Urethral Obstruction by Seminal Coagulum is Associated with Medetomidine–Ketamine Anesthesia in Male Mice on C57BL/6J and Mixed Genetic Backgrounds

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Male and female mice were anesthetized by intraperitoneal injection with a mixture delivering 0.5 mg/kg medetomidine and 50 mg/kg ketamine to achieve immobilization for whole-body radiographs and bone densitometry, as part of a phenotypic screen for bone and mineral disorders in mice carrying genetic modifications induced through mutagenesis with N'-ethyl-N'-nitrosourea. Morbidity and mortality occurred in 19 of 628 (3%) of male mice 24 to 72 h after a seemingly uneventful recovery from anesthesia. No morbidity or mortality occurred in 1564 female mice that were similar in age to the affected male mice and that underwent the same procedure. Of the 7 male mice that underwent postmortem examinations, 5 had urinary bladders grossly distended with urine and 1 had ascites. In addition, the pelvic or penile urethra in 5 of the examined male mice was obstructed with seminal coagulum associated with varying degrees of erosion of the urothelial lining and inflammation of the urethra. In 2 of these animals, from which plasma samples were recovered, azotemia, hyperphosphatemia, and hyperkalemia were present. The predilection for delayed morbidity and mortality in males after anesthesia suggests that anesthesia with 0.5 mg/kg medetomidine and 50 mg/kg ketamine is a potential risk factor for obstructive uropathy due to release of seminal coagulum. This adverse effect did not recur when we altered our anesthesia protocol to 10 mg/kg xylazine and 100 mg/kg ketamine.

Over 4 mo, 628 male and 1564 female mice were anesthetized with 0.5 mg/kg medetomidine and 50 mg/kg ketamine to achieve immobilization for X-ray and bone densitometry investigation of the skeleton phenotype. Overall 1% of these mice were found sick or dead without premonitory signs 24 to 72 h after an apparently uneventful recovery from anesthesia. All of the affected mice were male, most of which had a distended urinary bladder on dissection or necropsy. These observations prompted us to suspect urinary obstruction. Although the obstruction might have been due to the release of seminal coagulum (synonyms: copulatory plug,[5] mouse urologic syndrome[1]), the presence of seminal coagulum in the urinary bladder or urethra in male mice has to be interpreted cautiously because its release can be an agonal event without leading to obstruction[8] and can depend on the method of euthanasia. Ether overdose causes convulsions and urethral plug formation but sodium pentobarbital does not.[9] However, seminal coagulum has been recognized as a cause of urinary obstruction and sudden death in epileptic male EL mice,[11] in aging studies in the STR/1N[10] strain which have a degenerative joint disease and obese phenotype, and in the obese diabetic strain KK-A^y.[7]

This case study comprises pathology findings in 2 male mice found dead recently and 5 male mice euthanized when moribund. We were able to collect blood samples for clinical chemistries from 2 of the 5 animals euthanized. The clinicopathologic findings of obstructive uropathy and azotemia prompted us to reconsider the use of medetomidine–ketamine anesthesia.

Thus far, the alternative immobilization protocol adopted has prevented recurrence of this adverse effect.

Case Studies

Mice. The mice described in this report were cared for and used humanely according to the appropriate MRC Harwell project licenses approved by the UK Home Office in accordance with the Animals (Scientific) Procedures Act 1996. The work was performed in the Mary Lyon Centre under its Home Office Certificate of Designation. Mutant mice on several different genetic backgrounds were imaged, including offspring of C57BL/6J male mice mutagenized with N'-ethyl-N'-nitrosourea and crossed with C57BL/6J female mice in a bone and mineral disorder phenotypic screen; a novel N'-ethyl-N'-nitrosourea skeletal mutant, originally on a mixed C57BL/6J × C3H/HeH stock and now second backcross generation on a BALB/cAnNCrl background; and a recessive pedigree on a mixed C57BL/6J × C3H/HeH background.

Anesthesia protocols. Unmated male and female mice were anesthetized for a maximum of 15 min by intraperitoneal injection by using either of the following protocols.

