BIOLOGICAL MONITORING OF METABOLITES OF SARIN AND ITS BY-PRODUCTS IN HUMAN URINE SAMPLES

Masayasu Minami, Da-Mei Hui, Zhiyu Wang, Masao Katsumata, Hirofumi Inagaki and Qing Li,
Shou Inuzuka, Kunihiro Mashiko, Yasuhiro Yamamoto and Toshifumi Ootsuka,
Camille A. Boulet and John G. Clement

Department of Hygiene and Public Health, Nippon Medical School
Department of Emergency and Critical Care Medicine, Nippon Medical School
Defense Research Establishment Suffield, Medicine Hat, Alberta, T1A 8K6, Canada

ABSTRACT — More than 20,000 passengers of Tokyo underground trains were intoxicated with warfare toxic chemicals. Most of the patients examined had marked miosis and decreased serum cholinesterase activity. Transient increase of serum CPK activity after 3 days of the exposure was the another sign. We intensively analyzed the metabolites in the urine of 4 patients. The following analytic results indicated the exposure to sarin as well as contaminated compounds such as diisopropyl methylphosphonate (DIMP), ethyl methylphosphonate fluoride (EMPF, or ethylsarin), diethyl methylphosphonate (DEMP), and ethyl isopropyl methylphosphonate (EIMP).

1. Isopropanol (IPA) and ethanol (EtOH) were detected of large quantities in the urine samples, and were thought to be derived from sarin and the sarin counterpart, EMPF, DIMP, DEMP and EIMP.
2. Monoalkyl methylphosphonic acids (isopropyl methylphosphonic acid (IMPA) and ethyl methylphosphonic acid (EMPA)) also were excreted in large amounts with taking the similar excretion pattern of IPA and EtOH.
3. The metabolite only derived from sarin and ethylsarin is F anions whose integral output in the urine was less than the equimolar level of the excreted (IMPA+EMPA+IPA+EtOH).
4. Other corroborative findings were low lethality: of more than 5,510 patients treated, 11 were acutely dead.
5. Nine exposed males had higher sister chromatid exchange (SCE) rate (5.00±1.48/cell) than the control (3.81±0.697/cell), because dialkyl methylphosphonates seemed to have alkylating activity and producing DNA adducts. The SCE rate also increased after the in vitro exposure of lymphocytes to dialkyl methylphosphonates.

INTRODUCTION AND METHODS

Four seriously intoxicated patients hospitalized in ICU of Nippon Medical School (NMS) were intensively examined during the hospitalization period as to their clinical changes such as the diameter of pupils, laboratory data comprised of activities of serum cholinesterase (ChE) and acetylcholine esterase (AChE) of erythrocyte membrane [1] being prepared by the method of Dodge [2] and creatinine kinase (CK-MM and CK-MB) using the CK kits of Merck [3]. The information from the biologically monitored data being suggestive of the exposure to complex substances consisted of (1) IPA and EtOH which were biotransformed from the both of sarin and dialkyl methylphosphonates measured by the head-space equilibration method with a GC/FID apparatus, (2) metabolites of sarin and alkyl MPA esters such as IMPA, EMPA and MPA in the urine and serum samples determined with newly established procedures developed by us [Minami, M. et al., J. Chromatogr. B, in press] using GC/FPD equipment after the samples were pretreated by the purification steps, (3) picomolar urinary F anions determined by an improved method devised by us [Hui, D-M. et al., submitting for publication] with adopting the derivatizing procedure of Chiba et al. [4] using GC/FID.

We could study the frequencies of sister chromatid exchange (SCE) in lymphocytes [5] sampled from the 9 of the sarin exposed male patients having been hospitalized in NMS and other hospitals 3 to 5 months after...
the exposure and 36 control persons whose specimens obtained in the similar time period. The in vitro SCE tests also were administered to normal human lymphocytes with exposing the cells to DEMP, DIMP and EIMP.

