Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 4, pp. 781–788, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0151-7

Efficacy of HI-6 and HLö-7 in Preventing Incapacitation Following Nerve Agent Poisoning

BERT P. C. MELCHERS,[1] INGRID H. C. H. M. PHILIPPENS AND OTTO L. WOLTHUIS

Department of Experimental Pharmacology, TNO-Pharma, P.O. Box 5815, 2280 HV Rijswijk, The Netherlands

Received 11 January 1994

MELCHERS, B. P. C., I. H. C. H. M. PHILIPPENS AND O. L. WOLTHUIS. *Efficacy of HI-6 and HLö-7 in preventing incapacitation following nerve agent poisoning.* PHARMACOL BIOCHEM BEHAV **49**(4) 781–788, 1994.—The therapeutic efficacy of the oximes HI-6 and HLö-7 (132.5 μmol/kg), in combination with atropine, in soman- or tabun-intoxicated guinea pigs was compared, particularly with respect to recovery of shuttlebox performance and electroencephalograms (EEGs). After 1.5 × LD_{50} soman SC, therapy with HI-6 or HLö-7 resulted in survival of 87.5% of the animals in each group. In both groups postintoxication performance decrements and EEG abnormalities lasted approximately 2 weeks after intoxication. After 3 × LD_{50} soman all HLö-7-treated animals died within 5 h; 70% of the HI-6-treated animals were still alive after 8 h; however, only 10% survived more than 24 h. After 2 × LD_{50} tabun 36% of the HI-6-treated animals died; HLö-7 prevented lethality and led to faster recovery of performance and EEG than after HI-6. Even after 7.5 × LD_{50} tabun, followed by HLö-7, full recovery was reached within 1 week in the surviving animals (82%). In soman-intoxicated guinea pigs HI-6 is therapeutically slightly more effective than HLö-7. HLö-7 is far more effective, under similar conditions, against tabun intoxication than HI-6.

Soman Tabun Behavioral performance EEG Postintoxication incapacitation HI-6 HLö-7

THE standard therapy against an intoxication with acetylcholinesterase (AChE) inhibitors of the organophosphate (OP) type, including insecticides and nerve agents, consists of a combination of the cholinolytic atropine and an oxime. Such a therapeutic regime may be life saving, even in primates after poisoning with the highly toxic nerve gas soman (6,18). However, oxime therapy does not antagonize a severe postintoxication incapacitation: in spite of treatment, OP-poisoned subjects may be semicomatose, disoriented, and extremely confused (18). Obviously, such a severely incapacitated victim will have a small chance of survival under combat conditions. Hence, it is of importance to determine to what extent a neuropsychological incapacitation occurs following a severe intoxication with nerve agents and whether it is possible to prevent this incapacitation.

The oxime HI-6 is considered to be a very potent therapeutic agent against OP poisoning. It is not only effective in rodents (8) but also in primates, such as rhesus (6) and marmoset (18) monkeys. HI-6 is believed to be less effective against tabun intoxication, although the results of a study in rhesus monkeys intoxicated with 5 × LD_{50} tabun (6) suggest that this notion may not be true. HLö-7 is a more recently synthesized oxime. It is quite effective against several OP inhibitors (1,4,11), including soman and tabun. Because of its broader antidotal spectrum, HLö-7 may be superior to HI-6 (1,4,11).

The objective of this study is to compare the therapeutic efficacy of HI-6 and HLö-7 after intoxication with soman or tabun. In particular, these experiments are focused on the efficacy whereby these oximes, supplemented by atropine, counteract the performance deficits of a learned task, as well as the electroencephalographic (EEG) changes that follow intoxication with these nerve agents. The choice of the animal species is a factor to be considered when testing therapeutic regimes against OP intoxication. It was decided to test these effects in guinea pigs for the following reasons: blood of humans (and monkeys) contains no OP-scavenging carboxylesterases. Guinea pig blood contains only low concentrations of these enzymes and, as a result, guinea pigs have lower LD_{50}

[1] To whom requests for reprints should be addressed.

values for OPs than rats and mice, which have high blood concentrations of carboxylesterases (12). Responsiveness to carbamate pretreatment in guinea pigs is also more similar to that in monkeys than responsiveness in rats (10). Thus, the guinea pig appears to be a better model for man than the rat or the mouse. The use of guinea pigs in behavioral studies has become more attractive because we have recently succeeded in developing a technique to reliably measure behavioral performance in this species (16).

METHOD

Guinea Pigs

Dunkin-Hartley albino guinea pigs [CrL : (HA)BR, Charles River] with a starting weight of 350–450 g were used. Within that range, the tabun-treated animals had a lower average starting weight than the soman-treated animals. During the experiments the animals were housed in Makrolon® cages, one animal per cage. Ambient temperature was kept at 20–22°C. Relative humidity was not regulated but was monitored and always exceeded 50%. Food and water were available ad lib.

Injections

Multiples of the SC LD_{50} of soman (1.5–10) (LD_{50} = 24.7 μg/kg) or tabun (2–10) (LD_{50} = 115 μg/kg) were injected, followed after 1 min by separate IM injections of atropine sulphate (15 mg/kg) and either HI-6 or HLö-7. To compare the efficacy of the oximes, an equimolar dose of 132.5 μmol/kg was administered.

