

Original article

MRGPRX2 mediates immediate-type pseudo-allergic reactions induced by iodine-containing iohexol

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ABSTRACT

Background: Iohexol is a typical iodinated radiocontrast medium and widely used in clinical angiography. Hypersensitivity reactions induced by iohexol are common side effects known to increase the risk for patients. Iodine is the main functional group of iohexol, and it can induce delayed anaphylaxis. However, iohexol also induces immediate-type allergies, but the underlying mechanism is still not clear. MRGPRX2 is a key receptor present on mast cells, which mediates pseudo-allergic reactions induced by various drugs.

Methods: We aimed to verify the relationship between iohexol-induced anaphylactic reactions and MRGPRX2. MRGPRX2-mediated pseudo-allergic reactions induced by iohexol were investigated *in vivo* and *in vitro* using a mouse model of local and systemic anaphylaxis and mast cell degranulation assays, respectively.

Results: Iohexol caused pseudo-allergic reactions in wild-type (WT) mice by activating mast cells to release histamine and cytokines. However, it did not induce a similar phenomenon in Kit^{W-sh/W-sh} (MUT) mice. Iohexol stimulated intracellular calcium ion (Ca²⁺) influx in MRGPRX2-HEK293, MrgprB2-HEK293, and LAD2 cells but not in NC-HEK293 cells. After knockdown of MRGPRX2 expression in LAD2 cells, the degree of iohexol-induced degranulation was reduced. In addition, after structural modification of iohexol by removal of iodine, a reduction in iohexol-induced effects, such as local and systemic anaphylaxis in mice and degranulation of LAD2 cells, could be observed. Iohexol was shown to induce immediate-type pseudo-allergic reactions *via* MRGPRX2, which was dependent on the presence of iodine.

Conclusions: Conclusively, inhibition of MRGPRX2-mediated mast cell degranulation and cytokine release is important to prevent iohexol-induced immediate-type pseudo-allergic reactions.

1. Introduction

Iodinated radiocontrast media (RCM) are widely used in clinical angiography such as angiography of blood vessels, coronary arteries, and the urinary tract, or CT scans and myelograms [1]. Hypersensitivity reactions induced by RCM are common side effects that increase the risk for patients. RCM induce small intestinal edema [2,3] and cutaneous anaphylaxis after being injected into the human body [4]. Many studies have reported that RCM can induce delayed anaphylaxis [5], which occurred within an hour after infusion, and might be up to 7 days later while hypersensitivity reactions to iodinated contrast media are rare [6].

RCM can cause hypersensitivity reactions, either of the immediate-type within 5–10 min of RCM injection, or of the delayed-type, which become apparent more than an hour after RCM exposure [7].

RCM are composed of small, iodine-containing molecules that are not defined as antigens or haptens. In addition, most hypersensitivity reactions induced by RCM are of the immediate-type [8], leading to increased serum histamine levels over a short period of time, which return to normal within 10 min [9,10]. These characteristics suggest that RCM may induce pseudo-allergic reactions, which occur within a short period after the drugs have been administered, and are significantly dose-dependent [11,12]. These are referred to as

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non-immune-mediated allergic reactions with direct stimulation of mast cells (MCs) or basophils [13], leading to MC degranulation and anaphylactic reactions.

Mas-related G protein-coupled receptor member X2 (MRGPRX2) is a key receptor mediating drug-induced pseudo-allergic reactions. Iohexol is a non-ionic iodinated contrast medium that is widely used in clinical practice. Iohexol accounts for a large proportion of RCM-associated anaphylactic reactions [14]. Iodine is the key functional group of iohexol in clinical angiography, and it is deemed to be the main inducer of anaphylactic reactions. However, the mechanisms and cellular targets are still not clear in iohexol-induced immediate-type anaphylactic reactions.

To ensure the safe use of RCM, iohexol was chosen as a representative non-ionic iodinated contrast medium in this study, which was conducted to investigate whether iohexol could activate MCs and induce pseudo-allergic reactions via MRGPRX2. *In vivo* and *in vitro* experiments were performed to explore the mechanisms underlying the pseudo-allergic reactions induced by iohexol. In addition, we modified the structure of iohexol for the removal of iodine to prove the dependence on iodine in iohexol-induced immediate-type pseudo-allergic reactions.

2. Materials and methods

2.1. Drugs and reagents

Iohexol, Compound 48/80, p-nitrophenyl N-acetyl- β -D-glucosamide, Triton X-100 reagents and Pluronic F-127 were purchased from Sigma-Aldrich (St. Louis, USA). Fluo-3, AM ester was obtained from Biotium (California, USA). Tyrode's solution buffer was prepared fresh on the day of use (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). All aqueous solutions were prepared by using ultrapure water produced by an MK-459 Millipore Milli-Q Plus ultra-pure water system at the proper concentrations prior to use.

