Loss of Mouse P2Y<sub>4</sub> Nucleotide Receptor Protects against Myocardial Infarction through Endothelin-1 Downregulation

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Nucleotides are released in the heart under pathological conditions, but little is known about their contribution to cardiac inflammation. The present study defines the P2Y4 nucleotide receptor, expressed on cardiac microvascular endothelial cells and involved in postnatal heart development, as an important regulator of the inflammatory response to cardiac ischemia. P2Y4-null mice displayed smaller infarcts in the left descending artery ligation model, as well as reduced neutrophil infiltration and fibrosis. Gene profiling identified inter alia endothelin-1 (ET-1) as one of the target genes of P2Y4 in ischemic heart. The reduced level of ET-1 was displayed smaller infarcts in the left descending artery ligation model, as well as reduced neutrophil infiltration and fibrosis. Gene profiling identified inter alia endothelin-1 (ET-1) as one of the target genes of P2Y4 in ischemic heart. The reduced level of ET-1 was correlated with reduction of microvascular hyperpermeability, neutrophil infiltration, and endothelial adhesion molecule expression, and it could be explained by the decreased number of endothelial cells in P2Y4-null mice. Expression analysis of metalloproteinases and their tissue inhibitors in ischemic heart revealed reduced expression of matrix metalloproteinase (MMP)-9, reported to be potentially regulated by ET-1, and MMP-8, considered as neutrophil collagenase, as well as reduction of tissue inhibitor of MMP-1 and tissue inhibitor of MMP-4 in P2Y4-null mice. Reduction of cardiac permeability and neutrophil infiltration was also observed in P2Y4-null mice in LPS-induced inflammation model. Protection against infarction resulting from loss of P2Y4 brings new therapeutic perspectives for cardiac ischemia and remodeling. The Journal of Immunology, 2015, 194: 1874–1881.

Cardiac injury is a great challenge owing to the limited regenerative ability of the heart. Myocardial infarction (MI) is the most common source of cardiac injuries leading to ischemic death of a large number of cardiomyocytes (1). After this phase, dying cells trigger an inflammatory reaction with the recruitment of leukocytes into the infarcted area. Neutrophils and macrophages clear the wound from dead cells and matrix debris (2). However, MI is a multifactorial process that leads also in the first hours to a postischemic edema that develops as a result of increased microvascular permeability. Myocardial edema contributes to vessel collapse and impaired heart function, including reperfusion arrhythmias and stunning, and could affect ventricular remodeling by altering myocardial stiffness (3). Among the proteins regulating the inflammatory response to cardiac ischemia, endothelin-1 (ET-1) plays an important role. Indeed ET-1 is involved in neutrophil trafficking and increases cardiac microvascular permeability (4, 5). After the edema step, the healing process proceeds to the formation of granulation tissue, which is characterized by the presence of fibroblasts, macrophages, myofibroblasts, new blood vessels, and extracellular matrix proteins (6, 7), ultimately resulting in scar formation, which is characterized by acellular and cross-linked collagen-rich regions (8–11).

Cardiac endothelial cells and cardiomyocytes are an important source of extracellular nucleotides within the heart, especially under ischemia (12). Extracellular nucleotides are involved in the protection of rat cardiomyocytes from hypoxic stress through activation of P2Y2 nucleotide receptors (13). UTP administration to rats reduced infarct size and enhanced myocardial function (14). The implication of P2Y2 receptor in the cardioprotection mediated in vivo by UTP has been recently demonstrated (15).

We investigated in the present study the potential role of the P2Y4 receptor in cardiac inflammation and myocardial infarction. The human P2Y4 receptor is a UTP receptor coupled to the phosphoinositide/Ca2+ pathway (16). Recombinant rat and mouse orthologs of human P2Y4 receptor are activated by UTP and ATP (17–19), as described for the ubiquitously expressed mouse P2Y2 receptor (20). Interestingly, P2Y2 and P2Y4 receptors were abundantly detected in the rat developing heart (21). P2Y4-null mice have been generated in our laboratory (22) and we demonstrated P2Y4 expression in cardiac endothelial cells but not in...
cardiomyocytes (23). We showed recently that this receptor is involved in postnatal heart development (23), as well as in exercise tolerance and exercise-induced cardiac hypertrophy (24).

