Introduction

Atrial fibrillation is the most common sustained arrhythmia. Since atrial fibrillation is known to increase the risk of stroke, dementia, and heart failure (1), better understanding of the pathophysiology of atrial fibrillation is desired. Atrial remodeling, characterized by shortening of action potential duration and fibrosis, is considered an important factor for the initiation and progression of atrial fibrillation (2).

Accumulation of recent findings strongly suggests that atrial inflammation plays a pivotal role in atrial remodeling. This hypothesis is supported by the observations that inflammatory cytokines (IL-6 and C-reactive protein) were elevated in patients with atrial fibrillation and that anti-inflammatory treatment reduced the incidence of atrial fibrillation (3–6). Recent studies have indicated that macrophages infiltrate the atrium in atrial fibrillation (7, 8). However, little is known about the role of macrophages in atrial remodeling or the mechanism of macrophage infiltration of the atrium in patients with atrial fibrillation.

Atrial fibrillation is a multifactorial disease, and several risk factors have been identified. Among them, hypertension is a well-known risk factor (9, 10), and enlargement of left atrial diameter is one of the most consistently observed clinical features in atrial fibrilla-

Stretch of Atrial Myocytes Stimulates Recruitment of Macrophages via ATP Released Through Gap-Junction Channels

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Abstract. Atrial inflammation is critical to atrial fibrillation initiation and progression. Although left atrial dilatation is a risk factor for atrial fibrillation, the mechanism linking atrial dilatation and inflammation is unclear. We evaluated the mechanisms underlying infiltration of macrophages induced by stretch of atrial myocytes. Murine macrophages were co-cultured with HL-1 murine atrial myocyte–derived cells. Mechanical stretch applied to atrial myocytes induced transwell macrophage migration. The gap junction–channel blocker carbenoxolone and the non-specific ATP-signaling modifiers apyrase and pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate inhibited the enhanced migration. Mechanical stretch of atrial myocytes induced transient increase in extracellular ATP concentration, which was inhibited by carbenoxolone. siRNA knockdown of panxxin-2 inhibited ATP release and macrophage migration. Mice underwent transverse aortic constriction or sham procedure. Transverse aortic constriction procedure induced macrophage infiltration. Daily carbenoxolone administration significantly inhibited macrophage infiltration in the atrium. Thus, mechanical stretch of atrial myocytes induces macrophage migration by ATP released through gap-junction channels, at least in part, in vitro. Administering a gap junction family–channel blocker inhibited this inflammatory change in vivo.

Keywords: atrial fibrillation, inflammation, macrophage, ATP, gap-junction channel
tion (11). These findings indicate that mechanical stretch induces atrial remodeling. Although a number of studies have reported that mechanical stretch induced atrial fibrosis (12), the causative relationship between mechanical stretch and inflammation in the atrium has not been fully elucidated.

ATP is well known to act as a transmitter substance at autonomic neuromuscular junctions in addition to serving as a ubiquitous intracellular source of energy. Recently, extracellular ATP has been highlighted as an intercellular signal transduction factor (13). Notably, gap junction–family channels, including pannexin and connexin, have been considered conduits of ATP release from the cytosol to the extracellular space (14, 15). In red blood cells and cardiomyocytes, ATP is released by deformation of cell shape and/or mechanical stretch via gap junction–family channels (16–18). A recent paper also demonstrated that apoptotic cells recruited macrophages via extracellular ATP released as a “find-me” signal through pannexin-1 (19).

Thus, in the present study we aimed to examine whether mechanical stretch induced atrial inflammation and to elucidate the underlying mechanism. In particular, we focused on the infiltration of macrophages, the major effector cells of organ inflammation. Our data indicated that the stretch of atrial myocytes induces macrophage recruitment and extracellular ATP released through gap junction–family channels plays a role, at least in part, in vitro. A gap-junctional channel inhibitor, carbenoxolone, inhibits macrophage recruitment in vivo.

Materials and Methods

Cell culture

HL-1, a mouse atrial myocyte cell line, was kindly provided by Dr. Claycomb (University of Louisiana, New Orleans, LA, USA) (20). HL-1 cells were seeded on dishes or chambers coated with gelatin/fibronectin. Cells were cultured in Claycomb medium (SAFC, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, 0.1 mM norepinephrine, and 2 mM L-glutamine. For migration assays, HL-1 cells were seeded on silicone chambers (STREX, Osaka). J774.1, a murine macrophage cell line, was cultured in RPMI-1640 medium (Life Technologies, Carlsbad, CA, USA) supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin.

