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# Antithrombotic actions of the thrombin inhibitor, argatroban, in a canine model of coronary cyclic flow: comparison with heparin

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**1** The antithrombotic action of argatroban, a synthetic thrombin inhibitor, was studied in a canine model of coronary cyclic flow having some of the characteristics of acute unstable angina. Heparin was studied as a reference anticoagulant.

**2** Localized endothelial damage was induced in the circumflex coronary artery of anaesthetized open-chest foxhounds and a critical stenosis was applied by use of a Lexan constrictor placed around the artery at the site of endothelial damage. An electro-magnetic flow probe was placed distal to the lesion, and cyclic flow variations (CFVs) were observed, as thrombi formed at the site of the arterial lesion and were dislodged. Test compounds were administered by i.v. infusion commencing 1 h after the appearance of CFVs, and maintained for 1 h. On termination of the treatments, coronary flow was observed for a further 60 min. A series of blood samples were taken at predetermined times throughout each experiment in order to determine the coagulation parameters, thrombin time (TT) activated partial thromboplastin time (aPTT) and for the determination of fibrinopeptide A (FpA) levels before, during and post-treatment.

**3** Argatroban and heparin showed antithrombotic effects in this model. Argatroban dose-dependently increased the minimum coronary flow at the nadir of the CFVs from $5.4 \pm 1.7$ to $9.1 \pm 2.1$ ml min$^{-1}$ (30 $\mu$g kg$^{-1}$ min$^{-1}$, $P=0.041$) and from $2.9 \pm 0.9$ to $16.3 \pm 4.5$ ml min$^{-1}$ (100 $\mu$g kg$^{-1}$ min$^{-1}$, $P=0.023$, $n=8$ dogs at each dose level). Heparin (5 and 15 iu kg$^{-1}$ min$^{-1}$) also increased minimum flow, but the increase was not statistically significant at the 5% level, although the $P$ value in animals treated with 15 iu kg$^{-1}$ min$^{-1}$ ($P=0.0521$, $n=6$ dogs) fell just outside this limit. Although neither compound significantly decreased the overall CFV frequency, argatroban (100 $\mu$g kg$^{-1}$ min$^{-1}$) significantly ($P<0.01$) decreased the number of large amplitude CFVs (minimum coronary flow $<10$ ml min$^{-1}$) by 63%, and heparin (15 iu kg$^{-1}$ min$^{-1}$) caused a 50% decrease in this parameter ($P<0.05$).

**4** The thrombin times were increased by a factor greater than 10 during antithrombotic treatment, irrespective of the compound or doses used. Heparin treatment induced 17 and $>30$ fold increases in aPTT at 5 and 15 iu kg$^{-1}$ min$^{-1}$ respectively. However, argatroban produced only 2 and 3 fold increases in aPTT at 30 and 100 $\mu$g kg$^{-1}$ min$^{-1}$, despite significant antithrombotic effects. FpA levels were reduced in the presence of both argatroban and heparin.

**5** These data show that, when administered as an intravenous infusion, argatroban is a potent antithrombotic agent in a canine model of coronary cyclic flow.

**Keywords:** Argatroban; heparin; canine coronary thrombosis; cyclic flow reductions; coagulation

## Introduction

Unstable angina is an acute coronary syndrome characterized by the presence of a thrombus superimposed upon a segment of a stenosed coronary artery, where the stenosis is due to the presence of an asymmetrical atheromatous plaque (see Fuster *et al.*, 1990; Badimon *et al.*, 1993 for reviews). Angioscopic studies have revealed the thrombus to be greyish-white and thus rich in platelets (Mizuno *et al.*, 1992); in addition, patients with unstable angina present persistent activation of the coagulation pathway as denoted by elevated prothrombin $F_{1+2}$ fragments and fibrinopeptide A (FpA) levels in their plasma (Merlini *et al.*, 1994). Thus, thrombin generation could play an important pathological role in unstable angina.

Argatroban ((**2R**,**4R**)-4-methyl-1-[$N^2$-((**RS**)-3-methyl-1,2,3,4,-tetrahydro-8-quinoline sulfonyl)-L-arginyl]-2-piperidine carboxylic acid hydrate) is a potent and selective synthetic thrombin inhibitor with $K_i$ values against thrombin ranging from 5 to 39 nM (Okamoto *et al.*, 1981; Kikumoto *et al.*, 1984; Tapparelli *et al.*, 1993). It is also a potent anticoagulant *in vitro* (Tamao *et al.*, 1986), and has been shown to inhibit the platelet aggregatory and vasoconstrictor actions of thrombin, which are dependent upon the proteolytic properties of this enzyme (Nakamura *et al.*, 1985; Hara *et al.*, 1986). Argatroban has antithrombotic properties in a wide variety of animal models of both platelet-rich and erythrocyte-rich thrombosis (see Bush, 1991 for review), and, as expected for an active site inhibitor, its action is independent of the presence of antithrombin III, unlike heparin (Kumada & Abiko, 1981). Also, we have recently shown that its antithrombotic activity in a rat arterial thrombosis model is attainable with a much lower degree of systemic anticoagulation than that required for heparin to produce an equivalent antithrombotic effect (Berry *et al.*, 1994a).