The first protocol involved a mixture of 0.5 mg/kg medetomidine hydrochloride (Domitor, Pfizer Animal Health, Sandwich, UK) and 50 mg/kg ketamine hydrochloride (Ketaset, Fort Dodge Animal Health, Southampton, UK). The second protocol used a mixture of 10 mg/kg xylazine (Sedaxylan, CEVA Animal Health, Chesham, UK) and 100 mg/kg ketamine. The effects of both α2-adrenergic agonist anesthetics, xylazine and medetomidine, were reversed by intraperitoneal injection of 5 mg/kg atipamezole hydrochloride (Antisedan, Pfizer Animal

Health). Dosages for these drugs were adapted from those used to achieve general anesthesia in mice,[2] with consideration that our goal was to achieve a relatively short (less than 15 min) period of immobilization for mouse imaging rather than surgical anesthesia. After imaging, the mice were allowed to recover in a heated box and then returned to their home cage either alone or with same-sex cagemates that had undergone the same procedure at the same time. Subsequently mice were monitored once daily.

Imaging. Whole-body radiographs (Faxitron X-ray Corporation, Qados, Sandhurst, UK) and bone densitometry measurements (DEXA machine, Lunar Piximus II, Fitchburg, WI) were performed on anesthetized mice according to the manufacturers' instructions. Both instruments were on the open bench.

Husbandry. Specific-pathogen–free mouse stocks in our high-health status facility were rederived by embryo rederivation.[3] Male and female mice were housed separately in individually ventilated racks (Techniplast UK, Kettering, UK) on a 12:12-h light:dark cycle (lights on, 0700 to 1900) at 19 to 23 °C and 45% to 65% relative humidity on grade 6 sawdust bedding (Datesand, Manchester, UK). All cage equipment and supplies were autoclaved prior to use. Husbandry manipulations were performed in a laminar-flow cabinet (Walker Safety Cabinets, Glossop, UK). Mice were fed irradiated rat and mouse Number 3 breeding diet (Special Diets Services, Witham, Essex, UK) and provided water (reverse-osmosis water treated with 8 to 13 ppm chlorine) ad libitum.

Microbiologic status. Quarterly sentinel screening for viruses, bacteria, and parasites followed recommendations of the Federation of European Laboratory Animal Sciences Associations.[6] Sentinel cages (1 per 56 cages) were challenged with samples of dirty bedding from a column of 7 cages each week, thereby completely sampling the 56-cage rack over a period of 8 wk. Live mice were submitted to an outside laboratory (Harlan UK Ltd Technical Services Department, Loughborough, UK) for microbiologic testing. Sentinel screening at the Mary Lyon Center indicates the stock have been free of pathogens listed by the Federation of European Laboratory Animal Sciences Associations since the facility's opening in 2004.[3]

Pathology. Clinically affected mice were euthanized humanely by overdose of sodium pentobarbital (Pentoject, Animalcare, York, UK) administered intraperitoneally. Samples (approximately 200 μL each) of blood from the jugular vein were collected into lithium heparin pediatric tubes (Kabe Labortechnik GmbH, Nümbrecht-Elsenroth, Germany) and the plasma stored at –20 °C. Mice were weighed and necropsied immediately after death. The intestines and lungs were inflated with 10% neutral buffered formalin, and the remaining soft tissues (mesentery with pancreas and mesenteric lymph node, liver, kidneys, adrenals, thymus, spleen, heart, lungs, trachea, esophagus, stomach, brain, penis, urinary bladder and male accessory glands) were immersion-fixed in 10% neutral buffered formalin. Tissues were embedded in wax and sectioned at 3 μm. The penis was sectioned in longitudinal plane, and all sections were stained with hematoxylin and eosin. Some of the mice that were found dead were opened and, if autolysis was not too advanced, the mouse was submitted for necropsy examination. A note regarding enlargement of the urinary bladder was made for all mice that were opened.

Clinical chemistry. Frozen plasma samples from 2 C57BL/6J males were thawed and evaluated individually on an automated analyzer (model AU400, Olympus Diagnostics, Southall, UK).[4] Plasma clinical chemistry reference values (mean ± 2 SD) for various inbred strains, including C57BL/6J, were generated in our laboratory by using 20 clinically normal male and 20 normal female mice (age, 14 to 16 wk) as part of the European Mouse Disease Clinic program (www.eumodic.org).

Statistical analysis. The incidence of delayed morbidity and mortality in male compared with female mice undergoing anesthesia and between cohorts of male mice anesthetized by different protocols were assessed by using the Fisher exact test, with a P level of less than 0.05 considered statistically significant. Individual clinical chemistry values that were either greater than the mean + 2 SD or less than the mean – 2 SD were considered to be abnormal.

