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REPORTS OF ORIGINAL INVESTIGATIONS

Investigation into spinal anesthetic failure with hyperbaric bupivacaine: the role of cold exposure on bupivacaine degradation

Enquête sur les échecs de la rachianesthésie réalisée avec de la bupivacaïne hyperbare : étude du rôle de l'exposition au froid sur la dégradation de la bupivacaïne

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Abstract

Purpose *Hyperbaric bupivacaine (0.75% in dextrose) is used for spinal obstetric anesthesia. Occasional clusters of anesthetic failures occur in this setting, not readily attributable to clinical factors. We hypothesized that cold temperature exposure is related to bupivacaine instability.*

Methods *An electronic survey was distributed to Canadian anesthesiologists to determine consistencies in spinal anesthesia practice, and to invite submission of failed bupivacaine samples for analysis. Another survey for hospital pharmacists focused on bupivacaine logistics. Ultraviolet (UV) spectrometry, differential scanning calorimetry, and high performance liquid chromatography were used to evaluate the effect of temperature on bupivacaine chemical stability. Mass spectrometry (MS) was used to observe bupivacaine and dextrose degradation in laboratory samples of hyperbaric 0.75% bupivacaine in dextrose. Hyperbaric bupivacaine that failed to produce adequate anesthesia in labour and delivery patients was subject to tandem MS/MS analysis on commonly observed ions to look for ion patterns consistent with bupivacaine degradation products and to compare with laboratory samples subjected to cold temperatures.*

Results *Canadian obstetric anesthesiologists report similar practices and use hyperbaric bupivacaine for spinal anesthesia. Pharmacists surveyed indicated facility storage at room temperature but variable temperatures during shipping. No standard procedure for failure reporting was identified. Analysis of bupivacaine showed a slight decrease in bupivacaine concentration or UV spectral changes after incubation at temperatures* $\leq 4°C$*. Mass spectrometric analysis of hyperbaric bupivacaine from failed spinal anesthesia cases showed complex and inconsistent patterns of ion formation, and different from the ion patterns observed for cooled vs uncooled bupivacaine solutions. Temperature-related changes were noted for dextrose in cooled samples in which dextrose-related ions were formed.*

Conclusions *Canadian clinical practice and handling of hyperbaric bupivacaine is consistent. Most respondents indicated an interest in a formal reporting and collection process. Cold exposure did not degrade bupivacaine. A complex and possibly inconsistent reaction involving dextrose was identified that requires further analysis of a larger sample size to elucidate the mechanisms.*

Résumé

Objectif *La bupivacaïne hyperbare (0,75 % dans du dextrose) est utilisée pour l'anesthésie obstétricale rachidienne. Il arrive parfois que plusieurs anesthésies rapprochées soient inefficaces dans cette situation, et ces*

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échecs ne sont pas nécessairement attribuables à des facteurs cliniques. Nous avons émis l'hypothèse qu'une exposition de la bupivacaïne au froid expliquerait son instabilité.

Méthode *Un sondage électronique a été distribué aux anesthésiologistes canadiens afin de déterminer les similitudes dans la pratique de la rachianesthésie, et nous avons invité les médecins à nous envoyer des échantillons de bupivacaïne à des fins d'analyse lorsque leur anesthésie était inefficace. Un autre sondage, envoyé aux pharmaciens hospitaliers, mettait l'emphase sur la logistique entourant la manutention de la bupivacaïne. Nous avons utilisé une spectrométrie de rayons ultraviolets (UV), une analyse calorimétrique différentielle et une chromatographie liquide à haute performance afin d'évaluer l'effet de la température sur la stabilité chimique de la bupivacaïne. Une spectrométrie de masse (SM) a été utilisée pour observer la dégradation de la bupivacaïne et du dextrose dans des échantillons de laboratoire de bupivacaïne hyperbare 0,75 % dans le dextrose. La bupivacaïne hyperbare qui n'a pas procuré une anesthésie adéquate chez des patientes en travail ou en accouchement a été sujette à une analyse de SM/SM en tandem sur les ions fréquemment observés afin d'identifier des modèles ioniques correspondant aux produits de dégradation de la bupivacaïne et les comparer à des échantillons de laboratoire soumis au froid.*

Résultats *Les anesthésiologistes obstétricaux canadiens font état de pratiques semblables et utilisent de la bupivacaïne hyperbare pour réaliser une rachianesthésie. Les pharmaciens interrogés ont indiqué que la bupivacaïne était entreposée à température ambiante au sein de leur établissement mais qu'elle était exposée à des températures variables pendant l'expédition. Aucune procédure standardisée n'a été identifiée pour rapporter les échecs d'anesthésie. L'analyse de la bupivacaïne a montré une légère réduction dans la concentration de bupivacaïne ou des changements spectraux UV après une période d'incubation à des températures* $\leq$ *4°C. L'analyse par spectrométrie de masse des échantillons de bupivacaïne hyperbare utilisés lors d'échecs de la rachianesthésie a révélé des types de formation des ions complexes et incohérents, lesquels différaient des modèles des ions observés dans les solutions de bupivacaïne refroidies vs non refroidies. Les changements liés à la température ont été notés sur le dextrose dans les échantillons refroidis dans lesquels des ions liés au dextrose se sont formés.*

