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Concentration of Epinephrine and Anesthetic in Local Anesthetics Exposed to Environmental Extremes

Maj Paula Morse

ABSTRACT

The purpose of this study was to evaluate the concentrations of epinephrine and anesthetic in dental anesthetic carpules after storage in simulated deployed environments. Two test environments, the first simulating the hot environment of Djibouti, Djibouti and the second simulating the cold environment of Bishkek, Kyrgyzstan, were used to store carpules of Articaine 4% with 1:100,000 epinephrine and Lidocaine 2% with 1:100,000 epinephrine. The Djibouti environment cycled between 41°C/39% humidity and 31°C/50% humidity. The Bishkek environment cycled between -4°C and -8°C. Control groups were stored at 22.2°C and 50% humidity. Epinephrine and anesthetic concentrations in Articaine and Lidocaine local anesthetics were analyzed using high-performance liquid chromatography (HPLC) at baseline, 1 week, 2 weeks, and 1 month of storage. The levels of anesthetic and epinephrine were statistically analyzed per anesthetic type and temperature with Dunnett's tests comparing each week of storage to baseline (alpha=0.05). No significant difference in the levels of anesthetic or epinephrine was found based on storage temperature or time for either type of anesthetic ($p>0.05$). Storage of cartridges of anesthetic over time at hot or cold temperatures does not significantly affect the levels of anesthetic or epinephrine.

INTRODUCTION

Various local anesthetic agents are used routinely in operative dentistry as a means for temporarily eliminating sensitivity to noxious stimuli by inhibiting the initiation

and propagation of nerve impulses. Some of these agents include the addition of either levonordefrin or epinephrine as vasoconstrictors, both of which act to prolong the anesthetic effect by decreasing the vascular permeability and subsequent absorption of the anesthetic. If either the concentration of the anesthetic or the vasoconstrictor is decreased, clinical efficacy could be reduced. The possibility of chemical degradation during prolonged storage in various environmental conditions warrants investigation.

Local anesthetics are not always stored according to manufacturers' instructions, especially when shipped to various military deployment locations. Storage at high or low temperatures may result in a change in the molecular concentrations of epinephrine and anesthetic. Extreme environmental temperatures are encountered in emergency medical service environments such as ambulances and other emergency response vehicles. Multiple studies have been conducted regarding the stability of lidocaine and epinephrine as emergency medicine substituents stored in non-insulated or non-temperature-controlled environments. Among the studies, it was shown that recorded temperatures ranged from -20°C to 80°C (Madden et al., 1999; Helm et al., 2003; Gill et al., 2004; and Dubois 2000). Not all studies documented time spent within extreme temperature ranges and no study recorded relative humidity levels or durations of exposure to varying humidity. No studies were found regarding temperature variations and humidity levels in Djibouti or Kyrgyzstan deployment locations.

Johansen et al. (1993) evaluated lidocaine hydrochloride and epinephrine as separate agents (as they are used in emergency medicine) after each was exposed to extreme temperatures of -20°C, 70°C, and a combination of the two temperatures. Exact methodology was not elucidated in this study, so it was impossible to determine how long

each medication was exposed to these temperatures. Also, the variations in humidity were not reported. Regardless, the following conclusion was offered: "[d]rug temperatures beyond the manufacturers' recommended storage ranges do not affect the chemical structures of...lidocaine...and epinephrine." Their conclusion was based on the concentrations obtained for each drug through gas chromatography mass spectrophotometry. An analysis of available literature by Kupper et al. (2006) regarding epinephrine summarized that a ". . . minimal decrease of the substance. . ." will occur if stored at 40°C for 1 year, but no appreciable change occurred at 70°C for 3 months. Their study did not report effects of cold on epinephrine concentrations, but it did state that, "[l]idocaine is very temperature-resistant. . ." as reported originally by Castner in 2000 at the University of Ulm, Germany in a study that was not published in English.