Results
Clinical features and prevalence. Over 4 mo period, 628 male and 1564 female mice anesthetized by using a mixture yielding 0.5 mg/kg medetomidine and 50 mg/kg ketamine recovered uneventfully and were returned to their home cages. Approximately 24 to 48 h after recovery, 19 mice either were found dead without premonitory signs or were hunched, piloerect, immobile, and isolated from their cagemates. All affected mice were male and ranged in age from 11 to 28 wk; the male sex bias was statistically significant at $P < 0.001$. The affected C57BL/6J mice and those with the recessive pedigree came predominantly from different litters, whereas 4 male mice with the novel skeletal mutation were from the same litter. New lots of medetomidine and ketamine were substituted, but the problem persisted. We therefore discontinued the medetomidine–ketamine anesthetic regimen, and the subsequent 626 male and 585 female mice were anesthetized with a mixture yielding 10 mg/kg xylazine and 100 mg/kg ketamine. None of the mice treated with xylazine–ketamine showed delayed (24 to 72 h) morbidity or mortality. The incidence of delayed morbidity or mortality in male mice treated with medetomidine–ketamine was significantly ($P < 0.001$) higher than that in male mice treated with xylazine–ketamine. This result remained statistically robust ($P < 0.031$) when the analysis was repeated by using only those 5 mice that were confirmed to have a urethral plug at necropsy.

Gross and histopathology. Only male mice anesthetized with medetomidine–ketamine showed delayed morbidity and mortality, and the following description of gross and clinical pathology is confined to this group. The urinary bladders in 4 of the 7 male mice that underwent complete necropsy were distended by clear urine, an additional mouse had a distended bladder and small intestinal intussusception, another mouse had ascites, and the remaining mouse had no gross abnormality of abdominal cavity. None of these animals showed no evidence of injury or skin lacerations to the genital area.

The 4 male mice with enlarged bladders and the 1 male mouse with ascites each had seminal coagulum plug in the pelvic or penile urethra and associated with variable degrees of erosion of the urothelial lining, neutrophil exudation, congestion of veins in the suburothelial stroma, and (in some cases) transmural inflammation. In addition, some mice had edema of the adjacent fascia and mild infiltration of inflammatory cells. In 3 of these male mice (2 with distended bladders, 1 with ascites), renal cortical tubules but not the renal pelvis were dilated.

One male mouse with a distended bladder had no seminal coagulum plug, but unilateral papillary necrosis and a small intestinal intussusception were identified (data not shown). The cause of morbidity in 1 of the male mice could not be established by necropsy examination.

The 3 male mice that were opened but too autolysed for a useful necropsy all had enlarged urinary bladders.

Clinical pathology. Although attempted in all mice that were euthanized, blood collection was successful in only 2 of 5 cases. Both plasma samples were from male C57BL/6J mice that subsequently were confirmed to have seminal coagulum plugs. Both plasma samples were markedly azotemic, with urea values of 51.8 and 49.5 mmol/L (reference mean, 5.4 mmol/L; reference range, 3.4 to 7.4 mmol/L) and creatinine levels of 160 and 129 µmol/L (reference mean, 34.6 µmol/L; reference range, 25.0 to 44.2). Both mice were hyperphosphatemic (8.34 and 9.86 mmol/L; reference mean, 2.3 mmol/L; reference range, 1.6 to 3.0 mmol/L) and hyperkalemic (8.0 and 8.8 mmol/L; reference mean, 5.4 mmol/L; reference range, 3.4 to 7.4 mmol/L). In addition, 1 mouse was hypocalcemic (2.0 mmol/L; reference mean, 2.3 mmol/L; reference range, 2.1 to 2.6 mmol/L) with low protein (38.5 g/L; reference mean, 47.4 g/L; reference range, 42.2 to 53.3 g/L) and albumin 18.5 g/L (reference mean, 26.0 g/L; reference range, 23.4 to 28.7 g/L; calcium value corrected for low albumin). Sodium, chloride, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total cholesterol, triglycerides, and glucose levels were within reference range for these 2 male mice.

Discussion

To obtain X-rays and bone densitometry data, we needed a method to immobilize mice for a brief (15 min) period and could not use inhalation anesthesia with our imaging equipment. Our initial choice of a medetomidine–ketamine combination resulted in an unexpected adverse effect of morbidity and mortality of male mice 24 to 72 h after recovery from anesthesia. Detailed necropsies were performed on 7 of 19 male mice in this cluster, and the most consistent pathologic findings of full necropsies were a distended urinary bladder and urethral seminal coagulum plug (in 4 of 7 mice). One mouse with a urethral seminal coagulum plug had a normal-sized bladder with unremarkable, histologically contracted bladder muscle, but the ascites from this mouse was not collected to evaluate the clinical possibility of intra-abdominal urine leakage. Of the remaining 12 mice, which were found dead, the 3 that were opened but not submitted for necropsy had distended bladders.

