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# A nephroprotective iodinated contrast agent with cardioprotective properties: A pilot study

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## Abstract

**Background and Purpose:** Evaluation and treatment of acute ischemic syndromes, in the heart and brain, require vessel visualization by iodinated X-ray contrast agents. However, these contrast agents can induce injury, in both the kidneys and target organs themselves. Sulfobutylether beta cyclodextrin (SBECD) added to iohexol (SBECD-iohexol) (Captisol Enabled-iohexol, Ligand Pharmaceuticals, Inc, San Diego, CA) is currently in clinical trials in cardiovascular procedures, to determine its relative renal safety in high-risk patients. Preclinical studies showed that SBECD-iohexol reduced contrast-induced acute kidney injury in rodent models by blocking apoptosis. The current study was undertaken to determine whether SBECD-iohexol is also cardioprotective, in the male rat ischemia-reperfusion model, compared to iohexol alone.

**Methods:** After anesthesia, the left coronary artery was ligated for 30 min and the ligation released and reperfusion followed for 2 h prior to sacrifice. Groups 1–4 were injected in the tail vein 10 min prior to ischemia with: (1) vehicle; (2) iohexol; (3) SBECD; and (4) SBECD-iohexol. Infarct size, hemodynamics, and serum markers were measured.

**Results:** An eight-fold increase in serum creatine kinase in the iohexol-alone group was observed, compared with no increase in the SBECD-iohexol group. The mean arterial pressure and rate pressure product were depressed in the iohexol-alone group, but not in the SBECD-iohexol group, or controls. No difference in infarct size or serum creatinine among the groups was observed.

**Conclusion:** The results of this study suggest that SBECD-iohexol is superior to iohexol alone, for both the preservation of cardiomyocyte integrity and preservation of myocardial function in myocardial ischemia.

KEYWORDS
cardiotoxicity of contrast, contrast for interventional procedures, iodinated contrast safety, protection from contrast, safe

## INTRODUCTION

The diagnosis and treatment of acute ischemic syndromes, both in the heart and brain, require the intra-arterial injection of iodinated X-ray contrast agents for vessel visualization. However, these contrast agents can have deleterious side effects, both on the kidneys as the agents are cleared from the body, and on the target organs themselves.[1–6] There clearly is a need for an iodinated contrast agent that is safer for both the kidneys and target organs of visualization, including both the heart and brain.

The importance of endovascular procedures in the treatment of large vessel intracranial proximal arterial occlusion in acute

ischemic stroke is now well established.[7-17] Multiple modifiable factors, however, can affect the outcomes of patients undergoing these procedures.[18-23] The neurotoxicity of iodinated contrast has long been recognized. Encephalopathy occasionally occurs as a complication of coronary and brain angiography.[4,5,24-34] While encephalopathy is relatively rare, the known neurotoxicity of contrast for neuronal tissue could play a role during angiographic procedures in more subtle ways.

Acute kidney injury induced by contrast agents (CI-AKI) occurs in a significant number of interventional cardiology procedures in high-risk patients. In addition, a recent report suggests that 13% of patients undergoing elective diagnostic coronary angiography show elevated serum troponin T levels in the absence of clinical or other signs of procedural ischemia, and that the volume of contrast agent used was an independent predictor of that elevation.[35]

Thus, multiple organ systems, including those that are the targets of vascular visualization, can be vulnerable to exposure to iodinated contrast agents, highlighting the need for safer such agents.

As we previously reported in this journal, SBECD (trade name Captisol, Ligand Pharmaceuticals, Inc, San Diego, CA) was shown to mitigate CI-AKI in laboratory animals.[36] A reformulation of iohexol with SBECD is now in phase II clinical trials in the United States, for prevention of CI-AKI in vulnerable patients undergoing elective coronary angiography. This trial is intended to determine whether those nephroprotective properties of the reformulation, found in multiple species of laboratory animals, can be translated to the clinic. SBECD is widely used in marketed drug formulations to increase solubility, including remdesivir, one of the few approved treatments for Covid-19.[37] However, in our studies, only one molecule of SBECD per 40 molecules of iohexol is necessary for nephroprotection, ruling out a solubilization mechanism in this case.[36]

We previously showed that the mechanism by which this nephroprotective effect was achieved was the inhibition of contrast-induced apoptosis in renal cell tissue cultures.[36] Since apoptosis plays a major role in myocardial ischemic tissue damage,[38-40] we wondered whether SBECD-iohexol might protect the ischemic myocardium in a rat model of myocardial ischemia-reperfusion. In short, we wondered whether SBECD-iohexol might be cardioprotective as well as nephroprotective.

