



Cisatracurium induces mast cell activation and pseudo-allergic reactions via MRGPRX2

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ABSTRACT

Background: Pseudo-allergic reactions occur when patients receive muscle relaxants during perioperative anesthesia. These reactions may result in a serious threat to the patient's life, particularly to a child's life. Cisatracurium, a relatively new NMBA, has resulted in bronchospasms and cardiovascular collapse. However, the mechanisms underlying the anaphylactoid reactions caused by cisatracurium have not been fully elucidated. **Methods:** In the present study, the MRGPRX2-related pseudo-allergic reactions induced by cisatracurium were investigated using hindpaw swelling and extravasation assays in vivo and mast cell degranulation assays.

Results: Cisatracurium caused anaphylactoid reactions in wild-type mice. However, cisatracurium did not induce a similar phenomenon in KitW-sh/W-sh mice. Furthermore, mast cell-related G protein-coupled receptor B2-knockout mice did not display an inflammatory response upon treatment with cisatracurium. Cisatracurium induced LAD2 cell degranulation, leading to the dose-dependent release of β -hexosaminidase, histamine and TNF- α . However, cisatracurium only induced the release of low levels of these mediator LAD2 cells transfected with MRGPRX2 siRNA. Cisatracurium also stimulated intracellular Ca²⁺ influx in MRGPRX2-HEK293 cells compared with that in NC-HEK293 cells. Interestingly, cytokine release was not observed in LAD2 cells even with high dose of cisatracurium.

Conclusions: Cisatracurium activated MRGPRX2 and triggered mast cell degranulation, leading to anaphylactoid reactions. Therefore, strategies targeting MRGPRX2 might potentially block cisatracurium-induced pseudo-allergic reactions.

1. Introduction

Mast cell activation and systemic histamine release are major side effects of muscle relaxants [1]. Anaphylactic reactions to anesthetics and associated agents used during the perioperative period have been reported with increasing frequency in most developed countries [2]. Neuromuscular blocking agents (NMBAs) have been reported to account for the majority of anaphylactic reactions that occur during perioperative period [3]. Therefore, approximately of perioperative hypersensitivity reactions are believed to be pseudo-allergic (anaphylactoid) reactions. Quaternary and tertiary ammonium (QA) ions are thought to be the key structures in NMBAs, and opiates [4], and environmental chemicals also contain QA which is responsible for anaphylactoid reaction [5,6]. This shared structure might partially explain

the cross-sensitization between different NMBAs observed in the majority of the patients allergic to NMBAs, and a history of previous exposure to NMBAs is only found in < 50% of patients who present an adverse reaction to NMBAs during anesthesia [7,8].

Cisatracurium is a stereoisomer of atracurium and was initially characterized as having low histamine-releasing effects and excellent cardiovascular stability. Cisatracurium does not induce apparent histamine release upon a bolus administration and causes fewer allergic-like reactions than other NMBAs [9]. However, severe anaphylactic reactions to cisatracurium resulting in bronchospasms and cardiovascular collapse have been reported worldwide [10–13]. Clarification of the molecular mechanism of cisatracurium-induced anaphylactoid reactions is urgently needed.

According to a recent study by McNeil and colleagues, human

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MRGPRX2 is homologous to MRGPRB2 in mice, and these receptors are targets of many small-molecule drugs associated with pseudo-allergic or anaphylactoid reactions [14]. As reported in the study by Katherine Lansu et al., opioids trigger anaphylactoid reactions via the MRGPRX2 receptor [15]. Recently, Zhang et al. revealed that some antifungal agents, aminoglycosides, and sulfonamides also activate mast cells via MRGPRX2 [16]. Thus, MRGPRX2 is a target receptor that interacts with many types of drugs with different chemical structures. Substances activate MRGPRX2, subsequently inducing Ca^{2+} influx, mast cell degranulation, and allergic mediator release. Drugs that often induce anaphylaxis in clinical practice [17], such as atracurium [18], cause allergic-like reactions through the MRGPRX2-induced activation of mast cells.

In the present study, mouse hindpaw swelling and extravasation, intracellular Ca^{2+} mobilization, β -hexosaminidase, histamine, and de novo synthesized chemokine release assays were performed to investigate whether cisatracurium induces allergic-like reactions and to clarify the mechanism by which cisatracurium induces allergy-like reactions through MRGPRX2 activation in mast cells.

2. Materials and methods

2.1. Drugs and reagents

Compound 48/80 was purchased from Sigma-Aldrich (St. Louis, USA). Cisatracurium was obtained from Meilunbio Co., Ltd. (Dalian, China) and was purified to $\geq 98\%$. Fluo-3, AM ester and Pluronic F-127 were obtained from Biotium (California, USA). TM buffer was prepared on the day of experiments (6.954 g/L NaCl, 0.353 g/L KCl, 0.282 g/L CaCl_2 , 0.143 g/L MgSO_4 , 0.162 g/L KH_2PO_4 , 2.383 g/L HEPES, 0.991 g/L glucose and 1 g/L BSA, pH 7). The reagents *p*-nitrophenyl *N*-acetyl- β -D-glucosamide and Triton X-100 were obtained from Sigma-Aldrich Co., LLC (Shanghai, China) and were prepared at the proper concentrations before use. Stop buffer consisted of 0.1 M sodium carbonate and sodium bicarbonate, pH 11. All aqueous solutions were prepared using ultrapure water produced by a MK-459 Millipore Milli-Q Plus ultrapure water system.