Important criteria that help distinguish obstructive antemortem urethral plugs from agonal nonobstructive plugs are urethritis,[11] azotemia[11] and hydronephrosis or renal cortical dilation.[7,11] We found that the seminal coagulum plugs were associated with varying degrees of urothelial erosion, congestion, and inflammation, which findings are consistent with prolonged pressure injury. In some male mice, the renal cortical tubules were dilated. Azotemia, hyperphosphatemia, and hyperkalemia in 2 mice lend support to the idea that urinary outflow obstruction was clinically significant and contributed to morbidity. Furthermore, no other primary organ pathology was noted that could account for clinical signs in these 5 mice with urethral plugs.

The finding of urethral obstruction at even low (3%) incidence is considered important because it is exceeds the background level at our facility, where only 1 other case of urethral obstruction was recorded among more than 2000 necropsies of male mice. Furthermore, none of the male mice anesthetized by using xylazine–ketamine developed urethral obstruction. This low incidence contrasts with the 25% and 40% incidences of urethral obstruction in male B6C3F₁ and ICR mice, respectively.[1] In male B6C3F₁ mice, urethral obstruction occurred at an average age of 12 mo, where it was associated with housing in wire cages. In those mice, urethral obstruction was suggested to be associated with inflammation and infection of the lower urinary tract, perhaps secondary to fighting and trauma to the external genitalia.[1]

The diet of mice in the present study was standardized but can be an important variable in the incidence of obstructive uropathy in mice, with high-fat diet reducing the incidence in the EL[11] and STR/N[10] strains and a high-calorie diet increasing the incidence in KK-A^y mice.[7] The underlying mechanisms are unclear but may involve the central nervous system, urogenital system, or systems that influence the composition of seminal fluids.[11] With the exception of epileptic convulsions that are thought to cause abnormal ejaculaion,[11] the triggering events in seminal coagulum release leading to urethral obstruction in rodents are not well documented. Morbidity and sudden death 24 to 72 h after anesthesia with medetomidine–ketamine suggests this combination may be a trigger, at least at the dosages used. To our knowledge, a connection between obstructive uropathy and the use of these anesthetic drugs in male rodents has not been reported in the literature. In our experience, it appears to account for much of the 3% delayed posttreatment morbidity and mortality in our male mice. This association is of scientific as well as welfare concern because each offspring of a male mouse mutagenized with *N*′-ethyl-*N*′-nitrosourea carries a unique set of genetic mutations and is a unique genetic resource. Our standard practice is to freeze sperm from all of the offspring of all MRC Harwell ENU-mutagenized males for the DNA archive to use in gene-driven screens so that the mutant mouse line can be resurrected from a parallel sperm archive (www.har.mrc. ac.uk/services/dna_archive/). Freezing sperm is not possible with animals that are found dead and is difficult to schedule when a sick mouse requires prompt euthanasia.

Having identified an unexpected adverse effect associated with a particular regulated procedure, the terms of a UK Home Office project license require cessation of the procedure. We chose another anesthetic regimen, a xylazine–ketamine combination, and empirically the adverse effect has not recurred. However, this association does not establish causality and raises difficult questions as to why two α2-adrenergic agonist anesthetics should differ in this regard. We did not undertake a controlled study in which the 2 anesthetic procedures were used concurrently in the same group of animals, but the imaging procedures, husbandry, health status, and genetic backgrounds of the mice in the phenotypic screens were similar throughout. Nor was there any evidence for association postanesthetic complications with drug contamination because the lots of medetomidine and ketamine used immediately were discarded and replaced after a death occurred, such that multiple batches of medetomidine and ketamine were used over the 4-mo period. An experimental investigation is required to elucidate the mechanism of and establish cause and effect in this anesthetic-associated obstructive uropathy in male mice and therefore is beyond the scope of this retrospective case study.

In conclusion, our intention in this report is to raise awareness of obstructive uropathy as a potential adverse effect for specific anesthetic combinations that might be applicable to other drug combinations and to facilitate the development of safer immobilization protocols.

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