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Inhibition of platelet function by administration of MRS2179, a $P2Y_1$ receptor antagonist

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Abstract

The effects of a potent $P2Y_1$ receptor antagonist, N^6-methyl-2′-deoxyadenosine-3′,5′-bisphosphate (MRS2179) on adenosine-5′-diphosphate (ADP)-induced platelet aggregation in vitro, ex vivo and on the bleeding time in vivo were determined. In suspensions of washed platelets, MRS2179 inhibited ADP-induced platelet shape change, aggregation and Ca^{2+} rise but had no effect on ADP-induced inhibition of adenylyl cyclase. Binding studies using the new radioligand [^{33}P]MRS2179 showed that washed human platelets displayed 134 ± 8 binding sites per platelet with an affinity (K_d) of 109 ± 18 nM. Finally, intravenous injection of MRS2179 resulted in inhibition of rat platelet aggregation in response to ADP and prolonged the bleeding time, in rats or mice, as compared to controls. These results suggest this potent $P2Y_1$ receptor antagonist to be a promising tool to evaluate the in vivo effects of pharmacologically targeting the $P2Y_1$ receptor with a view to antithrombotic therapy.

Keywords: Platelet aggregation; $P2Y_1$ receptor; MRS2179 (N^6-methyl-2′-dexyadenosine-3′,5′-bisphosphate); Antiplatelet drug; Thrombosis

1. Introduction

The central role of adenosine-5′-diphosphate (ADP) as an aggregating agent (Hellem, 1960; Gaarder et al., 1961), not only in the physiological processes of haemostasis but also in the development and extension of arterial thrombosis (Maffrand et al., 1988), has been established for a long time (Cattaneo and Gachet, 1999). Activation of platelets by ADP leads to rapid Ca^{2+} entry and mobilization of intracellular Ca^{2+} stores and simultaneously to inhibition of adenylyl cyclase. The platelet receptors through which extracellular adenosine nucleotides elicit these physiological responses are: the $P2Y_1$ metabotropic receptor responsible for the mobilization of ionized Ca^{2+} from internal stores, which is coupled to Gq protein/Phospholipase C (PLC) activation and initiates aggregation, and an as yet unidentified P2 receptor termed $P2Y_{ADP}$, $P2T_{AC}$ or $P2_{CYC}$, which is coupled to adenylyl cyclase inhibition and is essential for full aggregation in response to ADP (Cattaneo and Gachet, 1999). The latter receptor is the target of the antiplatelet thienopyridine compounds ticlopidine and clopidogrel (Hechler et al., 1998a; Geiger et al., 1999) and of the ATP analogs, the Astra compounds, 2-propylthio-β,γ-difluoromethylene ATP (AR-C66096MX) and N^6-(2-methylthioethyl)-2-(3,3,3-trifluoropropylthio)-β,γ-dichloromethylene ATP (AR-C69931MX) (Humphries et al., 1994; Fagura et al., 1998; Daniel et al., 1998).

Both $P2Y_1$ and P2cyc are known to be essential for normal ADP-induced aggregation to occur (Hechler et al., 1998b; Jin and Kunapuli, 1998). The $P2Y_1$ receptor is necessary for ADP to induce platelet aggregation, since its inhibition in vitro by selective antagonists totally abolishes ADP-induced aggregation, shape change and Ca^{2+} mobilization (Hechler et al., 1998b). Moreover, $P2Y_1$-null mice display strong resistance to the thromboembolism

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induced by intravenous injection of ADP, a mixture of collagen and adrenaline (Léon et al., 1999a; Fabre et al., 1999) or thromboplastin (Léon et al., 2001). Thus, like the unidentified P2 receptor sensitive to the antiaggregant thienopyridine, the $P2Y_1$ receptor represents a potential pharmacological target for antithrombotic drugs. Adenosine-2′,5′-bisphosphate (A2P5P) and adenosine-3′,5′-bisphosphate (A3P5P), which are selective $P2Y_1$ receptor antagonists (Boyer et al., 1996), have been modified structurally to obtain N^6-methyl-2′-deoxyadenosine-3′,5′-bisphosphate (N^6MABP or MRS2179), to date the most potent and selective known antagonist of the $P2Y_1$ receptor (Boyer et al., 1998).

The aim of the present study was to determine whether $P2Y_1$ receptor antagonists such as MRS2179 affect platelet functions or haemostasis and thrombosis, in vitro, ex vivo or in vivo. N^6MABP was found to inhibit ADP-induced aggregation of human and rat platelets both in vitro and ex vivo and to prolong the bleeding time in rats and in mice.

