ELSEVIER

International Journal of Pharmaceutics 109 (1994) 35–43

international journal of pharmaceutics

Operational evaluation of wet/dry autoinjectors containing atropine in solution and powdered HI 6 or HLö 7

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(Received 19 November 1993; Accepted 25 January 1994)

Abstract

To counteract organophosphate poisoning, the combined administration of atropine and a cholinesterase reactivator has been a standard therapy. Because of potential large-scale emergencies that might occur upon dissemination of nerve agents, administration of life-saving drugs by autoinjectors for self- and buddy-aid can be mandatory. HI 6 and HLö 7 belong to the newer generation of 'H-oximes' with a broader antidotal spectrum, including soman, and are therefore considered as possible candidates to replace the currently marketed oximes, pralidoxime and obidoxime. Since HI 6 and HLö 7 are unstable in solution they must be administered by the newly developed binary wet/dry autoinjectors that allow rapid dissolution of solid compounds prior to injection. The purpose of this in vitro study was to evaluate the performance of two commercial autoinjector systems, containing solid HI 6 or HLö 7 together with atropine in solution, and to determine the delivery of the oximes. The Astra Tech HI 6 autoinjectors 'Meditec' contained 500 mg HI 6 dichloride and delivered 426 mg HI 6 (coefficient of variation, CV 5.7%). The HI 6 autoinjectors from STI 'Binaject' contained 600 mg HI 6 and delivered 533 mg (CV 1.0%). The somewhat large variation of the HI 6 remaining in the Meditec autoinjectors was markedly increased when the device was fired against an increasing back pressure, such as during i.m. administration. At a pressure of 0.6 kg/cm^2 the Meditec autoinjectors delivered only 273 mg HI 6 (CV 13.4%), whereas the Binaject autoinjectors still delivered 511 mg (CV 2.7%) against 1 kg/cm^2. Incomplete delivery from the Meditec autoinjectors was also found during administration of HI 6 to dogs, where the Binaject autoinjectors functioned reliably. Malfunctions of the Meditec autoinjectors were detected neither in vitro nor in vivo when they were filled with 225 mg HLö 7 dimethanesulfonate and atropine or with atropine only. The same holds true for the Binaject injectors. Special care has to be taken with regard to the appropriate shaking procedure during dissolution. In this respect, the manufacturers should improve the user's instructions.

Key words: Autoinjector, wet/dry; HI 6; HLö 7; Atropine; In vitro study

1. Introduction

Several asymmetrical bis-pyridinium aldoximes ('H-oximes') developed in the laboratory of Professor I. Hagedorn (Freiburg, Germany) have

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SSDI 0378-5173(94)00051-6

been shown to possess good antidotal properties against poisoning with a variety of extremely toxic organophosphorus compounds in various animal experiments. Among these H-oximes, HI 6 (1-(((4-(aminocarbonyl)pyridinio)methoxy)methyl)-2-((hydroxyimino)methyl)pyridinium dichloride monohydrate; CAS 34433-31-3) in combination with atropine is presently regarded as the most promising compound against poisoning by soman and sarin (Oldiges and Schoene, 1970; Clement, 1981, 1983; Wolthuis et al., 1981a,b; Boskovic et al., 1984; Clement et al., 1992; Lundy et al., 1992). HI 6 was also administered to patients intoxicated with organophosphate insecticides (Kusic et al., 1991). However, HI 6 is quite ineffective in reactivating acetylcholinesterase inhibited by the phosphoramidate tabun (Schoene and Oldiges, 1973; De Jong and Wolring, 1980; Cetkovic et al., 1984; De Jong et al., 1989).

