Abstract

Objectives: To assess and quantify medetomidine contamination level present in multidose vials of butorphanol in small animal general practices and determine if practice policies and procedures regarding drug handling, as determined by questionnaire, impact upon contamination level.

Methods: Samples of butorphanol were withdrawn from in use vials in participating practices in June and July 2013. Samples were analysed using high-performance liquid chromatography and mass spectrometry.

Results: 41 samples were obtained from 31 practices. Contamination was detected in 29 samples from 10 mL vials. In those contaminated samples the average level of contamination was 0.275 +/- 0.393 $\mu g.mL^{-1}$ (mean +/- SD). The maximum level of contamination was 2.034 $\mu g.mL^{-1}$. There was no significant correlation between volume of the vial used and the level of contamination. None of the survey factors predicted the presence or absence of measured contamination within the vials.

Clinical Significance: Contamination of butorphanol multidose vials with medetomidine was common. However, the level of contamination was insufficient to cause detrimental effects in dogs when butorphanol is administered alone. The potential for sporadic higher levels of contamination must be taken into account especially when using 50mL vials when sedating critically ill cases as this could result in clinical side effects.

Keywords

Butorphanol, medetomidine, contamination, veterinary

Introduction

Butorphanol is marketed as an analgesic and sedative agent and is frequently used in veterinary practice. Although it is most commonly used in combination with medetomidine; used alone it

provides mild sedation (Girard et al. 2010) without any major cardiovascular side effects (Trim 1983). This makes butorphanol a particularly useful sedative agent when dealing with patients with cardiovascular disease (Karas 1999).

Butorphanol is commonly administered with medetomidine in the same syringe resulting in profound sedation. In contrast to butorphanol, medetomidine has profound effects on the cardiovascular system and can reduce cardiac output by up to 70%. These effects are still pronounced at as little as 1/20th of the widely used data sheet recommended dose of 20 $\mu g.kg^{-1}$ in dogs and would be clinically significant in those animals with certain cardiovascular diseases (Pypendop & Verstegen 1998).

Butorphanol is supplied in either a 50mL or a 10mL multidose vial in the UK. While Strachan et al. (2008) have shown that significant bacterial contamination may occur with repeated punctures of multidose vials of propofol, alfaxalone and thiopentone, no studies have addressed the potential for contamination with other drugs. We believe that the common practice of drawing up both medetomidine and butorphanol in the same syringe leads to contamination due to negative pressure within the vials. Using a hypothetical mathematical model, clinically significant contamination of vials is evident even if small volumes (5 µL of medetomidine) are aspirated repeatedly. This could produce potentially unwanted side effects in animals when using butorphanol alone or as part of a neuroleptanalgesic combination.

This study aims to measure the concentration of medetomidine which is present as a contaminant in multidose vials of butorphanol in small animal general practices and to determine if individual practice policies and procedures regarding drug handling, as determined by questionnaire, impact upon the level of contamination present.

Materials and Methods

First opinion small animal practices were asked to participate in the study either during a visit from an ambulatory specialist cardiologist (CD) or by email sent to those practices commonly referring cases to the University Small Animal Teaching Hospital. All practices consented to their involvement in the study.

Samples of butorphanol were withdrawn from in use vials in participating practices between June and July 2013 during practice visits by a cardiologist (CD), and during a one-day collection by another investigator (AB). Vials were inverted and the volume was withdrawn with a needle and syringe. The volume required for analysis was 0.1mL, which was stored in a plain 1mL sample container and refrigerated prior to analysis. The original volume of the butorphanol vial was recorded along with the volume remaining at the time of sampling. The volume remaining was measured by withdrawing all remaining vial contents into a 10mL syringe and noting the volume.