2. Materials and methods

2.1. Chemicals

2-Methylthioadenosine-5′-diphosphate (2MeSADP) was from Research Biochemicals (Natick, USA). ADP, A3P5P, prostaglandin E_1 (PGE_1) and essentially fatty acid-free human serum albumin were from Sigma (Saint Quentin-Fallavier, France). Human fibrinogen was from Kabi (Stockholm, Sweden), fura-2/acetoxymethyl ester (fura-2/AM) from Calbiochem (Meudon, France) and the cyclic adenosine-3′,5′-monophosphate (cAMP) assay kit from Amersham (Les Ulis, France). Apyrase was purified from potatoes as previously described (Cazenave et al., 1983). [^{33}P]2MeSADP and [^{33}P]MRS2179 were provided by Du Pont NEN (Le Blanc Mesnil, France). MRS2179 and the precursor for the synthesis of [^{33}P]MRS2179 were synthesized by P. Raboisson (CNRS, Faculty of Pharmacy, Strasbourg, France). AR-C66096MX was a generous gift from ASTRA (Charnwood, UK). Anesthesic drugs xylazine (Rompun®) and ketamine (Imalgene 1000®) were from Bayer (Paris, France) and Mérial (Lyon, France), respectively. MRS2179 was checked for purity and stability by high performance liquid chromatography (HPLC) analysis using a Partisil 10 μ SAX column (Interchrom, Interchim, Montluçon, France) eluted with a linear gradient of 0–900 mM ammonium phosphate buffer, pH 3.8.

2.2. Washed human and rat platelet aggregation

Washed human and rat platelets were prepared as previously described (Cazenave et al., 1983) and resuspended at 3×10^5 platelets/μl in Tyrode's buffer containing 2 mM $CaCl_2$, in the presence of 0.02 U/ml of the ADP scavenger apyrase (adenosine-5′-triphosphate diphosphohydrolase, EC 3.6.1.5), a concentration sufficient to prevent the desensitization of platelet ADP receptors during storage (Baurand et al., 2000). Platelets were kept at 37°C throughout all experiments and aggregation was measured by standard methods (Hechler et al., 1998b; Cazenave et al., 1983). Briefly, a 450-μl aliquot of platelet suspension was stirred at 1100 rpm and activated by addition of agonists and for human platelets of human fibrinogen (0.8 mg/ml),

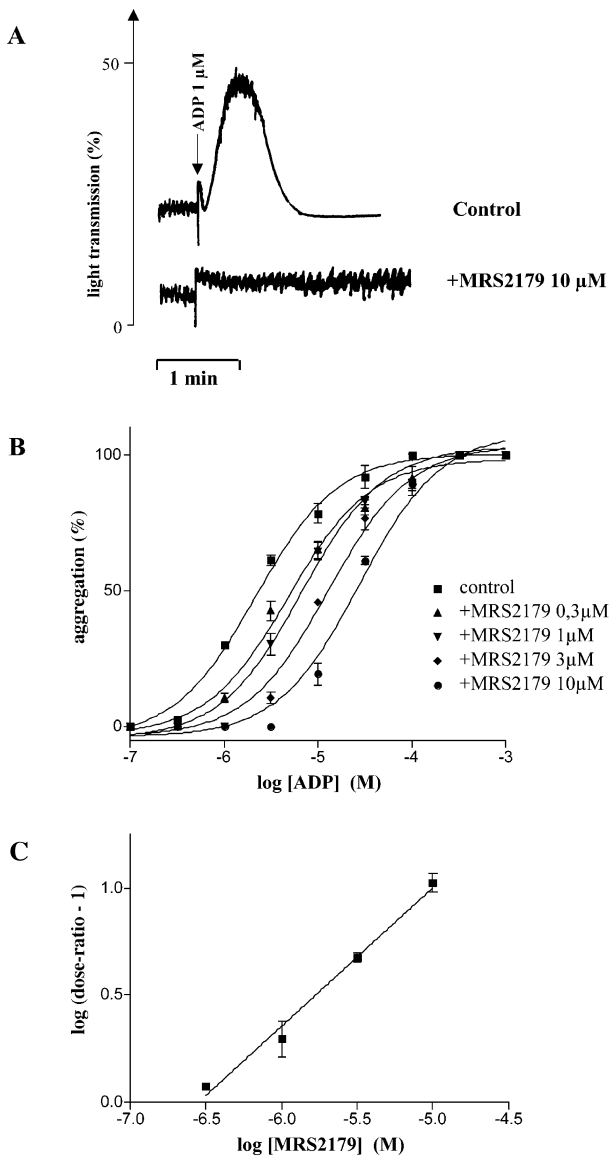

Fig. 1. Inhibition by MRS2179 of ADP-induced aggregation of washed human platelets. (A) Aggregation and shape change in response to 1 μM ADP (control) were inhibited by 10 μM MRS2179. Traces are from one experiment representative of five independent experiments giving identical results. (B) Aggregation was induced by increasing concentrations of ADP, in the presence of the indicated increasing concentrations of MRS2179 added 30 s before ADP (inset). (C) Schild regression analysis of the data shown in (B). Curves represent the mean of three independent experiments and give a pA_2 of 6.55 ± 0.05 and a Schild slope of 0.64. Bars show the S.E.M.

in a final volume of 500 μl. The extent of aggregation was estimated quantitatively by measuring the maximum curve height above the baseline.

