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Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider

Patricia V Turner,[1,*] Thea Brabb,[2] Cynthia Pekow,[3] and Mary Ann Vasbinder[4]

Administration of substances to laboratory animals requires careful consideration and planning to optimize delivery of the agent to the animal while minimizing potential adverse experiences from the procedure. For all species, many different routes are available for administration of substances. The research team and IACUC members should be aware of reasons for selecting specific routes and of training and competency necessary for personnel to use these routes effectively. Once a route is selected, issues such as volume of administration, site of delivery, pH of the substance, and other factors must be considered to refine the technique. Inadequate training or inattention to detail during this aspect of a study may result in unintentional adverse effects on experimental animals and confounded results.

Administration of substances to laboratory animals is often a critical component of experimental design. Administered substances may include: infectious disease agents; various therapeutics, such as vaccinations, antimicrobials, pharmacologic agents, anesthetics, and analgesics; chemical test agents; radiocontrast agents; electrolytes and other fluids; and nutritive support. Because substances may be administered repeatedly to the same animal or to multiple animals on the same study, the dosing methodology is an important consideration when planning an experiment and during protocol review by animal care and use committees and represents an essential opportunity for refining treatment of research subjects. Specific considerations for delivery of substances to animals are numerous and include factors such as absorption, distribution, metabolism and excretion of therapeutic or chemical agents; route, volume, and frequency of administration; duration of treatment; pH, stability, homogeneity, and osmolality of the substance to be administered; selection of vehicle or solvent for delivering substances that cannot be administered in a solid or particulate state; solution preparation, including considerations for sterility if the substance is being administered parenterally; and dosing apparatus and animal restraint necessary for specific routes of delivery. In addition, research teams should be aware of potential adverse effects related to substance administration to avoid confounding effects with other aspects of study design and to permit accurate interpretation of research findings.

Although understanding the basic pharmacology of any administered therapeutic or chemical agent is important for experimental planning, it is beyond the scope of this article to review principles of pharmacokinetics and pharmacodynamics, and readers are referred to several excellent texts dealing with these subjects.[22,102,106] This article is the first of a 2-part review of substance delivery to laboratory animals and summarizes recommended practices for various routes of administration to a range of species and factors to consider during experimental planning. The second part of this review examines dosing equipment and apparatus needed for substance delivery, considerations for selecting vehicles, and solute preparation and handling.[134]

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[1]Department of Pathobiology, University of Guelph, Guelph, Canada, [2]Department of Comparative Medicine, School of Medicine, University of Washington and [3]Research and Development, Veterans Affairs Puget Sound Health Care System, Seattle, Washington; [4]Platform, Technology and Sciences, Quality and Risk Management, Glaxo Smith Kline, Research Triangle Park, North Carolina.

Routes of Administration

Selection of a route. Substances are administered to laboratory animals by a wide variety of routes. A key factor determining the route selected is whether the agent is being administered for a local or systemic (either enteral [through the digestive tract] or parenteral [outside the digestive tract]) effect. Parenteral administration methods typically produce the highest bioavailability of substances because these methods avoid the first-pass effect of hepatic metabolism, which occurs commonly with orally administered chemicals and therapeutics. Parenteral routes also circumvent some of the unpredictability associated with enteral absorptive processes. Furthermore, regulatory requirements may influence the selection of a particular route, depending on the purpose of the study (for example, nonclinical safety testing, in which the route of delivery to animals should closely resemble the projected route of administration to humans).[37,38]

A substance may be given into the mouth (orally) or delivered directly into the stomach (gastric gavage); delivered into a blood vessel (intravenous); delivered onto, into, under, or across the skin or into a muscle (epicutaneous, intradermal, subcutaneous, transdermal, and intramuscular administration, respectively); instilled onto or into the eye (transcorneal or intraocular, respectively); into the brain (intracerebral) or the space surrounding the dura mater or that surrounding the distal spinal cord (epidural and intrathecal, respectively); administered into the peritoneal cavity (intraperitoneal), directly into the marrow cavity (intraosseous); sprayed into the nose for absorption across the nasal mucous membranes or into the lungs (intranasal) or delivered into the lungs by direct tracheal instillation (intratracheal) or inhalation; or administered by a

exposures, and species-specific anatomic features (for examples, see references 16, 41, 60, 64, 73, 91, and 127).