Lentivirus shRNA vector construction, gene transduction, and RT-PCR

shRNA constructs and lentivirus-based gene knockdown in cell lines were described in a previous report (21). In brief, the sense and antisense oligonucleotides (Fw: TGCTGTTTCACAAGGATCTGCTCGGTAAA GGCCACTGACTGACGGAGCAGACTGTTTGA GAA, Rv: CCTTGTCTCACAAGGATCTGCTCCGTCAA GTCACTGGCCAAAACGGAGCAGAATCTGTTTGA AGAAC) were annealed for knockdown of pannexin-2 (NM_001002005) using the Block-iT GATEWAY system (Life Technologies), followed by construction of lentiviral vectors. After 4 weeks of viral infection and selection by blasticidin, over 90% of cells demonstrated stable gene transduction and were used for subsequent analysis.

Murine mRNA was extracted from cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol, followed by reverse transcription using the High Capacity cDNA Reverse Transcription Kit (Life Technologies). For detection of pannexins, the following primer sets were used: CGAGATTGGGACCTAAGAGACGG (Fw) and GTGGGCA GGATTCCATACACTTTT (Rv) for pannexin-1 and TT GTTTGAGCACAAGTGTTCTGCCC (Fw) and TGCTC TTGCAGGAAGATGAAGGTT (Rv) for pannexin-2.

Transwell migration assay

Figure 1 is a schematic figure showing how HL-1 cells and macrophages were cultured and how stretch was applied only to HL-1 cells. HL-1 cells were cultured on a flexible silicon chamber. Macrophages were seeded onto a modified rigid Boyden chamber (8-μm pore size; Millicell, Millipore, Billerica, MA, USA). The handmade spacer was put on the edge of the culture dish, and the Boyden chamber with macrophages was set on the spacer, preventing direct contact between the membrane filter and the silicone chamber. Cyclic stretch (20% extension, 1 Hz) was applied to the silicone chambers for 24 h using a Cell Strain ST-140 instrument (STREX), so that stretch was applied only to HL-1 cells, but not to macrophages. After co-culture with or without stretch, the membrane filter of the Boyden chamber was mounted on the slide glass. Nuclei and cell membranes were stained with Hoechst 33342 (Dojindo Laboratories, Kumamoto) and PKH26 (Sigma-Aldrich, St. Louis, MO, USA), respectively, and then observed under a confocal microscope (LSM 510; Zeiss, Oberkochen, Germany). Cells in the upper and lower chambers were counted in randomly selected fields using Image J software (NIH, Bethesda, MD, USA). Migration index was calculated by the following formula:

Migration index (%) = cells in lower chamber / (cells in upper + lower chambers) × 100
Extracellular ATP measurement

HL-1 cells were seeded in silicone chambers (32 × 32 mm) and cultured to 90% confluence. Culture medium was exchanged for 2 mL (2 mm depth) of Hanks' balanced salt solutions (HBSS) 1.5 h prior to the experiment, and cells were kept free from mechanical stimulation to stabilize ATP concentrations. Chambers were then stretched to 20% extension, and stretched status was maintained for 20 min. Extracellular buffer was carefully collected and flash frozen with liquid nitrogen. The concentration of ATP in the samples was measured using the ENLITEN ATP assay kit (Promega, Madison, WI, USA).

ATP chemoattractant assay

Chemoattractant assay was performed using the slide chamber (μ-Slide VI-flow through; Ibidi, Verona, WI, USA). Macrophages were harvested from one chamber, and various concentrations of ATP were applied to the other reservoir. After 37-h incubation, the migration of macrophages was observed using a light microscope (CK2; Olympus, Tokyo). Migration of macrophages was expressed as distance moved from the initial position.