Procedures

EEG electrodes were fixed onto the skull and 2–3 days later shuttlebox training was started, following the same technique that was used before [see (16)]. After 7 days most animals (generally >90%) had reached their training criterion of at least 80% correct avoidance reactions (CARs). Following the last training session on day 7, control EEGs and respiratory frequency were registered. Body weights were recorded just before and on several days after the animals were injected with agent, atropine, and either HI-6 or HLö-7. The animals were closely observed on the day of injection and regularly during the days that followed, and overt signs, such as convulsions, tremors, dyspnoea, and salivation, were noted. One day and 1 week following injection the respiratory frequency was recorded. When the overt signs had disappeared, behavioral testing for incapacitation and EEG recordings was started. Finally, at the end of the experiment, the EEG was registered again.

The actual events in the two main experiments with 1.5 × LD_{50} soman SC or 2 × LD_{50} tabun SC are shown in Fig. 1. In these two experiments all parameters were measured in each animal. For reasons mentioned in the Discussion section, no control groups (i.e., only atropine and oxime but no OP intoxication) were added. The experiment with soman (1.5 × LD_{50}) consisted of two groups of eight animals. One group was treated with HI-6 and atropine; the other group received Hlö-7 and atropine. After soman intoxication the signs and symptoms lasted longer than after tabun and, therefore, the observation period after soman lasted longer. Behavioral testing and EEG recording took place when the animals seemed fully recovered upon close observation. It was intended to carry out similar measurements after intoxication with 3 × LD_{50} soman in two groups of 10 animals each. However, almost all animals died fairly rapidly and testing was omitted.

In the experiments with 2 × LD_{50} tabun the same procedures were followed; one group (n = 11) was treated with HI-6 and atropine, the other (n = 11) with HLö-7 and atropine. Although some HI-6-treated animals died (see the Results section), by close observation surviving animals seemed to have recovered on day 3 after intoxication and testing was resumed. Because in this experiment several of the HI-6-treated animals died, further testing of HI-6 against higher tabun doses made no sense. However, because none of the animals treated with HLö-7 died, we attempted to obtain an impression of the therapeutic quality of this oxime by carrying out two additional experiments, along the same procedures, in which the animals were intoxicated with 7.5 × LD_{50} (n = 11) or 10 × LD_{50} tabun (n = 8).

EEG Registrations

Under halothane-N_2O anaesthesia, a small hole was drilled into the skull, 3 mm lateral to the sutura sagitalis and 8.5 mm caudal from the sutura fronto-parietalis. The dura mater was left intact. A silver electrode was fitted into the hole with dental cement and a reference electrode—connected to earth—was fixed over the nasal cavity.

For the EEG recordings the animals were immobilized, with their hind paws resting on a solid floor, in a vertically mounted plastic tube (diam. 7 cm, length 16.5 cm). Their heads were situated just within the tube. This appeared comfortable, because the animals did enter the tube freely without struggling upon repeated testing. EEG signals were amplified (50,000×), filtered (between 0.1–30 Hz), and fed into the analog-digital converter of an IBM-compatible PC; sampling frequency was 50 Hz. Fast Fourier Transformation (FFT) was performed, on line, on five randomly chosen epochs of each 10 s out of the total recording period of 5 min, to obtain EEG power spectra. These EEG power spectra were obtained before the injections (control), a second time as soon as the overt signs had disappeared, and a third time at the end of the experiment.

Behavioral Performance

Behavioral performance of the animals was assessed essentially as described previously (16). In short, in an automated shuttlebox, consisting of two equal compartments of 23 × 23 × 23 cm, connected by a photocell-guarded gate, the animals had to learn to avoid a stream of air (about 6 l/s, air tube diameter 1 cm) aimed at their fur, within 10 s after a tone was presented. The animals received 20 trials per day at an interval between trials of 25 s (±20% random).

Respiratory Frequency

The respiratory frequency was measured in the home cage of each animal by a capacitance technique, similar to the one used before to detect respiration in an open field test (20): the Makrolon® home cage was placed on a metal plate and a capacitor was formed by connecting this metal plate and the standard metal grid cover of the cage to an electronic device that transformed the capacitance changes (due to the movements of the animal) into an analog signal. Once the animal had settled down and the capacitance was not disturbed by other than respiratory movements, registration started. Recording time was 5 min and, as for the EEG, five periods of 10 s were selected. The average respiratory frequency was obtained using FFT and was expressed as respiratory cycles/min (resp.c/min). The validity of this technique was checked