If it is cardioprotective, SBECD-iohexol could be neuroprotective as well, since apoptosis also plays a major role in tissue damage in acute ischemic stroke.[41-43] Since multiple modifiable risk factors can lead to improved stroke outcomes, it is possible that the use of a safer iodinated contrast agent could further improve stroke outcomes for patients undergoing endovascular evaluation and treatment.

## METHODS

### Materials

SBECD (trade name Captisol, Ligand Pharmaceuticals, Inc) and iohexol (Omnipaque 300, GE) were used in the concentrations shown in Table 1.

### Experimental procedure

The rat ischemia/reperfusion model has been well established and is a widely used animal model for studying myocardial ischemia.[44,45] A total of 24 male Sprague–Dawley rats (0.318–0.354 kg) were used in this pilot study and allocated into five groups, see Table 1.

All rats were anesthetized via an intraperitoneal injection of Nembutal. Anesthetic plane was maintained by administering additional half doses of Nembutal as needed. Once consciousness was lost, the trachea was isolated for mechanical ventilation with room air. Depth of anesthesia was monitored throughout the experiment by observing the toe pinch reflex at least every 15 min. Animal body temperature was maintained at approximately 37°C throughout the procedure. A lead II electrocardiogram was monitored throughout the experiment via electrodes placed in the skin of the right arm, left leg and chest of the animal. An indwelling catheter was placed into the left jugular vein for collection of a blood sample, and another indwelling catheter was placed into a lateral tail vein for test compound administration. A femoral artery was cannulated for measurement of systemic arterial pressure by Millar catheter. Left thoracotomy (through the fourth intercostal space) and pericardiotomy were performed. A 6-0 silk suture was introduced around the left anterior descending coronary artery (LAD) midway between atria and apex. A small piece of plastic tubing (PE50) that was larger in diameter than the artery was placed on top of the artery and left in place until induction of ischemia.

Hemodynamic parameters were monitored during the approximately 10-min equilibration period. After hemodynamic parameters reached steady-state, animals were monitored for 10 min to establish baseline values. After the baseline period, animals in Groups 1–4 received an intravenous infusion of either vehicle or test article (Figure 1) into the tail vein over a 2-min period; animals were monitored for an additional 8 min. At the end of the dose monitoring period for animals in Groups 1–4, or at the end of baseline for animals in Group 5, the suture was secured around both the tubing and the artery to totally occlude the left LAD and induce ischemia. The tubing was removed after 30 min of ischemia to initiate the 120-min reperfusion period. Animals in Group 5 received an intravenous infusion of the test article into the tail vein over a 2-min period at the beginning of reperfusion. Refer to Figure 1 for further details.

Blood samples were obtained from the jugular vein cannula following the ischemia/reperfusion period and serum levels of creatine kinase (CPK) and creatinine determined.

Infarct size was determined by staining the hearts with India Ink/2,3,5-triphenyltetrazolium chloride (TTC). Briefly, while rats were still anesthetized, hearts were religated and India ink was injected via a tail vein until normally perfused myocardium was adequately stained. Hearts were then removed and sectioned transversely into 1 mm thick slices. The right ventricle, apex, and atria were discarded, and the left ventricle slices were incubated at 37°C for 10–15 min in a 1% TTC in phosphate buffered saline. TTC solution stains the area at risk (AAR) for infarction with a reddish color and the infarcted tissues appear pale yellow. After staining, heart slices were transferred to 10% neutral

