Inter and intraindividual variations in plasma cholinesterase activity and substance concentration in employees of an organophosphorus insecticide factory

A Brock

Abstract
During a period of 10 months, inter and intraindividual variations in plasma cholinesterase (ChE) activity were studied in 331 employees of an organophosphorus insecticide factory, and in 193 healthy volunteers without occupational exposure to known ChE inhibitors. Repeated (n = 6) measurements of ChE activity and ChE substance concentration were performed in 410 subjects. The study showed substantial intraindividual variations of ChE activity and ChE substance concentration (up to 40%) in the employees and in the reference group. When effects due to sex, ChE-1 phenotype, body weight, and height were considered, one subgroup of employees of the organophosphorus insecticide factory showed a significantly lower average ChE activity than other subgroups; as ChE substance concentrations were found to be proportionally decreased, it was concluded that the low ChE activity was unrelated to occupational exposure. A combined determination of ChE activity and ChE substance concentration is recommended as a rational diagnostic tool when an unexpected decrease of plasma ChE activity is registered in people joining organophosphorus insecticide health surveillance programmes.

Organophosphorus insecticides are highly toxic substances to man as well as to pests. Persons with risk for substantial occupational exposure to such substances are employees of organophosphorus insecticide factories and pesticide operators within gardening and agriculture. Because even a few mg may possess appreciable toxic effects in man, and as absorbed organophosphorus insecticides are excreted as non-specific metabolites (for example, p-nitrophenol from parathion and methyl-parathion), most programmes for health surveillance are based on measurements of plasma cholinesterase activity (ChE, pseudocholinesterase, butyrylcholinesterase, acetylcholine acetylhydrolase, EC 3.1.1.8), which is strongly inhibited by all organophosphorus insecticides. In such health surveillance programmes (as in other areas of clinical medicine) decisions should be made from rational interpretations of observed variations based on knowledge of spontaneous inter and intraindividual variations in the population.

Biosynthesis of the 574 amino acid ChE subunit is controlled by the ChE-1 locus on chromosome No 3, where a number of alleles (ChE, ChE, ChE, ChE, ChE) define the actual genotype; the genotype ChEChE (u = usual) is found in about 95% of Caucasian populations. About 4% should be expected to be ChEChE (a = atypical); the atypical ChE has recently been shown to be an Asp-70 → Gly mutation owing to a change of the actual ChE codon from GAT to GGT. Within a Caucasian population one person in 100 000 will be expected to be of ChE-1 genotype ChEChE (s = silent) without detectable ChE catalytic activity in plasma. Such people, who are found much more frequently among the Inuits of the north-western parts of Canada than among a general population (Dr Alison Dinwoodie, University of Alberta, Canada, personal communication), are healthy and without any increased risk of death unless exposed to suxamethonium to which they are extremely sensitive.

Previous studies have analysed the well known, wide interindividual variation in plasma ChE activity in healthy subjects. Such variations, which are independent of electrophoretic heterogeneity, are statistically related to physiological factors such as body weight, height, and sex. Four of these studies also describe substantial intraindividual variations that are independent of age, body weight,
height, sex, and ChE-1 phenotype. The main problem in the interpretation of significant variations of plasma ChE activity (which is further influenced by a variety of other physiological and pathological conditions) is to decide whether a decrease in catalytic activity is caused by a reduced ChE substance concentration (for example, due to preanalytical errors, reduced ChE synthesis, or increased ChE degradation) or by a partial inhibition of the catalytic activity (for instance, due to absorbed organophosphorus insecticides).

This paper describes the inter and intraindividual variations in catalytic and immunoreactive plasma ChE activity in a group of employees of an organophosphorus insecticide factory. It also evaluates the variations by a multiple regression model that is appropriate for a healthy population group without occupational exposure to known ChE inhibitors.

Materials and methods

SAMPLE

During a period of 10 months (November 1987-September 1988) heparin stabilised venous blood samples were taken, at intervals of four to six weeks, from 331 employees of an organophosphorus insecticide factory (319 men, 12 women) and 193 healthy volunteers (122 men, 71 women) not exposed to known ChE inhibitors. Plasma was separated from the blood cells within 24-30 hours after withdrawal and stored at -20°C. Detailed data concerning the 193 volunteers have already been published.

