Warmed diluent speeds dantrolene reconstitution

[Le diluant réchauffé accélère la reconstitution du dantrolène]

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Purpose: To define more completely the aqueous solubility of dantrolene in order to devise faster reconstitution techniques for use during malignant hyperthermia episodes.

Methods: To determine Beer's law compliance and the extinction coefficient, we measured the spectrophotometric absorbance at 385 nm of known dantrolene solutions. We added small aliquots of sterile water USP (pH 5.3; 15–40°C) or buffered water (20°C; pH 6.8–9.1) to dantrolene, mechanically agitated and filtered the solutions, and spectrophotometrically determined concentration. To simulate clinical reconstitution conditions, we added sterile water, 60 mL, at temperatures between 15 and 40°C to dantrolene vials and measured the manual shaking time needed to create a) a suspension of small particles, and b) a clear solution.

Results: A plot of UV-vis absorbance at 385 nm vs dantrolene concentration was linear and went through the origin; the extinction coefficient is 16.1 mM⁻¹. At 20°C, dantrolene is nearly insoluble below pH 8.8. Dantrolene is 2.8 times more soluble in 0.1 M THAM (tris-(hydroxymethyl)aminomethane) than in sterile water at pH 9.1. Dantrolene is 6.7 times more soluble in 40°C than in 20°C water at pH 9.5 (the pH of reconstituted dantrolene). Under clinical conditions, water temperature altered the time to create a clear solution but not a suspension (60 sec).

Conclusion: Diluting dantrolene with 40°C water rather than operating-room temperature water (20°C or below) would speed dantrolene reconstitution.

Objectif : Définir plus adéquatement la solubilité aqueuse du dantrolène dans le but d'élaborer des techniques de reconstitution plus rapide, utilisables au cours des crises d'hyperthermie maligne.

Méthode : Nous avons déterminé la compliance à la loi de Beer et le coefficient d'extinction en mesurant l'absorption spectrophotométrique à 385 nm de solutions de dantrolène connues. Nous avons ajouté au dantrolène quelques aliquots d'eau stérile USP (pH 5,3 ; 15 – 40 °C) ou d'eau tamponnée (20 °C ; pH 6,8 – 9,1), agité mécaniquement et filtré les solutions et déterminé la concentration

par spectrophotométrie. Nous avons ajouté de l'eau stérile, 60 mL, aux bouteilles de dantrolène afin de simuler les conditions cliniques de reconstitution à des températures de 15 à 40 °C et nous avons mesuré le temps d'agitation manuelle nécessaire pour créer a) la suspension de petites particules et b) une solution cristalline.

Résultats : Un tracé de l'absorption UV-vis à 385 nm vs la concentration de dantrolène était linéaire et passait par l'origine ; le coefficient d'extinction est de 16,1 mM⁻¹. À 20 °C, le dantrolène est presque insoluble pour un pH plus bas que 8,8. Le dantrolène est 2,8 fois plus soluble dans 0,1 M de THAM (tris-(hydroxyméthyl)aminométhane) que dans de l'eau stérile dont le pH est de 9,1. Le dantrolène est 6,7 fois plus soluble dans de l'eau à 40 °C plutôt qu'à 20 °C pour un pH de 9,5 (le pH du dantrolène reconstitué). Dans des conditions cliniques, la température de l'eau modifie le temps nécessaire à la création d'une solution cristalline mais non celui d'une suspension (60 sec).

Conclusion : La dilution du dantrolène avec de l'eau à 40 °C plutôt que de l'eau à la température ambiante de la salle d'opération (20 °C ou moins) devrait accélérer la reconstitution du dantrolène.

RECONSTITUTING dantrolene fast enough to reverse malignant hyperthermia (MH) in an adult is a difficult task. Gronert *et al.* advise that this job usually requires the full-time efforts of three or four people.¹ The only *iv* dantrolene formulation available in North America (Dantrium®, Procter & Gamble Pharmaceuticals, Cincinnati, OH, USA; each vial contains dantrolene 20 mg, mannitol 3 g, and sodium hydroxide to ensure that the reconstituted solution has a pH of 9.5), is poorly water soluble. However, water is the recommended dantrolene diluent.

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Heat speeds dantrolene dissolution. If dantrolene does not dissolve readily, textbooks recommend heating the dantrolene vial either by immersing the vial in hot water or gently autoclaving the vial.¹ This could be hazardous, for one could easily apply excessive heat (particularly with autoclaving) and produce a dantrolene solution hot enough to cause burns.^{2,3} Also, both suggested heating techniques take time. An alternative way to facilitate dantrolene dissolution might be to preheat the sterile water diluent to an effective but thermally safe (i.e., $\leq 40^{\circ}\text{C}$) temperature. However, there is insufficient information on the temperature dependence of dantrolene solubility to determine whether such preheating might be clinically useful. In addition, the pH adjustments recommended by some authors to assist dantrolene preparation have little support.^{4,5} Therefore, we systematically studied the solubility of dantrolene as a function of temperature and pH.