We decided to study the potential antithrombotic effects of argatroban and heparin in a canine model of coronary unstable angina (Folts, 1991). This model is characterized by cyclic coronary flow variations (CFVs) due to intraluminal thrombus formation and embolisation (either spontaneously or mechanically induced) resulting from the combination of vessel damage and critical stenosis of a coronary artery. Given that, unlike heparin, argatroban has a short duration of action both in animals and in man (Iida *et al.*, 1986; Verstraete, 1990; Clarke *et al.*, 1991), in order to ascertain its real anti-thrombotic efficacy, we have administered argatroban as an intravenous infusion. Part of this work was presented to the British Pharmacological Society, December 1994 (Duval *et al.*, 1995).

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## Methods

### *Surgical preparation*

Male Foxhound dogs (22–35 kg, Lebeau, France) were chosen for use in this study for the structure of their coronary circulation. Animals were anaesthetized with sodium pentobarbitone (Sanofi, 35 mg $kg^{-1}$, i.v. for induction and 5 mg $kg^{-1}$ $h^{-1}$ throughout the experiment to maintain anaesthesia.) Animals were artificially ventilated via the trachea with room air, supplied by a Hugo Basile animal ventilator, stroke volume 600–700 ml, frequency 12–14 $min^{-1}$. Blood gas parameters ($Po_2$, $Pco_2$, pH) were measured at regular intervals and were maintained within normal limits. Body temperature was monitored rectally and maintained between 37–38°C by means of a thermostatically-controlled operating table. A carotid artery was cannulated for continuous blood pressure monitoring with a Gould P23id pressure transducer, and the right brachial vein was cannulated for drug administration. Electrodes were placed under the skin of the four limbs, for ECG and heart rate monitoring. A left thoracotomy was performed at the level of sixth intercostal space, and the heart was suspended in a pericardial cradle. A 1.5–2 cm segment of the circumflex coronary artery was carefully isolated proximal to the first major collateral vessel, and the segment was fitted with an electromagnetic flow probe (Carolina Medical Electronics). A cotton thread was placed around the segment distal to the probe. Arterial blood pressure, heart rate, ECG and coronary flow were recorded on a Grass RPS 7c8 chart recorder. None of the catheters used in this study was heparinised.

### *Thrombosis induction*

Cyclic flow variations (CFVs) were induced following a procedure similar to that described by Folts (1991). Briefly, after recording the reactive hyperaemic response to momentary coronary occlusion by means of the cotton thread, the segment of the coronary artery distal to the flow probe was clamped three times in a controlled fashion with artery forceps to cause an endothelial lesion. An appropriately sized Lexan constrictor (containing a tapered nylon monofilament (fly fishing line 3×) in the lumen) was placed around the damaged segment in order to reduce the coronary diameter to approximately 50%. The reactive hyperaemic response was checked, and, if critical stenosis (absence of such a response) was not achieved, the degree of stenosis was adjusted by use of the nylon filament. Under these condition CVFs occur within 2 to 15 min of placing the constrictor.

### *Experimental procedure*

After surgery, the haemodynamic parameters were allowed to stabilize for 30 min prior to thrombus induction, during which time three arterial blood samples were taken at −20, −10, and −2 min for the immediate determination of blood gases, pH, haematocrit and haemoglobin and the subsequent determination of platelet counts and baseline coagulation parameters (see below). After performing the endothelial lesion and placing the constrictor, CFVs were recorded for 60 min, prior to a 60 min treatment period where test compounds or their respective vehicles were administered by i.v. infusion. Once the treatments were terminated, CFVs were recorded for a further 60 min. During the period of stenosis, arterial blood samples were taken at 45, 60, 75, 90, 105, 120, 150 and 180 min for the determination blood gases etc. as above. The animals were killed at the end of each experiment by an overdose of anaesthetic.

CFVs were evaluated in terms of their frequency (number of CFVs $h^{-1}$), and their amplitude. The nadir of each CFV was measured, and used to determine the average minimum coronary flow (ml $min^{-1}$) for each animal as a measure of the extent of thrombus formation during each observation period. Additionally, the CFVs were classified into large and small amplitude, where large amplitude CFVs were defined as producing a nadir of $\leqslant 10$ ml $min^{-1}$.