All employees of the organophosphorus insecticide factory (Cheminova, Lemvig, Denmark) were subdivided by the Health Service and the local safety committee into four groups according to their actual risk for exposure to toxic organophosphorus compounds as follows: group 1 (high risk for exposure); persons occupied with production of diethyl-phosphorochloridothionate; persons handling repairs and maintenance all over the factory; group 2 (medium risk for exposure); persons occupied with production of dimethyl-phosphorochloridothionate, parathion, methyl-parathion, malathion, pirimiphos, dimethoate, and p-nitrophenol; persons occupied with unspecified assistance, drawing, and handling of waste water; persons employed in quality control and development; persons occupied with laboratory routines: group 3 (low risk for exposure); persons occupied with production of dimethyl- and diethyl-dithiophosphoric acid; persons taking care of lubrication, fire control, etc.; engineers, supervisors, etc.; group 4 (negligible risk for exposure to toxic organophosphorus compounds); persons occupied with production of phosphoropentasulfide; persons within planning and management.

Age, body weight, height, previous liver disease, use of oral contraceptives, and any regular intake of drugs were recorded from information given by the Health Service. Of the 331 employees, 318 completed the full programme (six samples from each subject) and so did 131 of the 193 volunteers (table 1).

| Table 1 | Population groups from which six blood samples were obtained. Risk group 1, high risk for exposure to organophosphorus insecticides; 2, medium risk; 3, low risk; 4, negligible risk. Reference group, subjects without occupational exposure to known ChE inhibitors |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Population group | No of subjects | Mean body weight (kg / SD) | Mean height (cm / SD) | Mean age (y / SD) |
| Employees | 318 | 79.6 (10.8) | 175.5 (6.5) | 40.2 (9.7) |
| Risk group 1 | 75 | 81.3 (9.7) | 176.0 (5.8) | 40.8 (7.5) |
| Risk group 2 | 200 | 78.4 (11.0) | 176.2 (6.6) | 39.9 (10.2) |
| Men | 188 | 79.2 (10.2) | 176.8 (6.0) | 39.9 (10.0) |
| Women | 12 | 66.2 (15.4) | 166.8 (9.0) | 30.1 (8.9) |
| Risk group 3 | 29 | 81.8 (12.3) | 179.2 (7.9) | 41.9 (9.4) |
| Risk group 4 | 14 | 82.5 (9.9) | 178.6 (4.8) | 46.1 (10.5) |
| Reference group | 131 | 71.8 (13.0) | 173.0 (8.7) | 39.0 (9.7) |
| Men | 75 | 79.5 (9.8) | 178.3 (6.1) | 38.9 (9.6) |
| Women | 56 | 61.5 (9.0) | 165.8 (6.0) | 39.2 (9.8) |
the 131 volunteers of the reference group, seven were classified as ChE"ChE", and the remaining 124 were classified as ChE"ChE".

Determination of immunoreactive ChE substance concentration was performed by an enzyme linked immunosorbent assay (ELISA) using polyclonal (rabbit) antihuman ChE antibodies combined with a highly specific monoclonal (mouse) antihuman ChE antibody and a peroxidase conjugated (rabbit) antibody against mouse immunoglobulins as signal carrier. Day to day reproducibility was assessed from parallel determinations of lyophilised quality control material (M + D Monitrol I-E and Monitrol II-E); CV = 4.97% (mean = 4.62 mg/l, SD = 0.23 mg/l, n = 73) for lot No LTD 210; and CV = 5.56% (mean = 3.42 mg/l, SD = 0.19 mg/l, n = 83) for lot No PTD 109.

STATISTICS

Results were evaluated from a multiple regression analysis, an analysis of variance covariance, and a repeated measure analysis of variance. All calculations were performed on an IBM PC/AT using the SPSS/PC+ software package (SPSS Inc, Chicago, IL).