In laboratory species, many of the commonly used methods of delivery require restraint, sedation, or general anesthesia. The use of such manipulations should be considered when selecting the administration route to refine procedures so that they are less invasive or aversive to the animals. In addition, each route has advantages and disadvantages that should be considered depending on the final effect to be achieved, and ultimately the route selected will markedly affect the pharmacokinetics of the substance. This pharmacokinetic effect of route of administration is exemplified by naloxone, a potent opioid antagonist. Given intravenously, naloxone rapidly reverses opioid-induced central nervous system depression,[28] but when given enterally, the drug can be used to treat opioid-induced bowel stasis without antagonism of the analgesic effects of systemically administered opioids.[52] Another consideration regarding once-daily administration of substances to animals is their chronobiology or circadian rhythm. Depending on the aims and objectives of the experiment, the timing of substance administration may need to be considered carefully, for example, to administer a therapeutic when an animal's system is most or least metabolically active to induce or minimize toxicity.[119]

Enteral administration. Administration of substances directly into the mouth, admixed in diet or other foodstuffs, or by orogastric or nasogastric gavage is common in laboratory animal medicine and research. Per rectum administration of substances by enema or suppository is less common in animals than in humans. The oral route is economical, convenient, relatively safe, and some animals can be trained to cooperate voluntarily, depending on the compound being administered (Figure 1 A through C). Although voluntary consumption of the material being administered is ideal, this dosing technique may not be reliable in all animals or dose groups or for long-term studies, because of individual preferences for flavors, palatability issues, and changes in behavior over time. For substances being tested for safety, oral dosing mimics the most commonly used mode of administration of substances to humans. When placing substances directly into the mouth, it is important to ensure that tablets or gelatin capsules containing test material are placed far back in the mouth and that the animal swallows, to ensure receipt of the full dose. The number and size of capsules or tablets administered should be proportional to the size of the animal being dosed, to minimize regurgitation. Gavage (esophageal or gastric) is often used in research settings, instead of mixing substances in water or food, to ensure precise and accurate dosing of animals (Figure 1 D).

Selection of appropriate tubing size for orogastric or nasogastric gavage is important to minimize discomfort while optimizing delivery of substances. Nasogastric tubes are used commonly in rabbits for enteral nutrition and in nonhuman primates for dose administration and typically comprise 3- to 8-French soft rubber pediatric feeding tubes.[18,104] Tubing is measured from the external nares to the last rib and marked. To minimize discomfort, a small amount of xylocaine jelly can be placed on the end of the tubing or a drop of 0.5% proparicaine hydrochloride ophthalmic solution is placed directly in the nares prior to introducing the tubing into the ventromedial meatus (Figure 2).

Except when given in the diet or admixed with food, oral administration of substances typically requires some form of restraint. In many species, including rodents and nonhuman primates, restraint can be the greatest adverse effect of a procedure.[25,78,135] Habituation or positive reinforcement training to restraint may reduce the stress associated with the procedure.[1,107,120] In addition, the administration of large volumes of substances by orogastric or nasogastric gavage may cause stress due to gastric distension in species that are unable to vomit, such as rodents.[21] Therefore, using the smallest volume possible is recommended for the oral route of administration, optimally 5 mL/kg for all species (Table 1). When rats underwent gavage at this volume, no difference was noted between the stress induced by gavage compared with that induced by restraint alone.[135] When large volumes must be administered by gavage, a slower delivery rate may be better tolerated by animals.

Limitations of oral dosage may include a slower onset of action compared with parenteral delivery, a potentially significant first-pass effect by the liver for those substances metabolized through this route with reduced efficacy, lack of absorption of substances due to chemical polarity or interference with absorption by ingesta, poor compliance with voluntary consumption because of poor palatability or local irritation, lack of systemic absorption from the digestive tract, degradation of substances by digestive enzymes and acid, and inability to use this route in animals that are unconscious or have clinically significant diarrhea or emesis.[11] Oral gavage requires moderate technical skill and confidence. Research personnel should have training and practice prior to study initiation to minimize adverse events associated with the technique and to ensure that it is performed accurately, rapidly, and humanely in experimental animals.

