Journal of Animal and Veterinary Advances 13 (15): 945-951, 2014
ISSN: 1680-5593

Assessment of Ketamine/Dexmedetomidine Anesthesia in Renal Ischemia-Reperfusion Injury in Nude Rats

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Abstract: In order to perform surgical manipulations for model development an appropriate anesthetic protocol is important to avoid mortality and morbidity that negatively impact a study. Reversible injectable anesthetics are favored in comparison to non-reversible anesthetics because of the presumed safety. Rats responses to injectable anesthetics are determined by the drug, route and individual differences. The group studies kidney regeneration but researchers must first surgically produce a model of chronic renal failure which requires anesthesia. In this study, researchers report the mortality results of using four different anesthetic protocols, Ketamine-Dexmedetomidine (KD), Intramuscularly (IM) with ketoprofen and Buprenorphine (BP); KD-Intraperitoneally (IP) with ketoprofen and buprenorphine; KD and BP IM and Pentobarbital (PB) and BP, IP. Male Nude Rats (250-350 g; n = 40) were anesthetized with anesthetic regiments noted above. After a laparotomy, a bilateral 60 min renal ischemia followed by reperfusion procedure was done. The animals were given antipamezole, a reversal agent when appropriate. PB with BP, IP significantly improved mortality rates at 20%. This study shows that kidneys exposed to ischemia/reperfusion result in renal tissue damage as well as decreased renal function. The results showed increased mortality using ketamine and significantly with favorable survivability using pentobarbital. Future, studies are needed to determine how pentobarbital provided mortality protection.

Key words: Renal ischemia-reperfusion, (ARF) Acute Renal Failure, (CRF) Chronic Renal Failure, appropriate, significantly

INTRODUCTION

Renal Ischemia and Reperfusion injury(I/R) leading to Acute Renal Failure (ARF) is common in several clinical situations including kidney transplantation, hemorrhagic shock, major vascular surgery and certain hypotensive states (Muller *et al*., 2002; Bagheri *et al*., 2011). As a consequence of arterial occlusion leading to a deprivation of oxygen carrying blood, cellular respiration is impaired with irreversible damage. This occurs virtually to every organelle and subcellular system of the affected cells of the organ (Serviddio *et al*., 2008). Efforts to decrease I/R have been the subject of many previously performed studies (Muller *et al*., 2002; Durrani *et al*., 2006; Senturk *et al*., 2008; Serviddio *et al*., 2008; Curtis *et al*., 2011). In the current studies, researchers propose to create a model of Chronic Renal Failure (CRF) in nude rats with a similar I/R procedure to test a regenerative medicine approach for CRF treatment. This model has its limitations. Researchers expected a 20% mortality rate within the first 3 days after the surgery due to complications from the induced ARF. It has been reported that I/R injury can cause up to 92% mortality rate in male rats and 25% mortality in female rats with the disparity due to unknown sexual dimorphism (Muller *et al*., 2002). The ARF signs caused by this procedure leading to death include hyperkalemia, decreased GFR and azotemia (Muller *et al*., 2002).

As with any surgical procedure and in keeping with the 3R's Methods of Refinement an appropriate anesthetic protocol is paramount for a successful surgery and recovery of the patient (Institute for Laboratory Animal Research, 1996). Ketamine, a dissociative anesthetic is often combined with other agents to provide a surgical plane of anesthesia (Fish *et al*., 2008). Ketamine-xylazine is the most commonly used and most reliable combination anesthetics for routine use with rats (Fox *et al*., 2002). Xylazine is an α 2-adrenoceptor agonists used in anesthesia and critical care as they not only decrease sympathetic tone and attenuate the stress responses to anesthesia and surgery but also causes a reversible sedation and analgesia (Fox *et al*., 2002). In the study, researchers used dexmedetomidine which is the most recent agent in this group. It was approved by FDA

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in 1999 for use in humans for analgesia and sedation. Dexmedetomidine has >10 times the affinity for the α-2 receptor than xylazine. Therefore, it is thought to be safer and a more predictable compound (Paris and Tonner, 2005). Most common adverse reactions associated with dexmedetomidine are hypotension and the resultant bradycardia (Arcangeli *et al*., 2009). Barbiturates such as pentobarbital were once the dominant drugs for rodent anesthesia (Fox *et al*., 2002). It causes a dose dependent respiratory and cardiovascular depression and is not reversible (Fox *et al*., 2002). Although, researchers eventually used pentobarbital, it had previously fallen out of favor in the group due to the presumed safety of the former mentioned anesthetics. Researchers did not evaluate gas anesthesia due to increased mortality associated with a previous protocol.