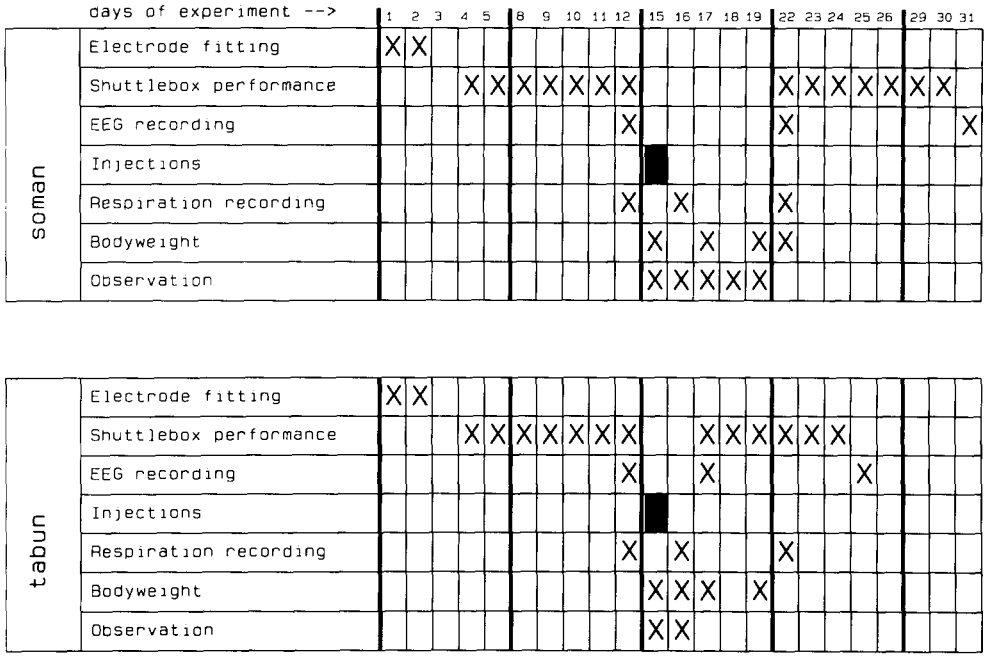

FIG. 1. Schematic overview of the sequence of events and measurements. The "X" signifies on that particular day of the experiment a certain action took place. The thick vertical lines represent intervals of 2 days (weekend). The black square represents the day on which the injections were administered.

by counting the respiratory frequency during observation of the animals.

Statistics

The multiple *t*-test according to Welch (14), including the Bonferroni correction for multiple comparisons, was used to test whether differences were significant. Significance is defined as $p < 0.05$, tested two-tailed. Variability within treatment groups was always calculated as SEM and is shown as vertical bars in the figures.

Compounds

Soman (*O*-pinacolyl methylphosphonofluoridate) and tabun (*O*-ethyl-*N*,*N*,-dimethyl phosphoramidocyanidate) were synthesized by Dr. H. P. Benschop of the Prins Maurits Lab., TNO Rijswijk. HI-6 {1-[[4-(aminocarbonyl)-pyridinio]methoxymethyl]-2-[(hydroxyimino)-methyl]pyridinium dichloride} was provided by Dr. J. G. Clement (Defence Research Establishement Suffield, Canada) and HLö-7 {1-[[4-(aminocarbonyl)-pyridinio]methyl]-2,4-bis[(hydroxyimino)methyl]pyridinium dimethanesulfonate} was a gift of Prof. Dr. Eyer (Ludwig-Maximillians-University of Munich).

RESULTS

Efficacy of HI-6 and HLö7 After 1.5 × LD_{50} Soman

General. Shortly after the injections of 1.5 × LD_{50} soman and the therapeutic drugs, the animals developed the usual signs of OP intoxication. There were no gross differences in the signs of intoxication between animals treated with HI-6 or HLö-7. Initially, in the HI-6 group eight out of eight and in the HLö-7 group six out of eight animals showed heavy chewing and salivation. Three animals in the HI-6 group and one animal in the HLö-7 group suffered from loss of posture, and all animals suffered from dyspnoea. Two animals in each treatment group developed heavy convulsions and in each group one animal died, leaving seven animals in each treatment group. Convulsions were only observed during the first day. Although the other symptoms of the survivors decreased in severity, they remained present for several days, which was in clear contrast with the effects seen after tabun intoxication (see below). Compared with preinjection values, average body weights in the HI-6 treatment group remained unchanged (541 ± 9 g, some gained, some lost a little weight). However, on average the animals did not gain weight during the first few days after intoxication. Thereafter, the weight gains appeared to be normal (i.e., about 5–10 g per day). The average body weight of all the animals treated with HLö-7 decreased significantly by 11% (from 578 ± 9 g before and 514 ± 11 g after 2 days) during the first 2 days after intoxication; thereafter their body weights increased, following the normal growth curve for guinea pigs in our laboratory. On day 7 after intoxication, their averaged body weights did not differ significantly from those of the HI-6 treatment group. It is not known whether the difference in weight loss was due to differences in food intake or defecation patterns (diarrhoea); these parameters were not noted. However, late during the day of intoxication some animals of either treatment group made attempts to drink and to chew on food pellets. The day after intoxication all animals were drinking and consumed some food pellets.

Compared to preinjection values, a significant increase in the average respiratory frequency of 29% was measured 24 h after HI-6 treatment (from 103 ± 6.1 to 133 ± 8.4 resp.c/min), whereas 24 h after HLö-7 treatment a significant decrease by 20% was found (from 100 ± 5.4 to 80 ± 4.0 resp.c/min). One week after intoxication respiratory fre-

quency in both treatment groups had returned to values that did not differ from those obtained before injection.

Shuttlebox performance. The performance curves during training and testing of guinea pigs in the shuttlebox are shown in Fig. 2. The animals reached criterion after 7 days of training. After the injections of soman, atropine and HI-6 or HLö-7, the animals exhibited signs that lasted at least 5 days and made behavioral testing impossible. When performance testing was resumed 7 days after intoxication, both groups showed a statistically significant performance decrement from which they recovered rapidly in the days that followed. There were no significant differences between the performance deficits following HI-6 or HLö-7. Although in this study a group injected with only saline was not added, we have sufficient evidence showing that an interval of 1 week should not cause any drop in performance. Hence, the drop in performance in Fig. 2 is real and signifies that the animals of both groups were still incapacitated 1 week after intoxication with soman. Compartment changes during the intertrial intervals, usually taken as a parameter of spontaneous activity (in rats), are extremely variable in guinea pigs and do not provide useful data. When failing to avoid, all animals escaped rapidly when the air stream turned on, indicating that the absence of the avoidance response was not due to motor inability.