HLö 7 diiodide (1-(((4-(aminocarbonyl)pyridinio)methoxy)methyl)-2,4-bis((hydroxyimino)-methyl)pyridinium diiodide; CAS 120103-35-7) bears an additional aldoxime function in position 4, which appears necessary for high reactivation rates. In fact, HLö 7 was superior to HI 6 in reactivating AChE blocked by soman, sarin or tabun (De Jong et al., 1989; Eyer et al., 1992). In addition, HLö 7 diiodide was the most active oxime in restoring rat diaphragm contractility blocked by DFP, tabun, sarin, or soman (Alberts, 1990). In combination with atropine, HLö 7 diiodide afforded higher protection ratios than HI 6 in mice poisoned with soman, sarin, or tabun. In atropine-protected guinea-pigs, however, HLö 7 was somewhat less effective than HI 6 against soman but more effective against tabun (Eyer et al., 1992; Lundy et al., 1992).

On a molar basis, HLö 7 is about 1.5-times more toxic to rodents than HI 6. On the other hand, the antidotal potency in mice, i.e., reduction of 95% to 50% mortality against soman, sarin, or tabun is at least 4-times greater with HLö 7 compared to HI 6 (Eyer et al., 1992). Hence, the therapeutic index may be even higher with HLö 7. At any rate, due to their broader antidotal spectrum, both oximes appear to be suitable to replace the currently marketed oximes, obidoxime and pralidoxime.

The major disadvantage of HLö 7 diiodide, its poor solubility in water, has been overcome by the development of the highly soluble dimethanesulfonate salt (Eyer et al., 1992). This property enables HLö 7 dimethanesulfonate to be used also in autoinjectors. Because of potential large-scale emergencies that might occur upon dissemination of nerve agents, intramuscular administration by autoinjectors for self- and buddy-aid can be mandatory. Although the use of chemical weapons has been officially banned, the recent Iran/Iraq conflict demonstrated the importance of having effective therapies for a variety of nerve agents.

For practical reasons, the dose under consideration, i.e., about 500 mg HI 6 dichloride monohydrate (Kusic et al., 1985) and about 200 mg HLö 7 dimethanesulfonate (Eyer et al., 1992), must be dissolved in a small volume for i.m. administration, not exceeding 3 ml. While water solubility of the oximes poses no problems, their stability in aqueous solution does. Particularly in concentrated solutions, HI 6 and HLö 7 are too unstable for storage (Eyer and Hell, 1985; Eyer et al., 1986, 1988, 1989; Fyhr et al., 1987).

Recently, a new design of autoinjectors was described in which compounds unstable in solution can be stored in powder form and dissolved by a diluent in an adjacent chamber upon activation of the device (Schlager et al., 1991).

To evaluate the bioavailability of HI 6 dichloride monohydrate (500 mg) and HLö 7 dimethanesulfonate (200 mg) from autoinjectors containing 2 mg atropine sulfate in 2–3 ml solution, we used beagle dogs, where the anticipated human doses to be administered i.m. have turned out to be without adverse effects (Klimmek and Eyer, 1986; Eyer et al., 1992). On commencing our studies with two systems from different manufacturers (Astra Meditec AB, Mølndal, Sweden; and STI International Ltd, Rochester, Kent, U.K.), we became aware that the Astra autoinjectors containing HI 6 only partially delivered its content during the 5 s injection period. When the cannula was withdrawn, roughly one third of the autoinjector content splashed away. This did not happen with the HLö 7 autoinjectors from Astra and with either oxime from the STI autoinjectors.

Therefore, we decided to evaluate the technical functions of the autoinjectors in more detail. For a recent publication on the solvation characteristics of HI 6 and its stability in the same wet/dry autoinjectors, the reader is referred to Schlager et al. (1991).

2. Materials and methods

2.1. Autoinjectors

The autoinjectors were obtained from Astra Tech AB (Mølndal, Sweden; Meditec autoinjectors), and from STI International Ltd (formerly Medimech International Ltd – Frindsbury, Rochester, Kent, U.K.; Binaject autoinjectors).