Conclusion *La pratique clinique canadienne et la manutention de la bupivacaïne hyperbare est homogène. La plupart des répondants ont indiqué être intéressés par un processus formel d'enregistrement et de récolte des données. L'exposition au froid n'a pas dégradé la bupivacaïne. Une réaction complexe et possiblement inconstante ayant un rapport avec le dextrose a été identifiée; elle requiert des analyses approfondies sur un échantillonnage plus important afin d'en élucider les mécanismes.*

Subarachnoid (spinal) block (SAB) is generally viewed as safe, produces sensory and motor blockade, and reduces the likelihood that the airway management associated with general anesthesia will be required during Cesarean deliveries.[1] Hyperbaric bupivacaine, 0.75% in 8.25% (w/v) dextrose, is often the drug of choice for such procedures.[1] Subarachnoid neuraxial (spinal) anesthesia failure despite apparent technical success is well described,[2,3] but is not well understood; it may include non-mutually exclusive reasons such as unseen or misunderstood technical failure, patient anatomical idiosyncrasies, and pharmacodynamic changes of the anesthetic agent.[2] When failure occurs, the patient may receive a general anesthetic or another spinal anesthetic.[4] A general anesthetic may expose the mother to an increased risk of aspiration and hemodynamic perturbations, it may not allow the mother to experience the birth of their child, and may expose the fetus to anesthetic agents unnecessarily. With multiple attempts at neuraxial techniques the patients relative risk of infection, bleeding, and neurologic complications may increase.[5]

We hypothesized that clusters of spinal anesthetic failures are due to exposure of hyperbaric bupivacaine solution to cold temperatures resulting in bupivacaine chemical instability, rather than inconsistencies in clinical practice. We conducted two surveys, one each with anesthesiologists and hospital pharmacists, to increase our current understanding surrounding bupivacaine transport, storage, preparation, and clinical practice when a failure occurs. We attempted to detect bupivacaine degradation products or physical alterations in pharmaceutical reference chemicals, commercial products, and in commercial products that failed to produce an anesthetic response in labour and delivery patients at a local hospital.

Methods

Anesthesiologist survey

A cross-sectional electronic survey was distributed to Canadian anesthesia academic department heads, directors of obstetric anesthesia, pharmacy and therapeutics leaders, and clinical chiefs for completion. The survey (eAppendix,

available as Electronic Supplementary Material [ESM]) queried spinal anesthesia practice and invited submission of failed bupivacaine samples for analysis at our institution. The survey was pretested by staff anesthesiologists and residents ($n = 4$). The survey was then pilot tested by ($n = 4$) obstetrical anesthesiologists within the Department of Anesthesiology, Royal University Hospital, Saskatoon to ensure the questions were appropriate. The finalized electronic survey was prepared using the University of Saskatchewan Fluid Survey Tool and was distributed via email (personal contacts). The (target population) sample frame was chosen by nonprobability design to select individuals who work in obstetrical anesthesia and are in positions that would be informed of neuraxial anesthetic failures within their institution. The target population was department heads, directors of obstetric anesthesia, anesthesia department pharmacy and therapeutics leaders, and clinical chiefs. Respondents were located across Canada in British Columbia, Alberta, Ontario, Quebec, New Brunswick, and Nova Scotia. Some snowball sampling occurred where respondents identified other relevant members of their department. This is a limitation as our true N value is unknown. The initial email invitation was followed at two-week intervals with two additional invitations to complete the survey. Of the 72 surveys we distributed, 65 were returned (90% response rate). Only a single response from each email was enabled using a unique invite code. The number of responses for each answer and the percentage of respondents who selected each response were determined. Emails were sent to obstetrical anesthesiologists and posters were placed throughout the labour and delivery unit of the authors' institution to inform practitioners that we were collecting samples of failed hyperbaric bupivacaine for analysis. When a spinal failure occurred, the vial was placed in a container and then hand-delivered to the local university mass spectrometry (MS) laboratory for analysis, which was in a connecting building.

Canadian hospital pharmacist survey

A separate survey was created for hospital pharmacists that focused on bupivacaine logistics (eAppendix, available as ESM). A 22-item electronic questionnaire was sent out via Survey Monkey to 87 consenting pharmacists at Canadian hospitals with obstetrics units, regarding the acquisition, transport, and storage of bupivacaine, as well as the reporting of spinal bupivacaine drug failures. The Survey Monkey platform was used in place of Fluid Surveys because of a change in the institution's subscription status. The pharmacist survey did not exactly mirror the anesthesiologist survey because of differences in the questions and how the pharmacists were contacted. Contact information was obtained by calling hospital pharmacies from public directories of hospitals with labour and delivery units. After consent, potential respondents were sent a link to the survey, available over approximately seven weeks. To promote survey completion, we followed up via phone or email. The response rate was 44% (38 responses out of 87 invitations). The number of times each response was selected and percentages for each response was determined using Survey Monkey.