Effects on the EEG. The EEG findings in guinea pigs intoxicated with soman are shown in Fig. 3A-C. One week after the intoxication with 1.5 × LD_{50} soman and subsequent oxime treatment, a significant increase in power at the δ frequency band around 2 Hz was found (Fig. 3B). At that time, the overt signs of intoxication had disappeared. However, there were no significant differences between the groups treated with HI-6 or HLö-7. At the end of the experiment, 16 days after the injections, the power peak around 2 Hz had disappeared and only a slight and insignificant overall increase in power persisted in both treatment groups.

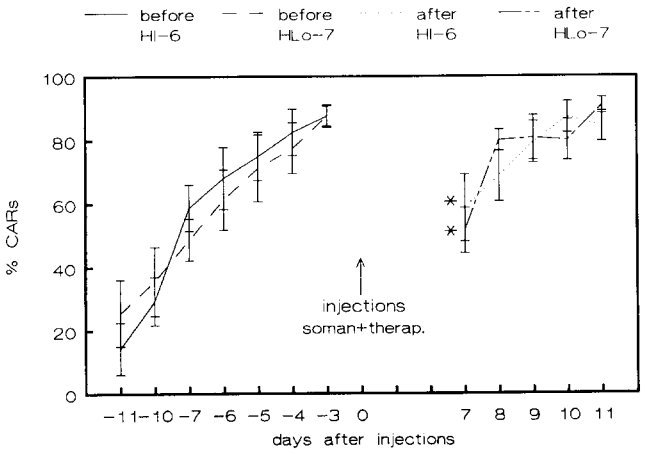

FIG. 2. The effects on performance (mean ± SEM) of guinea pigs of a shuttlebox task after 1.5 × LD_{50} SC soman, followed after 1 min by the IM injections with atropine (15 mg/kg) and either HI-6 or HLö-7 (in both cases 132.5 μmol/kg). The level of performance was assessed by the percentage of correct avoidance responses (CARs). The animals were first trained and then injected. With both treatment regimes a similar and significant (*) performance deficit was found 7 days after the injections. At that time, the overt symptoms had disappeared. Recovery was rapid in both treatment groups (each group n = 7).

Efficacy of HI-6 and Hlö7 Against 3 × LD_{50} Soman

In a separate experiment, animals were fitted with EEG electrodes, trained in the shuttlebox, intoxicated with 3 × LD_{50} soman, and injected IM after 1 min with atropine sulphate (15 mg/kg) plus either HI-6 (n = 10) or HLö-7 (n = 10), both oximes in a dose of 132.5 μmol/kg. The efficacy of this treatment was only marginal: one HI-6-treated animal and none of the HLö-7-treated animals survived. However, survival time in the group treated with HI-6 was significantly longer: all animals in the group treated with HLö-7 died within 5 h, whereas 8 h after intoxication 7 out of the 10 animals in the HI-6 treatment group were still alive.

Efficacy of HI-6 and HLö7 Against 2 × LD_{50} Tabun

General. The signs of intoxication following 2 × LD_{50} tabun and therapy lasted shorter but were more pronounced than after therapy following 1.5 × LD_{50} soman. When treated with either HI-6 or HLö-7 after the tabun intoxication, 9 out of the 11 animals in each treatment group developed violent convulsions and tremors, and most animals suffered from dyspnoea. Loss of posture was observed in two animals in the HI-6 treatment group and in none of the animals in the HLö-7 treatment group. Although gross differences in the signs of intoxication between the two groups were not detected, the signs seemed somewhat more pronounced in the HI-6 treatment group, an observation that corresponds with the fact that 4 out of the 11 HI-6-treated animals died. Two of these animals died during the night following intoxication, one died the following day, and one 2 days after intoxication. In contrast, in the HLö-7 treatment group none of the 11 animals died. As mentioned, the signs of intoxication disappeared in 2 days and testing could be resumed (see Fig. 1).

In contrast with the effects after soman, the guinea pigs intoxicated with tabun showed a significant loss in body weight after treatment with both oximes of about 11% on day 1 after intoxication: in the HLö-7 group the body weights reduced from 483 ± 9 to 428 ± 9 g; in the HI-6 group they reduced from 479 ± 10 to 426 ± 10 g. Thereafter, a quick recovery of body weight occurred in the HLö-7 treatment group: their average body weight had returned to the initial value after 4 days. Thereafter, they grew at the usual speed. This recovery was much slower in the HI-6 treatment group, in which it took about 1 week to reach preinjection body weight levels. It was remarkable to note that, except for the four HI-6-treated animals that ultimately died, all animals started to drink and eat several hours after intoxication.

The results of the measurements of respiratory frequencies were also different from the findings after soman intoxication. The only significant effect on this respiratory parameter was a 24% depression of the average respiratory frequency 24 h after HI-6 treatment. Respiration frequency was also slightly lowered after tabun intoxication followed by HLö-7 treatment, but this decrease was not statistically significant. When measured 1 week after intoxication, respiration frequencies in both treatment groups did not differ significantly from preinjection values.

Shuttlebox performance. The faster recovery of the animals made testing in the shuttlebox possible 2 days after intoxication with tabun and therapy with atropine and HLö-7 or HI-6. Performance of the shuttlebox task was hardly affected in the animals that were treated with HLö-7 and was significantly better than in the treatment group that received HI-6 (Fig. 4). However, in this case the performance deficit after HI-6 treatment recovered rapidly in the days that followed.