2.3. $[Ca^{2+}]_i$ measurements

Fura-2/AM loaded human platelets were prepared as previously described (Hechler et al., 1998b) and resuspended in Tyrode's buffer without $CaCl_2$. Where indicated, $CaCl_2$ (2 mM final concentration) was added just prior to stimulation with an agonist. Aliquots of fura-2-loaded platelets were transferred to a 10×10 mm quartz cuvette maintained at 37°C and fluorescence measurements were performed under continuous stirring, in a PTI Deltascan spectrofluorimeter (Photon Technology International, Princeton, NJ, USA) (Hechler et al., 1998a). The excitation wavelength was alternately fixed at 340 or 380 nm, fluorescence emission was determined at 510 nm and results were calculated as the fluorescence ratio (340:380) in arbitrary units.

2.4. Measurement of adenylyl cyclase activity

A 450-μl aliquot of washed platelets resuspended in Tyrode's buffer containing 2 mM Ca^{2+} and 1 mM Mg^{2+} was stirred at 1100 rpm in an aggregometer cuvette and the following reagents were added at 30-s intervals: (i) 10 μM Prostaglandin E_1, (ii) 1 μM AR-C66096MX or different concentrations of MRS2179 and (iii) 5 μM ADP or vehicle (Tyrode's buffer containing no Ca^{2+} or Mg^{2+}). The reaction was stopped 1 min later by addition of 50 μl of ice-cold 6.6 N perchloric acid. Perchloric acid extracts were centrifuged at $11{,}000 \times g$ for 5 min to eliminate protein precipitate and cyclic AMP was isolated from the supernatants using a mixture of trioctylamine and freon (28:22, vol/vol). The upper aqueous phase was lyophilized

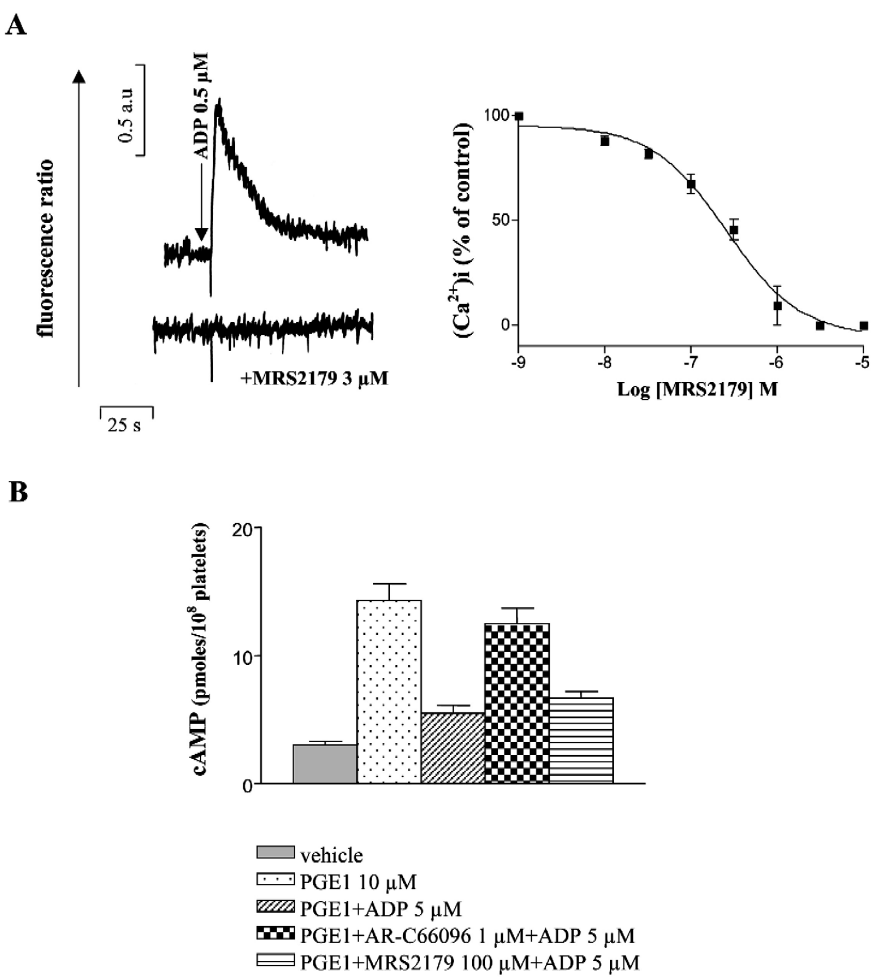

Fig. 2. Effects of MRS2179 on ADP transduction pathways. (A) 3 μM MRS2179, added 30 s before ADP, totally abolished the $[Ca^{2+}]_i$ rise induced by 0.5 μM ADP in washed human platelets in the presence of 2 mM external Ca^{2+} (left). Data are from one experiment representative of five independent experiments giving identical results. MRS2179 modified the $[Ca^{2+}]_i$ rise induced by 1 μM ADP in washed human platelets in a dose-dependent manner with $IC_{50} = 2.6 \times 10^{-7}$ M (right). The curve represents the mean of three independent experiments and bars show the S.E.M. (B) In the presence of 100 μM MRS2179 (hatched bars), ADP was still able to reduce Prostaglandin PGE_1-stimulated cAMP accumulation in washed human platelets.