In addition to choosing the correct anesthetic, analgesics and prophylactic antibiotics are given to improve post-operative outcomes (Fish *et al*., 2008). Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as ketoprofen and carprofen and opioids such as buprenorphine are common analgesics given for rodent surgery (Fish *et al*., 2008). The lab's I/R Model differs from many groups by creating CRF and not an ARF Model. Researchers hope to show in future studies the ability of primary injected renal cultured expanded cells from donor rats to improve renal functions in a rat model of renal impairment. Researchers will evaluate the functional recovery of these diseased kidneys by assessing renal function levels. Due to the high mortality rate associated with the I/R procedure mentioned earlier, researchers hypothesized that the variability in anesthetic regiments would have a direct effect in survivability of animals throughout the ARF period of recovery.

In this study, researchers determined the best anesthetic protocol for the I/R procedure by comparing the mortality rates while using 4 different anesthetic protocols. Researchers will also show renal function parameters Blood Urea and Nitrogen (BUN) and serum creatinine differences between the four groups.

MATERIALS AND METHODS

Animals: Male Athymic Nude Rats (RNU Rat) 200-350 g; n = 42 were obtained from a commercial vendor (Charles River Laboratories, Raleigh, NC). For this study, researchers used 4 groups of 10 rats and 2 naive rats for histologic samples. This is based on the number of animals presented for surgery on the respective days. All rats were healthy, pathogen free and group housed in an American Association of Laboratory Animal Care approved facility in a temperature-controlled room (22±2°C) with a 12:12 h light-dark cycle (lights on 6:00 am to 6:00 pm) and allowed free access to food and water. The rats are fed Certified Rodent Diet 5002 (Lab diet Purina, St. Louis, Mo), the rats were allowed at least 10 days to acclimate to the new environment post shipping prior to surgical interventions. These procedures were approved by the Wake Forest University School of Medicine Institutional Animal Care and Use Committee as part of a larger study.

Anesthesia protocol: The rats were divided into 4 batches of 10 animals. One batch per experimental day presented for I/R surgery. The initial anesthetic protocol (Plumb, 2008) consisted of ketamine (Ketoset®) and dexmedetomidine (Dexdomitor®) IP (50-60/0.05-0.15 mg kg^{-1}), Ketoprofen (Ketofen®) Subcutaneous (SC) (5 mg kg^{-1}), Buprenorphine (Buprenex®, Reckitt Benckiser Pharmaceuticals, Richmond VA) SC (0.01-0.05 mg kg^{-1}) and enrofloxacin (Baytril®) SC 10 mg kg^{-1}. The second batch of animals received of KD IM (50-60/0.05-0.15 mg kg^{-1}), ketoprofen subcutaneous (SC) (5 mg kg^{-1}), BP SC (0.01-0.05 mg kg^{-1}) and enrofloxacin SC (10 mg kg^{-1}). The third batch of animals received KD (50-60/0.05-0.15 mg kg^{-1}) and BP (0.01-0.05 mg kg^{-1}) IM. The fourth batch of animals received Pentobarbital (Nembutal® Ovation Pharmaceuticals, INC., Deerfield, IL) 50 mg kg^{-1} initially, 25 mg kg^{-1} maintenance with BP (0.01-0.05 mg kg^{-1}) IP. The animals all received warm, sterile, saline (10/mL/kg/h SC) during the procedure.

Surgical protocol: The goal is to produce a model of CRF but initially the animal must go through am ARF phase. The animal was confirmed anesthetized by toe pinch, the abdomen was shaved and sterilized using iodine solution. Sterile drapes were used to cover the animal exposing only the abdominal area. A midline incision was made to expose the abdominal contents. Using soft forceps, the intestines were exteriorized over warm damp sterile gauze to keep them moist during the procedure. The renal pedicle (artery and vein only) was then exposed on each side separately using a vascular clamp (Micro-serrefine curved 6 mm, Fine Science Tools Inc., Foster, CA) the renal pedicles were obstructed for a period of 60 min to induce the ischemia injury (Fig. 1). During the 60 min wait, the intestines were internalized and the abdomen was covered with the sterile warm damp gauze. After the 60 min time frame, the clamps were gently removed and re-perfusion of the kidney was visually confirmed by