Results

CHOLINESTERASE ACTIVITY

Table 2 shows the total ChE activity in six plasma samples (ChE-1-ChE-6) from the 449 subjects (employees 318; reference group 131) who completed the full programme. It shows the well known wide interindividual variation that may conceal a treat-

Table 2 Mean (SD) ChE activity in each of six plasma samples (ChE-1-ChE-6) from 318 employees of an organophosphorus insecticide factory and in 131 healthy subjects without occupational exposure to known ChE inhibitors (reference group)

<table>
<thead>
<tr>
<th>Population group</th>
<th>No of subjects</th>
<th>ChE-1 (kU/l (SD))</th>
<th>ChE-2 (kU/l (SD))</th>
<th>ChE-3 (kU/l (SD))</th>
<th>ChE-4 (kU/l (SD))</th>
<th>ChE-5 (kU/l (SD))</th>
<th>ChE-6 (kU/l (SD))</th>
<th>Standardised ChE mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employees</td>
<td>318</td>
<td>8.78 (1.79)</td>
<td>8.83 (1.81)</td>
<td>8.78 (1.85)</td>
<td>8.78 (1.81)</td>
<td>8.76 (1.89)</td>
<td>8.57 (1.85)</td>
<td>8.21 (1.64)</td>
</tr>
<tr>
<td>Risk group 1</td>
<td>75</td>
<td>8.56 (1.56)</td>
<td>8.65 (1.65)</td>
<td>8.63 (1.69)</td>
<td>8.64 (1.75)</td>
<td>8.65 (1.76)</td>
<td>8.48 (1.80)</td>
<td>7.92 (1.58)</td>
</tr>
<tr>
<td>Risk group 2</td>
<td>200</td>
<td>8.94 (1.88)</td>
<td>8.96 (1.88)</td>
<td>8.92 (1.93)</td>
<td>8.89 (1.84)</td>
<td>8.88 (1.95)</td>
<td>8.66 (1.91)</td>
<td>8.39 (1.66)</td>
</tr>
<tr>
<td>Men</td>
<td>188</td>
<td>8.99 (1.84)</td>
<td>9.04 (1.84)</td>
<td>9.00 (1.88)</td>
<td>8.95 (1.81)</td>
<td>8.95 (1.91)</td>
<td>8.73 (1.76)</td>
<td>8.39 (1.65)</td>
</tr>
<tr>
<td>Women</td>
<td>12</td>
<td>8.08 (2.43)</td>
<td>7.86 (2.21)</td>
<td>7.57 (2.29)</td>
<td>7.99 (2.13)</td>
<td>7.75 (2.29)</td>
<td>7.67 (2.47)</td>
<td>8.31 (1.88)</td>
</tr>
<tr>
<td>Risk group 3</td>
<td>29</td>
<td>8.21 (1.98)</td>
<td>8.24 (1.64)</td>
<td>8.25 (1.65)</td>
<td>8.16 (1.65)</td>
<td>8.09 (1.62)</td>
<td>8.03 (1.52)</td>
<td>7.60 (1.36)</td>
</tr>
<tr>
<td>Risk group 4</td>
<td>14</td>
<td>8.93 (1.71)</td>
<td>9.09 (1.86)</td>
<td>8.81 (1.79)</td>
<td>9.18 (1.94)</td>
<td>9.71 (1.97)</td>
<td>8.99 (1.83)</td>
<td>8.40 (1.78)</td>
</tr>
<tr>
<td>Reference group</td>
<td>131</td>
<td>8.26 (1.92)</td>
<td>8.15 (1.85)</td>
<td>7.92 (1.79)</td>
<td>8.03 (1.85)</td>
<td>8.00 (1.83)</td>
<td>8.17 (1.93)</td>
<td>8.25 (1.36)</td>
</tr>
<tr>
<td>Men</td>
<td>75</td>
<td>8.81 (1.91)</td>
<td>8.75 (1.84)</td>
<td>8.46 (1.84)</td>
<td>8.58 (1.82)</td>
<td>8.49 (1.83)</td>
<td>8.65 (2.02)</td>
<td>8.26 (1.49)</td>
</tr>
<tr>
<td>Women</td>
<td>56</td>
<td>7.53 (1.68)</td>
<td>7.34 (1.54)</td>
<td>7.20 (1.46)</td>
<td>7.29 (1.62)</td>
<td>7.33 (1.63)</td>
<td>7.53 (1.61)</td>
<td>8.25 (1.18)</td>
</tr>
</tbody>
</table>