2.2. Mouse model

C57BL/6 STOCK Kit^{W-sh/W-sh} mice (MUT) were purchased from the Model Animal Research Center of Nanjing University. C57BL/6 mice (WT) aged 8 weeks were purchased from the Experimental Animal Center of Xi'an Jiaotong University. Mice were breeding in 20–25 °C, with a relative humidity of 40 % and a day-night cycle of 12/12 h, and housed in individual cages in a large colony room with free access to water and were fed a standard dry food twice per day. All animal experiments involving equal treatments were conducted by experimenters who were blinded to the conditions. The mice were randomly divided into the following groups (6 mice per group): control, compound 48/80, and the experimental group. All animals were under pentobarbital sodium anesthesia when undergoing any operation.

2.3. Ethics statement

This study was carried out 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 involving mice were approved by the Animal Ethics Committee at Xi'an Jiaotong University, Xi'an, China (Permit Number: XJTU 2019-711).

2.4. Synthesis procedure

100 mg of iohexol was dissolved in MeOH (5 mL). The solution was cooled to 0 °C under N_2 atmosphere. Then 10 mg of NaBH_4 was added. The mixture was allowed to react at this temperature with stirring for 18 h. the resulting solution was quenched by 1 mL HCl (1 N) and evaporated. The residue was loaded onto chromatography column and eluted by water-MeOH (0–50 %). The desired fraction was collected and

lyophilized to give the product (about 10 mg).

Other methods we tried:

- 1 React iohexol (100 mg), paraformaldehyde (5.0 mmol), $\text{Pd}(\text{OAc})_2$ (0.0005 mmol) and Cs_2CO_3 (7.0 mmol) in DMSO at 80 °C for 12 h. Pour the reaction mixture into H_2O . Extract the reaction mixture with Et_2O . The reaction was messy and no desired product was obtained.
- 2 A mixture of iohexol (100 mg), 10 % Pd/C (10 mg) in MeOH (10 mL) was hydrogenated under hydrogen atmosphere for 12 h. No product was detected and the starting material retained.
- 3 Add iohexol (1 mmol) to an excess amount of zinc powder (0.260 g, 4 mmol) in saturated aqueous ammonium chloride (4 mL) and THF (2 mL). Stir the mixture at room temperature. Separate the unreacted zinc after the disappearance of Zn powder (3 h). The reaction was messy and no desired product was obtained.

2.5. Hind paw swelling and extravasation

Male C57BL/6 mice, and C57BL/6 STOCK Kit^{W-sh/W-sh} mice were anesthetized with an intraperitoneal injection of 80 mg/kg 1 % pentobarbitalum natricum. Fifteen minutes after the induction of anesthesia, each mouse was injected intravenously (i.v.) with 0.2 mL of 0.4 % Evans blue dye in saline. A Vernier caliper was used to measure the thickness of the paw before any injection. Five minutes later, 5 μl of iohexol (75, 150, 300 mg/mL) and IOH-1 (300 mg/mL) were administered using a microinjector into the left paw, and saline only into the right paw as a blank control. Fifteen minutes later, the paw thickness was measured again and recorded. The mice were then sacrificed by CO_2 , and a photo of each paw was obtained. The paw tissues were collected, dried for 24 h at 50 °C, and weighed. Evans blue was extracted by a 12 h incubation in 500 μl acetone-saline (7:3) at 37 °C. Tissues were cut into pieces, placed for 10 min in an ultrasonic machine, centrifuged for 20 min at 3000 rpm. The supernatant was equally distributed in 200 μl aliquots into 96 well cell culture plates, and the optical density value was read at 620 nm using a spectrophotometer. For studies using drug substances, the mice were injected with 5 μl of 30 $\mu\text{g/mL}$ compound 48/80 as a positive control.

2.6. Analysis of mouse serum

Iohexol and IOH-1 (4, 8, 16 g/kg) were made up by saline and only saline was used as negative control. And after tail vein injection, blood was collect and centrifuged to get serum. Mouse histamine, TNF- α , MCP-1 and CXCL2 ELISA Kit was purchased from Sino CO., LTD. (Beijing, China) and all the steps were finished following the instructions strictly.

2.7. Histology

Skin samples were fixed in 4 % paraformaldehyde and embedded in paraffin. Tissue sections (5 mm) were stained with hematoxylin and eosin (H&E). Images were taken immediately using a confocal scanning laser microscope (Nikon, Tokyo, Japan).

2.8. Skin mast cell purification

Adult male C57BL/6 mice age 8 weeks were sacrificed through CO_2 inhalation. Skin specimens were treated by using an enzymatic procedure in IMDM containing 1.5 mg/mL collagenase, 0.75 mg/mL hyaluronidase (Sigma-Aldrich, St Louis, Mo), and 0.1 % BSA at 37 °C for 1 h. The cells from each mouse were isolated by magnetic beads separation. Anti-mouse CD117 and Anti-R-Phycoerythrin (PE) Magnetic (BD Biosciences, New York, USA) was used for isolation and purification mouse peritoneal mast cell. The mast cells in the pellet were retrieved, and their purity was > 95 % as assayed by morphology. The mast cells were then seeded into 96-well plates and used.