Bupivacaine physicochemical analyses

Materials

Bupivacaine reference standard (AlphaAesar > 99% purity), 1, 2-dimethylanaline (> 98.5% purity), D-(+) glucose (dextrose) (> 99.5% purity) were purchased from Sigma Aldrich Canada. Mass spectrometry-grade methanol and water were from Fisher Scientific. Differential scanning calorimetry was performed in vented aluminum pans from TA Instruments (Newcastle, DE, USA). Commercial hyperbaric bupivacaine was Marcaine® 0.75% hyperbaric (Hospira).

High performance liquid chromatography (HPLC)

To determine whether the enantiomeric ratio of R, S-bupivacaine was affected by storage temperature, chiral HPLC of bupivacaine was performed after incubation of bupivacaine at various temperatures (43°C, RT, 4°C, −20°C). A Waters Alliance 2695 separations module with a Waters 996 PDA detector was used with a Chiralpack AS-H (Diacel), 4.6 × 15 mm column for separation, and Millennium32 (version 2.1) software for data analysis. The mobile phase was hexane:2-propanol (98:2 v/v) +0.3% acetic acid and 0.05% (v/v) trimethylamine run in isocratic mode at 0.75 $mL \cdot min^{-1}$ at ambient temperature. Retention times were 4.95 and 7.2 min for the two enantiomers. Bupivacaine was quantified using an external calibration curve (linear range 10-125 $\mu g \cdot mL^{-1}$, $r^2 = 0.99$). The enantiomeric ratio was determined by the quotient of integrated peak areas at 4.95 and 7.2 min.

Bupivacaine and 2, 6-diemethylanaline (DMA) were quantified by reverse-phase HPLC after storage at 21°C, 4°C, or −20°C for 72 hr. A Waters Alliance 2695 separations module with a Waters 996 PDA detector was used with an Xterra C18 3.5 μm column (4.6 × 100 mm) at 40°C. Data analysis was performed with Empower software (v.1154). The mobile phase was acetonitrile: H_2O (50:50 v/v) run in isocratic mode at 0.6 $mL \cdot min^{-1}$. The retention times for bupivacaine and DMA were 1.03

min and 3.86 min, with a linear range of 10-100 $\mu g \cdot mL^{-1}$ ($r^2 > 0.96$) for bupivacaine and 5-100 $\mu g \cdot mL^{-1}$ ($r^2 > 0.99$) for DMA. Data are expressed as mean (standard deviation [SD]) ($n = 12$).

Nuclear magnetic resonance (NMR)

A Bruker Advance III HD 600 MHz spectrometer three-channel NMR system with variable temperature capability and a Bruker 5 mm broadband observe probe was used to analyze bupivacaine reference standards in dextrose. Samples were prepared using deuterated water (D_2O) and stored at 21°C, 4°C, or −20°C for 24 hr prior to ^{1}H and ^{13}C NMR.

Differential scanning calorimetry

Aliquots of bupivacaine powder (7 mg) were hermetically sealed in aluminum pans and thermal cycling was performed in a differential scanning calorimeter (Q2000, TA Instruments). For solutions, vented pans were used. Samples were cooled from 40°C to −50°C at a rate of 1°C/min, held for five minutes at −50°C then heated to 40°C at 1°C for three freeze/thaw cycles. The crystallization and melting points as well as enthalpy were determined from the peak onset temperatures on the thermograms using the Universal Analysis data analysis software (V4.5A, TA Instruments).

Mass spectrometry

To evaluate degradation in the clinical samples of commercial hyperbaric bupivacaine (Marcaine®), MS analysis was performed. Hyperbaric bupivacaine samples that had no anesthetic effect were analyzed before and after incubation at 4°C overnight. Other samples that displayed the expected therapeutic effects from the same lot of failed samples were also analyzed. Bupivacaine reference standard and dextrose alone were also analyzed for comparison. Bupivacaine standards were stored at various temperatures (−80°C, −20°C, 4°C, or 23°C) for 72 hr to assess temperature-induced degradation.

