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

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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 SBECD 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: Iovue-M 200 (Braconn Diagnostics) and Visipaque 320 (GE Healthcare) were diluted to 150 mg iodine/mL with phosphate buffered saline (PBS) HEPES (Hyperionic phosphate-Buffered-Enzyme-Substrate-Compounds) 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 HP-CD (a hydroxypropyl-β-cyclodextrin, CTD, Inc.) was added and dissolved in various mole ratios.

Iohexol (dog studies): Aqueous formulations were prepared containing 350 mg iodine/Iohexol (Hovione Farmaca SIA), 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.

RESULTS AND DISCUSSION
Kidney Pathology
A single dose of iohexol caused significant pathology in 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 (i.e., SBECD mole ratio of 1.0:0.025). Iohexol treated kidneys indicate pathologic changes in both the renal cortex (A) and medulla (B) such as tubular vacuolation, tubular dilation (arrow), cast formation (thin arrow), loss of brush border (arrow head), and focal edema (E). Concurrent SBECD administration significantly attenuated the morphologic changes in both cortex (B) and medulla (D).

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 SBECD in the presence and absence of SBECD.

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

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 iohexol. The test formulations were dosed 20 min later as single 10 mL/kg injections into the tail vein at 1.5 g iodine/kg.

The animals were sacrificed with rapid intrahepatic anesthesia at 24 or 48 post dosing and the kidneys removed and stored in buffered formalin. They were mounted in paraffin and cut into 5 sections per slide. Kidney pathology scores were added using pathologist’s guide (H&E, 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
- edema/mosaic/necrosis
- loss of brush border
- vascularity
- tubular casts
- tubular cell degeneration

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 shown in some figures for efficiency of presentation. All error bars are SEM.

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

In addition to these transient quantitative changes, qualitative alterations in electrophysiologic morphology were observed for both formulations. These were generally corroborated with histopathological changes that included the formation of casts and vacuoles in the renal cortex, and likely associated with brief myocardial ischemia from interruption of arterial flow. The changes consisted of QRS complex widening 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 in both test formulations. Iohexol was used for further in vivo studies (mouse).

Cardiovascular Assessment
The Veropaque formulation contains ~154 mM 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.

CONCLUSIONS
- Substituted cyclodextrins diminished toxicity 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 a difference from 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 cardiac procedures. Development is in progress.