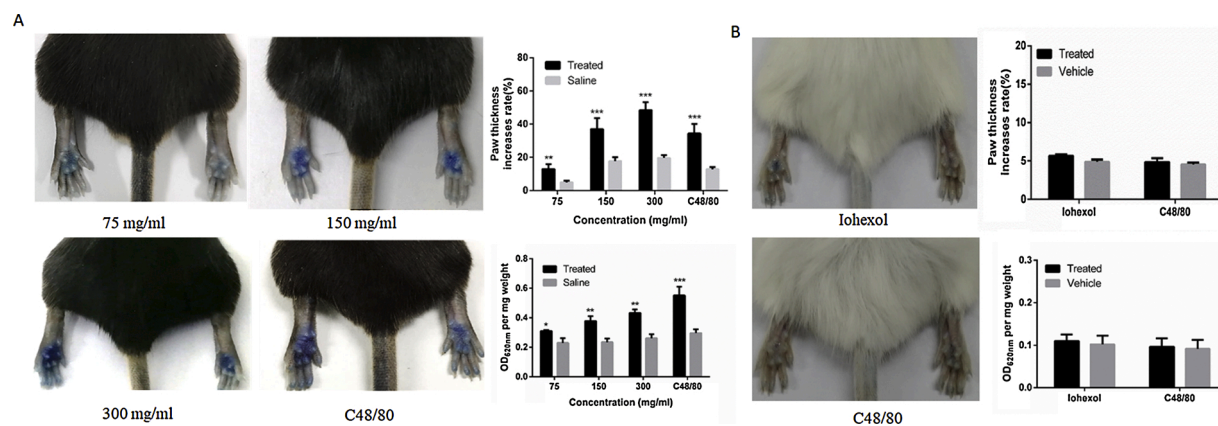


Fig. 1. Iohexol induced pseudo-allergic reactions in wild type C57 BL/6 mice via mast cells. (A) the quantification of paw thickness increase and EBD leakage after 15 min induced by iohexol. (B) iohexol did not induce pseudo-allergic reactions in MUT mice. (n = 6 per group; experiments were repeated 3 times. Data are expressed as mean $\pm$ S.E.M. and analyzed one-tail paired Student's *t*-test. Differences were considered significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$).

2.9. Cell lines

The Laboratory of Allergic Disease 2 (LAD2) human mast cells were kindly provided by NIH. The cells were maintained in StemPro-34 medium supplemented with 10 ml/l StemPro nutrient supplement, 1:100 penicillin-streptomycin, 2 mM L-glutamine and 100 ng/mL human stem cell factor in an atmosphere containing 5% CO₂ at 37 °C and the cells were maintained at a density of 2×10^6 cells/mL.

MrgprB2-expressing HEK293 and MRGPRX2-expressing HEK293 cells were constructed by HIV-1-based lentiviral vectors. And NC-HEK293 cells were transfection with blank lentiviral vectors. Both the cells cultured by DMEM with 10 %FBS and 1:100 penicillin-streptomycin in an atmosphere containing 5% CO₂ at 37 °C.

2.10. Intracellular calcium ion (Ca²⁺) mobilization assay

All drug substances 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, with the pH adjusted to 7.4 using 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, MrgprB2-HEK293 cells and NC-HEK293 cells were plated at 5×10^3 cells per well in a 96-well plate and incubated overnight at 37 °C with 5% CO₂. The cells were then washed twice in 100 μ l of CIB. Next, 100 μ l of incubation buffer was added, and the plate was incubated for 30 min at 37 °C with 5% CO₂. Then, the cells were again washed twice with CIB. The cells were used immediately for imaging. For the calcium imaging, the cells were magnified 200 times, and one photo per second was obtained under blue light. Cells were identified as responding if the [Ca²⁺]_i rose by at least 50 %, and each color line represents an individual cell.

MPMC were used after isolation, and LAD2 cells were incubated and washed in a 1.5 ml EP tube, then plated at 1×10^4 cells per well in a 96-well plate. The cells fluorescence changes were read by fluorescence microscope following the method described in HEK293 cell calcium influx.