**TABLE 1** Rat model of ischemia/reperfusion injury study design
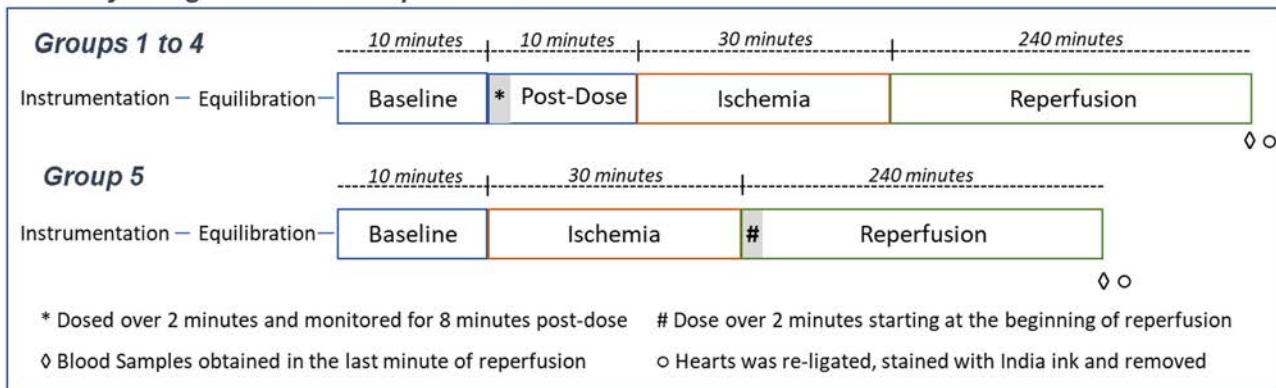
**A- Treatments**

Group 1: Vehicle (sterile saline for injection 5 ml/kg, U.S.P.; n=4)

Group 2: Iohexol 1500 mg/kg (n=4)

Group 3: SBECD 225 mg/kg (n=3)

Group 4: Iohexol 1500 mg/kg + SBECD 225 mg/kg (n=6)

Group 5: Iohexol 1500 mg/kg + SBECD 225 mg/kg (n=5)

**B- Study Design – Ischemia/Reperfusion**

| Groups 1 to 4 | 10 minutes | 10 minutes | 30 minutes | 240 minutes |
|---|---|---|---|---|
| Instrumentation — Equilibration— | Baseline | * Post-Dose | Ischemia | Reperfusion |

| Group 5 | 10 minutes | 30 minutes | 240 minutes |
|---|---|---|---|
| Instrumentation — Equilibration— | Baseline | Ischemia | # Reperfusion |

\* Dosed over 2 minutes and monitored for 8 minutes post-dose     # Dose over 2 minutes starting at the beginning of reperfusion

◊ Blood Samples obtained in the last minute of reperfusion     ○ Hearts was re-ligated, stained with India ink and removed

\*Dosed over 2 min and monitored for 8 min post-dose

#Dose over 2 min starting at the beginning of reperfusion

◊Blood samples obtained in the last minute of reperfusion

○Hearts were religated, stained with India ink, and removed

nNumber of animals in a group

Note: All animals underwent coronary artery occlusion for 30 min followed by 2 h of reperfusion. Rats were treated, according to the indicated schedule, with vehicle (saline 5 ml/kg, Group 1), iohexol (1500 mg/kg, Group 2), sulfobutylether beta cyclodextrin (SBECD) (225 mg/kg, Group 3), or a combination of SBECD-iohexol (1500–225 mg/kg, Groups 4 and 5).

buffered formalin for at least 24 h and then placed between two glass slides. Both sides of the slide were scanned using a flatbed scanner and scanned images were digitized and analyzed using Image J 1.46r software.

## RESULTS

### Hemodynamic parameters

Figure 1A shows a graph of mean arterial pressure (MAP) for Groups 1–4, showing that all groups declined throughout the study prior to sacrifice. However, MAP for the animals in the SBECD-iohexol group tracked much closer to the vehicle group than did the animals receiving iohexol alone, even in the preischemic phase of the study.

Figure 1B shows a graph of the rate–pressure product (RPP) for Groups 1–4. This measurement is held to be a surrogate measure of myocardial oxygen consumption and cardiac workload. The RPP for the iohexol alone group tracked considerably lower than the group treated with SBECD-iohexol, which was similar to the other two groups, even during the pre-ischemic phase of the study.

Group 5 animals, not shown here, which received SBEDC-iohexol after ligation rather than before, tracked with the vehicle group 1.

## Clinical chemistry

### Creatine kinase

The bar graph in Figure 2A shows the results obtained for serum CPK values immediately prior to sacrifice. An eight-fold increase in the mean serum CPK value was seen in the iohexol alone group versus all other groups, including the SBECD-iohexol group. This suggests significant damage to ischemic cardiomyocytes. This cardiomyocyte damage was significantly mitigated in the SBECD-iohexol group.

