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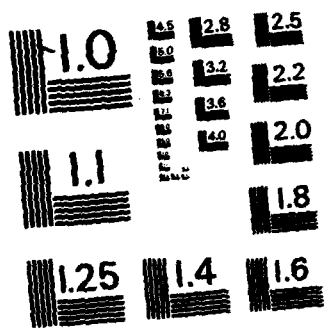
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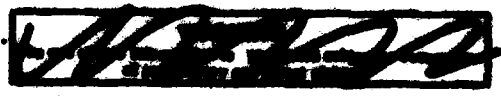
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ABSTRACT

A comparison of the chemical purity, toxicology and efficacy of HI-6 (COc1ccc(cc1N)C(=O)Nc2ccc(cc2)N) obtained from various sources was performed. There were no significant differences between HI-6 obtained from either Israel, Yugoslavia, The Netherlands or Canada regarding their efficacy, when combined with atropine, as an antidote of organophosphate poisoning. HI-6 obtained from Great Britain was significantly more toxic and less efficacious than any of the other HI-6 samples. In addition the results of this study showed that there was no significant difference between HI-6 prepared as a laboratory batch and HI-6 prepared commercially with regards to chemical purity, toxicology or efficacy.

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INTRODUCTION

Atropine plus oxime (PAM, TMB-4 or toxogonin) is an effective treatment for most cases of organophosphate poisoning. In the case of poisoning by soman (pinacolyl methylphosphonofluoridate) however, this treatment is relatively ineffective.

In the past decade new drugs have been synthesized that appear to be very effective against soman poisoning. In particular bispyridinium oximes such as HI-6, HS-6, HGG-12, HGG-42 originating from Professor Hagedorn's laboratory in Freiburg, Germany and BDB-27 from Dr. Binenfeld's group in Belgrade, Yugoslavia have shown potential as new oxime antidotes of organophosphate poisoning.

A great deal of interest has been shown in HI-6. This bispyridinium oxime was found to be an effective antidote with a low toxicity, and relatively inert

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pharmacologically compared with other bispyridinium compounds such as HGG-12 and HGG-42 (Clement, 1981; Lundy and Tremblay, 1979). Upon examining toxicity data on HI-6 (Table 1) it is apparent that there is a great deal of disparity. This could be the result of differences in either the purity of HI-6 from the various sources or the strain of mouse used in the toxicity studies.

The purpose of this investigation was to examine the chemical purity and the efficacy and toxicity of HI-6 obtained from various sources.

METHODS

Chemistry

Melting points were carried out on a Thomas-Hoover melting point apparatus equipped with a linear temperature programmer. Melting points were uncorrected.

Infrared spectra were obtained using a Beckman 4260 grating spectrophotometer. Samples were run as solid solutions in potassium bromide.

Nuclear magnetic resonance spectra were recorded using a Varian CFT 20 Fourier transform spectrometer with a pulse width of 8 μ sec for ^{13}C and 10 μ sec for ^1H respectively. Samples were run in D_2O at concentrations of 40 mg/mL for ^{13}C and ^1H .

High pressure liquid chromatography (HPLC) was performed using a Waters[®] HPLC system using the procedure of Benschop *et al.* (1981).

Thin layer chromatography was carried on by the method of Waysbort *et al.* (1981), on aluminum-backed cellulose plates (Merck 5552) using 40:60::1 m HCl:ethanol as solvent and visualization by both Rhodamine B (Stahl, 1969) or iodoplatinic acid (Stahl, 1969).

Toxicology and Efficacy Studies

Male mice (CD-1[®], 25 - 30 g) obtained from Canadian Breeding Farm and Laboratories Ltd., St. Constant, Quebec, Canada were used in this study. The animals were acclimatized for at least one week in our animal facilities prior to use. The mice had access to food and water *ad libitum* before and after drug administration. The 24 hr LD_{50} and ED_{50} values and potency ratios were calculated by probit analysis, using the procedure of Finney (1978). All drugs were injected in a volume of 1% of body weight.

In the ED₅₀ studies, the animals were injected with atropine (17.4 mg/kg) + HI-6 i.p., in the same 0.9% saline solution, 5 min prior to receiving sarin (510 µg/kg; s.c.). In the LD₅₀ studies, HI-6 was injected i.p. in 0.9% saline.

Sources of HI-6

Samples of HI-6 were solicited and received from the following individuals: Dr. H. Edery, Israel; Dr. L. Leadbeater, Great Britain; Dr. B. Bošković, Yugoslavia and Dr. H. Benschop, The Netherlands. Upon receipt the samples were stored desicated in the cold (-20°C).

Four Canadian samples of HI-6 were used in this study. All these were prepared by the method of Schoene (1967). DRES-20 was prepared in our laboratories and was recrystallized four times from ethanol/water. DRES-30, -31, -32 were all samples prepared commercially for DRES. DRES-32 was a 10 kg batch of HI-6 that was prepared commercially for DRES and had been recrystallized three times from ethanol/water. DRES-30 was a sample from a commercial preparation which suffered some decomposition during its processing and was recrystallized four times in an attempt to remove all impurities that might be present. DRES-31 was obtained from the same crude product as DRES-30 but it had been recrystallized in a small batch rather than a large one.

