Microbial growth in a mixture of hyperbaric bupivacaine and fentanyl prepared in a multi-dose syringe in the operating theatre environment

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Abstract

Background: A protocol has been devised in which a 20ml mixture of hyperbaric bupivacaine and fentanyl is prepared in a multi-dose syringe, from which aliquots are withdrawn into individual sterile syringes for use in spinal anaesthesia. The risk of microbial contamination of these multi-dose syringes is unknown and this study was designed to assess such risk.

Methods: In this pilot study, each syringe was prepared using non-aseptic technique to contain a mixture comprising fentanyl 10 μg.ml⁻¹, bupivacaine 4mg.ml⁻¹ and dextrose 64mg.ml⁻¹, with a total volume of 20ml. Syringes were then allocated to pairs. Aliquots were withdrawn hourly from one syringe of each pair for a twelve-hour study period, whilst the other syringe was sampled only at the beginning and end of the same period. All aliquots were withdrawn using standard aseptic technique in an operating theatre environment. For each syringe pair, both samples from the control syringe and four of the samples from the multi-dose syringe were submitted for microbiological culture.

Results: Of the 120 samples taken, one sample was excluded. Of the remaining 119 samples submitted for microbiological investigation, only one yielded growth. This sample had been taken from a multi-dose syringe at the beginning of the study period. Subsequent samples withdrawn from the same syringe were found to be sterile. The organism which had been cultured from this sample was Staphylococcus aureus.

Conclusion: It is possible that the culture medium which yielded the microbial growth was contaminated, which would explain why subsequent samples from the same syringe yielded no microbial growth. Alternatively, bupivacaine is known to be strongly antimicrobial against some pathogens and it is possible that there may have been initial contamination of the syringe by S. aureus, which was inhibited by the bupivacaine to produce subsequent sterile samples. Whilst this may suggest that the use of multi-dose syringes for spinal anaesthesia could be safe, in light of the inconclusive result, further investigation is warranted.

Introduction

Background

The latest American Society of Anesthesiologists (ASA) Practice Guidelines for Obstetric Anaesthesia state that neuraxial techniques are preferred to general anaesthesia for most Caesarean deliveries, owing to a radically decreased maternal mortality from airway complications.¹ Spinal anaesthesia is the commonest form of neuraxial blockade used for Caesarean section in South African operating theatres.

In his survey of the practice of regional anaesthesia in the developing world, Schnitagger found that hospitals may frequently run out of stock of local anaesthetics. In such cases, he found that practitioners often use ampoules intended for single use as multi-dose ampoules, sometimes using the same opened ampoule over several days.² However, guidelines published by the ASA recommend that single dose items be used for one patient only and that medications be drawn up into a syringe immediately before administration.³ Likewise, the position of SASA is to discourage ampoule-sharing between patients as a method to limit costs and other international bodies echo this sentiment.⁴ However, these guidelines have been established largely upon the assumption of good practice, rather than broad-based evidence. Furthermore, obstetric anaesthesia is a core anaesthetic service which is offered even...
in the most resource-limited of environments where these guidelines are not always practical.

And so we find ourselves in a dilemma: ampoule-sharing for spinal anaesthesia is a reality in resource-limited environments, despite guidelines which advise against this practice. These guidelines, in turn, are largely based upon the assumption of good practice rather than firm evidence. Practitioners in resource-limited environments should be supported by context-appropriate evidence in order to inform best practice within resource constraints. It is hoped that the current study will contribute to this pool of evidence.

