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The stability and microbial contamination of bupivacaine, lidocaine and mepivacaine used for lameness diagnostics in horses

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ABSTRACT

Local anaesthetics (LAs) are frequently used for diagnostic procedures in equine veterinary practice. The objective of this study was to investigate the physico-chemical stability and bacterial contamination of bupivacaine, lidocaine and mepivacaine used for lameness examinations in horses. The LAs were stored in 12 different groups at different temperatures (–18 °C to 70 °C), light intensities and in common veterinary field conditions for up to 16 months. The pH, presence of bacterial contamination and concentrations of LAs and methylparaben (a preservative present in lidocaine) were determined serially in both new and repeatedly punctured (RP) vials.

Mepivacaine remained chemically stable. A 1.9% increase in bupivacaine concentration was evident in one group, whereas a 1.9–3.7% decrease was noted in six groups. Risk factors associated with a change in concentration were light and RP vials. Lidocaine concentration decreased 6.3% in one group and increased 5.3–7.2% in two groups. Risk factors for degradation were heat and RP vials whereas storage in practice vehicles was a risk factor for increased concentrations. Methylparaben decreased 8.3–75.0% in seven groups, and RP vials, heat and storage in practice vehicles were risk factors for degradation. No contamination was present in any of the LAs and pH remained stable. Commercially available solutions of lidocaine, mepivacaine and bupivacaine stored under common veterinary field conditions are extremely stable and sterile for extended periods. The minor changes in concentration documented in this study are unlikely to affect anaesthetic efficacy during equine lameness examinations. When using products containing methylparaben, degradation of the preservative over time is to be expected.

Introduction

Lameness is the most common medical condition limiting activity in horses (Olivier et al., 1997; Vigre et al., 2002), and local analgesia is essential for pain localisation in lameness evaluation (Bassage and Ross, 2010). In horses, the most commonly used local anaesthetics (LAs) are of the amide type and consist of 2% lidocaine, 2% mepivacaine, or 0.5% bupivacaine (Bassage and Ross, 2010). Product labels for these LAs (AstraZeneca) recommend storage temperatures of maximum 25 °C and discourage freezing. The mepivacaine and bupivacaine labels state that these products should be used immediately after the first opening; this instruction is not mentioned on the lidocaine label. However, according to guidelines (CPMP/QWP/159/96) from the European Medicines Agency, aqueous sterile products containing preservatives such as lidocaine have a maximum in-use storage time of 28 days after opening.[1]

Large animal practitioners are confronted with challenging working conditions, often performing investigations in environmental conditions that are dramatically different from standard laboratory or hospital settings. Additionally, ambulatory practice often requires the transport of pharmaceutical substances under suboptimal conditions. These concerns have also been raised for medication stored in human ambulances, and the stability and efficacy of pharmaceutical substances could be altered by specific temperature and light conditions encountered in veterinary practice (Gill et al., 2004). Pain localisation in lameness examinations relies on consistently efficacious LAs, but the physico-chemical

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[1] See: European Medicines Agency. Product information: Reference documents and guidelines. http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000254.jsp&mid=WC0b01ac058008c34c referring to Committee for Proprietary Medicinal Products. Note for guidance on maximum shelf life for sterile products for human use after first opening or following reconstitution. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003476.pdf (accessed 11 October 2016).

stability of these products under veterinary field conditions has not been investigated.

To mitigate the risk of contaminating synovial structures, it is common practice for veterinarians to use a new vial of LA when performing intrasynovial analgesia (Bassage and Ross, 2010), whereas perineural analgesia is often performed with the remains of vials that have been previously punctured. LA vials that have been punctured can be stored in practitioners' vehicles or in veterinary hospitals for a number of days before they are reused. Moreover, rubber caps from reused vials are sometimes punctured several times, potentially causing bacterial contamination and consequently, increasing the risk of complications such as septic arthritis and limb cellulitis.

The aims of this study were to investigate the physico-chemical stability and sterility of LAs in both new and repeatedly punctured (RP) vials when stored under veterinary field conditions and in a controlled exposure protocol.

Materials and methods

Study design

A study under simulated clinical field conditions and a controlled exposure protocol were carried out. Twenty millilitre vials of commercially available solutions of the same batch of lidocaine (2% xylocain, AstraZeneca), mepivacaine (2% carbocain, AstraZeneca) and bupivacaine (0.5% marcain, AstraZeneca) were stored in 12 different groups (Table 1) for a period of 4 months (April 2014–August 2014). Groups 3, 7, 9 and 10 were further investigated for an additional 12-month period, leading to a total study period of 16 months (April 2014–August 2015). Unlike mepivacaine and bupivacaine, the commercial solution of lidocaine contained a preservative, methyl parahydroxybenzoate (methylparaben).

In all groups, one vial of each type of LA was opened at day 0 (B-vials). On every testing day, samples were collected from B-vials and from newly punctured vials (A-vials) that had been stored under same conditions as the B-vials. A- and B-vials were of the same batch.

Sampling was performed at 2, 7, 29, 56 and 78 days and 4 months after opening of the B-vials. Additional sampling after 16 months of storage was performed in B-vials from groups 3, 7, 9 and 10. The A-vials from group 3 and mepivacaine A-vials from groups 9 and 10 were also investigated after 16 months of storage. Vials stored according to manufacturer's recommendations (5–10 °C) were included in the study as control group (group 1; Table 1).

