STUDIES ON THE THERAPEUTIC EFFECT OF 2-PYRIDINE ALDOXIME METHIODIDE (2-PAM) IN MAMMALS FOLLOWING ORGANOPHOSPHORUS COMPOUND-POISONING (REPORT III): DISTRIBUTION AND ANTIDOTAL EFFECT OF 2-PAM IN RATS

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ABSTRACT — The metabolic fate of 2-PAM and its antidotal effect on organophosphorus compound poisoning in rats were studied. When 14C-2-PAM was administered intravenously, the amount of 14C reaching the brain was small. Following administration by intramedullary injection, 14C was present in high concentrations in the brain, and 72–90% of the 14C present in the brain corresponded to the unchanged form of 2-PAM. 2-PAM was rapidly excreted into the urine and feces following either intramedullary or intravenous administration. The half-life of 2-PAM in the brain following intramedullary administration was 1.52 hr. Intramedullary administration of 2-PAM to rats poisoned with fenitrothion or malathion enabled their survival and induced reactivation of brain cholinesterase.

KEY WORDS: 2-pyridine aldoxime methiodide, Intramedullary injection, Distribution, Half-life, Antidotal study

INTRODUCTION

2-Pyridine-aldoxime methiodide (2-PAM) and atropine have been used for the therapy of organophosphorus compound (OP) poisoning as antidote and as symptomatic treatment, respectively (Namba et al., 1970; Ueda and Hiraki, 1978). 2-PAM is considered not particularly effective for treatment of poisoning due to OPs of low acute toxicity, such as fenitrothion, though it is quite efficacious in the treatment of poisoning due to OPs of high acute toxicity, such as parathion (Namba et al., 1970; Quinby et al., 1963; Walsh et al., 1979).

We have been attempting to determine why the therapeutic effect of 2-PAM differs depending upon whether poisoning is the result of highly toxic OPs or OPs of low toxicity. Preceding the present study, we undertook studies of the efficacy of 2-PAM in vitro and in vivo. We found that the half-life of 2-PAM in the blood following intravenous administration is short, and that aging of the inactivated enzyme by O, O-dimethyl OP occurs rapidly (Uehara et al., 1993 (a),(b)).

Reports have been made concerning the metabolic pathway of 2-PAM, including its excretion, blood concentration and distribution. Urin-
ary excretion of 2-PAM in humans following intravenous administration is rapid: 65% by 2 hr, and 84.5% by 24 hr after administration (Loomis, 1963; Swartz et al., 1974). Similar results were obtained for experimental animals: 72% excretion by 24 hr after intravenous administration in mouse, and 50% and 80-90% by 24 hr after oral and intramuscular administration, respectively, in rats (Kalsen, 1959; Enander et al., 1962). Studies have also been made of the distribution of 2-PAM in mice, rats and dogs following intravenous administration of 2-PAM; concentrations of 2-PAM in kidney and liver are reported to be high, but only trace amounts have been detected in the brain (Loomis, 1963; Jager et al., 1958; Firemark et al., 1964; Watanabe, 1960).

In humans, 2-PAM was not detected in cerebrospinal fluid following its intravenous administration (Jager et al., 1958). 2-PAM is therefore believed not to penetrate the blood-brain-barrier (BBB), and the permeability of brain to 2-PAM is thought to be quite small. We surmised that the intramedullary route would prove relatively more effective in delivery to the brain, since 2-PAM can reach the central nervous system directly without passing through the BBB.

No reports are available concerning the distribution and excretion of 2-PAM following intramedullary injection. We therefore studied these aspects of 2-PAM, and its effect as an antidote to OP poisoning in rats.