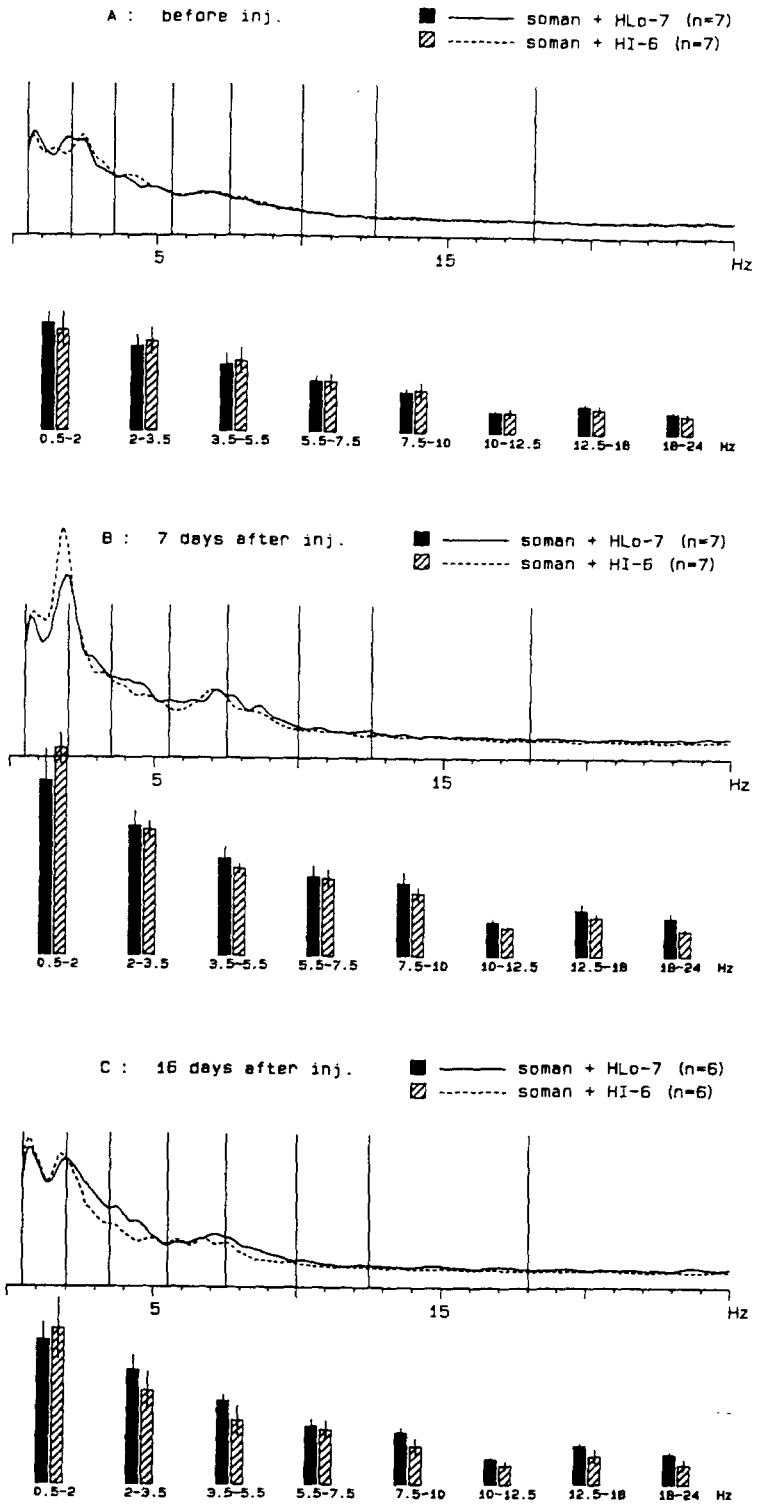

FIG. 3. (A–C) Power spectra of EEGs obtained 1 day before the injections of soman, atropine, and oximes (A), 7 days after the injections when the signs of intoxication had disappeared (B), and 16 days after the injections at the end of the experiment (C). It can be seen that the large power peaks at frequencies around 2 Hz, which were observed 7 days after the injections, have disappeared after 16 days. There were no significant differences between the effects of the HI-6 and HLö-7 treatments.

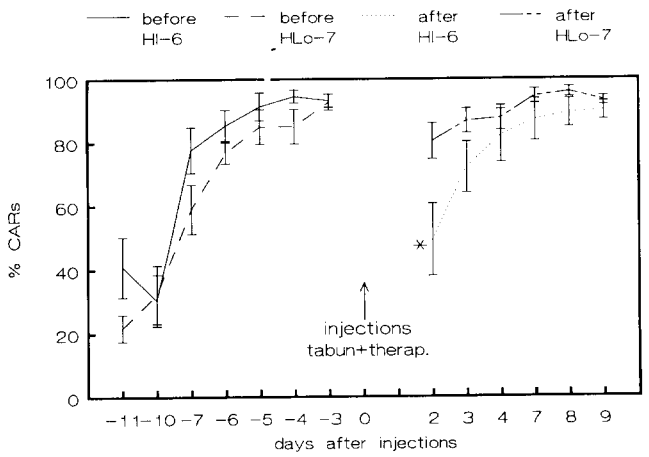

FIG. 4. The effects on shuttlebox performance (mean ± SEM) of guinea pigs of 2 × LD_{50} SC tabun, followed after 1 min by IM injections with atropine (15 mg/kg) and either HI-6 or HLö-7 (both 132.5 μg/kg). Compare with Fig. 2. Differences with the results after soman are that after tabun the signs of intoxication disappear in 2 days and, upon resuming behavioral testing, that after HI-6 treatment group a significant performance deficit was found that was not observed in the HLö-7 treatment group (n = 7 in HI-6 and n = 11 in HLö-7 treatment group).