2.11. Small interfering (si)RNA transfection of LAD2 cells

Specific knockdown was achieved using small interfering (si)RNAs targeting MRGPRX2, and non-targeting siRNAs as negative control (NC). The siRNA sequences were as follows: forward, 5'-GUACAACAGUGAAUGGAAATT-3' and reverse, 5'-UUUCCAUUCA CUGUUGUACTT-3' for MRGPRX2; and forward, 5'-UUCUCCGA ACGUGUCACGUTT-3' and reverse, 5'-ACGUGACACGUUCGGAGA ATT-3' for the control. For transfection, siRNA was delivered at a final

concentration of 80 nM using the Lipofectamine® 3000 reagent according to the manufacturer's instructions. The cells were incubated for 48 h to allow the knockdown of MRGPRX2. The efficiency of siRNA to knockdown MRGPRX2 expression was proved by western blot, RT-PCR [15] and Human Mas-related G-protein coupled receptor member X2, MRGPRX2 ELISA Kit (MyBioSource, LLC, San Diego, CA).

2.12. Mast cells release assay

1×10^6 LAD2 cells per well, were incubated in a 96-well plate overnight at 37 °C with 5% CO₂, while MPMC were used after isolation. The culture medium was removed, the iohexol and IOH-1 prepared by Tyrode solution (200, 400, 600 mg/mL) were added at the indicated concentrations, and the cells were incubated for 1 h for histamine, and β -hexosaminidase assay at 37 °C with 5% CO₂, while 12 h for TNF- α assay. 96-well plate was centrifuged at 200 g for 5 min at 4 °C and the supernatant was selected for assay. Human histamine, β -hexosaminidase assay ELISA Kit were purchased from Jianglai Industrial Limited By Share Ltd (Shanghai, China). Human TNF- α ELISA Kit was purchased from Sino Biological Inc (Beijing, China). All steps were performed strictly according to the manufacturers' instructions.

2.13. Statistical analysis

Experiments were repeated 3 times. Data are expressed as mean $\pm$ S.E.M. and analyzed one-tail paired Student's *t*-test. An independent samples analysis of variance was used to determine statistical significance in comparisons of the data using the SPSS software. 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. Iohexol induces pseudo-allergic reactions in mice via mast cells

Our *in vivo* results showed that iohexol induced local pseudo-allergic reactions in C57/BL6 wild-type (WT) mice. Additionally, the degree of swelling and EBD exudation in the left paws, which had been subjected to iohexol treatment, were higher than those observed in the right paws, which served as negative controls, and had been injected with a physiological saline solution. Furthermore, these effects were directly proportional to the iohexol concentration used (Fig. 1A). These experiments revealed that iohexol, a small molecule substance, induced local pseudo-allergic reactions in a dose-dependent manner.

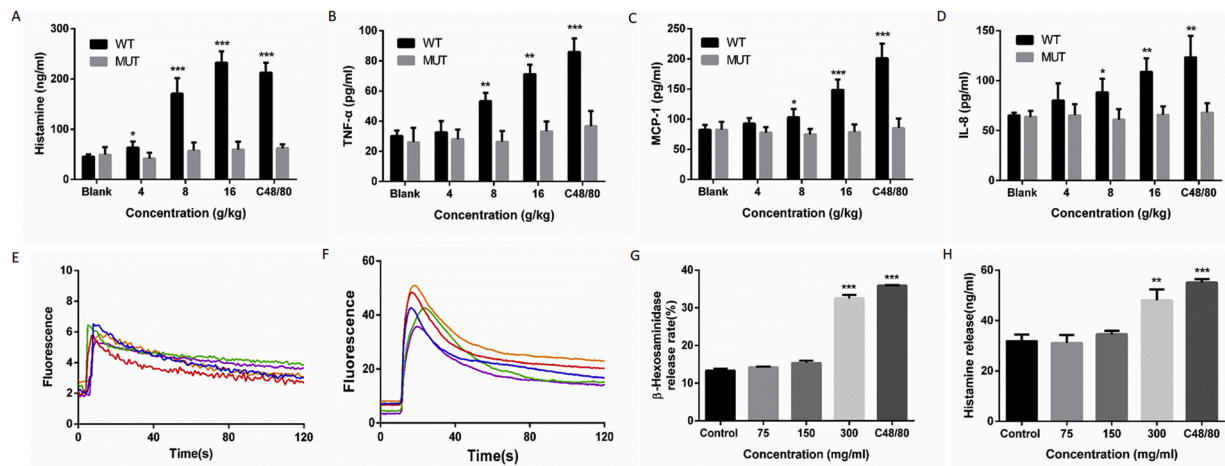


Fig. 2. Iohexol activated mast cells and induced inflammatory mediators release. (A–D) Iohexol induced WT mice serum histamine, TNF- α , MCP-1 and CXCL2 increased, while showed little effect on MUT mice ($n = 6$ per group; experiments were repeated 3 times. The data are presented as the means $\pm$ S.E.M. and analyzed one-tail paired Student's t -test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$). (E–F) Iohexol increased the intracellular Ca^{2+} concentration in MrgprB2-HEK293 cells and skin mast cells. Cells were identified as responding if the $[\text{Ca}^{2+}]_i$ rose by at least 50 %, and each color line represents an individual cell. (G–H) Iohexol induced skin mast cells release β -Hexosaminidase and histamine with a dose manner. ($n = 6$ per group; experiments were repeated 3 times. The data are presented as the means $\pm$ S.E.M. Analyzed by two-tailed unpaired Student's t -test and were calibrated by Bonferroni's test. Differences were considered significant at * $p < 0.0125$, ** $p < 0.0025$, and *** $p < 0.00125$).