In this study, we demonstrated that loss of mouse P2Y4 receptor is associated with an unexpected protection against infarction and a reduction of cardiac inflammation, permeability, and fibrosis.

Materials and Methods

Ischemia experiments: LAD ligation of P2Y4+/+ and P2Y4−/− mice

P2Y4−/− null mice were generated in the host laboratory (22). The P2Y4 gene is located on the X chromosome: male and female P2Y4−/− null mice are thus respectively designated as P2Y40/0 or P2Y4−/− female. Female mice were preferably used for ischemia experiments because they display lower ligation-induced mortality. P2Y4+/+ and P2Y4−/− female mice were anesthetized with avertin (240 mg/kg), intubated, and ventilated. A left side thoracotomy was performed, and the pericardium was incised. MI was then induced by the permanent ligation of the left anterior descending artery (LAD) proximal to its bifurcation from the main stem. For some experiments, 10 min before ligation, P2Y4+/+ and P2Y4−/− female mice were injected with vehicle or ET-1 (1 nmol/kg). Twenty-four hours after LAD ligation, hearts were washed and dissected, and slices were incubated immediately into 2 g/100 ml TCA triphenyltetrazolium chloride solution at 37°C for 15 min. Slices were then placed into 4% formal for tissue fixation and stored at 4°C overnight.

Echocardiographic analysis of P2Y4+/+ and P2Y4−/− ischemic mice

Transthoracic echocardiography was performed on mildly anesthetized spontaneously breathing mice (sedated by inhalation of 1% isoflurane, 1 l/min oxygen), using a Vevo 2100 high-resolution imaging system equipped with a 40 MHz transducer (VisualSonics, Toronto, ON, Canada). The mice were placed on a heated electrocardiogram platform. Left parasternal long axis view and left midapillary, apical, and basal short axis views were acquired. End-diastolic and end-systolic volumes and ejection fraction were evaluated on the left parasternal long axis view.

Immunohistochemistry experiments

P2Y4+/+ and P2Y4−/− ischemic mice were killed by cervical dislocation and dissected. Hearts were harvested, weighed, frozen directly in Tissue-Tek® compound (VWR Scientific). Heart sections were cut at 7 μm thickness and fixed with methanol. The sections were then washed in PBS and stained with H&E, DAPI, and Abs against myeloperoxidase (MPO). Thickness and fixed with methanol. The sections were then washed in PBS and stained with H&E, DAPI, and Abs against myeloperoxidase (MPO). Neutrophil density staining was quantified using ImageJ software in the heart sections of ischemic mice (7 μm of the heart using ImageJ software).

LPS-induced inflammation model

P2Y4+/+ and P2Y4−/− mice were injected i.p. with 100 mg/kg LPS suspended in sterile PBS. After 4 h, the peritoneal cavity was washed with 10 ml sterile PBS. Cell number was determined using a counting chamber. Thereafter, cytospin preparations were prepared using a Shandon CytoSpin III cytocentrifuge (Thermo Fisher Scientific, Leicestershire, U.K.) and Diff-Quick staining (Dade Behring, Deerfield, IL). For inflammation analysis, mice were co-injected with LPS (100 mg/kg) and ET-1 (1 nmol/kg) or a selective ETA receptor antagonist, BQ-123 (1 mg/kg).

Permeability experiments

Plasma leakage was measured in vivo using a modification of the technique employed by Hele et al. (25). Evan’s blue dye was used as a marker of albumin extravasation. In practice, vehicle, ET-1 (1 nmol/kg), or BQ-123 (1 mg/kg) were given 5 min prior to Evan’s blue (20 mg/kg, iv.) with or without LPS (30 mg/kg, iv.). After 15 min, animals were perfused with 50 ml sterile saline (0.9%). The vascular leakage, corresponding to the quantity of the dye in tissues, was quantified as the relative surface of fluorescence on five cryosections (7 μm) of the heart using ImageJ software.

Statistical analysis

Data are expressed as means ± SEM for in vitro and in vivo studies. Endpoint comparisons between two groups were performed using an unpaired two-tailed Student t test (Prism software version 5; GraphPad Software, La Jolla, CA). For parallel repeated-measures studies, two-way ANOVA was used with Bonferroni post hoc evaluations to determine the significance for individual time points (Prism software). For all studies, a p value <0.05 was considered significant.