The inhibitory effect of dialkyl methylphosphonates (DEMP, DIMP and EIMP) on authentic AChE and ChE was investigated by the assay system utilized by Maxwell [6] according to the principle proposed by Aldridge and Reiner [7]. All of the reagents unless otherwise indicated were of reagent grade, the authentic IMPA, EMPA, DEMP, DIMP and EIMP were synthesized by the method as previously described [8].

RESULTS

All of the patients had conspicuous miosis, pin-hole miosis at the admission time. The initial miosis coincident with the initial decrease of ChE and AChE activities and indicated the delayed recovery of AChE in erythrocytes because of their life span, about 90 days. In the hospital of NMS, most of the patients were not received oximes such as 2-pyridine aldoxime methiodide (PAM) therapy, and the activity of ChE and AChE slowly recovered, but in other hospitals, the activity improved within 2-3 days because of the early administration of PAM. Almost all of the patients examined had the transient increase of serum CPK activities on 3 or 4 days after the exposure, but there was no detectable peculiar change of the serum CK-MB. The time courses of large quantities of IPA and EtOH outputs into the urine are shown in Figs. 1(1) and 1(2), respectively.

Table 1 shows the classified SCE distribution according to the level above, or below the upper limit of the control group, i.e., 5.13/cell (mean+2SD of the control), and indicates that the exposed persons have comparatively higher SCE level than the control. The in vitro exposure of dialkyl methylphosphonates (DEMP, DIMP and EIMP) to lymphocytes increased SCE frequencies (Fig. 2). Those dialkyl methylphosphonates also have inhibitory effects on ChE.

<table>
<thead>
<tr>
<th>SCE &gt; 5.13</th>
<th>Control</th>
<th>Exposed</th>
</tr>
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<tbody>
<tr>
<td>38 persons</td>
<td>5 persons</td>
<td></td>
</tr>
<tr>
<td>1 person</td>
<td>4 persons</td>
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The splitting upper limit (5.13) is set at mean+2SD of the control. The probability is calculated by Fisher's exact method, p = 0.0029.

![Graph](image)

*Fig. 1-1.* Isopropanol excreted in urine after the gas exposure. The level exceed the Japanese reference value during abstinence.
DISCUSSION

We discuss the kinetical problem of sarin and ethyl sarin according to the scheme illustrated in Fig. 3. The concentration of the metabolite in urine surpasses above that in serum, suggesting that the biological half life of sarin and ethyl sarin is short as Shih et al. [9] have pointed out. The patients show no abnormal laboratory data concerning the renal function except AChE, ChE and CPK, and their heights and weights are within the normal Japanese range. We can roughly estimate the total exposure quantities of sarin from the time-integrated urinary values of IMPA with assuming daily creatinine output of the patients into urine being the

![Graph](image)

**Fig. 1-2.** Ethanol excreted in urine after the gas exposure. The level exceed the Japanese reference value during abstinence.

![Graph](image)

**Fig. 2.** Frequencies of sister-chromatid exchanges (SCE) in human peripheral blood lymphocyte induced by DIMP, DEMP and EIMP in vitro. The numbers in figure indicate the cell numbers counted.
Japanese reference value of adults: 0.7-1.5 g/day [10]. The roughly estimated respective sarin exposure values as to the patient being cardiopulmonary arrest on arrival and the other one having consciousness disorder on arrival were 0.13-0.25 mg/person and 0.016-0.032 mg/person, respectively.

Considering the estimated sarin exposure value, the former exceeds a little above the lethal dose published in the authorized data books [11, 12]. The contaminants, for example DIMP, DEMP and EIIMP should be taken into consideration, but none of those contaminant candidates is detectable in the urine samples from the patients with our method using GC/FPD system, because almost all of the dialkyl methylphosphonates seemed to be biotransformed rapidly into the metabolites such as EMPA, IMPA and MPA owing to the easily hydrolyzable property of those methylphosphonates by esterases such as acetylcholine esterase [our unpublished data]. Taking the hydrolyzing metabolism of the above mentioned dialkyl methylphosphonates and the lethal dose of sarin into account; comparatively high output of EMPA and IMPA in urine samples of the patients seemed to be derived from the biologically hydrolyzed products of the contaminant esters such as DIMP, DEMP and EIIMP.

REFERENCES