### *Platelet counts and coagulation parameters*

At each of the time points mentioned above, two blood samples (5 ml + 2 ml) were taken from the left carotid artery using 3.8% trisodium citrate (1 vol citrate: 9 vols blood) for the 5 ml sample and 3.8% trisodium citrate containing specific protease inhibitors and heparin supplied with the FpA assay kits (Diagnostica Stago, Asnières, France) for the 2 ml sample as anticoagulant. The blood samples were kept on ice until the end of the experiment. Platelet counts were performed on the three pre-thrombotic samples with an MS8 Melet Schloesing veterinary cell counter calibrated for dog platelets. The blood samples were centrifuged at 1000 *g* for 15 min and the plasmas were stored at −20°C until use. Coagulation parameters were determined using an automated coagulation laboratory workstation ACL3000 (Instrumentation Laboratory) with reagents supplied by Instrumentation Laboratory, and by following the manufacturer's instructions. In brief, thrombin times (TT) were determined by preincubation of 75 μl citrated plasma at 37°C for 2 min prior to the addition of 75 μl of a 4 NIH units $ml^{-1}$ thrombin solution. Activated partial thromboplastin times (aPTT) were measured by preincubating 53 μl plasma with 53 μl of a ready-to-use ellagic acid solution for 3 min at 37°C prior to the addition of 53 μl calcium chloride solution to initiate coagulation. In both cases, clotting times were determined in seconds. FpA levels were measured by enzyme linked immunoassay with kits purchased from Diagnostica Stago (Asnières, France), and following the manufacturer's instructions.

### *Statistical analyses*

All values are given as mean ± s.e.mean. In order to determine the effects of drug treatments on the parameters measured, tests for statistical significance between the pre-treatment values and those obtained during treatment were performed using Student's *t* test for paired samples. Comparisons of CFVs between groups were made using the Kruskal-Wallis test. Between group comparisons of coagulation parameters, mean arterial blood pressure, heart rate, blood pH, blood $Po_2$, haemoglobin content and haematocrit were performed by ANOVA followed by Dunnett's test. A value of $P<0.05$ was considered as being significant for all the parameters measured.

### *Drugs and treatment groups*

Argatroban dissolved in 0.9% NaCl acidified to pH 4.0 was obtained from Mitsubishi Chemical Corporation, Tokyo, and heparin (calcium salt) dissolved in 0.9% NaCl was purchased from Roche, France. Animals were divided into six treatment groups: (I) argatroban vehicle ($n=8$); (II) argatroban 30 μg $kg^{-1}$ $min^{-1}$ ($n=8$); (III) argatroban 100 μg $kg^{-1}$ $min^{-1}$ ($n=8$); (IV) heparin vehicle ($n=6$); (V) heparin 5 iu $kg^{-1}$ $min^{-1}$ ($n=6$) and (VI) heparin 15 iu $kg^{-1}$ $min^{-1}$ ($n=6$).

## Results

### *Cyclic flow variations (CFVs)*

The placing of a Lexan constrictor around the coronary artery led to a reduction of coronary flow from 61.4 ± 2.5 ml $min^{-1}$ to 39.1 ± 1.5 ml $min^{-1}$ ($n=42$ dogs). Table 1 shows that there was no significant difference between the treatment groups in the decrease in coronary flow caused by the stenosis. CFVs were observed in all the animals in the study during the pre-treatment periods, and there were no significant differences in

their frequency or in the values for minimum coronary flow in the different groups (Table 2). The CFVs could be classified into two types: large amplitude, where the lowest flow was $\leqslant 10$ ml min$^{-1}$ often accompanied by ST elevations in the ECG (data not shown), and small amplitude, where the lowest flow was $>10$ ml min$^{-1}$ not accompanied by ST elevations. Although all the dogs had large amplitude CFVs in the control period, 20 out of the 41 also had small amplitude CFVs. Comparison of the frequency of the large amplitude CFVs showed that they were evenly distributed across the treatment groups ($P=0.32$, $\chi^2$ test, Table 2).

### *Effects of argatroban and heparin on CFVs*

Neither argatroban nor heparin abolished the occurrence of CFVs at the doses used. However, as shown in Figure 1, argatroban did cause a reduction in the frequency of large amplitude CFVs, which was not statistically significant for the dose of 30 $\mu$g kg$^{-1}$ min$^{-1}$ ($P=0.082$, 2-tailed paired $t$ test), but was highly significant for 100 $\mu$g kg$^{-1}$ min$^{-1}$ ($P=0.0072$, 2-tailed paired $t$ test), where the number of CFVs h$^{-1}$ decreased from $7.8\pm0.7$ to $2.8\pm1.2$. Also, for the latter dose, the frequency of large amplitude CFVs was significantly lower in the treated animals than the corresponding vehicle-treated controls ($4.8\pm0.8$ CFVs h$^{-1}$, $P<0.05$, Kruskal-Wallis test). This decrease in large amplitude CFVs was accompanied by a concomitant increase in small amplitude CFVs, but which was not statistically significant for 100 $\mu$g kg$^{-1}$ min$^{-1}$ argatroban ($P=0.074$, 2-tailed paired $t$ test). Heparin at 15 iu kg$^{-1}$ min$^{-1}$ also led to a decrease in the frequency of large amplitude CFVs (from $5.3\pm0.55$ to $2.7\pm1.1$ CFVs h$^{-1}$, $P=0.038$, Figure 1).