According to the manufacturer, the Astra Meditec autoinjectors were filled with 475–525 mg HI 6 dichloride monohydrate and 2.26 mg atropine sulfate in citrate buffer (3.0 ml, pH 3.9). With a complete ejection, the injectors were specified to have a fairly consistent residual volume of approx. 10%. For filling the HLö 7 autoinjectors, HLö 7 dimethanesulfonate was provided by this laboratory (Eyer et al., 1992). The manufacturer was requested to fill the solid chamber with 200 mg HLö 7 dimethanesulfonate with the other components in the fluid chamber being left unchanged.

According to the manufacturer, the STI Binaject autoinjectors were filled to deliver 500 mg HI 6 dichloride monohydrate and 2 mg atropine sulfate in water for injection at a dispensed volume of 2.4 ml. The HLö 7 autoinjectors were filled with 237.5 mg HLö7 dimethanesulfonate (±5%) and 2.375 mg atropine sulfate (±3%) in 2.68 ml water for injection. With this filling specification the device was designed to dispense 200 mg of HLö 7 dimethanesulfonate and 2 mg of atropine sulfate in 2.4 ml.

2.2. Technical details

After disassembling the autoinjectors the extensions of the components were measured with a caliper (Table 1). The technical details of the devices appeared to be identical, whether filled with HI 6 or HLö 7. The spring force (D) was determined by fixing the springs between a balance adjustable in height and a fixed counter piston. By compressing the spring continuously the exerted force (G) was read from the balance and the length of compression determined by measuring the vertical interval (l) with 0.05 mm precision ($D = \Delta G/\Delta l$ (kg/cm)).

Table 1
Technical details of the Binaject (BJ) and Meditec (MT) autoinjectors

	BJ	MT
Housing		
Length (cm)	16.5	16.5
Maximal diameter (cm)	1.78	2.49
Weight (g)	41	60
Chamber system		
Total chamber volume for mixing (cm^3)	6.65	3.53
Sectional area of the piston (cm^2)	0.95	1.77
Cannula		
Total length (cm)	3.70	4.03
Length available for injection (cm)	2.56	2.10
Inner diameter (mm)	0.58	0.60
Outer diameter (mm)	0.89	0.90
Spring		
Thickness (mm)	1.30	1.33
Weight (g)	11.4	11.4
Length before activation (cm)	5.6	5.6
Length after activation (cm)	15.0	14.6
Outer diameter (cm)	1.04	0.97
Spring constant (kg cm^{-1})	1.00	1.43

2.3. Sample preparation

The contents of the fluid and solid chambers were investigated separately. The volume and pH of the fluid and the weight of the solid were determined. The solid remaining in the chamber was washed out and determined photometrically and by HPLC.

In order to analyse the dispensed amount, each autoinjector was fired in a flask (4.65 ml)

sealed with parafilm to avoid splashing. The ejected content was weighed, the volume and pH measured, and the density calculated ($\delta = m/V$ (g/ml)). After dilution in 20 mM phosphoric acid (1:5000 for HI 6, 1:2000 for HLö 7 (v/v)) the oximes were determined photometrically and by HPLC. Following ejection, the autoinjectors were disassembled and rinsed with water which was collected in a volumetric flask (250 ml) to determine the oxime remaining.

2.4. HPLC

HPLC was performed with an L-6200A pump (Merck-Hitachi, Darmstadt, Germany) on LiChrosphere® 60 RP-select-B (5 μm; E. Merck, Darmstadt, Germany) at a flow rate of 1.2 ml/min. The mobile phase consisted of methanol/PIC-B7/PIC-A/H_2O (12:4:0.5:83.5% v/v, HI 6; 30:2:0:68% v/v, HLö 7) (PIC-B7® and PIC-A® being ion-pairing reagents; Waters-Millipore, Eschborn, Germany). HI 6 was eluted after 5.4 min, HLö 7 after 10.3 min. The oximes were quantified with a UV/Vis, SPD-6AV detector (Shimadzu, Duisburg, Germany) and a D-2500 Chromato-Integrator (E. Merck, Darmstadt, Germany) calibrated with authentic standards. The detection wavelength of HI 6 was 300 nm, that of HLö 7 being 298 nm.