2.2. Mouse model

Mice were purchased from the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China). Adult male mice weighing 18–22 g were included in the study. Mrgprb2 knockout mice were provided by the Xinzhong Dong Laboratory at Johns Hopkins University. STOCK Kit^{W-sh}/HN1hrJaeBsmJNju mice were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). All mice were housed in the Experimental Animal Center of Xi'an Jiaotong University. The mice were housed in individual cages in a large colony room with free access to water and were fed a diet of standard dry food twice per day. The breeding environment was set to a temperature of 20–25 °C, a relative humidity of 40% and a day-night cycle of 12/12 h. All experiments involving equal treatments in animals were conducted by experimenters who were blinded to the conditions.

2.3. Ethics statement

This study was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. The experimental protocols in which mice were used were approved by the Animal Ethics Committee at Xi'an Jiaotong University, Xi'an, China (Permit Number: XJTU 2011-0045). All the animals were anesthetized with pentobarbital sodium.

2.4. Cell lines

Laboratory of Allergic Disease 2 (LAD2) human mast cells were kindly provided by A. Kirshenbaum and D. Metcalfe (NIH, USA). Cells were maintained in StemPro-34 medium supplemented with 10 mL/L of StemPro nutrient supplement, 1:100 penicillin: streptomycin, 2 mmol/L L-glutamine and 100 ng/mL human stem cell factor in a 5% CO_2 atmosphere at 37 °C. The culture medium was replaced every 2 weeks, and cells were maintained at a density of 2×10^6 cells/mL.

2.5. Peritoneal mast cell purification

Adult male mice aged 2–3 months were sacrificed through CO_2 inhalation. Twelve milliliters of ice-cold mast cell dissociation media (15 mL of MCDM, 1.5 mL of $10 \times$ HBSS, 450 μL of fetal bovine serum, 150 μL of 1 mol/L HEPES, and 12.9 mL of sterile water, pH 7.2) was used for two to three sequential peritoneal lavages, in which MCDM and cells were mixed and then centrifuged at 200g for 10 min at 4 °C. Cells from each mouse were resuspended in 2 mL of MCDM, layered over 4 mL of an isotonic 70% Percoll suspension (2.8 mL of Percoll, 320 μL of $10 \times$ HBSS, 40 μL of 1 M HEPES, and 830 μL of MCDM), and centrifuged at 500g for 20 min at 4 °C. Mast cells were then collected from the pellet. Cells were transferred to a new centrifuge tube and diluted with a two-fold excess of MCDM relative to the volume of the suspension. This solution was mixed thoroughly and then centrifuged at 200g for 10 min at 4 °C. Mast cells were collected as a pellet, and the purity was $> 95\%$, as assayed by morphology. Mast cells were resuspended in DMEM supplemented with 0.05% recombinant mouse stem cell factor (mSCF) and seeded into 96-well plates.

2.6. Hindpaw swelling and extravasation

Adult male mice weighing 18–22 g were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). Fifteen minutes after the induction of anesthesia, each mouse was intravenously (i.v.) injected with 0.2 mL of 0.4% Evans blue in saline. Vernier calipers were used to measure the thickness of the paw before the injection. After 5 min., 5 μL of the test drug was administered by a micro-injector in the left paw, and saline was administered in the right paw as a negative control. Fifteen minutes later, paw thickness was measured again and recorded. The mice were then sacrificed by decapitation, and a photo of each paw was taken. Paw tissues were collected, dried for 24 h at 50 °C, and weighed. The Evans blue dye (EBD) was extracted by incubating the tissues in 500 μL of acetone-saline (7:3) at 37 °C for 24 h. Tissues were cut into pieces and placed in an ultrasonic machine for 10 min, followed by centrifugation at 3000 rpm for 20 min. The supernatant was equally distributed into 200- μL aliquots into 96-well cell culture plates, and the OD was read at 620 nm using a spectrophotometer. For studies using drugs, the mice were injected with 5 μL of a 30 $\mu\text{g}/\text{mL}$ solution of compound 48/80 as a positive control.