A linear ion trap-triple quadrupole mass spectrometer (4000 QTRAP®, AB Sciex, Analyst 1.6 software, Mississauga, ON, Canada) was used in the positive ion mode with direct injection of bupivacaine at 50 $ng \cdot mL^{-1}$ in methanol (LC-MS grade). Electrospray ionization was used with the following source parameters: temperature 200°C, ionization voltage 5,500, and declustering potential 60 V. The scan range was set at mass/charge ratio (*m/z*) 50-300. Commonly observed ions in the full scan mode were subjected to collision induced dissociation- tandem mass spectrometric analysis (CID-MS/MS) with a collision energy ranging between 20-30 eV. MS^3 analysis of selected peaks was conducted with the similar conditions above, keeping the excitation energy (AF2) in the range of 20-60.

Statistical analyses

Descriptive statistics and quartile analysis were calculated using Excel. Drug concentration data in stability studies were normally distributed and analysis of variance was used to determine significant differences between groups where indicated, with significance set to $P < 0.05$ and post-hoc testing performed using Tukey's test.

Results

Anesthesiologist survey

See Table 1. Canadian obstetric anesthesiologists report similar practice and mainly use hyperbaric bupivacaine for spinal anesthesia. Cesarean deliveries were predominantly (97%) done under spinal anesthetic, while some were performed using a combined spinal and epidural technique. All respondents indicated bupivacaine 0.75% was their primary choice for neuraxial blockade. Most respondents dosed their local anesthetic according to patient characteristics (predominantly weight and height) and predicted surgical speed. Doses ranged between 10-12 mg. Most respondents indicated they add adjuvants to their local anesthetic, most commonly fentanyl (10-25 μg) and epimorph (100-250 μg), immediately prior to use. All respondents indicated that they perform their spinals with the patient in the sitting position.

Canadian hospital pharmacist survey

Standards of practice were similar, except for those displayed in Table 2. Hospital pharmacy storage, personnel responsible for adding other medicinal ingredients to the bupivacaine before administration, and failures occurring throughout all seasons were the same among respondents. Only half (50%) of respondents indicated their facility had a standard protocol for reporting failures, while 64% of respondents maintained some record of the failures. All respondents indicated their bupivacaine was shipped from within the country, though 37% receive shipments from another province. The number of failures reported in the past two years ranged from none to greater than six. Pharmacists surveyed reported variable transportation conditions during shipping.

Table 1 Selected results from the anesthesiologist survey

Variable	Response	% of respondents	# responses
Location	Canada	98.5	55
	Hong Kong	1.5	1
% practice combined spinal epidurals	0–25%	95.3	62
	> 75%	4.7	3
Fixed dose used for spinals			
Dose (mg)	Yes	72.3	47
	10–12	27.7	18
	No	72.3	47
Factors for dose adjustment	Patient factors	81.8	36
	Practitioner factors	43.4	23
Adjuvant(s) mixed with local anesthetic			
Fentanyl (dose mcg) variable	–	76.9	50
	10–15	51.4	36
	20–25	7.8	5
	10–25	9.1	6
Epimorph (dose mcg) variable	–	72.3	47
	100–125	33.8	22
	150	27.7	18
	200	3.1	2
	100–250	7.6	5
Epinephrine	–	4.6	3
Type of needle used for subarachnoid	Whitacre	78.5	51
	Other	18.5	12
Needle gauge	24–25	60	39
	26–27	55.3	36
	25 or 27	3.1	2
% failures when technically successful	0%	10.8	7
	1–10%	87.7	57
Failure management	Titrated epidural	23.1	15
	GA	40	26
	Repeat spinal	18.5	12
	Other/variable	9.3	6
Failures reported	Yes	53.1	34
	No	46.9	30
Interest in participating in centralized analysis	Yes	85.9	55
	No	14.1	9

High performance liquid chromatography

Commercial hyperbaric bupivacaine available in Canada contains a 50:50 enantiomeric ratio (R/S). Some, but not all, studies suggest that potency in spinal anesthesia may differ by enantiomeric form[6,7]; therefore, to eliminate this factor as a potential concern, chiral HPLC was performed. Nevertheless, the analysis showed no change in R/S ratio (50:50) as a function of temperature following sample incubation at 43°C, 23°C, 4°C, or −20°C followed by ambient temperature analysis (data not shown).

To identify any changes in bupivacaine concentration (initially 0.75% in dextrose) following exposure of the solutions to various temperatures, HPLC was performed to quantify bupivacaine and a potential degradation product, 2, 6-dimethylanaline (DMA) (Fig. 1). The WHO guidelines for accelerated stability studies recommend a temperature of 40°C ± 2°C[8]; 23°C is the typical ambient temperature at our facility, 4°C is refrigerator temperature, and −20°C is a common winter temperature at which our facility freezers were maintained. No significant change in the mean bupivacaine concentration occurred as a function of

Table 2 Selected hospital pharmacist survey results regarding standards of practice for bupivacaine failures