The samples were injected by an AS-4000A autosampler (Merck-Hitachi) (50 μl; lead/rear volume 30 μl/30 μl; lead and rear volume denote the first and last part of the sample discarded during injection).

The coefficient of variation (CV) was 0.9% for both oximes (five injections each).

2.5. Photometric determination

In addition to HPLC, the concentrations of oxime solutions were determined photometrically in 20 mM phosphoric acid, pH 2, using the following extinction coefficients: HI 6 $\epsilon_{mM\ 300\ nm} = 12.15\ cm^{-1}$; HLö 7 $\epsilon_{mM\ 298\ nm} = 16.3\ cm^{-1}$. The results agreed with the HPLC determination within 0.5% on average ($n = 15$) at 1.5% CV.

2.6. Determination of the back pressure

3.0 ml saline was injected as a bolus in a pig's knuckle, obtained from a butcher, surrounded by intact skin. The transient back pressure developing in the musculature was read from a manometer. The assembly shown in Fig. 1 consisted of two identical glass syringes connected by a snuggly fitting stainless-steel double piston. Syringe 1 and its connection to the manometer were filled bubble-free with water to exclude air compression. During injection by handling the device at syringe 1, the pressure developing in syringe 2 was transmitted to the manometer.

Similarly, the back pressure was determined under equilibrium conditions by injecting up to 3 ml saline into a rubber septum-sealed flask (4.65 ml air volume). The pressure development vs the injected volumes is shown in Fig. 2. The theoretical data were calculated from the equation:

$$\Delta p = p_o(V_o/(V_o - V_i) - 1)(\mathrm{kg/cm^2})$$

where Δp is the increase in pressure, p_o denotes atmospheric pressure, V_o is the volume of the

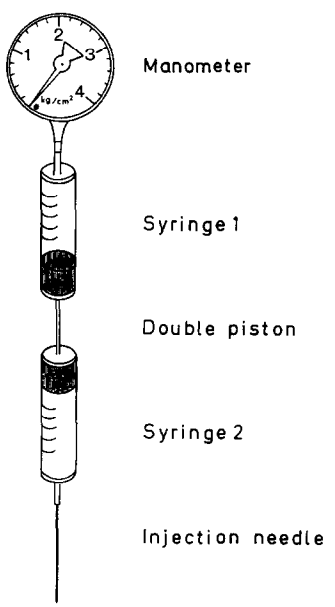

Fig. 1. Assembly for back pressure measurement. During injection by handling the device at syringe 1, the pressure developing in syringe 2 is transmitted to the manometer (for details see section 2).

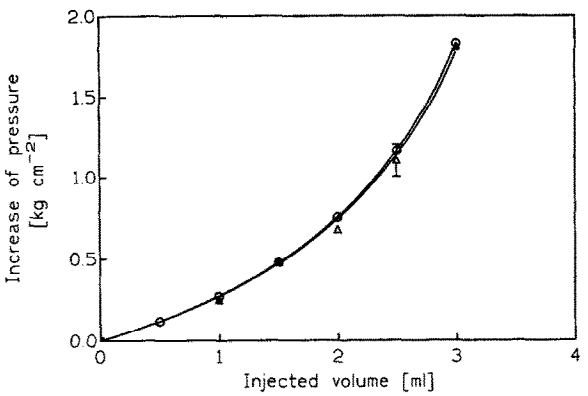

Fig. 2. Increase of pressure developing in a rubber septum-sealed flask (4.65 ml air volume) during injection of various volumes of saline ((△) means ± SE, $n = 3$; (○) calculated data, cf. section 2).

empty flask, i.e., 4.65 ml, and V_i represents the injected aqueous volume.

