STUDIES ON THE THERAPEUTIC EFFECT OF 2-PYRIDINE ALDOXIME METHIODIDE (2-PAM) IN MAMMALS FOLLOWING ORGANOPHOSPHORUS COMPOUND (OP)-POISONING (REPORT II) : AGING OF OP-INHIBITED MAMMALIAN CHOLINESTERASE

Sachiko UEHARA, Toshihiko HIROMORI, Takashi SUZUKI*, Terushige KATO and Junshi MIYAMOTO

Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., 1–98, 3-chome Kasugade-naka, Konohana-ku, Osaka 554, Japan
*Sumika Chemical Analysis Service, Ltd., 1–135, 3-chome Kasugade-naka, Konohana-ku, Osaka 554, Japan

Accepted June 4, 1993

ABSTRACT — We studied the ability of 2-PAM to reactivate cholinesterase (ChE) inhibited by organophosphorus compounds (OPs) and aging. We estimated the reactivation rate with 2-PAM following inhibition of ChE by fenitrothion, methylparathion or ethylparathion using erythrocytes of rat and rabbit and rat brain. The period of time during which inhibited ChE could be reactivated was shorter in the case of inhibition by fenitrothion or methylparathion than in the case of inhibition by ethylparathion. This results suggest that aging is related to the presence of the alkyl group in OPs, and occurs faster in the case of inhibition by OPs with an O,O-dimethyl moiety than in the case of inhibition by OPs with an O,O-diethyl moiety.

KEY WORDS: Organophosphorus compounds, ChE inhibition, 2-PAM, Reactivation rate, Aging

INTRODUCTION

This study was undertaken to determine those factors limiting the therapeutic efficacy of 2-pyridine aldoxime methiodide (2-PAM) as treatment for poisoning due to organophosphorus compounds (OPs), and to discover a means by which the efficacy of 2-PAM can be increased in treatment of poisoning due to the so-called OPs of low toxicity, e. g., fenitrothion and malathion; 2-PAM is, reportedly, relatively ineffective in the treatment of poisoning by OPs of low toxicity (Namba et al., 1970; Walsh et al., 1979). In a previous study (Uehara et al., 1993), we established an assay for determination of 2-PAM concentration in mammalian tissues using high-performance liquid chromatography, and calculated the half-life of 2-PAM in the blood of rats and rabbits following intravenous injection (0.87 hr in rats, and 0.90 hr in rabbits). These values are almost the same as that obtained for humans (0.9 hr; Jager et al., 1958). The half-life of 2-PAM is short compared with the period of time during which ChE is inhibited as a consequence of OP poisoning. The short half-life of 2-PAM has been considered one of the major factors limiting the in vivo therapeutic efficacy of its
intravenous administration to mammals, including humans.

There is in addition another factor that modulates the efficacy of 2-PAM as a therapeutic agent, the aging of the inactivated enzyme. Aging refers to the formation of dealkylated phosphorylated ChE; once aging occurs, ChE is only minimally reactivated by 2-PAM. Aging is related to the molecular structure of the OPs, especially to the presence of the alkyl group in OPs (Eto, 1974; Gray, 1984). There are some differences in the structure of the alkyl group between low toxic OP and high toxic OP, thus we thought the occurrence of aging might be different between in low toxic OP and in high toxic OP, and that would be one of the reasons 2-PAM is thought to be ineffective against low toxic OP poisonings. Thus, we studied the relationship between the chemical structure of OPs and the degree of aging in vivo.

MATERIALS AND METHODS

1. Chemicals

Fenitrothion (purity, 96.6%) was prepared by Sumitomo Chemical Co., Ltd. (Osaka). Ethylparathion (purity, >97.0%) and methylparathion (purity, >99.0%) were obtained from Wako Pure Chemicals Co., Ltd. (Osaka). PAM inj. SUMITOMO®, a formulation containing 500 mg 2-PAM in 20 ml distilled water, was kindly supplied by Sumitomo Pharmaceutical Co., Ltd. (Osaka). Sephadex G-25 (Pharmacia LKB Biotechnology, Uppsala) and other chemicals used were obtained from commercial vendors.

2. Methods

2–1. Animals

Sprague-Dawley male rats (6 weeks of age) were purchased from Charles River Japan, Inc. (Kanagawa), and Native Japanese White male rabbits (2.0–2.3 kg body weight) from Nihon Dobutsu Co. (Osaka). The animals were kept in isolation for a period of at least 1 week, for the purpose of quarantine and acclimatization prior to commencement of the study, in a room with controlled temperature (24±2°C) and relative humidity (55±15%) and equipped with a fluorescent lighting system (12 hr light/dark cycle). The rats were permitted free access to feed, while the rabbits were placed on a diet of approximately 100 g feed/day. The animals were permitted drinking water ad libitum.

2–2. Administration

Fenitrothion, ethylparathion and methylparathion were suspended in aqueous 10% Tween 80. In the case of rats, fenitrothion (400 mg/10 ml/kg), methylparathion (3 mg/10 ml/kg) or ethylparathion (5 mg/10 ml/kg) was administered orally following a 20 hr-fasting. In the case of rabbits, fenitrothion (65 mg/ml/kg) or ethylparathion (4 mg/ml/kg) was administered subcutaneously.

