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Targeted inhibition of the serotonin 5HT$_{2A}$ receptor improves coronary patency in an *in vivo* model of recurrent thrombosis

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Summary

Background—Release of serotonin and activation of serotonin 5HT$_{2A}$ receptors on platelet surfaces is a potent augmentative stimulus for platelet aggregation. However, earlier-generation serotonin receptor antagonists were not successfully exploited as antiplatelet agents, possibly owing to their lack of specificity for the 5HT$_{2A}$ receptor subtype.

Objective—To assess whether targeted inhibition of the serotonin 5HT$_{2A}$ receptor attenuates recurrent thrombosis and improves coronary patency in an *in vivo* canine model mimicking unstable angina.

Methods—In protocol 1, anesthetized dogs were pretreated with a novel, selective inverse agonist of the 5HT$_{2A}$ receptor (APD791) or saline. Recurrent coronary thrombosis was then initiated by coronary artery injury + stenosis, and coronary patency was monitored for 3 h. Protocol 2 was similar, except that: (i) treatment with APD791 or saline was begun 1 h after the onset of recurrent thrombosis; (ii) template bleeding time was measured; and (iii) blood samples were obtained for *in vitro* flow cytometric assessment of platelet responsiveness to serotonin.

Results—APD791 attenuated recurrent thrombosis, irrespective of the time of treatment: in both protocols, flow–time area (index of coronary patency; normalized to baseline coronary flow) averaged 58–59% ($P < 0.01$) following administration of APD791 vs. 21–28% in saline controls. Moreover, the *in vivo* antithrombotic effect of APD791 was not accompanied by increased bleeding, but was associated with significant and selective inhibition of serotonin-mediated platelet activation.

Conclusion—5HT$_{2A}$ receptor inhibition with APD791, even when initiated after the onset of recurrent thrombosis, improves coronary patency in the *in vivo* canine model.

Keywords

angina; platelets; serotonin; thrombosis

The pathogenesis of platelet-mediated coronary artery thrombosis is complex, with multiple *in vivo* agonists playing important causal and contributory roles [1,2]. Current clinical therapies target either cyclo-oxygenase 1, the ADP P2Y$_{12}$ receptor, or the glycoprotein (GP) IIb/IIIa receptor [3]. However, these therapies, even in combination, are not fully effective in preventing major thrombotic events [3], and are associated with an increase in bleeding [4].

Release of serotonin from platelet dense granules and activation of serotonin receptors on platelet surfaces has long been recognized to serve as a potent augmentative stimulus for platelet aggregation [5–8]. Clinical application of serotonin receptor antagonists for the prevention of thrombotic coronary events has, however, been confounded by their lack of selectivity for the 5HT$_{2A}$ receptor (i.e. the serotonin receptor subtype expressed on platelets) [9,10]. Accordingly, our aim was to assess the efficacy of APD791 (Arena Pharmaceuticals, Inc., San Diego, CA, USA), a newly developed, potent and highly selective inverse agonist of the 5HT$_{2A}$ receptor [9], in a well-established preclinical canine model of recurrent thrombosis mimicking unstable angina [11–15]. Our specific goals were to: (i) establish whether pretreatment with APD791 improves subsequent coronary patency; (ii) investigate whether the efficacy of APD791 is maintained when the agent is administered after the onset of recurrent thrombosis; (iii) show that APD791 acts on platelets *in vivo* and inhibits serotonin-mediated platelet aggregation; and (iv) investigate whether APD791 exacerbates bleeding.

Materials and methods

This study was approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School, and conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (1996).

Surgical preparation

Twenty-six adult mongrel dogs (weight: 12–14 kg) were anesthetized with intravenous sodium pentobarbital (30 mg kg^{-1}), intubated, and mechanically ventilated. Catheters were inserted in the left jugular vein for administration of fluids and supplemental anesthesia, and in the left carotid artery for measurement of heart rate and arterial pressure and collection of blood samples. After exposure of the heart through a left lateral thoracotomy, two adjacent segments of the left anterior descending coronary artery (LAD) were isolated, usually midway along its course: a Doppler flow probe (Transonic Systems, Inc., Ithaca, NY, USA) was applied to the distal LAD segment for continuous measurement of mean coronary blood flow (CBF), and the proximal segment served as the site of later injury + stenosis. Arterial pressure and CBF were monitored throughout each experiment, using a Micro-Med data acquisition system.

Protocol 1: APD791 pretreatment

Study design—To address our first aim (i.e. investigate whether pretreatment with APD791 attenuates recurrent coronary thrombosis), we randomly assigned 14 dogs to receive: (i) APD791 − 0.07 mg kg^{-1} intravenous bolus (dissolved in 1 mL of saline),

followed by a continuous intravenous infusion of 1.16 µg kg min^{-1} (volume of 100 µL min^{-1}) for the remainder of the protocol; or (ii) a volume-matched bolus + infusion of saline (control; $n = 7$ per group; Fig. 1).

At 15 min after the onset of treatment, the isolated LAD segment was squeezed with forceps, and a micromanometer constrictor was positioned around the site of injury and tightened such that mean CBF was reduced to approximately 35% of its baseline value. This triggers the development of cyclic variations in coronary blood flow (CFVs) caused by platelet activation–aggregation, and the resultant spontaneous accumulation/dislodgement of platelet-rich thrombi at the site of injury + stenosis (Fig. 2) [11–15]. CBF was then monitored for 3 h without further intervention.

At the end of the 3-h observation period, cardiac arrest was produced under deep anesthesia by intracardiac injection of KCl. As the severity of arterial injury is recognized to be a crucial determinant of patency in this model [11,14–17], the damaged LAD segment was collected from all dogs and stored in 10% neutral buffered formalin for later histologic evaluation.

Endpoints and analysis—Heart rate and mean arterial pressure were recorded at baseline (before randomization), during the initial 15 min after administration of APD791 or saline, and throughout the 3-h observation period following injury + stenosis. In addition, CBF was tabulated at baseline, during the 15 min after treatment, and immediately upon application of the stenosis (before the onset of CFVs).

Histologic analysis of all damaged and stenotic LAD segments was performed by one investigator (PW) without knowledge of the treatment group. Cross-sections (5 µm in thickness) were cut from each artery and stained with picrosirius red (to facilitate specific visualization of collagen fibers). For each vessel, the severity of injury was assigned a semiquantitative score: 1 = endothelial denudation with little or no damage to the tunica media; 2 = tears and dissections in the media, without exposure of the tunica adventitia; 3 = loss of media and/or deep tears, with focal points of adventitial exposure totaling < 10% of the arterial circumference; and 4 = loss of media with confluent and extensive (> 10%) adventitial exposure [14–17].

Plasma APD791 levels were measured in all dogs at baseline (before randomization), at 10 min after the onset of treatment, and at 1 h and 2 h poststenosis (1.25 h and 2.25 h after the start of treatment: Fig. 1). At each time-point, citrated blood samples were obtained and centrifuged at 1500 ×g for 15 min, and the plasma was frozen and stored at − 80 °C for quantitation of APD791 concentration [9].

Vessel patency during the 3 h following injury + stenosis was assessed by quantifying two variables: the duration (in minutes) of total thrombotic occlusion (CBF = 0); and percentage flow–time area, defined as the area of the flow–time tracing normalized for each dog to the baseline coronary flow × 180 min [14,15,17].

Statistics—Hemodynamics, CBF and plasma APD791 levels were compared by two-factor ANOVA (for group and time) with replication, and, if significant *F*-values were obtained, *post hoc* pairwise comparisons were made using the Newman–Keuls test. Zero flow duration, percentage flow–time area and arterial injury scores were compared between saline and APD791-treated groups by *t*-tests. All data are reported as means ± standard errors of the mean (SEMs), and *P*-values < 0.05 are considered to be statistically significant.