Simulated clinical field conditions

Vials stored in groups 8–10 were stored in vehicles belonging to the field practice (veterinarians and technicians) of the Large Animal Hospital of the University of Copenhagen (Denmark), simulating real time veterinary field conditions in Denmark. Vials from groups 8 and 9 were stored on the dashboard for maximum light and temperature exposure. Vials from group 10 were stored in the boot (trunk), shielded from light. Table 1 shows total light exposure and temperature range for these groups.

Controlled exposure protocol

A controlled exposure protocol was carried out in parallel to the simulated clinical field conditions study. Vials from groups 2 and 7 were exposed to fixed storage conditions of different temperatures (−18 °C and 70 °C). Group 4 was exposed to light intensities which met the recommendations of photostability testing from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Q1B Guideline[2]). Groups 11 and 12 were exposed to elevated temperatures (38 °C or 70 °C) and light intensities which met the ICH Q1B Guideline. Table 1 presents details of groups and storage conditions.

Temperature and light intensity

Light intensities and temperatures were measured continuously throughout the study period using a temperature data logger (mini data logger SL51T, Signatrol) and an illuminance recorder (TR-74Ui, T and D Corporation). The temperature logger was programmed to log temperatures every 30 min in groups placed at variable temperature (groups 3, 4, 5, 8, 9, 10, 11, 12) and every 2 h in groups placed at fixed temperatures (groups 1, 2, 6, 7). The illuminance recorder measured light intensities every 5 min throughout the study period in groups 8, 9 and 10. Total light intensity (Table 1) throughout the study period was calculated based on these measurements.

Preparation of samples

Vials were gently turned upside down several times before sampling. B-vials were wiped with alcohol gauze and the rubber cap was allowed to dry prior to sampling. Samples were drawn from the vials using sterile needles and syringes. Hands were cleaned according to the hand hygiene norm (prEN1500)[3] (Verwilghen, 2016) prior to sampling.

Preparation of samples for HPLC analysis

Lidocaine, mepivacaine and bupivacaine samples were diluted using a 10% methanol solution (1:400 for lidocaine and mepivacaine; 1:100 for bupivacaine).

Chemical stability

The concentration of the LAs and of methylparaben was measured using reverse phase chromatography on an Agilent (Agilent Technologies) high-performance liquid chromatography (HPLC) system consisting of a series 11 solvent delivery system, gradient pump (G1312A), autosampler (G1329A) with thermal stabilisation (G1330B), column oven (G1316A), variable wavelength detector (G1314A) operating at 230 nm, and a series 1200 online degasser (G1329B). The column was a Phenomenex (Torrance) Kinetex biphenyl column 100 Å 100.0 mm × 4.6 mm, PN 00D-4622-E0. A binary gradient elution method was used. Mobile phase A consisted of 0.1% formic acid in deionised water (MilliQ). Mobile phase B consisted of 0.1% formic acid in MeOH. The gradient program was 20.0%B at 0.00 min increasing to 80.0%B at 3.50 min, constant 80.0%B until 4.50 min, down to 20.0%B at 5.00 min, constant at 20.0%B until 11.0 min (re-equilibration of column) yielding an analysis time of 11 min/sample. The flow was 0.6 mL/min.

Analysis was performed in triplicate and the chromatograms were compared with control samples (group 1). The concentrations were calculated based on the areas under the curve obtained using HPLC. All samples were refrigerated until processing within 24 h of sampling.

Initial concentration

The concentration of the batch of each commercial LA solution was measured as the mean of 12 different newly punctured vials of each batch, and all were analysed in triplicate. The concentration of the batch was defined as the initial concentration. Table 2 shows initial and stated concentrations of the LAs and methylparaben.

Validation parameters for HPLC method

Standard curves for the three LAs and methylparaben were measured using a range of approximately 50–125% of the nominal concentrations; the minimisation of uncertainty was prioritised over achieving the exact targeted concentrations. The design and validation followed the recommendations for validation of analytical procedures of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Q2 Guideline[4]).

The calibration curve was fitted to a linear function using linear least squares (Supplementary Table 1). The validated range was described by a linear calibration function without any significant deviations from linearity as evidenced by plots of the residuals. The three LAs demonstrated good repeatability, but only the highest concentration (C5) displayed a good repeatability when methylparaben was analysed. The lower concentrations (C1–C4) of methylparaben gave quite high relative standard deviations (RSDs) due to the relatively low concentration and uncertainties from integration. C1 was well above the limit of quantitation for the three LAs, while C1 for methylparaben was approximately twice the limit of quantitation, and therefore was considered only just adequate for quantitative analysis (Supplementary Tables S1 and S2). The following definition was used for the limit of quantitation:

$$(\text{Average area of analytical signal}) = 10 \cdot (\text{standard deviation of analytical signal})$$

The latter formula corresponded to an RSD of 10%. This was considered acceptable, as the primary objective of the study was the investigation of the three LAs and not the analysis of methylparaben.

[2] See: ICH. Stability Testing: Photostability Testing of New Drug Substances and Products. http://www.ich.org/products/guidelines/quality/quality-single/article/stability-testing-photostability-testing-of-new-drug-substances-and-products.html (accessed 11 October 2016).

[3] See: European Standards. CSN EN 1500. http://www.en-standard.eu/csn-en-1500-chemical-disinfectants-and-antiseptics-hygienic-handrub-test-method-and-requirements-phase-2-step-2/ (accessed 12 October 2016).

[4] See: ICH. Validation of Analytical Procedures: Text and Methodology. http://www.ich.org/products/guidelines/quality/quality-single/article/validation-of-analytical-procedures-text-and-methodology.html (accessed 11 October 2016).