MATERIALS AND METHODS

1. Chemicals

2-[Pyridyl-2,6,14C] PAM: Radioactive 2-PAM was synthesized as follows. A mixture of [2,6-14C]pyridine-2-aldoxime (699.3 MBq, 127 mg) purchased from Amersham International plc. (England) and methyl iodide (1.41 g) in isopropanol (2.4 ml) was stirred under reflux for 18.5 hr. Following the addition of methyl iodide (114 mg), the mixture was refluxed for 6.5 hr and cooled. The precipitate that formed was filtered, washed twice with 0.3 ml of isopropanol each time, and dried under reduced pressure. The crude crystal (259 mg) was dissolved in 2.3 ml of 70% isopropanol with reflux, and recrystallized by cooling to room temperature to obtain pure 2-[pyridyl-2,6-14C] PAM (492.1 MBq, 181 mg) as a yellow crystal. The radiochemical purity was greater than 99% as demonstrated with silica gel thin-layer chromatography (TLC; solvent system: ethanol/water/aq.ammonia=10/1/2 by volume, Rf 0.39; n-butanol/acetic acid/water=5/2/2 by volume, Rf 0.23; localized by iodine vapor and GM-radiodetector) and high-performance liquid chromatography (solid phase: μ-Bondapak C18 with a diameter of 10 μm; column: 4 mm in inner diameter, 30 cm in length; solvent system: water/acetonitrile/PI C B-7 =20/25/18 by volume; flow rate: 0.8 ml/min; room temperature; monitored by UV (292 nm) photometer and radiodetector; retention time: 12.1 min). The chemical structure of the final product was confirmed using nuclear magnetic resonance spectroscopy.

Organophosphorus compounds and other chemicals:

Fenitrothion (purity, 96.6%) and malathion (purity, 95.4%) were prepared by Sumitomo Chemical Co., Ltd. (Osaka). 2-PAM (purity, 99.9%) and PAM inj. SUMITOMO®, a formulation containing 500 mg 2-PAM in 20 ml distilled water, were kindly supplied by Sumitomo Pharmaceutical Co., Ltd. (Osaka). Other chemicals were obtained from commercial vendors.

2. Administration of organophosphorus compounds (OPs) and 2-PAM to animals

Animals: Sprague-Dawley male rats (6 weeks of age) were purchased from Charles River Japan, Inc. (Kanagawa). 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 and water.

Administration of OPs and 2-PAM: Fenitrothion and malathion were suspended in 10% Tween 80 aqueous solution and administered intravenously via the tail vein at a rate of 0.2 ml/min. In the distribution study, 14C-2-PAM was administered intravenously (100 mg/4 ml/kg) or by intramedullary injection (10 mg/400 μl/kg) 10 min after intravenous administration of fenitrothion (30 mg/2 ml/kg) or the vehicle (2 ml/kg).
In the antidote study, 2-PAM was administered intravenously (50 mg/2 ml/kg) or by intramedul-
lar injection (10 mg/400 μl/kg) after the adminis-
tration of fenitrothion (60 mg/2 ml/kg or 75 mg/2
ml/kg) or malathion (105 mg/2 ml/kg).

The intramedullary injection was made ac-
cording to the method of Satoh et al. (1983)
into the subarachnoid space through the foramen
intervertebrale between L3 and L4.

3. Distribution study

Quantification of 14C in tissues: Following the
administration of 14C-2-PAM, three rats at each
time point studied were exsanguinated via the
aorta abdominalis under ether-anesthesia, blood
was collected and organs were removed. A few
grains of sodium heparin was added, and then a
portion of the blood was centrifuged to obtain
plasma and erythrocytes. Whole blood, plasma,
erthrocytes, liver, cerebrum, cerebellum, medul-
la oblongata and medulla spinalis were com-
busted in an oxidizer (Model 306; Packard,
USA). Oxisorb-CO2® and Oxi-prep-2® (New
England Nuclear, USA) were used respectively as
14CO2 absorbent and scintillator. The radioac-
tivity in each sample was quantified using a
Tri-Carb 460CD Liquid Scintillation Spectro-
meter (Packard, USA).

