Operational Evaluation of Three Commercial Configurations of Atropine/HI-6 Wet/Dry Autoinjectors[1]

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Commercially manufactured wet/dry autoinjectors containing atropine in solution and powdered HI-6 were evaluated using HPLC for consistency of drug delivery with various solvation times and stability of drugs postsolvation at a temperature of 40°C. Three configurations of autoinjector were tested. System A (SYS A), with a specified mixing time of 5 sec, delivered a volume of 3.0 ml containing 1.86 mg of atropine sulfate and 443 mg of the bispyridinium oxime HI-6 dichloride. System B1 (SYS B1) and System B2 (SYS B2), with specified mixing times of 40 sec, delivered volumes of 2.3 ml containing 2.13 and 2.06 mg atropine citrate and 424 and 545 mg HI-6 dichloride, respectively. Average coefficients of variation for SYS A were 3.4% for atropine and 5.8% for HI-6 and for SYS B1 and B2 were 5.2% for atropine and 7.0% for HI-6 determinations. Stored from 3 to 14 days at 40°C after the autoinjector contents were mixed, SYS A delivered 1.77 mg atropine sulfate and SYS B1 and B2 delivered 2.02 mg atropine citrate. The delivery of HI-6 dichloride decreased with a half-life of 34 days for SYS A, 39 days for SYS B1, and 32 days for SYS B2. This resulted in a decrease to 90% of the respective day 0 amount after 4 (SYS A) or 5 (SYS B1 or B2) days.

KEY WORDS: autoinjector; wet/dry; HI-6; atropine.

INTRODUCTION

The combination therapy of the anticholinergic drug atropine and the cholinesterase reactivator pralidoxime chloride (2-PAM) has been a standard therapy for organophosphorus poisoning for decades (1,2). Currently the U.S. Army uses two separate autoinjectors to administer these emergency drugs, the Atropen to deliver 2 mg of atropine sulfate and the Combopen to deliver 600 mg of 2-PAM chloride. Both the Atropen and the Combopen contain solutions of drug that can be immediately injected with little user preparation required.

Although an effective antidote against most nerve agents, 2-PAM offers little protection against soman (3). The bispyridinium oxime HI-6 has been found to be less toxic and a more effective antidote for soman poisoning in animals (4–8) but has poor gastrointestinal absorption since it is a bisquaternary ammonium compound. It is readily absorbed when injected intramuscularly; however, the compound is unstable in concentrated solutions, which precludes its use in currently fielded autoinjectors (9–11).

Recently, a new design of autoinjector has been developed in which a compound unstable in solution, such as HI-6, can be stored in powder form and dissolved at the time of need using diluent contained in an adjacent chamber of the autoinjector. Because of the method of drug packaging, the autoinjector has been termed a "wet/dry" type. The development of the wet/dry autoinjector has made it feasible to reconsider the possible deployment of HI-6 as an emergency antidote packaged in an autoinjector system. However, the use of a wet/dry autoinjector must not compromise an individual's ability to immediately administer the antidote once symptoms of nerve agent poisoning occur. Solvation of the dry component should be instantaneous and cause no delay in a life-threatening situation.

The purpose of this study was to evaluate the performance of wet/dry autoinjectors containing atropine (in solution) and HI-6 (as powder) manufactured by two companies, Astra Meditec AB (Molndal, Sweden) and Medimech Limited (Rochester Kent, United Kingdom). The autoinjector systems were obtained in three different configurations. Astra Meditech manufactured one system (SYS A), which contained 2 mg atropine sulfate and 500 mg HI-6 dichloride (dry form). Medimech manufactured two systems, each to deliver 2 mg of atropine citrate and either 400 mg (SYS B1) or 500 mg (SYS B2) of HI-6 dichloride (dry form). Injection generally requires five steps: (i) breaking the barrier between the wet and the dry components, (ii) shaking the autoinjector to mix the components, (iii) removing a safety mechanism, (iv) placing the autoinjector against the injection site, and (v) firing the autoinjector while holding it in place for 10 sec. The focus of the evaluation was on the consistency and quantity of drug deliveries for the three configurations with various solvation times and on the stability of the drugs postsolvation. The stability study was performed to estimate the length of time for which the drugs retain greater than 90% potency when solutions are held at a temperature of 40°C.