Mice, electrocardiography, ultrasound echocardiography, and transverse aortic constriction surgery

All animal experiments were approved and performed under the regulations of the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University. C57BL/6j mice were purchased from CLEA Japan, Tokyo. Transverse aortic constriction operation was performed on 8 – 10-week-old male mice. Surface electrocardiogram (ECG) was recorded under anesthesia with 0.5% – 1.2% isoflurane mixed with 100% oxygen. Mice that demonstrated abnormal ECG were excluded from the study. Ultrasound echocardiography was performed with the Vevo770 Imaging system using a 30MHz probe (Visual Sonics, Tronto, Canada) under anesthesia. LV contractility and dimension were evaluated in short axis view at the level of the papillary muscles.

For transverse aortic constriction surgery, mice were anesthetized by intraperitoneal (ip) injection of chloral hydrate, the transverse aorta was constricted with a 7-0 suture using a blunted 27-gauge needle, and the needle was removed after ligation. After aortic constriction, the chest was closed with a 4-0 silk suture, and mice were recovered from anesthesia. The surgeon kept unaware of the study group. Mice were sacrificed 10 days after transverse aortic constriction or sham procedure for histological observations. In subgroups of mice, carbenoxolone or vehicle was administered daily from 3 days prior to transverse aortic constriction surgery until the day of sacrifice.

Immunohistochemistry

For immunohistochemistry, hearts were immersed in OCT compound (Sakura Finetek, Tokyo), followed by flash freezing in liquid nitrogen. After cryosection at 10-μm thickness, immunohistochemistry was performed with the antibody for F4/80 at 1:100 dilution (ab-6640; Abcam, Cambridge, UK). The number of macrophages was counted in each slice and summarized for quantitative evaluation.

Statistical analyses

All data are shown in terms of mean ± S.E.M. values. The Mann-Whitney U-test was performed for compari-
son of continuous variables. Statistical analyses were performed with Statview (version 5). $P < 0.05$ was considered statistically significant.

**Results**

**Migration of macrophages induced by mechanical stretch of myocytes**

We first examined if stretch of cardiomyocytes stimulated recruitment of macrophages in vitro. We performed a migration assay using a custom-made co-culture system under mechanical stretch. We applied cyclic stretch only to atrial myocytes and evaluated transwell migration of macrophages in a modified Boyden chamber. Migration index (%) was calculated as the ratio of cells in the lower chamber to the total number of cells in the lower and upper chambers (Fig. 2A). Mechanical stretch significantly increased migration of macrophages compared to co-culture without stretch (19.7% ± 1.2% vs. 11.0% ± 2.1%, $P < 0.01$; Fig. 2B). Cyclic stretch on silicon chambers without myocytes resulted in no change in macrophage migration (data not shown). This finding suggests that some humoral factors secreted from stretched HL-1 cells stimulate macrophage recruitment, which is supported by our experiments with conditioned medium from stretched HL-1 cells (data not shown).

Since several reports demonstrate the role of ATP in macrophage recruitment, we applied non-specific ATP signal modifiers to the co-culture medium: apyrase that degradates ATP and ADP, and pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonate (PPADS), a non-selective P2 receptor antagonist. Administration of these agents inhibited migration of macrophages (Fig. 2B). We confirmed that apyrase or PPADS had no effects on macrophage migration in the absence of stretch of HL-1 cells: migration index was 7.7% ± 1.2% for the control ($n = 5$), 13.4% ± 2.1% for apyrase ($n = 5$, ns vs. control) and 10.7% ± 1.6% for PPADS ($n = 5$, ns vs. control). We then measured extracellular ATP concentration under mechanical stretch of myocytes (Fig. 2C). In this experiment, we applied 20% extension of silicon chambers containing HL-1 cells and maintained the stretched status for 20 min. Extracellular ATP concentration was 4.0 ± 1.0 nM at baseline. Serial measurements of ATP concentration revealed transient increase of ATP, with its peak at 3 min after starting stretch (23.7 ± 6.7 nM, $P < 0.001$ vs. control). The control group, which underwent serial sampling without stretch, showed no significant change in ATP concentration.

To confirm whether extracellular ATP itself worked as a chemoattractant for macrophages, we performed the migration assay with serial concentrations of ATP. Macrophages migrated toward the reservoir containing ATP (Fig. 3). When the concentration of ATP was increased up to 100 μM, migration of macrophages increased in a dose-dependent fashion. Higher concentration of ATP (1 mM), however, induced cell death rather than migration.