Separation of 14C-2-PAM in tissues: Thirty
minutes after the administration of 14C-2-PAM,
three rats were exsanguinated via the aorta abdo-
minalis under ether-anesthesia. Blood was col-
clected and the liver, cerebrum, cerebellum,
medulla oblongata and medulla spinalis were
removed. The tissues from the three rats were
combined and homogenized in 3–20 volumes of
methanol using a homogenizer (Politron® , Kine-
matica, Inc., Switzerland); the resulting mixture
was then centrifuged at 1,000 g for 10 min. This
procedure was repeated twice, and the super-
natants obtained following each centrifugation
were combined and concentrated in vacuo. For
blood and liver samples, the methanol extracts
were subjected to thin-layer chromatography
(TLC, Silica gel 60F254, Merck). The samples
from cerebrum, cerebellum, medulla oblongata
and medulla spinalis were extracted twice with a 1
to 1 mixture of n-hexane and acetonitrile (v/v),
and the acetonitrile layers were subjected to
TLC. The solvent system used was ethanol/
water/ammonia (10/1/2, v/v). The amounts of
14C-2-PAM and other metabolites in each tissue
sample were calculated using a Radiochromatyz-
er (ALOKA, Japan).

Whole-body autoradiography: Thirty minutes
after the administration of 14C-2-PAM, the rats
were sacrificed under deep ether-anesthesia, and
autoradiography was conducted according to the
method of Ullberg et al. (1981). The rats were
frozen and cut into 30 μm-thick sections with a
microtome (CRYO-MICROTOME, LKB,
Sweden). The dried sections were placed in
contact with X-ray film (SB-5, Kodak) and ex-
posed for 2–7days.

Excretion of 14C-2-PAM: After the adminis-
tration of 14C-2-PAM, three rats were singly
placed individually in metabolic cages (Metabolici-
a CO2, Sugiyamagen Irika, Japan) so that their
feces and urine might be separately collected at 2,
4, 24, 48 and 72 hr after administration. Urine
samples were mixed with 10 ml of Emulsifier
scintillator 290TM (Packard, USA). The feces
were homogenized in water and combusted in an
oxidizer as described above. The rats were sac-
crificed 72 hr after the administration of 14C-2-
PAM, and their organs and blood were com-
busted in an oxidizer. The radioactivity in each
sample was quantified using a Liquid Scintillation
Spectrometer (Packard).

4. Half-life of 2-PAM in the brain

After intramedullary injection of 2-PAM (10
mg/kg), the concentration of 2-PAM in the brain
were measured 0.5, 1, 2, 3, 4, 5 and 6 hr
thereafter; the half-life of 2-PAM was then
calculated using a method described previously
(Uehara et al., b)). Three rats were studied at
each time-point tested.

5. Antidote study

Effect of 2-PAM as an antidote for OP poison-
ing: Immediately after intravenous administra-
tion of fenitrothion or malathion, 2-PAM was
administered intravenously or by intramedullary
injection, and observation was made for develop-
ment of signs of toxicity, including death. Five
rats were used for each dosing group.

Measurement of cholinesterase (ChE) activity:
Rat blood was obtained from orbital plexus under
ether-anesthesia. A few grains of sodium hepar-
in were added, and blood samples were then
centrifuged at 800 g for 10 min at 4°C to separate
erthrocytes from plasma. The erythrocytes
were washed with physiological saline. After decapitation, the brain was excised and homogenized in 0.1% Triton X-100 to obtain an 8%(W/V) homogenate. Following centrifugation of the homogenate at 800 g for 10 min at 4°C, the supernatant was used for the enzyme assay. The ChE activities in plasma, erythrocytes and brain were determined using the method of Ellman et al. (1961). Five rats were used for each dosing group.

RESULTS

1. Distribution and excretion of 14C

Whole-body autoradiography: Following the intravenous administration of 14C-2-PAM, high radioactivity was detected in the liver, heart, kidneys, urinary bladder and submandibular gland, while almost no radioactivity was detected in the central nervous system (Fig.1(A)). Following intramedullary administration of 14C-2-PAM, high radioactivity was detected in the medulla spinalis, cerebrum, cerebellum and medulla oblongata. Rapid migration of radiocarbon from the injection site to the brain was observed (Fig.1(B)).