Dr. Edery reported that the Israeli sample contained an insoluble impurity and this was confirmed. The insoluble material was not identified.

RESULTS

Chemistry

In appearance samples DRES-20, -30 and -32 were identical, consisting of white chunky crystals. DRES-31 was composed of white needles. The Dutch sample was composed of off-white chunky crystals, while the Yugoslavian sample consisted of yellow chunky crystals. The sample from the UK was a yellow-brown powder. The Israeli sample was an off-white amorphous powder.

All samples melted with decomposition in the range 132 - 138.5°C except for the Israeli sample which melted/decomposed at 174 - 175°C (Table 2). This result identified the sample as anhydrous HI-6 as reported by Binenfeld *et al.* (1978). This fact

was confirmed by infrared spectroscopy since all samples in the study had identical infrared spectra except for the Israeli sample which had an infrared spectrum identical to that reported by Binenfeld *et al.* (1978) and to an authentic sample of anhydrous HI-6 prepared in our laboratories. Thus, all samples of HI-6 in this study were HI-6 monohydrate except for the Israeli sample.

Thin layer chromatography of all the samples gave a spot for HI-6 in the range R_f 0.462 – 0.469 (Table 2). Only the UK sample showed a second spot when the plates were visualized with rhodamine B. Further analysis by tlc confirmed that this second spot in the UK sample was not due to any of the following compounds, isonicotinamide, pyridine 2-aldoxime, 1,1'-[oxybis(methylene)] bis(2-[(hydroxyimino)methyl]pyridinium dichloride or 1,1'-[oxybis(methylene)] bis(4-aminocarbonyl)pyridinium dichloride.

High pressure liquid chromatography showed no significant impurities in any of the DRES samples or the Israeli sample. Tiny traces of an impurity with a retention time slightly longer than HI-6 were seen in the Dutch and Yugoslavian samples. The sample from the UK contained small amounts of three different materials. None of the impurities were identified.

Nuclear magnetic resonance spectra (both ^1H and ^{13}C) of all samples except the UK sample were identical. The ^{13}C spectrum of the UK sample differed from the other ^{13}C spectra in that the bases of all peaks were considerably broadened and noisy. There were, however, no discrete peaks that could be assigned to impurities. The ^1H spectrum of the UK sample showed similar changes when compared with the ^1H spectra of the other samples. The ^1H spectrum has been previously reported by Binenfeld *et al.* (1978) and the ^{13}C spectrum has been previously reported by Waysbort *et al.* (1981).

Toxicology

Table 3 lists the LD_{50} values of the various samples of HI-6 in non-fasted CD-1 mice. The various samples of DRES HI-6 and Netherlands HI-6 did not differ significantly in toxicity, however they were all significantly different (at least $p < 0.05$) from the Yugoslavian and Great Britain HI-6 LD_{50} values. Not enough HI-6 was available to estimate the toxicity of the Israeli HI-6.

In Table 4 the effect of sex and size of mouse on the HI-6 LD_{50} value are shown. The smaller mice (22 g) tended to have a higher LD_{50} value, however when examined

using the potency ratio, the values were not significantly different. Female mice had a significantly ($p < 0.001$) lower LD_{50} value than male mice in the same weight range but was not significantly different from that in larger, older male mice.

Efficacy

Efficacy of the various HI-6 samples in protecting mice against the lethal effects of sarin is presented in Table 5. Sarin was used as the challenge agent since it was found that this was the agent HI-6 was most effective against (Clement, 1983). In addition, since HI-6 ED_{50} vs sarin was small, we could conserve on the amount of HI-6 used in estimating the ED_{50} values.

The ED_{50} values of the four DRES samples (DRES-20, -30, -31 and -32) were not significantly different from one another and were not significantly different from the samples obtained from Israel, Yugoslavia or The Netherlands. The DRES-32 sample proved to be the most efficacious with the Great Britain sample being $6 \times$ less effective than DRES-20 and DRES-32. The Great Britain HI-6 ED_{50} value was significantly different ($0.01 > p < 0.001$) from all other ED_{50} values when examined using the potency ratio test.

DISCUSSION

The results of this study indicate that HI-6 from various sources (Israel, Yugoslavia, The Netherlands and Canada) is very similar in most respects with regards to its chemical properties and its toxicology and efficacy. Only the HI-6 sample from Great Britain was significantly more toxic and considerably less efficacious than HI-6 from other sources.

The variation in the toxicity of HI-6 (as reported in Table 1) is most likely due to strain differences in mice used in toxicity evaluations rather than significant differences in the purity of the material. Due to the marked difference between the efficacy of the sample of HI-6 from Great Britain and that from Canada caution should be exercised in direct comparison of efficacy data. Utilizing the HI-6 from Great Britain, the relative effectiveness of HI-6, compared to other oximes, as an organophosphate poisoning would not probably be lower than that found using an alternate source of HI-6.

Finally, the results demonstrate that the chemical purity, toxicology and efficacy of HI-6 prepared as small laboratory batch compared to the HI-6 prepared commercially were not significantly different.