Protocol 2: APD791 'delayed' treatment

Study design—To address our three remaining aims (determine whether administration of APD791 after the onset of recurrent thrombosis inhibits CFVs, show that APD791 acts on platelets *in vivo* and inhibits serotonin-induced platelet activation, and assess whether APD791 exacerbates bleeding), we subjected 12 additional dogs to coronary artery injury + stenosis, as described for protocol 1. After CBF had been monitored for 1 h, dogs received either: (i) APD791 at the same dose (bolus + infusion) administered in protocol 1; or (ii) saline. CBF was monitored for an additional 2 h after the onset of treatment ($n = 6$ per group).Template bleeding time, *ex vivo* serotonin-stimulated platelet function and plasma APD791 concentrations were assessed in all dogs at three time-points: before injury + stenosis (baseline), immediately before randomization and treatment (i.e. at 1 h after the onset of CFVs), and at 1.5 h after the start of APD791 or saline administration (Fig. 1). At the end of the 3-h observation period, animals were killed, and the damaged LAD segments were harvested for histologic evaluation.

Endpoints and analysis—Heart rate and mean arterial pressure were recorded at baseline and throughout the 3-h observation period; CBF was tabulated at baseline and after application of the stenosis.

Histologic analysis of the LAD at the site of injury + stenosis and quantitation of plasma APD791 levels were conducted as in protocol 1.

Vessel patency was quantified by measuring zero flow duration and flow–time area as described for protocol 1. In this case, however, data obtained during the 1-h pretreatment phase were normalized and expressed as a percentage of baseline coronary flow $\times$ 60 min, whereas results obtained during the 2-h post-treatment period were normalized and expressed as a percentage of baseline flow $\times$ 120 min.

Template bleeding times were assessed from standardized incisions made on the anterior surface of the tongue (Surgicutt automated incision-making instrument: incisions 1 mm in depth and 5 mm in length; ITC, Edison, NJ, USA). Blood was wicked onto preweighed blotting paper at 20-s intervals, and bleeding times were calculated as the time from making of the incision until transfer of blood to the blotting paper ceased [18]. In addition, shed blood 'volumes' were estimated by allowing the blotting paper obtained at each sample period to dry for 24 h, reweighing, and calculating the difference in blotting paper weight after vs. before the bleeding time test.

APD791 inhibition of *ex vivo* serotonin-stimulated platelet function was assessed from citrated blood samples collected at each of the three sample times. Two assays were performed. For detection of serotonin-stimulated F-actin polymerization (assessed by phalloidin binding), blood was diluted 1 : 4 in HEPES–Tyrode's buffer, stimulated for 25 s at 22 °C with 1 μ_M serotonin, fixed with 1% ultrapure formaldehyde, and diluted 1 : 20 in HEPES–Tyrode's buffer. The samples were then permeabilized with 0.1% Triton X-100 and stained with Alexa Fluor 488-conjugated phalloidin (500 n$_M$; Invitrogen Molecular Probes, Carlsbad, CA, USA) and, as a platelet identifier, phycoerythrin (PE)-conjugated CD41 (DAKO). In control experiments, 1 μ_M serotonin was replaced by 10 μ_M ADP. The samples were analyzed by flow cytometry (FACSCalibur; Becton Dickinson, Franklin Lakes, NJ, USA), and the data were expressed as the increase in phalloidin fluorescence induced by either serotonin or ADP. For determination of serotonin-induced increases in platelet cytosolic calcium levels (assessed by Fluo-4 fluorescence), blood was diluted 1 : 10 in HEPES–Tyrode's buffer and incubated for 30 min at 22 °C with 5 μ_M Fluo-4 (Invitrogen Molecular Probes), 1 m$_M$ probenecid (Invitrogen Molecular Probes; to reduce extracellular leakage of Fluo-4, as recommended by the manufacturer), and CD41–PE (DAKO) (as a

platelet identifier). Platelet Fluo-4 fluorescence was monitored by flow cytometry before and immediately after stimulation with 1 μ_M serotonin, using time as a parameter. In control experiments, 1 μ_M serotonin was replaced by 10 μ_M ADP. The increase in Fluo-4 as a result of increased intracellular calcium was calculated as the ratio of postactivation/preactivation fluorescence. For both assays, data obtained before and after treatment were normalized, for each dog, to the corresponding baseline values.

Statistics—Arterial injury scores were compared between groups by *t*-test. For all other variables, two-factor ANOVA with replication followed by the Newman–Keuls test was applied. As in protocol 1, data are reported as means ± SEMs, and *P*-values < 0.05 are considered to be statistically significant.

Protocol 3: in vitro assessment of APD791

To provide further insights into the effect of APD791 on platelet-dependent clot formation in models devoid of vascular smooth muscle, two *in vitro post hoc* experiments were performed. The clot strength of human blood was determined in the thromboelastogram (TEG) PlateletMapping System [19,20]. Paired aliquots of heparinized whole blood were incubated with APD791 (100 n_M) or vehicle for 10 min at 37 °C, and then placed in a TEG pin-and-cup system with 10 μ_M serotonin, reptilase, and activated factor XIII (*n* = 3 paired samples). The maximum amplitude of torsion (index of platelet-dependent clot strength) was quantified for all samples. Platelet-mediated thrombosis under conditions of shear and in the absence of exogenous serotonin was assessed in canine blood using the Platelet Function Analyzer (PFA)-100 [19,21]. Aliquots of citrated blood (*n* = 3 pairs) were incubated with APD791 (100 n_M) or vehicle for 10 min at 37 °C, and then pipetted into collagen–ADP cartridges. For each sample, the time (in seconds) required for the complete platelet-mediated thrombotic occlusion of the membrane aperture was recorded.

Statistics—For both of the *in vitro* experiments, the primary endpoint (maximum amplitude of torsion; closure time) was compared between APD791-treated and vehicle-treated samples by paired *t*-test. Data are reported as means ± SEMs, and *P*-values < 0.05 are considered to be significant.

Results

Protocol 1: APD791 pretreatment

Hemodynamics and CBF—There were no differences in heart rate or mean arterial pressure between saline-treated and APD791-treated groups at any time during the protocol (that is, for mean arterial pressure, *P* = 0.508 between groups, and *P* = 0.540 for group–time interaction; Fig. 3).

CBF was comparable between groups at baseline, averaging 12.9 ± 2.4 vs. 12.1 ± 1.7 mL min^{-1} in dogs later assigned to receive saline vs. APD791 (*P*-value not significant). Moreover, there was no difference in the severity of stenosis between the two cohorts: CBF was reduced to 33% ± 2% vs. 34% ± 2% of baseline values upon application of the constrictor.

Arterial damage—Arterial damage was consistently characterized by medial tearing and dissection with minimal adventitial exposure (i.e. injury scores of 2–3; Fig. 2), with no differences in mean injury score between control and APD791-treated dogs (2.7 ± 0.4 and 2.5 ± 0.2; *P*-value not significant).

Plasma APD791 levels—As expected, plasma concentrations of APD791 were below the threshold of quantitation in all dogs at baseline, and remained below the threshold of detection for control dogs following administration of saline. In dogs assigned to receive APD791, plasma APD791 levels showed a rapid and sustained increase, averaging 25.5 ± 4.1, 28.7 ± 4.6 and 31.2 ± 4.5 ng mL^{-1}, respectively, at 10 min, 1.25 h and 2.25 h after the start of treatment.

Vessel patency—As expected, all dogs exhibited spontaneous recurrent thrombosis following coronary injury + stenosis, and no animals developed permanent thrombotic occlusion during the 3-h observation period.

