Adverse Skin Reactions to Iodinated X-ray Contrast Agents in Healthy Rats

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Purpose: The aim of this preclinical study on healthy Sprague-Dawley rats was to determine whether differences exist in the induction of adverse skin reactions after the intravenous administration of a monomeric and 2 dimeric iodinated nonionic contrast agents.

Materials and Methods: After intravenous injection of iopromide (monomeric), iodixanol (dimeric), and iotrolan (dimeric) at a dose of 4 g iodine/kg of body weight, mechanical ear volume measurements (10 minute after injection) and intravital microscopy (baseline, 5 minutes after injection) of the ear with the near-infrared dye indocyanine green were performed to determine the volume change and plasma extravasation. Histopathological analysis (20 minutes, 1 hour, and 3 hours after injection) was performed to diagnose alterations in the skin. Blood plasma was analyzed to identify elevated levels of histamine (5 minutes after injection) and inflammatory markers (a multianalyte profile of 58 markers; 1 hour and 3 hours after injection).

Results: Only iodixanol induced immediate angioedema formation, with a 100% incidence rate and with slight mast cell infiltration in the ear, muzzle, and paws. The ear showed a 53% volume increase and strong extravasation of plasma proteins into the interstitium, which correlated with highly (11-fold) increased plasma histamine levels 5 minutes after injection. Elevated levels of tumor necrosis factor-α (7.1-fold), macrophage inflammatory protein (MIP)-1α (3.2-fold), and MIP-2 (7.7-fold) were identified 1 hour after the iodixanol injection. Increased levels (fold-change) of MIP-1β (14; 6.3), monocyte chemotactic protein-1 (3.3; 3.7), monocyte chemotactic protein-3 (2.4; 3.0), stem cell factor (1.7; 2), vascular endothelial growth factor (2; 2.1), and interferon gamma-induced protein-10 (4.1; 39.1) were identified 1 hour and 3 hours after the iodixanol administration, respectively. The level of these molecules remained unchanged after the iopromide and iotrolan injections (except for stem cell factor).

Conclusions: A reversible anaphylactoid-like reaction in healthy Sprague-Dawley rats was observed after the iodixanol administration but not after the monomeric iopromide or dimeric iotrolan injections. Therefore, we conclude that the induction of adverse skin reactions is not per se because of a class effect of dimeric contrast agent.

Key Words: iodinated contrast agents, adverse reactions, skin

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Four classes of iodinated contrast agents (CAs) exist, which exhibit a basic structure of tri-iodinated benzene rings,[1,2] namely, 2 ionic (including high-osmolar monomeric and low-osmolar dimeric molecules) and 2 nonionic compound classes. A correlation between the incidence of adverse reactions and the CA class has been clinically observed over the years, indicating a significantly higher incidence rate of adverse reactions associated with the high-osmolar ionic CAs (9%–54%) compared with nonionic CAs (2%–17%).[2–4] The risk for adverse reactions has decreased because of the compulsory use of nonionic molecules.[3,5–8] The nonionic CA classes are composed of low-osmolar monomeric molecules (400–800 mOsm/kg H_2O and low viscosities of 4–6 mPa) and iso-osmolar dimers (290 mOsm/kg H_2O, viscosities of 8–12 mPa).

Adverse reactions are diverse with respect to their clinical manifestations and severity and, in most cases, exhibit allergic-like characteristics but are not mediated by immunoglobulin E.[9] Classic allergic reactions are only exhibited in rare cases,[10–12] although both types of reactions (allergic and allergic-like) give rise to the same clinical symptoms. The clinical manifestations can affect the skin (eg, erythema, urticaria, angioedema, exanthema, or toxic epidermal necrosis), the gastrointestinal tract (eg, nausea and vomiting), the respiratory organs (eg, coughing, sneezing, or bronchospasm), and the cardiovascular system (eg, dizziness, vasodilation, tachycardia, cardiac arrest, or anaphylactic shock).[7,12–15] Adverse reactions can be categorized as mild, moderate, or severe,[1] and they can occur immediately (<1 hour) or can be delayed (1 hour-1 week).[9] The most frequent CA-induced reactions are mild for both the immediate and delayed manifestations,[2,16] and they are usually short in duration and self-healing. Symptoms with higher degrees of severity are associated with moderate reactions and require appropriate therapy, to which patients usually respond well. In contrast, severe and life-threatening reactions occur much less frequently, and their induction is not clearly significantly different between the use of ionic and nonionic CAs in the overall study population.[17] A comparison of enhanced and unenhanced computed tomographic procedures with a focus on delayed reactions demonstrated that only skin reactions appeared more frequently among patients who received CAs and constitute the majority of true contrast-related reactions.[18–20] The use of nonionic dimeric CAs has been reported to induce more frequent[21,22] or more severe[23] delayed skin reactions. In contrast, no significant differences have been reported with respect to the frequency of delayed adverse reactions between nonionic monomers[24,25] or between nonionic monomers and the ionic dimer ioxaglate.[26,27]

In the present study, we wanted to determine the tolerability of nonionic iodinated CAs under standardized conditions in a preclinical experiment in healthy rats to analyze in more detail whether differences exist in the induction of adverse skin reactions between the monomeric and the dimeric CA classes.