2.7. β -Hexosaminidase assay

This assay was performed in a 96-well plate. LAD2 cells, maintained at a density of 2×10^5 cells/well, were incubated overnight at 37 °C in a 5% CO_2 atmosphere. The cell density of each well was strictly monitored and maintained. Following the incubation, the culture medium was removed and drugs were added at the indicated concentrations. The cells were then incubated for 30 min at 37 °C in a 5% CO_2 atmosphere. The 96-well plate was then centrifuged. Negative control cells were lysed with 0.1% Triton X-100 in TM buffer to analyze the total β -hexosaminidase content. The amounts of β -hexosaminidase released into the supernatants and in the cell lysates were quantified by monitoring the hydrolysis of *p*-nitrophenyl *N*-acetyl- β -D-glucosamide in 0.1 M citric acid/sodium citrate buffer (pH 4.5) for 90 min at 37 °C. The reaction was stopped by the addition of stop buffer. The percentage of

β -hexosaminidase release was assessed by determining the absorbance of the samples at 405 nm using a microplate spectrophotometer. The cells treated with 30 μ g/mL compound 48/80 were used as positive controls.

2.8. Histamine release assay

LAD2 cells were maintained at a density of 1×10^6 cells/well and incubated overnight at 37°C in a 5% CO₂ atmosphere. The cells were counted to normalize each sample. Histamine (HA) was purchased from Sigma, HA 2-HCl (A, A, B, B-D4, 98%) was obtained from Cambridge Isotope Laboratories, Inc. (MA, USA), and HPLC-grade methanol and acetonitrile were purchased from Thermo Fisher Scientific (Pittsburgh, USA). Mass spectrometry grade formic acid was obtained from Sigma. An LCMS 8040 mass spectrometer (Shimadzu Corporation, Kyoto, Japan) was used for the applied LC-ESI-MS/MS method. Histamine concentrations were evaluated using a system employing an HILIC column (Venusil HILIC, 2.1 mm $\times$ 150 mm, 3 μ m, Agela Technologies, Tianjin, China) and isocratic elution with acetonitrile: water containing 0.1% formic acid and 20 mM ammonium formate (77:23, v/v) at a flow rate of 0.3 mL/min.

2.9. Chemokine release assay

LAD2 cells were plated in 96-well plates at a density of 1×10^6 cells per well and incubated at 37°C in a 5% CO₂ atmosphere overnight. The culture medium was then removed, drugs were added at the indicated concentrations, and the cells were incubated for 12 h at 37°C in a 5% CO₂ atmosphere. The Human Chemokine Array Kit was purchased from R&D Systems China Co., Ltd. (Shanghai, China), and the Human TNF- α ELISA kits were purchased from Tiangen Biotech (Beijing) Co., Ltd. (Beijing, China). The protocols were strictly conducted according to the manufacturer's instructions.

2.10. Intracellular Ca²⁺ mobilization assay

All the drugs were diluted to the required concentrations in calcium imaging buffer (CIB; 125 mM NaCl, 3 mM KCl, 2.5 mM CaCl₂, 0.6 mM MgCl₂, 10 mM HEPES, 20 mM glucose, 1.2 mM NaHCO₃, and 20 mM sucrose, brought to pH 7.4 with NaOH). The incubation buffer consisted of 0.5 μ L of Fluo-3, 2 μ L of Pluronic F-127 and 997.5 μ L of CIB. MRGPRX2-HEK293 cells or NC-HEK293 cells were plated at 1×10^4 cells per well in a 96-well tissue culture plate and incubated overnight at 37°C. Cells were then washed twice with CIB followed by the addition of the required incubation buffer. After the 30 min incubation at 37°C, Cells were again washed twice with CIB and used immediately for imaging. For calcium imaging, cells were imaged at 200 $\times$ magnification and a rate of one photo per second under blue light. The cells were described as responding to the treatment if [Ca²⁺] increased by at least 50% after the substances were injected.

Mouse peritoneal mast cells were plated in 96-well plates at a density of 1×10^4 cells per well in DMEM with 0.05% recombinant mouse stem cell factor (SCF) and incubated for 2 h to measure mast cell responses. After the drugs were added to the wells, the fluorescence changes in the cells were monitored under a fluorescence microscope using the method described for the HEK293 cell calcium influx.

2.11. Transfection of LAD2 cells with siRNAs

MRGPRX2 knockdown was achieved using specific small interfering (si) RNAs; a control siRNA was used as a control. Smart pools of double-stranded siRNAs that targeted MRGPRX2, as well as nonspecific siRNAs, were obtained from Shanghai GenePharma Co., Ltd. The siRNA sequences were as follows: forward, 5'-GUACAACAGUGAAUGGAAATT-3' and reverse, 5'-UUUCCAUUCACUGUUGUACATT-3' for MRGPRX2; and forward, 5'-UUCUCCGACGUGUCACGUTT-3' and reverse,

5'-ACGUGACACGUUCGGAGAATT-3' for the control. For transfection, siRNAs were delivered at a final concentration of 80 nM using Lipofectamine® 2000 reagent, according to the manufacturer's instructions. The cells were incubated for 48 h to allow MRGPRX2 knockdown. These cells were then used for the β -hexosaminidase assay and histamine release assay.

2.12. Determination of the half-maximal effective concentration (EC₅₀)

Human MRGPRX2-expressing HEK293 cells were plated in a 96-well plate at a density of 2×10^4 cells per well and incubated overnight at 37°C in a 5% CO₂ atmosphere. The FLIPR Calcium 5 Assay Kit was purchased from Molecular Devices, LLC (Sunnyvale, California, USA), and the EC₅₀ value was analyzed using FlexStation 3.