Vessel patency was maintained better in dogs pretreated with APD791: that is, zero flow duration was reduced from 57 ± 14 min in controls to 1 ± 1 min in the APD791-treated group ($P < 0.01$), whereas percentage flow–time area was increased from $28\% \pm 3\%$ in controls to $58\% \pm 3\%$ in the cohort that received APD791 ($P < 0.01$; Fig. 4).

Protocol 2: APD791 'delayed' treatment

Hemodynamics and CBF—As in protocol 1, heart rate and arterial pressure did not differ between saline-treated and APD791-treated groups (for mean arterial pressure: $P = 0.492$ between groups, and $P = 0.926$ for group–time interaction; Fig. 5). In addition, coronary flow was comparable between groups at baseline, and was reduced to 33–36% of baseline in all dogs following placement of the constrictor (P-value not significant).

Arterial damage—As in protocol 1, all injured arterial segments displayed medial tearing and dissection with minimal adventitial exposure (injury scores of 2–3; P-value not significant between groups).

Plasma APD791 levels—Plasma APD791 concentrations were below the threshold of quantitation in all dogs before randomization, remained undetectable in saline-treated dogs, and averaged 20.4 ± 3.4 ng mL^{-1} at 1.5 h after the onset of treatment in animals that received APD791.

Vessel patency—As in protocol 1, all animals developed CFVs (with no instances of permanent thrombotic occlusion) following coronary injury + stenosis.

During the 1-h pretreatment phase, coronary patency was comparable in dogs later randomized to receive APD791 vs. saline: mean percentage flow–time area was $26\% \pm 2\%$ vs. $21\% \pm 3\%$, and zero flow duration (expressed as a percentage of the 60-min pretreatment period) averaged $24\% \pm 6\%$ vs. $28\% \pm 8\%$, respectively (P-value not significant; Fig. 6). In the saline-treated cohort, there was no significant change in patency during the final 2 h of the protocol (following treatment) when compared with the pretreatment period. In contrast, APD791 given 1 h after injury + stenosis evoked a significant increase in percentage flow–time area (to $59\% \pm 4\%$) and a decrease in zero flow duration (to $0.2\% \pm 0.1\%$) during the final 2 h of observation ($P < 0.01$ vs. pretreatment, and $P < 0.01$ vs. control for both endpoints; Fig. 6).

Template bleeding times and blood 'volumes'—Baseline values of template bleeding time and shed blood 'volume' averaged 120 s and 35 mg, respectively, with no differences in dogs later assigned to receive APD791 or saline. There was no significant increase in bleeding following administration of APD791: at 1.5 h after the onset of treatment, bleeding time was $105\% \pm 12\%$ of baseline, and blood 'volume' averaged $88\% \pm 26\%$ of baseline (Fig. 7).

APD791 inhibition of ex vivo serotonin-stimulated platelet function—The increase in platelet F-actin polymerization (as revealed by phalloidin staining) and platelet intracellular calcium concentration (as reported by Fluo-4 fluorescence) in response to *ex vivo* addition of serotonin averaged 90%–115% of baseline values in blood samples collected at 1 h after the onset of thrombosis (immediately before randomization; Fig. 8A,B). Serotonin-stimulated platelet function remained unchanged following administration of saline. In contrast, blood obtained from dogs following APD791 treatment displayed significant inhibition of *ex vivo* serotonin-stimulated platelet F-actin formation (Fig. 8A) and platelet intracellular calcium levels (Fig. 8B). Moreover, this *ex vivo* inhibition of platelet activation in APD791-treated dogs was agonist-specific: that is, negative control experiments showed no difference in ADP-stimulated phalloidin staining and Fluo-4 fluorescence between the APD791-treated and saline-treated groups (Fig. 8C,D).

Protocol 3: in vitro assessment of APD791

In vitro addition of APD791 was associated with a prolongation of PFA-100 closure time (index of platelet-mediated thrombosis under conditions of shear) in canine blood samples, and a reduction in the maximum amplitude of torsion (index of platelet-mediated clot strength) in human blood samples ($P < 0.01$ and $P < 0.05$, respectively, vs. matched vehicle controls; Fig. 9).

Discussion

We report here that the serotonin $5HT_{2A}$ receptor antagonist APD791 improves arterial patency in the canine model of recurrent coronary thrombosis, with efficacy being achieved even when treatment is 'delayed' (i.e. initiated after the onset of recurrent thrombosis). In addition, we provide novel *ex vivo* and *in vitro* data establishing that APD791 acts on platelets (specifically, inhibits serotonin-stimulated increases in platelet F-actin polymerization and intracellular calcium concentration, and attenuates platelet-dependent clot strength and platelet-dependent thrombosis under conditions of shear), and show that its effects are specific to serotonin. Finally, we show that APD791 was not associated with increased bleeding.

Comparison with previous studies

Protocols 1 and 2 provide robust, quantitative evidence that APD791 improves flow–time area and reduces the duration of total thrombotic occlusion, irrespective of whether treatment is initiated before or after the onset of recurrent thrombosis. These data are consistent with historical evidence that serotonin receptor antagonists (i.e. sarpogrelate) have a favorable effect on vessel patency in thrombotic models [7,8,22–27]. However, the novel aspect of the present study is the selectivity of APD791: in contrast to sarpogrelate, which displays 10-fold and 100-fold selectivity for the for the $5HT_{2A}$ vs. $5HT_{2c}$ and $5HT_{2B}$ receptors, respectively [28], APD791 shows > 2000-fold selectivity for the $5HT_{2A}$ subtype and no detectable affinity for other G-protein-coupled receptors [9].

APD791 and patency: antiplatelet effect vs. inhibition of vasoconstriction?

We propose that APD791 evokes the observed, significant improvement in coronary patency by blocking platelet $5HT_{2A}$ receptors, thereby abrogating the proaggregatory effects of serotonin released from activated platelets. In this regard, pharmacologic characterization of APD791 has revealed that the agent has significant antiplatelet effects: that is, incubation of platelet-rich plasma (canine and human) with APD791 and oral administration of APD791 to conscious dogs both attenuated serotonin-induced platelet aggregation as assessed *ex vivo* by platelet aggregometry [9]. Additional evidence is provided in the current study: our *ex vivo* assay conducted on blood obtained in protocol 2 following *in vivo* treatment (i)

demonstrates that APD791 acts on circulating platelets and inhibits serotonin-stimulated platelet activation; and (ii) establishes that the effects of APD791 are specific to serotonin. This concept is further corroborated by our supplemental *in vitro* experiments showing that incubation of whole blood with APD791 attenuates platelet-dependent thrombosis as determined by both TEG and PFA-100 closure times. It is of note that the latter observation of a prolonged closure time in APD791-treated aliquots was obtained in the absence of exogenously added serotonin, thereby suggesting that serotonin released from activated platelets contributed to the formation of the occlusive clot in collagen–ADP cartridges.

Although these data support a significant role for platelet inhibition, it could be argued that the better maintenance of coronary patency achieved with APD791 may reflect blunting of serotonin-induced vasoconstriction via inhibition of 5HT$_{2A}$ receptors on vascular smooth muscle [10,29]. There is no question that, in arterial segments displaying endothelial damage, serotonin is a potent vasoconstrictor [30]. Moreover, APD791 has been shown to attenuate serotonin-induced constriction of isolated rabbit aortic rings (endothelium removed) in a dose-dependent manner [9]. We cannot exclude the possibility that the benefits of APD791 in our model are due in part to inhibition of serotonin-induced vasoconstriction. In addition, because of differences in the concentrations of serotonin and APD791 used in the platelet vs. vasoconstriction assays [9], we cannot extrapolate from these data to speculate on the contribution of APD791-mediated attenuation of vasoconstriction to the improved patency seen with APD791 *in vivo*. However, the *in vitro* evidence of attenuated platelet-mediated thrombosis in models devoid of vascular smooth muscle, and the fact that the cyclic flow variations seen *in vivo* following coronary injury + stenosis are well-documented to reflect recurrent platelet-dependent thrombosis rather than vasospasm [11–15,31], suggest that inhibition of serotonin-induced platelet aggregation plays a major role in the improved patency achieved with APD791.