The decrease in the number of large amplitude CFVs was accompanied by increases in the minimum coronary flow during the treatment period. In the animals receiving 30 $\mu$g kg$^{-1}$ min$^{-1}$ and 100 $\mu$g kg$^{-1}$ min$^{-1}$ argatroban, minimum coronary flow increased from $5.4\pm1.7$ ml min$^{-1}$ to $9.1\pm2.1$ ml min$^{-1}$ ($P=0.041$, 2-tailed paired $t$ test), and from $2.9\pm0.9$ to $16.3\pm4.5$ ml min$^{-1}$ ($P=0.023$, 2-tailed paired $t$ test) respectively. Heparin at 5 and at 15 iu kg$^{-1}$ min$^{-1}$ led to marked increases in minimum coronary flow in 2 and in 3 out of 6 animals respectively. However, the overall increases failed to reach statistical significance (from $3.8\pm0.9$ to $13.5\pm5.2$ ml min$^{-1}$, $P=0.144$, and from $5.7\pm2.0$ to $19.3\pm5.4$ ml min$^{-1}$, $P=0.052$, for 5 and 15 iu kg$^{-1}$ min$^{-1}$ heparin respectively, 2-tailed paired $t$ test).

Neither the decrease in the frequency of large amplitude CFVs nor the increase in minimum coronary flow observed during argatroban (100 $\mu$g kg$^{-1}$ min$^{-1}$) treatment were maintained once the treatments were stopped. The large amplitude CFV frequency increased to $5.1\pm1.3$ h$^{-1}$, and the minimum coronary flow decreased to $6.2\pm2.8$ ml min$^{-1}$; these data were not statistically significant from the pretreatment values. However, the minimum coronary flow in animals receiving heparin at 15 iu kg$^{-1}$ min$^{-1}$, which was $19.3\pm5.4$ ml min$^{-1}$ during treatment, was maintained at $17.5\pm2.9$ ml min$^{-1}$ after cessation of treatment, which was significantly different from the pretreatment value ($P=0.018$, 2-tailed paired $t$ test). Similarly, the large amplitude CFV frequency was $2.8\pm1.1$ h$^{-1}$ which was also significantly different from the pre-treatment values ($P=0.022$).

**Table 1** Reductions in coronary blood flow caused by the placing of a Lexan constrictor around the circumflex coronary artery prior to the appearance of CFVs

| *Group* | *Basal flow* | *Post-stenotic flow* | *% decrease* |
|---|---|---|---|
| I | 54.8 ± 5.4 (8) | 35.8 ± 3.0 (8) | 33.2 |
| II | 56.1 ± 4.3 (8) | 39.0 ± 3.8 (8) | 30.2 |
| III | 59.1 ± 8.3 (8) | 35.0 ± 3.4 (8) | 37.4 |
| IV | 59.8 ± 4.8 (6) | 41.3 ± 3.1 (6) | 30.2 |
| V | 66.8 ± 4.1 (6) | 41.7 ± 2.5 (6) | 36.7 |
| VI | 76.4 ± 6.4 (6) | 44.7 ± 5.2 (6) | 41.7 |

Coronary flow is expressed as mean (±s.e.mean) flow in ml min$^{-1}$ for ($n$) animals per treatment group. Treatment groups were assigned as follows: (i) argatroban vehicle; (ii) argatroban 30 $\mu$g kg$^{-1}$ min$^{-1}$; (iii) argatroban 100 $\mu$g kg$^{-1}$ min$^{-1}$; (iv) heparin vehicle; (v) heparin 5 iu kg$^{-1}$ min$^{-1}$ and (vi) heparin 15 iu kg$^{-1}$ min$^{-1}$.

**Table 2** Pretreatment CFV frequency and minimal coronary flow values in stenosed coronary arteries

| *Group* | *Overall CFV frequency ($h^{-1}$)* | *Large amplitude CFV frequency ($h^{-1}$)* | *Small amplitude CFV frequency ($h^{-1}$)* | *Minimal coronary flow (ml min$^{-1}$)* |
|---|---|---|---|---|
| I (8) | 8.1 ± 1.1 | 5.6 ± 0.4 | 2.5 ± 0.9 | 6.8 ± 1.8 |
| II (8) | 9.0 ± 0.7 | 7.6 ± 0.9 | 1.4 ± 0.9 | 5.4 ± 1.7 |
| III (8) | 8.6 ± 1.1 | 7.8 ± 0.7 | 0.9 ± 0.7 | 2.9 ± 0.9 |
| IV (6) | 11.0 ± 1.6 | 9.5 ± 1.2 | 1.5 ± 1.0 | 4.7 ± 1.2 |
| V (6) | 7.8 ± 0.9 | 6.3 ± 0.8 | 1.3 ± 0.4 | 3.8 ± 0.9 |
| VI (6) | 7.3 ± 1.2 | 5.3 ± 0.6 | 2.0 ± 1.4 | 5.7 ± 2.0 |

Treatment groups were assigned as in Table 1. Results are expressed as mean (±s.e.mean). The number of animals per treatment group is shown in parentheses.