Samples were analysed using high-resolution mass spectrometry and High-performance liquid chromatography using an ACE C18 AR column (150 x 4.6 mm with 5 µm particle size) interfaced with an Agilent 6460 QQQ LC-MS system. The mobile phase consisted of 0.1% v/v formic acid in water:01% v/v formic acid in acetonitrile (65:35) at a flow rate of 0.4 ml. The ESI voltage was +4.0 kV and the medetomidine was monitored in via the transition between the molecular ion at m/z 201 and the fragment ion at m/z 95 using a collision energy of 15 V with argon as the collision gas. The samples were diluted extensively to prevent distortion effects in the chromatography from the much larger butorphanol peak, thus a calibration curve was prepared over the range 8.8×10^{-5} – 0.0132 $\mu g.mL^{-1}$ and had a correlation coefficient of 0.999. The precisions for repeat analysis (n=5) of the points at 8.8×10^{-5}, 8.8×10^{-4} and 0.0132 $\mu g.mL^{-1}$ were ±8.5%, ±2.5% and ±0.72% respectively and the limit of detection determined from the regression line was 2.8×10^{-5} $\mu g.mL^{-1}$.

Practice principles were asked to complete a short survey concerning sedation practices (Appendix 1). This was conducted either on paper or using an internet survey tool (Surveymonkey Inc, California, USA). All statistical analysis was performed using SPSS statistics (IBM SPSS Statistics for

Windows, Version 22.0. IBM, Armonk, NY). Correlations between the level of contamination and the volume of the vial used were examined using Pearson correlation. Contamination was classified as a binary outcome and tabulation of results and Pearson Chi-square was used to investigate the link between contamination and survey response. Additionally, Mann–Whitney *U*-tests were used to compare the level of contamination between groups as defined by survey responses.

There was deemed to be potential for contamination in the vial if the responses to the survey indicated that medetomidine was drawn into a syringe before butorphanol when drawing up drug combinations. Statistical significance was defined as a p value less than 0.05.

Results

41 samples were obtained from 31 participating practices with a maximum of 2 samples per practice. Samples from 10 mL vials accounted for 39 of the samples and 2 samples came from 50 mL vials. The average volume remaining in 10 mL vials when sampling occurred was 3.1 +/- 2.7 mL (mean +/- SD). Contamination was detected in 29 samples from 10 mL vials. In those contaminated samples the average level of contamination was 0.275 +/- 0.393 $\mu g.mL^{-1}$ (mean +/- SD). The maximum level of contamination documented was 2.034 $\mu g.mL^{-1}$ and the distribution of values is shown in figure 1. Of the two 50mL vials sampled, one was uncontaminated and contamination in one was measured at 1.483 $\mu g.mL^{-1}$; in this vial a 7mL volume remained. There was no significant correlation between the volume of the vial used and the level of contamination (figure 2) unless the 50mL vials were included in the analysis, when a statistically significant but weak correlation was evident ($R = 0.51$, $p = 0.04$).

Practices were questioned as to whether dexmedetomidine was used this would have affected the chemical analysis (HPLC phase) and samples would have been treated differently, however all practices used medetomidine exclusively. In 22 practices (71%) the drugs were drawn up by a veterinary surgeon compared with 8 practices (26%) where nurses drew up the medications and 1

practice (3%) where both were responsible. The use of separate syringes was only reported by 1 practice. A formal SOP existed in 6 (20%) of the practices. 67% of respondents drew up medetomidine before butorphanol when drawing up a dog sedation and 64% would draw up medetomidine before butorphanol when preparing a cat premedication. Combining these there was potential for contamination of the butorphanol vial with medetomidine in 24 (78%) of practices in this study. None of the survey factors reported above were significantly associated with the presence or absence of measured contamination within the vials or the level of contamination.

Discussion

The results of the practice survey presented here demonstrate that medetomidine is commonly combined with butorphanol in the same syringe. In 78% of the practices surveyed, there was potential for contamination of the butorphanol multidose vials as medetomidine was drawn up before butorphanol in the same syringe. This practice undoubtedly contributes to the relatively high incidence of contamination of butorphanol multidose vials seen here.