MATERIALS AND METHODS

Sample Preparation

According to the manufacturer, SYS A contained 500 mg of HI-6 dichloride and 2.0 mg of atropine sulfate in citrate buffer (3.0 ml, pH 3.9) with methyl and propyl parahydroxybenzoate as preservatives. In SYS A, the HI-6 was fully pulverized by a proprietary process, and the specified mixing time was 5 sec. SYS B1 and SYS B2 were made to deliver 400 and 500 mg of HI-6 dichloride, respectively, and were filled with citrate buffer (2.35 ml, pH 4) containing atropine citrate (1 mg/ml) with 193 m*M* glycerin and 42.5 m*M* phenol. In SYS B1 and SYS B2, the HI-6 was used without

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further processing, and the specified mixing time was 40 sec. Each autoinjector was fired into a tared graduated cylinder, the ejected contents were weighed, and the approximate volumes were measured. Volumetric measurements were made difficult by air bubbles in the solutions attributed to the force of injection, so gravimetric measurements were made and volumes were calculated using the solution density (1.05 g/ml, SYS A; 1.08 g/ml, SYS B1 and B2; except 1.04 g/ml, SYS A and 1.06 g/ml, SYS B1 and B2, for the samples with no mixing). For analysis, sample solutions were diluted 1:100 (v/v) in water by taking a 100-μl aliquot of the solution and bringing its volume to 10.00 ml. A 1:20,000 dilution was prepared by taking a 50-μl aliquot of the 1:100 dilution and bringing its volume to 10.00 ml. All aliquots were pipetted using a Gilson P-100 Pipetman (±0.15 μl) (Rainin Instrument Co., Inc., Emeryville, CA) and were weighed to confirm sampling accuracy.

HPLC Analysis

Analyses for atropine and HI-6 were performed using Waters Associates (Millipore Corp., Bedford, MA) HPLC systems (Model 710B or 712 WISP, Model 510 or 6000A pump, and Waters Data Module) with Kratos Spectroflow 783 or Applied Biosystems 783A programmable absorbance detectors, respectively. The chromatographic separation, a modified version of a published method (12), utilized a Waters μBondapak C18 column (P/N27324, 10 μm, 3.9 mm × 30 cm) at ambient temperature with a flow rate of 1.0 ml/min and an injection volume of 20 μl. Atropine was determined by measuring the absorbance at 225 nm in the 1:100 dilution with an isocratic mobile phase of 21% acetonitrile (HPLC grade) in 1.0 m*M* tetramethylammonium chloride (Mallinckrodt, Inc., Paris, KY) with 5.0 m*M* PIC B7 Low UV ion pairing reagent (Waters Chrom. Div., Millipore Corp., Bedford, MA). HI-6 was determined by measuring the absorbance at 302 nm in the 1:20,000 dilution with a mobile phase of 23% acetonitrile in the same aqueous phase as above. The coefficients of variation (CV) of the analyses with $n = 5$ were 1.0% for HI-6 and 0.3% for atropine. The response was linear for 5–1000 ng HI-6 and 40–400 ng atropine. Duplicate sample peak areas were averaged and compared to the average peak area of known standards. Standard solutions equivalent in composition to the wet components but of known concentration were provided by the manufacturers (0.67 mg/ml of atropine sulfate for SYS A, 1.00 mg/ml of atropine citrate for SYS B) along with powdered HI-6 dichloride. An HI-6 solution of known concentration was prepared in the atropine standard solution. Standards supplied by the manufacturers were normalized against atropine sulfate (Boehringer Ingelheim, Ingelheim, Germany) and HI-6 dichloride (received from Walter Reed Army Institute of Research, synthesized by SRI International, Menlo Park, CA), both >99% pure. Aliquots of the standard solutions were stored frozen at −70°C and diluted on the day of analysis in the same manner as described for samples. Under the chromatographic conditions described above, retention times were approximately 13 min for atropine and 5.3 min for HI-6.

Statistical Analysis

Statistical analyses were performed using Number Cruncher Statistical System (Dr. Jerry L. Hintze, Kaysville, UT) and Lotus 1-2-3 (Lotus Development Corp., Cambridge, MA) software. Analysis of variance (ANOVA) was used to test for significant differences with $P = 0.05$; the critical value was $F(2,27) = 3.35$ for the solvation study and $F(3,36) = 2.87$ for the stability study (13). Statistical analyses of the kinetics calculations were performed by Jim Lennox, Applied Pharmacology Branch, USAMRICD (14).