2.7. Statistics

Arithmetic means ± SE are presented throughout. Coefficients of variation (CV) are given as 100 SD/mean (%). One-way analysis of variance (ANOVA) was applied to test for significant differences (p values two-tailed), using InStat software (Graphpad, San Diego, CA, U.S.A.).

3. Results

3.1. Technical details of the two types of autoinjectors

The devices from the same manufacturer filled with HI 6 or HLö 7 appeared to be identical. The cannula of the Meditec autoinjector was 9% longer than that of Binaject and had a 3.5% larger inner diameter. Hence, according to the law of Hagen-Poiseuille, the resistance of the cannula in the Binaject device should be 5% greater. The spring force of the Meditec device was 43% stronger. Since the sectional area of the piston was 185% greater in the Meditec autoinjector, pressure generation in the Meditec device was presumed to be smaller (ignoring differences in friction). These data suggested that the delivering pressure was greater in the Binaject autoinjector (for details see Table 1).

3.2. Contents of fluid and solids

The autoinjectors were disassembled to determine separately the fluid and the weight of solids. According to the manufacturer, the HI 6 Meditec autoinjector should contain about 500 mg HI 6 dichloride. We found this specified amount of HI 6 and a solvent volume of 2.73 ml. The HI 6 Binaject autoinjectors were prepared to deliver 500 mg HI 6. We found about 600 mg HI 6 and a solvent volume of 2.38 ml.

For the HLö 7 autoinjectors Astra Meditec was requested to prepare the device with 200 mg HLö 7 dimethanesulfonate. We found about 235 mg HLö 7 and a solvent volume of 2.84 ml. The HLö 7 Binaject autoinjector was specified to contain 237 mg HLö 7 dimethanesulfonate and 2.68 ml water. We found 234 mg HLö 7 and a solvent volume of 2.61 ml (for details see Table 2).

3.3. Delivery of the autoinjector contents without back pressure

According to the manufacturer's instruction, both HI 6 autoinjectors should be shaken for 5 s after activation of the assembly. Interestingly, the Binaject autoinjector must be shaken with the red activator pin down and the injection side up-

Table 2
Contents of fluids and solids in the Binaject (BJ) and Meditec (MT) autoinjectors

Auto-injector	pH	Volume (ml)	Amount (dry) (mg)	Amount (remaining) (mg)	Amount (total) (mg)
HI 6					
BJ	6.5	2.380	563.81	28.30	592.11
BJ	6.5	2.380	580.23	27.68	607.91
MT	3.9	2.729	487.30	18.74	506.04
HLö 7					
BJ	6.5	2.600	223.99	11.20	235.19
BJ	6.5	2.620	226.15	5.81	231.96
MT	3.9	2.840	223.56	11.90	235.46
MT	3.9	2.835	223.56	12.00	235.56

wards! We found that mixing was incomplete even after 30 s shaking when the injection side was held downwards. The reason for this puzzling phenomenon is the presence of a sieve mounted between the fluid and the solid. Only with the fluid on top does manual shaking allow rapid dissolution of the solid. Although the non-verbal illustration for application shows the correct mixing technique, the casual user might spontaneously shake the autoinjector with the injection side downwards (several uninformed persons were asked to shake the injector according to the description; they all used the wrong technique!). The shaking technique recommended by the Meditec illustration appeared to us to be even more enigmatic. In an attempt to standardize the mixing process, the autoinjectors were shaken mechanically at 300 strokes per min up and down with a 6 cm lift. Both autoinjector types showed incomplete dissolution of HI 6 after 60 s shaking, regardless of the autoinjector position. Therefore, we decided to shake the autoinjectors by hand with 60 strokes in 1 min. Complete dissolution was observed throughout.