2.13. Experimental repetition and statistical analysis

All in vitro and in vivo experiments were repeated at least 3 times. Data are expressed as mean $\pm$ S.E.M. and analyzed using two-tailed unpaired student's *t*-test. An independent sample variance analysis was used to determine the significance of differences in statistical comparisons using SPSS. Differences were considered significant at **p* < 0.05, ***p* < 0.01, and ****p* < 0.005. Multiple doses were calibrated by Bonferroni's test. Differences were considered significant at **p* < 0.0125, ***p* < 0.0025, and ****p* < 0.00125.

3. Results

3.1. Cisatracurium induces mast cell degranulation in a dose-dependent manner

Cisatracurium caused human mast cell (LAD2) degranulation in vitro, leading to the dose-dependent release of β -hexosaminidase and histamine (Fig. 1A and B). Cisatracurium was applied at doses of 100, 200, or 400 μ g/mL, TM buffer was used as a negative control, and 30 μ g/mL C48/80 was used as a positive control. The rate of β -hexosaminidase release caused by 100, 200, 400 μ g/mL cisatracurium were $13.23 \pm 1.12\%$, $28.51 \pm 2.43\%$, and $56.34 \pm 2.14\%$, respectively, and the amounts of histamine released were 42.23 ± 5.73 , 87.54 ± 9.14 , and 148.25 ± 7.36 ng/mL, respectively. Cisatracurium also induced TNF- α release with a dose-dependent. The release amount of TNF- α was 69.34 ± 4.32 , 234.83 ± 12.58 , 322.53 ± 15.63 ng/mL, respectively (Fig. 1C). HEK293 cells overexpressing MRGPRX2 were used to determine the EC₅₀ of the tested drug. The EC₅₀ value of cisatracurium was 103.0 ± 15.34 μ g/mL (Fig. 1D). Cisatracurium substantially activated the overexpressing HEK293 cells and increased the intracellular calcium ion (Ca²⁺) concentrations.

3.2. Cisatracurium induces a local inflammatory reaction via MRGPRB2

In this study, 5 μ L different concentration of cisatracurium were administered by a microinjector in the left paw and saline was administered in the right paw as a negative control. The results showed that paw swelling and EBD exudation in the drug-treated STOCK Kit^{W-sh}/HNhrJaeBsmJNju mice were similar to those in the negative control (*p* > 0.05) (Fig. 2A). Cisatracurium did not induce a pseudo-allergic reaction in the mast cell-deficient mice, therefore, we concluded that mast cell activation was critical for the cisatracurium-induced pseudo-allergic reaction.

According to the results of subsequent in vivo analyses, higher degrees of swelling and EBD exudation were observed in the left paws of the wild-type (WT) mice injected with the drug than those in the right paws, which were injected with a physiological saline solution, and these effects were directly proportional to the drug concentrations (Fig. 2B). Additionally, the 0.4 mg/mL cisatracurium dose induced higher degrees of swelling and EBD exudation than the C48/80. Based

