

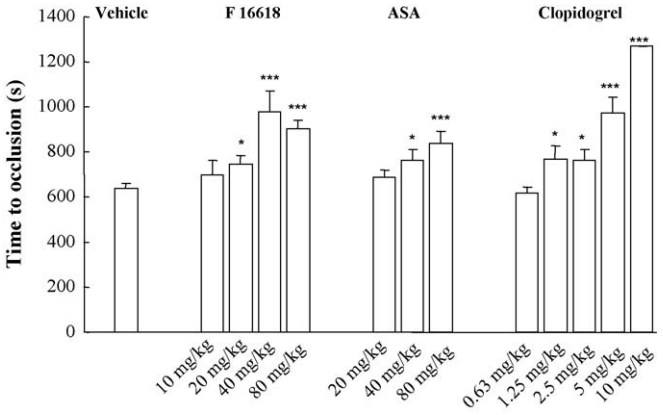

Fig. 4. Effects of F 16618, aspirin and clopidogrel orally administered on time to occlusion. Data are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle.

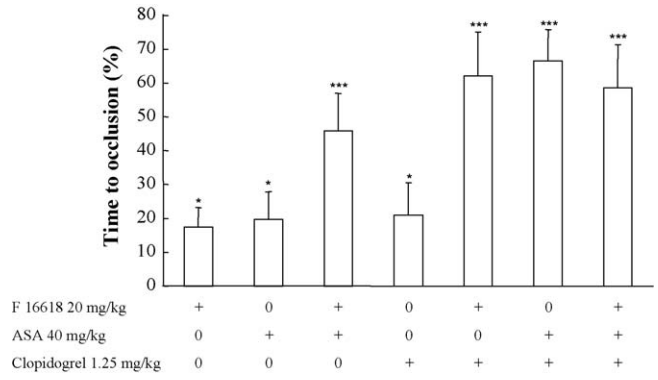

Fig. 5. Effects of association of different antithrombotic agents on time to occlusion. F 16618 was orally given at the dose of 20 mg/kg, aspirin at 40 mg/kg and clopidogrel at 1.25 mg/kg. Data are means ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$ versus vehicle.

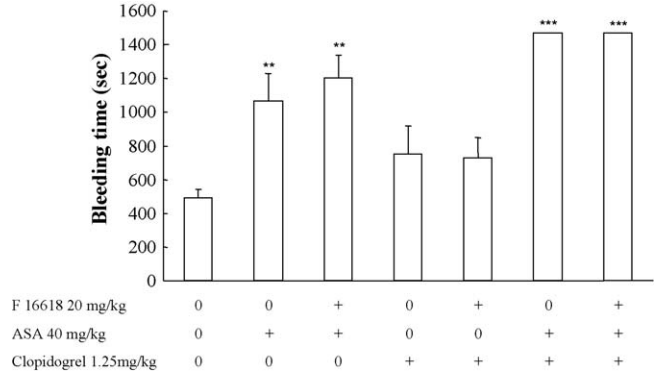

Fig. 6. Effects of association of different antithrombotic agents on bleeding time. F 16618 was orally given at the dose of 20 mg/kg, aspirin at 40 mg/kg and clopidogrel at 1.25 mg/kg. Data are means ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle.

4. Discussion

The aims of the present work were to describe the antithrombotic activity of F 16618 administered by intravenous or oral routes and to evaluate whether this pharmacological activity could be associated to those of agents of other well-known antiaggregant therapeutic classes. F 16618 exerted a potent antithrombotic activity by intravenous route from 1.25 mg/kg and by oral route from 20 mg/kg, without affecting the main hemodynamic parameters and the bleeding time. Furthermore, the antithrombotic activity was potentiated in presence of aspirin or clopidogrel. Therefore, F 16618 constitutes a new interesting antithrombotic agent.

Experimental models of thrombosis conducted in animals are useful to study the mechanisms involved in thrombus formation and to compare the potency of various antithrombotic drugs. Therefore, it is important to use, as a first approach, an experimental model of arterial or venous thrombosis in animals, the similarity of which to human arterial thrombosis has been well-established [26]. An arterio-venous shunt model in the rat is widely used for evaluating antithrombotic drugs [14,27] since it is sensible to both the modulation of the coagulation system and/or platelet function [19,28]. In this model of low-shear rate, simple and reproducible hemodynamic and rheological conditions of the experiments are important and must be controlled [18]. The thrombotic occlusion is initiated by a silk thread placed inside an extracorporeal shunt between the carotid artery and the jugular vein. Hemodynamic and blood gases are controlled throughout the experimental protocol. It has been reported that the thrombus formed under these conditions consists of platelets, erythrocytes and fibrin [20]. Platelets appear to play a particularly important role in thrombus formation [29]. Indeed, the thrombus in this experimental procedure is characterized by the presence of many platelets.

