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Acute and subacute toxicity studies of CMICE-013, a novel iodinated rotenone-based myocardial perfusion tracer, in Sprague Dawley rats and Gottingen minipigs

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ABSTRACT

Purpose: Extensive acute and subacute toxicities studies are required to evaluate the toxicological profile of the novel cardiac perfusion imaging tracer ^{123}I-CMICE-013 to support applications for clinical trials.
Methods: Sprague-Dawley rats and Gottingen minipigs received injections of non-radioactive 127I-CMICE-013 at two dosage levels of 1 and 5 μg/kg, and vehicle buffer as control. In the acute toxicity studies, each animal was injected on two occasions 24 h apart and then underwent a 14-day recovery period; in the subacute study, animals received daily injections for 14 days continuously. The health status and mortality of test animals were monitored daily and body weight, food consumption, physiological and biochemical parameters were measured at various time points during the study. Animals were euthanized at the end of the studies and dissected for pathologic examination of organs and tissues.
Results: The acute and subacute administrations of injections of the non-radioactive CMICE-013 in rats and minipigs were well tolerated. Little to no dosing-related adverse effects were observed in animal body and organ weights, hematology, coagulation, clinical chemistry, urinalysis, ophthalmoscopy, electrocardiograms, heart rates, blood pressure, macroscopic and microscopic examination of the preserved animal tissues including the brain.
Conclusion: The lack of adverse effects from acute and subacute dosing suggest that the CMICE-013 injection solution has a reasonable safety margin within the designed concentration range to be utilized in imaging applications. The dosage level of 5 μg/kg was considered the no adverse effect level for both rats and minipigs based on our acute and subacute studies.
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1. Introduction

The radioactive tracer ^{123}I-CMICE-013 is a novel myocardial perfusion imaging agent. It chemically resembles rotenone, a pesticide that is known for its specific and potent inhibition of mitochondrial complex I (MC-I), the key component of the ATP-generating mechanism. With iodination at its 7′ position and hydroxylation at its 6′ position, ^{123}I-CMICE-013 retained specific affinity for MC-I binding but had a decrease in structural stability *in vivo* (Wei et al., 2013). MicroSPECT imaging in rats demonstrated that ^{123}I labeled (radiochemical purity ≥ 95%) tracer with high specific activity (400–3000 mCi/μmol) had significant myocardial uptake and delineated myocardial defects in animals with

Abbreviations: MPI, myocardial perfusion imaging; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; HDW, hemoglobin distribution width; PLT, platelets; WBC, white blood cells; NEUT, neutrophils; LYMPH, lymphocytes; MONO, monocytes; EOS, eosinophils; BASO, basophils; LUC, large unstained cells; RETIC, reticulocytes; PT, Prothrombin Time; APTT, Activated Partial Thromboplastin Time; Na, sodium; K, potassium; Cl, chloride; CREA, creatinine; TP, total protein; ALB, albumin; GLOB, globulin; A/G, albumin/globulin ratio; BIL-T, bilirubin (total); CA, calcium; PHOS, phosphorus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatise; GLUC, glucose; CHOL, cholesterol (total); TRIG, triglycerides; NOEL, no observed effect level; NOAEL, no adverse effect level.

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myocardial infarctions and temporary ischemic injury. ^{123}I-CMICE-013 was comprised of four isomers with similar imaging results (Wei et al., 2014). Myocardial perfusion imaging in an ischemic porcine model showed that SPECT imaging with ^{123}I-CMICE-013 generated excellent quality images with favorable heart-to-organ contrast, comparable to the standard tracers such as ^{99m}Tc-labeled tetrofosmin and sestamibi, and ^{201}Tl (Wells et al., 2015).

Toxicity and radiation safety studies in animal models were required to ensure that the tracer has an acceptable safety profile, prior to approval of ^{123}I-CMICE-013 for clinical application. A desirable biokinetic and radiodosimetric character is critical for ensuring minimum radiation exposure to the patients. In the healthy rat model, CMICE-013 showed swift myocardium uptake, rapid liver clearance, and lack of lung activity. Radiodosimetric analysis based on rat data estimated that the tracer contributed medium to mild equivalent human radiation doses to various tissues, such as stomach (1.4–1.6 $\times$ 10^{-2} mSv/MBq), thyroid (1.2–1.4 $\times$ 10^{-2} mSv/MBq), segments of gastrointestinal tracks (6.6–8.4 $\times$ 10^{-3} mSv/MBq), pancreas (6.5–8.0 $\times$ 10^{-3} mSv/MBq), adrenal (5.4–6.7 $\times$ 10^{-3} mSv/MBq), spleen (5.3–6.6 $\times$ 10^{-3} mSv/MBq), liver (5.8–7.5 $\times$ 10^{-3} mSv/MBq), kidney (4.9–6.1 $\times$ 10^{-3} mSv/MBq) and gonads (3.9–6.8 $\times$ 10^{-3} mSv/MBq), an overall modest profile compared to other iodine-123 tracers (Duan et al., 2014). Furthermore, with a moderate whole-body effective human dose of 7.0–7.2 $\times$ 10^{-3} mSv/MBq, ^{123}I-CMICE-013 is comparable to conventional ^{99m}Tc-based MPI agent in terms of radiodosimetry (Duan et al., 2014). A preliminary study of the acute and subacute toxicity of the non-radioactive form of the tracer ^{127}I-CMICE-013 demonstrated no significant adverse effects (Fernando et al., 2014). With the measured specific activity of ^{123}I-CMICE-013 (2000 mCi/μmol), we estimated that the maximum possible mass of the CMICE-013 compound that an adult patient would receive during a single MPI application is approximately 0.02 μg per kg of body mass. Therefore, a high dose level at 5 μg/kg (250-fold in excess) and a low dose level at 1 μg/kg (50-fold in excess) were established for this study. No deaths or adverse reactions were observed among the animals in the acute dosing of CMICE-013. Subacute dosing showed no significant CMICE-013-associated effects on animal behavior, physical wellbeing, parameters of biochemistry, hematology, and histopathology. Attention was paid to the potential cardiotoxicity of the rotenone-based tracer. Echocardiography was used to assess the cardiac function and anatomy of animals before and after dosing. No significant changes were observed between CMICE-013 and vehicle-treated animals.