Administration of medetomidine results in a decrease in heart rate and cardiac output and an initial increase in arterial blood pressure. A number of previous studies have determined the dose of medetomidine which results in cardiovascular effects. Beths (2008) determined the effective dose (ED_{50}) of intravenous medetomidine which affected both heart rate and systolic arterial blood pressure. The HR decreased at an ED_{50} of medetomidine of 0.187$\mu g.kg^{-1}$, and the SABP increased at an ED_{50} of 2.05 $\mu g.kg^{-1}$. There was minimal effect on HR and SABP at doses below 0.1 $\mu g.kg^{-1}$. Pypendop & Verstegen (1998) evaluated the cardiovascular effects of medetomidine at different IV doses ranging from 1 to 20 $\mu g.kg^{-1}$. Medetomidine given at a dose of 1 $\mu g.kg^{-1}$ IV, resulted in both cardiac index and heart rate decreasing to approximately half of normal.

The licensed dose for butorphanol is 0.2 to 0.3 $mg.kg^{-1}$ given intravenously, intramuscularly or subcutaneously in dogs when administered alone (Butorphanol Datasheet). When butorphanol is

combined with a sedative, the dose is reduced to 0.1 $mg.kg^{-1}$. Butorphanol may be used alone for sedation in some patients; particularly those with significant cardiovascular disease where the side effects of sedatives such as medetomidine could cause significant morbidity. Clearly cardiac disease is a broad category comprising multiple conditions of differing aetiologies and pathogenesis, and the potential for clinically significant morbidity after medetomidine may differ depending on the condition. Lamont et al (2002) reported that the administration of medetomidine to cats with dynamic left ventricular outflow tract obstruction and ventricular hypertrophy may result in elimination of outflow tract obstruction. While α-2 agonists have been advocated as suitable sedatives in some cardiac disease patients such as cats with hypertrophic cardiomyopathy and some cases of canine aortic stenosis, we should still be cautious about their use and be aware if we are administering the drugs. An unexpected reduction in heart rate would be interpreted rather differently in the presence of α-2 agonist administration. We also do not fully understand, based on clinical evidence, the effects of α-2 agonists in cardiac disease of different aetiologies and caution is advised as inappropriate coronary vasoconstriction induced by dexmedetomidine, might cause myocardial hypoxia (Murrell & Hellebrekers 2005).

Based on the average level of contamination detected in this study, a case receiving butorphanol from a contaminated vial at the licensed dose would receive only 0.006 $\mu g.kg^{-1}$ of medetomidine. If the multidose vial was contaminated at the maximum level observed here, that dose of medetomidine would be 0.04 $\mu g.kg^{-1}$. Although contamination is consistently present, the amount of medetomidine a patient would receive, even at the maximum contamination level detected would likely not be clinically significant and is unlikely to cause cardiovascular effects in dogs as it represents approximately one fifth of the ED_{50} for effects on heart rate. Nevertheless all the studies of cardiovascular effects of medetomidine have been conducted on healthy dogs with normal cardiovascular reserves and vascular tone. In dogs with cardiac compromise the effect could be more significant.

However, we have demonstrated a weak correlation between the volume withdrawn from a vial and the level of contamination when 50 mL vials were considered. This result must be treated with caution due to the low sample size. This correlation does however seem plausible as one would expect contamination to increase the more times the vial is punctured. Each time the vial is contaminated during puncture, a small volume is removed; so additional punctures contaminate a lower volume. This potentially results in an exponential increase in medetomidine concentration within the vial. As such, there is a theoretical possibility that a 50 mL vial could be more contaminated by a factor of at least 5 times the maximum demonstrated here with 10 mL vials. This level of contamination would be at the ED_{50} for medetomidine's effects on heat rate and caution should be exercised when using butorphanol from larger vials as a single agent for sedation in critically ill patients.