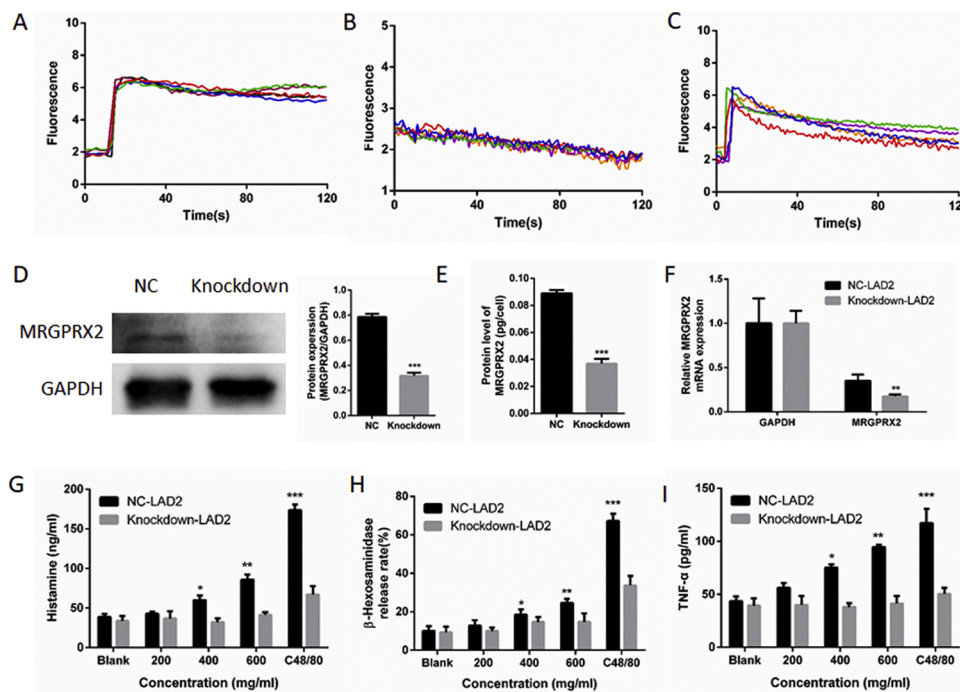


Fig. 3. Iohexol activated LAD2 cells via MRGPRX2. (A–B) Iohexol increased the intracellular Ca^{2+} concentration in LAD2 cells and MRGPRX2-HEK293, while did not increase the intracellular Ca^{2+} concentration in NC-HEK293 cells. (C) Iohexol increased the intracellular Ca^{2+} concentration in LAD2 cells. (D–F) Analysis by Western blot, ELISA Kit and RT-PCR for MRGPRX2 protein expression after knockdown by siRNA transfection. (G–I) After siRNA transfection, Iohexol induced knockdown-LAD2 cells release β -Hexosaminidase, histamine and TNF- α was significantly lower than NC-LAD2 cells. Cells were identified as responding if the $[\text{Ca}^{2+}]_i$ rose by at least 50 %, and each color line represents an individual cell. (experiments were repeated 3 times. The data are presented as the means $\pm$ S.E.M. and analyzed one-tail paired Student's t -test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$).

In order to confirm the observed effects of Iohexol, Kit^{W-sh/W-sh} (MUT) mice were used as PCA model in a further study. Knowledge on the functions of MCs in MUT mice is lacking. The MUT mice included in this study exhibited similar paw swelling and EBD exudation as those of the negative controls ($p > 0.05$; Fig. 1B). Iohexol did not induce pseudo-allergic reactions in MC-deficient mice. In addition, Iohexol was administered systemically to WT and MUT mice via tail vein injection, and the results of serum inflammatory medium analysis showed that Iohexol could induce the release of histamine, TNF- α , MCP-1, and CXCL2 in WT mice in a dose-dependent manner. However, Iohexol could also trigger histamine release in MUT mice (Fig. 2A–D).

Moreover, Iohexol activated WT skin MCs and MrgprB2-HEK293 cells, which led to increased intracellular Ca^{2+} concentrations (Fig. 2E

and F). Iohexol caused degranulation of WT skin MCs *in vitro*, which led to the dose-dependent release of β -hexosaminidase and histamine (Fig. 2G and H).