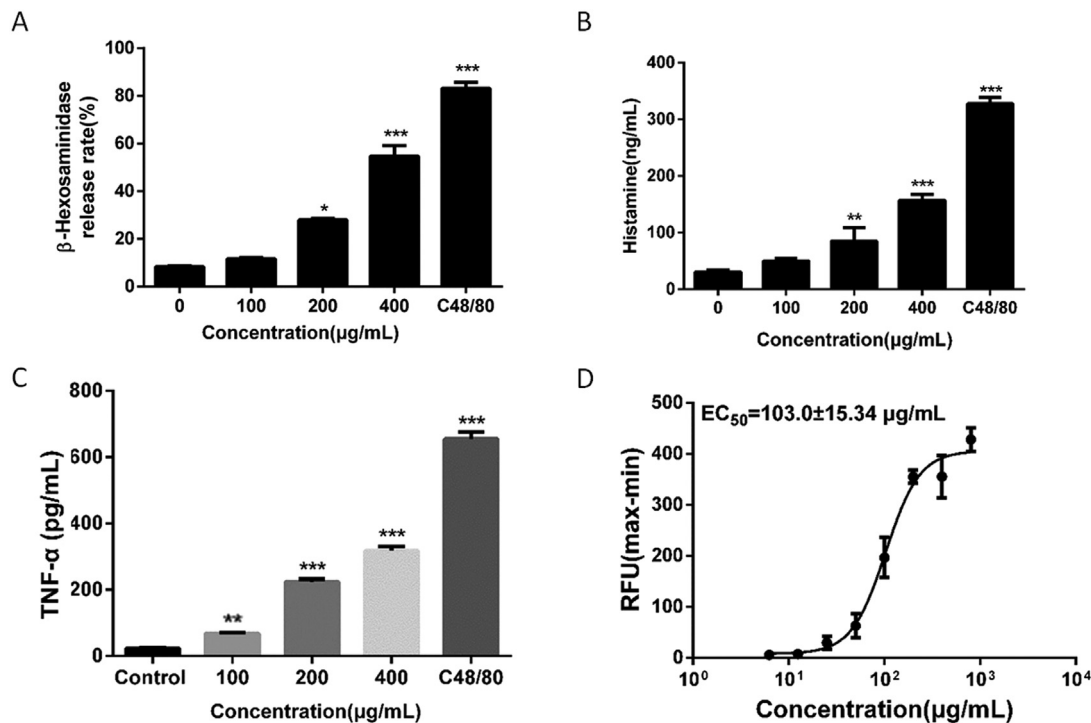


Fig. 1. Cisatracurium induces LAD2 mast cell degranulation. (The dose of cisatracurium was set as 100, 200, 400 μg/mL and TM buffer was set as negative control and 30 μg/mL C48/80 as positive control.). (A) β-Hexosaminidase release from LAD2 cells treated with different concentrations of cisatracurium. (B) Histamine release from LAD2 cells treated with different concentrations of cisatracurium. (C) TNF-α release on LAD2 cells treated with serial concentration of cisatracurium. (D) The EC₅₀ value of cisatracurium. Data are expressed as mean ± S.E.M. and analyzed using two-tailed unpaired student's *t*-test and Bonferroni's test. Differences were considered significant at **p* < 0.0125, ***p* < 0.0025, and ****p* < 0.00125.

on these findings, cisatracurium induced local inflammatory reactions in the WT mice. Since the human *MRGPRX2* gene is homologous to the mouse *Mrgprb2* gene, the WT and *Mrgprb2*-knockout mice were compared to further elucidate the target of drug-induced allergic reactions. The paws of the knockout mice injected with this drug did not swell and showed no obvious EBD exudation or local inflammatory reactions (Fig. 3A). The primary peritoneal mast cells from the WT and *Mrgprb2*-

knockout mice were also treated with the drug. Cisatracurium elevated intracellular Ca²⁺ concentrations in > 80% of the primary peritoneal mast cells from the WT mice, whereas approximately 10% of the primary peritoneal mast cells from the *Mrgprb2*-knockout mice showed elevated Ca²⁺ levels. The differences between the WT and the *Mrgprb2*-knockout mice were significant (Fig. 3B). Thus, cisatracurium interacts with MRGPRX2 and subsequently triggers a mast cell response that

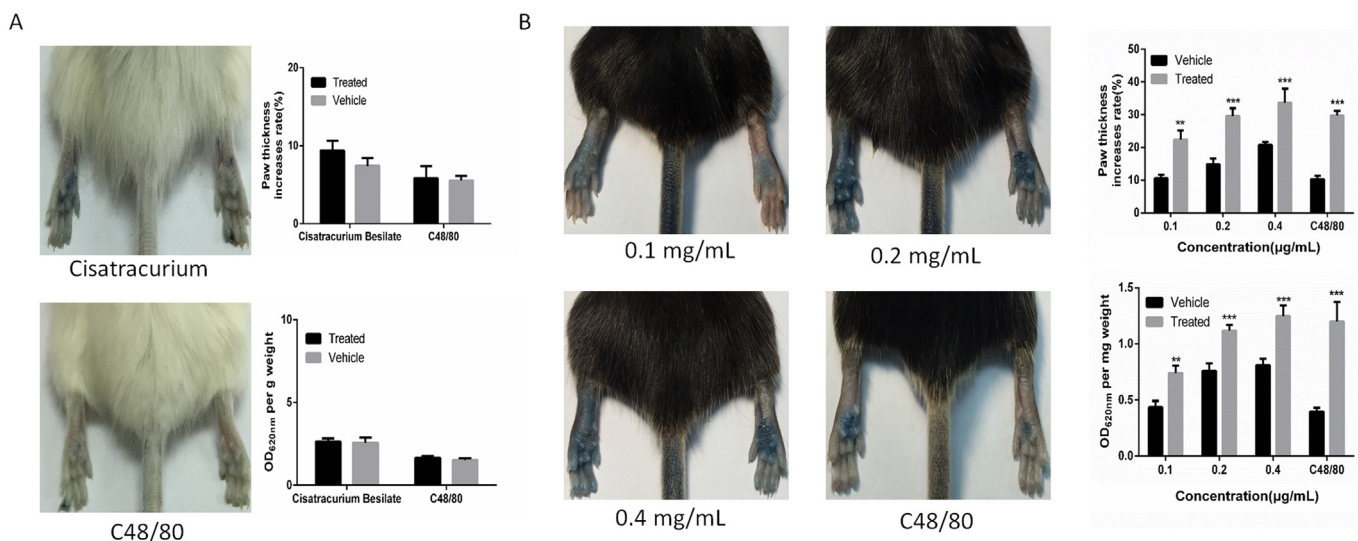


Fig. 2. Cisatracurium increases anaphylactoid reactions in mice (*n* = 6 mice per group and experiments were repeated > 3 times; data are presented as the mean ± s.e.m., two-tailed unpaired Student's *t*-test). 0.1, 0.2, 0.4 mg/mL of cisatracurium were administered by a microinjector in the left paw and saline was administered in the right paw as a negative control. The histograms showed quantification of Evans blue leakage into the paw and increase in paw thickness after 15 min. (A) Cisatracurium did not increase anaphylactoid reactions in the Kit^{W-sh/W-sh} mice. (B) Cisatracurium induced pseudo-allergic reactions in the C57 WT mice. Differences were considered significant at **p* < 0.05, ***p* < 0.01, and ****p* < 0.005. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