Table 3 Intraindividual variation of ChE activity (n = 6) in employees of an organophosphorus insecticide factory and in healthy subjects without occupational exposure to known ChE inhibitors (reference group)

<table>
<thead>
<tr>
<th>Population group</th>
<th>No of subjects</th>
<th>Maximum variation (mean and range)</th>
<th>Individual SD range (kU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employees</td>
<td>318</td>
<td>12.6 (3-34-38) (9)</td>
<td>0.11-1.02</td>
</tr>
<tr>
<td>Risk group 1</td>
<td>75</td>
<td>12.7 (4-3-32) (0)</td>
<td>0.12-0.90</td>
</tr>
<tr>
<td>Risk group 2</td>
<td>200</td>
<td>12.9 (3-34-38) (9)</td>
<td>0.11-1.02</td>
</tr>
<tr>
<td>Risk group 3</td>
<td>29</td>
<td>10.9 (3-34-21) (5)</td>
<td>0.15-0.63</td>
</tr>
<tr>
<td>Risk group 4</td>
<td>14</td>
<td>11.3 (3-37-18) (6)</td>
<td>0.18-0.62</td>
</tr>
<tr>
<td>Reference group</td>
<td>131</td>
<td>14.0 (3-0-41) (8)</td>
<td>0.10-1.12</td>
</tr>
</tbody>
</table>
activity. In four of the employees and in three of the
referrers the maximum intraindividual variation
exceeded 30%. The maximum intraindividual varia-
tion (expressed relative to the mean activity) was
independent of ChE activity. 10

A quantitative estimate of the intraindividual
variance of the 318 employees was obtained from a
repeated measures analysis of variance, which also
gives a quantitative estimate of the influence by risk
group on the intraindividual variation of ChE
activity from one sample to another (second sample v
first sample, third sample v first and second, fourth
sample v first, second, and third, etc.). The repeated
measures analysis of variance showed an average
SD_{inv} of 0.43 kU/l without influence of actual risk
group (F = 1.05, p > 0.10). In the same way, the
average SD_{inv} of the reference group was 0.44 kU/l.

CHOLINESTERASE SUBSTANCE CONCENTRATION

Of the 449 subjects who completed the full
programme, immunoactive ChE substance concen-
trations were measured in 316 of the 318
employees, and in 94 of the 131 subjects of the
reference group. Table 4 shows the immunoactive
ChE substance concentrations of all 410 subjects
studied. Like ChE activities, ChE substance concen-
trations are influenced by body weight, height, sex,
and ChE-1 phenotype; 15 therefore, "standardised"
mean ChE substance concentrations (model: ChE_{stand} = \text{ChE}_{mean} − (\text{weight}−75) \times 0.048 + (\text{ChE}_{type} − 1) \times 1.10 + (\text{height}−180) \times 0.064 + (\text{sex}−1) \times 0.85) are preferable when comparing groups and subgroups. Like standardised ChE activity, standard-
dised ChE substance concentration of employees in
risk group 3 differed significantly (t = 2.12, 0.01 < p < 0.05) from that of men in the reference
group; no other male groups differed from each other.
Female employees showed the same standardised
ChE substance concentration as women in the reference group.

Table 5 shows the maximum intraindividual vari-
tions and intraindividual variations expressed as
individual SD (n = 6). As in the reference group, 13
the distribution of intraindividual variations of the
employees of the organophosphorus insecticide fac-
tory was skewed to the right. Average maximum
variation was 18.7% (range: 3–43%), independent of
actual risk group, and similar to that of the reference
group (mean: 20%, range: 6–43%). Within the 316
employees, a repeated measures analysis of variance
showed an average SD_{inv} of 0.41 mg/l (reference
group: SD_{inv} = 0.44 mg/l).