APD791 and bleeding

APD791 did not prolong template bleeding time or increase the 'volume' of blood shed from the incision. This lack of effect of APD791 on bleeding may reflect the fact that serotonin by itself is a weak stimulus for platelet activation; rather, serotonin augments platelet activation induced by primary agonists (e.g. thrombin, ADP, and collagen) [5,6], and thus inhibition of 5HT$_{2A}$-mediated responses affects secondary amplification of platelet activation–aggregation in a growing thrombus. Clinical studies are required to confirm whether APD791 has a similar, benign bleeding profile in patients.

Summary, clinical relevance and future directions

There has been recent clinical interest in the use of sarpogrelate for the treatment of intermittent claudication and ischemic stroke [32–34]. However, efforts to utilize earlier-generation serotonin receptor antagonists as coronary antithrombotic agents have been limited and largely unsuccessful [10,35], possibly owing to their lack of specificity for the 5HT$_{2A}$ receptor subtype and, in some instances, a concomitant increase in bleeding [10,23,26]. The improved coronary patency achieved with APD791, and its lack of effect on hemodynamics and bleeding, suggest that this potent and selective serotonin 5HT$_{2A}$ receptor inhibitor may be effective in the treatment of unstable angina. In addition, our *ex vivo* assays of serotonin-stimulated platelet function may be applied to monitor the *in vivo* efficacy of APD791 in patients.

We did not, in our current protocols, assess whether any of the other complex molecular effects of serotonin on platelet function (including including serotonin-induced GPIb-α shedding and receptor-independent 'serotonylation'-triggered platelet α-granule release [36,37]) were altered by APD791 treatment. In addition, we cannot, on the basis of the

present study, comment on the relative effectiveness of APD791 vs. agents that target cyclo-oxygenase-1, GPIIb/IIIa or the ADP P2Y$_{12}$ receptor. Nonetheless, our data raise the possibility that targeted inhibition of the serotonin 5HT$_{2A}$ receptor may provide a mechanistically distinct alternative or adjunct to currently approved antiplatelet therapies.
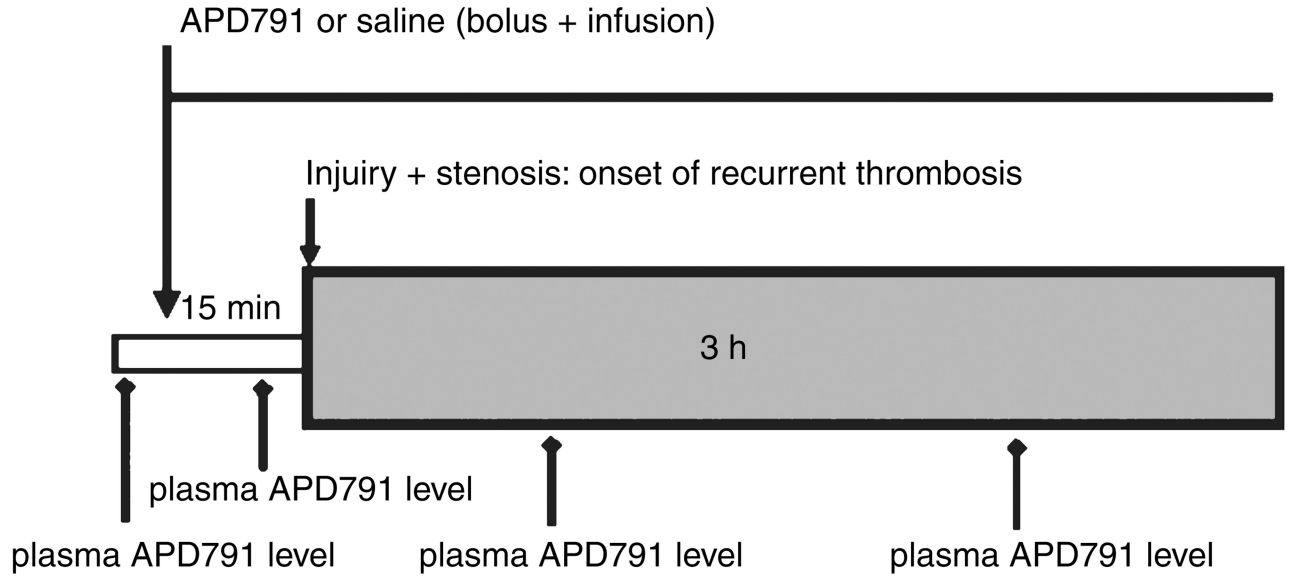
Acknowledgments