**Gap-junction hemichannels as a conduit of ATP in vitro**

We further investigated the conduit of ATP. Given that several findings indicated gap junction–family chan-
nels as a conduit of ATP release through the cell membrane (14 – 18), we applied a non-selective gap junction channel–pore blocker, carbenoxolone, to the culture medium. Administration of carbenoxolone reduced migration of macrophages in a dose-dependent manner (20 μM, 13.8% ± 2.0% and 100 μM, 9.2% ± 1.5%, P < 0.05 and P < 0.001 vs. stretched condition, respectively; Fig. 4A). Carbenoxolone had no effects on macrophage migration in the absence of stretch of HL-1 cells: migration index was 7.7% ± 1.2% for the control (n = 5) and 10.0% ± 0.9% for carbenoxolone (n = 5, ns vs. control).

Carbenoxolone (20 μM) also significantly suppressed the transient increase of extracellular ATP (3.1 ± 0.4 nM, P < 0.001 vs. stretched condition; Fig. 4B).

We then evaluated the expression of gap junction–family channels in HL-1 cells. As previously reported, HL-1 cells expressed Cx40, 43, and 45 (data not shown). Regarding pannexins, HL-1 cells expressed pannexin-2 but not pannexin-1 (Fig. 4C). Since recent data have indicated members of the pannexin family as candidates for conduit of ATP release (16 – 19), we knocked down pannexin-2 using siRNA. Stable knockdown of pannexin-2 in atrial myocytes attenuated both migration of macrophages and transient ATP release (Fig. 4: D, E). siRNA for pannexin-2 had no effects on macrophage migration in the absence of stretch of HL-1 cells: the migration index was 7.7% ± 1.2% for the control (n = 5), and 9.5% ± 1.1% for pannexin-2 siRNA (n = 5, ns vs. control). These findings indicated that gap junction–family channels, including pannexin-2, were crucial to the mechanism of recruitment of macrophages under mechanical stretch in vitro.

Infiltration of macrophages in stretched atrium induced by transverse aortic constriction

We next examined a gap junction–channel blocker, carbenoxolone, in macrophage infiltration into the atrium.

Fig. 3. Migration assay with extracellular ATP. Migration distance was calculated with various ATP concentrations in reservoirs (n = 4 in each experiment). *P < 0.05 (vs. 0 nM).

Fig. 4. Migration of macrophages and gap junction–channel expression. A) Migration index in control, stretch, and stretch with carbenoxolone administration conditions (n = 5 – 20 independent experiments). B) Extracellular ATP measurement under stretch with or without carbenoxolone (n = 6 and 13, respectively). C) Expression of pannexin mRNA in HL-1. PCR results from cloned cDNA are shown as positive controls. D) Migration index in control, stretch, and stretch of HL-1 cells subjected to pannexin-2 knockdown (n = 5 – 20 independent experiments). E) Extracellular ATP measurement under stretch with HL-1 or pannexin-2 knockdown HL-1 cells. *P < 0.05, **P < 0.01, ***P < 0.001 (vs. stretched condition). CBX, carbenoxolone; panx1, pannexin-1; panx2, pannexin-2.
in vivo using a mouse model of pressure overload with transverse aortic constriction. Transverse aortic constriction induced an increase of atrium/body weight ratio at 10 days after operation as reported previously (Fig. 5A) (23). Macrophages were rarely detected in sham-operated mouse atrium. By contrast, transverse aortic constriction induced significant infiltration of macrophages into the atrium (Fig. 5B). To elucidate the involvement of gap junction–family channels in this mechanism, we applied 50 nmol/g carbenoxolone daily from 3 days before transverse aortic constriction until the day of sacrifice. Administration of carbenoxolone at this dose did not affect PR interval or QRS duration (Fig. 5C). Fractional shortening assessed by echocardiography also exhibited no difference between before and after acute administration of carbenoxolone (38.8% ± 2.2% vs. 38.2% ± 2.3%, \( P = \text{ns} \)).

Administration of carbenoxolone significantly reduced the infiltration of macrophages (432 ± 54 vs. 288 ± 53 cells/mm² in vehicle and carbenoxolone groups, respectively, \( P < 0.05 \); Fig. 5: B, D).