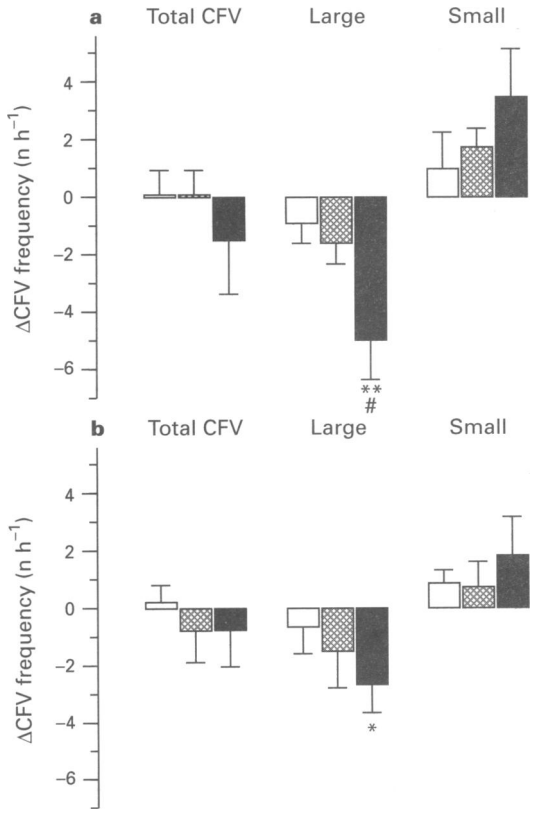


**Figure 1** Effects of 30 or 100 $\mu$g kg$^{-1}$ min$^{-1}$ argatroban (a) and of 5 or 15 iu kg$^{-1}$ min$^{-1}$ heparin (b) infusions on CFV frequency (all, large amplitude and small amplitude CFVs) in the canine coronary thrombosis model. Each column shows the mean (±s.e.mean) difference in frequency for each treatment group compared to the CFV frequency before treatment ($n=8$ for the argatroban study, $n=6$ for the heparin study). In each case: vehicle groups, open columns; low dose-treated group, cross-hatched columns and high dose-treated groups, solid columns. Statistical significance compared to control values is denoted as follows: $^*P<0.05$, $^{**}P<0.01$ versus pre-treatment frequency, Student's paired $t$ test, and $^{\#}P<0.05$ versus vehicle, Kruskal-Wallis test.

*Coagulation parameters*

Both compounds significantly ($P<0.01$, Dunnett's test) increased the TT values obtained in plasma taken at various times throughout the experiments (Figure 2). The TT was increased 13 fold by 30 $\mu g\ kg^{-1}\ min^{-1}$ argatroban 30 min after the start of the treatment. The TTs were in excess of 200 s for 100 $\mu g\ kg^{-1}\ min^{-1}$ argatroban and for both doses of heparin. At the end of the treatment periods, the TT values rapidly declined in the animals receiving argatroban, whereas in those treated with heparin the TT values were still elevated even 60 min after the end of the treatment period.

Both doses of heparin led to marked increases in the aPTT (Figure 3). In the case of 5 iu $kg^{-1}\ min^{-1}$, the increase was slow in onset, and the aPTT ratio over control reached a value of 16 at the end of the treatment period, whereas with 15 iu $kg^{-1}\ min^{-1}$, the aPTT was greater than 300 s 30 min after the start of the infusion. Moreover, the increase in aPTT was maintained throughout the 60 min post-treatment observation period. Argatroban produced a moderate, albeit significant, dose-dependent increase in the aPTT; 30 and 100 $\mu g\ kg^{-1}\ min^{-1}$ produced 2 and 3 fold increases respectively. The increase was rapid in onset, stable throughout the treatment period, and returned to baseline rapidly once the treatments were stopped.

Table 3 shows the effects of argatroban and heparin on the circulating FpA levels. The results are expressed as the mean FpA (±s.e.mean), for each treatment group, where the data obtained from blood samples taken in each observation period were pooled for each dog. In the control period before the induction of CFVs, the mean FpA levels were 3.4±0.19 ng $ml^{-1}$. During the pre-treatment period of stenosis, the FpA levels were 4.2±0.21 ng $ml^{-1}$ which were significantly greater than the prestenosis levels ($P=0.006$, 2-tailed paired $t$ test, $n=42$ dogs). Both argatroban and heparin