3.2. Iohexol activates LAD2 cells via MRGPRX2

Results obtained during our study suggested that Iohexol could activate mouse MCs via MrgprB2 *in vitro*, and thus induced pseudo-allergic reactions *in vivo*. Since human MRGPRX2 is homologous to mouse MrgprB2, we used LAD2 cells to verify the agonistic action of Iohexol on MRGPRX2 *in vitro*. Iohexol treatment elicited an increase in the intracellular Ca^{2+} concentration of LAD2 cells (Fig. 3A). Iohexol did not activate NC-HEK293 cells, and the intracellular Ca^{2+} concentration

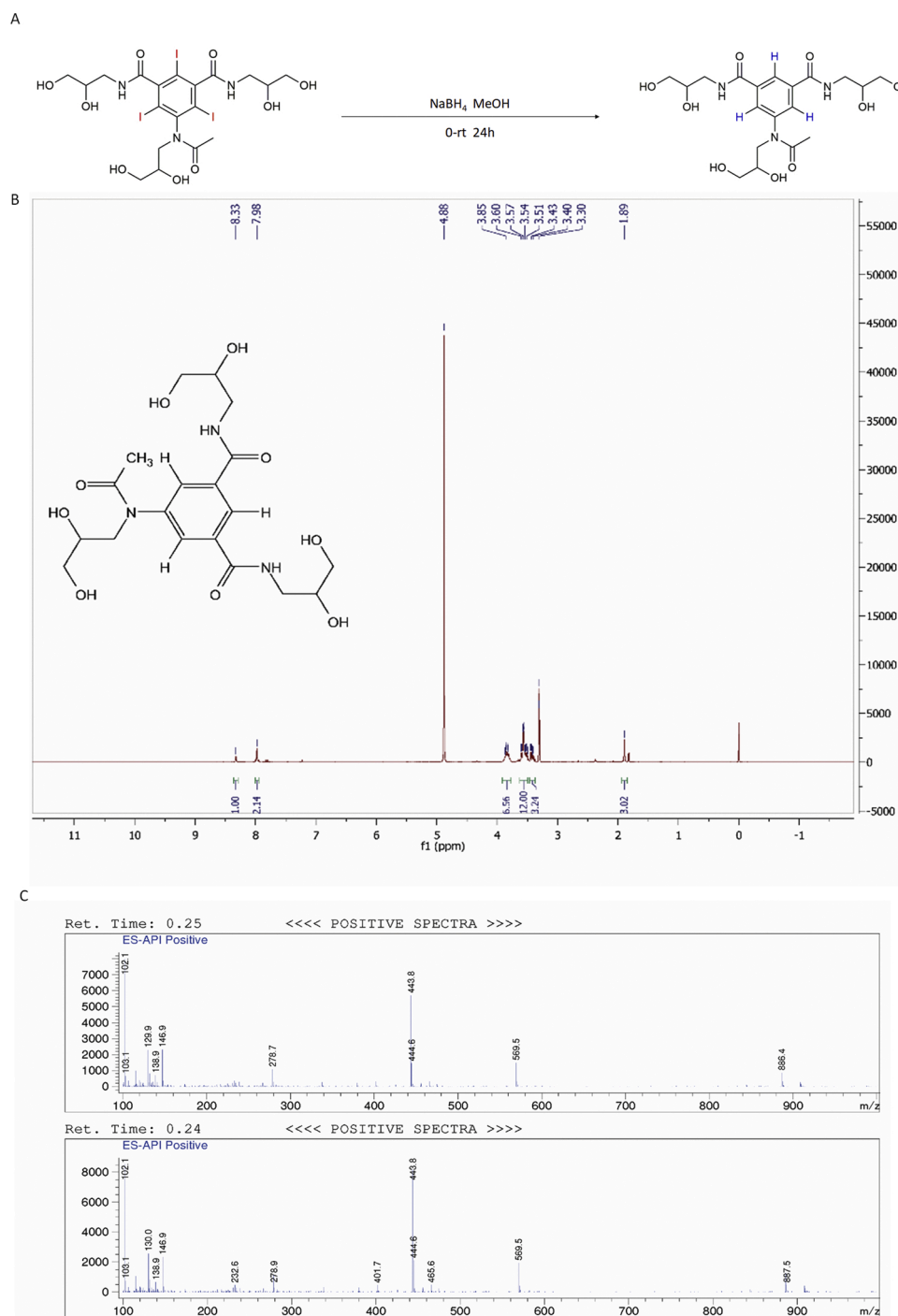


Fig. 4. Synthesis of IOH-1. (A) The synthesis route of IOH-1. (B) The NMR analysis of synthetic product. (V) The MS chromatogram of synthetic product.

remained unchanged (Fig. 3B). In contrast, iohexol treatment resulted in an increase in the intracellular Ca^{2+} concentration in MRGPRX2-HEK293 cells (Fig. 3C). Furthermore, iohexol induced LAD2 degranulation, which caused the release of β -hexosaminidase, histamine, and TNF- α in a dose-dependent manner. After knockdown of MRGPRX2 expression (Fig. 3D–F), the iohexol-induced release of β -hexosaminidase, histamine, and TNF- α was reduced (Fig. 3G–I).