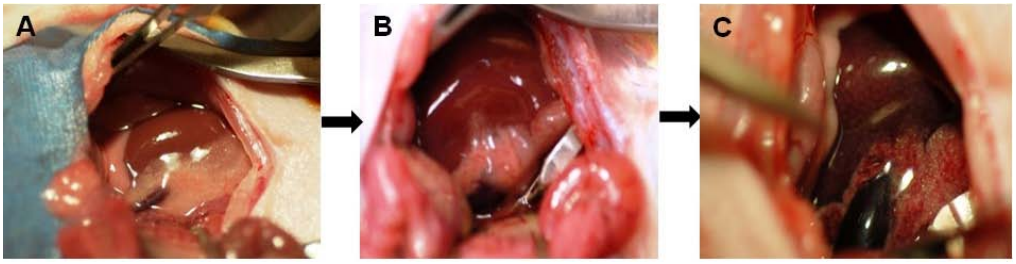

Fig. 1: Renal ischemia the renal pedicle (artery and vein only) was then exposed on each side separately using a vascular clamp (Micro-serrefine curved 6 mm, Fine Science Tools Inc., Foster, CA) the renal pedicles were obstructed for a period of 60 min to induce the ischemia injury

a return to normal color of the kidneys. The abdomen was closed in layers with 3-0 polyglycolic acid interrupted sutures (Ethicon, Somerville, NJ) for the muscle layer and continuous subcuticular manner for the skin. After conclusion of the procedure for anesthetic reversal, IM atipamezole (Antisedan®) using the same volume as dexmedetomadine (425 mg mL^{-1}) IM (Plumb, 2008) was given if the animal was in batch 1-3. Enrofloxacin was given SC to the appropriate batch. After the animal awakened, it was transferred to the housing room. The animals were maintained on heating pads during the 60 min ischemia time, the first 24 h, post-surgery and as needed thereafter. The animals were monitored twice daily. The analgesic regimen was repeated every 8-12 h and every 24 h with buprenorphine and ketoprofen, respectively where applicable, thereafter as needed. Mortality in this study in addition to acute death is defined as animals showing reluctance to move, abnormal posturing, anorexia or generally failure to thrive. Those animals deemed to be suffering were euthanized via CO_2 with a secondary method. The CRF protocol occurs 2 weeks post-operatively and will not be discussed. The current study describes findings during the ARF phase of this project.

Clinical Chemistries: Blood samples, 0.8-1.5 mL from animals were collected after anesthetic drug injections on day 0 and from post mortem animals. Blood Urea Nitrogen (BUN) and Creatinine (Cr) levels were determined from these samples using Beckman Synchon Clinical System CX5CE.

Renal histopathology: For 2 animals in each batch that were euthanized the kidneys were removed and fixed in 10% non-buffered formalin followed by staining with hematoxylin-eosin. The slides were reviewed blindly with regards to batch group and in addition to 2 naive nude rats. Representative images were taken at 200x magnification to assess the structural integrity of the glomeruli and tubules, presence of fibrosis and overall architectural change. The percentages of histopathologic changes(tubular injury) was scored using a semi quantitative scale designed to evaluate the degree of tubular necrosis (Rabb *et al*., 1994). Higher scores indicated more severe damage as follows: 0 = normal kidneys, 1 = minimal injury (<5% involvement), 2 = mild injury (5-25% involvement), 3= moderate injury (25-75% involvement) and 4 = severe injury (>75% involvement).

Statistical analysis: Statistical comparisons of data were performed using Student's t-test with a 95% confidence limit. Data are presented as mean±standard deviation. Differences with $p<0.05$ were considered significant.

RESULTS

Mortality: In batch 1, nude rats undergoing an I/R procedure that were given the anesthetic protocol of KD IP, BP, ketoprofen and enrofloxacin resulted in severe acute renal failure with a mortality of 60%. As illustrated in Fig. 2, 6 of 10 animals died within 4 days after the surgery. Mortality rates of KD IM, BP, ketoprofen and enrofloxacin were 60%. KD IM with BP resulted in 40% mortality rate. The use of PB and BP resulted in a significantly improved mortality rate of 20% (Fig. 2).

