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Sterility of Sustained-release Buprenorphine

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Sustained-release formulations of controlled substances are commonly used to provide analgesia in research animals. These formulations represent refinements that offer the advantage of prolonged, multiday pain relief with a single injection, thereby decreasing handling stress in animals and saving time for scientists. Compounding pharmacies produce sustained-release buprenorphine for veterinary use (i.e., buprenorphine SR-LAB); one of these pharmacies has shortened the original 6-mo shelf-life to 28 d to comply with United States Pharmacopeia standards for ensuring sterility. This limitation risks increasing the waste of controlled substances, which require an expensive destruction process that is legally enforced in our state. To assess whether the sterility of buprenorphine SR-LAB is preserved for at least 6 mo in a general laboratory setting, we tested 5 bottles for the presence of endotoxin and bacterial and fungal contamination monthly for 6 mo. Overall, results of the study showed that the bottles remained sterile over the 6-mo duration as no endotoxin was detected and the bottles did not become contaminated with bacteria or fungi. In conclusion, when stored securely and used with aseptic handling techniques, buprenorphine SR-LAB can be maintained in a sterile state for 6 mo in a general laboratory setting.

Abbreviation: buprenorphine SR-LAB, sustained-release buprenorphine for veterinary use

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Sustained-release formulations of buprenorphine for analgesic use in animals (i.e., buprenorphine SR-LAB) are produced for the veterinary market by compounding pharmacies. The extended release properties of these formulations serve as a refinement that benefits animals and their caregivers by providing multiday pain relief after a single injection.[4,8,9,11,14,15] Compared with the administration of traditional analgesic medications, sustained-release analgesics provide continuous pain relief—typically for multiple days—and lessen the time, cost, stress, and hazards associated with handling an easily stressed and potentially painful animal daily (or more often).[2-4,14,15] Compounding pharmacies provide crucial services in human and veterinary medicine and must comply with United States Pharmacopeia standards as enforced by state pharmacy regulators.[5] For example, as a consequence of inspection by a state pharmacy board, a veterinary compounding pharmacy preparing buprenorphine SR-LAB was required to label and limit the use of buprenorphine SR-LAB to 28 d after first puncture of the vial to best ensure sterility, although the formulation was labeled with a 6-mo expiration date (shelf-life). The majority of use of buprenorphine SR-LAB at our institution is for rodents, and the 28-d use rule is problematic due to considerable amounts of remaining drug at this time point. For example, a 5 mL-vial of buprenorphine SR-LAB with a concentration of 0.5 mg/mL contains enough drug to treat 100 mice each weighing 25 g at the dose of 1 mg/kg. If the buprenorphine SR-LAB is considered to be expired after 28 d, any remaining drug is wasted, poses a potential for abuse, requires strict secure storage, and involves considerable expense for its legal destruction. These hurdles increase the likelihood that researchers will abandon attempts to use this formulation in animals, violate state-mandated standards, and engage in drug conserving and sharing schemes that are not compliant with federal law. As such, the change in shelf life complicates the use of buprenorphine SR-LAB in rodents, which in turn discourages the use of an effective analgesic that improves animal welfare. Although sterility studies for analgesic drugs such as carprofen, buprenorphine, and meloxicam have been conducted,[7,10,13,16] the long-term sterility of sustained-release buprenorphine currently is unknown. Therefore, this study was conducted to test the sterility of buprenorphine SR-LAB over the stated 6-mo shelf-life.

Materials and Methods

Study design and sample collection. We used 5 vials of buprenorphine SR-LAB (5-mL vials, 0.5 mg/mL; ZooPharm, Windsor, CO), which were stored at room temperature (21 to 23 °C) in a locked, light-tight drug safe for the duration of the study (6 mo). A sample size of 5 bottles was sufficient to detect microbial contamination at the threshold of 40,000 cfu/mL and endotoxin at a noninferiority margin above 2.5 EU/mL as significant at a P value of less than 0.0975 according to a one-sided paired t-test and principles of noninferiority.

Vials were handled monthly at 6 time points (0, 2, 3, 4, 5, and 6 mo) for sample collection and twice weekly between time points to examine color and clarity of the drug within the vials. To assess color and clarity, vials were observed against a white background (printer paper) and in front of a light source (room light). The 0-mo time point was when the vial was punctured initially. At each twice-weekly access, a sterile tuberculin needle and syringe (1 mL syringe with 25-gauge 5/8-in. needle; catalog no. 26046, EXELINT, Redondo Beach, CA) was aseptically inserted into and removed from each vial in simulation of a drug withdrawal. At each time point (0, 2, 3, 4, 5, and 6 mo), each vial was accessed, and a 700-µL sample of drug was collected aseptically. Samples were not collected and tested at the 1-mo time point. The bottles were accessed in a general laboratory space on a benchtop. Clean, powder-free nitrile exam gloves were worn, the rubber stopper of the vial was wiped with a single-use alcohol wipe (70% isopropyl alcohol prep pad; catalog

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no. MDS09735, Medline Industries, Mundelein, IL), and a sterile tuberculin needle and syringe was used.