MATERIALS AND METHODS

Animals

We used healthy Sprague-Dawley hairless rats (Rj:SDH-Dsg4) (males; 300–350 g), a mutant outbred strain obtained from Centre D'Elevage R. Janvier (Le Genest-St Isle, France). A mutation in the *desmoglein-4* gene of chromosome 18 yields a hairless phenotype that manifests as abnormalities in hair and vibrissae growth. The animals were kept under standard laboratory conditions at a temperature of 22°C and a dark/light rhythm of 12 hours. Standard rat chow and water were provided ad libitum. The animals were handled and treated according to German animal regulations.

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TABLE 1. Iodine Concentration, Osmolality, and Viscosity of the Contrast Agents

Brand Name	Compound	Concentration (mg iodine/mL)	Molecular Structure	Osmolality (mOsm/kg H_2O) at 37°C	Viscosity (mPa/s) at 37°C
Visipaque 320	Iodixanol	320	Dimeric	290	11.8
Ultravist 300	Iopromide	300	Monomeric	610	4.6
Isovist 300	Iotrolan	300	Dimeric	290	8.4

Contrast Agents

Commercially available x-ray CAs were compared in this study. The following compounds were used in their commercial formulation for injection into humans: iopromide 300 (Ultravist 300; Bayer Vital GmbH, Leverkusen, Germany), iodixanol 320 (Visipaque 320; GE Healthcare Buchler GmbH & Co KG, Braunschweig, Germany), and iotrolan 300 (Isovist 300; Bayer Yakuhin Ltd, Osaka, Japan). All CAs were prewarmed to 37°C before intravenous injection. Detailed information on the CAs is provided in Table 1.

Experimental Procedure

The Sprague-Dawley rats were injected in the tail vein with iopromide, iodixanol, or iotrolan at a dose of 4 g iodine/kg of body weight (b.w.). The control animals received equal volumes of saline. For intravital microscopy, indocyanine green (ICG) was used as a diagnostic tool to visualize the plasma proteins as well as flow and was administered at a dose of 5-mg/kg b.w. The number of animals per group, the investigation time points, and the experimental procedures are summarized in Table 2.

Macroscopic and Mechanical Measurement of Ear Volume

The rats were macroscopically observed after intravenous CA application. The thorax, head, and paws were photographically documented before and 10 minutes after contrast injection. The ear thickness was measured using a skin thickness gauge (in-house development Bayer Healthcare). The distance between 2 measuring cylinders on a support bar and a mobile measuring bar was determined when a connected force sensor exceeded 0.5 N. To rule out inaccuracy in the ear volume determination due to individual differences in the size and weight of the rats at baseline, the measured ear thickness of each rat before CA injection was defined as 100%, and the intraindividual volume increase was determined for each animal after CA administration.

Intravital Microscopy

The rats were anesthetized intraperitoneally with Rompun (2% xylazine; Bayer Vital GmbH, Leverkusen, Germany) and Ketavet 100 mg/mL (Pfizer Pharmacia GmbH, Berlin, Germany) (mix ratio, 1:2) at a dose of 1-mL/kg b.w.

An Axio Imager Z1 (Zeiss, Jena, Germany) with a 2.5× EC-Plan-Neofluar objective, an AxioCam MRm camera, and AxioVision software (version 4.5) was used to visualize the blood plasma flow through detection of the near-infrared (NIR) dye ICG (Pulsion Medical Systems AG, Munich, Germany; peak spectral absorption at approximately 800 nm), which reversibly binds to plasma proteins.

The rat was positioned with the ear fixed to a black neoprene sheet under the 2.5× magnification objective. Microscopy was performed with an optical Cy7 (absorption, 795 nm) filter and a halogen light bulb to visualize the NIR dyes. The imaging period was 5 minutes after the ICG injection, with the generation of 1 image every 2 seconds at a shutter speed of 500 milliseconds. An infusion cannula was positioned into the tail vein for consecutive drug application.

Indocyanine green (5-mg/kg b.w.) was applied intravenously for baseline plasma flow detection for 0 to 5 minutes, followed by a single intravenous injection of CA at a dose of 4 g iodine/kg of b.w. after the completion of the ICG elimination. At the 5-minute post contrast injection, ICG was readministered with an imaging period of 5 minutes to intraindividually compare the plasma flow before and after contrast application.

Histamine Analysis

The rats were anesthetized intraperitoneally with Rompun (2% xylazine; Bayer Vital GmbH) and Ketavet 100 mg/mL (Pfizer Pharmacia GmbH) (mix ratio, 1:2) at a dose of 1 mL/kg of b.w. Blood (500 μL) was drawn from the carotid artery 5 minutes after the contrast injection, and the plasma was obtained with sodium chloride/heparin (Sarstedt AG, Nümbrecht, Germany) through centrifugation (10 minutes, 2500 g at 4°C). A histamine enzyme-linked immunosorbent assay (RE59221; IBL international, Hamburg, Germany) for plasma was performed in accordance with the manufacturer's instructions. The test principle is based on competition: specifically, a fixed amount of enzyme-labeled antigen and an unknown amount of antigen (histamine) in the sample compete for the binding sides of the antibodies coated on the wells. After the incubation and washing steps as well as the subsequent substrate reaction, the intensity of the developed color is inversely proportional to the amount of the antigen in the sample. The reading was performed using a SpectraMax M4 (Molecular Devices, Biberach an der Riss, Germany).