For analysing the dispensed amount, each autoinjector was fired in a flask sealed with parafilm to avoid splashing. The results are listed in Tables 3 (HI 6) and 4 (HLö 7). The Binaject autoinjectors dispensed 91.4 ± 0.4% of the HI 6 filled in, the device from Meditec 85.7 ± 2.6%. The difference in delivery was significant ($p = 0.035$). The total content of HI 6 was more constant in the Binaject autoinjectors (CV 0.7%) compared to Meditec (CV 3.0%). The delivery from the HLö 7 autoinjectors was 90.4 ± 1.3% for Binaject and 93.5 ± 1.5% for Meditec. The difference was not significant ($p = 0.054$). The content uniformity of HLö 7 was similar for both injectors, CV 1.2% each. The pH of the oxime solutions from Binaject was around 3 and for Meditec 3.5. It should be noted that ejection without back pressure was complete within about 2 s.

Table 3
Delivery of HI 6 after activation of the autoinjectors without back pressure (Binaject, BJ; Meditec, MT)

Auto-injector	Density (kg l^{-1})	Volume (ml)	Amount (ejected) (mg)	Amount (remaining) (mg)	Amount (Σ) (mg)
BJ 1	1.067	2.623	539.25	43.62	582.87
BJ 2	1.068	2.550	531.20	57.75	588.95
BJ 3	1.073	2.589	537.11	50.53	587.64
BJ 4	1.073	2.620	537.65	39.85	577.50
BJ 5	1.073	2.568	529.37	55.77	585.14
BJ 6	1.069	2.566	525.27	55.42	580.69
Mean	1.071	2.586	533.31	50.49	583.80
±SE	0.0012	0.0123	2.26	2.97	1.76
MT 1	1.040	2.950	452.88	39.34	492.22
MT 2	1.048	2.792	427.54	91.81	519.35
MT 3	1.055	2.576	391.87	105.19	497.66
MT 4	1.051	2.895	443.09	51.23	495.55
MT 5	1.056	2.882	412.93	65.20	478.12
Mean	1.050	2.819	425.66	70.55	496.58
±SE	0.0028	0.066	10.84	12.30	6.64

Table 4
Delivery of HLö 7 after activation of the autoinjectors without back pressure (Binaject, BJ; Meditec MT)

Auto-injector	Density (kg l^{-1})	Volume (ml)	Amount (ejected) (mg)	Amount (remaining) (mg)	Amount (Σ) (mg)
BJ 1	1.035	2.470	203.58	30.13	233.71
BJ 2	1.031	2.614	217.45	17.99	235.44
BJ 3	1.038	2.666	211.71	19.12	230.84
BJ 4	1.040	2.573	218.01	20.37	238.38
BJ 5	1.034	2.522	210.12	24.63	234.75
Mean	1.036	2.569	212.17	22.45	234.62
±SE	0.0016	0.034	2.65	2.23	1.22
MT 1	1.029	2.875	213.35	10.41	223.76
MT 2	1.033	2.782	209.99	12.42	222.41
MT 3	1.033	2.879	204.39	15.59	219.98
MT 4	1.034	2.874	214.40	11.92	226.32
MT 5	1.031	2.635	198.28	22.02	220.30
Mean	1.032	2.809	208.08	14.47	222.55
±SE	0.0008	0.047	3.01	2.07	1.17

3.4. Delivery of the autoinjector contents against various back pressures

The in vivo observation of incomplete delivery of HI 6 from the Meditec autoinjectors prompted us to study the effect of an increasing back pressure that may build up during i.m. injection. In order to obtain a rough idea as to the pressures that might transiently occur, we injected 3.0 ml saline in a pig's knuckle from the butcher. During injection we observed peak pressures of about 1.8 kg/cm^2 which declined within a few seconds.

