BRIEF REPORT

Effect of Fluctuating Extreme Temperatures on Tranexamic Acid

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Abbreviations:
FTMS: Fourier Transform Mass Spectrometry
TXA: tranexamic acid
USP: United States Pharmacopeia

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Abstract

Introduction: Tranexamic acid (TXA) is an antifibrinolytic agent shown to reduce morbidity and mortality in hemorrhagic shock. It has potential use in prehospital and wilderness medicine; however, in these environments, TXA is likely to be exposed to fluctuating and extreme temperatures. If TXA degrades under these conditions, this may reduce antifibrinolytic effects.

Problem: This study sought to determine if repetitive temperature derangement causes degradation of TXA.

Methods: Experimental samples underwent either seven days of freeze/thaw or heating cycles and then were analyzed via mass spectrometry for degradation of TXA. An internal standard was used for comparison between experimental samples and controls. These samples were compared to room temperature controls to determine if fluctuating extreme temperatures cause degradation of TXA.

Results: The coefficient of variability of ratios of TXA to internal standard within each group (room temperature, freeze, and heated) was less than five percent. An independent t-test was performed on freeze/thaw versus control samples ($t = 2.77$; $P = .17$) and heated versus control samples ($t = 2.77$; $P = .722$) demonstrating no difference between the groups.

Conclusion: These results suggest that TXA remains stable despite repeated exposure to extreme temperatures and does not significantly degrade. These findings support the stability of TXA and its use in extreme environments.

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Introduction

Hemorrhage is a leading cause of death in both military and civilian trauma.[1,2] Tranexamic acid (TXA) is an antifibrinolytic agent which supports thrombosis by preventing blood clots from dissolving.[3] Briefly, TXA binds to lysine receptor sites on plasmin and prevents plasmin from binding and degrading fibrin.[4] Large studies have shown a mortality benefit of TXA when used in certain patients with hemorrhagic shock following traumatic injury.[5,6] The survival benefit of TXA led the United States Joint Trauma System (Fort Sam Houston, Texas USA) to add TXA treatment to its Damage Control Resuscitation Clinical Practice Guideline, and the World Health Organization (WHO; Geneva, Switzerland) model list of essential medicines now includes TXA.[7,8] As such, the utilization of TXA for hemorrhagic shock following traumatic injury is expected to continue to increase.

The antifibrinolytic effects of TXA are known to be time-dependent and most effective if administered within three hours of injury.[5,9] Consequently, TXA is now being issued to US Special Operations Forces for use in the battlefield as an effort to reduce time to administration.[7] Given its success in the field, the clinical applications of TXA are also being assessed within the prehospital and wilderness medicine realms. Like military personnel, prehospital providers are stationed in environments with wide temperature variances. Temperatures can range from -20°C in mountainous regions to 40°C in deserts.[10,11] The time constraints of TXA administration necessitate that it be readily available within these conditions. Multiple studies have already shown large fluctuations in storage temperatures of prehospital medications.[12–14] Medication storage temperatures within air-medical helicopters may range from 13°C-31°C.[12] Ambulance storage temperatures can reach greater than 40°C in summer and as low as -14°C in the winter.[13] A one-year-long observational study of Emergency Medical Service vehicles in Arizona, Florida, Kansas, Oregon, and New York (USA) found that medication storage temperatures in all five locations

	Aliquot 1	Aliquot 2	Aliquot 3	Average (Range)	CV (%)
Control	0.87	0.93	0.86	0.89 (0.07)	4.27
Freeze	0.88	0.85	0.90	0.88 (0.05)	2.87
Heated	0.89	0.97	0.97	0.94 (0.08)	4.90

Table 1. Ratios of Signal Intensity of TXA Experimental Samples versus Internal Standard
Abbreviations: CV, coefficient of variability; TXA, tranexamic acid.

reached temperatures outside of the United States Pharmacopeia (USP; Bethesda, Maryland USA) controlled room temperature standards.[14] This is concerning as manufactures recommend that TXA be stored at room temperature.[5] From these data, it can be inferred that providers in extreme environments are exposing TXA to temperatures well outside suggested guidelines.

Previous work has demonstrated that the chemical structure and effectiveness of TXA is preserved when stored at extreme, but stable temperatures.[7] Unfortunately, constant temperatures do not reflect actual prehospital or wilderness expedition storage settings, and little is known regarding the stability of TXA during fluctuating conditions. Temperature cycling has been shown to increase degradation of certain chemical compounds, especially proteins.[7] As TXA is a synthetic derivative of the amino acid lysine, it therefore may be more susceptible to degradation from fluctuating temperatures.[7] Further, parenteral medications in solution, such as TXA, have an increased predisposition to temperature-induced degradation.[7,15,16] This is worrisome as the antifibrinolytic properties of TXA may be compromised if the chemical structure is altered, resulting in ineffective hemorrhage control. In this study, the researchers sought to determine whether extreme cyclic temperature variation, similar to those that exist in prehospital and wilderness settings, would alter TXA stability.