TABLE 2. Summary of the Experimental Procedures

Experimental Procedure (Total No. Rats)	CA and Control	Iodine Concentration, g iodine/kg b.w.	Time of Investigation	No. Animals Per CA Group
Macroscopic observation and mechanical measurement of ear volume, n = 54	Iopromide Iodixanol Iotrolan Saline	4	10 min p.i.	18
Intravital microscopy, n = 16	Iopromide Iodixanol Iotrolan Saline	4	0–5 min before CA, 5–10 min p.i.	4
Histamine ELISA, n = 24	Iopromide Iodixanol Iotrolan Saline	4	5 min p.i.	6
Histopathology, n = 72	Iopromide Iodixanol Iotrolan Saline	4	20 min p.i., 1 h p.i., 3 h p.i.	18/CA, n = 6/time
Plasma cytokine analysis, n = 48	Iopromide Iodixanol Iotrolan Saline	4	1 h p.i., 3 h p.i.	6/CA, n = 6/time

b.w. indicates body weight; CA, contrast agent; ELISA, enzyme-linked immunosorbent assay; p.i., after injection.

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Histopathological Examination

The rats were anesthetized with isoflurane (Baxter Deutschland GmbH, Unterschleißheim, Germany) before being killed (20 minutes, 1 hour, and 3 hours after injection), and skin tissue samples from the ear, paws, and muzzle were removed. The skin samples were fixed in 10% neutral buffered formalin. After fixation and routine dehydration, the tissue samples were embedded in paraffin and sectioned (4–6 μm) for hematoxylin and eosin staining. The histopathological evaluation was performed by a certified veterinary pathologist in a randomized sequence in accordance with the best practice guidelines of the Society of Toxicologic Pathology to investigate the occurrence of treatment-related lesions and to rule out the accidental inclusion of animals with a preexisting abnormal skin pathology. Ulceration, hyperkeratosis, acanthosis, acantholysis, subepidermal fissuring, hyperemia, edema, interface dermatitis, folliculitis, increased cellularity, and an elevated number of mast cells were investigated. The incidence rate and qualitative grade were determined as follows: not present, negative value; minor, 1; mild, 2; moderate, 3; distinct, 4; and profound, 5.

This analysis was performed on the basis of comparison between the groups and versus controls and was documented through photography.

Plasma Cytokine Analysis

The rats were anesthetized with 4% isoflurane and killed. Blood was drawn from the vena cava, and the plasma was obtained with sodium citrate through centrifugation for 10 minutes at 1800 × g. The plasma was analyzed with Luminex technology by Myriad RBM (Austin, TX). A rodent multianalyte profile (rodentMAP) (https://rbm.myriad.com/products-services/rodentmap-services/rodentmap/) was used to quantitatively identify 58 inflammatory biomarkers.