Effects on the EEG. As in the experiments with soman, the EEG measurements in guinea pigs after the oxime treatment of the tabun intoxication showed clear power peaks in the δ frequency band (Fig. 5A–C). The average power in this peak, around 2 Hz, in the HLö-7 treatment group was significantly larger than in the HI-6 treatment group. Overall power of the EEG at the lower frequencies was reduced, in contrast with the effects found after soman intoxication. However, it should be noted that, at variance with the experiments with soman, in this case EEG recordings were already performed 2 days after tabun intoxication, because the overt signs of intoxication had disappeared faster. The reduction in power in the lower EEG frequencies at this point in time was more pronounced in the HI-6 treatment group and differed significantly from the effect found in the HLö-7 treatment group. At the end of the experiment (10 days after the injection), the EEGs had recovered and were indistinguishable from those obtained before the injections.

Efficacy of HLö7 Against 7.5 × or 10 × LD_{50} Tabun

Eleven trained guinea pigs, fitted with EEG electrodes, were SC injected with 7.5 × LD_{50} tabun, followed after 1 min by the previously used combination of HLö-7 and atropine. Because 4 out of 11 animals after HI-6 treatment in the previous experiment with 2 × LD_{50} had died, a HI-6 treatment group was not added. After 7.5 × LD_{50} tabun, 9 out of 11 guinea pigs treated with HLö-7 survived and regained posture after about 30 min. Their body weights returned to normal after 1 week. Two days after intoxication, respiratory frequency was normal. Performance of these animals in the shuttlebox still showed a significant deficit 2 days after intoxication, but recovery was rapid in the days that followed.

The EEGs showed a large loss of power 2 days after intoxication. As after 2 × LD_{50} tabun, the EEGs had returned to normal values again when measured 1 week after intoxication.

An additional group of eight animals was injected with 10 × LD_{50} tabun, followed after 1 min by IM injections of 132.5 μmol/kg HLö-7 and 15 mg/kg atropine sulphate. As a result, five animals died within 5 min; the other three animals regained posture after 30 min but still exhibited tremors during several days. Because of the small number of survivors, behavioral testing or EEG recordings were not carried out.

DISCUSSION

The objective of these experiments was to directly compare the efficacy of equimolar doses of the oximes HI-6 and HLö-7 with particular emphasis on their effects on performance deficits and EEG changes after intoxication. Control animals receiving only the cholinesterase inhibitors were not added because they would all die. Neither were animals added receiving only atropine and oxime. Based on earlier experiments in rats (19), such a combination may cause a short-lasting light form of incapacitation in nonintoxicated subjects, undetectable 24 h later. Moreover, the effects caused by atropine, being a competitive antagonist of ACh at muscarinic receptor sites, would be counteracted by the accumulation of ACh after OP intoxication.

The oximes were administered in conjunction with atropine, 1 min after intoxication with soman or tabun in a dose of 132.5 μmol/kg. This dose is relatively moderate compared to the oxime doses of 484 μmol/kg (Hlö-7) and 722 μmol/kg (HI-6) that were administered to soman- or tabun-intoxicated guinea pigs in an earlier comparative study of these two oximes (4). This dose was chosen to mimic more closely the situation in man, where the oxime dose that can be administered is evidently limited. However, extrapolation purely on a weight basis from the oxime dose used here to man, 132.5 μmol/kg would still amount to about 3.5 g of oxime for a person of 70 kg, supposing that the affinity of the oximes for therapeutic relevant binding sites will be similar. The present results show that even these doses do not prevent a severe postintoxication incapacitation following either of the nerve agents, although they do protect against the lethality.

Following soman intoxication (1.5 × LD_{50}) and treatment with either oxime, animals remained incapacitated for about 5 days to such an extent that behavioral testing was impossible. After this period, a significant performance deficit in the shuttlebox was still present, as well as significant aberrations in the EEG. The EEGs showed a significant increase in δ activity. Such an increase is often associated with behavioral impairment (5,17). A similar effect was found in rats 6 days after soman intoxication followed by treatment with atropine and diazepam (15). Aberrations in the δ band were also found in humans after accidental nerve agent exposure (3). However, because all symptoms appeared to be reversible, the oxime treatment appeared to have protected against the irreversible neuronal damage often found after soman intoxication (2,13): both performance levels and EEGs had recovered at 10 days after intoxication. To determine whether all neuronal damage was prevented, detailed histological (electronmicroscopical) analysis of the brain would have been necessary.

There were no statistically significant differences between the magnitude and speed of recovery of the performance deficits and EEGs in the groups treated with either HI-6 or HLö-7. HI-6 might be regarded as being slightly more effective against soman than HLö-7: after 1.5 × LD_{50} soman, HI-6 protected better against body weight loss; HI-6 prevented the decrease in respiratory frequency, and, following 3 × LD_{50} soman, survival times after HI-6 were longer. It should be emphasized that an effect on respiratory frequency, taken as a sole param-