Contrarily, Valenzuela et al. (1989) revealed the results of their study to be that epinephrine was not stable after being exposed to summer temperatures for 4 weeks. In this study, epinephrine was 1 of 23 emergency medications stored in a simulated "summer" environment and analyzed by gas chromatography. The authors found that ". . . epinephrine manifested a change in its ionized state" , but they did not specify what conditions were used to simulate the summer environment. The clinical ramifications of this change were not determined in this study. Additionally, Gammon et al. (2008) used high-performance liquid chromatography, suggested by Barron (2003) to be ". . . the 'gold standard' in the pharmaceutical industry for stability and quantitative testing operations", to test the stability of numerous emergency medications, including epinephrine and lidocaine, in a cyclical thermal exposure of -6°C to 54°C. The authors concluded that lidocaine ended with a concentration of less than 90% of the starting concentration. They

did not state that epinephrine had a statistically significant decrease in concentration; however, the reported level for epinephrine at day 28, which was the concluding sample point of the study, was 86.24 percent with a standard deviation of 0.44%. This suggests that epinephrine was not, in fact, stable over the duration of the study.

The only dental anesthetic-specific article regarding the degradation of vasoconstrictors evaluated the stability via changes in concentrations of epinephrine and levonordefrin before, during, and after storage in a cartridge heater for 3 months (Fry and Ciarlone, 1980). The use of a spectrophotofluorometer demonstrated decreases in concentrations of all epinephrine samples while equivocal results vis-à-vis levonordefrin were reported, with two samples demonstrating little change and one sample demonstrating a 50% increase in concentration.

No studies were found which reported on anesthetic stored as dental cartriges (neither the previously-used volumes of 1.8mL nor the presently-used volumes of 1.7mL) exposed to temperature variations. Given this, it follows that no studies were found regarding the stability of local anesthetics exposed to deployed military environments. Nor were there studies exploring the possible degradation in extreme environments of epinephrine as it is used in combination with dental anesthetics.

Several studies did evaluate the concentration of lidocaine and/or epinephrine in various substances using HPLC (Jancic-Stojanovic et al., 2010; De Orsi et al., 2009; Shah et al., 2009; Abdelmageed et al., 2008; Kwok et al., 2006; Zehetmayer et al., 1997; Achilli et al., 1996), establishing the efficacy of using HPLC to test concentrations of lidocaine and epinephrine. However, none of them tested the anesthetic itself using HPLC; the

anesthetic was always mixed with other substances. Because of this, it was necessary to create and validate a novel HPLC method to test both the anesthetic and epinephrine levels in articaine and lidocaine.

The purpose of this study was to evaluate the stability of the most commonly used injectable local anesthetics (Articaine and lidocaine) (Malamed, 2006) among United States dentists when subjected to extreme, cyclical environmental conditions. The environmental conditions simulated were those of common deployment locations for military personnel in Djibouti and Kyrgyzstan. The null hypothesis was that there would be no significant difference in concentrations of 1) local anesthetic, and 2) epinephrine in two different local anesthetics used in dentistry before and after exposure to temperature and humidity variations analogous to those found in Djibouti and Kyrgyzstan for 1 week, 2 weeks, and 4 weeks as determined by liquid chromatography mass spectrometry.

METHODS AND MATERIALS

The protocol was approved by the Institutional Review Board at Wilford Hall Ambulatory Surgical Center, JBSA-Lackland, Texas. A control group and two test groups were created for each of the following local anesthetics, in which the levels of the local anesthetics and also epinephrine were tested: 1) Lidocaine 2% with 1:100,000 epinephrine (Novocol Pharmaceutical of Canada, Cambridge, Ontario), 2) Articaine 4% with 1:100,000 epinephrine (Novocol Pharmaceutical of Canada, Cambridge, Ontario). Each of the control groups and each of the test groups included 5 cartridges for each of

the specified time intervals: baseline, 1 week, 2 weeks, and 1 month. Cartridges from within each test group came from the same lot number and same box of local anesthetic, which was supplied from the manufacturer with 5 blister packs of 10 cartridges each. The 5 cartridges within each time interval group were selected from 5 different blister packs from the same box of local anesthetic. Units of measurement were in micrograms per milliliter (µg/mL) for epinephrine and percentages for anesthetic. The local anesthetics were analyzed with a Waters 2695 Alliance HPLC (Milford, MA) equipped with a Waters 474 fluorescent detector.