Results

P2Y4−/− null mice are protected against infarction in the LAD ligation model

We performed a mouse model of myocardial ischemia, based on the ligation of the LAD, in P2Y4+/+ and P2Y4−/− female mice. Analysis of whole hearts from base to apex revealed smaller infarcts in P2Y4−/− hearts 24 h after ligation (Fig. 1A). We observed a 25% reduction of infarct size, defined as weight of the infarcted area divided by weight of left ventricle weight, in P2Y4−/− mice compared with P2Y4+/+ mice (Fig. 1B). Effectively, 24 h after ligation, infarct size was 20.2 ± 1.6 and 14.7 ± 1.4% in P2Y4+/+ and P2Y4−/− hearts, respectively (means ± SEM; p < 0.05) (Fig. 1B). These ratios were obtained for 21 P2Y4+/+ mice and 17 P2Y4−/− mice.

We then performed echocardiography experiments to compare cardiac parameters such as left ventricular end-diastolic volume (LVED), left ventricular end-systolic volume (LVES), and ejection fraction (EF) in P2Y4+/+ and P2Y4−/− ischemic mice (Fig. 1C-E). LVED and LVES values were significantly increased 7 and 14 d after ligation in P2Y4+/+ ischemic mice, but not in P2Y4−/− ischemic mice (Fig. 1C, 1D). EF was slightly but not significantly increased at 7 and 14 d in P2Y4−/− ischemic mice (Fig. 1E).

ELISA

Sera of P2Y4 wild-type and knockout mice were collected and ET-1 (Enzo Life Sciences) or VEGF (R&D Systems) level was measured by ELISA, according to the manufacturer’s procedure.

Allylamine-induced heart fibrosis

Male P2Y4+/+ and P2Y4−/− mice were given 0.1% allylamine-HCl as drinking fluid ad libitum. These mice demonstrated a sustained decreased fluid consumption. For histopathological study, mice were given allylamine for 3, 7, 14, or 30 d and were killed by cervical dislocation. The hearts were rapidly removed and frozen.

Quantification of fibrosis in ischemic or allylamine-treated mice

Heart sections of ischemic or allylamine-treated mice were cut, fixed in Bouin’s solution, and stained with the Masson trichrome. The fibrosis was quantified as the relative surface of the blue staining on five cryosections (7 μm) of the heart using ImageJ software.

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in ischemic heart. Microarray experiments revealed tens of differences in gene expression in the ischemic heart of P2Y4-null compared with wild-type mice (data not shown). Among the inflammatory genes that were regulated, ET-1 was downregulated with a ratio of 0.5. We have focused on this particular target gene because, as described below, we identified in parallel an inflammatory defect in P2Y4-null mice. It is known that ET-1 is involved in cardiac inflammation, heart failure, and ventricular remodeling.

Therefore, we evaluated ET-1 serum level in P2Y4+/+ and P2Y4−/− mice during infarction (Fig. 2A). Serum concentration of ET-1 was lower in P2Y4−/− mice than in P2Y4+/+ mice both in basal conditions and 6 h after coronary ligation (Fig. 2A).

We investigated then the potential effect of reduced ET-1 expression in P2Y4−/− mice in the LAD ligation model. ET-1 or PBS was administered via a single i.v. administration 10 min before the ischemic period onset, and infarct size was assessed after 24 h. Injection of ET-1 increased infarct size in both P2Y4+/+ and P2Y4−/− mice (Fig. 2B). Interestingly, it appeared that infarct size was comparable in P2Y4−/− ischemic mice injected with ET-1 and P2Y4−/− ischemic untreated mice (72.1 ± 3.3 versus 98.2 ± 1.8%, respectively) (Fig. 2B).

Defective neutrophil infiltration and endothelial adhesion molecule expression in P2Y4-deficient mice during myocardial infarction

To investigate whether loss of P2Y4 affected inflammation during ischemia, we quantified neutrophil infiltration in the infarction area 24 h after ligation (Fig. 3A, 3B). We observed that loss of P2Y4 significantly reduced MPO+ cells infiltrating the infarcted heart: from 885 ± 117 neutrophils/mm2 in P2Y4+/+ heart to 456 ± 90 neutrophils/mm2 in P2Y4−/− heart (Fig. 3B). We also observed a reduction of TNF-α serum level in P2Y4−/− mice 24 h after ligation (data not shown).