and the dry residue dissolved in the buffer provided with the commercial radioimmunoassay kit for cyclic AMP measurement (Amersham) (Hechler et al., 1998b).

2.5. Binding studies

Competitive binding of [^{33}P]2MeSADP (850 Ci/mmol) to washed platelets at 37°C for 5 min was determined as described in earlier work (Gachet et al., 1995). Binding of [^{33}P]MRS2179 (2000 Ci/mmol) to washed human platelets in Tyrode's buffer containing 0.01% human serum albumin fatty acid free, was measured at 20°C for 30 min in 3 ml polypropylene tubes in a final volume of 1 ml and saturation was determined by isotopic dilution. The reaction was started by addition of washed platelets to the reaction mixture and all experiments were carried out in triplicate. Non specific binding, determined by incubation in the presence of 1 mM unlabeled A3P5P amounted to about 10–15% of total binding.

Saturation and displacement experiments were performed using a single concentration of radiolabeled ligand, [^{33}P]MRS2179 (0.5 nM, 200.000 dpm) or [^{33}P]2MeSADP (0.2 nM, 200.000 dpm), in the presence of increasing concentrations of the appropriate unlabeled ligand. The reactions were terminated by addition of ice cold Tyrode's buffer and rapid filtration through Whatman GF/C glass fiber filters under vacuum, after which the tubes and filters were rinsed twice. Radioactivity bound to the platelets on the filters was measured by scintillation counting (Wallac 1409 β-counter, count rate (DPM/CPM ± S.E.M.): 1.070 ± 0.0018; Turku, Finland) and data were analysed with the program EBDA-LIGAND (Munson and Rodbard, 1980). The dissociation constant (K_d) of the radioligand and the inhibition constant for the drug (K_i) were calculated using the GraphPad software package (GraphPad, San Diego, CA).

2.6. Ex vivo studies

Male Wistar rats weighing 300 g (Iffa-Credo, l'Arbresle, France) were anesthetized by intraperitoneal injection of 200 μl xylazine base (0.2 mg/kg) and ketamine (1 mg/kg). At time zero, MRS2179 (50 mg/kg) or vehicle was injected into the penis vein. Blood (6.3 ml) was drawn