The antithrombotic effect of aspirin administered by oral route has been evaluated in this model. Aspirin from 40 mg/kg lengthened the time leading to occlusion, however, at this dose the bleeding time was widely prolonged. Similar results were reported in an arterio-venous shunt in a guinea-pig model [30]. This pharmacological effect has been primarily attributed to irreversible blockade of the cyclooxygenase-1 enzyme in platelets which leads to an attenuation in the production of thromboxane A_2 [31]. In addition, recent reports suggest that aspirin therapy may inhibit the function of the glycoprotein IIb/IIa receptor [32]. Clopidogrel, on the other hand, inhibits platelet aggregation by blocking adenosine diphosphate (ADP)-dependent platelet activation through the blockade of platelet $P2Y_{12}$ receptor [33]. In experimental studies in rats, the maximal inhibition of platelet aggregation induced by 2.5 μM ADP was achieved 4 h after oral administration [34]. Consequently, in this present study, clopidogrel has been administered 3 h 30 before anesthesia corresponding to 4 h following gavage at the opening of the arterio-venous shunt. Clopidogrel appears to be a potent antithrombotic agent since the time to occlusion was doubled at 10 mg/kg (threshold effective dose, 1.25 mg/kg), revealing the important role of ADP in this experimental model. However, clopidogrel (1.25 mg/kg) significantly affected the bleeding time as expected.

Recently, in the same model, we have shown that selective PAR1 antagonists (ER-121958 and SCH-203099) administered intravenously exerted an antithrombotic activity, which is comparable to that obtained with conventional antithrombotic agents [14]. In the present study, F 16618, a new non-peptidic small molecule PAR1 antagonist [22] also demonstrated a lengthening of time leading to occlusion, and this both when administered by i.v. route or orally, in the latter case at doses from 20 mg/kg. At higher doses (by i.v. and oral routes), F 16618 slightly lost its effectiveness. This bell-shaped dose-response relationship has been already observed in the presence of another PAR1 antagonist [35]. May be the relative solubility of F 16618 could explain that its effect is less important for high doses. In the presence of F 16618, the bleeding time was not altered, even at the highest dose tested (2.5 mg/kg). In addition, at this dose, F 16618 was devoid of significant effects on main hemodynamic parameters (mean arterial pressure and heart rate). This absence of secondary effects with F 16618 allowed combinations of different products. Furthermore, it was important to associate aspirin and clopidogrel to test for additional effects. Indeed, the activation of platelets is accompanied by the release of ADP and thromboxane A_2, amplifying adhesion and platelet aggregation. Thus, aspirin and clopidogrel possessed a complementary role, which induces a synergy of antithrombotic activities. In line with this, the present results demonstrate that the association of clopidogrel with aspirin significantly increases the time to occlusion. The combination of a PAR1 antagonist with clopidogrel or aspirin is particularly promising. Thrombin (generated by the coagulation cascade), ADP (released from platelet dense granules) and thromboxane A_2 (synthesized and released by activated platelets) are considered as secondary haemostatic agonists, although thrombin is the most potent known platelet agonist [1]. Each agonist, to variable degrees, stimulates a range of common platelet responses. Thus it appears logical that F 16618 when orally administered in combination with currently available antithrombotic drugs enables, an additional antithrombotic activity in the arterio-venous shunt model, even if the combination of F 16618 with clopidogrel appears more promising than that of F 16618 with

aspirin. The most important point was that the bleeding observed with clopidogrel or aspirin was not further enhanced in the presence of F 16618. A safety and tolerability phase II study with SCH-530348 (PAR1 antagonist) recently showed that this compound was generally well-tolerated and did not cause increased bleeding according to the thrombolysis in myocardial infarction scale [36].

In conclusion, by using an extracorporeal arterio-venous shunt model in the rat, our results demonstrated that F 16618 is a potent new antithrombotic compound in an absence of significant effects on bleeding time and on global hemodynamic parameters (mean arterial pressure and heart rate), even at the highest dose studied. Furthermore, when added to the commonly prescribed antithrombotic drugs, aspirin and clopidogrel, F 16618 lead to an additional antithrombotic activity. F 16618 constitutes an example of a promising new antithrombotic strategy, which could be used as complement or as alternative to currently available treatments.

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