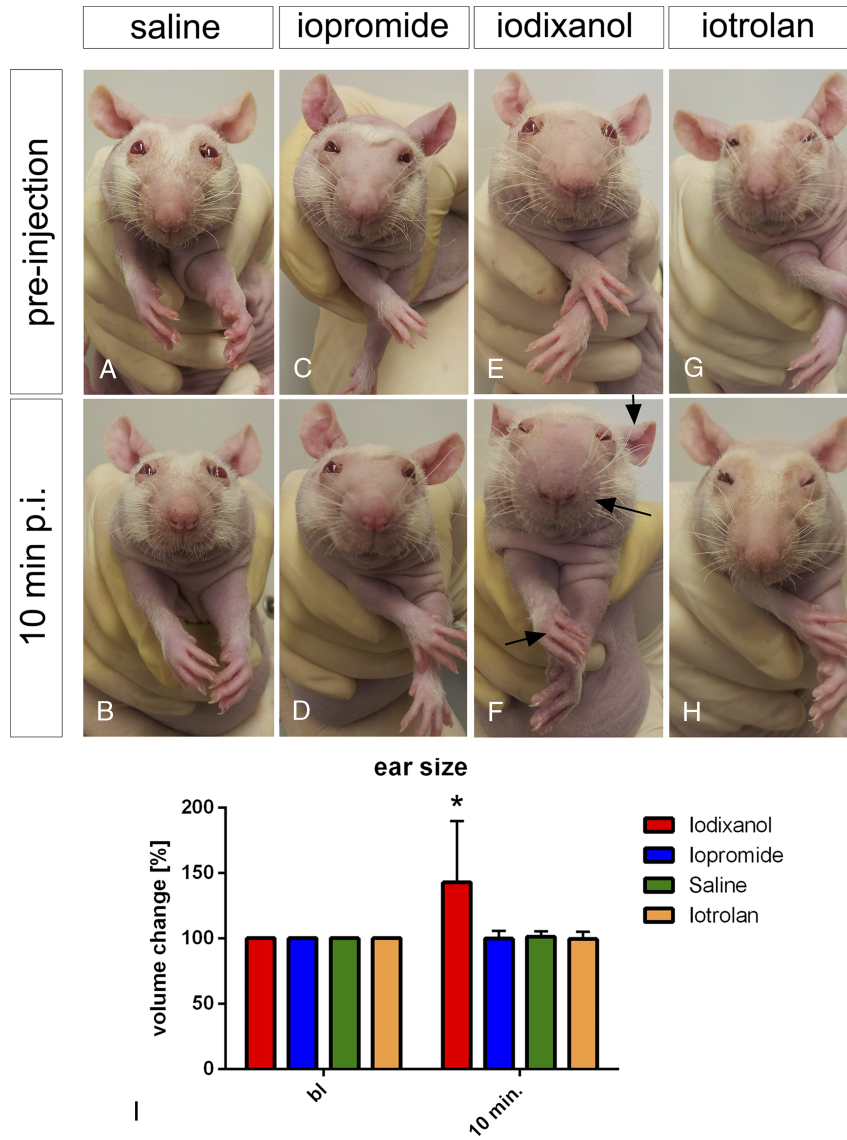


FIGURE 1. Macroscopic examination of rats before (A, C, E, G) and after (B, D, F, H) the injection of saline (A, B), iopromide (C, D), iodixanol (E, F), and iotrolan (G, H). F, Swelling of the ears, muzzle, and paws after the iodixanol administration is indicated by the arrows. I, Mechanical measurement revealed a 53% volume increase with statistical significance (marked as asterisk) in the ears 10 minutes after the iodixanol (red) administration.

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Statistics

The Kruskal-Wallis test (1-way analysis of variance on ranks) was used to compare the groups (unpaired data), and the Mann-Whitney test was used for nonparametric comparisons between the 2 groups. GraphPad Prism (GraphPad Software, La Jolla, CA) was used for all statistical analyses, and a significance level of $P < 0.05$ was used.

RESULTS

CA-Induced Behavior and Macroscopic Volume Alteration

Macroscopic swelling of the muzzle, ears, and limbs as well as reddened skin were detected immediately after the intravenous administration of iodixanol (Fig. 1F), compared with baseline (Fig. 1E). Iodixanol-treated rats exhibited a nonactive behavior with a bent posture. No macroscopic or behavioral alterations compared with baseline (Fig. 1, A, C and G) were observed after saline (Fig. 1B), iopromide (Fig. 1D), or iotrolan (Fig. 1H) injection. Mechanical measurements identified a statistically significant 53% mean volume increase in the ears of the iodixanol-treated rats (Fig. 1I). The ear volume in the saline-, iopromide-, and iotrolan-treated animals remained unchanged. The observed iodixanol-induced swelling was reversible after approximately 2 hours.

Vessel Leakage Into the Skin Tissue

As a result of the ear, paw, and muzzle swelling after the iodixanol administration, we analyzed the observed process in more detail through intravital microscopy of the ear vessels, applying the small NIR dye ICG, which reversibly binds to plasma proteins. After the intravenous injection of ICG into the tail vein, all animals exhibited comparable baseline plasma flow and vessel morphology (Fig. 2, A0–D0, baseline). After the CAs were administered, followed by an additional injection of ICG saline (Fig. 2, B1–B5), iopromide (Fig. 2, A1–A5) and iotrolan (Fig. 2, C1–C5) administration did not induce changes in the vessel view and plasma-ICG flow, respectively, compared with baseline. In contrast to saline, iopromide, and iotrolan, the injection of iodixanol (Fig. 2, D1–D5) led to severe extravasation of ICG-bound proteins into the interstitium of the ear tissue in 4 of 4 rats. Analysis of the blood plasma through enzyme-linked immunosorbent assay (5 minutes after injection) revealed a statistically significant 11-fold higher histamine level after the iodixanol administration (Fig. 3) compared with the levels resulting from iopromide, iotrolan, and saline.

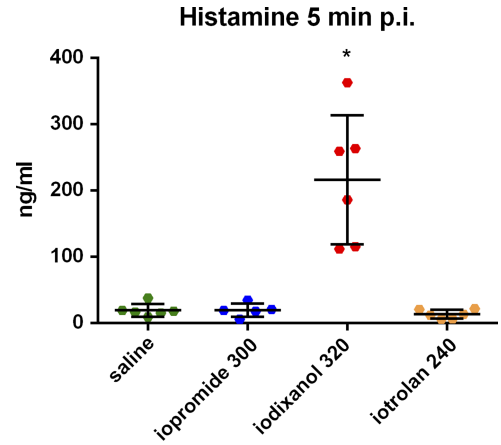


FIGURE 3. Plasma histamine levels after saline (green), iopromide (blue), iodixanol (red), or iotrolan (yellow) administration. Statistically significant (marked as asterisk) elevated histamine levels were identified 5 minutes after the iodixanol injection (6 per group).

Edema Formation

Because of the observed extravasation of plasma proteins, the ear tissue was investigated in more detail via histopathological analysis. From 11 examined parameters, 3 experiment-related alterations were detected at 20 minutes, 1 hour, as well as 3 hours after injection and included slight edema formation (Fig. 4; Table 3) and hyperemia (Table 4) in the ear and muzzle tissue as well as a minor-to-mild increase in the number of mast cells (Table 5; Fig. 4). With a 100% incidence rate (6/6 animals) at every sampling time point, we observed edema formation after the iodixanol administration, which was graded as mild to moderate (mean severity grading, 2.17) at 20 minutes after injection, minor to mild (mean severity grading, 1.5)