Variable	Response	# of responses	% of respondents
Transport method from manufacturer to pharmacy	Temp controlled ground	11	40.7
	Non-temp controlled ground	4	14.8
	Temp controlled air	1	3.7
	Non-temp controlled air	0	0
	Unknown	11	40.7
Presence of standard protocol to report failures	Yes	11	50
	No	11	50
Maintenance of record of failures	Yes	14	63.6
	No	8	36.4
Info pertaining to failures recorded	Date of failure	11	78.6
	Batch number	8	57.1
	Lot number	11	78.6
	Additives incorporated	0	0
	Not recorded	1	7.1
	Other	3	21.4
Procedure when failure detected	Entire batch disposed	1	4.6
	All product containing same lot # disposed	3	13.6
	Failed batch sent back to manufacturer	4	18.2
	Only failed sample disposed	2	9.1
	Failed batch sent for testing	2	9.1
	Other/unknown	10	45.45
Quantity bupivacaine received at a time	< 500 ampoules	15	57.7
	500–1,000 ampoules	4	15.4
	1,000–5,000 ampoules	1	3.9
	Unknown	6	23.1
Interested in participating in a centralized analysis to collect failed batches	Yes	9	50
	No	9	50

The included questions are those relevant to standard practices surrounding bupivacaine failures. Some responses have been amalgamated from the original survey response options

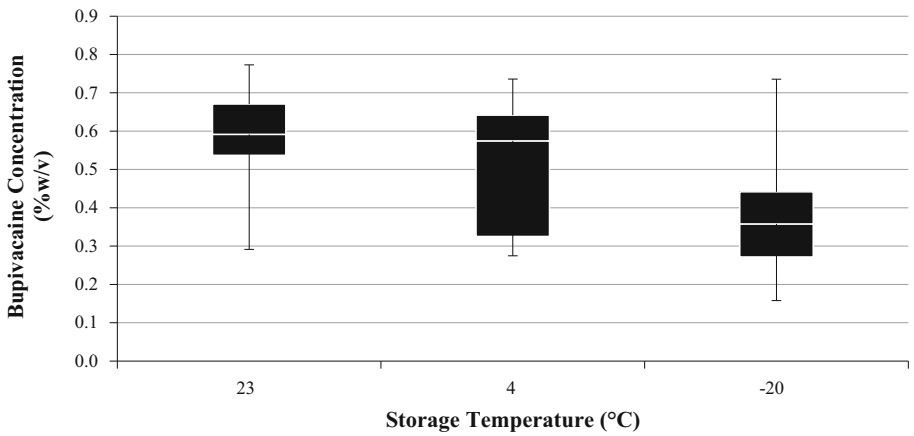

Fig. 1 High performance liquid chromatography analysis of 0.75% w/v bupivacaine in 8.25% dextose, pH 7 of samples stored 72 hr at the indicated temperatures (n = 12). The plots describe mean (white line), interquartile range (box), and standard deviation (bars)