As a subsequent expansion of our preliminary investigation, the current study aimed to determine the acute and subacute toxicity of non-radioactive CMICE-013 in healthy rats and minipigs by examining the potential impacts in multiple aspects such as behavior, body weight, food consumption, electrocardiograms, blood pressure, ophthalmoscopy, clinical pathology (hematology, coagulation, clinical chemistry and urinalysis), organ weight, and anatomic pathology.

2. Materials and methods

2.1. Compliance and reference

The toxicity studies were conducted in the CiToxLAB facility in Laval, QC. The design and execution of experimentation including compliance to Good Laboratory Practice (GLP) regulation and care and use of laboratory animals were in accordance with 1) ICH M3 (R2): Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, and 2) FDA Redbook 2000: General Guidelines for Designing and Conducting Toxicity Studies.

2.2. Test animals

Thirty-six (36) Sprague-Dawley rats (18 males, 18 females) supplied by Charles River Inc. (St-Constant, QC) were used in the acute and subacute toxicity studies. The animals were between 7 and 8 weeks old at the start of treatment. The body weights ranged from 251 to 341 g for males and from 185 to 240 g for females.

Thirty-six (36) Gottingen minipigs (18 males, 18 females) supplied by Marshall Bioresources (North Rose, NY) were used in the acute and subacute toxicity studies. The animals were between 3 and 4 months of age at the start of treatment. The body weights ranged from 7.5 to 10.8 kg for males and females.

2.3. Preparation of CMICE-013 dosing formulations

The non-radioactive version of CMICE-013 (^{127}I-CMICE-013) was synthesized using methods described in our previous paper (Wei et al., 2013). Briefly, rotenone powder (≥95%) was first dissolved in trifluoroacetic acid (TFA). Under constant stirring, NaI (in 0.1 M NaOH) and iodogen (1,3,4,6-tetrachloro-3a,6a-diphenylglucoluril) in TFA were then added slowly. The reaction was incubated at 60 °C for 45 min. After thorough vacuum-assisted evaporation of TFA, the concentrated mixture was mixed with pure water and dichloromethane to allow liquid–liquid extraction. The dichloromethane phase (containing CMICE-013) was collected and dried, whereas the aqueous phase was discarded. After a second solvent evaporation, an intermediate product was then injected into a Phenomenex Luna C18(2) reverse-phase column (Torrance, CA) for purification. The preparative liquid chromatography was performed on Waters HPLC system (Milford, MA) under a flow rate of 6 mL per min using an isocratic mix of water and 95% ethanol (50/50). The desired CMICE-013 product was collected at approximately 35 min for final evaporation.

Following synthesis, the product powder was re-dissolved and diluted to form a concentrated CMICE-013 solution, which was later sterile-filtered and formulated into final product solution that had 5% ethanol (v/v), 10 mM sodium acetate, and a pH at 5.0. A vehicle solution was also prepared with the same excipients at the same concentration and pH. The concentration of CMICE-013 in the solutions was verified by HPLC before the products were finally dispensed into crimped vials, properly labeled, checked for defects, and stored at −80 °C. Vials of CMICE-013 and vehicle solutions were transferred to the test facility under proper refrigeration conditions and were only thawed before single use. The thawing process was performed in a covered sonication water bath at 23–25 °C.

2.4. Administration of dosing formulations

The detailed dosing regimens are listed in Table 1. The current study adopted the two dosing levels - 1 and 5 μg/kg from the preliminary evaluation as the low and high doses. To test for acute toxicity, rats and minipigs were injected intravenously with ^{127}I-CMICE-013 or vehicle control on two occasions (24 h apart), followed a 14-day recovery period. The current clinical practice of myocardial perfusion imaging requires that the same patient needs to be scanned during both rest and stress (physically or pharmacologically induced) conditions, which requires one tracer injection per scan on the same day or 24 h apart. Therefore, the acute dosing has been designed to mimic the two injections of radioactive ^{123}I-CMICE-013 at rest and stress testing. As for the subacute study, both species of animals were given daily injections of ^{127}I-CMICE-013 or vehicle control for 14 consecutive days. The volume of dosage administered to each animal was calculated and adjusted based on the most recent practical body weight of each animal.

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