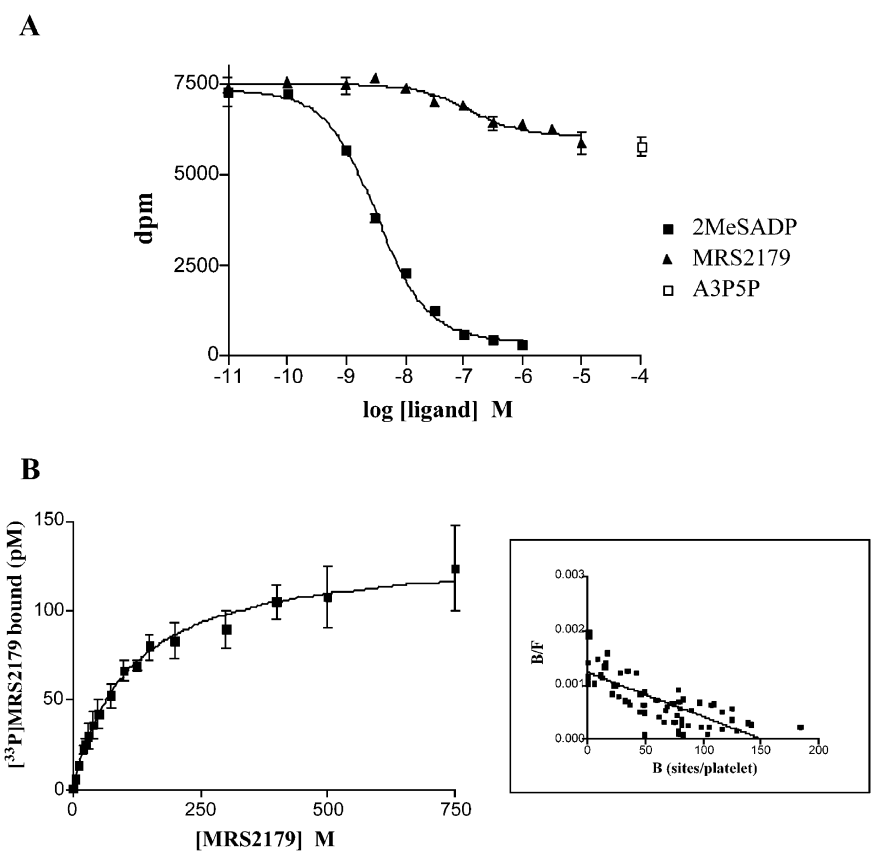

Fig. 3. Binding of [^{33}P]2MeSADP and [^{33}P]MRS2179 to washed human platelets. (A) Competition curves for the binding of [^{33}P]2MeSADP to washed platelets measured after incubation for 5 min at 37°C. Displacement experiments were performed using a single concentration of [^{33}P]2MeSADP (0.1 nM) in the presence of increasing concentrations of unlabeled 2MeSADP, MRS2179 or A3P5P. Curves are from one experiment representative of three independent experiments giving identical results. (B) Equilibrium specific binding of [^{33}P]MRS2179 to washed human platelets was determined after incubation for 30 min at 20°C, using a single concentration of [^{33}P]MRS2179 (0.5 nM) and increasing concentrations of the appropriate unlabeled ligand. Points represent the mean of five independent experiments performed in triplicate.

5 min later from the abdominal aorta into syringes containing 0.7 ml 3.15% sodium citrate and immediately centrifuged (70 s at $1570 \times g$) at room temperature. Citrated platelet-rich plasma (cPRP) was removed and platelets were adjusted to $5 \times 10^5/\mu l$ with platelet-poor plasma (PPP). Platelet aggregation was measured in citrated platelet-rich plasma from control and MRS2179-treated rats as described above.

2.7. In vivo studies

The bleeding time was measured 1 min after injection of MRS2179 (50 mg/kg) or vehicle into the jugular vein of mice. CL57BL/6 mice were bred at Iffa Credo. Male mice weighing 20–30 g were anesthetized by intraperitoneal injection of 150 μl of a mixture of 0.2% xylazine base and 1% ketamine in physiological saline. The mice tail was amputated 3 mm from the tip and was immediately immersed in isotonic 0.9% NaCl buffer at 37°C. The bleeding time was defined as the time required for arrest of bleeding.

3. Results

3.1. Platelet aggregation is inhibited by MRS2179

Addition of MRS2179 (10 μM) to washed human platelets 30 s before ADP (1 μM) inhibited platelet aggregation and shape change (Fig. 1A), while MRS2179 alone did not induce shape change or aggregation even at high concentrations (up to 100 μM, data not shown). The nature of the inhibition was determined by generating a series of concentration–response curves for ADP in the presence of different concentrations of MRS2179. MRS2179 caused a parallel shift to the right of the concentration–response curve, but high concentrations of ADP could completely override high concentrations of MRS2179 (Fig. 1B). Schild analysis of the inhibition gave a pA_2 value of 6.55 ± 0.05 ($n = 5$) and a slope of 0.64, which could be explained by the fact that we observed an integrated aggregation process involving the activation of two receptors ($P2Y_1$ and P2cyc) and their transduction machinery. Identical results were obtained using washed rat platelets, MRS2179 inhibiting ADP-induced platelet aggregation with a parallel shift to the right of the concentration–response curve and a pA_2 value of 6 (data not shown).

3.2. MRS2179 affects only the $P2Y_1$ receptor transduction pathway

ADP induces simultaneous mobilization of intracellular Ca^{2+} stores and inhibition of adenylyl cyclase, through activation of the $P2Y_1$ and P2cyc receptors, respectively. The intracellular Ca^{2+} rise induced in washed human platelets by 0.5 μM ADP was totally inhibited by 3 μM MRS2179, in the presence (Fig. 2A, left) or absence (data not shown) of 2 mM external Ca^{2+}. MRS2179 modified the $[Ca^{2+}]_i$ increase in response to 1 μM ADP in a dose-dependent manner with $IC_{50} = 0.26$ μM (Fig. 2A, right). A similar inhibition of ADP-induced $[Ca^{2+}]_i$ rises was observed in washed rat and mouse platelets (data not shown).

Conversely, 100 μM MRS2179 had no influence on basal levels of cAMP in human, rat or mouse platelets, or on the cAMP levels induced by 10 μM Prostaglandin E_1 (data not shown). The ability of ADP to inhibit Prostaglandin E_1-stimulated cAMP accumulation was likewise not affected by 100 μM MRS2179, in either human (Fig. 2B) or rodent platelets (data not shown), whereas AR-C66096, a selective P2cyc receptor antagonist, totally reversed the inhibitory effect of ADP.