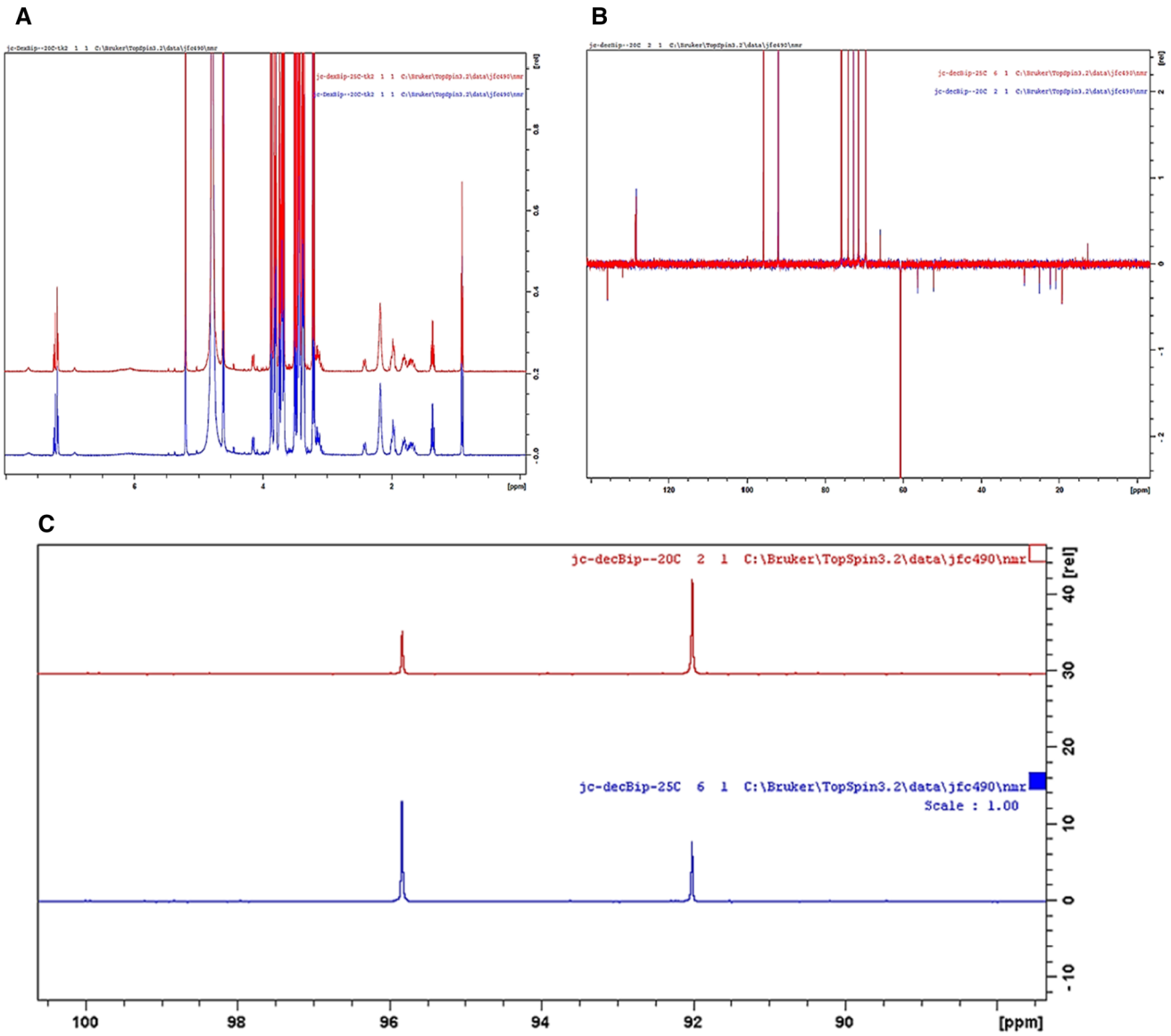

Fig. 2 Nuclear magnetic resonance (NMR) analysis of laboratory-prepared hyperbaric bupivacaine solution. ^{1}H (A) and ^{13}C (B) NMR obtained for bupivacaine (0.75% w/v) and dextrose (8.25% w/v) in D_2O after 24 hr at either 21°C or −20°C. For A) red: 2°C; blue: −25°C. For B) blue: 21°C; red: −25°C. C) Very subtle differences are seen, predominantly with dextrose (conversion of α- to β- with decrease in temperature as seen in expansion of B shown in Fig. 2C)

storage temperature and no appearance of DMA was noted in the samples.

Nuclear magnetic resonance

Nuclear magnetic resonance analysis indicates chemical shifts of functional groups according to their molecular environment and is therefore a powerful technique to identify pharmaceutical impurities or degradation products. Nuclear magnetic resonance of the bupivacaine reference samples prepared as 0.75% (w/v) in 8.25% dextrose in deuterated water (D_2O) and stored at 21°C, 4°C, or −20°C for 24 hr showed minor changes in the dextrose (α-β conversion) but no changes in the ^{1}H or ^{13}C bupivacaine NMR signal compared with samples stored at ambient temperature (Fig. 2).

Differential scanning calorimetry (DSC)

Bupivacaine hydrochloride can be found as a monohydrate and can form at least two distinct polymorphic forms.[9-12] Polymorphs may differ in solubility or degradation rate. Differential scanning calorimetry was performed to look for polymorphism, which would be revealed by changes in melting or freezing points as well as enthalpy of phase changes following thermocycling. For bupivacaine powder,

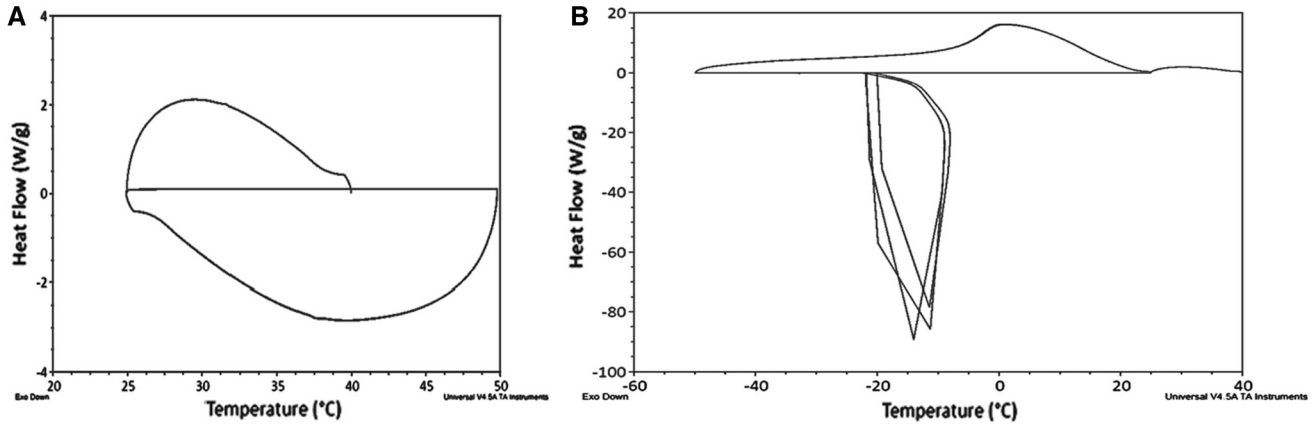

Fig. 3 Differential scanning calorimetry analysis of bupivacaine melting and freezing curves with thermocycling (three times) in dry powder form above ambient temperature (superimposed, no changes); A) in 8.25% (w/v) dextrose solution (hysteresis; slight changes in freezing point upon thermocycling); and B) in the range of temperatures that may be encountered during product shipping