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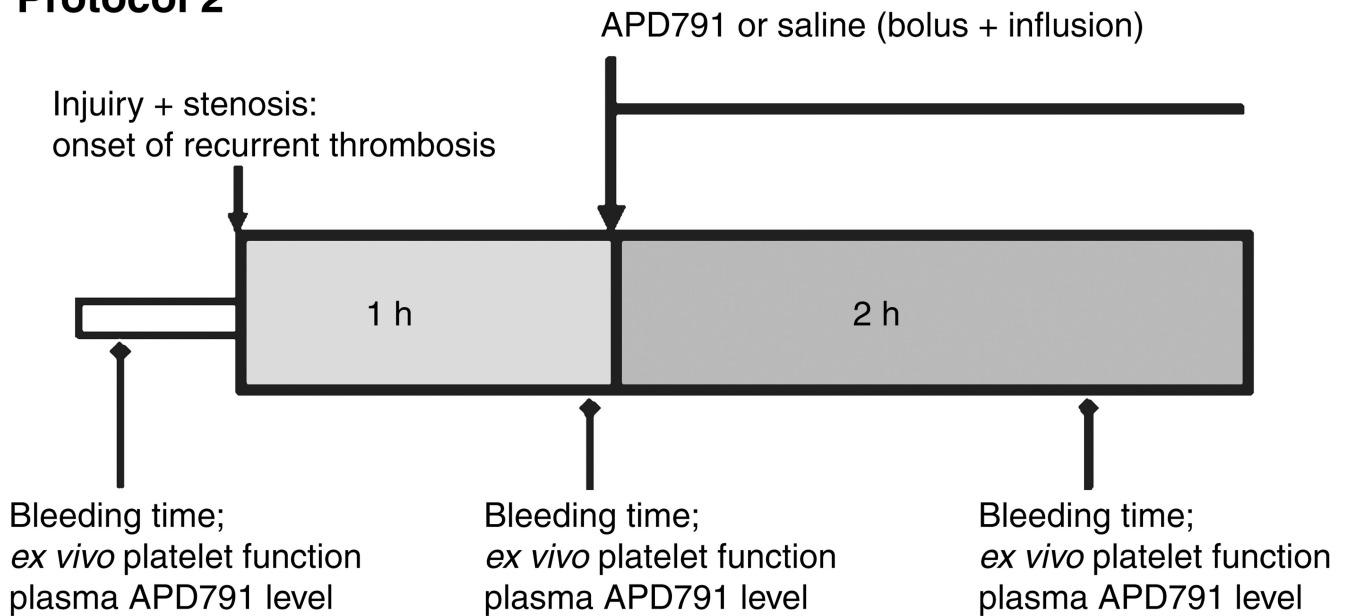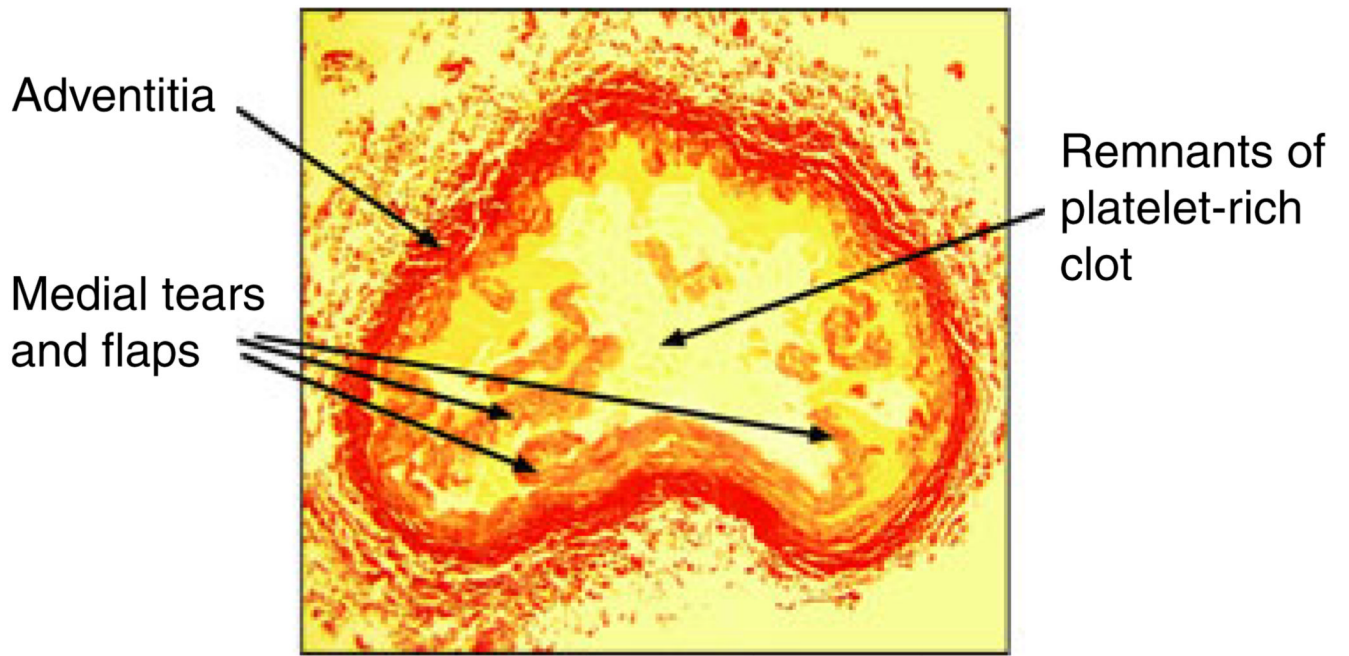
Protocol 1

APD791 or saline (bolus + infusion)

Injuiry + stenosis: onset of recurrent thrombosis

15 min

3 h

plasma APD791 level

plasma APD791 level

plasma APD791 level

plasma APD791 level

Protocol 2

APD791 or saline (bolus + influsion)

Injuiry + stenosis:
onset of recurrent thrombosis

1 h

2 h

Bleeding time;
ex vivo platelet function
plasma APD791 level

Bleeding time;
ex vivo platelet function
plasma APD791 level

Bleeding time;
ex vivo platelet function
plasma APD791 level

Fig. 1.
Study design.

Fig. 2.
Histologic section of damaged arterial segment. The micrograph shows medial tearing and remnants of platelet-rich thrombus in the lumen.
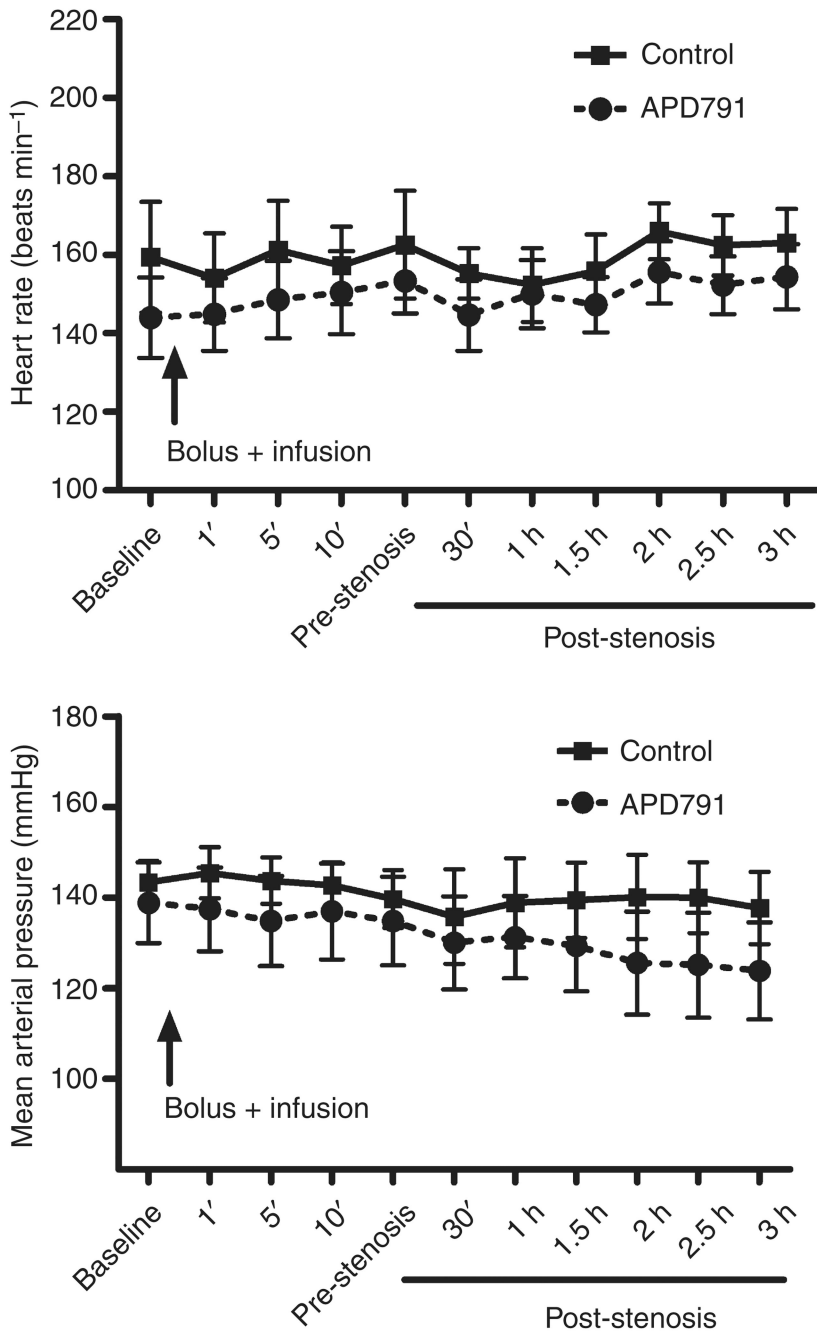
Fig. 3.
Protocol 1: hemodynamics.