Content of 14C-2-PAM in tissues: Tables 1 and 2 show the 14C content in various tissues following intravenous or intramedullary injection of 14C-2-PAM. Following intravenous administration, the 14C content in the brain was smaller than that in the blood or liver. There were no big differences in 14C concentration between the fenitrothion-treated and the vehicle-treated rats. Following intramedullary injection, the 14C content in the brain (including cerebrum, cerebellum and medulla oblongata) reached its maximum level at 1 hr; 14C content in blood, however, was very low. Table 3 shows the concentrations of the unchanged form of 14C-2-PAM in various tissues following intravenous or intramedullary administration of 14C-2-PAM. Following intravenous administration to the fenitrothion-treated rats, the concentrations of 14C-2-PAM in the blood, liver and brain were respectively 15.1, 133.9 and 2.6-3.6 μg 2-PAM eq/g tissue, and 40-64% of 14C in blood, liver and brain were present in the unchanged form of 14C-2-PAM. Following intramedullary administration to the fenitrothion-treated rats, the concentrations of 14C-2-PAM in the blood, brain and medulla spinalis were respectively 0.7, 87-363 and 1064 μg.

![Fig. 1 Whole-body autoradiography of rat after administration of 14C-2-PAM.](image)

(A) 14C-2-PAM (100 mg/kg) was administered intravenously (i.v.) 10 min after the injection of fenitrothion (30 mg/kg, i.v.), and the autoradiography was conducted 30 min after the administration of 14C-2-PAM.

(B) 14C-2-PAM (10 mg/kg) was administered by intramedullary injection (i.m.) 10 min after fenitrothion (30 mg/kg, i.v.), and autoradiography was conducted 30 min after the administration of 14C-2-PAM.
Table 1. Concentration of \(^{14}\)C in various tissues following intravenous administration of \(^{14}\)C-2-PAM.

<table>
<thead>
<tr>
<th>tissue</th>
<th>(\mu)g 2-PAM eq/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
</tr>
<tr>
<td>blood</td>
<td>34.6 ± 1.62</td>
</tr>
<tr>
<td></td>
<td>35.8 ± 3.93</td>
</tr>
<tr>
<td>plasma</td>
<td>43.3 ± 1.76</td>
</tr>
<tr>
<td></td>
<td>42.3 ± 6.90</td>
</tr>
<tr>
<td>erythrocyte</td>
<td>26.7 ± 3.82</td>
</tr>
<tr>
<td></td>
<td>27.5 ± 2.36</td>
</tr>
<tr>
<td>liver</td>
<td>429.7 ± 55.3</td>
</tr>
<tr>
<td></td>
<td>302.8 ± 40.3</td>
</tr>
<tr>
<td>cerebrum</td>
<td>20.9 ± 7.87</td>
</tr>
<tr>
<td></td>
<td>8.11 ± 1.50</td>
</tr>
<tr>
<td>cerebellum</td>
<td>12.0 ± 3.91</td>
</tr>
<tr>
<td></td>
<td>6.30 ± 1.20</td>
</tr>
<tr>
<td>medulla oblongata</td>
<td>24.7 ± 9.26</td>
</tr>
<tr>
<td></td>
<td>6.86 ± 2.08</td>
</tr>
</tbody>
</table>

The data are the mean values ± standard deviation of three trials. 
\(^{14}\)C-2-PAM (100 mg/kg) was administered intravenously (i.v.) 10 min after the administration of fenitrothion (30 mg/kg, i.v., upper figures) or vehicle (lower figures).

Table 2. Concentration of \(^{14}\)C in various tissues following intramedullary injection of \(^{14}\)C-2-PAM.