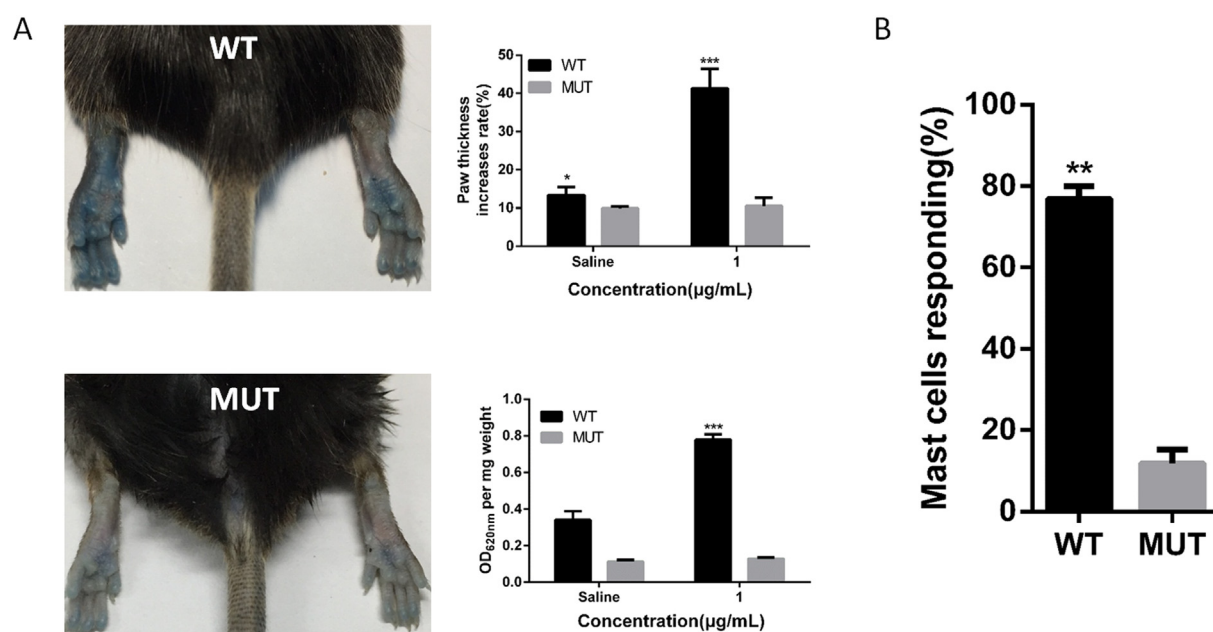


Fig. 3. MRGPRB2 mediates mast cell responsiveness and anaphylactoid reactions upon cisatracurium administration ($n = 6$ per genotype, experiments were repeated > 3 times; data are presented as the mean $\pm$ s.e.m., two-tailed unpaired Student's t -test). Wild-type (WT) C57/BL6 mice and *Mrgprb2*^{-/-} (MUT) mice were injected into 0.4 mg/mL of cisatracurium in the left paw and saline was administered in the right paw as a negative control. The histograms showed quantification of Evans blue leakage into the paw and paw thickness increase. (A) Difference between WT and MUT mice in Evans blue leakage and paw thickness increase. (B) Percentage of responding cells from the WT and *Mrgprb2*^{-/-} (MUT) peritoneal mast cells after drug application, as assayed using Fluo-3 imaging. Differences were considered significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

produces pseudo-allergic reactions.

3.3. Cisatracurium induces pre-synthesis inflammatory modulator release

Chemokine release is a quite dangerous situation for patients, and it may induce delayed allergic reactions. We analyzed chemokine release using the ELISA spot technique, and the results showed that cisatracurium induced degranulation in vitro. However, the cisatracurium-induced chemokine release was similar to the negative control ($p > 0.05$) (Fig. 4A), with the exception of TNF- α release (Fig. 1C). Chemokines, such as IL-8, MCP-1 and MIP-1d, were not released in response to cisatracurium. Cisatracurium might induce the release of pre-synthesis inflammatory modulators, such as histamine and TNF- α , in the perioperative period.

3.4. MRGPRX2 mediates cisatracurium-induced mast cell activation

Cisatracurium (100 μ g/mL) increased the intracellular Ca^{2+} concentrations in the HEK293 cells overexpressing MRGPRX2 but did not alter the intracellular Ca^{2+} concentrations in the HEK293 cells transfected with an empty plasmid (Fig. 5A).