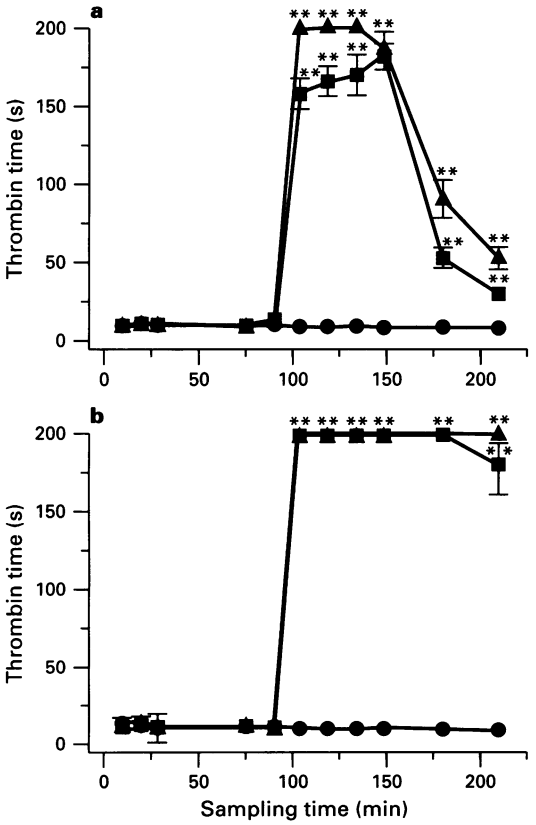


**Figure 2** Effects of 30 or 100 $\mu g\ kg^{-1}\ min^{-1}$ argatroban (a) and of 5 or 15 iu $kg^{-1}\ min^{-1}$ heparin (b) infusions on thrombin time in the canine coronary thrombosis model. Each point shows the mean (±s.e.mean) thrombin time in seconds in blood samples taken at various times throughout the experiments ($n=8$ for the argatroban study, $n=6$ for the heparin study). In each case: vehicle groups, (●); low dose groups, (■); and high dose-treated groups, (▲). Statistical significance compared to control values is denoted as follows: $^{**}P<0.01$ versus pre-treatment values, Student's paired $t$ test.

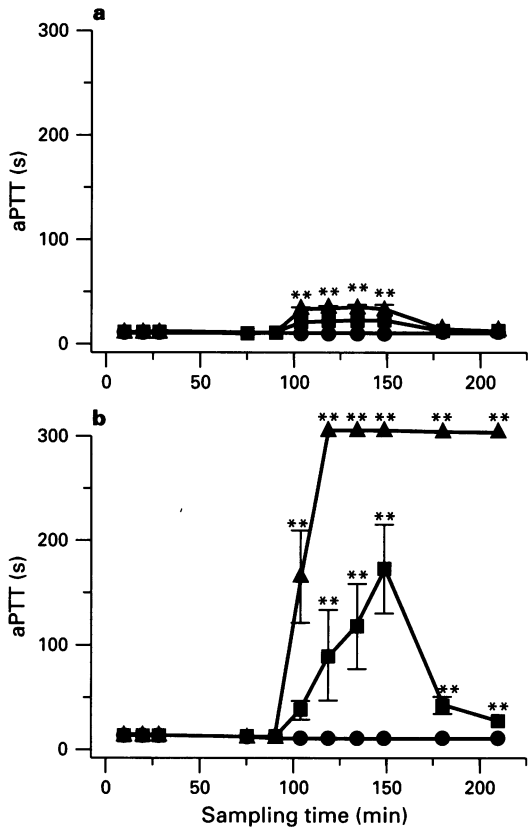


**Figure 3** Effects of 30 or 100 $\mu g\ kg^{-1}\ min^{-1}$ argatroban (a) and of 5 or 15 iu $kg^{-1}\ min^{-1}$ heparin (b) infusions on the aPTT in the canine coronary thrombosis model. Each point shows the mean (±s.e.mean) aPTT in seconds in blood samples taken at various times throughout the experiments ($n=8$ for the argatroban study, $n=6$ for the heparin study). In each case: vehicle groups, (●); low dose-treated groups, (■); and high dose-treated groups, (▲). Statistical significance compared to control values is denoted as follows: $^{**}P<0.01$ versus pre-treatment frequency, Student's paired $t$ test. In the case of 30 $\mu g\ kg^{-1}\ min^{-1}$ argatroban, the asterisks are omitted for clarity, but the increases were significant ($P<0.01$) during the treatment period.

**Table 3** Effects of argatroban and heparin on the plasma FpA levels in blood samples taken during the 180-min post-lesion and stenosis observation period

| *Group* | | *Pre-treatment* | *Treatment* | *Post-treatment* |
|---|---|---|---|---|
| I | (8) | 4.1 ± 0.5 | 3.7 ± 0.3 | 3.8 ± 0.4 |
| II | (8) | 4.1 ± 0.5 | 2.7 ± 0.3** | 4.2 ± 0.7 |
| III | (8) | 3.3 ± 0.4 | 2.4 ± 0.3** | 3.0 ± 0.3 |
| IV | (6) | 4.7 ± 0.5 | 4.5 ± 0.5 | 5.7 ± 1.1 |
| V | (6) | 4.0 ± 0.4 | 2.2 ± 0.3** | 2.3 ± 0.6* |
| VI | (6) | 4.7 ± 0.5 | 1.9 ± 0.3** | 1.9 ± 0.2** |

Treatment groups were assigned as in Table 1. Results show mean (±s.e.mean) plasma FpA levels (ng $ml^{-1}$), where data for each dog were pooled during each observation phase prior to determination of the group means. The number of animals per treatment group is shown in parentheses. Statistical significance compared to control values is denoted by asterisks where $^{*}P<0.05$, $^{**}P<0.01$ versus the pre-treatment period, Student's $t$ test for paired samples.