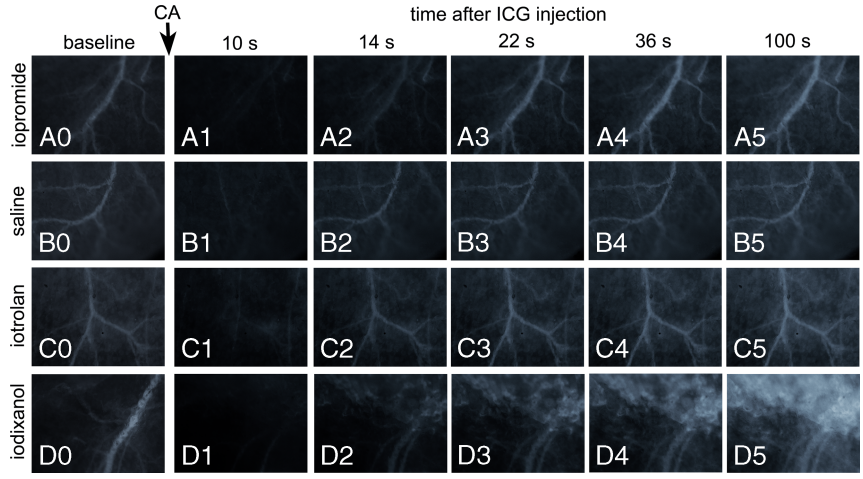


FIGURE 2. Microscopic images demonstrating plasma flow in the ear of Sprague-Dawley rats stained with the NIR dye ICG before (A0–D0) and 5 minutes after (A1–A5, B1–B5, C1–C5, D1–D5) the iopromide (A0–A5), saline (B0–B5), iotrolan (C0–C5), or iodixanol (D0–D5) injection. The images were taken 10 seconds (A1–D1), 14 seconds (A2–B2), 22 seconds (A3–D3), 36 seconds (A4–D4), and 100 seconds (A5–D5) after the ICG administration. Extravasation of ICG-bound plasma proteins in the ears of the iodixanol-treated rats (4/4 rats) was identified 14 to 100 seconds after the ICG injection (D2–D5).

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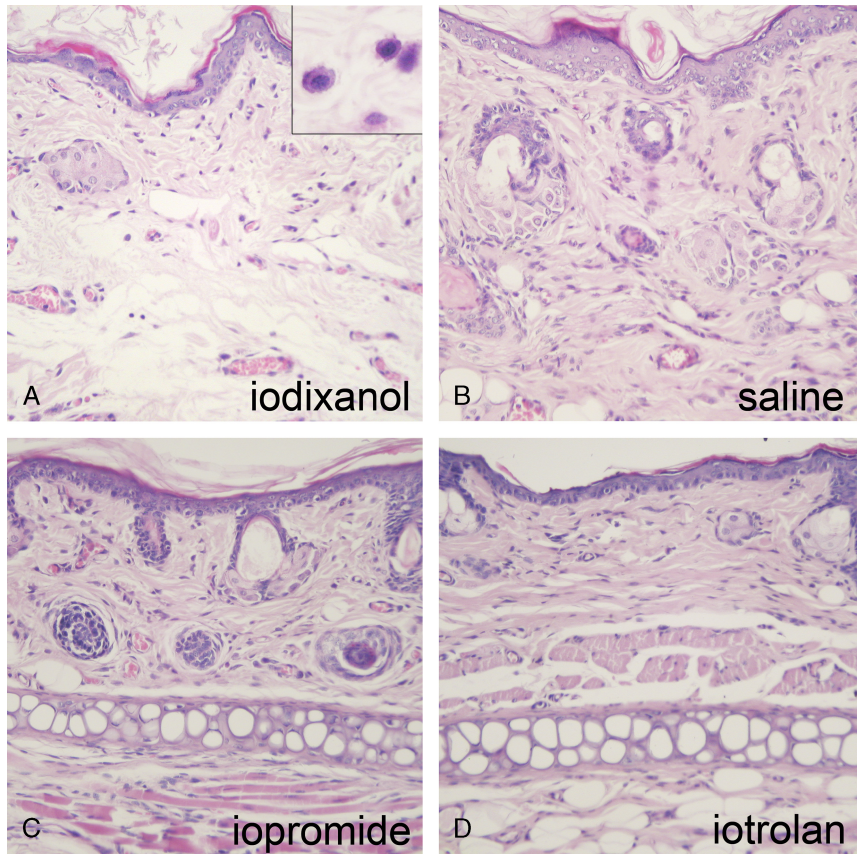


FIGURE 4. Hematoxylin and eosin–stained skin sections of the ear 20 minutes after the iodixanol (A), saline (B), iopromide (C), or iotrolan (D) administration revealed edema formation in the iodixanol-treated rats (A). The magnification in A shows mast cell infiltration in the skin.

at 1 hour after injection, and minor (mean severity grading, 1.0) at 3 hours after injection (Table 3). Minor hyperemia was detected in 2 of 6 animals after the iodixanol administration 20 minutes and 1 hour after injection as well as in 1 of the 6 animals at 3 hours after injection (Table 4). In the saline-, iopromide-, and iotrolan-treated rats, no edema formation or hyperemia was histologically detectable (Fig. 4, B–D). Skin samples of the back served as reference tissue because no macroscopic volume alterations were observed after the application of any CA. Histologically, no edema formation was identified in the back skin samples after the saline, iopromide, iotrolan, or iodixanol administration at any of the 3 time points (data not shown).