**Discussion**

To the best of our knowledge, this study is the first to demonstrate that mechanical stretch on myocytes induced recruitment of macrophages. Our data indicate that extracellular ATP, at least in part, plays a role in this mechanism in vitro. ATP was released through gap-junction hemichannels, possibly pannexin-2 but not pannexin-1, in HL-1 cells. In vivo, carbenoxolone, a gap junction–channel blocker, significantly attenuated infiltration of macrophages in pressure-overloaded mouse atrium.

Since atrial dilatation is one of the risk factors for atrial fibrillation (11), mechanical stretch is considered a facilitating factor of atrial remodeling. To date, several mechanisms have been proposed to explain atrial remodeling under stretch. The involvement of the renin–angiotensin

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**Fig. 5.** Infiltration of macrophages in transverse aortic constriction–operated mouse atrium. A) Atrium/body weight ratio (left) and heart/body weight ratio (right) in sham- and transverse aortic constriction–operated mice (n = 20 for each group). B) Immunohistochemistry of mouse atrium 10 days after transverse aortic constriction surgery with administration of vehicle (left) or 50 nmol/g carbenoxolone (right panel). Brown spots indicate F4/80-positive cells. Scale bar represents 100 μm. C) Electrocardiographic parameters on the day of the transverse aortic constriction procedure. Administration of carbenoxolone (up to 50 nmol/g) did not change the PR interval or QRS duration. D) Number of infiltrated macrophages in transverse aortic constriction–treated atria with administration of vehicle or carbenoxolone (n = 13 and 16, respectively). \(* P < 0.05, **P < 0.001, \text{CBX}, \text{carbenoxolone.} \)
system and TGFβ signaling has been extensively investigated (12, 24). A recent study reported that mast cells played a role in atrial inflammation and fibrosis through PDGF signaling using a similar animal model (23). Our present results have added stretch-induced macrophage infiltration as a novel factor in the initial mechanism of atrial remodeling. Since macrophages are the major source of cytokines to induce inflammation and fibrosis, we speculate that infiltration of macrophages is one of the initial steps of atrial remodeling.

Neonatal rat ventricular myocytes were recently reported to release nucleotides including ATP and UDP in response to mechanical stretch, which stimulated Gα12/13 signaling in an autocrine fashion (17). Our study also revealed that a gap-junction hemichannel is a major conduit of ATP after mechanical stretch of atrial myocytes. An important difference between these 2 studies was the subtypes of pannexin involved. In contrast to the predominant expression of pannexin-1 in ventricular myocytes (17, 25), we showed that the atrial myocyte cell line HL-1 expressed pannexin-2 but not pannexin-1. The differential expression of pannexin isoforms may explain the different response to mechanical stretch between ventricle and atrium. Pannexin-2, therefore, could be a potential future therapeutic target specific for the pathogenesis of atrial diseases including atrial fibrillation.

A recent report showed that extracellular ATP was a major factor to recruit macrophages in apoptotic cells (19). Thus, we focused on the role of extracellular ATP in our experimental settings. Extracellular ATP indeed plays a role, at least in part, in vitro. We found that knockdown of pannexin-2 suppressed ATP release and migration of macrophages. Pannexin-2 knockdown almost completely inhibited ATP release, whereas its inhibition of macrophage migration was partial compared to 100 μM carbenoxolone. This discrepancy suggests that carbenoxolone also inhibited a mechanism involved in macrophage recruitment other than ATP release. As shown in a previous study using ventricular myocytes (17), other nucleotides in addition to ATP may be released from stretched atrial myocytes. Thus, although we did not evaluate the contribution of other nucleotides, the possible release of nucleotides other than ATP via a different pathway from that involving pannexin-2 may be an alternative and additive explanation. Since apyrase degrades ADP and PPADS is a non-specific inhibitor of purine receptors, including the ADP-response of purine receptors, ADP may be a candidate, which should be addressed in the future studies. An effect on hemichannels in macrophages is another possibility because pannexin-1 is expressed in macrophages, and increased cell permeability through pannexin-1 is important in inflammatory response to mechanical stretch, up to 24 nM. On the other hand, we needed 10 μM to induce migration of macrophages. We assume this discrepancy in ATP concentration is due to technical limitation. We performed sampling of ATP around 2-mm away from the HL-1 cell surface. This is needed because damage of the cells and agitation of the buffer and resultant shear stress on HL-1 cells are significant inducers of ATP release. Thus, the measured ATP concentration should be much lower than that on the cell surface. On the other hand, in the ATP chemoattractant assay we added ATP solution 25-mm distant from macrophages in the slide chamber to make the ATP concentration gradient. Thus, the concentrations at the macrophage cell surface were expected to be much lower. These different experimental conditions may explain the discrepancy.