The setting
Chris Hani Baragwanath Hospital (CHBH) is home to a busy tertiary referral obstetric unit, which performs an average of over 600 Caesarean sections per month. For purposes of cost and time-saving, a protocol was devised whereby hyperbaric bupivacaine and fentanyl are mixed in a 20 ml syringe to yield concentrations of fentanyl 10 μg.ml⁻¹, bupivacaine 4mg.ml⁻¹ and dextrose 64mg.ml⁻¹. A new sterile syringe is then used to withdraw 2.0 – 2.2 ml of the drug mixture for use in spinal anaesthesia for each Caesarean section. Considering current tender prices of drugs, this protocol results in a drug cost per patient of R4,48 as opposed to R11,90, which is the cost if a new ampoule of each drug is opened for each patient. With the number of Caesarean sections performed each year, the cost difference becomes appreciable. However, there was a concern as to possible microbial contamination of the contents of this multi-dose syringe when left in the operating theatre over a number of hours. This study was designed to investigate this risk.

Literature review
A systematic literature review was conducted to give a sense of what the risk of microbial contamination of this drug mixture may be. No previous studies have examined the risk of microbial contamination of this particular drug mixture, and therefore the literature was searched for related studies. When looking at the risk of microbial contamination of each component of the drug mixture, the literature offers conflicting suggestions. Bupivacaine displays antimicrobial effects against certain pathogens, whilst others (notably Pseudomonas aeruginosa) are resistant to its effects.6-16 Dextrose may support microbial growth by provision of nutritional substrate at the concentrations in our protocol 17-20 whilst it appears that fentanyl neither inhibits nor promotes microbial growth.12,21 Also of relevance is that the risk of contamination of multi-dose vials, in general clinical practice, appears to be exceedingly rare.22-27 What was clear from the literature, however, was that the introduction of microbes into the intrathecal space is highly undesirable, with some authors suggesting that any microbes introduced into the CSF will almost certainly produce a fulminant meningitis.28 Previous work has estimated the incidence of post dural puncture meningitis at between 0 – 7.2/1 000 00029-34 whilst the incidence of epidural abscess following spinal anaesthesia is probably far lower.30 However, these estimates were made in the developed world where, presumably, the occurrence of ampoule-sharing is low.

Study design
Since the protocol under consideration is not in line with current international recommendations, it was not surprising that our review of the literature failed to produce any similar prior studies. Therefore, a prospective longitudinal pilot study was designed in order to give an indication of whether the use of this multi-dose syringe protocol poses an overt risk to the patient. An ethics waiver was granted by the Human Research Ethics Committee of the University of the Witwatersrand.

The methodology of this initial study was intentionally kept as simple as possible so as to avoid confounding factors. The objective was to use the data collected during the pilot study to inform methodology and required sample size for a future definitive study. In this pilot study, syringes were prepared according to the CHBH protocol in one of the obstetrics theatres at that hospital. Syringes were allocated to pairs, with each pair having a so-called “control syringe” from which a sample was withdrawn at Time = 0 hours and again at Time = 12 hours, both of which were sent for microbiological investigation at the National Health Laboratory Service (NHLS) laboratory at CHBH. The other syringe of the pair was designated as the “multi-dose syringe,” from which aliquots were withdrawn hourly for the same 12 hour study period in order to simulate clinical sampling rates as closely as possible. Samples withdrawn from the multi-dose syringe at Time = 0, 4, 8 and 12 hours were sent for the same microbiological investigation whilst remaining samples were discarded. All sampling was carried out in the same operating theatre. Throughout study periods, the syringes were left on the anaesthetic work surface in the theatre to imitate the clinical situation. Theatre temperature trends and theatre traffic were also recorded during all study periods.

Results
Twenty syringe pairs were studied, yielding a total number of 120 samples. Of these, one sample was excluded as it was deemed that aseptic technique had been breached during collection. Of the remaining 119 samples, one sample yielded positive microbial
growth, with *Staphylococcus aureus* (S. aureus) being identified as the organism responsible. This sample had been taken from a multi-dose syringe at time = 0 hours. Subsequent samples from the same syringe yielded no growth.

Measurement of theatre temperatures indicated a consistent pattern of diurnal variation. However, there was considerable discrepancy between temperatures recorded for correlating times between the twelve-hour study periods. The only sample which yielded microbial growth was taken when the theatre temperature was 22.6 °C, with the lowest recorded temperature during the study period being 22.4 °C.