The cells were transfected with siRNAs to investigate the target of cisatracurium. MRGPRX2 was down-regulated in LAD2 human mast cells transfected with a siRNA, LAD2 cells transfected with non-functional siRNA were used as a control. The results of western blot (Fig. 5B) and RT-PCR (Fig. 5C) verified the knockdown MRGPRX2 was completed. And the rate of β -hexosaminidase release and the amount of histamine released by LAD2 mast cells were determined 30 min after drug administration (Fig. 5D and E). At identical drug concentrations, lower amounts of β -hexosaminidase and histamine were released by the MRGPRX2-knockdown LAD2 cells than those released by the NC-LAD2 cells, with a significant decrease in drug-induced degranulation.

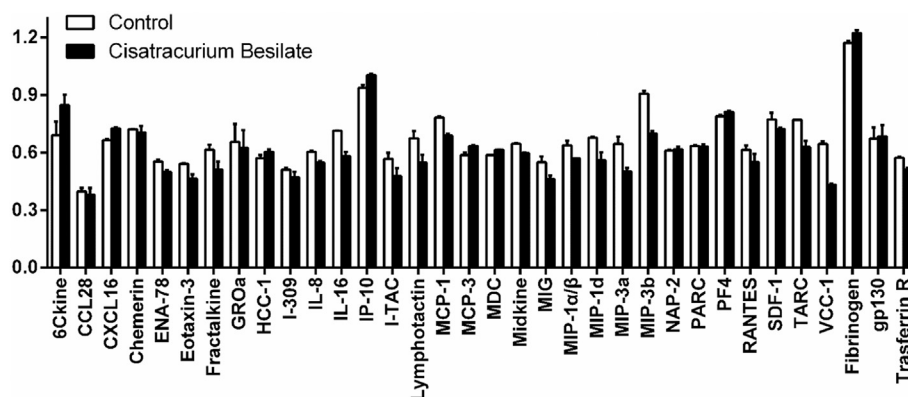


Fig. 4. Chemokine release by LAD2 cells treated with cisatracurium.

A significant difference was not observed between the experimental group and the control group.

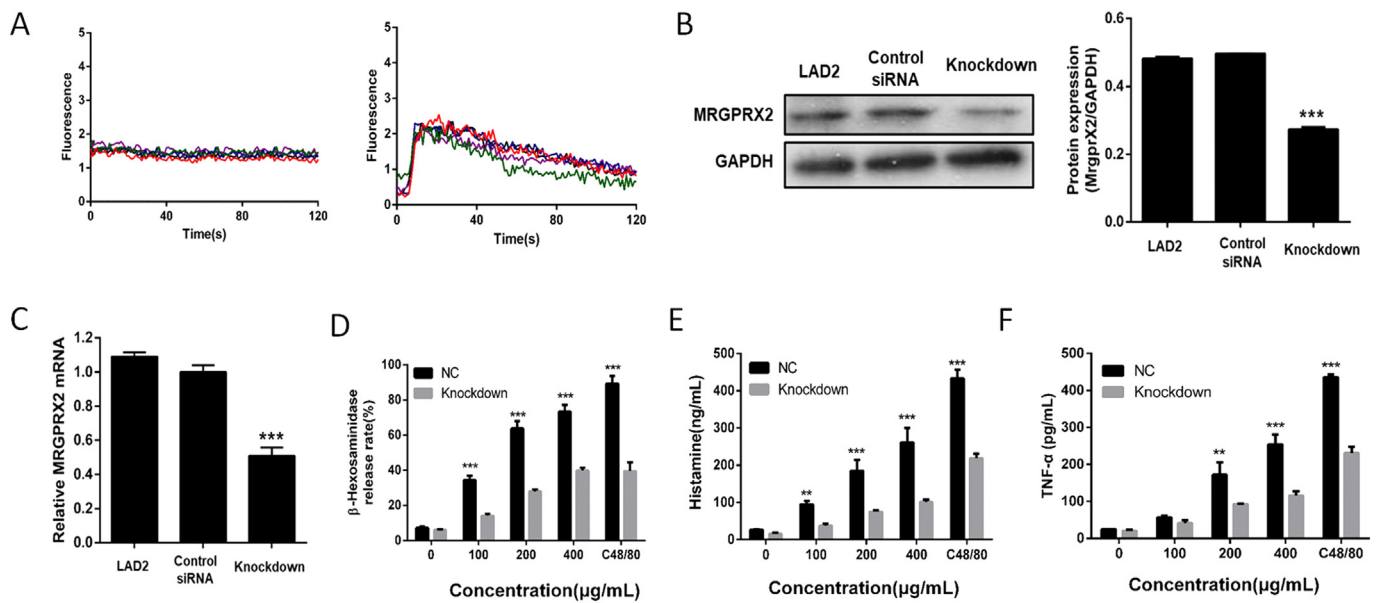


Fig. 5. Cisatracurium induces mast cell degranulation via MRGPRX2. (A) Cisatracurium did not increase intracellular Ca^{2+} concentrations in NC-HEK293 cells but increased intracellular Ca^{2+} concentrations in cells overexpressing MRGPRX2. Cisatracurium was added at 5 s and each trace was a response from a unique cell. (B–C) western blot and RT-PCR verified protein and mRNA level of MRGPRX2 (D–E) β -Hexosaminidase and histamine release from NC-LAD2 and MRGPRX2 knockdown LAD2 cells treated with cisatracurium. (F) TNF- α release from LAD2 and MRGPRX2 knockdown mast cells treated with cisatracurium. Differences were considered significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$.