**Table 4** Mean arterial pressure and heart rate values during the 180-min post-lesion and stenosis observation period

| | | *Mean arterial pressure* | | | *Heart rate* | | |
|---|---|---|---|---|---|---|---|
| *Group* | | *Pre-* | *During* | *Post-* | *Pre-* | *During* | *Post-* |
| I | (8) | 120±2 | 121±2 | 121±2 | 163±7 | 166±6 | 169±7 |
| II | (8) | 109±2 | 109±3 | 107±3 | 162±5 | 163±5 | 161±4 |
| III | (8) | 112±3 | 114±6 | 114±6 | 163±6 | 165±6 | 165±6 |
| IV | (6) | 106±4 | 107±4 | 107±4 | 170±2 | 176±3 | 178±4 |
| V | (6) | 103±2 | 108±3 | 105±4 | 163±7 | 166±7 | 168±7 |
| VI | (6) | 117±4 | 122±4 | 122±4 | 173±5 | 176±5 | 178±7 |

Treatment groups were assigned as in Table 1.

led to reductions in the FpA levels during the treatment period, and as with the coagulation parameters the effect was rapidly reversed after stopping argatroban, but not after heparin.

### *Systemic haemodynamic parameters*

Neither argatroban nor heparin had any effect on the mean arterial pressure or the heart rate at the doses used in this study (Table 4).

## Discussion

The data presented above show that argatroban and heparin have antithrombotic activity in a canine model of unstable angina. However, neither treatment led to the abolition of cyclic flow variations. Nevertheless, the reduction in the frequency of large amplitude CFVs and the increase in minimum coronary flow during the treatment periods correspond to improved myocardial perfusion indicating that the compounds afforded some degree of protection to the myocardium from ischaemia. The observation that the thrombin inhibitors used in this study did not abolish the CFVs during the treatment period could be due to two factors. The first (and simpler) explanation could be that the doses used were not sufficient, and that higher doses would indeed abolish the CFVs. In fact, Eidt *et al.* (1989) showed, in a similar model of canine coronary thrombosis, that a bolus dose of argatroban, which gave an 8 fold increase in the aPTT (see below), was capable of abolishing CFVs. Moreover, the efficacy of argatroban in models of arterial platelet-rich thrombosis has been well established (Jand *et al.*, 1990; Berry *et al.*, 1994a), and it has been shown to accelerate experimental thrombolysis and prevent reocclusion in a dog model of femoral arterial thrombosis (Mellott *et al.*, 1990). In addition, the direct acting thrombin inhibitor, D-methyl-phenylalanyl-prolyl-arginal (LY294468), has also been shown to have similar prothrombolytic activity (Jackson *et al.*, 1993). The second explanation is that, in the dog, thrombin is only one of several endogenous platelet agonists contributing to thrombus formation. This is reinforced by the observation (Eidt *et al.*, 1989) that thrombin inhibitors were less effective when treatment was started 3 h after thrombus induction. Moreover, Fitzgerald & Fitzgerald (1989) showed in a canine model of coronary thrombosis that, although argatroban at a dose of 40 $\mu g\ kg^{-1}\ min^{-1}$ accelerated tissue plasminogen activator-induced thrombolysis and prevented reocclusion, CFVs were observed which were abolished by adjunct treatment with a thromboxane $A_2$ antagonist. Similarly, recombinant hirudin, although having a marked antithrombotic effect at 1 $mg\ kg^{-1}$ bolus followed by a 17 $\mu g\ kg^{-1}\ min^{-1}$ infusion, did not abolish CFVs except in the presence of the thromboxane antagonist, vapiprost, in a model of canine coronary thrombosis where endothelial damage was induced electrically (White *et al.*, 1994). 5-$HT_2$ receptor antagonists, inhibitors of thromboxane synthesis and thromboxane antagonists have each been shown to inhibit CFVs in canine coronary thrombosis models (Bush *et al.*, 1984a,b; Ashton *et al.*, 1986) confirming that mechanisms other than thrombin are involved. Nevertheless, the fact that argatroban, which is highly specific towards both the amidolytic and platelet aggregating actions of thrombin (Kikumoto *et al.*, 1984; Hara *et al.*, 1986; Tamao *et al.*, 1986), and that other thrombin inhibitors have significant antithrombotic activity in this and similar models, reinforces the hypothesis that thrombin is one of the primary central mediators of arterial thrombosis.

The systemic anticoagulation (TT and aPTT prolongation) profiles of argatroban and heparin were considerably different in this study. Both doses of heparin increased the TT to more than 200 s, and large increases were also observed with argatroban. In the case of the aPTT, 5 $iu\ kg^{-1}\ min^{-1}$ heparin led to a 15 fold increase in this parameter, despite the fact that no significant antithrombotic effects were observed at this dose. On the other hand, argatroban even at 100 $\mu g\ kg^{-1}\ min^{-1}$, produced only a modest, albeit significant, 2 fold increase in the aPTT (cf. Eidt *et al.*, 1989), and significant antithrombotic effects were observed at both doses studied. It is important to note that the doses of argatroban used in this study were chosen for their capacity to increase the aPTT by no more than a factor of 2–3 based upon published data in man (for example, Clarke *et al.* (1991) obtained a 1.6 fold increase in the aPTT with 1 $\mu g\ kg^{-1}\ min^{-1}$), and are therefore clinically relevant (cf. Eidt *et al.*, 1989). Thus, it is conceivable, as discussed above, that higher doses of argatroban producing a 4 fold or greater increase in aPTT would have more pronounced antithrombotic effects. The difference in sensitivity of the aPTT to argatroban in the dog compared to man, if matched by a similar difference in antithrombotic activity, could also have important implications for the extrapolation of doses active in animal models to dose-finding in clinical studies. This merits further investigation.