The number of theatre cases commenced at each sampling time was recorded. There was no appreciable difference in number of cases done during each study period, with the range being between five and seven cases commenced during each 12 hour study period.

**Discussion**

**Discussion of results**

Of 119 samples submitted for microbiological investigation, one yielded growth of S. aureus. The sample which yielded this growth was taken at the beginning of a study period from a multi-dose syringe (Time = 0 hours). However, subsequent samples taken from the same syringe at Time = 4, 8 and 12 hours yielded no growth, either of S. aureus or any other microbe. There are several possible explanations for these results.

Possible explanations as to why one sample was found to be contaminated include:

1. **True contamination**: It is assumed that contamination sufficient to yield a positive culture result could be clinically significant. Our literature review has suggested that bupivacaine is a potent antimicrobial, which is particularly effective against S. aureus.6,13,15,16 This could explain why subsequent samples from the same syringe yielded negative culture results.

2. **False positive**: Contamination of the culture medium may have occurred at some stage before or after inoculation, rather than as a result of contamination of the contents of the syringe. This would explain why all other samples taken from the same syringe yielded negative culture results.

Explanations of why more samples were not found to be contaminated include:

1. **Lack of other contamination**: It is possible that there simply was no contamination of the remaining syringes. This would be in keeping with the literature which suggests that contamination of multi-dose drug containers is exceedingly rare in general.

2. **Microbes may have been unable to survive in the drug mixture**: Some microbes may have been inoculated into the syringes under investigation, but were unable to survive or multiply in the drug mixture, mainly as a result of the antimicrobial properties of bupivacaine. The concern with this explanation, however, is that there are some pathogens which seem to be resistant to the antimicrobial effects of bupivacaine, such as *Pseudomonas aeruginosa*,6,7,14,15 which may be allowed to multiply in this drug mixture should contamination take place.

3. **Contaminants may not have yielded positive culture results**: Thioglycollate broth was selected for use as it is an all-purpose growth medium which is useful in isolating a wide range of organisms and is good at yielding recovery from low numbers of microbes in the initial inoculum. The broth was incubated in an aerobic incubator only, as per laboratory protocol,35 and this could conceivably have decreased the growth of any anaerobes present. However, there are some species which are fastidious and may not grow under the laboratory conditions presented.

**Discussion of secondary outcomes**

This pilot study was not sufficiently powered to provide robust evidence to comment upon the secondary outcomes. It was interesting to note, however, that the sample which yielded microbial growth was taken at one of the lowest theatre temperatures recorded. This could be coincidence (and would certainly be if this is a spurious result), or could be related to the decreased antimicrobial properties of bupivacaine at lower temperatures.36 There was no appreciable difference between theatre traffic in any of the sampling intervals.

**Discussion of legal implications**

The drug mixture under consideration contains fentanyl, which is a schedule six drug. As stated in the General Regulations in terms of the Medicines and Related Substances Act, 1965 (Act No 101 of 1965), published by the Department of Health in 2003, all schedule six substances need to be logged in a register where they are dispensed.37 Whilst the Regulations do not state that each ampoule must be registered to a single patient, the name of each patient who does receive a portion of an ampoule must appear in the register.

**Limitations of this study**

Since research of this nature has not been done before,
this pilot study was designed in order to inform both study methodology and sample size needed for a future study. The design of the pilot study intentionally removed as many confounders as possible in order to examine the question at hand. However, in removing these variables, the clinical context in which the results of this study are applicable have been narrowed in the following ways.