A

Coronary flow (mL min⁻¹): Control

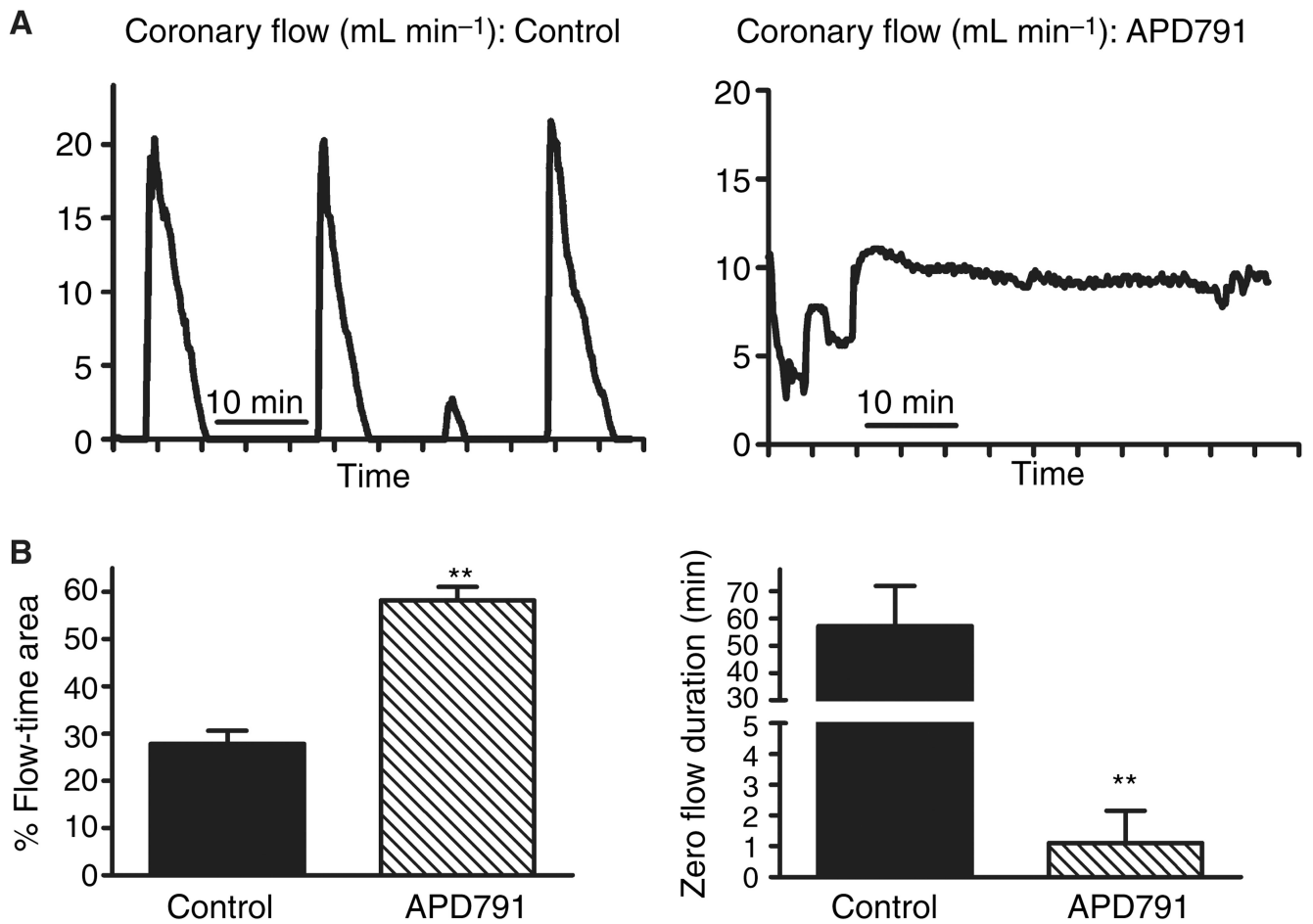Coronary flow (mL min⁻¹): APD791

B

Fig. 4.
Protocol 1: vessel patency. (A) Original recordings of mean coronary flow following injury
+ stenosis from one control animal (showing the typical pattern of cyclic variations in flow)
and one dog pretreated with APD791. (B)Mean values of percentage flow–time area and
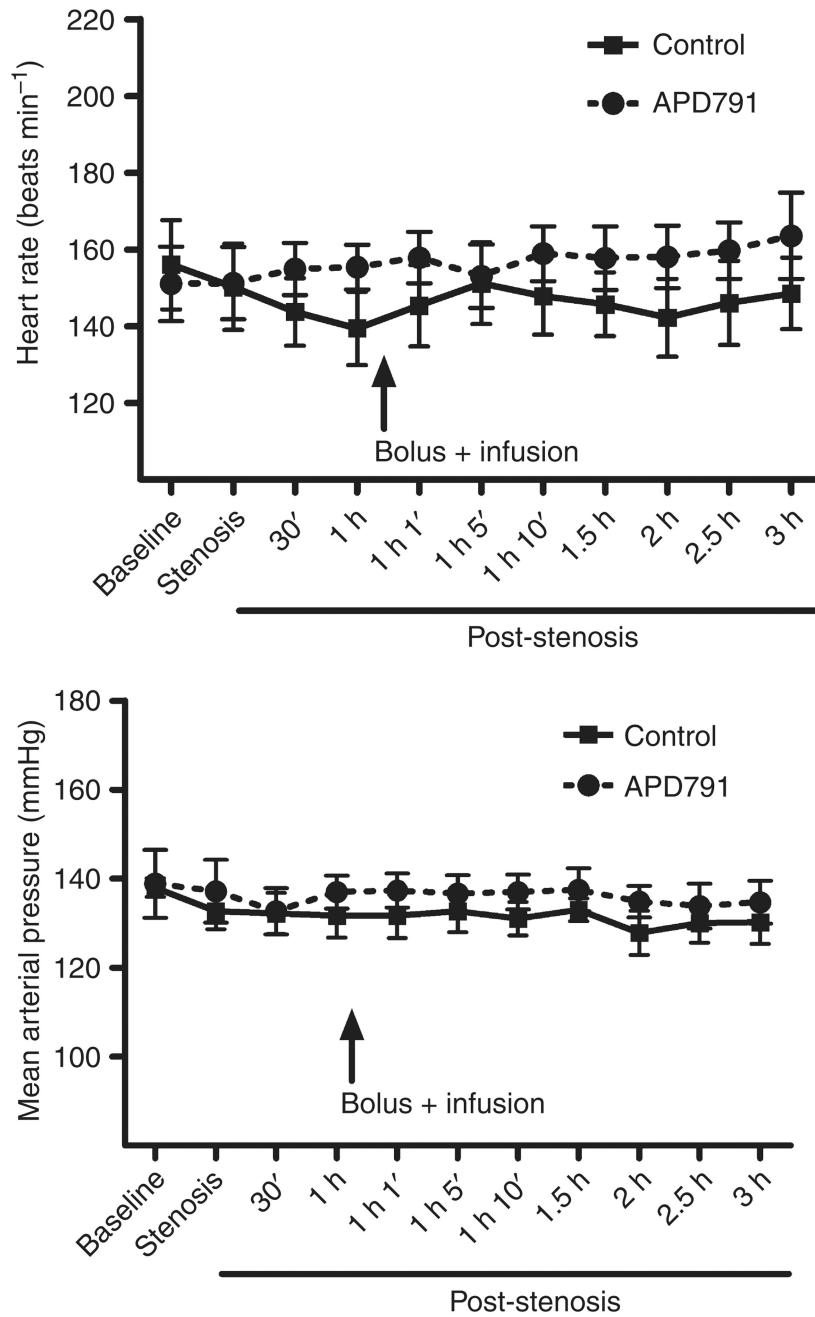zero flow duration for control and APD791-treated groups. **$P < 0.01$ vs. control.

Fig. 5.
Protocol 2: hemodynamics.