Methods

Materials

The dantrolene used to confirm compliance with Beer's law was obtained from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. We obtained the dantrolene used in temperature, pH and THAM dissolution experiments (Dantrium® Intravenous, Procter & Gamble Pharmaceuticals, Cincinnati, OH, USA) at the time of clinical expiration (April 30, 1999) from City Avenue Hospital, Philadelphia, PA, USA and performed these experiments May 11-12, 1999. We obtained phosphate buffer (sodium potassium phosphate), concentrated HCl, THAM (tris-(hydroxymethyl)aminomethane), and an Accumet pH meter from Fisher (ACS grade; Fisher Scientific, Malvern, PA, USA). THAM and phosphate buffer solutions were diluted with house distilled water that was additionally purified by passage through a Barnstad water polisher. All other water used was sterile water, USP. Cloudy suspensions and solutions containing particles were filtered through 0.2 μm syringe filters with cellulose acetate membranes (Fisher Scientific, Malvern, PA, USA).

Measurement of ultraviolet-visible spectra of dantrolene solutions and calculation of dantrolene concentration

We obtained ultraviolet-visible (UV-vis) spectra in quartz cuvettes using a Hitachi U2001 spectrophotometer (Pleasanton, CA, USA) scanning from 190 nm to 1100 nm at a scan speed of $800\text{ nm}\cdot\text{min}^{-1}$ using the appropriate buffer as a reference. To determine compliance with Beer's law and dantrolene's extinction coefficient, we measured the maximal absorbance at 385 nm of solutions of pure dantrolene (12–120 μM) dissolved in water. We calculated the concentra-

tions of the solutions in parts 1 and 2 using absorbance data at 385 nm.

Part 1. Dantrolene solubility with varying pH

We determined the effect of solute pH on dantrolene solubility by adding 0.5–1.0 mL aliquots (up to a total volume of 10 mL) of 0.1 M THAM (pH 7.1–9.1), 0.1 M phosphate (pH 6.8 and 7.2), or sterile water, USP (pH 5.3) to 0.33–0.35 g Dantrium®. Solutions were mechanically agitated to speed dissolution. Aliquots were added until a clear solution formed or a maximum volume of 10 mL was reached. Solutions that were cloudy with 10 mL diluent were filtered. Solution pH was redetermined after reconstitution. We compared the optical densities for dantrolene dissolved in sterile water vs 0.1 M THAM at pH 9.1.

Part 2. Dantrolene solubility with varying water temperature

In order to assess whether dantrolene stability depended on the temperature of the water used for reconstitution, we measured absorbance at 385 nm immediately and 24 hr after reconstitution for all solutions mixed in part 2. Solutions were stored at 20°C after reconstitution. The diluent was sterile water, USP (pH 5.3).

A. LABORATORY CONDITIONS

We determined the minimum fluid volume in which the contents of a Dantrium® vial dissolved when the initial temperature of the sterile water diluent was 20, 25, 30, 37, or 40°C . We performed one experiment at each diluent temperature. In each experiment, we incrementally added 2–5 mL aliquots of sterile water, USP, to a previously unopened Dantrium® vial, thoroughly shook the vial manually and with a mechanical agitator for at least 30 sec after each aliquot, and then visually inspected the vial to determine whether the vial contents had dissolved. If the vial contents did not fully dissolve in 60 mL of water, the resulting solution was filtered to remove insoluble material.

B. SIMULATED CLINICAL CONDITIONS

Sterile water, USP, 60 mL, was added at 15, 20, 25, 30, 37, or 40°C to Dantrium® vials and the duration of manual shaking needed to dissolve the contents was determined. We did not use a mechanical agitator as one is usually not available in the operating room. The solutions were observed continuously to determine: a) when a clear solution formed; and b) when a suspension with small but not large particles formed. Manual shaking was discontinued for five seconds every 30 sec to facilitate visual inspection. We decided that dissolution

FIGURE 1 Dantrolene solubility *vs* pH. Maximal dantrolene concentrations in 0.1 M phosphate (pH 6.8 and 7.2) and 0.1 M THAM (pH 7.1–9.1) were determined spectrophotometrically. When dantrolene is reconstituted as recommended with 60 mL of water, the dantrolene concentration is about 1 mM.

times longer than three minutes were clinically impractical and did not shake vials longer than 180 sec. Vials in which a clear solution had not formed by 180 sec were observed again after 60 min storage at 20°C.

Results

A plot of UV-vis absorbance at 385 nm *vs* dantrolene concentration was linear and went through the origin, indicating compliance with Beer's law. We obtained an extinction coefficient of 16.1 mM⁻¹.

Part 1. Dantrolene solubility with varying pH

Dantrolene is nearly insoluble at pH values below 8.8 at 20°C (Figure 1). At 20°C and pH 9.1, dantrolene is 2.8 times more soluble in 0.1 M THAM than in sterile water (2.52 *vs* 0.88 mM).

Part 2. Dantrolene solubility with varying water temperature

Absorption at 385 nm did not change over 24 hr in any of the part 2 solutions.