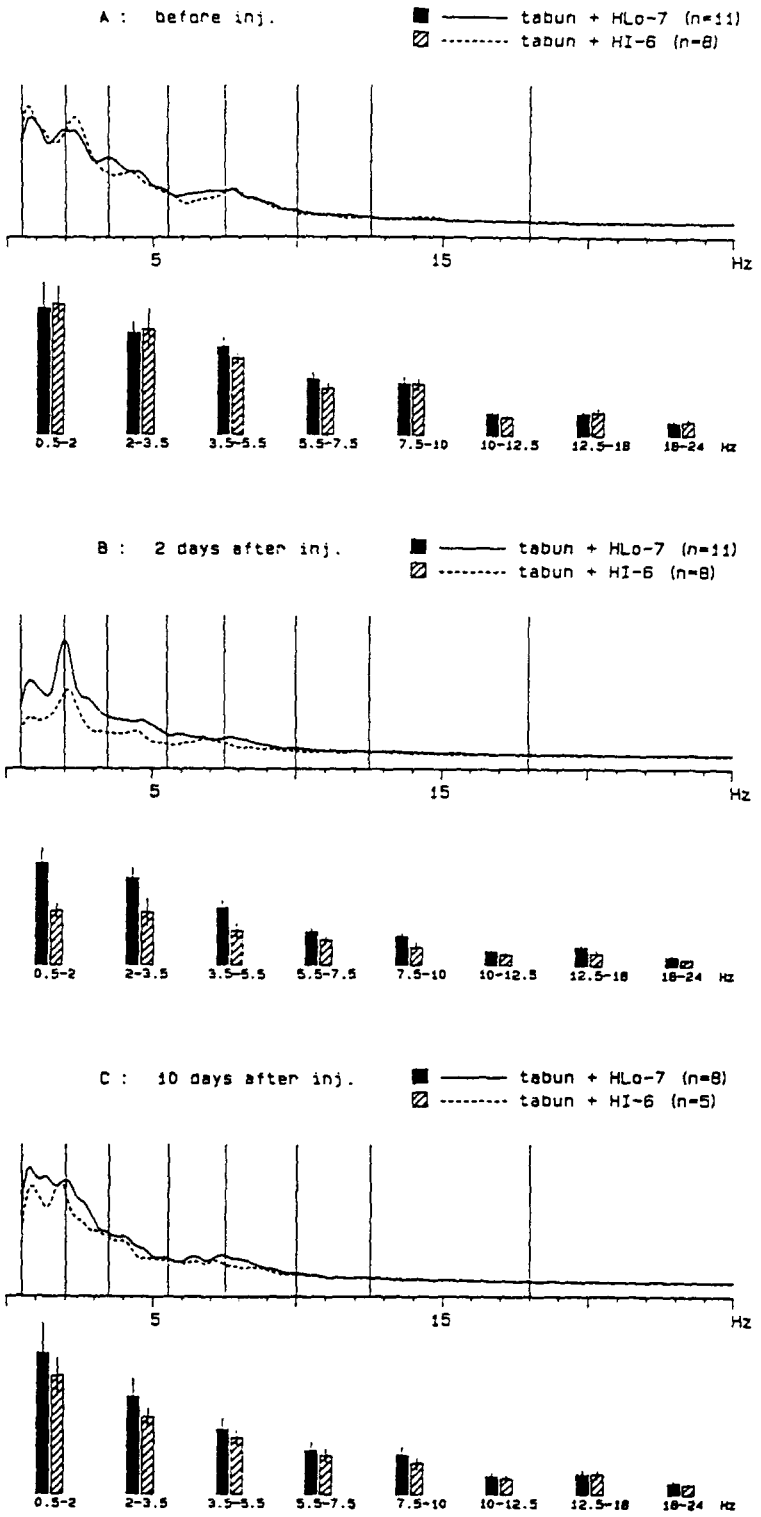

FIG. 5. (A-C) Power spectra from EEGs obtained 1 day before the injection of tabun, atropine, and oximes (A), as well as 2 days after the injections when the signs of intoxication had disappeared (B), and finally 10 days after the injections at the end of the experiment (C). Similar power peaks can be seen around 2 Hz as after soman. However, in contrast with the soman experiments, the power of the EEG at these lower frequencies is reduced. This reduction after HI-6 treatment is significantly more pronounced than after HLö-7. Ten days after the injections a complete recovery of the EEGs can be observed.

eter, does not necessarily signify that the animals have respiratory problems. In fact, a higher frequency may be accompanied by more shallow breathing and a slower frequency by deeper breathing. In both cases, the O_2/CO_2 exchange may be the same.

After intoxication with tabun ($2 \times LD_{50}$) and treatment with either HLö-7 or HI-6, a severe postintoxication incapacitation was also found. However, the animals recovered more rapidly than after soman and behavioral testing could be performed after 2 days. At that time, shuttlebox performance was still significantly disturbed in the HI-6-treated animals. The animals in the HLö-7-treated group recovered faster, and 2 days after intoxication no significant decrease of performance was found in the HLö-7-treated group. EEGs were still disturbed in both treatment groups, and there were clear power peaks found at the δ frequency band as well as an overall loss of power. The latter was more pronounced in the HI-6-treated animals. Notwithstanding the convulsions that developed in most animals following the tabun intoxication, the EEGs were recovered to normal 10 days after the injections. These convulsions may lead to permanent damage to the central nervous system (2,13).

Although both treatment groups suffered from a loss in body weight, recovery in the HLö-7 treatment group was faster. Respiratory frequencies in both treatment groups were reduced, but only the reduction in the HI-6 treatment group was large enough to reach statistical significance. In view of the significant lethality after $2 \times LD_{50}$ tabun and HI-6-treatment, it was not thought worthwhile to test the efficacy of HI-6 after intoxication with higher doses of tabun. This was different in the case of HLö-7. Even after $7.5 \times LD_{50}$ tabun, treatment with HLö-7 afforded a significant protection. Shuttlebox performance showed a significant deficit 2 days after intoxication. This recovered rapidly to normal in a few days. The EEGs were still abnormal 2 days after intoxication with $7.5 \times LD_{50}$ tabun, but were normal after 1 week.