ET-1 is known to regulate neutrophil adhesion and expression of endothelial adhesion molecules such as ICAM-1 and E-selectin. Quantitative PCR experiments were performed to quantify expression of MPO, ICAM-1, and E-selectin (5). Quantitative PCR experiments were performed to quantify expression of MPO, ICAM-1, and E-selectin in P2Y4+/+ and P2Y4−/− ischemic heart (Fig. 3C–F). Lower expression of MPO, ICAM-1, and E-selectin was observed in the infarcted area of P2Y4−/− ischemic hearts compared with P2Y4+/+ ones (Fig. 3C–3F). A significant effect of ET-1 injection was observed only on ICAM-1 expression in P2Y4−/− ischemic heart (Fig. 3D).

Impaired fibrosis in P2Y4-deficient mice

We have stained sections of P2Y4+/+ and P2Y4−/− infarcted hearts with the Masson trichrome method (Fig. 4A). As shown in Fig. 4B and 4C, Masson trichrome blue staining revealed a highly significant decrease of the fibrotic area in the infarcted heart of P2Y4−/− mice compared with P2Y4+/+ mice 14 and 30 d after LAD ligation.

Members of the MMP/TIMP system such as MMP-2, MMP-8, MMP-9, and TIMP-1 are reported to be involved in cardiac remodeling (26). Therefore, we evaluated TIMP-1, TIMP-2, TIMP-3, and TIMP-4 in P2Y4−/− mice during infarction (Fig. 2A). Serum concentration of ET-1 was lower in P2Y4−/− mice than in P2Y4+/+ mice both in basal conditions and 6 h after coronary ligation (Fig. 2A).

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Similarity between the effects of P2Y4 knockout and endothelin antagonist on LPS-induced cardiac microvascular permeability and neutrophil infiltration

SYBR Green experiments were then performed using three independent preparations of RNAs isolated from the heart of male P2Y4+/- and P2Y4-/- mice injected or not with LPS. ET-1 is upregulated in response to LPS, with a lower level of ET-1 in LPS-treated P2Y4+/- mice compared with LPS-treated P2Y4+/- mice (Fig. 5A). ET-1 is also quantified by ELISA in the corresponding mouse sera (Fig. 5B). In basal conditions, we observed that ET-1 concentration was lower in P2Y4+/- than in P2Y4-/- mice. Fig. 5B shows that LPS injection increased more significantly the serum concentration of ET-1 in P2Y4+/- than in P2Y4-/- mice.

Intraportal injection of LPS induced a massive recruitment of immune cells such as neutrophils (15- ± 6-fold) in the peritoneal cavity of P2Y4+/- mice (Fig. 5C). A similar injection of LPS produced a significant but smaller (6- ± 2-fold) neutrophil infiltration in P2Y4+/- mice. Interestingly, when mice were coinjected with LPS (100 mg/kg) and ET-1 (1 nmol/kg), neutrophil recruitment was comparable in P2Y4+/- and P2Y4-/- mice (Fig. 5C). Injection of mice with the selective ETA receptor antagonist BQ-123 (1 mg/kg) markedly attenuated neutrophil recruitment in LPS-injected P2Y4+/- and P2Y4-/- mice (Fig. 5C).

We then investigated vascular permeability in the heart of P2Y4+/- and P2Y4-/- mice using the protocol described by Hele et al. (25). Evan’s blue dye was used after exposure to LPS (30 mg/kg, iv.) alone or combined with ET-1 (1 nmol/kg) or BQ-123 (1 mg/kg). After injection of LPS, vascular leakage was markedly increased in P2Y4+/- compared with P2Y4-/- mice (Fig. 5D). As described above for neutrophil recruitment (Fig. 5C), when mice were coinjected with LPS and ET-1 (1 nmol/kg), cardiac permeability was no longer significantly different in P2Y4+/- and P2Y4-/- mice (Fig. 5D). Preinjection of mice with the selective ETA receptor antagonist BQ-123 (1 mg/kg) markedly attenuated vascular permeability in LPS-injected P2Y4+/- and P2Y4-/- mice (Fig. 5D, 5E).