the temperature range of the thermocycling (+40°C to −50°C) was not associated with any melting points. The reported melting point of bupivacaine is (104-108°C).[12] No polymorphic changes were noted on the thermograms for the powdered drug. For bupivacaine 0.75% (w/v) in dextrose 8.25% (w/v) solutions, which contain water, the melting point was at −10°C to −15°C, a freezing point depression consistent with the presence of dextrose. The thermocycling generated nonsuperimposable exothermic peaks with a heat flow of −80 to −90 g/W. The onset of melting of the ice was consistently −5°C for all cycles, generating a broad, amorphous peak (Fig. 3).

Mass spectrometry

Bupivacaine was ionized as a protonated species $[M+H]^+$ that was observed at a *m/z* value of 289. Proposed structures of the observed ions are presented in the inset of Fig. 4. Commonly observed ions in failed samples and therapeutically effective samples from the same lot had *m/z* values of 276, 265, 245, 240, 234, 217, 157, 146, 130, 115, and 102 (Fig. 4). It should be noted that the above-observed ions were not consistent in all the samples. Additionally, neither the reported degradation product of bupivacaine, 2, 6-dimethylaniline (*m/z* = 121) nor its sodium adduct (*m/z* = 144) were observed during MS analysis of failed samples and therapeutically effective samples from the same lot. The CID-MS/MS analysis of bupivacaine standard showed product ions observed at *m/z* 140, 98, 84 (Fig. 4B) and MS^3 analysis of confirmed their proposed structures which was dominant by the sequential losses of CH_2 groups.

In samples obtained from the labour and delivery unit of the authors' institution that did not produce an anesthetic effect (clinically failed), two additional peaks with an *m/z* value of 198 and 195 were observed after storage at 4°C for 24 hr. The CID-MS/MS and MS^3 analyses revealed that ions with *m/z* values of 276, 245, 240, 234, 217, 203, 198, and 195 were related to dextrose.[13] Specifically, the ion at *m/z* 198 was dextrose adduct with a water molecule $[M+H+H_2O]^+$ while the ion at *m/z* 203 was a sodium adduct of dextrose.[14] Nevertheless, additional ions at 134 and 115 were observed from different clinically failed samples, which may be due to atmospheric exposure of the samples prior to analysis. Overall, the results showed absence of bupivacaine degradation products upon cold exposure.

Discussion

Canadian clinical practice and product handling of hyperbaric bupivacaine in the clinical setting is fairly uniform. For example, all respondents indicated that they perform their spinals with the patient in the sitting position. Unlike isobaric or hypobaric bupivacaine, patient posture should not impact the distribution of hyperbaric bupivacaine,[15,16] but may have an impact on time to analgesic onset.[16] The pharmacist survey suggested there are variable transport conditions, and therefore variable temperature exposures, for bupivacaine.

In the current study, there was no observation of bupivacaine degradation upon cold stress in the laboratory based on UV spectra, NMR, or HPLC. This finding did not support our study hypothesis of bupivacaine failures being caused by bupivacaine degradation upon cold exposure. Differential scanning calorimetry showed that hyperbaric bupivacaine solution had minor hysteresis in the melting point upon freeze-thaw, which may be due to either of the two components. Dextrose exhibits consistent changes in the M/S profile upon cold exposure of the hyperbaric bupivacaine. Residual bupivacaine solution from vials used