Weight measurements: Figure 3 shows that each batch had weight loss when comparing between pre-surgical and immediately post mortem weight measurements in animals that underwent I/R surgery. Batch 1 (ketamine/dexmedetomidine IP with ketoprofen and enrofloxacin) weighed 0.262+/-0.03g presurgical and 0.237+/-0.031 g post mortem; batch 2 (ketamine/dexmedetomidine IM with buprenorphine, ketoprofen and enrofloxacin) weighed 0.258+/-0.032 g presurgical and (0.251+/-0.035g) post mortem; batch 3 (ketamine/dexmedetomidine with buprenorphine weighed 0.292+/-0.036 presurgical and 0.273+/-0.041 g post mortem;

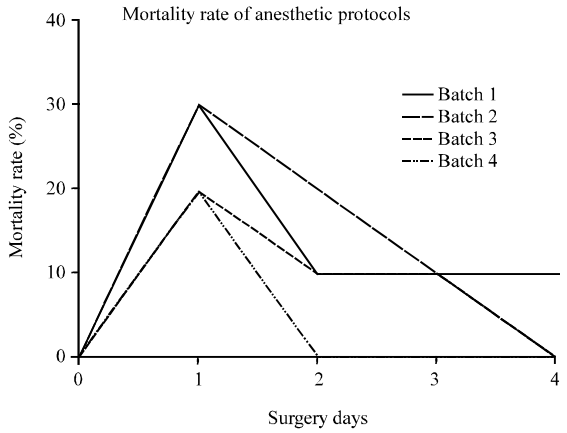

Fig. 2: Mortality rates after I/R injury of different anesthetic protocols. Batch 1 (ketamine/ dexmedetomidine IP with ketoprofen and enrofloxacin) and batch 2 (ketamine/ dexmedetomidine IM with buprenorphine, ketoprofen and enrofloxacin) resulted in 60% mortality; batch 3 (ketamine/dexmedetomidine with buprenorphine) resulted in 40% mortality; batch 4 (pentobarbital and buprenorphine) resulted in 20% mortality (n = 10/batch). All anesthetic protocols showed a decrease in mortality rate post operatively. Pentobarbital shows a significantly lower mortality rate after day 1 *($p<0.05$)

Fig. 3: Post-mortem weight loss. Batch 1 (ketamine/dexmedetomidine IP with ketoprofen and enrofloxacin); batch 2 (ketamine/dexmedetomidine IM with buprenorphine, ketoprofen and enrofloxacin); batch 3 (ketamine/dexmedetomidine with buprenorphine; batch 4 (pentobarbital and buprenorphine). Batch 1 showed the greatest amount of weight loss between groups and is significantly different when compared to batch 2 (*$p<0.05$)

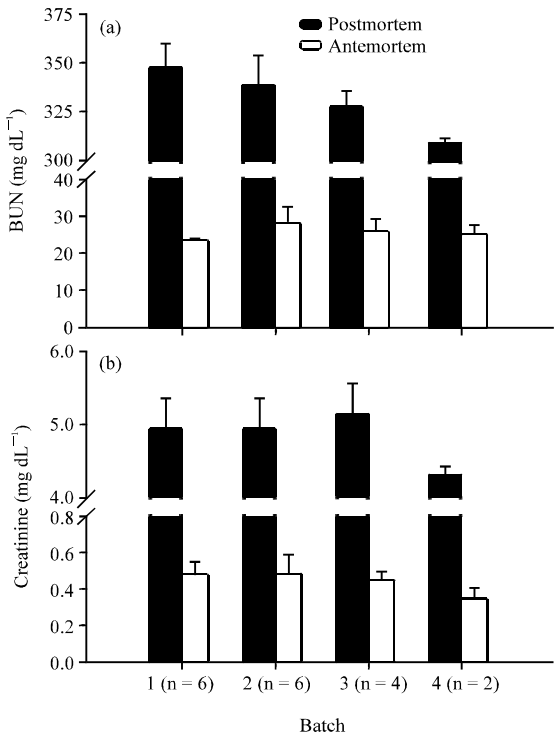

Fig. 4: Effect of different anesthetic protocols on kidney function: a) baseline vs. post-mortem Blood Urea Nitrogen (BUN); b) baseline vs. post-mortem Creatinine (Cr). Batch four differed from the other three significantly in both the BUN and creatinine levels (*$p<0.05$)

batch 4 (pentobarbital and buprenorphine) weighed (0.251+/-0.023 g) presurgical and 0.219+/-0.026 g post mortem. Batch 1 showed the greatest amount of weight loss between groups. There is no statistical difference between batch 4 and the other treatments.

Clinical chemistries: Baseline, antemortem BUN and creatinine levels were comparable between all batches (Fig. 4a and b). Post-mortem measurements differed significantly from the base line measurements and published normal values (Fox *et al*., 2002). Additionally, the post-mortem measurement from batch four differed from the other three significantly in both the BUN and creatinine levels.