It is becoming apparent that the comparison of the antithrombotic and anticoagulant effects of compounds acting by different mechanisms on the clotting cascade proteases can pose problems as to the choice of which marker to use. Carteaux *et al.* (1995) recently showed that the direct thrombin inhibitors, napsagatran (Ro 46-6240) and hirudin, were antithrombotic in a guinea-pig model of CFVs with moderate increases in the aPTT at doses which inhibited thrombus formation by 80%. A similar antithrombotic dose of heparin (10 $iu\ kg^{-1}$) produced a much greater increase in the aPTT. However, all three compounds produced similar increases in the activated clotting time (ACT) in freshly drawn whole blood. Nevertheless, all the compounds in that study produced large increases in both the aPTT and ACT at three times greater doses, and yet thrombus formation was not abolished. The choice of clotting parameter, however, may not be the only complicating factor in data interpretation, the choice of species is also an important issue. This is illustrated by a recent study in man which shows that both the aPTT and ACT dose-response curves for argatroban are very flat, ranging from 1.3 and 1.4 fold increases respectively at 1.25 $\mu g\ kg^{-1}\ min^{-1}$ to 2.8 and 2.5 fold increases at 40 $\mu g\ kg^{-1}\ min^{-1}$ (Schwarz *et al.*, 1996), and thus neither clotting parameter may have adequate sensitivity in man for the use of low molecular weight direct thrombin inhibitors.

The circulating levels of FpA are considered to be a systemic marker of thrombin activation and thus thrombus formation in man (Grant & Prentice, 1994). Thus the increases observed after thrombus induction are consistent with this consideration. The reductions in FpA levels observed during the treatment periods are also consistent with systemic anticoagulant therapy. Heparin caused a marked decrease in FpA levels even at the dose which had no antithrombotic activity. Moreover, the levels of FpA during and after treatment with 15 $iu\ kg^{-1}\ min^{-1}$ heparin were 40% below those found before thrombus induction. The effects of both doses of argatroban were more modest. Thus, in the context of the model used here,

this marker appears to measure most closely the degree of anticoagulation rather than the thrombotic state. However, it is likely that the surgical procedures used in the preparation of the animals resulted in significant thrombin activation, and hence fibrinogen degradation. This may have masked the localized FpA generation in the stenosed coronary arteries, which could in part explain the decrease to below the prestenosis FpA values during heparin treatment, limiting the predictive value of FpA measurements in such models when compared with the clinical setting.

The anticoagulant effects of argatroban were rapidly reversed on cessation of treatment, whereas those of heparin persisted, probably due to the differential elimination rates of the two compounds as mentioned above (*op. cit.*). These data also underline the need to take into account the duration of action of test compounds when comparing efficacy in animal models where the pathogenic stimulus may outlast the presence of one or more compound in the circulation. Indeed, the rapid reversibility of argatroban represents a potential advantage in clinical settings where anticoagulation may need to be reduced rapidly such as for the prevention of bleeding at puncture sites.

The fact that heparin is inactive or only weakly active as an antithrombotic agent in this model at 'supramaximal' anti coagulant doses (at least in terms of the aPTT) could be due to several factors. Heparin is inhibited by platelet factor 4, to which it binds with a higher affinity than to antithrombin III (Levine & Wohl, 1976). The formation of the prothrombinase complex, with binding of factor Xa to the activated platelet in association with factor Va, protects factor Xa from inactivation by the heparin-antithrombin III complex (Miletich *et al.*, 1978). Clot-bound thrombin is resistant to the inhibitory effects of the heparin-antithrombin III complex (Mirshahi *et al.*, 1989; Weitz *et al.*, 1990). Argatroban is a potent inhibitor of clot-associated thrombin with only a 3 fold shift to the right of the log-concentration inhibition curve compared to its effects on fluid phase thrombin, compared to a 228 fold shift to the right for heparin (Berry *et al.*, 1994b). In addition, clot-associated thrombin is capable of inducing platelet activation, which is also resistant to heparin: antithrombin III (3000 fold shift to the right of the log-dose inhibition response curve versus thrombin in solution), and which argatroban inhibits almost effectively as it does thrombin in solution (Berry *et al.*, 1995). In the context of platelet-rich thrombosis models such as the one described here, this latter observation could be important for the explanation of the apparent superiority of argatroban over heparin in terms of the balance between antithrombotic effects and systemic anticoagulation.

In conclusion, the results in this study suggest that argatroban has a potential as an antithrombotic agent in the context of coronary arterial thrombosis.

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