<table>
<thead>
<tr>
<th>tissue</th>
<th>(\mu)g 2-PAM eq/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>blood</td>
<td>4.42 ± 0.91</td>
</tr>
<tr>
<td>plasma</td>
<td>3.55 ± 0.54</td>
</tr>
<tr>
<td>erythrocyte</td>
<td>2.61 ± 1.86</td>
</tr>
<tr>
<td>cerebrum</td>
<td>139.6 ± 19.5</td>
</tr>
<tr>
<td>cerebellum</td>
<td>26.4 ± 1.91</td>
</tr>
<tr>
<td>medulla oblongata</td>
<td>148.8 ± 12.7</td>
</tr>
<tr>
<td>medulla spinalis</td>
<td>714.0 ± 17.8</td>
</tr>
</tbody>
</table>

The data are the mean values ± standard deviation of three trials. 
\(^{14}\)C-2-PAM (10 mg/kg) was administered by intramedullary injection (i.m.) 10 min after the administration of fenitrothion (30 mg/kg, i.v.).
2-PAM eq/g tissue, and 84-90% of $^{14}$C in the brain and medulla spinalis were present in the unchanged form of 2-PAM.

Rapid elimination of radioactivity was observed following the intravenous administration of $^{14}$C-2-PAM. The % excretions of total $^{14}$C into the urine and feces within the 72 hr period following administration were respectively 85% and 9% (Fig. 2). The % of total $^{14}$C excretion within the 4 hr period following intramedullary injection was smaller than that within the same period following intravenous administration, while 77% of administered $^{14}$C was excreted into the urine and 12% into the feces.

**Table 3.** Concentration of unchanged form of $^{14}$C-2-PAM in various tissues following intravenous (IV) or intramedullary (IM) administration to rats.

<table>
<thead>
<tr>
<th>tissue</th>
<th>$^{14}$C-2-PAM (IV)</th>
<th>$^{14}$C-2-PAM (IM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FNT (-)</td>
<td>FNT (+)</td>
</tr>
<tr>
<td>blood</td>
<td>13.620 (70.6)</td>
<td>15.082 (64.0)</td>
</tr>
<tr>
<td>liver</td>
<td>34.705 (20.6)</td>
<td>133.897 (39.9)</td>
</tr>
<tr>
<td>cerebellum</td>
<td>2.020 (57.7)</td>
<td>3.188 (54.6)</td>
</tr>
<tr>
<td>cerebrum</td>
<td>3.188 (74.4)</td>
<td>3.644 (59.4)</td>
</tr>
<tr>
<td>medulla oblongata</td>
<td>1.936 (53.9)</td>
<td>2.552 (54.1)</td>
</tr>
<tr>
<td>medulla spinalis</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

FNT (-): 10% Tween 80 aqueous solution (2 ml/kg, i.v.) was administered 10 min before administration of $^{14}$C-2-PAM.

FNT (+): Fenitrothion (30 mg/2 ml/kg, i.v.) was administered 10 min before administration of $^{14}$C-2-PAM.

![Graph](image)

**Fig. 2** Cumulative % excretion of $^{14}$C-2-PAM into the urine and feces.

The data show the cumulative excretion ratio of $^{14}$C into the urine and feces following administration of $^{14}$C-2-PAM (100 mg/kg, i.v., left or 10 mg/kg, i.m., right) to rats previously treated with fenitrothion (30 mg/kg, i.v.) or vehicle (2 ml/kg).
within the 72 hr period following injection. There were no differences in the $^{14}$C excretion ratio between the fenitrothion-treated and the vehicle-treated rats. Levels of residual $^{14}$C present in the tissues 72 hr after administration were quite low regardless of the route of administration.

2. **Half-life of 2-PAM in the brain**

Following intramedullary administration of 2-PAM to rats, the concentration in the brain reached its maximum level at 1 hr. The results of HPLC study showed that the half-life of 2-PAM in the brain was 1.52 hr (Fig. 3).