### Creatinine

Figure 2B shows the results of serum creatinine measured immediately prior to sacrifice. All the groups had values that were within the error of the measurement, to be expected for this short-duration study.
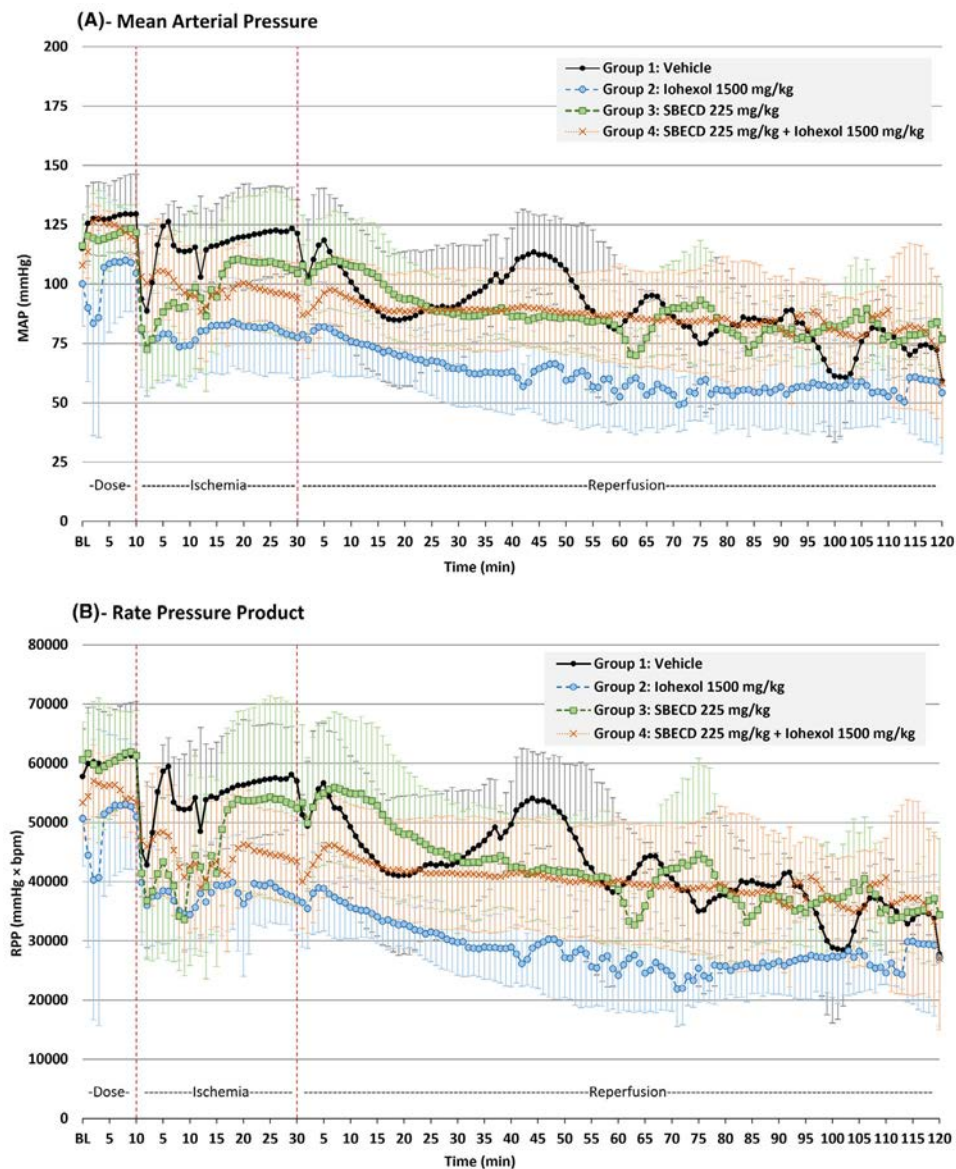
**FIGURE 1** Mean arterial pressure (MAP) (A) and rate pressure product (RPP) (B) in the rat model of ischemia/reperfusion (30 min/2 h) injury. Rats were treated with vehicle (saline, Group 1; $n = 4$), iohexol (1500 mg/kg, Group 2; $n = 4$), sulfobutylether beta cyclodextrin (SBECD) (225 mg/kg, Group 3; $n = 3$), or a combination of SBECD-iohexol (1500–225 mg/kg, Group 4; $n = 6$). Animals in Groups 1–4 received an intravenous infusion of either vehicle or test article into the tail vein over a 2-min period after the baseline (BL) period. Animals were monitored for the duration of the study. Values are expressed as mean ± standard deviation

## Infarct size

The AAR for each group is shown in Figure 3A. This represents the percentage of the left ventricular volume that experienced ischemia during coronary artery ligation. Figure 3B shows the percentage of the AAR determined to be infarcted.