With an incidence rate of 6 of 6, 5 of 6, and 6 of 6 rats at 20 minutes, 1 hour, and 3 hours after injection, respectively, a minor-to-mild increase in the number of mast cells (severity grade, 1.17, 1.2, and 1.17, respectively) infiltrating the ear tissue was observed, mainly after the iodixanol administration (Fig. 4A; Table 5). Occasionally, with a low incidence rate of 1 of 6 rats, a minor elevated number of mast cells was observed after the iopromide injection after 20 minutes, 1 hour, and 3 hours, and 2 of 6 rats showed minor mast cell infiltration after the saline treatment 3 hours after injection; the iotrolan-treated animals did not exhibit mast cell infiltration.

Systemically Elevated Cytokine Levels in the Blood Plasma

Because of the immediate edema formation observed after the iodixanol administration, the blood plasma was analyzed for inflammatory responses. At 1 hour and 3 hours after injection, we performed a quantitative multiplex analysis to assess alterations in the levels of inflammatory molecules in the plasma. We identified several cytokines (tumor necrosis factor α [TNF-α], macrophage inflammatory protein [MIP]-1α, [MIP]-2, monocyte chemotactic protein [MCP]-1, MCP-3, MIP-1β, stem cell factor [SCF], interferon gamma-induced protein [IP]-10, and vascular endothelial growth factor [VEGF]-A) that were elevated in the rat plasma after the CA administration at different time points (Fig. 5). We identified a statistically significant 7.1-fold increase

TABLE 3. Edema Formation

	Iodixanol		Saline		Iopromide		Iotrolan	
	Incidence	Mean Severity	Incidence	Mean Severity	Incidence	Mean Severity	Incidence	Mean Severity
20 min after injection	6/6	2.17	—	—	—	—	—	—
1 h after injection	6/6	1.5	—	—	—	—	—	—
3 h after injection	6/6	1.0	—	—	—	—	—	—

Em dash symbol indicate not present; 1, minor; 2, mild; 3, moderate; 4, distinct; 5, profound.

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TABLE 4. Hyperemia

	Iodixanol		Saline		Iopromide		Iotrolan	
	Incidence	Mean Severity	Incidence	Mean Severity	Incidence	Mean Severity	Incidence	Mean Severity
20 min p.i.	2/6	1.0	—	—	—	—	—	—
1 h p.i.	2/6	1.0	—	—	—	—	—	—
3 h p.i.	1/6	1.0	—	—	—	—	—	—

Em dash symbol indicate not present; 1, minor; 2, mild; 3, moderate; 4, distinct; 5, profound.

in TNF-α (Fig. 5A), a 3.2-fold increase in MIP-1α (Fig. 5B), and a 7.7-fold increase in MIP-2 (Fig. 5C) 1 hour after the administration of iodixanol versus saline, which decreased at 3 hours after injection to the baseline level (TNF-α) and to 1.8-fold (MIP-1α) and 2.4-fold (MIP-2) of the baseline levels. After the saline, iopromide, and iotrolan injections, the TNF-α, MIP-1α, and MIP-2 levels remained unchanged. At both time points of investigation (1 hour and 3 hours after injection), we detected elevated levels of MIP-1β (Fig. 5D), MCP-1 (Fig. 5E), MCP-3 (Fig. 5F), SCF (Fig. 5G), and VEGF-A (Fig. 5H). Using the saline controls for comparison, we monitored significantly increased levels of MIP-1β (14-fold, 6.3-fold), MCP-1 (3.3-fold, 3.7-fold), MCP-3 (2.4-fold, 3.0-fold), SCF (1.7-fold, 2-fold), and VEGF (2-fold, 2.1-fold) 1 hour and 3 hours after the iodixanol injection, respectively. No elevated levels were detected for these molecules after the iopromide injection. Iotrolan administration induced a 1.5-fold increase in the SCF at 1 hour after injection. An additional 4.1- and 39.1-fold increase in the IP-10 protein levels at 1 hour and 3 hours after injection, respectively, was detected after the administration of iodixanol only. The plasma analysis revealed 7.3-fold elevated levels of TNF-α at 5 minutes after the iodixanol administration (data not shown) but not after the saline, iopromide, or iotrolan injections.

DISCUSSION

Skin reactions are reported to be typical of delayed and immediate adverse reactions. A series of clinical studies demonstrated that delayed skin reactions are more prevalent after the administration of nonionic dimeric molecules[18] and can occur up to 3 to 4 times more frequently[21,22] than with monomeric agents. In contrast, other studies have reported no significant difference in the occurrence of late adverse effects associated with nonionic dimers[28] but have revealed an increased severity of reactions related to dimeric CA iodixanol.[23] Discriminatory study outcomes are not surprising when we consider the variation in patient cohorts with respect to medical records, medication use, and genetic divergence.

In our preclinical study on healthy Sprague-Dawley rats, we compared the tolerability of the 2 iodinated CA classes with respect to clinical observations under standardized conditions to measure differences in the induction of adverse reactions. According to the Food and Drug Administration toxicology guidelines,[29] the most appropriate method for extrapolating an animal dose to the equivalent human dose is the normalization of doses to the body surface area. Therefore, we applied a dose of 4 g iodine/kg of b.w. to the rats, which corresponds to the dose used for humans in clinical applications of 0.65 g iodine/kg of b.w.