3. **Antidote study**

*Effect of 2-PAM administered by intramedullary injection on OP poisoning*: Soon after intravenous administration of fenitrothion, rats showed muscular fibrillation, salivation, miosis, clonic convulsion, ataxic gait, paretic gait, decreased respiration, and dyspnea with gasping. All rats died within 12 min of administration (Table 4). When 2-PAM was administered intravenously after that of fenitrothion, muscular fibrillation, salivation and miosis were alleviated, but the other symptoms of fenitrothion administration persisted; all rats died despite the administration of 2-PAM. When 2-PAM was administered by intramedullary injection after intravenous administration of fenitrothion, salivation and dyspnea with gasping were alleviated, and all rats survived. Muscular fibrillation and miosis diminished with both intravenous and intramedullary administration of 2-PAM. Intramedullary injection of 2-PAM alone induced clonic convulsion, ataxic gait and hyperexcitability.

Intravenous administration of malathion induced miosis, clonic convulsion, paretic gait, salivation, decreased respiration, dyspnea with gasping and nostril discharge. All rats died within 6 min of administration (Table 5). Intravenous administration of 2-PAM had no effect as an antidote following malathion; on the other hand, intramedullary injection of 2-PAM inhibited dyspnea with gasping and nostril discharge, and all treated rats survived. When 2-PAM was administered by both intravenous and intramedullary injection, muscular fibrillation also disappeared.

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![Graph](image)

*Fig. 3* Half-life of 2-PAM in rat brain. Data points represent means ± S.D. 2-PAM was administered by intramedullary injection to rats (10 mg/kg). The 2-PAM concentrations were determined at the specified time points using HPLC, and the half-life was then calculated.
Table 4. Effect of 2-PAM administered by intramedullary injection in acute poisoning with intravenously administered fenitrothalon (FNT) in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>number of animals which showed toxic symptoms</th>
<th>death (mortality, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>muscular fibrillation</td>
<td>salivation</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>I FNT</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>II FNT + 2-PAM (i.v.)</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>III FNT + saline (i.m.)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>IV FNT + 2-PAM (i.m.)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>V FNT + 2-PAM (i.m./i.v.)</td>
<td>5</td>
<td>(5)</td>
</tr>
<tr>
<td>VI vehicle + 2-PAM (i.m.)</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

The animals of the various groups were treated as indicated; N shows the number of animals used.
I Fenitrothalon (FNT, 75 mg/kg) was intravenously (i.v.) administered.
II 2-PAM (50 mg/kg, i.v.) was administered 1–2 min after FNT (i.v.).
III Physiological saline (400 μL/kg) was administered by intramedullary injection (i.m.) 1–2 min after FNT (i.v.).
IV 2-PAM (10 mg/kg, i.m.) was administered 1–2 min after FNT (i.v.).
V 2-PAM (10 mg/kg, i.m.) was administered 1–2 min after FNT (i.v.) and 2-PAM (50 mg/kg, i.v.) was administered 10 min after FNT (i.v.).
VI 2-PAM (10 mg/kg, i.m.) was administered 1–2 min after the vehicle (i.v.).

Toxic symptoms observed included the following; the figures in parentheses indicate that the symptom disappeared following administration of 2-PAM.

Table 5. Effect of 2-PAM administered by intramedullary injection in acute poisoning with intravenously administered malathion (MLT) in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>number of animals which showed toxic symptoms</th>
<th>death (mortality, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>muscular fibrillation</td>
<td>salivation</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>I MLT</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>II MLT + 2-PAM (i.v.)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>III MLT + saline (i.m.)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>IV MLT + 2-PAM (i.m.)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>V MLT + 2-PAM (i.m./i.v.)</td>
<td>5</td>
<td>(5)</td>
</tr>
<tr>
<td>VI vehicle + 2-PAM (i.m.)</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

The animals of the various groups were treated as indicated; N shows the number of animals used.
I Malathion (MLT, 105 mg/kg) was administered intravenously (i.v.).
II 2-PAM (50 mg/kg, i.v.) was administered immediately after MLT (i.v.).
III Physiological saline (400 μL/kg) was administered by intramedullary injection (i.m.) immediately after MLT (i.v.).
IV 2-PAM (10 mg/kg, i.m.) was administered immediately after MLT (i.v.).
V 2-PAM (10 mg/kg, i.m.) was administered immediately after MLT (i.v.) and 2-PAM (50 mg/kg, i.v.) was administered 10 min after MLT (i.v.).
VI 2-PAM (10 mg/kg, i.m.) was administered immediately after the vehicle (i.v.).

