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Surgical preparation of rats and mice for intravital microscopic imaging of abdominal organs

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ABSTRACT

Intravital microscopy is a powerful research tool that can provide insight into cellular and subcellular events that take place in organs in the body. However, meaningful results can only be obtained from animals whose physiology is preserved during the process of microscopy. Here I discuss the importance of preserving the overall state of health of the animal, methods of anesthesia, surgical techniques for intravital microscopy of various abdominal organs, methods to maintain and monitor the physiology of the animal during microscopy and associated peri- and post-operative recovery considerations.

1. Introduction

Intravital microscopy has afforded researchers the ability to better understand cellular and subcellular processes within multiple organs in the body [1–4]. The utilization of the rat and mouse models can be advantageous in that organ structure and function in these species mimics humans. Therefore, models of disease specific to these organs may be studied in the rat and mouse, potential treatment modalities may be tested thereafter, and, the application of such treatment modalities may potentially be transferred to humans [2,4–7]. However, meaningful results can only be obtained from animals whose physiology is preserved during microscopy. Here, I describe methods of anesthesia, surgical techniques, and methods to maintain and monitor animal physiology that we have developed for intravital microscopy of the kidney, liver, spleen and pancreas of rats and mice.

The methods described here were optimized to provide access to abdominal organs such that the tissues are sufficiently immobilized to support high-resolution imaging, while preserving the animal and organ physiology. These methods were designed in accordance with guidelines provided by Indiana University's Institutional Animal Care and Use Committee (IACUC). Prior to any animal studies, appropriate institutional approval must be obtained. In the United States, each research institution must have an IACUC that reviews proposals for research, teaching or testing activities involving vertebrate animals, and approval is required prior to initiating any such activities. IACUCs follow federal regulations as they pertain to the Animal Welfare Act, National Research Council's Guide for the Care and Use of Laboratory Animals, and the Public Health Services Policy on Humane Care and Use of Laboratory Animals.

Intravital microscopy studies may consist of a single imaging session or of a sequence of multiple imaging sessions, as for longitudinal studies in which an animal is repeatedly imaged over periods of days or weeks. Since the animal is euthanized at the end of a single-session imaging study, the surgery is considered a non-survival surgery. Studies involving multiple imaging sessions involve survival surgeries, which incur additional considerations for anesthesia, surgical preparation, and pain management, as described below.

2. Anesthesia and pain management

There are several key factors to consider when choosing an anesthetic agent and pain management during intravital imaging: 1) physiologic state of the animal, 2) whether the session is non-survival or survival, 3) rat versus mouse, 4) personal preference of the investigator that is either performing or supervising the procedure, and 5) length of the procedure/duration of action of the agent. The overall goal is to achieve a surgical plane of anesthesia prior to manipulating the animal. A surgical plane of anesthesia is described as a state of medically-induced unconsciousness in

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which the animal does not produce protective reflexes to stimuli [8]. In general, we tend to utilize Isoflurane as our number one agent of choice because it is easy to titrate to effect, which is critical in physiologically unstable animals, and emergence and recovery from the surgical plane of anesthesia is relatively rapid compared to injectable agents that are available (Figs. 1 and 2). The physiologic state of the animal refers to the temperature, blood pressure, heart rate and respiratory rate. Regardless of whether a rat or mouse model is used, it is important to inspect the animal's physiologic state regularly throughout the course of the imaging session. Observation of respiratory rate, color of the mucosa and response to stimulation (ear pinch) are appropriate for determining proper depth of anesthesia and are indirect indicators of blood pressure and heart rate. However, monitoring of blood pressure and heart rate with an intra-arterial catheter or tail vein blood pressure cuff transducer system, and rectal thermometer, to measure core temperature, afford the investigator a more accurate determination of the physiologic state of the animal [1,5,9].

2.1. Single imaging session studies: non-survival surgeries

Intravital imaging using non-survival surgery requires that the animal will be euthanized once the imaging session is complete. The animal, therefore, does not need to emerge from anesthesia. The vital characteristic of any anesthetic modality in the non-survival setting, therefore, is that it must induce a state of unconsciousness in the animal in which painful stimuli are not sensed by the animal. It is in this surgical plane of anesthesia that the animal must be maintained prior to skin incision and even at the time of euthanasia. Analgesia in the non-survival setting is, therefore, not imperative if the surgical plane of anesthesia is maintained throughout.

2.1.1. Rat anesthesia in non-survival studies

In non-survival studies in the rat, agent selection is influenced most heavily by the individual investigator's personal preference and underlying physiologic state of the animal (Table 1). The following agents are those most commonly used in our facility. The advantages and limitations of each are discussed.