With the small number of animals in each group in this pilot study, the differences among the groups did not reach statistical significance for infarct size. However, the wide range of values for Group 4, the SBECD-iohexol group, suggests that some animals in that group did have a smaller infarct size than those in the other groups.

## DISCUSSION

The safety of FDA-approved iodinated contrast agents for clinical procedures involving X-ray technology, and the necessity of their use in modern clinical medicine, is generally accepted. But since these agents can cause kidney injury in vulnerable patients, and since these vulnerable patients are frequently the ones who require intravascular injection of contrast, considerable effort has gone into reducing the risk of this complication. Thus, SBECD-iohexol, now in clinical trials, could represent a major improvement in the safe use of iodinated contrast in vulnerable populations, based on a reduction of
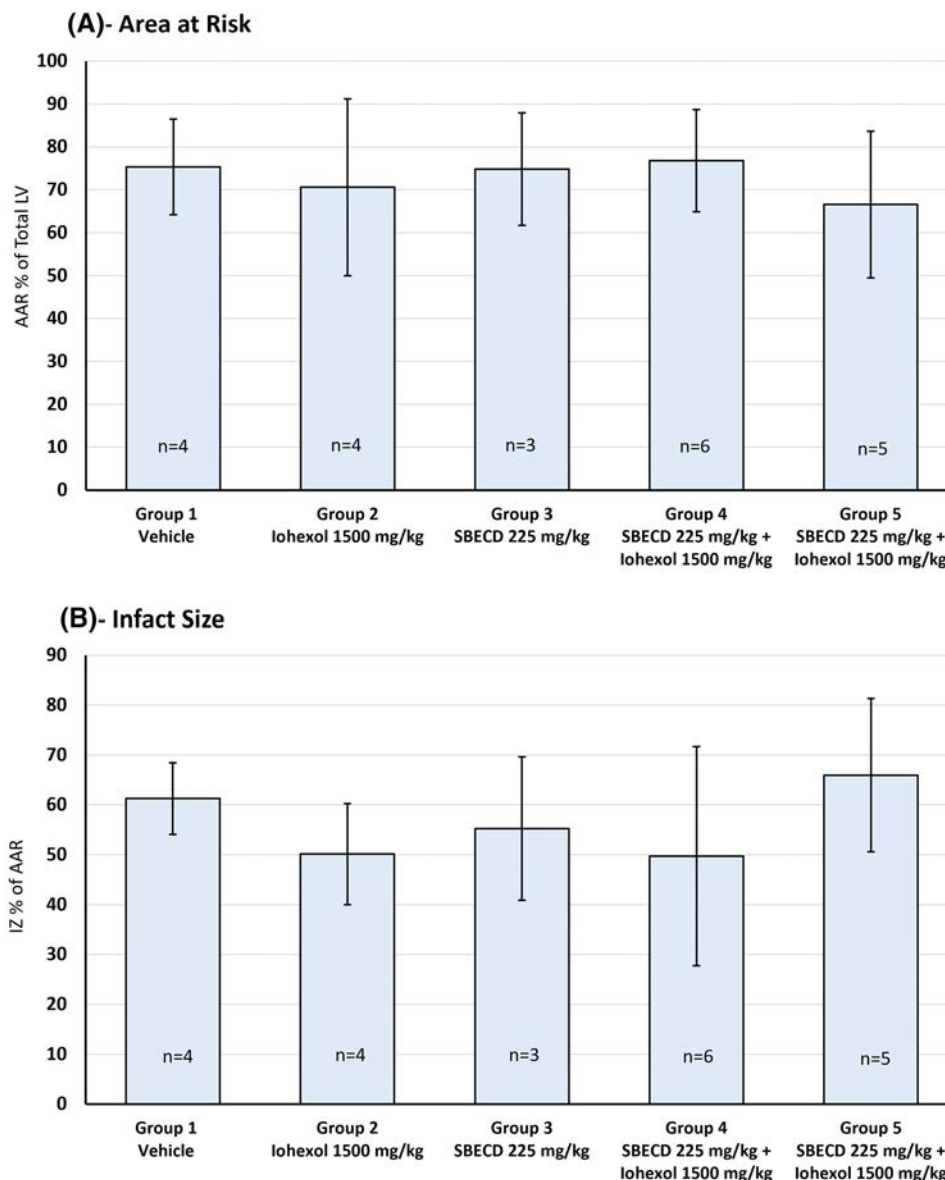
**(A)- Creatine Kinase**
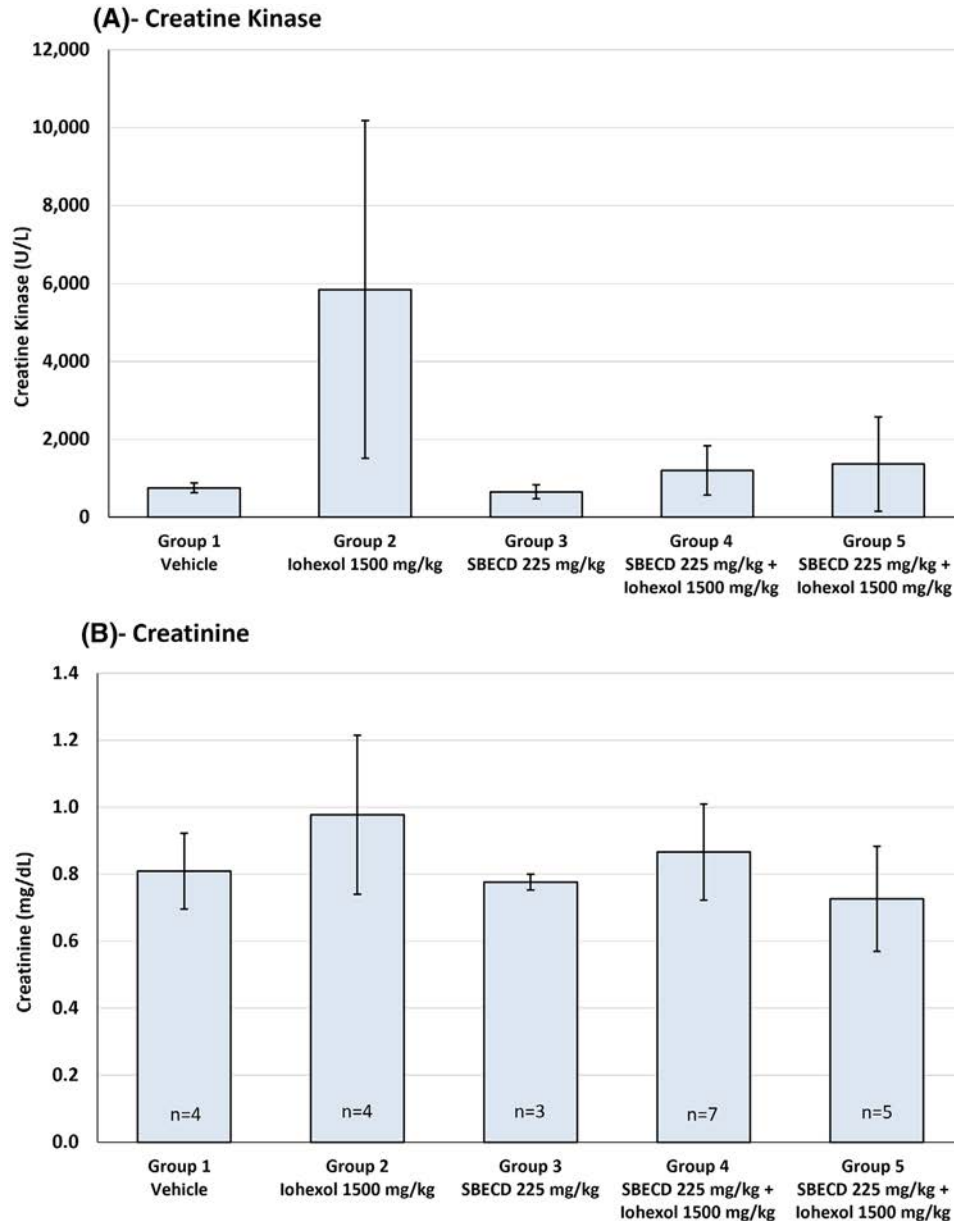
**(B)- Creatinine**