Calibration curves for each of the local anesthetics and epinephrine were created and used for comparison with samples drawn from the control and test groups as described below. Control groups were stored according to manufacturer instructions. According to the MSDS for each anesthetic, storage should be, "...in a cool, dry place. Avoid extremes in temperature." Additionally, it is recommended by the manufacturer of the chosen test anesthetics that the anesthetic cartridges be stored out of the light. According to the United States Pharmacopeial Convention Inc. (USP), "controlled room temperature" is defined as, "[a] temperature maintained thermostatically that encompasses the usual and customary working environment of 20° – 25°C (68° – 77°F); that results in a mean kinetic temperature calculated to be not more than 25°C; and that allows for excursion between 15° – 30°C (59° – 86°F)..." (Brown and Campagna, 2005). Nothing is mentioned about humidity in this definition. No studies have been conducted documenting the storage parameters employed for local anesthetics by dentists. Given that lack of information, it was chosen to store local anesthetic at room temperature (22.2°C) and humidity (50%) for the control groups as these parameters fall within the

definition of “controlled room temperature”. An environmental chamber (Electro-tech Systems, Inc., Glenside, PA) capable of controlling both parameters was used in this study. Also, the cartridges of anesthetic were placed into the original blister packs, resealed around the entire periphery, and stored inside the original anesthetic box which was also resealed.

The first test group for each local anesthetic was stored in simulated environmental conditions representative of average temperature and humidity for Djibouti, Djibouti in July, the hottest month of the year. Average high and low daily temperatures have been documented at 41°C and 31°C, respectively. Average low and high humidities have been documented at 39% and 50%, respectively, during July (www.wunderground.com). The same incubator capable of controlling both parameters was utilized as before. Both the temperature and humidity cycled daily from low to high and high to low, respectively, to mimic the climate in Djibouti, Djibouti.

The second test group for each local anesthetic was stored in simulated environmental conditions representative of the average temperature for Bishkek, Kyrgyzstan in January, the coldest month of the year. Average high and low daily temperatures have been documented at 1°C and -8°C, respectively (wunderground.com). A TempCon Refrigerator/Freezer (Olaf, KS) capable of maintaining the temperature between 1°C and -8°C was utilized. It was not capable of controlling humidity, therefore, humidity was not controlled or measured in this group.

Cartridges assigned to specified time intervals in each group were removed from the environmental chamber after the designated amount of elapsed exposure time. After withdrawal from the chamber, the samples were stored out of the light at average room

temperature and humidity until the one-month point for the group in question. One aliquot was drawn from each cartridge of local anesthetic and divided into two separate Autosampler Vials (Waters, Milford, MA), one for testing anesthetic levels and one for testing epinephrine levels. Each vial was run through the HPLC, after which new caps with intact diaphragms were placed on each Autosampler Vial. Concentrations of each substance were compared to the appropriate calibration curve.

A mean and standard deviation was determined for both anesthetic (ug/mL) and epinephrine (percentage) at each time period per temperature and anesthetic type. See tables below. The levels of anesthetic and epinephrine were analyzed per anesthetic type and temperature with Dunnett's tests comparing each week of storage to baseline (alpha=0.05).

RESULTS

No significant difference in the levels of anesthetic or epinephrine was found based on storage temperature or time for both types of anesthetic ($p>0.05$).

Anesthetic	Anesthetic (µg/mL) (standard deviation)											
	Room Temperature				Hot Temperature				Cold Temperature			
	Baseline	Week 1	Week 2	Week 4	Baseline	Week 1	Week 2	Week 4	Baseline	Week 1	Week 2	Week 4
Lidocaine 1:100,000 Epinephrine	2.018 (0.013)	2.018 (0.032)	2.040 (0.020)	2.037 (0.033)	1.947 (0.027)	1.957 (0.017)	1.983 (0.047)	1.964 (0.064)	1.966 (0.60)	1.996 (0.053)	1.995 (0.053)	1.973 (0.043)
Articaine 1:100,00 Epinephrine	4.004 (0.034)	3.938 (0.061)	4.027 (0.057)	3.978 (0.024)	3.998 (0.018)	3.956 (0.080)	3.898 (0.073)	3.986 (0.053)	3.928 (0.052)	3.967 (0.053)	3.962 (0.065)	3.924 (0.027)