Histology: A significant amount of renal tubular damage signified by loss of structural detail occurred in all groups undergoing renal ischemia/reperfusion. The renal tubules showed a significant increase in lumen diameter, sloughing off of cells into the lumen and overall architectural destruction.

Kidneys from the naive nude rat showed no histopathological changes with numerical damage scores

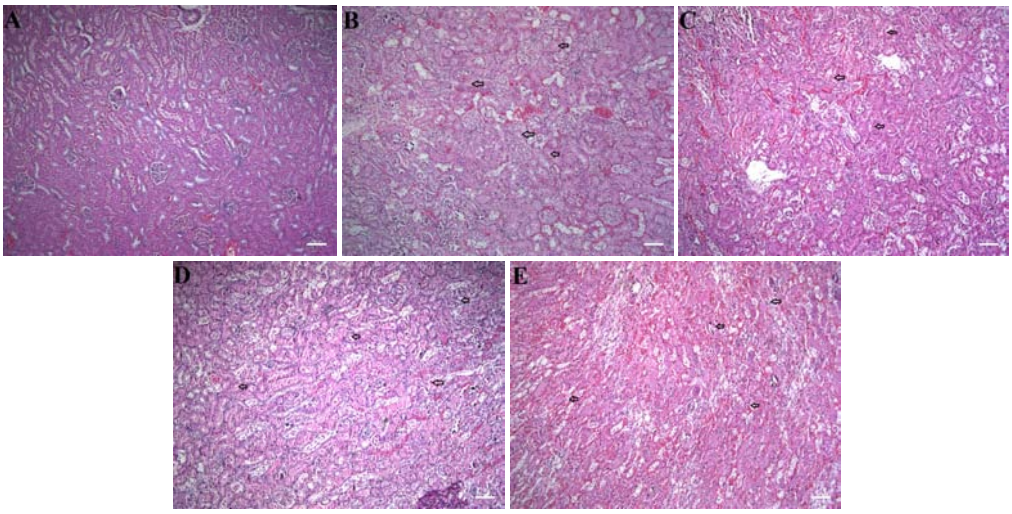

Fig. 5: Renal histology (A-E) representative samples of renal histology stained with Hematoxylin and Eosin (H&E) between the different anesthetic protocols of Renal/Ischemia reperfusion injury in nude rats 200X: A) control; (B-E) batch 1-4, respectively. Arrows in slides B-E indicate sloughing of cells, tubular congestion with necrosis

of 0. No significant differences were present in any of the I/R batched animals as all presented with numerical damage value of 3 (25-75%) (Fig. 5).

DISCUSSION

Determining a beneficial anesthetic protocol to improve survivability of nude rats while undergoing bilateral renal ischemia and reperfusion surgery for a chronic renal failure model was the goal of this study. The use of injectable anesthetics in rodent surgery is commonly preferred over inhalant anesthetics. This is due to ease of use and decreased equipment which allows a number of animals to be maintained under anesthesia at the same time in the case 10 rats (Fox *et al*., 2002). Based on the results, the injectable anesthetic pentobarbital with the analgesic buprenorphine proved to be best for use in nude rats with I/R injury. It has been reported that barbiturates have a depressant effect on effective renal plasma flow and glomerular filtration rate by decreasing blood pressure (Walker *et al*., 1986). That same group however showed that hypotension not caused by barbiturates did not result in a decrease of renal plasma flow or glomerular filtration rate (Conrad *et al*., 1984). Researchers found that an interruption in the renin-angiotensin system caused dilation in renal vasculature leading to a reduction in perfusion pressure (Walker *et al*., 1986). This decrease in perfusion pressure is what researchers propose is the cause for the decreased mortality seen with the PB group. Ketamine can induce a rise in arterial pressure usually measured as hypertension in rodents (Fox *et al*., 2002). Unlike xylazine, dexmedetomidine has fewer side effects of hypotension that would have offset the hypertension when used together with ketamine (Fox *et al*., 2002). In this study, researchers hypothesized that using IM administration of KD would slowly release the anesthetic which would result in decreased side effects and mortality compared with IP administration. The route of administration is usually considered due to the volume that can be administered (Fox *et al*., 2002). An increased volume of anesthetics can be administered intraperitoneally over intramuscularly due to the decreased muscling of rodents (Fox *et al*., 2002). Researchers could not confirm whether the route of administrations had an effect on mortality or not. For successful surgery the anesthetic protocol should include adequate levels of analgesia and asepsis (Institute for Laboratory Animal Research, 1996). Initially, researchers used ketoprofen and enrofloxacin for analgesia and antibiotics, respectively. Regarding ketoprofen, multiple studies in humans and other species have shown that there is a risk of inducing renal failure with nonspecific COX inhibitors. Renal blood flow decrease may lead to renal ischemia and damage (Junot *et al*., 2008). Due to ketoprofen's side effects it should be not be used with I/R surgery and could have possibly contributed to the high mortality rate. The favored protocol used only BP as an analgesic which does not have reported renal side effects. Enrofloxacin was used in this study because of the presumed safety in guarding against any post-operative infections. If asepsis is maintained throughout surgery there is no need for a long course of antibiotics (Slatter, 2003). Researchers did not experience any post-operative