**FIGURE 2**    Effect of iohexol (1500 mg/kg, Group 2), sulfobutylether beta cyclodextrin (SBECD) (225 mg/kg, Group 3), or a combination of SBECD-iohexol (1500–225 mg/kg, Groups 4 and 5) on the circulating levels of creatine kinase (A) and creatinine (B) after ischemia/reperfusion (30 min/2 h) injury in the rat. Animals in Groups 1–4 received an intravenous infusion of either vehicle or test article into the tail vein over a 2-min period after the baseline period; animals were then monitored for an additional 8 min. Animals in Group 5 received an intravenous infusion of the test article into the tail vein over a 2-min period at the beginning of reperfusion. Values are expressed as mean ± standard deviation. $n$ = number of animals in each group

CI-AKI alone. This benefit would apply to all uses of iodinated contrast, including in endovascular diagnosis and therapy by neurologists, radiologists, interventional radiologists, neuroradiologists, as well as cardiologists. In addition, it is possible that SBECD-iohexol can improve clinical outcomes in other ways. For instance, in the current study, there was an eight-fold increase in serum creatine kinase in the animals treated with iohexol alone compared to the group receiving SBECD-iohexol. Likewise, there was diminished cardiac function in the iohexol-alone group compared to the SBECD-iohexol group. This suggests that adding SBECD to iohexol, in the proper ratio, is cardioprotective for ischemic heart tissue, an addition to its nephroprotective effects.

In animal models of brain ischemia, produced by ischemia/reperfusion and cardiac arrest/reperfusion, as well as in direct brain ischemia models, apoptosis has been shown to be an important factor in ultimate tissue damage.[41-43] We previously showed that iodinated contrast agents, including iohexol (Omnipaque, GE), iodixanol (Visipaque, GE), and iopromide (Ultravist, Bayer), induced apoptosis in cultured HK-2 cells, and that for all of these agents, the addition of SBECD in the appropriate ratio blocked that apoptosis.[36] Thus, it is

## (A)- Area at Risk



## (B)- Infact Size



**FIGURE 3** Myocardial infarction in the rat model of ischemia/reperfusion (30 min/2 h) injury. (A) Area at risk (AAR; % of left ventricle [LV]) and (B) infarct size (IZ, % AAR) in rats treated with vehicle (saline, Group 1), iohexol (1500 mg/kg, Group 2), sulfobutylether beta cyclodextrin (SBECD) (225 mg/kg, Group 3), or a combination of SBECD-iohexol (1500–225 mg/kg, Groups 4 and 5). Animals in Groups 1–4 received an intravenous infusion of either vehicle or test article into the tail vein over a 2-min period after the baseline period; animals were then monitored for an additional 8 min prior to initiation of ischemia. Animals in Group 5 received an intravenous infusion of the test article into the tail vein over a 2-min period at the beginning of reperfusion. Values are expressed as mean ± standard deviation. *n* = number of animals in each group

reasonable to hypothesize that SBECD-iohexol might be protective for the brain in acute ischemic stroke as well.

The toxicity of iodinated contrast agents for many cell types has been widely studied (brain, intervertebral disc, and cardiomyocytes),[5,26,28,46–49] though renal cells have received the most attention. For example, in a recent study of patients undergoing elective coronary angiography, 13% of asymptomatic patients exhibited an increase in troponin T, indicating cardiomyocyte damage after a purely diagnostic coronary angiogram. The volume of contrast used was an independent variable predicting this elevation. No appreciable difference among several iodinated contrast agents was noted.[35]

Neurologic complications, including encephalopathy, can occur after myocardial infarction and coronary intervention, without obvious arterial occlusion. Encephalopathy may be related to the volume of contrast used.[24,25]

In a general way, the apoptotic process appears to be present in many chronic neurodegenerative processes as well as the more acute syndromes mentioned above.[50–53] Because of this, there have been many attempts to identify agents and methods to reduce apoptosis in brain, heart, and other vulnerable tissues.[54–58] Whether or not reformulations with SBECD will affect these diseases must await further study.

In conclusion, the results of this study suggest that SBECD-iohexol is superior to iohexol alone, for both the preservation of cardiomyocyte integrity and preservation of myocardial function in myocardial ischemia. Further studies of this new contrast agent in additional tissues, particularly in neuronal tissue, are warranted. If the current clinical trials of SBECD-iohexol (Captisol Enabled-iohexol, Ligand Pharmaceuticals, Inc) are successful and this new contrast agent is approved, it may contribute to improved outcomes, not only in cardiovascular procedures, but also in neurointerventional procedures.

## ACKNOWLEDGMENTS AND DISCLOSURE

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