We demonstrated that adverse skin reactions are not induced per se by a class effect of dimeric CAs. In the rats, we observed an immediate anaphylactoid-like reaction, consisting of swelling in the skin of the ear, muzzle, and paws, beginning shortly after the intravenous administration of dimeric iodixanol only. At the same time, we identified a strong extravasation of plasma proteins into the interstitium and edema formation, presumably provoked by the detected high plasma histamine levels. However, in clinical reports, the majority of iodixanol-induced skin reactions are manifested by maculopapular exanthema, which is followed by the appearance of urticaria and angioedema.[12] Angioedema mainly emerges as an immediate reaction but may also have delayed occurrence.[30,31] Because the immediate symptoms (edema formation, extravasation, and increased plasma histamine levels) did not occur in the iotrolan-treated animals, we conclude that iodixanol-induced reactions are not induced by the physicochemical properties of dimeric CAs but are rather the effect of the iodixanol substance itself. Similarly, in patients receiving iotrolan, the plasma histamine levels remained unchanged compared with those in patients receiving iopromide, in whom slight but not significantly elevated histamine levels were identified.[32] However, iotrolan was voluntarily withdrawn from intravascular applications in 1995 because of the higher incidence rate of delayed skin reactions observed in Japan.[33] The incidence rate of delayed adverse reactions to nonionic dimers appears to be twice as high in Japan as it is in Europe.[2] Ethnic differences, other medication procedures, or simply the more careful patient observation protocols practiced in Japan may account for this observation.[12] Nevertheless, when we consider the nonionic dimers iotrolan and iodixanol individually, the 7.3% incidence rate of delayed skin reactions among patients receiving iotrolan injections[33] is lower than the incidence rate of 12.2% among patients receiving iodixanol injections[21] in comparison with the rate of 3% to 4% among patients receiving nonionic monomeric agents. Unfortunately, the lack of comparative clinical studies assessing the onset of adverse reactions to iotrolan and iodixanol allows no direct conclusions to be made regarding their comparative tolerability.

The underlying pathomechanism in the induction of allergy-like/allergic reactions still remains a matter of speculation. Immediate anaphylactoid reactions with histamine and tryptase release[29] by mast cells and basophils in patients or activation of the complement

TABLE 5. Mast Cell Infiltration in the Skin

	Iodixanol		Saline		Iopromide		Iotrolan	
	Incidence	Mean Severity	Incidence	Mean Severity	Incidence	Mean Severity	Incidence	Mean Severity
20 min after injection	6/6	1.17	—	—	1/6	1	—	—
1 h after injection	5/6	1.2	—	—	1/6	1	—	—
3 h after injection	6/6	1.17	2/6	1	1/6	2	—	—

Em dash symbol indicates not present; 1, minor; 2, mild; 3, moderate; 4, distinct; 5, profound.

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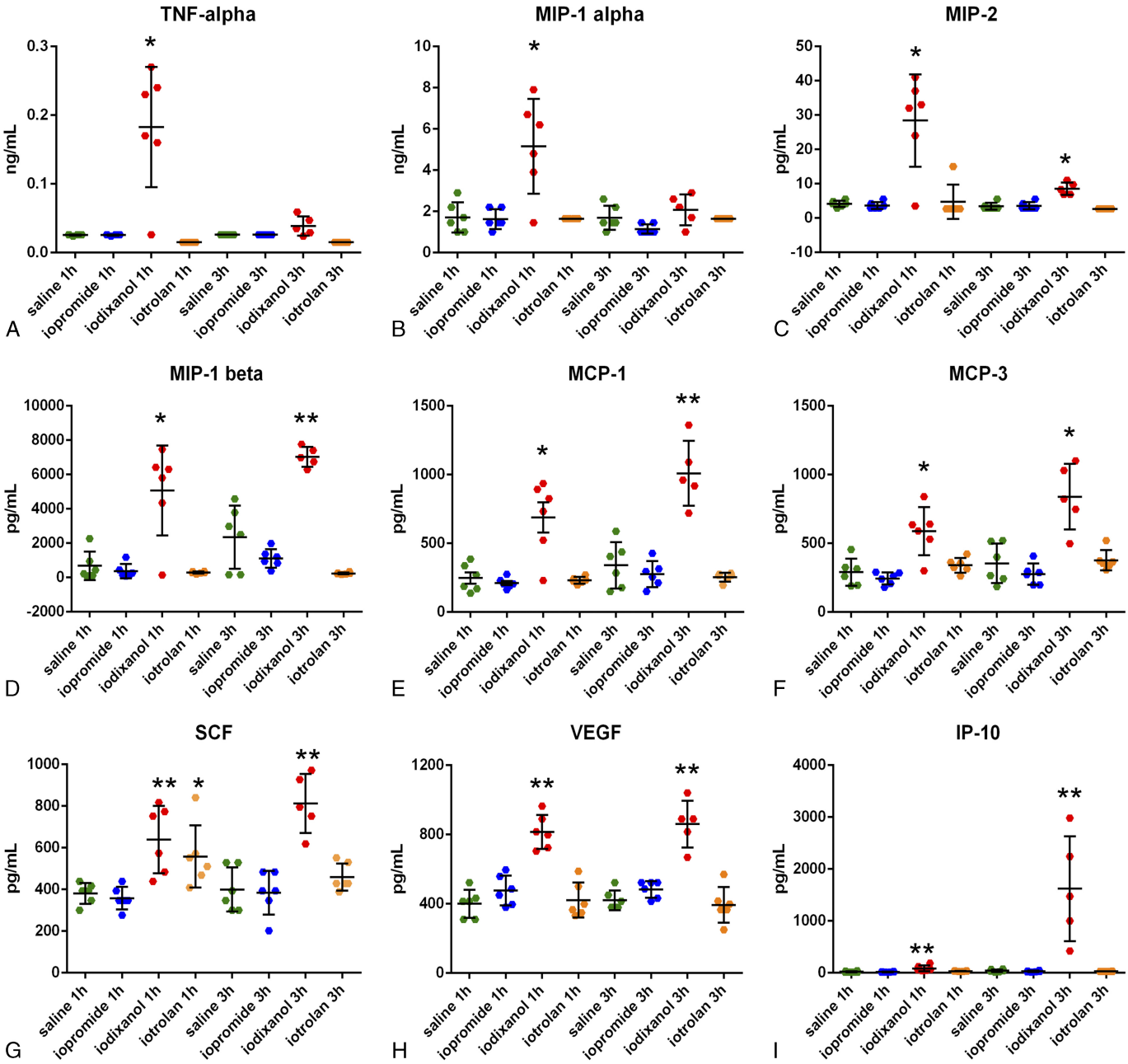