infections throughout the use of enrofloxacin (batch 1 and 2) or when enrofloxacin was discontinued (batch 3 and 4). Although, no additional renal impairment is caused by enrofloxacin, renal impairment markedly prolongs elimination half-life that result suggests that renal impairment could affect the pharmacokinetics of enrofloxacin (Hwang *et al*., 2009).

Renal function will remain within the normal range unless >50% of the nephrons are destroyed (Baum *et al*., 1975). In similar studies, renal injury is initiated as early as 4 h following 45 min ischemia in rats. Peak injury occurred at 24 h and evidence of renal damage were present even after 1 week following injury (Williams *et al*., 1997). Based on the measurements of serum creatinine, BUN and histology researchers contend that renal damage did occur. The goal of the research is to have a chronic renal failure model so, interventions that would decrease acute renal failure were not attempted. Many studies have reported on the protective effects of antioxidants in different organs and renal I/R injury (Huang *et al*., 1995; Sehirli *et al*., 2003; Sener *et al*., 2004, 2006). Further, studies should be conducted to determine what level of interventions can decrease acute renal damage, thus decreasing mortality while still producing a chronic renal failure model.

It has been reported that there is sexual dimorphism in I/R Models with female rats showing decreased mortality (Muller *et al*., 2002). In the studies, researchers used male nude rats. Further, studies can also be conducted to determine whether mortality together with choice of anesthetic is influenced by sex. To assess the degree malfunction of the kidney caused by the different anesthetic protocols BUN and Cr levels was determined. The results of baseline CR and BUN did not show any differences between the four anesthetic protocols. However, this might not be indicative of the prolonged effect of the anesthetic protocols on the kidney function. Creatinine and BUN were also measured in animals that did not recover from the I/R injury. This showed that even though animals given pentobarbital as the anesthetic choice ultimately declined in health both functional measurements of kidney function were significantly better than other methods tested. Blood measures were not taken in animals that survived but it is hypothesized that the creatinine and BUN levels were also increased in those animals but did not show any clinical signs and/or symptoms of kidney malfunction. A longer term study will be needed to assess if there is any adverse effect of the anesthetic protocols on the chronic kidney function. In future studies, renal function should also be found by measuring the Glomerular Filtration Rate (GFR) (Schneider *et al*., 2003). Another limitation of the research is the strain of rat used. It has been reported that survival ability of I/R injury depends somewhat on the strain chosen (Raman *et al*., 2011). Researchers do not know if Nude rats are more susceptible to I/R injury this should be studied further.

CONCLUSION

Current literature on bilateral ischemia and reperfusion show varied anesthetic protocols. Most literature is focused on reducing the effect of I/R injury in contrast to the long term goals of producing a model of chronic renal failure (Muller *et al*., 2002; Durrani *et al*., 2006; Serviddio *et al*., 2008; Curtis *et al*., 2011). Improving survivability was the purpose of this study due the severity of the I/R injury and the high mortality rate associated with it. This study shows that making presumed improvements to Ischemia/Reperfusion injury of the kidney with the choice of safer anesthetics like ketamine and dexmedetomidine combinations, NSAIDs and route of administrations of drugs can sometimes have deleterious effects in terms of survivability. As stated earlier, researchers determined pentobarbital and buprenorphine to be the best anesthetic protocol to decrease mortality following I/R surgery. This choice is not a definitive selection. Additional studies are needed to compare different anesthetics as well as different dosages of the anesthetics used in this present study in the hopes of further improving the survivability. The efforts put forth in this study will aid to reduce the total number of animals needed to complete the group's research.

ACKNOWLEDGEMENT

Researchers thank Adam Wilson and Tiffaney Bledsoe for technical support.

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