3.3. [^{33}P]MRS2179 is a suitable radioligand to estimate numbers and affinities of $P2Y_1$ sites

Studies of the binding of ADP to its platelet receptors are currently performed using [^{33}P]2MeSADP, a radioligand which binds to both $P2Y_1$ and P2cyc (Gachet et al., 1995; Hechler et al., 1998a; Léon et al., 1999b). MRS2179,

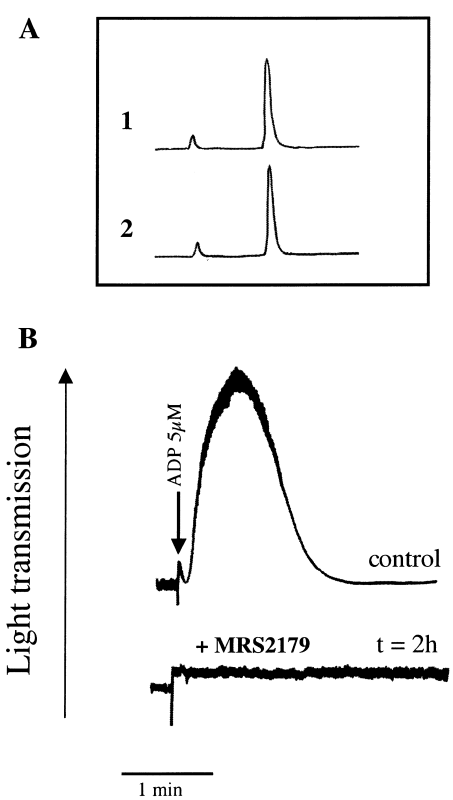

Fig. 4. Stability of MRS2179 in the presence of apyrase and in the presence of washed platelets. (A) HPLC profiles of MRS2179 before (1) and after 2-h incubation with apyrase 0.1 U/ml (2). (B) Platelet aggregation was still inhibited when MRS2179 100 μM was added 2 h before stimulation by ADP 5 μM.

on the other hand, appeared to us to be a good candidate for use as a $P2Y_1$ specific radioligand. Firstly, in order to verify that MRS2179 displaced [^{33}P]2MeSADP only from $P2Y_1$ receptors, we compared its effects to those of A3P5P, a well known $P2Y_1$ receptor antagonist (Hechler et al., 1998b; Léon et al., 1999b). The specific binding of [^{33}P]2MeSADP to washed human platelets was competitively and partially displaced by MRS2179 (K_i = 111 nM) and A3P5P ($K_i = 0.25 \pm 0.06$ μM, data not shown) at approximately 20% of [^{33}P]2MeSADP sites (Fig. 3A). In preliminary assays, the binding of [^{33}P]MRS2179 to washed platelets was tested at three different temperatures +4°C,

