TCT-138

Veropaque, A Novel Contrast Formulation, Mitigates Contrast Induced Acute Kidney Injury

S. Biswas, PhD[1], ES Rowe, PhD MBA[1,2], G Mosher, Ph.D.[2], L Insisienmay[1], MK Ozias, MS[1], MR Gralinski, PhD[3] and VD Rowe, MD[1,2]
[1]MidAmerica Neuroscience Research Foundation, [2]Verrow Pharmaceuticals, Inc., [3]CorDynamics, Inc.

Verrow would like to acknowledge Dr. Geoffrey O. Hartzler, a friend, investor, and board member for his insightful input and support of this project.

Disclosures: G Mosher is an employee of Verrow Pharmaceuticals and ES Rowe and VD Rowe have equity positions in Verrow Pharmaceuticals.

Introduction

Contrast-induced acute kidney injury (CI-AKI) continues to be an important complication of contrast administration, particularly in high risk patients. We've recently discovered the utility of substituted cyclodextrins (SCD) for mitigating the renal toxicity of several classes of nephrotoxic agents including antibiotics, anticancer agents and contrast agents (CA). This discovery is the basis for the development of Veropaque, a kidney sparing contrast agent containing iohexol and a SCD.

Here we report on preclinical animal studies using two SCDs and several CAs administered at clinically relevant doses to evaluate kidney pathology and function, mortality, and cardiovascular effects.

Materials

Iopamidol & Iodixanol: Isovue-M 200 (Bracco Diagnostics) and Visipaque 320 (GE Healthcare) were diluted to 150mg Iodine/mL with phosphate buffered saline (PBS) then solid SBECD (sulfobutylether β-cyclodextrin, CyDex Pharmaceuticals) was added and dissolved in various mole ratios.

Iohexol (rodent studies): Omnipaque 300 (GE Healthcare) was diluted 1:1 with PBS then solid SBECD or HPCD (2-hydroxypropyl β-cyclodextrin, CTD, Inc.) was added and dissolved in various mole ratios.

Iohexol (dog studies): Aqueous formulations were prepared containing 350 mg iodine/mL iohexol (Hovione FarmaCiencia SA), 0 or 50 mg/mL SBECD, 0.105 mg/mL edetate calcium disodium hydrate and 1.21 mg/mL TRIS buffer (pH 6.8-7.7). The formulation containing SBECD is Veropaque.

Methods

Rodent Pathology Model: Female C57BL/6 mice (8-10 weeks) and Sprague Dawley male rats (9-11 weeks) were made renally compromised (RC) with a 10 mg/kg intraperitoneal (IP) injection of L-NAME (N-nitro-L-arginine methyl ester) followed in 10 min with 10 mg/kg indomethacin. The test formulations were dosed 20 min later as single 10 mL/kg injections into the tail vein at 1.5g iodine/kg.

The animals were sacrificed with rapid inhalation anesthesia at 24 or 48h post dosing and the kidneys removed and stored in buffered formalin. They were mounted in paraffin blocks, cut into 5 µM sections and stained with H&E and periodic acid Schiff (PAS). The sections were examined by light microscopy and scored for pathology in a blinded fashion.

Blood samples for creatinine determination were taken predose (iv) and at sacrifice (cardiac puncture). The plasma was isolated and stored at –70°C until assayed. Creatinine was measured colorimetrically with QuantiChrom Creatinine Assay Kit (BioAssay Systems, Hayward, CA).

Pathology Evaluation: Kidney sections were evaluated at 400x magnification. Five randomly selected fields in each section were assessed for the occurrence of:

- dilated tubules
- loss of brush border
- tubular casts
- edema/mononuclear infiltration
- vacuoles
- tubular degradation

and scored in a blinded manner on a scale of 0-4. A total of 4 sections were analyzed per kidney. The 20 assessments for each parameter were averaged and reported as an average score per field. Total pathology score, a summation of the average scores for the six parameters, is used in some figures for efficiency of presentation. All error bars are SEM.

Rodent Survival Model: Male Sprague Dawley rats (9-11 weeks, 8/group) received IP L-NAME and indomethacin as above followed by IV placebo, iohexol or Veropaque at 2.5 g I/kg. Their survival was then monitored for 14 days.

Instrumented Cardiovascular Dog Model: Male Beagle dogs were anesthetized with propofol, intubated, and placed on isoflurane gas anesthesia. Morphine (0.5mg/kg) was used for pain management in this open chest procedure.

A Swan-Ganz catheter for measurement of right heart pressure was inserted into the jugular vein, advanced to the pulmonary artery 'wedge' position. A solid-state high fidelity pressure catheter (Millar) for left ventricular pressure and aortic pressure was inserted into the carotid artery. Both were secured with suture.

The surface lead ECG was recorded continuously via electrodes placed on the right arm, left leg and chest of the dog. ECGs were continuously recorded throughout the experiment, reporting PR, QRS, QT, QTc(VdW) and heart rate. Monophasic action potential was recorded (when possible) via left ventricular epicardial probe.