A number of clinical practices which should have reduced the likelihood of vial contamination were investigated by the practice survey. While a practice stating that they routinely drew up the drugs in separate syringes should have eliminated contamination, this was not associated with a reduction in the incidence of contamination. This finding seems counterintuitive and probably reflects the fact that the survey was filled out by one senior member of the practice, while a much greater number of staff members drew up the drugs, not always abiding by the procedures deemed appropriate by the senior staff. As only 1 practice in the survey drew up the drugs in separate syringes the sample size is too small to draw firm conclusions. There was also no evidence here that the staff member drawing up the drugs (veterinary surgeon/nurse) or the presence of a standard operating procedure for drawing up the drugs had an effect on vial contamination.

We did not ascertain how long the vials sampled had been in use for in the practices and this represents a limitation. The UK summary of product characteristics (SPC) states that vials should be discarded after 28 days and if vials were used beyond this it could have affected the level of contamination present. However it is not clear exactly the effect this would have. Excessively long

duration use of vials could potentially have led to degradation of contaminating medetomidine. There are no data available that describe the stability of medetomidine in butorphanol specifically. However, the most common cause of degradation of drugs is extremes of pH and since both drugs are of a similar pH (Zoetis and Orion Pharma; personal communications) and remain in the same vehicle it is unlikely there would be significant degradation. Also, there is no obvious chemical incompatibility between the two drugs based on structure.

Using vials for over 28 days could also have led to more punctures being made and a higher level of contamination. The authors acknowledge the number of broaches would have been useful to document for each vial but it was not practicable during data collection and volume remaining was used as a surrogate. Irrespective of limitations surrounding the number of broaches and potential prolonged vial usage beyond SPC recommendations it is important to note that all the vials were still in use in practices. Drugs from these vials would have been administered to patients after sampling and as such the study documents the clinical level of contamination that is occurring in a sample of UK practices. Finally, we took the samples in good faith from veterinary professionals and while we did not record vial broach dates, we would expect that the SPC advice was followed in the majority of the cases yet the majority of samples were contaminated.

While in this study we did not demonstrate clinically significant levels of contamination the sample size was relatively small and a risk of greater contamination on a one-off basis exists with potentially very severe consequences. Contamination of the butorphanol vial was widespread, albeit not clinically significant, and practices should consider implementing procedures which would be considered best-practice such as withdrawing drugs into separate syringes. One should also bear in mind the potential for contamination when using other potent drugs in multidose vials. While we did not investigate any other vial types in this study, a level of contamination, which may or may not be clinically significant, would be expected where vial withdrawal techniques are similar.

In conclusion, contamination of butorphanol multidose vials with medetomidine was common in small animal general practices. However the level of contamination found in the study was insufficient to cause detrimental effects in dogs when administering butorphanol as a single sedative agent. In the absence of further data, veterinary surgeons should be cautious when using butorphanol alone for sedation in critically ill cases; particularly when using the larger 50mL vials as contamination in these vials could reach a clinically significant level.

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Appendix 1 - Practice Questionnaire

The following questions are related to the drawing up of drugs for sedation/premedication for general anaesthesia in your practice

a. Who usually draws up the drugs?

☐Veterinary Surgeon ☐Veterinary nurse

b. Are separate syringes used to draw up different drugs?

☐Yes ☐No

c. If you have to draw up the following drugs in the same syringe, what order would you draw them up? Place in the box, one (1) for the first drug, two (2) for the second drug and three (3) for the third drug. (For example, cat sedation: [1] Medetomidine, [3] Butorphanol, [2] Ketamine)

Dog sedation/premedication: ☐Medetomidine ☐Butorphanol

Cat sedation/premedication: ☐Medetomidine ☐Butorphanol☐Ketamine

d. Is there a policy or SOP in place for this protocol?

☐Yes ☐No

Figure 1. Histogram showing the distribution of contamination in the 29 vials of contaminated butorphanol in the study (10 mL vials)

Figure 2. Scatter plot showing the correlation between volume of the vial used and contamination (10 mL vials)