Solvation Study

The time required for powdered HI-6 completely to dissolve or otherwise disperse in the autoinjector was evaluated by measuring the amounts of HI-6 and atropine in solutions discharged after no mixing, 5 sec, and 10 sec (SYS A) or 40 sec (SYS B1, SYS B2) of mixing. Mixing was accomplished by manually shaking the autoinjectors at an approximate rate of 2 shakes/sec. Times were chosen relative to those specified by the companies and were measured beginning when the wet/dry barrier was broken (i.e., the solvation step) and ending when the device was fired. Ten autoinjectors were used for each mixing time.

Stability Study

The stability of the mixed HI-6/atropine solution in the autoinjectors was evaluated in all three systems at 40°C using times of 3, 7, and 14 days after solvation. The autoinjectors were shaken for either 10 sec (SYS A) or 40 sec (SYS B1, SYS B2) to dissolve the HI-6 and then stored in an oven (The Chemical Rubber Co., Cleveland, OH) at 40°C (±1°C) for the above times ($n = 10$ for each storage time). At the prescribed storage interval, the autoinjectors were fired and the solutions were analyzed by HPLC.

RESULTS AND DISCUSSION

Solvation Study

The original, filled autoinjectors were very consistent by weight, having average weights of 61.55 g (range, 61.34–61.91; $n = 29$; SYS A), 40.78 g (range, 40.44–40.95; $n = 30$; SYS B1), and 40.87 g (range, 40.66–41.10; $n = 30$; SYS B2) with an average coefficient of variation (CV) of 0.24%. The volumes of ejectate delivered by the autoinjectors in the solvation study were quite consistent, with average values of 3.05 ml (range, 2.88–3.17 ml; $n = 29$; avg. CV = 2.5%; SYS A) and 2.40 ml (range, 2.08–2.59 ml; $n = 60$; avg. CV = 3.9%; SYS B1 and B2).

The atropine content of the ejectate from the SYS A autoinjectors was not found to change significantly with mixing time as is shown in Fig. 1. The amounts of atropine delivered by the SYS B1 and SYS B2 autoinjectors were found to decrease slightly but significantly as the mixing time increased ($F = 30.0$, SYS B1; $F = 4.5$, SYS B2). This might have been due to an expected volume expansion as HI-6 was solubilized in the atropine, resulting in an apparent time-dependent decrease in the concentration of atropine in the ejectate. When adequately mixed, there was not a significant difference in the amount of atropine delivered by autoinjectors in 400-mg (SYS B1) versus 500-mg (SYS B2) HI-6 configurations.

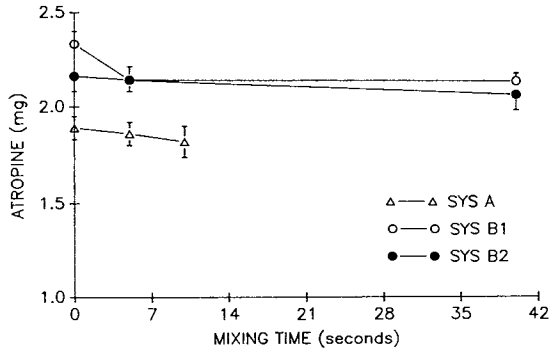

Fig. 1. Effect of component mixing time on the delivery (mg salt) of atropine. Autoinjectors were activated to solvate HI-6 and the contents ejected after the specified mixing time (sec). Error bars represent the standard deviation of the mean determined from 9 or 10 autoinjectors for each system (SYS).

The amounts of HI-6 found in all autoinjector ejectates increased significantly as the mixing time increased (F = 47.4, SYS A; F = 60.5, SYS B1; F = 68.8, SYS B2) and is shown in Fig. 2. As would be expected, solutions from the autoinjectors filled with 500 mg HI-6 (SYS A and SYS B2) contained proportionally more HI-6 compared to that delivered by SYS B1 at equal mixing times. However, SYS B2 with no mixing delivered amounts of HI-6 which were highly variable and low on average. With no mixing, SYS A delivered 352 mg (±32 mg), SYS B1 delivered 344 mg (±23 mg), and SYS B2 delivered 303 mg (±75 mg) of HI-6 dichloride. An explanation for this might be that the greater quantity of HI-6 packed in the SYS B2 autoinjectors slowed the solubilization of the crystals. However, this did not occur with the SYS A autoinjectors, perhaps because of more rapid solubilization of the pulverized HI-6, the larger volume of wet component or other differences in system design. While the amounts of HI-6 delivered increased as the mixing time was changed from 5 to 40 sec, this difference was not statistically significant for either SYS B1 or SYS B2. After the maximum mixing time (10 sec, SYS A; 40 sec, SYS B1 and SYS B2) the average amounts of atropine delivered were 1.82 mg (SYS A), 2.13 mg (SYS B1), and 2.06 mg (SYS B2), and the average amounts of HI-6 delivered were 460 mg (SYS A), 424 mg (SYS B1), and 545 mg (SYS B2).