Each formulation (iohexol or Veropaque) was bolused into the left main coronary artery as 5 doses of 4 mL each, administered at ~1mL/sec with 10 seconds between doses. Thirty minutes after the last dose, the procedure was repeated with the second formulation. The overall process was repeated in two additional animals.

Results and Discussion

Kidney Pathology

A single dose of iohexol caused significant pathological changes to the kidneys of the RC rodents. The photomicrograph in Figure 1 illustrates the typical pathology seen at 24h in RC mice that received a single 1.5 g iodine/kg dose of iohexol or Veropaque (iohexol:SBECD mole ratio of 1:0.025). Iohexol treated kidneys indicate pathological changes in both the renal cortex (A) and medulla (C) such as tubular vacuolation, tubular dilatation (big arrow), cast formation (thin arrow), loss of brush border (arrow heads) and focal edema (E). Concurrent SBECD administration significantly attenuated the morphological changes in both cortex (B) and medulla (D).

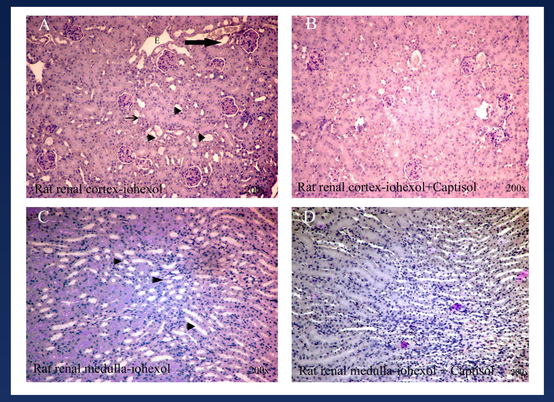


Figure 1. Light microscopy of renal tissue of mouse (H&E, PAS, 200x)

The corresponding kidney pathology scores are presented in Figure 2 for the deep renal cortex/outer medulla. The presence of the SBECD in Veropaque provides dramatic reduction in the pathology at 24h. Although not presented here, similar protection is also observed in the outer cortex and in both regions at 48h, though the overall toxicity is reduced at 48h. The individual pathology scores can be added to give a total pathology score as shown in Figure 3, which shows the results for the outer renal cortex for both iohexol and iodixanol in the presence and absence of SBECD.

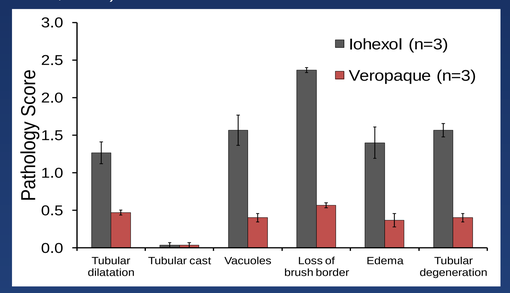


Figure 2. Pathology in the deep renal cortex and outer renal medulla of mice at 24h

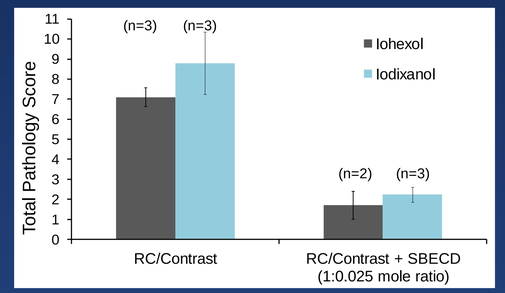


Figure 3. Total renal pathology scores in the outer renal cortex of mice at 48h

The nephroprotection can also be demonstrated in a rat model. Figure 4 illustrates the effects of the contrast agent: cyclodextrin mole ratio for iohexol and iopamidol. A dramatic reduction in pathology is observed in the presence of SBECD, and in a dose dependent manner. As mole ratios increase greater than about 1:0.05 (not shown), the pathology score begins to increase due to the known effects of higher doses of the SCD on the kidney tissues.

Several cyclodextrins have been evaluated and shown to provide nephroprotection of varying degrees. Figure 5 illustrates the reduction in outer renal cortex pathology scores from iohexol in the presence of increasing amounts of a different cyclodextrin, HPCD. Veropaque (iohexol with SBECD) is shown for comparison. SBECD provides slightly greater efficacy than HPCD, generating a pathology score comparable to the RC control values.

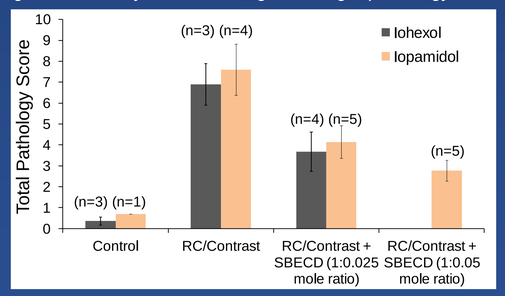


Figure 4. Total renal pathology scores in the outer renal cortex of rats at 24h

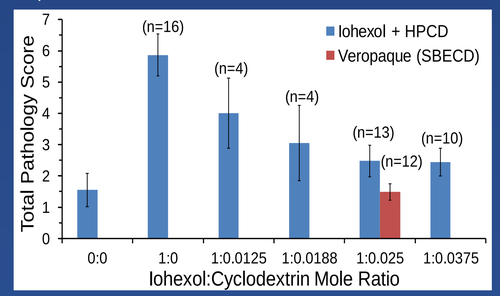


Figure 5. Effect of iohexol:cyclodextrin mole ratio on the total pathology score in the outer renal cortex of rats at 48h