Toxic symptoms observed included the following; the figures in parentheses indicate that the symptom disappeared following administration of 2-PAM.
**Reactivation of ChE by 2-PAM**: Intramedullary administration of 2-PAM after the administration of fenitrothion reactivated brain ChE from 11% of the result for the normal group to 23% at 15 min and 28% at 60 min (Table 6), whereas induced no reactivation of ChE in the erythrocytes or plasma. Intravenous administration of 2-PAM resulted in no reactivation of brain ChE.

Intramedullary injection of 2-PAM after the administration of malathion reactivated the brain ChE from 28% of the result for the normal group to 79% at 15 min, while intravenous administration of 2-PAM resulted in no reactivation of brain ChE (Table 7).

**Table 6.** Reactivation effect of 2-PAM administered by intramedullary injection on ChE inhibited by fenitrothion (FNT) in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>number of animals</th>
<th>brain (U/g tissue)</th>
<th>plasma (UI)</th>
<th>erythrocytes (UI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 min</td>
<td>60 min</td>
<td>15 min</td>
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<tr>
<td>I</td>
<td>normal</td>
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<td></td>
<td>5</td>
<td>12.3±0.52</td>
<td></td>
<td>732±84.5</td>
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<tr>
<td>II</td>
<td>vehicle+2-PAM (i.m.)</td>
<td>5</td>
<td>12.5±0.69</td>
<td>615±31.1</td>
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<td></td>
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<td></td>
<td>12.4±0.09</td>
<td>563±29.6</td>
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<tr>
<td>III</td>
<td>FNT+ saline (i.m.)</td>
<td>5</td>
<td>1.4±0.05*</td>
<td>146±24.8*</td>
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<tr>
<td>IV</td>
<td>FNT+2-PAM (i.m.)</td>
<td>5</td>
<td>2.8±0.74</td>
<td>144±26.6</td>
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<td>V</td>
<td>vehicle+2-PAM (i.v.)</td>
<td>5</td>
<td>11.8±0.34</td>
<td>626±72.4</td>
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<tr>
<td>VI</td>
<td>FNT+2-PAM (i.v.)</td>
<td>5</td>
<td>1.3±0.14*</td>
<td>266±69.1*</td>
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</table>

The data are the mean values ± standard deviation of five trials.
The figures in parentheses show the ratio (%) to the result for the normal group. The cholinesterase activity was determined using the rate assay. The animals of the various groups were treated indicated; a) indicates that animals that died within 15 min of FNT (i.v.) were also taken into account.

I 2-PAM (10 mg/kg) was administered by intramedullary injection (i.m.) 1–2 min after the vehicle (i.v.).
II Physiological saline (400 μl/kg, i.m.) was administered 1–2 min after FNT (i.v.).
IV 2-PAM (10 mg/kg, i.m.) was administered 1–2 min after FNT (i.v.).
V 2-PAM (50 mg/kg, i.v.) was administered 1–2 min after the vehicle (i.v.).
VI 2-PAM (50 mg/kg, i.v.) was administered 1–2 min after FNT (i.v.).
Table 7. Reactivation effect of 2-PAM administered by intramedullary injection on ChE inhibited by malathion (MLT) in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>number of animals</th>
<th>brain (U/g tissue)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>I</td>
<td>normal</td>
<td>5</td>
</tr>
<tr>
<td>II</td>
<td>vehicle + 2-PAM (i.m.)</td>
<td>5</td>
</tr>
<tr>
<td>III</td>
<td>MLT + saline (i.m.)</td>
<td>5</td>
</tr>
<tr>
<td>IV</td>
<td>MLT + 2-PAM (i.m.)</td>
<td>5</td>
</tr>
<tr>
<td>V</td>
<td>vehicle + 2-PAM (i.v.)</td>
<td>5</td>
</tr>
<tr>
<td>VI</td>
<td>MLT + 2-PAM (i.v.)</td>
<td>5</td>
</tr>
</tbody>
</table>