Thiobutabarbital (Inactin), administered intraperitoneally, at a dose of 100–160 mg/kg, is an agent that we utilize in the male rat in only non-survival settings given its relatively long duration of action [9–11]. It is a barbiturate that is described as short-acting, however the male rat remains in a surgical plane for more than 4–6 h. Therefore, redosing is not required for non-survival studies of that length. It's especially useful in cases in which the underlying physiology of the rat has not been compromised.

Female rats present a unique problem from an anesthetic standpoint in our experience, because they do not consistently respond to Thiobutabarbital. Previously, sodium pentobarbital has been used in females [9], however, given its lack of availability, it is more likely that Isoflurane or ketamine cocktail will now be utilized.

Sodium Pentobarbital is a popular injectable agent for imaging procedures, in physiologically stable rats, that are less than 60 min long [12,13]. It is a shorter acting barbiturate, injected intraperitoneally at 30–60 mg/kg, that usually requires re-dosing every 45–60 min. However, pentobarbital in female rats has been used in non-survival sessions of long-duration because it has a significantly longer duration of action compared to males [12]. Unfortunately, it's availability in the US is rather limited currently and for that reason it may not be a viable option.

Isoflurane is an inhalational agent that is most valuable in rats that have compromised physiologic states due to disease models employed [14–17]. Use of Isoflurane does require a closed anesthesia circuit with a pressurized vaporizer, metered oxygen flow, and utilizing a gas waste scavenging system. It can be more cumbersome to manage the circuit on the microscope stage. However, it is quite easy to adjust the level of agent administered, and therefore, appropriately titrate the depth of anesthesia in those animals who would be susceptible to overdose with a standard injectable agent.

Ketamine cocktails, are also viable injectable options for non-survival imaging in the rat. Ketamine and xylazine mixture,

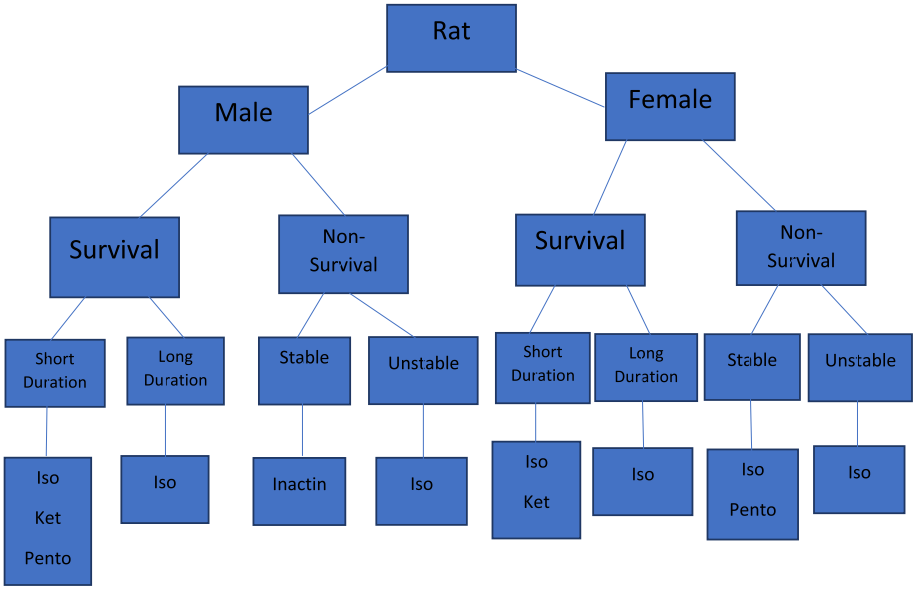

Fig. 1. Flow diagram for selection of anesthetic agent in rats. Isoflurane is generally the agent of choice because it is easy to titrate to effect for cases of long or short duration, or in rats that are physiologically unstable. In addition, emergence and recovery from Isoflurane are rapid in comparison to injectable agents. Inactin is ideal in non-survival cases in male rats that are physiologically stable because it has a long duration of action that eliminates the need for redosing. Iso = Isoflurane 1–2% vapor with a flow of Oxygen at 1 L/min. Ket = Ketamine cocktail 60–100 mg/kg Intraperitoneal injection (typically with Xylazine 5–10 mg/kg +/- Acepromazine 2.5 mg/kg). Pento = Sodium Pentobarbital 30–60 mg/kg Intraperitoneal injection. In survival cases Isoflurane is combined with Buprenorphine HCl subcutaneously at a dose of 0.01–0.05 mg/kg for analgesic purposes.