Intravenous administration. The intravenous route of delivery is the most efficient means of delivering substances to animals because it bypasses the need for solute absorption. With this method, substances are administered as a bolus or infusion directly into blood vessels on either an acute or chronic basis (Figure 3). Precision electronic infusion pumps equipped with alarms to indicate flow interruptions and microdrop infusion sets are used to ensure accurate chronic intravenous delivery of many substances; however, less expensive precision and spring-operated disposable pumps have become available for this purpose in recent years and may represent a more economical alternative for experimental intravenous substance delivery, depending on the nature of the material to be administered and the duration of treatment.[2,32,117]

Although fluids and parenteral nutrition typically are infused on a continuous basis over several hours or days, the decision to administer other substances by the intravenous route often depends on the pharmacokinetics of the substance, as well as the maximum tolerated dose, the time interval over which delivery is required (referred to as dosing intensity), and the need to minimize variations in peak and trough blood levels in the substance being administered. The actual technique involves aseptic preparation of skin for percutaneous venous injection or surgical exposure of blood vessels for substance administration. Intentional intraarterial administration of substances should be avoided routinely and used only for specific experimental conditions, because of the potential for severe complications with this route, including blindness, cerebrovascular stroke, permanent motor deficits, and limb gangrene.[75,114,116,142] Suggested sites and volumes for intravenous injection and infusion of substances are given in Table 1.

Researchers designing experiments requiring single or repeated intravenous treatments should consider technique refinements that may enhance animal comfort, including the use of the smallest needle or catheter size possible to minimize injection trauma, butterfly needles for single injections to minimize perivascular trauma, indwelling catheters and vascular access ports for animal comfort and locomotor freedom, topical

Figure 1. (A) Rat voluntarily consuming nutritional supplement from a syringe. Photo courtesy of Colette Wheler. (B) Macaque voluntarily drinking medication from a syringe. Photo courtesy of Andrew Winterborn. (C) Pig voluntarily accepting medication when administered in a marshmallow. (D) Oral gavage of fish. Photo courtesy of Gerald Johnson.

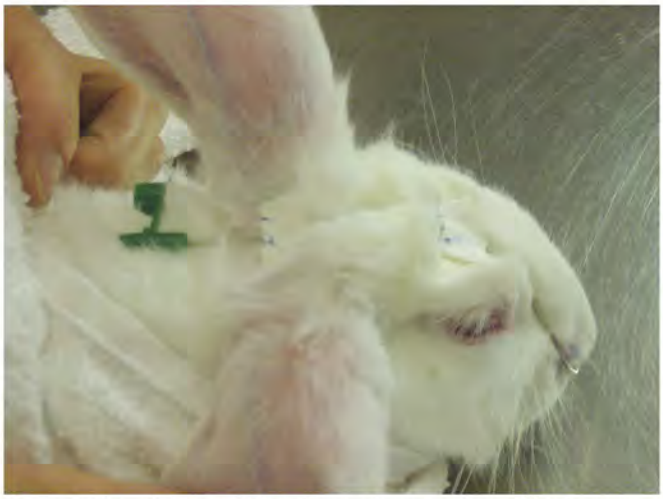

Figure 2. Chronic nasogastric catheter placement in a rabbit for enteral nutrition. Photo courtesy of Colette Wheler.

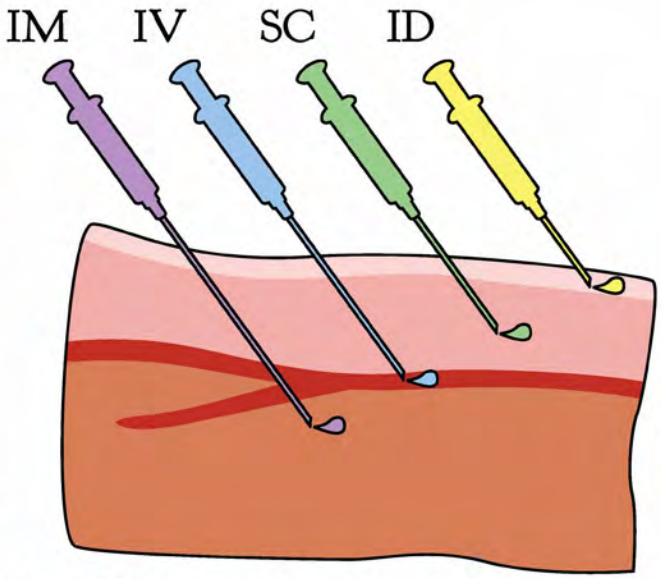

Figure 3. Different routes of skin administration of substances. Depicted are intramuscular (IM), intravenous (IV), subcutaneous (SC), and intradermal (ID) routes. Illustration courtesy of Gianni Chiappetta.