Discussion

Cardiac endothelial cells and cardiomyocytes are an important source of extracellular nucleotides within the heart, especially under ischemia or pressure overload. Release of UTP has been observed in human heart during myocardial infarction (28). The involvement of P2Y2 receptors in UTP-mediated cardioprotection in vivo has been previously demonstrated (13–15). P2Y4, described as a UTP receptor (16), displays a very restricted tissue distribution including heart and lung, and its physiological role has been poorly investigated. We have previously demonstrated that adult P2Y4-null mice display a microcardia phenotype related to a cardiac angiogenic defect (23) and a reduced exercise tolerance (24).

The present study identifies a protection against myocardial infarction in P2Y4-null mice. The discrepancy with the previously reported cardioprotective effect of UTP (13–15) can be explained by cell-specific actions of UTP. The cardioprotective effect of UTP is mediated by P2Y2 receptors expressed on cardiomyocytes, whereas P2Y4 is expressed on cardiac endothelial cells but is absent on cardiomyocytes.

To investigate further the mechanisms involved in the cardioprotection resulting from P2Y4 deletion, gene profiling experiments were performed and identified differences in gene regulation between P2Y4+/- and P2Y4-/- hearts in ischemia and inflammation models. Among P2Y4 target genes, we focused our CD31 staining was performed in both remote and infarcted areas of P2Y4+/- and P2Y4-/- infarcted hearts, and it revealed a reduction of the number of cardiac microvessels in P2Y4-/- compared with P2Y4+/- ischemic heart 30 d after LAD ligation (Fig. 4H, 4I). We also observed a reduction of VEGF expression in heart and serum of P2Y4-/- ischemic mice compared with P2Y4+/- ischemic mice (Fig. 4J, 4K).

To investigate further the role of P2Y4 in cardiac fibrosis, we performed the allylamine-induced fibrosis model (Fig. 4F, 4G). Allylamine was added in the drinking fluid of male mice during 3, 7, 14 or 30 d. Myocardial fibrosis was then quantified by Masson trichrome staining. In allylamine-treated male mice, the magnitude of fibrosis was lower in P2Y4-/- hearts than in wild-type hearts at all tested periods (Fig. 4F). The strongest difference was observed after 7 d: 3.0 ± 0.8 of fibrosis area for P2Y4+/- hearts versus 8.5 ± 2.5% for P2Y4-/- hearts (means ± SEM; p < 0.001) (Fig. 4F). MPO+ neutrophils were blindly counted in the hearts of allylamine-treated mice at days 3, 7, 14, and 30 (Fig. 4G). A significant reduction of neutrophils was observed after 30 d in P2Y4-null hearts compared with P2Y4+/- hearts (Fig. 4G).