A. LABORATORY CONDITIONS

Dantrolene optical density at 385 nm increased linearly with temperature between 20°C and 40°C at pH 9.5 (Figure 2). Dantrolene is 6.7 times more soluble in 40°C water than in 20°C water.

B. SIMULATED CLINICAL CONDITIONS

The time required to produce a suspension of small particles (about 60 sec) was not altered by the tem-

FIGURE 2 Dantrolene solubility *vs* temperature. Maximal dantrolene concentrations in sterile water, USP (20–40°C) were determined spectrophotometrically. The y-axis scale of this figure is the same as that in Figure 1. When dantrolene is reconstituted as recommended with 60 mL of water, the dantrolene concentration is about 1 mM.

TABLE Time required to dissolve the contents of commercially available dantrolene vials (Dantrium®) following the addition of 60 mL sterile water, USP, at water temperatures between 15°C and 40°C

Time (in seconds) to achieve a:	Temperature (°C)					
	15	20	25	30	37	40
Small particle suspension	70	90	60	50	60	—
Clear solution	> 180	> 180	100	60	70	30

Vials were shaken manually and observed continuously. Every 30 sec, shaking was stopped for a five-second observation period. All data were single trials. At 40°C, vial contents went into solution without an observable intermediate suspension stage.

perature of water added to the Dantrium® vial (Table). In contrast, the time required to produce a clear solution was strongly temperature dependent. Clear solutions did not form within 180 sec with 60 mL water at 15°C or 20°C. These suspensions were still cloudy when visually inspected 60 min later.

Discussion

Dantrolene solubility increases linearly with increasing temperature between 20 and 40°C. Because infusing *iv* fluids hotter than 40°C could cause red blood cell lysis and local tissue burns, we did not test higher water temperatures.^{2,3} However, reconstituting dantrolene with 40°C water greatly speeds drug preparation.

During simulated clinical conditions, a clear solution of dantrolene never formed after the addition of 60 mL sterile water (the recommended volume) at

15°C or 20°C. It is not known if injecting a dantrolene suspension rather than a solution would present patient safety issues. In general, however, drugs administered in aqueous solution are available at cellular sites of action more quickly than drugs administered as fine particles in suspension.⁷

Warm sterile water for injection is rarely available in the operating room. Hospitals frequently store the sterile water for dantrolene reconstitution in the operating suite, which has an average air temperature of 18–21°C.⁶ Procter & Gamble recommends that dantrolene be reconstituted with water at “room temperature”; one doubts that the manufacturer fully appreciates the frosty ambiance of most North American operating rooms. An *iv* fluid warming device could be used to warm the sterile water for dantrolene dissolution to 40°C. Alternatively, many operating rooms maintain 37°C warming cabinets for the storage of saline and sterile water irrigating solutions and cotton blankets. Sterile water for injection, USP could be stored in one of these warming cabinets.

Heat also increases the aqueous solubility of mannitol, the other active ingredient in Dantrium®. A vial of Dantrium® contains 3,000 mg mannitol and 20 mg dantrolene; thus, the ingredients are 99% mannitol by weight. According to the mannitol package insert, mannitol crystals precipitate if the solution is chilled.^A Heating is the recommended way to redissolve crystal-containing mannitol containers.^A

Paradoxically, the time saved by using 40°C water to reconstitute dantrolene could decrease heat gain in MH patients. During fulminant MH, core temperature can increase as much as 1°C every five minutes.¹ In a 70-kg adult, this translates to about 14 kcal·min⁻¹ heat production.^B The 420-mL of water needed to mix a 2-mg·kg⁻¹ dose for a 70-kg patient contains 8.4 kcal more heat at 40°C than at 20°C. Using 40°C water would be thermally neutral if one saved 36 sec in dantrolene preparation time (5.1 sec/vial) and would result in net heat loss if more time were saved.

At 20°C, dantrolene is nearly insoluble at pH values below 8.8. pH adjustments of the dantrolene diluent are unlikely to improve the drug preparation time.

Dantrium is packaged to have pH 9.5 after reconstitution with sterile water USP, and higher pH values might cause tissue damage. Although dantrolene is more soluble in THAM than in sterile water, the effect is small and THAM is quite expensive. THAM does not appear to be a cost-effective adjuvant in dantrolene preparation. In addition, the effects of THAM buffer on the stability and potency of dantrolene would need to be determined before the use of a THAM buffer diluent could be recommended.

Prompt dantrolene injection is the cornerstone of effective MH therapy. Precious time can be lost if one is not prepared for the practical problem of dantrolene reconstitution. We recommend that the sterile water for dantrolene reconstitution either be stored in a 40°C operating room warming cabinet or that equipment be available for warming the water rapidly to 40°C, since dantrolene is far more soluble in warm water than cold.

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^A Mannitol *iv* package insert, Abbott Laboratories, North Chicago, IL, USA, ©Abbott, 1994, revised 2/1998.

^B For this rough approximation, we assumed that total body water is 100% rather than 67% of body weight. One kilocalorie increases the temperature of 1 kg by 1°C. If body temperature can increase 0.2°C per minute during fulminant MH (1°C every five minutes), then a 70-kg MH patient can produce 14 kcal of heat per minute.