anesthetic creams and ointments prior to needle placement to minimize injection pain, and external pump packs to minimize the restriction of animal movement associated with tethering. Excellent recent reviews of techniques, equipment, and refinements for using catheters and vascular access ports in animals have been published.[16,53,89,124,125,128] A more detailed discussion of dosing equipment for intravenous delivery can be found in

Intraosseous administration of substances, particularly crystalloid fluids, is used in human pediatric medicine and emergency avian and rabbit medicine as an alternative for the

Table 1. Recommended volumes and sites of Administration of substances to laboratory animals

Route	Species	Optimal volume (range)	Site(s)	References
Gavage				
	All	5 mL/kg (to 20 mL/kg)	Mammals: intragastric	21, 82, 134
			Fish: esophageal[a]	127
	Fish	2 g/kg (gel capsules)		16
Intravenous				
	All	Up to 5 mL/kg (bolus)	Rodents: tail or saphenous vein Rabbits: ear or cephalic vein Larger species: jugular, cephalic, femoral, or saphenous vein	82
			Fish: caudal vein or artery[a,b]	16
		2 mL/kg hourly (to 4 mL/kg/h) continuous infusion)[c]		82, 89
Subcutaneous				
	Mammals	Maximum of 5 mL/kg per site	Intrascapular, neck, shoulder, flank	82
	Fish	1 mL/kg	Midline and just anterior to dorsal fin	53
Intradermal				
	All	0.05-0.1 mL per site	Skin	82
Intramuscular				
	All	Maximum of 0.05 mL/kg per site (rodents, rabbits, small nonhuman primates, fish)	Mammals: triceps, quadriceps, dorsal lumbar, semimembranosus, semitendinosus muscles	82
			Fish: base of dorsal fin or between dorsal fin and lateral line	16
Epidural				
	Mammals	0.15–0.2 mL/kg[d] (6 mL total volume in patients up to 35 kg)		47, 73, 138
Intraperitoneal				
	All	Maximum of 10 mL/kg	See text	82
Intranasal				
	Rodents	Minimum of 35µL per animal[e] (50 µL)		82, 121
	Dog, cats, nonhuman primates, rabbits	200 to 500 µL per animal		82

The physicochemical properties of the substance to be administered will markedly affect the volumes that are tolerated. For example, lower volumes than those listed in this table may need to be used for highly viscous or irritating substances.
[a]Sedation or light anesthesia may be needed for larger species.
[b]Renal first-pass effect is possible when injecting by using this route.
[c]Rates considerably lower than 2 mL/kg hourly may result in catheter patency issues in rodents.
[d]Larger volumes may result in more rostral spinal effects. Intrathecal injection volumes and doses are typically 50% of those used for epidural delivery.
[e]In mice, volumes less than 35 µL have been reported to be distributed primarily to the upper respiratory tract, whereas a 50-µL volume was predominantly deposited in the lower respiratory tract.

or collapsed veins.[31,80,129] The medullary cavity contains noncollapsing venous sinuses that directly enter into the central venous circulation and substances administered intraosseously are generally detectable immediately after administration. The technique is difficult to perform without advanced training and is potentially invasive, with considerable risk for postprocedural osteomyelitis, fat embolization, iatrogenic fracture and growth plate injury, and pain. Intraosseous administration typically is conducted in fully anesthetized animals.

Substances administered intravenously or intraosseously must be delivered aseptically and should be sterile; free of particulates that may induce foreign body emboli; and minimally irritating to vascular endothelia, to prevent vasculitis and thrombosis, and to erythrocytes, to minimize lysis. Certain oily substances, such as cremaphor, and various alcohols, surfactants, and other vehicles and excipients may induce hemolysis when introduced intravenously; these substances should be avoided, whenever possible, or first evaluated in vitro for safety.[4,79,90] The intravenous route of substance delivery, although efficient, can be risky in animals, and persons conducting this technique require training and practice to ensure competency. Careful control of hemostasis must be instituted when the catheter or needle is removed, to minimize blood loss and painful hematoma formation. When fluids or infusions are administered chronically, animals should be monitored closely for signs of fluid overload and pulmonary edema, such as dyspnea and cyanosis.[77] Chronically implanted catheters and vascular access ports require regular cleaning and maintenance to ensure patency and prevent infection.