FIGURE 3. Defective neutrophil infiltration and endothelial adhesion molecule expression in P2Y4-deficient mice during myocardial infarction. (A and B) Quantification of MPO+ neutrophils in the hearts of P2Y4+/- and P2Y4-/- mice 24 h after MI. Heart sections were stained with an Ab against MPO. Counting was performed using ImageJ software blindly at original magnification ×20 in 10 fields in the infarct area. Results represent the means ± SEM of 10 P2Y4+/- and 10 P2Y4-/- hearts. Scale bar, 50 µm. *p < 0.05. (C–F) Quantification of MPO, ICAM-1, E-selectin, and VCAM-1 in the hearts of P2Y4+/- and P2Y4-/- mice 24 h after MI. Quantitative RT-PCR experiments were performed using specific primers of mouse MPO, ICAM-1, E-selectin, and VCAM-1. cDNA was obtained from RNA extracted from the ischemic area or remote region of wild-type and P2Y4 knockout mice injected or not with ET-1 (1 nmol/kg). PCR data were normalized for each gene to RPL13 and HPRT housekeeping genes. *p < 0.05, ***p < 0.001.
FIGURE 4. Impaired fibrosis in P2Y4-null ischemic mice. (A) Typical hearts of P2Y4+/- and P2Y4-/- mice 30 d after MI. Frozen sections (7 μm) of whole hearts of P2Y4+/- and P2Y4-/- mice were stained with Masson trichrome. Images were taken at original magnification ×2.5 (whole hearts) and original magnification ×20. (B and C) Quantification of fibrosis in P2Y4+/- and P2Y4-/- hearts 14 and 30 d after MI. Fibrosis expressed as collagen staining in blue was quantified by color image analyzer ImageJ in whole hearts (B) and in remote (noninfarcted) and infarcted areas (C) 14 and 30 d after ligation. Results are expressed as the ratio between collagen area (stained in blue) and the total area. Results represent the means ± SEM of five P2Y4+/- mice and five P2Y4-/- mice. *p < 0.05, **p < 0.01. (D–G) Quantification of MMP-8, MMP-9, TIMP-1, and TIMP-4 in the hearts of P2Y4+/- and P2Y4-/- mice 24 h after MI. Quantitative RT-PCR experiments were performed using specific primers of mouse MMP-8, MMP-9, TIMP-1, and TIMP-4. cDNA was obtained from RNA extracted from the ischemic area or remote region of wild-type and P2Y4 knockout mice. PCR data were normalized for each gene to RPL13 and HPRT housekeeping genes. *p < 0.05, **p < 0.01. (H and I) Vessel quantification in P2Y4+/- and P2Y4-/- hearts 30 d after MI. CD31 staining was quantified by color image analyzer ImageJ in remote and infarcted areas 30 d after ligation. Results represent the means ± SEM of 8 P2Y4+/- mice and 8 P2Y4-/- mice. Scale bars, 50 μm. ***p < 0.001. (J and K) Quantification of VEGF in heart and serum of P2Y4+/- and P2Y4-/- ischemic mice. Heart cDNA and sera of P2Y4+/- and P2Y4-/- mice were collected 24 h after LAD ligation for quantitative PCR analysis (J) or ELISA measurements (K) of VEGF. (Figure legend continues)
Neutrophil infiltration in the peritoneal cavity of LPS-injected P2Y4 represent the means later, neutrophils harvested after peritoneal lavage were counted for ET-1 expression in the heart of P2Y4 mice. Defective neutrophil accumulation linked to a reduced ET-1 increase was observed in LPS-injected P2Y4-null mice and was restored by ET-1 injection. Administration of the ETA antagonist BQ-123 had an inhibitory effect on neutrophil level in peritoneal cavity, confirming that ET-1 is markedly involved in neutrophil trafficking. Lower ET-1 level in P2Y4-null mice is thus compatible with a protection against infarction. Reduced levels of ET-1, which are mainly produced by endothelial cells (32), could be explained by the defective cardiac angiogenesis observed in P2Y4 wild-type heart observed in the present study.

Identification of ET-1 as a P2Y4 target gene led us to study in vivo neutrophil trafficking in P2Y4-null mice. Indeed, ET-1 is known to increase vascular permeability and neutrophil adhesion on cardiac endothelial cells through increased expression of adhesion molecules such as ICAM-1 and E-selectin (5). Exposure to LPS is associated with changes in vascular reactivity and elevated plasma levels of ET-1 (33). A defective neutrophil accumulation linked to a reduced ET-1 increase was observed in LPS-injected P2Y4-null mice and was restored by ET-1 injection. Administration of the ETA antagonist BQ-123 had an inhibitory effect on neutrophil level in peritoneal cavity, confirming that ET-1 is markedly involved in neutrophil trafficking (5). Nucleotides and especially ATP were previously described as major mediators of inflammation. Chen et al. (34) described that ATP release and autocrine feedback through P2Y2 and A2 receptors provide signal amplification, controlling gradient sensing and migration of neutrophils. In the present study, we observed a defect in neutrophil infiltration in the P2Y4-null mice compared with P2Y4 wild-type mice in all tested models, that is, LAD ligation, LPS-induced inflammation, and allylamine-induced fibrosis models. These data define P2Y4 as a major regulator of neutrophil recruitment in inflammatory and ischemic conditions. Reduced neutrophil infiltration was correlated with reduced endothelial adhesion molecule expression in P2Y4-null compared with P2Y4 wild-type ischemic heart. Defective expression of ET-1 and endothelial adhesion molecules, which play a pivotal role in neutrophil infiltration, appears to be the consequence of reduced angiogenesis observed in P2Y4-null mice.