The data are the mean values ± standard deviation of five trials. The figures in parentheses show the ratio (%) to the result for the normal group. The cholinesterase activity was determined using the rate assay. The animals of the various groups were treated as indicated ; a) indicates that rats died within 6 min of MLT (i.v.).

I: Intact animal.
II: 2-PAM (10 mg/kg) was administered by intramedullary injection (i.m.) immediately after the vehicle (i.v.).
III: Physiological saline (400 μl/kg, i.m.) was administered immediately after MLT (i.v.).
IV: 2-PAM (10 mg/kg, i.m.) was administered immediately after MLT (i.v.).
V: 2-PAM (50 mg/kg, i.v.) was administered immediately after the vehicle (i.v.).
VI: 2-PAM (50 mg/kg, i.v.) was administered immediately after MLT (i.v.).

DISCUSSION

In order to investigate the therapeutic effect of 2-PAM in poisoning with OP of low toxicity, a study of the distribution of 2-PAM and an antidote study using $^{14}$C-2-PAM administered intravenously or by intramedullary injection were performed.

2-PAM is a quaternary ammonium salt which can hardly pass through the blood-brain-barrier (BBB)(Gray, 1984). The results of our study of the distribution of 2-PAM confirmed that 2-PAM hardly reaches the brain after intravenous administration. For purpose of direct delivery to the brain, we used intramedullary injection of 2-PAM.

Following intramedullary administration, the concentration of 2-PAM in the brain reached its maximum level in 1 hr. The content of the unchanged form of 2-PAM in the brain after intramedullary injection was more than 80% of the administered 2-PAM. It therefore appeared that the amount of 2-PAM reaching the brain was sufficient for reactivation of inhibited brain ChE. $^{14}$C-2-PAM was rapidly metabolized following either intramedullary or intravenous administration. The results of the autoradiography showed that 2-PAM had very limited access to the brain after intravenous administration, while it clearly had reached the brain 30 min after intramedullary injection. These results confirmed that 2-PAM administered by intramedullary injection reaches the brain via the cerebrospinal fluid. The half-life of the drug in the brain following intramedullary administration was 1.52 hr, and it was longer than that in the blood after intravenous administration (0.87 hr; Uehara et al., (b)). Making use of these findings, we studied the efficacy of 2-PAM as an antidote to fenitrothion and
malathion poisoning in rats. Both intravenous and intramedullary routes of 2-PAM treatment were tested. Intravenous administration of 2-PAM following fenitrothion or malathion poisoning proved ineffective, and all tested animals died. Intramedullary administration, however, resulted in alleviation of signs of toxicity; no treated animals died, and brain ChE in animals receiving intramedullary 2-PAM was reactivated.

Taken together, our results indicate that 2-PAM can reanimate ChE inhibited by OPs of low toxicity, and that 2-PAM is effective as an antidote for OP poisoning. We believe the following are reasons why the effect of 2-PAM as an antidote to OP poisoning was limited. In the case of poisoning due to OP of low toxicity, a large exposure to OP must occur for the development of signs of toxicity. The quantities of 2-PAM used for treatment may therefore prove insufficient. In addition, the half-life of 2-PAM in the blood is short, its permeability to the BBB is small, and aging of ChE inhibited by O,O-dimethyl OP occurs rapidly.

We therefore suggest that, when OP poisoning occurs, the pharmacodynamics of the OP compound involved and the route of administration of 2-PAM to be used should be considered in deciding upon treatment.

REFERENCES