2–3. Sample preparation and aging experiments

Erythrocyte (rat and rabbit) and brain (rat) samples were prepared at 3 (only rat), 6, 12, 24 and 48 hr after drug administration as follows. Immediately following sacrifice of rats by exsanguination under ether-anesthesia, the blood was collected and the brain was removed. In the case of rabbits, blood was collected from the ear vein. All the procedures to follow were undertaken at 0–4°C. A few grains of sodium heparin were added to the blood samples to prevent coagulation, and centrifugation was then carried out at 800 g for 8 min at 4°C to separate erythrocytes from plasma. The erythrocytes were washed with physiological saline and used for the experiments. The rat brain was homogenized in 0.1% Triton X-100 to obtain an 8% (W/V) homogenate using an homogenizer (Politrion®, Kinematica Inc., Switzerland). The supernatant obtained following centrifugation of the homogenate at 800 g for 10 min was used for the experiments. Physiological saline (0.5 ml) was added to 0.5 g of the erythrocyte sample or to 0.5 ml of the brain sample, and then 0.1 ml of 2-PAM solution (final concentration in the sample was 8.61×10−3 M) or physiological saline was added. Samples were then incubated for 30 min at 37°C. Following incubation, the erythrocyte sample was centrifuged and the resulting pellet was washed with physiological saline. Following incubation, the brain sample was gel-filtered to eliminate excess 2-PAM. The ChE activity of erythrocytes was measured using the end-point method; that of brain was measured using a rate assay based on the method of Ellman et al. (1961).

The reactivation rate was then calculated according to the method of Jong et al. (1984), as
follows:

\[
\text{Reactivation rate (\%) = \frac{E/I\text{A}(E/EA) - EI}{E - EI} \times 100}
\]

where 
- \(E\) = Activity of the intact enzyme
- \(EA\) = Activity of the intact enzyme incubated with 2-PAM
- \(EI\) = Activity of the inhibited enzyme
- \(E/I\text{A}\) = Activity of the reactivated enzyme with 2-PAM

For rats, erythrocyte samples and brain samples obtained from an intact rat were used for determination of \(E\) and \(EA\), while for rabbits, an erythrocyte sample obtained prior to the administration of OPs was used.

Five rats and three rabbits were used for each experiment.

Student's t-test was used for data analysis.

RESULTS

Fig. 1 shows the results of study of reactivation of rat erythrocyte ChE. The reactivation rate after administration of fenitrothion and methylparathion significantly decreased with time after 12 hr compared to the value of 3 hr and almost no reactivation was occurring at 48 hr. However, the reactivation rate was still high at 48 hr after the administration of ethylparathion.

Fig. 2 shows the results of study of reactivation of rat brain ChE. The reactivation rate following the administration of methylparathion significantly decreased with time after 6 hr compared to the value of 3 hr, and after administration of fenitrothion, the reactivation rate was significantly decreased after 24 hr compared to the value of 3 hr, and in both cases almost no reactivation of brain ChE was occurring at 48 hr. On the other hand, the reactivation rate of brain ChE at 48 hr after the administration of ethylparathion was more than 80%.

Fig. 3 shows the results of study of reactivation of rabbit erythrocyte ChE. There was no significant difference between reactivation rate of fenitrothion and that of ethylparathion 6 hr and 12 hr after the administration. However, a significant difference between fenitrothion and ethylparathion at 24 and 48 hr after administration was present, at which time the enzyme inhibited by fenitrothion was undergoing essentially no reactivation.

![Graph](image)

**Fig. 1** *In vitro* reactivation by 2-PAM of rat erythrocyte ChE inhibited by organophosphorus compounds.

*: Indicates a significant difference from the value of 3 hr (P<0.05).
Fig. 2 *In vitro* reactivation by 2-PAM of rat brain ChE inhibited by organophosphorus compounds

*: Indicates a significant difference from the value of 3 hr (P<0.05).

Fig. 3 *In vitro* reactivation by 2-PAM of rabbit erythrocyte ChE inhibited by organophosphorus compounds.

#: Indicates a significant difference from the value of ethylparathion at the same time (P<0.05).
DISCUSSION

We studied the effect of the aging of the inhibited enzyme in order to determine the therapeutic efficacy of 2-PAM in the treatment of mammals poisoned by OP.

Our results demonstrated the presence of clear differences in in vivo reactivation rates by 2-PAM between $O,O$-dimethyl OPs (fenitrothion, methylparathion) and $O,O$-diethyl OP (ethylparathion). The period of time during which inhibited ChE could be reactivated was shorter with use of fenitrothion or methylparathion than with use of ethylparathion. It is now generally accepted that aging of the phosphorylated ChE is due to dealkylation of the dialkoxyphosphonyl enzyme (Eto, 1974). Following dealkylation, the phosphorylated enzyme is stable and resists nucleophilic attack by oxime because of its negative charge. Given these findings, we suspect that aging is related to the presence of the alkyl group in OPs and occurs more rapidly in the case of inhibition by $O,O$-dimethyl OPs than in that by $O,O$-diethyl OPs.

When malathion, an $O,O$-dimethyl OP, was administered subcutaneously to hens, only 5% of the inhibited brain ChE could be reactivated at 18 hr after dosing (Witter et al., 1963). Gough et al. (1977) reported reactivation of ChE by intravenously administered 2-PAM in rabbits which had previously undergone intravenous administration of OPs. In that study, azodrin and SD-1652 were used as OPs; these are respectively an $O,O$-dimethyl OP and an $O,O$-diethyl OP. Almost no reactivation occurred 3 hr after the administration of azodrin, whereas reactivation was clearly taking place even 48 hr after the administration of SD-1652. Our results were similar though the OPs we tested were different.

Based on these results, 2-PAM should therefore be used as soon as possible after poisoning by $O,O$-dimethyl OPs such as fenitrothion, since aging takes place faster than after poisoning by $O,O$-diethyl OPs.

REFERENCES