Administration to skin and muscle. Some substances can be administered directly to the skin surface (epicutaneous administration) for a topical affect. The extent of absorption of materials through the skin and into the systemic circulation (that is, percutaneous or transdermal delivery) depends on: the surface area over which the substance is applied; the concentration of the substance administered; the lipid solubility of the material or vehicle; whether the skin surface is intact; the skin thickness at the site of application; the length of time that the material is in contact with the skin surface; and the degree of skin hydration and surface occlusion, in that covered and well-hydrated skin absorbs substances faster than does uncovered or dry skin.[87] For fish, specialized chambers can be constructed to expose the skin or gills specifically to test substances.[16,53] When administering substances topically to the skin of mammals, overlying hair is clipped to minimize matting and maximize contact with the material to be applied, and the skin surface is cleaned prior to application. Absorption of substances across the epidermis occurs through paracellular and transcellular mechanisms into the stratum corneum, to the stratum spinosum, and then to the basal layers of the skin and later, the dermis, as well as into the subcutaneous space through hair follicles and accessory glands.[42,93]

Caution must be exercised to avoid applying caustic or irritating material directly onto the skin, and some substances may induce local sensitization reactions. Consideration should be given to the potential for systemic toxicity when administering substances topically, particularly if the site is readily accessible for grooming.[46] Application of thin layers of cream or ointment to the skin at more frequent intervals may be more efficacious with less potential for systemic toxicity than is less frequent application of thicker layers.

Transdermal or percutaneous delivery represents a similar route of administration except that materials are applied to the skin surface deliberately, usually by means of a patch, for absorption across the epithelial barrier into the systemic circulation. Typically, this method produces very constant blood levels of the substance being administered. Percutaneous delivery is an attractive alternative to other parenteral routes, avoiding the need for repeated animal restraint, painful injections, and sharps hazards. In addition, materials can readily be removed from the skin surface if dosing needs to be interrupted or if adverse effects are noted. Transdermal delivery of substances may be acute or chronic, and current techniques for delivering substances by this route have been reviewed recently.[7,45,100] The skin is prepared as for topical delivery. When a transdermal delivery system will be used, the agent and delivery system (for example, patch) must be applied in advance of when the desired effect needs to occur, based on the pharmacokinetics of substance absorption. The product should be applied in such a way to protect it from ingestion and contamination, and the signs of toxicity after inadvertent ingestion by the animal should be known. Commercially available human transdermal products can be difficult to use in animals because of the much larger doses of substances impregnated into products intended for adult human use. Cutting transdermal patches to scale-down the dose being administered is not recommended; however, covering a portion of the patch to limit substance administration may be used. Animals should be observed closely for toxicity, and as for topical delivery methods, skin sensitization may occur over time with transdermal product use.[84] Animals must be prevented from removing and ingesting patches.

Nonirritating substances may be given subcutaneously, which represents a rapid, inexpensive, and simple method of parenteral substance administration (Figure 3). Substances administered subcutaneously often are absorbed at a slower rate compared with other parenteral routes, providing a sustained effect. The exact mechanism of absorption is unknown but is thought to be due to uptake of macromolecules within the subcutis by small capillaries underling the skin, with minimal lymphatic absorption.[56] Substances delivered subcutaneously can be aqueous or oily fluids, depots of oily materials for slow absorption, solid pellets, or injected into suitably sized osmotic minipumps or other implantable pumps, which subsequently are surgically inserted into a subcutaneous pocket. Because the subcutaneous space is largely a virtual space, it can be an excellent site for large volume fluid delivery in small or dehydrated animals, avoiding technical difficulties and problems sometimes seen with direct intravenous administration, such as fluid overload and pulmonary edema, because excess subcutaneous fluid is excreted rapidly by the kidneys. Compared with intravenous delivery, the subcutaneous route is a simple one to master; however, training and competency of personnel should be monitored to ensure that substances are delivered accurately and that inadvertent intravenous injection is avoided. Careful consideration should be given to using an appropriately sized needle, and humane and aseptic periinjection techniques. The skin overlying the site selected for injection should be loose to minimize discomfort, and the needle should be inserted at a shallow angle to minimize damage to underlying tissues. Passing a small-gauge needle through a thick rubber stopper to fill an attached syringe prior to injection may dull the needle point, enhancing injection discomfort. Contaminated substances injected subcutaneously typically will result in abscess formation. Recommended volumes and locations for subcutaneous injections are presented in Table 1. Inadvertent subcutaneous administration is a common complication of intradermal injections, and small, sharp needles are required for success with intradermal delivery.[82]

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