Anesthetic	Epinephrine (%) (standard deviation)											
	Room Temperature				Hot Temperature				Cold Temperature			
	Baseline	Week 1	Week 2	Week 4	Baseline	Week 1	Week 2	Week 4	Baseline	Week 1	Week 2	Week 4

Lidocaine 1:100,000 Epinephrine	9.876 (0.013)	9.877 (0.053)	9.864 (0.023)	9.841 (0.031)	9.584 (0.032)	9.573 (0.031)	9.607 (0.027)	9.563 (0.034)	9.772 (0.024)	9.751 (0.030)	9.777 (0.060)	9.757 (0.0.39))
Articaine 1:100,00 Epinephrine	10.091 (0.011)	10.094 (0.023)	10.075 (0.013)	10.075 (0.009)	10.090 (0.014)	10.089 (0.016)	10.072 (0.028)	10.106 (0.016)	10.102 (0.014)	10.106 (0.014)	10.080 (0.018)	10.091 (0.014)

DISCUSSION

Although the manufacturer's instructions state that the tested anesthetics should be stored "...in a cool, dry place…[a]voiding extremes in temperature," the results of this study would suggest that storage in environments similar to the ones tested would have little effect on the concentrations of either the epinephrine or the anesthetic.

The null hypothesis was not rejected in this study. No significant difference was found in the levels of local anesthetic or epinephrine in 4% Articaine with 1:100,000 epinephrine or 2% lidocaine with 1;100,000 epinephrine after each was exposed to temperature and humidity variations similar to those found in Djibouti and Kyrgyzstan for 1 week, 2 weeks, and 4 weeks. The results of this study suggest no statistically-significant breakdown of anesthetic or lidocaine occurred regardless of the environment. The results of this study should decrease the concern that delivery of anesthetics to deployed environments or storage in non-room temperature environments will alter the concentration of epinephrine and anesthetic, as determined by high-performance liquid chromatography.

In contrast to the findings of this study, when Valenzuela (1989) evaluated the emergency medicine epinephrine, it was concluded that changes in the ionized state of

epinephrine were detected by gas chromatography after 4 weeks at “summer” temperature. Additionally, Fry and Ciarlone (1980) found decreases in epinephrine concentrations after storage of dental anesthetic cartridge containing epinephrine in a cartridge heater for 3 months. Fry and Ciarlone did not evaluate the concentration of anesthetic. As this was the only dental anesthetic-specific article which evaluated the concentration of any components within a dental anesthetic cartridge, it would be prudent to conduct additional studies. Finally, Gammon (2008), found that subjected of lidocaine (as used in emergency medication) to cyclical temperature changes between -6°C and 54°C resulted in a concentration of less than 90% of its starting concentration.

The conclusions of Johnsen et al. (1993) and Kupper (2006) corroborate the findings of this current study. Johnsen et al. evaluated changes in concentrations of lidocaine and epinephrine as separate agents after exposure to extreme temperatures (-20°C and 70°C) via gas chromatography and found no differences. Kupper et al. evaluated epinephrine after exposure to hot temperatures and stated that a “. . . minimal decrease of the substance” will occur if stored at 40°C for 1 year, but no appreciable change occurred at 70°C for 3 months.

Given the inconsistencies among these studies, lack of uniform protocol design, and scant studies available addressing lidocaine and Articaine as used in dentistry with epinephrine mixed into each carpule, it would be prudent for additional studies to be conducted.

This study was limited to a period of 4 weeks and the packaging was kept intact. If the time of exposure were increased, it is possible that a change in concentration of either anesthetic or epinephrine could be found. If the individual cartridges were removed from the packaging during storage, the effect of the temperature extreme may be more apparent. Additionally, there are other components in a dental anesthetic cartridge aside from lidocaine and epinephrine, none of which were tested in this study. Future studies could evaluate additional components or the effect of longer exposure time.

CONCLUSION

Based on the results of this study, storage of cartridges of anesthetic over time at hot or cold temperatures does not significantly affect the levels of anesthetic or epinephrine.

DISCLOSURE

The views expressed in this study are those of the authors and do not reflect the official policy of the United States Air Force, the Department of Defense, or the United States Government. The authors do not have any financial interest in the companies whose materials are discussed in this article.

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