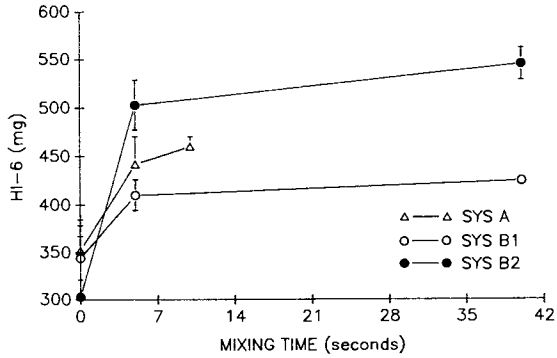

Fig. 2. Effect of component mixing time on the delivery (mg) of HI-6 dichloride. Autoinjectors were activated to solvate HI-6 and the contents ejected after the specified mixing time (sec). Error bars represent the standard deviation of the mean determined from 9 or 10 autoinjectors for each system (SYS).

Stability Study

Groups of 10 autoinjectors were numbered, their contents were mixed thoroughly, and they were stored at 40°C for 3, 7, or 14 days. Solutions from autoinjectors stored for 7 days changed from colorless to gray, which intensified with 14 days of storage. The average volumes delivered were 2.96 ± 0.14 ml (n = 29, SYS A), 2.24 ± 0.23 ml (n = 30, SYS B1), and 2.42 ± 0.14 ml (n = 30, SYS B2). Storage at 40°C for up to 2 weeks did not appear to influence system containment integrity.

The amounts of atropine delivered by the SYS A and SYS B1 autoinjectors were not found to change significantly with time in the stability study as shown in Fig. 3 and, on average, were 97.0% (1.77 mg) and 93.7% (1.99 mg), respectively, of the day 0 amounts. The SYS B2 autoinjectors delivered amounts of atropine which decreased significantly (to 1.86 mg at 14 days) with increasing storage time (F = 12.4). While statistically significant, the apparent decrease in atropine delivery in SYS B2 did not appear to be strictly time dependent and was not a considerable detriment to total drug dose delivered.

The amount of HI-6 in solution with storage at 40°C in the stability study decreased significantly with time in all systems (F = 39.3, SYS A; F = 29.4, SYS B1; F = 91.8, SYS B2) as shown in Fig. 4. The delivered amount of HI-6 showed significant decreases of 14, 19, and 27% (SYS A), 13, 16, and 24% (SYS B1), and 6, 16, and 31% (SYS B2) of day 0 amounts after 3, 7, and 14 days of high-temperature storage, respectively. After 14 days, 337 mg (SYS A), 324 mg (SYS B1), or 377 mg (SYS B2) of HI-6 dichloride was delivered on average.

Apparent first-order kinetic rate constants were determined from plots of the natural log of HI-6 concentration versus time in the stability study and are given in Table I. Using these rate constants, half-life values were calculated for HI-6 in citrate buffer, pH 4, at 40°C. They are consistent with the reported half-life of 20 days for a 250 mg/ml solu-

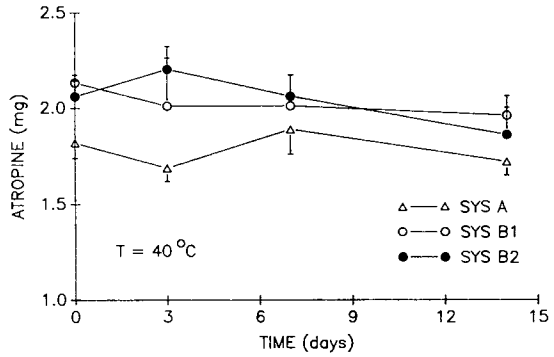

Fig. 3. Effect of high-temperature storage time on the delivery of atropine (mg salt) by three autoinjector systems. Autoinjectors were activated to solvate HI-6 and then placed in a laboratory oven maintained at 40°C, except for the time 0 samples. At the indicated time, autoinjector contents were ejected and analyzed for atropine (salt) content (see Materials and Methods). Error bars represent standard deviation of the mean atropine determination from 10 autoinjectors.