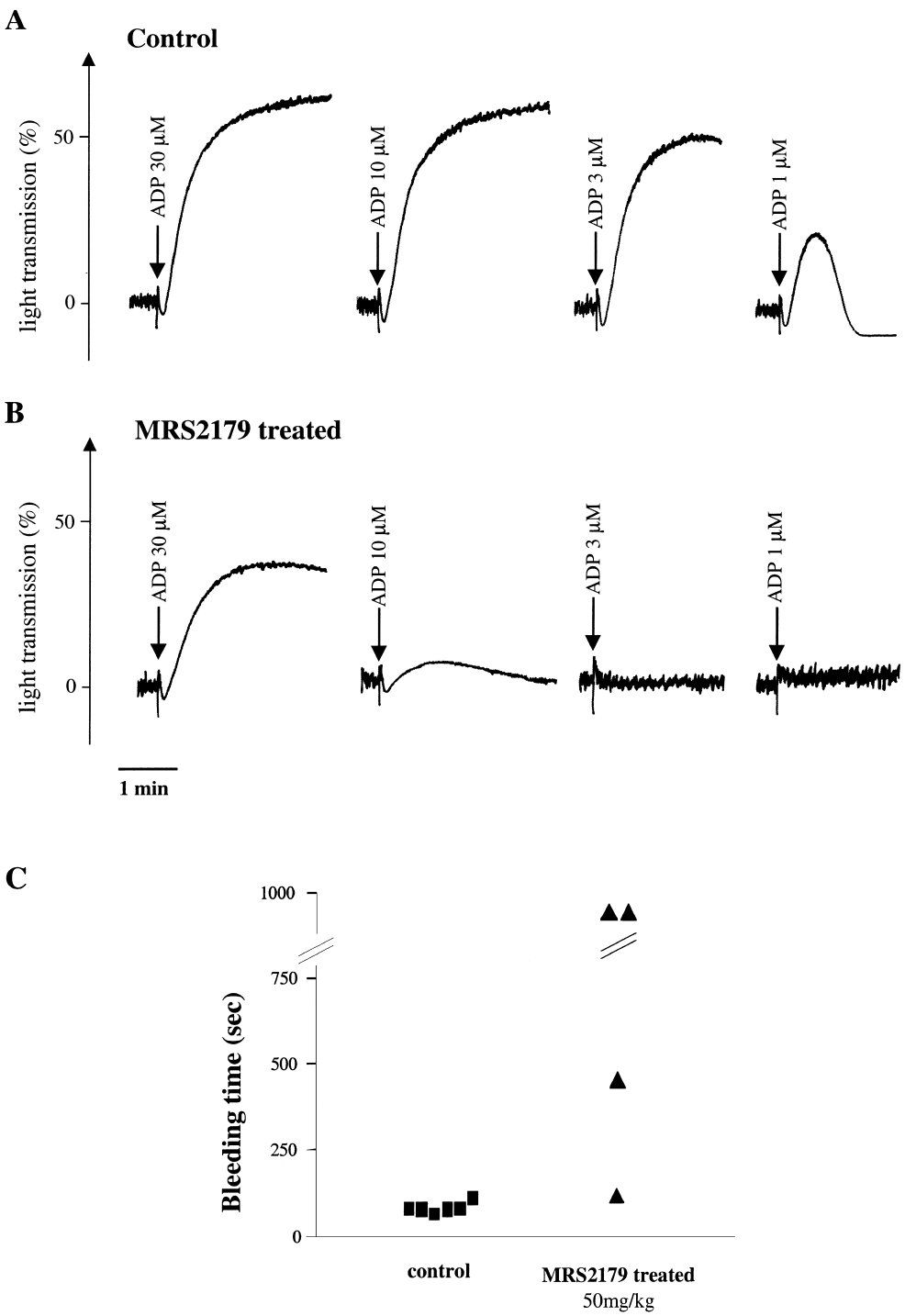

Fig. 5. Effects of MRS2179 on platelet aggregation and haemostasis in rats and mice. Platelet aggregation in response to increasing concentrations of ADP (1–30 μM) in citrated platelet-rich plasma from rats injected with MRS2179 (50 mg/kg) (A) or vehicle (B). (C) Bleeding time in mice measured 30 s after injection of MRS2179 (50 mg/kg) or vehicle.

+20°C, +37°C. The best condition was at 20°C and the binding was proportional to the concentration of platelets and maximal binding, stable for at least 30 min, was obtained after 30 s to 1 min at 20°C (data not shown). All subsequent saturation experiments were performed using a platelet concentration of 6×10^5/ml and an incubation time of 30 min at 20°C. The specific binding of [^{33}P]MRS2179 to washed human platelets was saturable (Fig. 3B) with a linear Scatchard plot (insert), 134 ± 8 binding sites per platelet and an affinity (K_d) of 109 ± 18 nM ($n = 3$). This result was confirmed by measuring the binding of [^{33}P]MRS2179 to astrocytoma cells (1321 NI) transfected with the $P2Y_1$ receptor or with the vector alone. In the cells transfected with $P2Y_1$, which displayed normal pharmacological selectivity and signaling properties, the number of binding sites was $162{,}500 \pm 7500$ per cell with a K_d of 138 ± 8 nM ($n = 3$), whereas the control cells showed no binding of [^{33}P]MRS2179. A similar affinity was observed for the murine platelet $P2Y_1$ receptor (data not shown).

3.4. MRS2179 inhibits platelet aggregation ex vivo

In order to test the stability of MRS2179 in the presence of ectonucleotidases, we measured the possible degradation products after a 2-h incubation with apyrase 0.1 U/ml. The HPLC profiles of MRS2179 before (Fig. 4A1) and after incubation (Fig. 4A2) were identical. Thus, MRS2179 is chemically stable in the presence of apyrase. Incubation of MRS2179 in citrated platelet-rich plasma for 2 h did not affect MRS2179 inhibitory activity regarding ADP-induced platelet aggregation (data not shown). Moreover, when MRS2179 (100 μM) was incubated for 2 h with washed platelets, ADP-induced aggregation was still inhibited (Fig. 4B), confirming the stability of MRS2179. MRS2179 was subsequently injected intravenously into anesthetized rat to study ex vivo ADP-induced aggregation. Blood samples were taken at various time points after injection of 50 mg/kg to follow the activity of the compound. In citrated platelet-rich plasma from MRS2179-treated rats, ADP-induced platelet aggregation was inhibited for ADP concentrations of up to 10 μM, 5 min after injection of MRS2179 (Fig. 5A,B). At this dose (50 mg/kg), there persisted an aggregation response to 30 μM ADP, which was nevertheless reduced as compared to the control (Fig. 5A,B).

3.5. MRS2179 prolongs the bleeding time

The bleeding time, which reflects in vivo primary haemostasis, was significantly prolonged in MRS2179-treated mice as compared to control mice, 30 s after injection of MRS2179 (50 mg/kg) (Fig. 5C). The mean bleeding time (±S.D.) was 595 ± 189 s for MRS2179-treated mice (range 120–1200 s, $n = 4$) and 83.5 ± 5.7 s for control mice (range 68–110 s, $n = 6$) and the difference between the two groups was statistically significant ($P < 0.01$, unpaired t-test). The bleeding time was also prolonged in MRS2179-treated rats (data not shown).