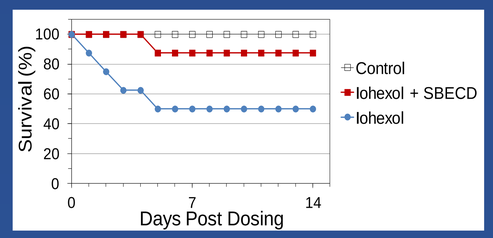


Figure 6. Survival of RC rats receiving single IV 2.5g I/kg doses of iohexol or iohexol + SBECD.

14-Day Survival Study

The nephrotoxicity of contrast agents can lead to mortality in rodents when larger doses are administered. Only half (4 of 8) survived a single dose of 2.5g I/kg iohexol as shown in Figure 6. The presence of SBECD at an iohexol: SBECD mole ratio of 1:0.025 reduced the nephrotoxicity and raised survival to 88% (7 of 8)

Kidney Functionality

The functionality of the kidney in terms of plasma creatinine levels, is also maintained in both rats and mice in the presence of a SCD (Figure 7). Iohexol dosed at 1.5g I/kg to RC rodents caused an increase in plasma creatinine especially in the mouse model. No increase was observed when the SCD was present with the iohexol.

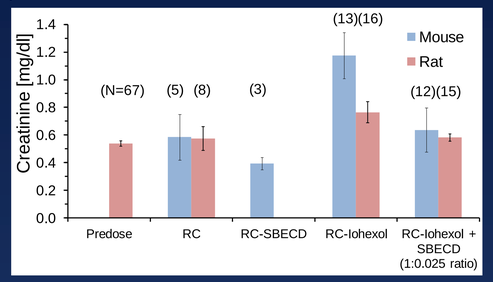


Figure 7. Plasma creatinine levels at 24h (mouse) or 48h (rat) post treatment in RC rodents

Cardiovascular Assessment

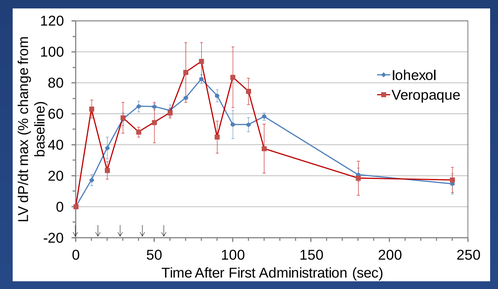
The Veropaque formulation contains ~154mM sodium from the SBECD, and its effects on the cardiac electrophysiology was compared to iohexol after direct injection into the left coronary artery of instrumented dogs.

There were no notable effects of intracoronary iohexol administration on most measured cardiovascular parameters. Variables including LV contractility (Fig 8) and QTc interval (Fig 9) were notably, yet transiently, altered following both iohexol and Veropaque, a finding consistent with the literature for iohexol†.

In addition to these transient quantitative changes, qualitative alterations in electrocardiographic morphology were observed for both formulations. These were generally concomitant with physical injection of the formulations into the coronary artery, and likely associated with brief myocardial ischemia from interruption of arterial flow. The changes consisted of QRS complex widening along with ST segment depression. Scattered premature ventricular contractions were also noted. Within 5 min after the end of each injection, ECG morphology returned to normal for each formulation.

(†Jacobsen, et al, Repeated intracoronary injections of contrast media, additive hemodynamic and electrophysiologic effects in a dog model. Investigative radiology, 28(10) (1993) p. 917-924.)

Figure 8. Left ventricular contractility changes following bolus dosing



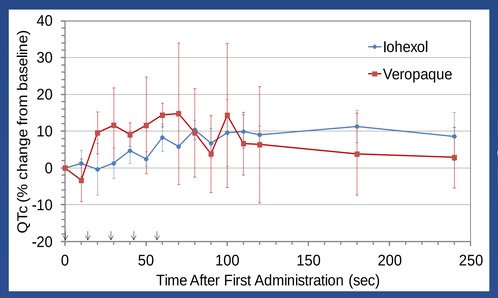


Figure 9. QTc interval changes following bolus dosing

Conclusions

- Substituted cyclodextrins protect the kidney from the nephrotoxicity of contrast agents in two animal models, at clinically relevant doses of several contrast agents including iohexol, iodixanol, and iopamidol.
- Direct intra-coronary injection of the Veropaque formulation into instrumented dogs showed no differences from injection of iohexol alone.
- The kidney protection occurs at mole ratios below 1:1 suggesting a mechanism other than complexation of iohexol with cyclodextrin.

Based on these and other data, we believe that Veropaque has the potential to markedly decrease the incidence of CI-AKI in high risk patients undergoing cardiology procedures. Development is in progress.

TCT2012