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Table 1
Toxicity of HI-6 in Mice

LD ₅₀ (mg/kg; i.p.)	Strain	Sex	Reference
390	Albino	M	Bošković, 1979
295	CC57	F	Kepner and Wolthuis, 1978
224	Albino	M	Wilhelm <i>et al.</i> , 1979
660	CD-1®	M	Clement, 1982
550	CD-1®	M	Clement, 1982
514	ALAS	M	Clement, 1981; Clement and Lockwood, 1982
588	CD-1®	M	Clement, 1983
723	Albino	M	Maksimović <i>et al.</i> , 1980

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TABLE 2
A COMPARISON OF VARIOUS SAMPLES OF HI-6*

Test	Sample							
	DRES-20	DRES-30	DRES-31	DRES-32	UK	Holland	Israel	Yugoslavia
Appearance	white, chunky crystals	white, chunky crystals	white needles	white, chunky crystals	brown powder	slightly yellow chunky crystals	off-white powder insoluble impurity	yellow chunky crystals
Melting point (°C)	134.5 - 135	132.5 - 134	132 - 133.5	135 - 136	132 - 134	137 - 138.5	174 - 175	134 - 134.5
TLC (RF)	0.469	0.469	0.469	0.469	0.469 (0.601)	0.462	0.462	0.469
Infrared spectrum	monohydrate	monohydrate	monohydrate	monohydrate	monohydrate	monohydrate	anhydrous	monohydrate
HPLC	no impurity	not done	no impurity	no impurity	3 impurity peaks	trace impurity at longer retention time than HI-6	no impurity	trace impurity at longer retention time than HI-6
NMR 'H	as reported	as reported	as reported	as reported	peaks broadened and noisy	as reported	as reported	as reported
NMR '3C	as reported	as reported	as reported	as reported	peaks broadened and noisy	as reported	as reported	as reported
LD ₅₀ (mg/kg)	588 (546 - 632)	577 (555 - 608)	—	561 (536 - 589)	495 (468 - 528)	563 (535 - 592)	—	653 (605 - 688)
ED ₅₀ vs GB (mg/kg)	0.99 (0.43 - 1.66)	1.53 (0.93 - 2.63)	1.72 (0.93 - 2.94)	0.93 (0.45 - 1.48)	6.2 (3.3 - 9.4)	1.36 (0.93 - 1.73)	1.09 (0.55 - 1.78)	1.33 (0.49 - 1.81)

* Summary Table only, for more details see text and other tables.

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Table 3
LD₅₀ Value of Various Samples of HI-6*

HI-6 Sample	LD ₅₀ (mg/kg)	95% Limits
Great Britain	495	(468 - 528)
Yugoslavia	653	(605 - 688)
Netherlands	563	(535 - 592)
DRES-30	577	(555 - 608)
DRES-20	588	(546 - 632)
DRES-32	561	(536 - 589)

* Oxime injected i.p. in 0.9% saline. Volume of injection was 1% of body weight.

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Table 4
Effect of Weight and Sex on the Toxicity of HI-6 (DRES-32)

Average Body Weight (gm)	Sex	LD ₅₀ (mg/kg; 95% limits)
22	M	619 (586 - 660)
30	M	561 (536 - 589)
35	M	578 (544 - 616)
22	F	548 (530 - 565)*

* Significantly different ($p < 0.001$) from male mice weighing 22 g.

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Table 5
ED₅₀ of Various Samples of HI-6
versus Sarin Poisoning in Mice

HI-6 Sample	ED ₅₀ (mg/kg)	95% Limits
Israel	1.09	0.55 - 1.78
Great Britain	6.2	3.3 - 9.4
Yugoslavia	1.33	0.49 - 1.81
Netherlands	1.38	0.93 - 1.73
DRES-31	1.72	0.93 - 2.94
DRES-30	1.53	0.93 - 2.63
DRES-20	0.99	0.43 - 1.66
DRES-32	0.93	0.45 - 1.48

Male non-fasted CD-1 mice (25 - 30 g) were injected i.p. with HI-6 + atropine (17.4 mg/kg) 5 min before receiving sarin (510 µg/kg, s.c.). Mortality was assessed 24 hr after sarin administration.

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13. ABSTRACT		
<p>A comparison of the chemical purity, toxicology and efficacy of HI-6 ([[[4-(aminocarbonyl)pyridino]methoxy]methyl]-2-[(hydroxyimino)methyl]-pyridinium dichloride) obtained from various sources was performed. There were no significant differences between HI-6 obtained from either Israel, Yugoslavia, The Netherlands or Canada regarding their efficacy, when combined with atropine, as an antidote of organophosphate poisoning. HI-6 obtained from Great Britain was significantly more toxic and less efficacious than any of the other HI-6 samples. In addition the results of this study showed that there was no significant difference between HI-6 prepared as a laboratory batch and HI-6 prepared commercially with regards to chemical purity, toxicology or efficacy.</p>		

KEY WORDS

HI-6 ([[[4-(aminocarbonyl)pyridino]methoxy]methyl]-2-[(hydroxyimino)methyl]-pyridinium dichloride)

Oxime

Analysis

Sarin

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