Furthermore, cisatracurium induced significantly higher amounts of pre-synthesis TNF- α release from the LAD2 cells than those released from the MRGPRX2-knockdown LAD2 cells (Fig. 5F). Based on these results, cisatracurium induces degranulation via MRGPRX2.

4. Discussion

The mortality of allergic reactions during anesthesia is estimated to be 6%, and between 1/10000 and 1/1250 anesthetized patients die from this type of reaction [19,20]. Consequently, NMBA are responsible for more than two-thirds of anaphylactic or anaphylactoid reactions and for 80% of cases of anaphylactic shock in patients under anesthesia [21]. Cisatracurium has been widely used in clinics and replaced cis-trans-isomeratracurium because it provides a stronger intensity and longer duration effect [22]. Furthermore, as a non-depolarizing muscle relaxant, cisatracurium induces fewer allergic reactions than other NMBA [23]. Atracurium and rocuronium are all common NMBA and induced pseudo-allergic reactions via MRGPRX2. Our study showed that cisatracurium had a higher EC_{50} value than atracurium, and rocuronium [14]. And the concentration of cisatracurium in serum in clinical use is lower than its EC_{50} [9]. However, cisatracurium induced pseudo-allergic reactions is highly correctly with concentration in serum. And Hofmann rearrangement that may be the reason why the pseudo-allergic reaction in rare case cause in clinical usage. Even cisatracurium is safer than other NMBA, cisatracurium also induces pseudo-allergic reactions, which are not documented in the packaging insert for some brands of cisatracurium. Recent studies have revealed isolated instances of modestly increased histamine levels after cisatracurium administration, which might cause anaphylactic reactions after anesthesia. Some patients have developed unusually severe, life-threatening bronchospasms refractory to bronchodilators after the induction of anesthesia with thiopentone and cisatracurium. Since the mechanism by which NMBA induce allergic-like reactions is controversial, the reactions are usually classified as IgE- or non-IgE-mediated reactions [24]. Several diagnostic techniques for detecting NMBA-induced anaphylaxis have been developed based on IgE-mediated allergic reactions. However, NMBA-induced anaphylaxis via IgE-independent pathways has been neglected, although a large proportion of

these cases has been noted [25]. Recently, the G protein-coupled receptor MRGPRX2 has been identified as an NMBA-induced anaphylactoid reaction receptor. Locally injected cisatracurium does not induce pseudo-allergic reactions in mast cell-deficient mice (Fig. 2A). Furthermore, similar local inflammatory reactions were also diminished in *Mrgrprb2* KO mice (Fig. 3A). Thus, we surmise that cisatracurium induces pseudo-allergic reactions via the MRGPRB2 receptor in C57BL/6 mice. Since MRGPRB2 and MRGPRX2 are orthologues, cisatracurium also triggers LAD2 cell degranulation and pre-inflammatory mediator release via MRGPRX2. And a strange phenomenon appeared in this study, when the dose of cisatracurium injected in left paws, the right paws of mice also showed exudation of Evans blue dye and the exudation level was dose-dependent with cisatracurium. We suggested that large concentration of cisatracurium injected into paws caused pseudo-allergic reaction and induced hemangiectasis. So cisatracurium could get into the blood circulation and caused systemic anaphylaxis. So saline was injected into the right paws, Evans blue dye exudation showed a dose-dependent. Therefore, if a patient who overexpresses MRGPRX2 is administered a high dose of cisatracurium with a fast injection speed, an anaphylactoid reaction may be induced. Thus, cisatracurium might induce allergy-like reactions via MRGPRX2 in clinical settings. Fortunately, cisatracurium does not appear to induce chemokine release, which might cause severe harm to the patient. Pre-synthesis inflammatory mediators, such as histamine, only appeared after cisatracurium was administered in the perioperative period. Based on the findings of the present study, anesthesiologists should be more aware of the anaphylactoid reactions caused by histamine and only use cisatracurium in patients undergoing surgery who do not pose a risk of allergy in the postoperative recovery period. Cisatracurium-induced allergy-like reactions might be related to its quaternary ammonium structure and not to its spatial conformation. These results might offer a novel approach to the design of new types of muscle relaxants to avoid allergenic properties. Therefore, strategies targeting human MRGPRX2 might also offer a promising treatment to prevent muscle relaxant- and cisatracurium-induced anaphylactoid reactions.

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