To simulate increasing back pressures under equilibrium conditions we injected step by step up to 3 ml saline into a rubber septum-sealed flask (4.65 ml air volume) and determined the pressure. Fig. 2 shows the exponential increase in pressure upon injection of various fluid volumes. The observed data are in close agreement with those calculated. Hence, it was possible to determine the back pressure that can be counteracted by the autoinjectors.

As shown in Table 5, the Binaject autoinjectors delivered nearly completely the HI 6 solution (2.487 ± 0.02 ml) against a pressure of about 1.2 kg/cm^2. Without back pressure the delivered volume was 2.586 ± 0.012 ml. In contrast, only 1.828 ± 0.120 ml HI 6 was dispensed from the Meditec autoinjectors against a pressure of 0.6 kg/cm^2. Without back pressure the delivered volume was 2.819 ± 0.066 ml. Correspondingly, the delivered amount of HI 6 from Binaject was 511 mg as specified but only 273 mg from Meditec, i.e., only 56%! This difference was very significant ($p < 0.001$).

Surprisingly, the delivery of HLö 7 from the Meditec autoinjectors was considerably more complete. The dispensed volume was 2.418 ± 0.037 ml, corresponding to a pressure of about 1.1 kg/cm^2. Without back pressure the delivered volume was 2.809 ± 0.047 ml. The dispensed HLö 7 amounted to 177.5 mg from Meditec and to 201.6 mg from Binaject, as specified (cf. Tables 4 and 6). The difference was significant ($p = 0.013$).

It should be noted that the delivery against an increasing pressure stopped within less than 5 s with all autoinjectors tested. After extraction of the cannula the Meditec autoinjectors immediately delivered the remaining material, whereas the Binaject autoinjectors appeared to be empty.

Table 5
Delivery of HI 6 from the activated autoinjectors against a back pressure of 1.2 kg/cm^2 (Binaject, BJ) or 0.6 kg/cm^2 (Meditec, MT)

Auto-injector	Density (kg l^{-1})	Volume (ml)	Amount (ejected) (mg)	Amount (remaining) (mg)	Amount (Σ) (mg)
BJ 1	1.071	2.440	495.41	79.44	574.85
BJ 2	1.069	2.501	510.59	68.30	578.89
BJ 3	1.074	2.498	526.57	66.60	593.17
BJ 4	1.069	2.445	499.46	81.62	581.08
BJ 5	1.070	2.550	521.70	54.25	575.95
Mean	1.071	2.487	510.75	70.04	580.79
±SE	0.0009	0.020	6.05	4.93	3.28
MT 1	1.054	1.838	264.99	207.20	472.19
MT 2	1.058	2.110	321.16	161.24	482.40
MT 3	1.055	1.840	272.48	200.71	473.19
MT 4	1.053	1.525	232.93	273.24	506.17
Mean	1.055	1.828	272.89	210.60	483.49
±SE	0.0011	0.120	18.23	23.22	7.90

Table 6
Delivery of HLö 7 from the activated autoinjectors against a back pressure of 1.1 kg/cm^2 (Binaject, BJ; Meditec, MT)

Auto-injector	Density (kg l^{-1})	Volume (ml)	Amount (ejected) (mg)	Amount (remaining) (mg)	Amount (Σ) (mg)
BJ 1	1.075	2.462	204.88	28.62	233.50
BJ 2	1.023	2.413	199.05	33.75	232.80
BJ 3	1.038	2.510	208.80	24.25	233.48
BJ 4	1.029	2.500	210.02	25.11	235.13
BJ 5	1.053	2.258	185.07	43.80	228.87
Mean	1.044	2.429	201.56	31.11	232.76
±SE	0.0093	0.046	4.55	3.59	1.04
MT 1	1.016	2.380	176.40	45.37	221.77
MT 2	1.030	2.450	178.72	41.63	220.35
MT 3	1.032	2.291	170.45	49.71	220.15
MT 4	1.032	2.480	179.18	41.01	220.19
MT 5	1.036	2.490	182.90	38.59	221.49
Mean	1.029	2.418	177.53	43.26	220.79
±SE	0.0034	0.037	2.05	1.94	0.35