Methods

Tranexamic acid from a 100mg/mL (10mL) stock vial (Lavigne Inc; Pine Brook, New Jersey USA; lot# ACC715, expiration date 7/2019, experiment performed 6/2018) was separated into nine separate 0.5mL aliquots and placed in amber vials (Sigma-Aldrich; Saint Louis, Missouri USA). Three vials were each separated into three different groups, which followed different temperature protocols for seven consecutive days: room temperature (control) group, freeze/thaw group, and heating group. The control group was kept at room temperature (26°C); the heating group was heated to 50°C from 7:00AM-7:00PM, then placed at room temperature (26°C) from 7:00PM-7:00AM; and the freeze/thaw group was placed at -20°C from 7:00AM-7:00PM, then placed at room temperature (26°C) from 7:00PM-7:00AM. These temperatures were chosen based on previous studies which tested drugs at extreme temperatures, known prehospital temperature derangements, and presumed wilderness expedition extremes.[11–13,15,16] The seven-day time period and 12-hour temperature cycles were based on a previous study which assessed the effects of freeze/thaw cycles on epinephrine and was meant to reflect the conditions likely encountered in a one-week expedition.[17]

After the seventh day, aliquots of TXA were diluted to 1mg/mL with water and an internal standard of stable isotope-labeled TXA ($C_6{}^{13}C_2H_{15}{}^{15}NO_2$) was used (Toronto Research Company; Toronto, Canada) for comparison. The internal standard (1mg) was suspended in water to achieve a 1mg/mL solution. The samples and the internal standard solution were then diluted 100x in water to make a 10mcg/mL solution. These solutions were then diluted 10x in 50% acetonitrile, 0.1% formic acid for a final concentration of 1mcg/mL.

Fourier Transform Mass Spectrometry (FTMS) was used to assess the level of degradation of TXA in control and temperature-stressed samples. The Mass Spectrometry Resource Laboratory at the University of Rochester (Rochester, New York USA) conducted all FTMS measurements using a Q Exactive Plus Hybrid Quadripole-Orbitrap mass spectrometer (Thermo Scientific; Waltham, Massachusetts USA). Diluted samples were ionized using positive ion electrospray ionization, and selected ion monitoring was used to analyze each sample for ion production with a mass to charge ratio (m/z) between 140.0000 and 180.0000. The capillary temperature was 320°C. To assess for degradation, the ratio of the signal intensity on mass spectrometry for the sample to internal standard was calculated for each aliquot of TXA. If there was degradation of TXA, the ratio of drug versus analyte would be decreased in comparison to room temperature controls.

Results

The coefficient of variability of ratios of TXA to internal standard within each group (room temperature, freeze, and heated) was less than five percent (Table 1). This suggested there was no significant degradation of TXA due to cyclic extreme temperatures. Further, an independent t-test was performed on freeze/thaw versus control samples ($t = 2.77$; $P = .17$) and heated versus control samples ($t = 2.77$; $P = .722$) demonstrating no difference between the groups. This indicated that neither extremely high nor low temperature cycling led to degradation of TXA.

Discussion

This study demonstrated no significant degradation of TXA at extreme fluctuating temperatures. Close approximation of the ratios and minimal degradation detected indicate that TXA concentrations may be preserved between temperature-stressed and control samples. The USP guidelines for TXA set acceptance criteria for clinical use for concentrations within 90%-110% of the original labeled concentration.[18] As this study assessed for degradation, an area of future research is the analytical measurement of changes in TXA concentration after exposure to extreme temperatures and determination if these remain within USP guidelines. While manufacturer guidelines should still be followed for storage, these results suggest that TXA maintains its chemical structure after exposure to extreme fluctuating temperatures. These findings support the availability of TXA in the prehospital or wilderness environments where potential benefits of early TXA treatment can be out-weighed by the unlikely degradation of medication due to extreme temperature fluctuations.

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Limitations

There are limitations to this study. The researchers did not include an assay to assess the functional fibrinolytic activity of TXA after exposure to fluctuating temperatures. While there may be little detected degradation, the chemical structure of TXA may be altered and antifibrinolytic activity may be reduced. Another limitation is that possible evaporation occurred of the TXA solution from the vials of heated TXA. However, this appears not to have affected measurements for TXA degradation as ratios of TXA to internal standard would be expected to be lower if this had an impact on the results. Finally, the fluctuating temperature cycles of this study were developed to reflect one-week of extreme storage conditions, but they do not forecast changes that could occur to TXA over long-term exposure to fluctuating temperatures. There is opportunity for further research to assess changes in TXA concentration as a result of different temperature conditions or over longer periods of time. This approach may require different analytical methods than used in this study, such as high-performance liquid chromatography. Importantly, the described methodology could also be applied to other prehospital and wilderness medications to assess quantitative changes in concentration from temperature derangement.

Conclusion

Tranexamic acid has many developing uses in prehospital and wilderness medicine. The timeliness of administration necessitates it be present in extreme environments where exposure to fluctuating extreme temperatures is possible. After multiple exposures to temperatures ranging from -20°C to 50°C, TXA showed no significant degradation. These results suggest that TXA maintains stability even after short-term exposure to extreme temperatures.

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