We demonstrated in vivo that carbenoxolone, a non-specific gap junction–channel blocker, inhibited atrial inflammation in a pressure overload mouse model. Carbenoxolone blocks gap junction–family channels including pannexins and connexins non-selectively, thus we cannot identify the channel that mainly contributed to the recruitment of macrophages in mice with transverse aortic constriction operation. Several previous papers proposed that connexin hemichannels act to release ATP and other nucleotides (27 – 31). In normal hearts, connexins form gap junctions predominantly at intercalated disks, and the proportion of hemichannels is quite low. However, pressure overload induced lateralization of connexins, resulting in increased expression of connexin hemichannels (32 – 34). Since connexin hemichannels are considered mechanosensitive (35), lateralized connexin hemichannels may have some role in ATP release in addition to pannexin-2 in pathological conditions such as hypertension and congestive heart failure, which...
should be examined in the future.

A recent paper showed that overexpression of connexin43 in atrial tissue prevents atrial fibrillation in the porcine burst-pacing model (36). Another group also performed gene transfer of connexins (connexin40 or connexin43) using a similar animal model (37). They described that overexpression of connexins recovered conduction velocity in their atrial fibrillation model, contributing to the termination of atrial fibrillation (37). In our study, carbenoxolone that inhibits pannexins at low concentrations and connexins at high concentrations attenuated macrophage infiltration to the atrium, while in their study connexin overexpression attenuated atrial fibrillation. Thus, connexin and pannexin may play a distinct role in atrial fibrillation. In addition, Igarashi et al. observed that their atrial fibrillation model showed atrial inflammation and translocation of connexins to make hemichannels. Overexpression of connexins restored localization of connexin in the intercalated disk from translocation in the lateral side, while it did not reduce atrial inflammation. This finding suggests that connexins may have no significant effects on atrial inflammation. Furthermore, in our experiments, carbenoxolone up to 50 nmol/g did not prolong PR interval or QRS duration, which may suggest minor effects of carbenoxolone on connexin in our experimental conditions. Nevertheless, to clarify the role of connexins and pannexins in development of atrial fibrillation in vivo, experiments using connexin and pannexin knock-out mice are necessary.

**Study limitations**

We observed macrophage infiltration of the atrium in pressure-overloaded mice. Since carbenoxolone, which blocks gap-junction channels and macrophage mobilization, also inhibited fibrosis and arrhythmogenicity, we speculated that infiltration of macrophages caused fibrosis and arrhythmogenicity at least in part. However, to clarify the causative relationships among macrophage infiltration, we need further studies to show the time course of and relationship among these changes.

Macrophages are divided into M1 (classically activated) and M2 (alternatively activated) types, which have differential roles in inflammation. Although macrophages have been demonstrated to infiltrate into fibrillated atrium, the role and relative contribution of M1 and M2 macrophages in atrial fibrillation are totally unknown and were not addressed in this study.

In this study, we assessed acute effects, but not chronic effects, of carbenoxolone on hemodynamics. In our study and the study by others (38), acute application of carbenoxolone did not affect fractional shortening obtained with ultrasound echocardiography or contraction of cardiomyocytes, while carbenoxolone was reported to increase systolic, mean, and diastolic blood pressure (39). Thus, if carbenoxolone has any effects on the hemodynamics, there may be an underestimation of its effects on macrophage recruitment.

HL-1 cells exhibit spontaneous beating, which could potential trigger pannexin-2 opening and ATP release. The concentration of ATP in the medium was 4.0 ± 1.0 nM without stretch, which could be ATP released through pannexin-2 during spontaneous beating. However, ATP was not decreased by application of carbenoxolone or siRNA against pannexin-2. Thus, the effect of ATP release through spontaneous beating HL-1 cells, if any, should be minimal for our analysis.

**Conclusions**

Gap junction–family channels play a critical role to recruit macrophages in stretched atrial myocytes. Extracellular ATP may be one of the significant factors involved in this mechanism in vitro. Administration of a gap junction family–channel blocker inhibited the infiltration of macrophages in vivo.

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