4. Discussion

A previous solvation study on HI 6 autoinjectors from Meditec and Binaject (formerly Medimech) (Schlager et al., 1991) showed that the Meditec autoinjectors delivered 445 mg HI 6 and the Binaject autoinjectors 500 mg HI 6 after 5 s manual mixing (2 shakes/s). Increasing the mixing time to 10 s increased the amount of HI 6 delivered from the Meditec autoinjector to 460 mg HI 6 (CV 6.3%). The Binaject autoinjector delivered 545 mg HI 6 (CV 7.0%) when the mixing time was extended to 40 s. These figures were roughly confirmed in our study with 426 mg HI 6 (CV 5.7%) for Meditec and 533 mg HI 6 (CV 1.0%) for Binaject when the mixing time was

60 s throughout (1 shake/s). Possibly, the longer dissolution time in our study resulted in smaller variation.

Somewhat unexpected was the large variation of the HI 6 remaining in the autoinjectors from Meditec. This drawback was even worse when the autoinjectors were fired against an increasing back pressure. In fact, the Meditec autoinjector filled with HI 6 was only able to eject up to a back pressure of 0.6 kg/cm^2, thereby delivering only 273 mg HI 6 (CV 13.4%). After retraction of the cannula from the rubber septum of the sealed flask, the autoinjectors delivered a further portion. Such behavior was also found when the Meditec autoinjectors were used for i.m. administration in beagle dogs (Spöhrer et al., 1994). Because the transient pressure gradient during i.m. administration into the hind leg muscles (M. quadriceps) was unknown, we tried to simulate the pressure development in a pig's knuckle and found pressure maxima of up to 1.8 kg/cm^2 when the injection site was right within the musculature.

The Binaject autoinjector filled with HI 6 showed reasonably complete delivery up to a back pressure of nearly 2 kg/cm^2, and the dispensed amount against 1 kg/cm^2 was 511 mg HI 6 (CV 2.7%). Since the flow stopped within 2–3 s, reaching true pressure equilibrium, the viscosity of the solution should play no role. In fact, the viscosity of the more concentrated solution of the Binaject autoinjectors as revealed from the density may be even higher. Hence, one obvious reason for the weaker delivery of HI 6 from the Meditec autoinjectors might be the greater sectional area of the piston (185%) that was hardly compensated by the greater spring force (43%).

However, such an explanation is not completely satisfactory on considering the results with HLö 7. The delivery was equally perfect for the autoinjectors from both manufacturers. Even at a back pressure of roughly 1 kg/cm^2 the delivery was quite acceptable: $86.6 \pm 2.3\%$ for Binaject and $80.4 \pm 1.2\%$ for Meditec. Hence, a substance-specific factor may additionally influence the delivery process of the Meditec device. Since the devices used for both oximes appeared to be identical an alternative explanation should also be considered: The piston of the Astra Meditec autoinjectors possesses two O-ring seals, one of which is in permanent contact with the solvent. It appears possible that longer storage of the manufactured autoinjectors, as may be the case for the commercially available HI 6 autoinjectors, leads to some swelling of the seals, thereby increasing the friction. This is not expected to occur with the Binaject device, where the piston is not in contact with the solvent during storage.

In conclusion, the Binaject autoinjectors were found to deliver the indicated amounts of HI 6 (500 mg) more reliably than the Meditec autoinjectors. With HLö 7 the delivery (200 mg) was acceptable with both autoinjector types. Special care has to be taken, however, with regard to the appropriate shaking procedure. In this respect, the manufacturers should improve the user's instructions. In addition, this study has shown that operational evaluations of autoinjectors should be carried out also against an increasing back pressure to simulate more closely the real situation of i.m. administration.

Acknowledgement

This work is part of the Ph.D. Thesis of U.S.

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