

DELIVERY OF FLUORESCENT PROBES VIA RENAL SUBCAPSULAR INJECTION

APPLICABLE IN RATS AND MICE

Given that this procedure is a survival procedure, aseptic principles and maintenance of normal core body temperature are vital. In addition, analgesia must be provided. Certain injectable agents such as barbiturates possess analgesic properties. However, in the case of inhalational fluranes, additional analgesia must be provided. As a guideline, we have provided our steps involved in this survival procedure below. All surgical instruments are autoclaved prior to use. Surgeon wears clean gown, cap, face mask and sterile gloves. The animals will be at a surgical plane of general inhalational or injectable anesthesia when the surgical procedures are performed. They will be placed on a warming pad in the right lateral decubitus position. Prior to skin incision, the animal is given 1.0 mg/kg subcutaneous dose of buprenorphine HCl in mice and 0.05 mg/kg subcutaneous dose of buprenorphine HCl in rats for analgesic purposes. A rectal thermometer is placed and connected to a thermo-coupling device so that the core temperature remains steady between 36.5 C and 37.5C. The abdomen and groin regions are shaved with clippers and all loose hair is disposed of. With a sterile gauze, warm germicidal soap and water solution are applied to clean the abdomen and it is dried with sterile gauze. The abdomen is then wiped with alternating washes of betadine solution and 70% ethanol solution and allowed to dry. This step is repeated 3 times, and then the abdomen is draped with a sterile fenestrated drape. A vertical left flank incision is performed approximately 0.5cm in length in mice and 1.0cm in rats. The left kidney is identified and externalized. The vascular pedicle is isolated with blunt dissection (cotton tipped applicator) and the renal artery and vein are clamped with a non-serrated vascular clamp. The anterior or posterior side of the kidney may be injected. A 1-mL syringe, with a 30-gauge needle, containing approximately 0.1mL of desired probe/agent is then used to inject the contents underneath the kidney capsule without penetrating the kidney parenchyma. Entrance may be at either superior or inferior pole of the kidney, depending on the position of the rat, and the needle is advanced along the surface toward the opposite pole. As the needle is withdrawn, the agent is injected and a bubble should raise indicating that the potential space between the kidney and the capsule is being filled. After the needle is withdrawn from beneath the capsule, 1 minute of light compression, with a cotton tip applicator at the injection site, aids in maintaining the bubble (prevents escape of the agent). The vascular pedicle clamp is then removed, and the kidney is inspected for any evidence of bleeding. The abdomen is then closed in 2 layers with simple interrupted sutures. Just prior to closure of the fascial layer, the animal will receive 1-mL of warm saline for fluid resuscitation purposes. Once the incision is closed the animal emerges from anesthesia. Once fully emerged the animal is moved to a recovery cage on a warming blanket with soft bedding. Once fully recovered the animal is returned to animal housing facility.

DELIVERY OF FLUORESCENT PROBES VIA RETROGRADE LEFT RENAL VEIN INJECTION

Given that this procedure is a survival procedure, aseptic principles and maintenance of normal core body temperature are vital. In addition, analgesia must be provided. Certain injectable agents such as barbiturates possess analgesic properties. However, in the case of inhalational fluranes, additional analgesia must be provided. As a guideline, we have provided our steps involved in this survival procedure below. All surgical instruments are autoclaved prior to use. Surgeon wears clean gown, cap, face mask and sterile gloves. The animals will be at a surgical plane of general inhalational or injectable anesthesia when the surgical procedures are performed. They will be placed on a warming pad in the supine position. Prior to skin incision, the animal is given 1.0 mg/kg subcutaneous dose of buprenorphine HCl in mice and 0.05 mg/kg subcutaneous dose of buprenorphine HCl in rats for analgesic purposes. A rectal thermometer is placed and connected to a thermo-coupling device so that the core temperature remains steady between 36.5 C and 37.5C. The abdomen and groin regions are shaved with clippers and all loose hair is disposed of. With a sterile gauze, warm germicidal soap and water solution are applied to clean the abdomen and it is dried with sterile gauze. The abdomen is then wiped with alternating washes of betadine solution and 70% ethanol solution and allowed to dry. This step is repeated 3 times, and then the abdomen is draped with a sterile fenestrated drape. A midline laparotomy is performed approximately 1.5 cm in length in the rat, 1.0 cm in the mouse. The left kidney and associated vascular pedicle are identified and dissected out with blunt dissection (cotton tip applicator). A suture tie is looped around the left renal artery and vein without constricting the vessels. Then, a non-serrated vascular clamp is placed on the left renal artery and vein near the junction of the renal vein and the inferior vena cava. At which point the suture tie ends are retracted medially to slightly elevate and lengthen the renal vein, and a 30-gauge needle, connected to a 1-mL syringe, is used to quickly inject 0.5 mL of probe/agent of choice retrograde into the renal vein (towards the kidney). As the needle is removed from the vessel, a cotton-tipped applicator is used to lightly compress the vein at the puncture site for a maximum of 3 minutes. The vascular clamp is removed from the left renal vein and the puncture site is inspected for any evidence of hemorrhage. Hemorrhage that does not resolve after an additional 1-minute application of pressure with a cotton tip applicator will result in euthanasia of the animal. The kidney is inspected for return of normal perfusion. Failure of the kidney to reperfuse will result in euthanasia of the animal. The abdomen is then closed in 2 layers with simple interrupted sutures. Just prior to closure of the fascial layer, the animal will receive 1 mL of warm saline for fluid resuscitation purposes. Once the incision is closed the animal emerges from anesthesia. Once fully emerged the animal is moved to a recovery cage on a warming blanket with soft bedding. Once fully recovered the animal is returned to the animal housing facility.