A

Coronary flow (mL min^{-1}): APD791 'Delayed' Treatment
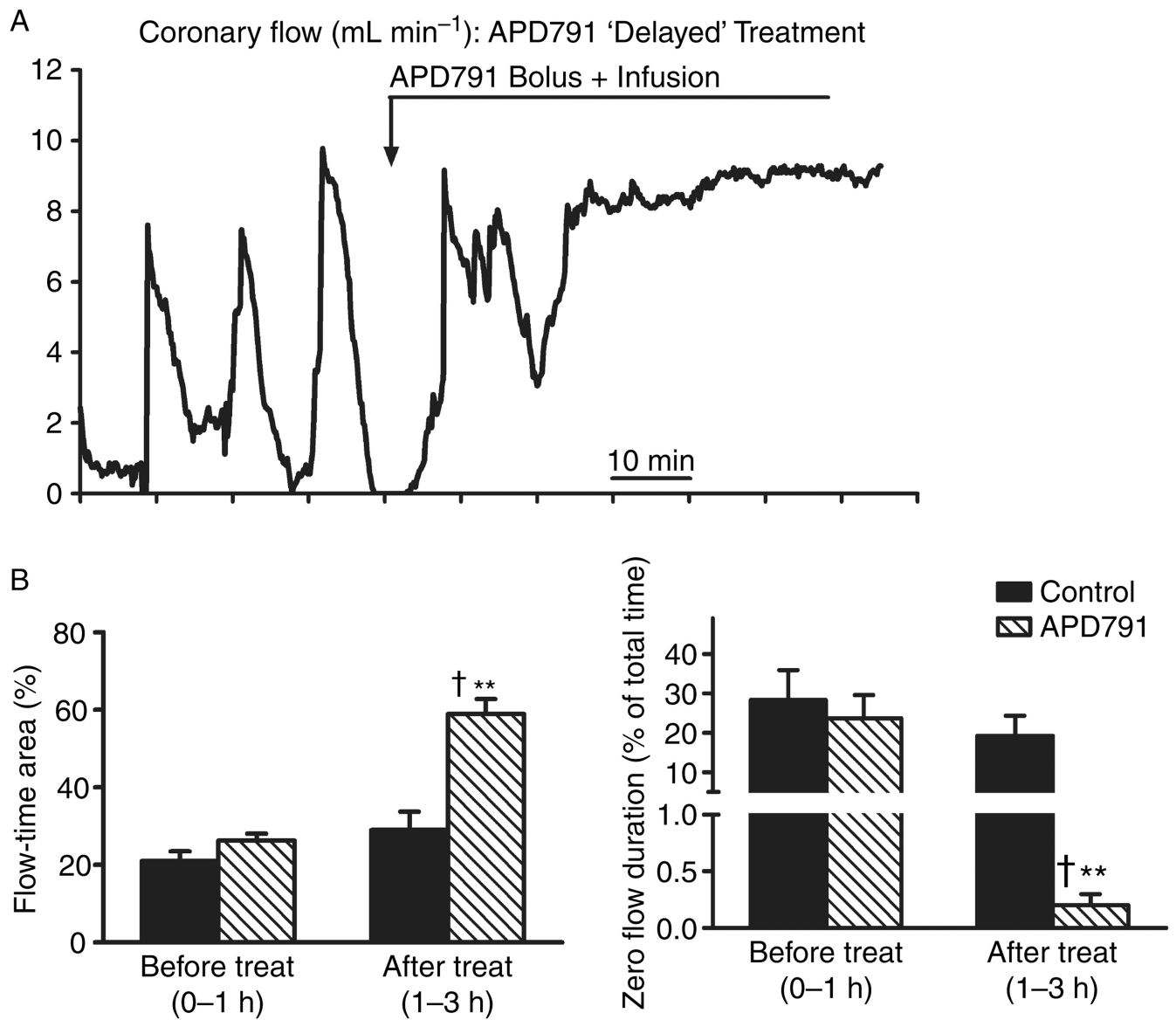
APD791 Bolus + Infusion

10 min

B

Fig. 6.
Protocol 2: vessel patency. (A) Original recording of mean coronary flow from one animal, obtained before and after administration of APD791. (B) Mean values of percentage flow–time area and zero flow duration, measured before and after treatment, for control and APD791-treated groups. †$P < 0.01$ vs. before treatment; **$P < 0.01$ vs. control.
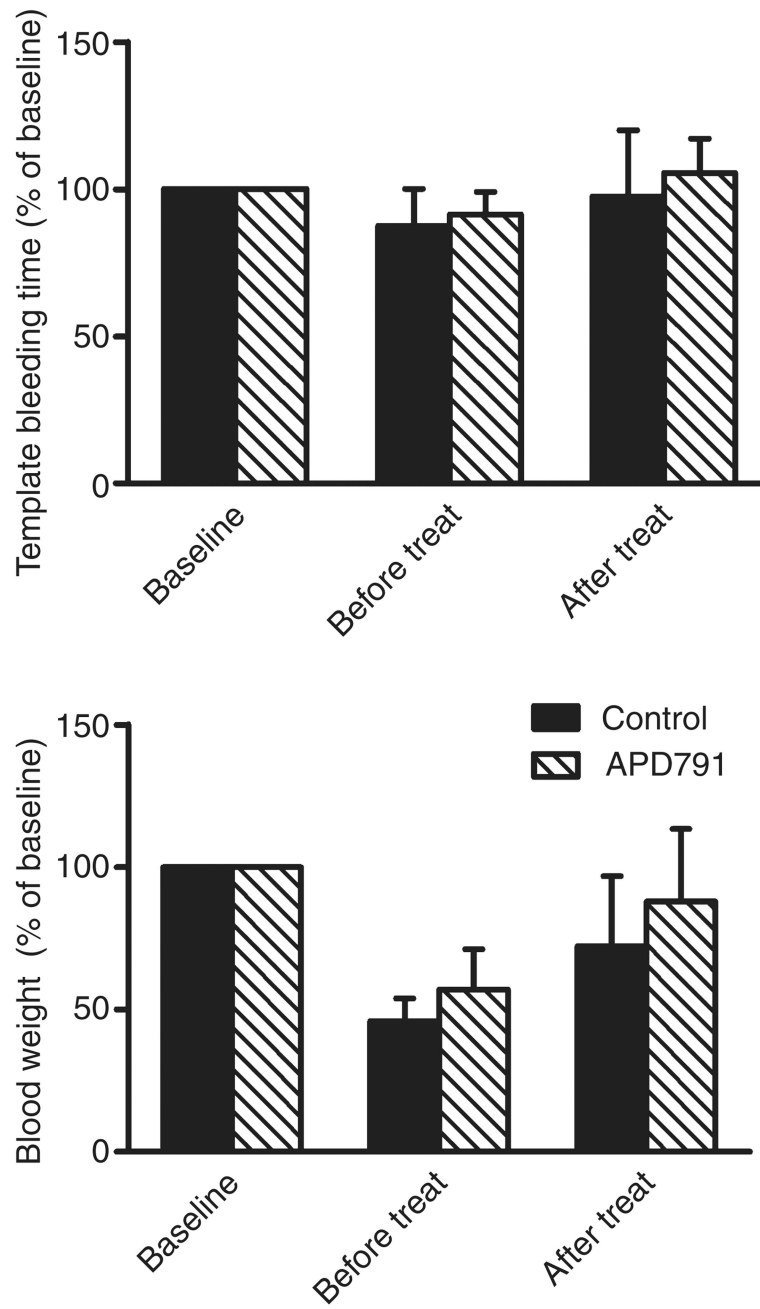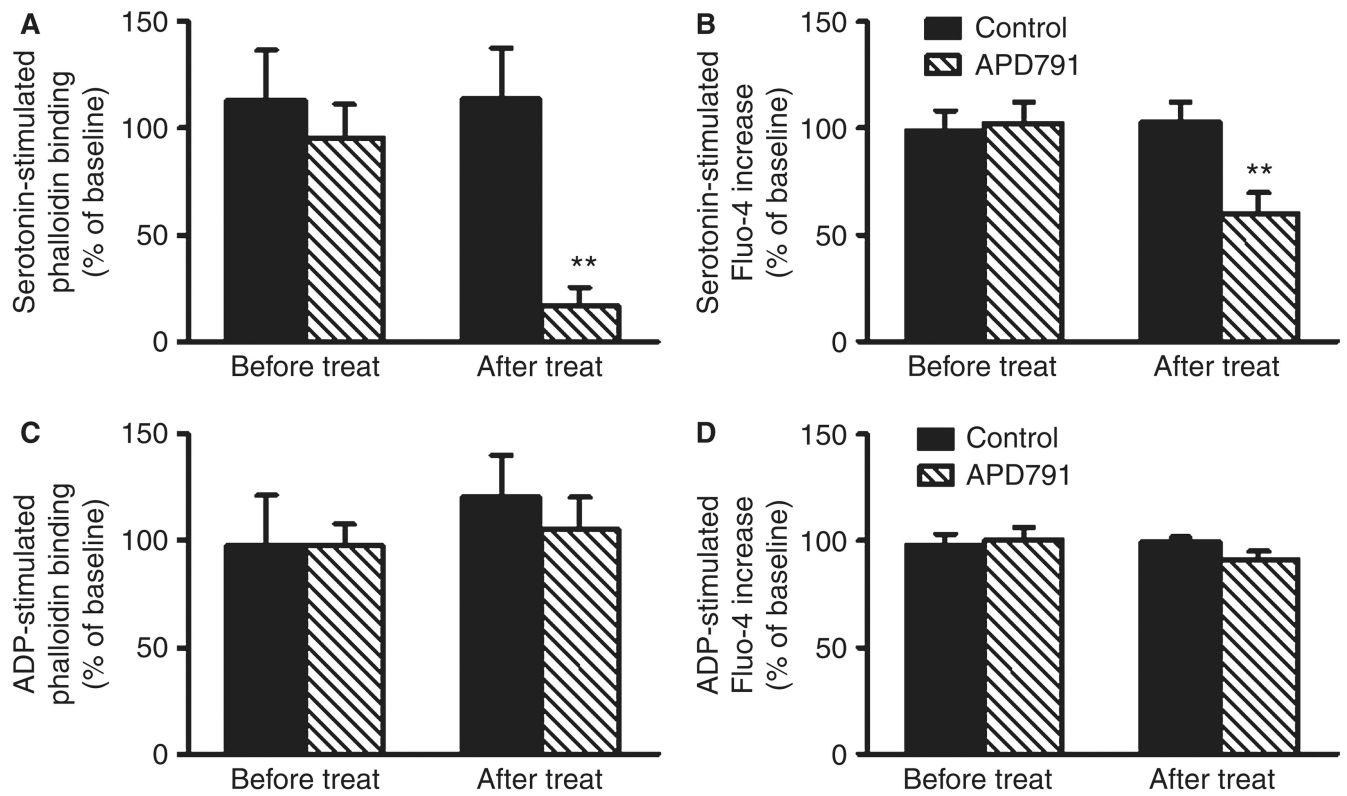
Fig. 7.
Protocol 2: template bleeding time and 'volume'. Mean values of template bleeding time and weight of shed blood for control and APD791-treated groups, measured before and after treatment and normalized to baseline.