FIGURE 5. Levels of inflammatory proteins 1 hour and 3 hours after the saline (green), iopromide (blue), iodixanol (red), or iotrolan (yellow) administration, as determined by multiplex Luminex technology. After the iodixanol injection, statistically significant elevated levels of TNF-α (A), MIP-1α (B), MIP-2 (C), MIP-1β (D), MCP-1 (E), MCP-3 (F), SCF (G), VEGF (H), and IP-10 (I) were identified, and there were elevated SCF levels after the iotrolan injection (6 per CA group and time).

system[34] are only partially associated with immediate reactions.[16,35] In contrast, T-cell activation and infiltration have been observed more frequently in delayed skin reactions.[16,36] In the present rat study, we identified numerous inflammatory chemokines and cytokines that were elevated in the blood plasma of rats treated with iodixanol; these molecules were sequentially released and induced over time. In addition, we observed a slight but exceptionally early infiltration of mast cells into the affected skin regions. Concurrently, an immediate release of prestored TNF-α at 5 minutes after injection was observed. At 1 hour after injection, we detected increased plasma levels of MIP-1α, TNF-α, as well as MIP-2, and at both time points tested (1 hour and 3 hours after injection), the markers MIP-1β, MCP-1, MCP-3, SCF, and VEGF were elevated. Significant increases in IP-10 levels were measured time-delayed in the plasma 3 hours after the iodixanol administration. In contrast to iodixanol, the iopromide and iotrolan treatment did not induce an elevation of plasma cytokines or chemokines. Only elevated levels of SCF were significantly induced by iotrolan, involving the activation of potential immune processes. Until now, it is not possible to predict from our data the exact immunological mechanisms in the skin on the basis of the

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increase in plasma chemokines, nor is it feasible to define the cell type associated with their expression and release. However, the activation processes of chronological or simultaneous immune cascades are apparently induced through iodixanol administration. Dermal mast cells, monocytes, fibroblasts, keratinocytes, and endothelial cells are able to release these early mediators. We assume that the occurrence of an initial activation of mast cells is caused by the immediately induced anaphylactoid-like reaction associated with high levels of histamine and TNF-α release and by the exceptionally early infiltration of mast cells into the skin lesions. Furthermore, T cells, macrophages, and neutrophils may be involved in releasing the second cascade of inflammatory mediators. In vitro studies of endothelial cells have revealed the secretion of proinflammatory products after iodixanol and TNF-α stimulation.[37] Moreover, an increase in cytokine secretion from human isolated monocytes and dendritic cells has been previously measured after iodixanol treatment.[38] Skin tests performed on patients with delayed skin reactions are often positive for activated basophils and activated $CD4^+$ and $CD8^+$ lymphocytes.[16,36] T-cell activation is often verified in patients only after the onset of clinical manifestations. Potential immunological processes occurring during the time interval between the appearance of skin reactions and contrast administration are, therefore, mostly uncaptured. In addition, many of the increased chemokines evaluated in the present rat study are able to recruit and activate T cells in T-cell–mediated immunological processes. Future analysis of the activation of T cells in the affected skin regions in rats is indispensable, as is the investigation of immunological processes in rats lacking mast cells.

We are aware that the adverse reactions associated with CAs in humans are not directly comparable with the observations made in our animal study, especially because we observed a 100% incidence rate in our animal model after the iodixanol injection. This observation was not specific to the Sprague-Dawley rat strain described herein but was also found in initial experiments with Wistar Han rats. Animals are artificial models used to simulate adverse reactions. If we assume corresponding types of adverse reactions in humans and rodents, the spontaneous histamine and TNF-α release as well as angioedema formation observed after iodixanol injection in rats resemble the immediate-type reaction in human patients.

It has been reported that patients are more susceptible to adverse reactions when they are afflicted with severe allergies,[39] bronchial asthma, or cardiac disease[40] as well as if they have a history of previous reactions to CA[17,41] or stimulated immune activity[42] at the time of CA exposure, as is the case in patients with autoimmune diseases. In these cases, the threshold for immune activation may be reduced. In the present study, we attempted to avoid the variance in patient medical histories by using a healthy rat population. We observed a reversible immediate anaphylactoid-like reaction after the iodixanol administration, which did not occur after either monomeric iopromide or dimeric iotrolan injection. Therefore, we conclude that a higher induction of adverse skin reactions is not per se because of a class effect of dimeric CAs but is rather specific to iodixanol.

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