BISQUATERNARY OXIMES AS ANTIDOTES AGAINST TABUN AND SOMAN POISONING

Antidotal Efficacy in Relation to Cholinesterase Reactivation

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INTRODUCTION

Quaternary pyridinium aldoximes such as 2-PAM were first introduced by Ginsburg and Wilson (1955) as nucleophilic reactivators of organophosphoryl (OP)-inhibited AChE. During the last forty years there were several attempts to improve the reactivation and antidotal efficacy of oximes against OP poisoning, e.g., by introducing a second pyridinium oxime group as in Toxogonin (TOX) or TMB-4. These oximes displayed remarkable efficacy against poisoning by certain OP nerve agents such as sarin and tabun (Heilborn and Tolagen, 1963). However, rapid aging of soman-inhibited AChE caused considerable difficulties in the antidotal treatment of soman poisoned animals (Coult and Marsh, 1960). The Hagedorn oximes HI-6 and HL0-7 which were introduced in the seventies were the first oximes which also displayed antidotal efficacy against soman in mice and guinea pigs (Loeffler, 1976 and Schoene et al., 1973). Due to its relatively low toxicity and remarkable reactivation potency HI-6 was extensively studied and was proposed to replace the first generation oximes in the military antidotal kit. However, some contradictory data were obtained for the antidotal efficacy of HI-6 against tabun poisoning. Boskovic et al.(1984) have demonstrated limited antidotal action of HI-6 (combined with atropine and diazepam) against tabun in mice, rats and dogs. Schoene et al.(1973) and Clement (1983, 1987) showed that HI-6 is practically devoid of any antidotal efficacy against tabun in mice. In contrast, Hamilton and Lundy (1989) have demonstrated that HI-6 is an effective antidote against tabun when administered in conjunction with atropine and diazepam in rhesus monkeys. It was further noted that bispyridinium salts such as SAD-128 and HH-54 which do not have an oxime moiety in their

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molecular structure could protect against soman poisoning in mice (1976). Moreover, it was
demonstrated that restoration of diaphragm activity by HI-6 in soman poisoned rats could
not be attributed to AChE reactivation (Wolthuis et al., 1978). Therefore, alternative
mechanisms which are unrelated to AChE reactivation were proposed for the antidotal action
of oximes. Indeed, moderate antimuscarinic (Amitai et al., 1980) and antinicotinic activity
(Kuhnen-Clausen et al., 1983) was demonstrated for certain bispyridinium oximes and salts.
Based on the observed anticholinergic activity of existing oximes we have designed bis-
quaternary oximes, designated AB-oximes, in which one pyridinium ring was substituted by
a quinuclidinium moiety. Some of these AB-oximes are relatively non-toxic in rodents and
displayed high efficacy against certain OP agents in mice and guinea pigs (Amitai et al.,
1985 and 1993). The study reported here describes the antidotal efficacy of AB-8, AB-13,
TOX, HI-6 and HLo-7 against tabun and soman in beagle dogs and baboon monkeys. Prior
to the antidotal evaluation we have determined the therapeutic doses of these oximes by
monitoring their dose-dependent toxic signs in dogs and monkeys. Using TOX as a reference
oxime, a calculated unit of equivalent dose (CED) was defined so that this unit is equivalent
in terms of toxic effect for all oximes. In addition, cholinesterase (ChE) activity was
measured both in vitro and in vivo in order to ascertain the relationship between the antidotal
efficacy and ChE reactivation during recovery from OP intoxication.

MATERIALS AND METHODS

Materials

The OP agents and the AB oximes were prepared by Dr. H. Leader and B. Manistersky
of the Department of Organic Chemistry, IIBR. Tabun and soman were synthesized according
to standard procedures. AB-8 and AB-13 were prepared as described previously (Amitai et
al., 1987). HI-6, HLo-7 and toxogonin were kindly provided by Dr. Kullmann, In San I 3
MOD, Bonn, Germany.

Animals

Mice, male and female, of CD-1 strain (Charles River, UK), Dogs, male Beagles,
9-12 months old (Harlan Olac CPB, Netherlands), Monkeys, male and female papio anubis
baboons of African origin.

Methods

Determination of Calculated Equivalent Dose (CED) of Oximes in Dogs and Mon-
keys. CED was defined as a dose unit of an oxime which is equivalent in its toxic effect to
TOX. This unit is the ratio between the oxime’s MTD (minimal toxic dose) and TR
(therapeutic ratio) of TOX. The TR is the ratio between the therapeutic dose and the MTD
of TOX. Assuming that for each oxime, MTD=ED\text{10}/k, where k is a constant equal to all
oximes, it can be shown that MTD may be replaced by ED\text{10} of the tested oxime (t), i.e.,

\[ \text{CED}_t = \frac{\text{ED}_{10,t}}{\text{TR}_t} \times \frac{\text{ED}_{10,r}}{\text{TR}_r}. \]

Where TR and ED\text{10,}\text{r} are the therapeutic dose and ED\text{10} of the reference (r) oxime
TOX. Since rigorous estimation of MTD requires a huge number of animals the advantage
of its replacement by ED\text{10} is obvious. The ED\text{10} of each oxime was estimated from the
experimental data, assuming the log-logistic function for the toxicity dose response curve.