Fig. 8.
Protocol 2: APD791 inhibition of *ex vivo* serotonin-stimulated platelet function. Mean values of serotonin-stimulated phalloidin binding and Fluo-4 fluorescence (A, B) and ADP-stimulated phalloidin binding and Fluo-4 fluorescence (C, D) for control and APD791-treated groups, measured before and after treatment and normalized to baseline. **$P < 0.01$ vs. control.
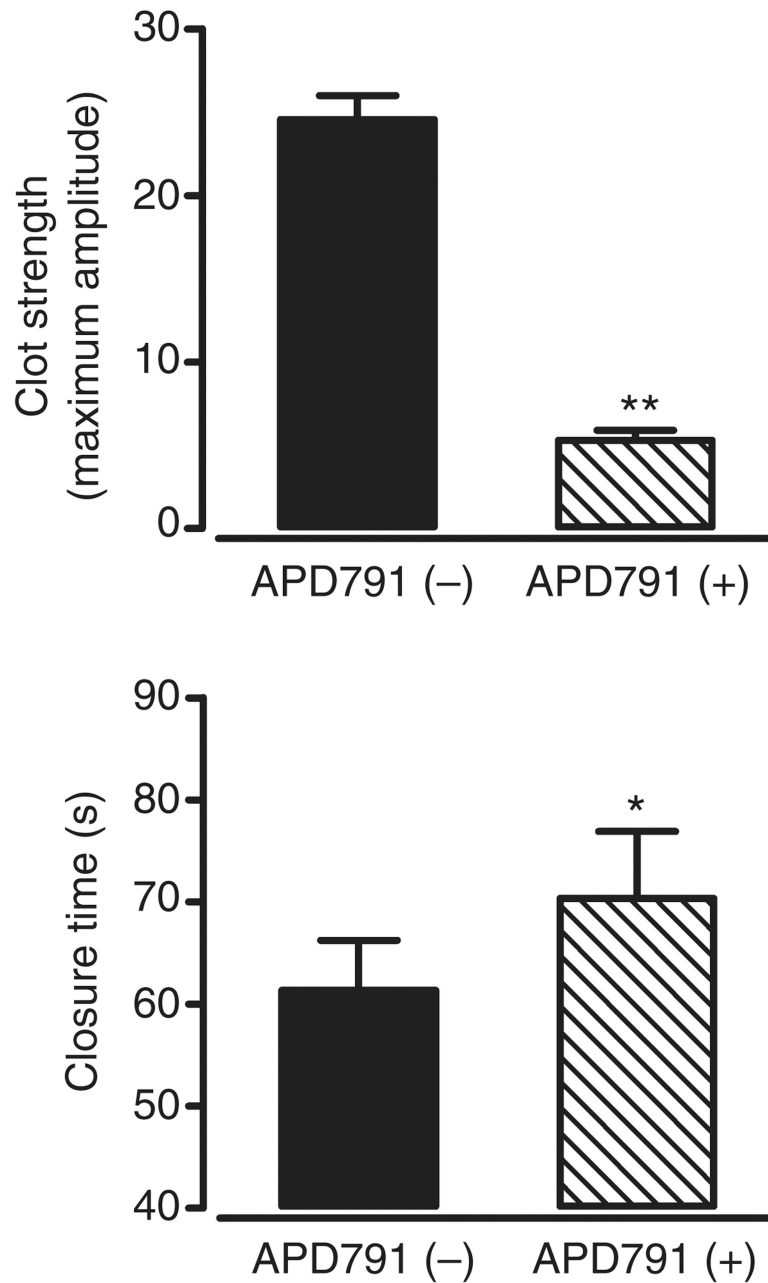
Fig. 9.
Protocol 3: effect of APD791 on *in vitro* platelet-dependent clot formation. Mean values of clot strength [determined using the thromboelastogram (TEG) PlateletMapping System] and closure time (assessed using the PFA-100) in whole blood aliquots incubated with APD791 [APD791 (+)] or vehicle [APD791 (−)]. *$P < 0.05$ and **$P < 0.01$ vs. matched APD791 (−) aliquots.