Sterility testing. Drug samples (one per vial) were tested for the presence of endotoxins and bacterial and fungal microorganisms at each of the 6 time points (0, 2, 3, 4, 5, and 6 mo). The assay kit used for endotoxin detection (catalog no. L00451-20, ToxinSensor Single Test Kit, GenScript, Piscataway, NJ) is a qualitative, end-point endotoxin assay kit with a sensitivity level of 0.25 EU/mL. For this assay, 200 µL of drug sample was injected into a sterile assay vial that contained limulus amebocyte lysate standardized to detect the presence of endotoxins at greater than 0.25 EU/mL. The assay vials were incubated in a 37 °C water bath for 60 min prior to obtaining results. For the endotoxin assays, cell culture–grade, endotoxin-free water (catalog no. SH30529.01, HyClone Laboratories, Logan, UT) was used as a negative control, and LPS (0.75 mg/mL; catalog no. L2630-10MG, Sigma-Aldrich, St. Louis, MO) was used as a positive control.

To detect bacterial and fungal organisms, 500 µL of drug sample was injected into a sterile vial (catalog no. STERCL2MLX1, Research Laboratory Supply, Pompano Beach, FL) and sent to Veterinary Diagnostic Laboratories (University of Georgia, Athens, GA) for aerobic and anaerobic bacterial culture and fungal culture. This diagnostic lab is fully accredited by the American Association of Veterinary Laboratory Diagnosticians. For aerobic culture, samples were inoculated into trypticase soy agar containing 5% sheep blood and incubated at 35 °C at 5% CO_2 for 72 h. For anaerobic culture, samples were inoculated into thioglycollate broth and *Brucella* agar with 5% sheep blood and hemin–vitamin K and incubated at 35 °C under anaerobic conditions for 72 h. For fungal culture, samples were inoculated into Sabouraud dextrose agar and incubated at room temperature for 30 d.

Results

We assessed the sterility of buprenorphine SR-LAB monthly for 6 mo, except for month 1. The first time point was when the bottle was initially punctured (month 0). At each monthly time point, sterility testing included an endotoxin assay to detect presence of endotoxins at greater than 0.25 EU/mL and bacterial (aerobic and anaerobic) and fungal cultures of samples collected from each of the 5 bottles of buprenorphine SR-LAB. For all 5 bottles, at all time points tested, endotoxins were not detected, and the aerobic and anaerobic bacterial cultures were negative. For all 5 bottles, at all time points, except at month 2, fungal cultures were negative. At the month 2 time point, a single sample (from 1 bottle) showed a positive fungal culture result. The culture detected a single colony of *Cladosporium* spp.; subsequent fungal cultures from that bottle (months 3 through 6) were negative. The color and clarity of each of the 5 bottles, which were checked twice weekly throughout the study, remained clear and colorless for 6 mo.

Discussion

Sterility testing showed that neither endotoxins (>0.25 EU/mL) nor bacteria were detected in any of the 5 bottles of buprenorphine SR-LAB throughout the study, thus suggesting that the solitary culture of a single colony of *Cladosporium* spp. at month 2 was a postcollection contaminant. The bottle from which the sample was collected tested negative for all fungal cultures during subsequent testing (3 through 6 mo), and the bottle contents remained clear and colorless throughout the study. Species of *Cladosporium* are ubiquitous, saprobic, dematiaceous fungi that represent some of the most common fungi found in air and are commonly isolated from soil, food, paint, textiles, and other organic matter.[1,12] This common organism may have contaminated the sample when it was injected into the sterile collection vial for transport to the diagnostic lab or when it was inoculated onto culture plates at the diagnostic lab.

We chose the endotoxin sensitivity level for our assay according to the acceptable endotoxin limit for sustained-release buprenorphine (2.5 EU/mL). Endotoxin limits are calculated for individual drug products in light of the threshold of pyrogenicity for humans and rabbits (5 EU/kg) and the maximum volume of drug that would be given in 24 h.[6] Our endotoxin assay was more sensitive than the acceptable limit for sustained-release buprenorphine, and our data show no positive results of endotoxin even at levels below what is considered acceptable.

Our study was limited to a single lot and formulation of sustained-release buprenorphine for veterinary use (buprenorphine SR-LAB). We chose to test this formulation because at our institution, most of the laboratories that use sustained-release buprenorphine as part of their analgesic regimens in animals use this formulation. In addition, the veterinary staff at our institution have used this formulation for clinical applications. However, we had a limited amount of drug available for this study and had to conserve some volume in case we needed to retest a sample. Because the manufacturer ensures sterility of the drug for 28 d (approximately 1 mo), we did not conduct sterility testing at month 1. Future studies should be conducted to test additional lots and other formulations and storage conditions of veterinary sustained-release buprenorphine. These future investigations should include different test methods to further validate our current results.

Our findings indicate that buprenorphine SR-LAB can be maintained as a sterile drug for 6 mo in a general laboratory setting when aseptic technique is used each time the drug is accessed. As part of the aseptic technique, we recommend wearing clean exam gloves, wiping the rubber stopper with an alcohol wipe, and only puncturing the bottle with a sterile needle and syringe at each use.

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