3.3. Iodine might be a key player in iohexol-induced pseudo-allergic reactions

Next, iohexol was structurally transformed into an iodine-free

compound, which was named IOH-1, and was quite pure (Fig. 4). IOhexol induced local pseudo-allergic reactions in WT mice, including a significant degree of swelling and EBD exudation. IOH-1, on the other hand, induced little swelling and EBD exudation (Fig. 5A). In addition, IOH-1 induced a significantly lower degree of skin microvascular dilatation than iohexol (Fig. 5B). Both the increase in intracellular Ca^{2+} concentration and the release of β -hexosaminidase and histamine from skin MCs and LAD2 cells were lower in response to IOH-1 than to iohexol treatment (Fig. 5C–H).

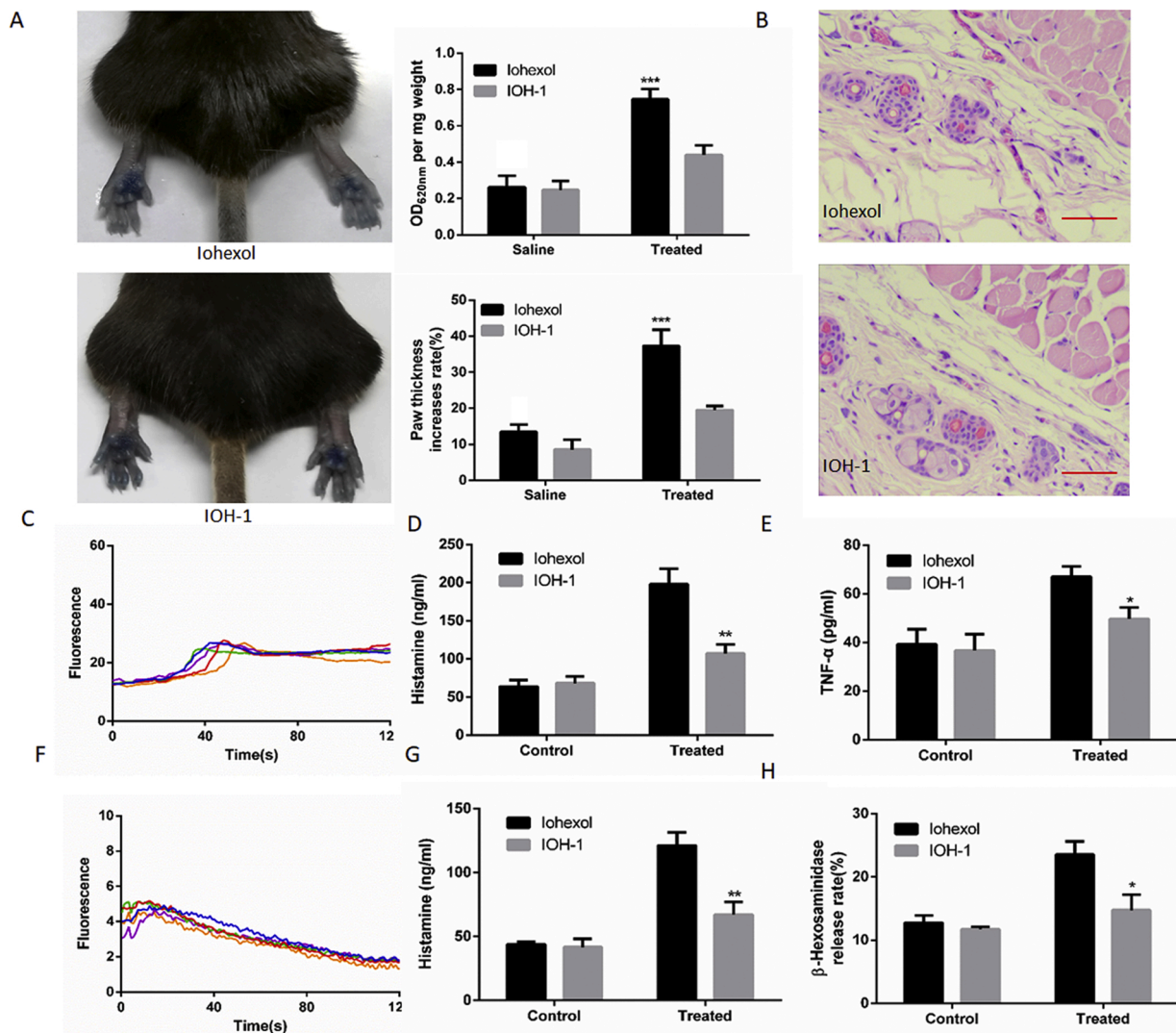


Fig. 5. Iodine was a key persad in iohexol-activated mast cells. (A) iohexol induced the quantification of paw thickness increase and EBD leakage in wild type C57 BL/6 mice, while IOH-1 showed little effect. (B) the effect of iohexol-induced mice hemangiectasis was higher than IOH-1. (C-E) IOH-1 showed little effect on activating mice skin mast cells than iohexol, induced less histamine and β -Hexosaminidase release. (F-H) IOH-1 showed little effect on activating LAD2 cells than iohexol, induced less histamine and β -Hexosaminidase release. (Cells were identified as responding if the $[Ca^{2+}]_i$ rose by at least 50 %, and each color line represents an individual cell. experiments were repeated 3 times. The data are presented as the means $\pm$ S.E.M. and analyzed one-tail paired Student's *t*-test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$).

4. Discussion

RCM provides the benefit of clearly identifying lesions and accurately visualizing the actual characteristics of a lesion and is thus widely used in clinical practice. However, all RCM can potentially cause hypersensitivity reactions, which become apparent more than one hour after RCM exposure, and can be either of the immediate- or delayed-type [16]. Most hypersensitivity reactions caused by RCM injection are of the immediate-type. The degree of the allergic reactions elicited is relatively mild [1]. Immediate-type hypersensitivity reactions occur within a short period of time after RCM injection and act in a significantly dose-dependent manner. In addition, the degree and occurrence rate of an allergic reaction are related to the dose rate. A study by Park et al. clearly demonstrated that a lowering of both the ICM dose as well as the injection speed was accompanied by a significant drop in the incidence of immediate ICM-mediated hypersensitivity reactions in a large patient cohort [17]. All the characteristics of RCM-induced hypersensitivity reactions suggest that they might be best defined as pseudo-allergic reactions.

MCs are the key and primary effectors of an inflammatory response.