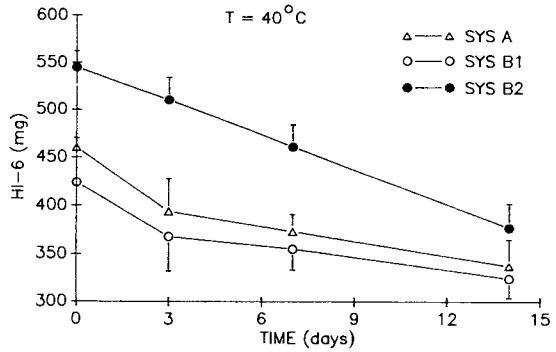

Fig. 4. Effect of high-temperature storage time on the delivery of HI-6 dichloride by three autoinjector systems. Autoinjectors were activated to solvate HI-6 and then placed in a laboratory oven maintained at 40°C, except for the time 0 samples. At the indicated time, autoinjector contents were ejected and analyzed for HI-6 dichloride content (see Materials and Methods). Error bars represent standard deviation of the mean atropine determination from 10 autoinjectors.

tion, at pH 4.0, stored at 45°C (15). Using our estimates of half-life, times were calculated for the concentration of HI-6 to decrease to 90% of the respective day 0 concentration. The stability results suggest that both atropine and HI-6 should have a shelf life, after solubilization of HI-6, of at least 5 days under temperate climatic conditions.

CONCLUSIONS

The SYS A wet/dry autoinjector will, on average, after mixing for the specified time of 5 sec, deliver a volume of 3.0 ml containing 1.86 mg of atropine sulfate and 445 mg of HI-6 dichloride. Increasing the mixing time to 10 sec will increase the amount of HI-6 dichloride delivered to 460 mg. The average coefficient of variation was 3.0% for atropine and 6.3% for HI-6 determinations. Stored from 3 to 14 days at 40°C after the contents have been mixed, the autoinjector will deliver 97% of the day 0 amount of atropine sulfate. With a half-life of 34 days, the amount of HI-6 delivered will decrease from 86% of day 0 after 3 days to 73% of day 0 after 14 days.

The SYS B1 wet/dry autoinjector specified to deliver a 400 mg HI-6 dichloride load will, on average after mixing for 40 sec, deliver a volume of 2.3 ml containing 2.13 mg of atropine citrate and 424 mg of HI-6 dichloride. Increasing the HI-6 dichloride load to 500 mg (SYS B2) results in a decrease in delivery of atropine citrate to 2.06 mg and an increase in delivery of HI-6 dichloride to 545 mg. The averages of the coefficients of variation were 5.2% for atropine delivery and 7.0% for HI-6 delivery. From 3 to 14 days at 40°C after the autoinjector contents have been mixed, it will deliver an overall average 96% of the day 0 amount of atropine citrate. The delivery of HI-6 dichloride will decrease with a half-life of 39 days for SYS B1 and 32 days for SYS B2, falling to 76% and 62% of the respective day 0 amounts after 14 days. Regardless of the load, the delivery of HI-6 dichloride falls to 90% of the respective day 0 amount after 4 (SYS A) or 5 (SYS B1 or SYS B2) days. This study establishes the viability of the wet/dry autoinjector configuration as a functional delivery device for the emergency administration of compounds such as HI-6 that are unstable in aqueous solutions.

Table I. Stability of HI-6 in Autoinjectors Stored at 40°C

	SYS A	SYS B1	SYS B2
Apparent first-order rate constant (day^{-1})	1.96E-2 (R = 0.85)	1.77E-2 (R = 0.84)	2.18E-2 (R = 0.96)
Half-life (days)	34.0	38.5	31.9
95% confidence limits	29.4–40.5	32.9–47.1	29.7–34.6
Time to decrease to 90% of day 0 (days)	4.0	5.2	5.0
95% confidence limits	2.9–4.9	4.1–6.3	4.5–5.5

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