1. This study was carried out by a single investigator. Since anaesthetic personnel differ in their performance of aseptic technique, it is possible that the findings of this study were dependent upon the individual carrying out the procedure.
2. This study was carried out in a single theatre and the results obtained in this particular theatre may not be able to be extrapolated to other theatres.
3. Each drug studied was supplied by a single manufacturer and the ampoules of each of these drugs came from a single batch.
4. In this study, thioglycollate broth was the sole culture medium used. This is an all-purpose growth medium which is useful in isolating a wide range of organisms. However, there are fastidious organisms which may require other speciality broths in order to grow.
5. Samples were sent to one laboratory only.
6. Samples were processed according to the National Health Laboratory Services Standard Operating Procedures, which specifies incubation in an aerobic incubator. There is a possibility that anaerobic organisms may not have grown because of this environment.

Suggestions for future studies
The purpose of this pilot study was to establish whether there is a clear and undeniable risk to patients in using this protocol, in which case further investigation would be unwarranted and the protocol should be immediately abandoned. However, this clear risk was not established with the simple methodology used in this pilot study. To avoid ambiguity in results, the methodology of future work will necessarily be more complicated and sample numbers will be greater. In view of the outcomes of this pilot, the following recommendations are made for future work.

Recommendations to make results more universally applicable
1. Involvement of multiple investigators: Since aseptic technique is important, but not standardised, it would seem prudent to include samples from drug mixtures prepared by many different anaesthetists.
2. Carry out investigations in multiple theatres:

Samples taken from syringes prepared in multiple theatres which service different patient populations could provide a broader spectrum of results.

3. Use drugs produced by various manufacturers: The drugs studied should be obtained from as many different suppliers as possible, since differences in manufacturing processes may alter results.
4. Use more diverse microbiological investigations: To ensure that all possible organisms causing contamination are cultured, a variety of growth media should be used, and these should be incubated in both aerobic and anaerobic incubators.

Recommendations to decrease the chance of ambiguous results
1. Increased sample size: The results obtained in this study suggest that 581 syringe pairs would be needed in order to assess a 5% difference in contamination rates between control- and multi-dose syringes (two-tailed test, $\alpha = 0.05$ and $\beta = 0.10$).
2. Transfer of samples in a sterile environment: It is of great benefit to prepare the drug mixtures and withdraw samples from the syringes in the theatre environment as this exposes the contents to conditions akin to those in the clinical context. However, transferring the sample into culture media in the theatre environment offers additional opportunity for spurious contamination of the culture medium. Therefore, the samples withdrawn from the syringes should be sealed in an air-tight container and then transported to the laboratory. Here, the samples can be inoculated into growth medium under a laminar flow hood, which would decrease the risk of extrinsic contamination.
3. Processing of samples in multiple laboratories: Ideally, multiple samples should be withdrawn at each time period and sent to different laboratories. This would give a comparison in the event of a positive culture result. Practicability and funding would dictate how many laboratories could be utilised.

Conclusions
A protocol has been devised in which a multi-dose syringe is prepared with a mixture of hyperbaric bupivacaine and fentanyl which is then drawn up into multiple smaller syringes, each used for spinal anaesthesia in different patients. This initial study failed to show that the use of such a protocol poses a microbiological risk to patient well-being. However, there is a great deal more work which needs to be done in order to assess whether there is a risk, and a far broader study will need to be undertaken.
Currently accepted guidelines which advise against the use of ampoule-sharing in the anaesthetic environment are based largely upon the assumption of good practice rather than evidence. 3-5 However, the reality in resource-limited settings is that drugs used for spinal anaesthesia are often in short supply and, as a result, practitioners regularly use single-dose ampoules for more than one patient. 1 Therefore, it is of utmost importance to establish appropriate, evidence-based guidelines to optimise resource utilisation without compromising patient safety.

This pilot study is the first step taken towards establishing this evidence in connection with the protocol for preparing a drug mixture for spinal anaesthetic in a multi-dose syringe. It is hoped that future work based upon this pilot study will assist in establishing such appropriate guidelines, which take cognisance of the reality of constraints in resource-poor environments, whilst striving to ensure patient safety and well-being.

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