IgE-dependent and -independent mechanisms are the two main pathways for MC activation to induce hypersensitivity reactions. MCs express numerous G protein-coupled receptors (GPCRs), which are the largest group of membrane receptor proteins, and the most common targets of drug therapy. Many US Food and Drug Administration-approved drugs activate human MCs and induce hypersensitivity reactions through a novel GPCR, known as MRGPRX2. Iodine, which is contained in many drugs and cosmetics, can induce an anaphylactic reaction [18]. The results of our study showed that iohexol, a typical drug of RCM, induced pseudo-allergic reactions *in vivo* and MC degranulation *via* MRGPRX2 *in vitro*, which were dependent on the presence of iodine.

Iohexol was identified as a new agonist for MRGPRX2, which causes immediate-type pseudo-allergic reactions by inducing hemangiectasis, wheal, itch, and other allergic symptoms. Furthermore, a recent study confirmed that MC activation through the MrgprB2/MRGPRX2 receptor contributes to non-histaminergic pruritus. Compared with IgE-Fc ϵ R1 signaling, MrgprB2/MRGPRX2-activated MCs release more tryptase and excite a distinct itch-sensory neuron population [19]. The symptoms induced by the interaction of iohexol and MrgprB2/MRGPRX2 need to

be investigated more deeply.

Anaphylaxis is a complex physiological process. Even though iohexol could activate MCs via MRGPRX and induce degranulation and cytokine release, MRGPRX2-mediated pseudo-allergic reactions were different among individuals, and not all patients experienced pseudo-allergic reactions after being injected with iohexol. One reason is that the capacity of the drugs to activate MRGPRX2 are positively correlated with the concentration and dose [20]. However, pseudo-allergic reactions might be compensated for by the body; for example, human SCF can impact pseudo-allergic reactions by inhibiting degranulation of MCs [21]. However, it has already been reported that naturally occurring SNPs in the *MRGPRX2* gene can influence responsiveness to MRGPRX2 ligands [22]. In addition, MRGPRX2 expression in skin MCs from different subjects shows different degrees of responsiveness to C48/80 or substance P, which has been shown to act as an agonist of MRGPRX2. This might further be related to the expression level of MRGPRX2.

5. Conclusion

In clinical, iohexol injection can be used at a high dose and allergic reactions are one of common side effects. Our research suggested that the dosage regimen and dose rate might be the key factors to reduce the pseudo-allergic reactions induced by iohexol. Inhibition of MRGPRX2 mediated mast cells degranulation reaction and cytokine release is meaningful to prevent iohexol induced immediate-type pseudo-allergic reactions.

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Authors' contributions

Delu Che, Feng Yuan and Chi Zhang drafted the manuscript; Maji Sun, Dongying Wu, Lin Cheng and Bin Pan participated in the experiments of this study. Delu Che and Feng Yuan participated in the proof-reading of this paper. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors have no financial conflicts of interest.

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