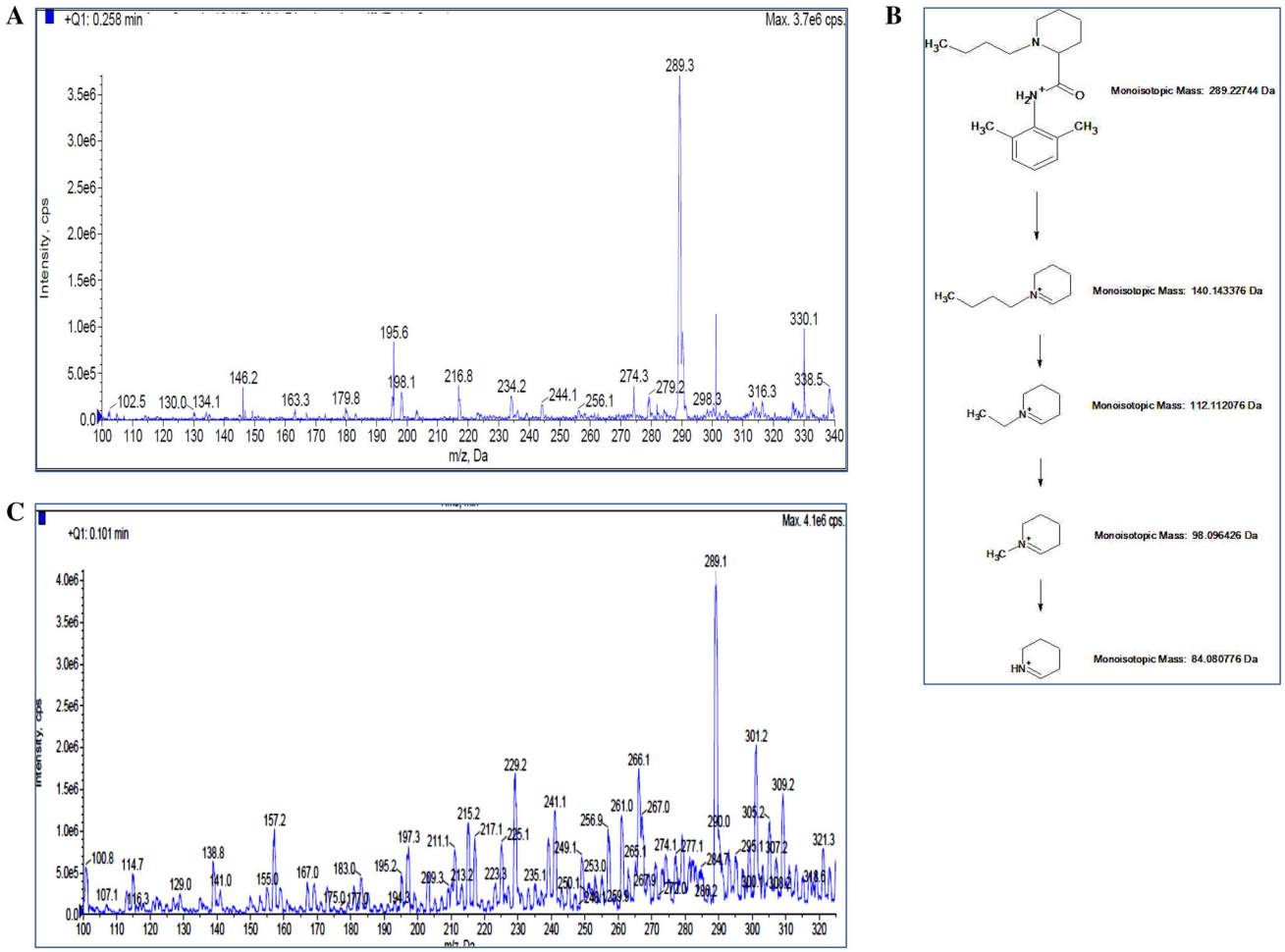

Fig. 4 Representative mass spectrometry (MS) results. A) Commercial product bupivacaine vials were received from three different Canadian hospitals and analyzed before and after incubation at 4°C overnight. B) The collision induced dissociation MS/MS analysis of pure bupivacaine pharmaceutical standard (*m/z* 289) showed product ions observed at *m/z* 140, 98, 84, with the predictive structures. C) Commercial product bupivacaine that did not produce an anesthetic effect in patients at the authors' institution were collected, stored at room temperature, and analyzed by MS, yielding complex patterns in the spectra

for failed spinal anesthesia had a complex pattern on MS analysis that was different from laboratory cold-exposed samples. The same samples were then transferred to storage at 4°C and reanalyzed by MS. The effects of cold exposure and sample handling produce a complex and inconsistent alteration compared with fresh product vials, and cold exposure seems to involve mainly the dextrose. Further analysis will be required on a larger sample size and at greater resolution to elucidate the mechanisms. There is a need and substantial interest among anesthesiologists and hospital pharmacies for a centralized process for analyzing bupivacaine failures to better understand this issue. Future studies demand the use of a high-resolution MS instrument and elemental analysis to aid quantitative chemical identification of the various unexplained ions observed during MS analysis of the clinically failed samples. Finally, the issue of spinal failure with hyperbaric bupivacaine is not new, with dozens of cases reported to Health Canada in the past decade. Given the frequency of spinal anesthesia using this product and the expected failure rate, the number of reported cases to Health Canada is likely to be a gross underestimate. As Health Canada is the most appropriate agency to report this type of failure, we encourage all physicians to report similar spinal anesthesia failures as per updated guidelines.[17,18] A more robust reporting of the problem could encourage Health Canada to more fully investigate this issue.

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