4. Discussion

The data presented here demonstrate that the $P2Y_1$ receptor antagonist MRS2179 strongly inhibits ADP-induced platelet aggregation in vitro and ex vivo. Schild analysis of the concentration–response curve for ADP in the presence of MRS2179 gave a pA_2 value of 6.55 ± 0.05, which represents an antiaggregant potency approximately 10-fold greater than that of the parent molecule, adenosine-3′,5′-bisphosphate (A3P5P) (Hechler et al., 1998b). MRS2179 is not a pure competitive antagonist of aggregation as indicated by a Schild slope of 0.64. This could be explained by the fact that aggregation results from the activation of both $P2Y_1$ and P2cyc receptors and multiple transduction pathways leading to binding of fibrinogen and finally to platelet aggregation. However, MRS2179 has been shown to be a pure competitive antagonist of the $P2Y_1$ receptor in transfected cell lines where PLC activity was measured (Boyer et al., 1998). Similarly, A3P5P, a well-known $P2Y_1$ receptor antagonist, found to be non-competitive on aggregation was nevertheless specific and competitive antagonist of the $[Ca^{2+}]_i$ increases induced by ADP (Hechler et al., 1998b). At the intracellular level, as expected for a $P2Y_1$ receptor antagonist, MRS2179 totally inhibited the $[Ca^{2+}]_i$ mobilization induced by ADP but had no effect on its inhibition of adenylyl cyclase. Binding experiments were performed to determine whether [^{33}P]MRS2179 might be a suitable radioligand to study the $P2Y_1$ receptor. To date, numbers of ADP binding sites have been measured using [^{33}P]2MeSADP, a known agonist of both $P2Y_1$ and P2cyc, the two ADP receptors expressed by platelets. The number of $P2Y_1$ binding sites on human platelets was evaluated by saturation experiments in the presence or the absence of A3P5P, a $P2Y_1$ receptor antagonist and was about 100 sites per platelets (Baurand et al., 2000). In the presence of unlabeled MRS2179, we observed a reduction in the number of [^{33}P]2MeSADP binding sites on human platelets which corresponded to the A3P5P sensitive sites. Conversely, specific binding of [^{33}P]MRS2179 to washed human platelets was saturable and revealed approximately 100 sites per platelet with a K_d in the 100 nM range, which corresponded to the $P2Y_1$ binding sites determined as previously described with [^{33}P]2MeSADP. Thus, MRS2179 seems to be an interesting tool to determine precisely the number of $P2Y_1$ binding sites on platelets.

The increased resistance to thromboembolism in mice lacking the $P2Y_1$ receptor (Léon et al., 1999a; Fabre et al., 1999) suggested this receptor to be a potential target for new antiplatelet drugs. To further study the involvement of

the $P2Y_1$ receptor in thrombotic states and to evaluate the antithrombotic potential of $P2Y_1$ receptor antagonists, MRS2179 was injected at a dose of 50 mg/kg into rats. Ex vivo, ADP-induced aggregation in citrated platelet-rich plasma was inhibited for ADP concentrations of up to 10 μM. This inhibition, which resulted in a shift of the ADP dose–response curve to higher concentrations, was transitory and persisted for a few minutes after injection, probably due to a rapid in vivo metabolization rather than a plasmatic degradation since we showed that MRS2179 is resistant to degradation by plasma or by apyrase. Further studies are required to establish dose–response curves and the pharmacokinetic of this compound. In vivo, the bleeding time was significantly prolonged in rats or mice treated with MRS2179 as compared to control animals. Moreover, other studies showed that in vivo administration of the $P2Y_1$ receptor antagonist MRS2179 to mice resulted in resistance to thromboembolism induced by a mixture of collagen and adrenaline and by tissue factor (Léon et al., 1999a, 2001). Altogether, these results provide considerable encouragement for development of new $P2Y_1$ receptor antagonists and for the evaluation of their properties in animal models of arterial thrombosis.

The ADP selective antiplatelet drugs so far existing are the thienopyridine compounds ticlopidine and clopidogrel which are selective inhibitors of the P2cyc receptor and marketed antithrombotic agents. These compounds have proved to be efficient in numerous animal models of arterial thrombosis as well as in a great number a large multicentric clinical trials evaluating their effects on ischemic heart diseases, peripheral vascular disease and stroke (Savi and Herbert, 2000). Similarly, the ATP analogues of the AR-C series, which are potent competitive P2cyc receptor antagonists, have proved to be efficient antithrombotics in animal models and some of them are in phase II of clinical trials for the treatment of acute coronary syndromes (Humphries, 2000).

Our present results show that besides the clopidogrel or AR-C compounds sensitive P2cyc receptor, the $P2Y_1$ receptor is a promising potential target for new antithrombotic drugs.

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