Cardiac fibrosis induced by ischemia was strongly reduced in P2Y4-null mice compared with P2Y4 wild-type mice. The magnitude of that reduction was larger than the decrease in infarct size and is likely to be the consequence of the decreased infiltration of neutrophils and reduced expression of members of the MMP/TIMP system such as MMP-9 and neutrophil collagenase, MMP-8. MMPs and TIMPs play major roles in cardiac ischemia through their action in extracellular matrix remodelling (27). MMP-8 contributes to the generation of chemotactic tripeptide of the extracellular matrix Pro-Gly-Pro that binds CXCR2 and mediates neutrophil migration (27). MMP-9, also named gelatinase B, is an important player in cardiovascular diseases such as atherosclerosis, stroke, and heart failure (35). Interestingly, regulation of MMPs such as MMP-9 by ET-1 has previously been reported (36). The initial increase in TIMP-1 and TIMP-4 mRNAs observed in wild-type mice at 24 h after MI was previously reported and decreased in the ischemic myocardium after 1 wk (27). Lower levels of TIMPs in P2Y4-null mice could contribute to the observed reduced fibrosis. However, even if reduced MMP-9 and MMP-8 levels in P2Y4-null mice are compatible with reduction of ET-1 and neutrophil infiltration, the link with lower fibrosis quantified after several weeks is complex and depends on MMP/TIMP time courses during ischemia experiments.

The fact that there was no significant beneficial effect on EF in P2Y4-null ischemic mice may suggest a contractile adaptation to attention on ET-1, a gene regulated in P2Y4-null ischemic and infarmed hearts. We confirmed by quantitative PCR and ELISA that ET-1 level was decreased in P2Y4 hearts compared with P2Y4 hearts in both LAD ligation and LPS-induced inflammation models. Interestingly, this protein displays effects on inflammation and vasoconstriction (29), particularly during myocardial infarction. ET-1 plays an important role in myocyte hypertrophy after myocardial infarction (30, 31). Studies have shown that plasma ET-1 levels are elevated in the coronary circulation during ischemia and that the coronary artery is very sensitive to ET-1

Results represent the means ± SEM of eight mice. *p < 0.05, **p < 0.01. (L and M) Quantification of cardiac fibrosis and neutrophil infiltration in allylamine-induced fibrosis model. Fibrosis was quantified by ImageJ. Results are expressed as the ratio between collagen area and the total area (D). MPO+ neutrophils were blindly counted at original magnification ×20 in 10 fields on transversal sections in the hearts of P2Y4 mice and P2Y4 mice (E). Results represent the means ± SEM of 6 P2Y4 mice and 6 P2Y4 mice. *p < 0.05, **p < 0.01, ***p < 0.001.
preserve cardiac output. The echocardiography experiments performed in is on the utilization of wild-type mice. These data suggest an attenuated remodeling in P2Y4-null mice that is compatible with the reduction of inflammation, permeability, and fibrosis observed in these mice.

ET-1 can also enhance vascular permeability in selected rat vascular beds, including the pulmonary, renal, and mesenteric circulation (37). It appeared that LPS-induced cardiac microvascular hyperpermeability, measured after van’s blue injection, was reduced in P2Y4-null mice compared with wild-type mice. Moreover, injection of ET-1 could restore vascular hyperpermeability in the coronary circulation of P2Y4-null mice to a level comparable to that of wild-type mice. As for neutrophil infiltration, BQ-123 administration had an inhibitory effect, confirming ETA receptor involvement in the observed permeability increase. The reduced cardiac permeability observed in P2Y4-null mice appears thus to be the consequence of lower ET-1 expression.

These data suggest an attenuated remodeling in P2Y4-null mice deficient in this nucleotide receptor suggests a therapeutic potential for P2Y4 antagonists to limit the early ischemia observed in mice deficient in pp60Src show a normal angiogenic response to VEGF, but no VEGF-mediated permeability response, and they consequently develop minimal infarcts. In a similar way, the P2Y4 receptor appears to be able to regulate both cardiac angiogenesis (23) and permeability.

The present study defines P2Y4 as an important regulator of cardiac inflammation and fibrosis in response to ischemia. Our data identify ET-1, a protein involved in cardiac ischemia and associated in chronic heart failure, as P2Y4 target genes in the heart. The protection against ischemia observed in mice deficient in this nucleotide receptor suggests a therapeutic potential for P2Y4 antagonists to limit the early damage of myocardial infarction.

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Disclosures

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References