It should be noted that these results are less favourable than the results obtained by others (4,11). The oxime dose that we used was not the optimal dose; as mentioned above, in our experiments the doses of oximes used were four to five times lower than those used in the experiments of Eyer et al. (4). This was done in an attempt to use oxime doses that might be more realistic for the application in humans. However, the findings of Eyer et al. (4), showing that higher oxime doses may give better therapeutic results, do implicate that there is still room for the development of more effective oximes.

Taken together, these results indicate that in guinea pigs HI-6 is slightly more effective against soman, whereas against tabun the oxime HLö-7 is clearly more effective. However, at the dose used here, therapy with neither oxime fully protected against the performance deficits and the EEG changes that can be measured 7 days after intoxication with $1.5 \times LD_{50}$ soman or 2 days following $2 \times LD_{50}$ tabun.

ACKNOWLEDGEMENTS

This study was supported by the German "Bundesministerium der Verteidigung." The authors wish to thank Dr. P. L. B. Bruijnzeel, Dr. H. P. M. van Helden, and Dr. R. W. Busker for their helpful comments.

REFERENCES

1. Clement, J. G.; Hansen, A. S.; Boulet, C. A. Efficacy of HLö-7 and pyrimidoxime as antidote of nerve agent poisoning in mice. Arch. Toxicol. 66:216–219; 1992.
2. De Groot, D. M. G.; Bierman, E. P. B.; Van Huijgenvoort, A. H. B. M. Involvement of acetylcholine and glutamate in soman-induced brain damage. Micron Micros. Acta 21:247–248; 1990.
3. Duffy, F. H.; Burchfiel, J. L.; Bartels, P. H.; Gaon, M.; Van Sim, M. Long-term effects of an organophosphate upon the human electroencephalogram. Toxicol. Appl. Pharmacol. 47:161–176; 1979.
4. Eyer, P.; Hagedorn, I.; Klimmek, R.; Lippstreu, P.; Löffler, M.; Oldiges, H.; Spöhrer, U.; Steidl, I.; Szinicz, L.; Worek, F. HLö-7 dimethanesulfonate, a potent bispyridinium-dioxime against anticholinesterases. Arch. Toxicol. 66:603–621; 1992.
5. Grossman, S. P. Essentials of physiological psychology. New York: John Wiley and Sons; 1973:425–427.
6. Hamilton, M. C.; Lundy, P. M. HI-6 therapy of soman and tabun poisoning in primates and rodents. Arch. Toxicol. 63:144–149; 1989.
7. Inns, R. H.; Leadbeater, L. The efficacy of bispyridinium derivatives in the treatment of organophosphate poisoning in the guinea pig. J. Pharm. Pharmacol. 35:427–433; 1983.
8. Kepner, L. A.; Wolthuis, O. L. A comparison of the oximes HS-6 and HI-6 in the therapy of soman intoxication in rodents. Eur. J. Pharmacol. 48:377–382; 1978.
9. Koplovitz, I.; Stewart, J. R.; Olson, C. T.; Dill, G.; Menton, R. Multispecies evaluation of the efficacy of HI-6, a nerve agent antidote. Proceedings of Medical Defense Bioscience Review 1991. US Army Medical Research and Development Command, August 7–8; 1991:599–601.
10. Leadbeater, L.; Inns, R. H.; Rylands, J. M. Treatment of poisoning by soman. Fundam. Appl. Toxicol. 5:S225–S231; 1985.
11. Lundy, P. M.; Hansen, A. S.; Hand, B. T.; Boulet, C. A. Comparison of several oximes against poisoning by soman, tabun and GF. Toxicology 72:99–105; 1992.
12. Maxwell, D. M.; Brecht, K. M.; O'Neill, B. L. The effect of carboxylesterase inhibition on interspecies differences in soman toxicity. Toxicol. Lett. 39:35–42; 1987.
13. McDonough, J. M.; Jaax, N. K.; Crowley, R. A.; Mays, M. Z.; Modrow, H. E. Atropine and/or diazepam therapy protects against soman-induced neural and cardiac pathology. Fundam. Appl. Toxicol. 13:256–276; 1989.
14. Natrella, G. A. Experimental statistics. National Bureau of Standards, Handbook 91 Government Printing Agency, Washington, DC; 1963.
15. Philippens, I. H. C. H. M.; Melchers, B. P. C.; De Groot, D. M. G.; Wolthuis, O. L. Behavioral performance, brain histology, and EEG sequela after immediate combined atropine/diazepam treatment of soman-intoxicated rats. Pharmacol. Biochem. Behav. 42:711–719; 1992.
16. Philippens, I. H. C. H. M.; Melchers, B. P. C.; Wolthuis, O. L. Active avoidance in guinea pigs: Effects of physostigmine and scopolamine. Pharmacol. Biochem. Behav. 42:285–289; 1992.
17. VanderWolf, C. H. Limbic-diencephalic mechanism of voluntary movement. Psychol. Rev. 78:83–113; 1973.
18. Van Helden, H. P. M.; Van der Wiel, H. J.; De Lange, J.; Busker, R. W.; Melchers, B. P. C.; Wolthuis, O. L. Therapeutic efficacy of HI-6 in soman-poisoned marmoset monkeys. Toxicol. Appl. Pharmacol. 115:50–56; 1992.
19. Wolthuis, O. L.; Philippens, I. H. C. H. M.; Vanwersch, R. A. P. Side effects of therapeutic drugs against organophosphate posioning. Neurotoxicol. Teratol. 11:221–225; 1989.
20. Wolthuis, O. L.; Vanwersch, R. A. P. Behavioral changes in the rat after low doses of cholinesterase inhibitors. Fundam. Appl. Toxicol. 4:S195–S208; 1984.