Discussion

The use of plasma ChE activity measurements for
biological monitoring should not be confused with
relating the enzymatic activity to clinical or toxi-
cological effects. Earlier systematic studies of ChE
after exposure to the toxic organophosphorus com-
 pound DFP (diisopropylfluorophosphate) showed up
to 95% reduction of plasma ChE activity after
parenteral administration of 0.5–3.0 mg. 16–18 No close
relation between toxic symptoms and changes in
plasma ChE activities were seen. Although distinct
organophosphorus insecticides inhibit plasma ChE
to a varying extent, and although plasma ChE differs
in amino acid sequence from the target enzyme for
organophosphorus insecticides (acetylcholinesterase
EC 3.1.1.7), the use of plasma ChE activity for
monitoring exposure to organophosphorus insect-
cides offers great advantages compared with eryth-
rocyte acetylcholinesterase, which (contrary to
plasma ChE) appears quite instable, even when stored
at −20°C (A Brock, unpublished observations).

When comparing plasma ChE activity in unmatch-
groups or subgroups, influence by factors other
than organophosphorus inhibitors has to be con-
sidered. The effects by ChE-1 phenotype and sex are
well known, 7,14 but also (as in other liver synthesised
plasma enzymes 19) varying body weight and height
has appeared to affect the interindividual variation of
plasma ChE activity, 10 as well as plasma ChE sub-
stance concentration. 13 Even when considering all

Table 4 Immunoreactive ChE substance concentration
(mean of six samples) in 316 employees of an
organophosphorus insecticide factory and in 94 healthy
subjects without occupational exposure to known ChE
inhibitors (reference group)

<table>
<thead>
<tr>
<th>Population group</th>
<th>No of subjects</th>
<th>ChE substance concentration (mean mg/l (SD))</th>
<th>Standardised ChE substance concentration (mean mg/l (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employees</td>
<td>316</td>
<td>5.47 (1.14)</td>
<td>5.13 (1.09)</td>
</tr>
<tr>
<td>Risk group 1</td>
<td>74</td>
<td>5.42 (1.06)</td>
<td>4.96 (1.06)</td>
</tr>
<tr>
<td>Risk group 2</td>
<td>199</td>
<td>5.53 (1.19)</td>
<td>5.23 (1.11)</td>
</tr>
<tr>
<td>Men</td>
<td>187</td>
<td>5.58 (1.16)</td>
<td>5.23 (1.11)</td>
</tr>
<tr>
<td>Women</td>
<td>12</td>
<td>4.77 (1.36)</td>
<td>5.20 (1.16)</td>
</tr>
<tr>
<td>Risk group 3</td>
<td>29</td>
<td>5.12 (0.99)</td>
<td>4.78 (0.91)</td>
</tr>
<tr>
<td>Risk group 4</td>
<td>14</td>
<td>5.62 (1.09)</td>
<td>5.26 (1.13)</td>
</tr>
<tr>
<td>Reference group</td>
<td>94</td>
<td>5.01 (1.11)</td>
<td>5.28 (0.91)</td>
</tr>
<tr>
<td>Men</td>
<td>43</td>
<td>5.42 (1.23)</td>
<td>5.28 (1.09)</td>
</tr>
<tr>
<td>Women</td>
<td>51</td>
<td>4.66 (0.88)</td>
<td>5.28 (0.74)</td>
</tr>
</tbody>
</table>

Table 5 Intraindividual variation of ChE substance
collection (n = 6) in employees of an organophosphorus
insecticide factory, and in healthy subjects without
occupational exposure to known ChE inhibitors (reference
group)

<table>
<thead>
<tr>
<th>Population group</th>
<th>No of subjects</th>
<th>Maximum variation (mean and range)</th>
<th>Individual SD range (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employees</td>
<td>316</td>
<td>18.7 (3.2–43.0)</td>
<td>0.06–1.20</td>
</tr>
<tr>
<td>Risk group 1</td>
<td>74</td>
<td>17.1 (5.4–38.0)</td>
<td>0.11–0.89</td>
</tr>
<tr>
<td>Risk group 2</td>
<td>199</td>
<td>19.4 (3.2–43.0)</td>
<td>0.06–1.20</td>
</tr>
<tr>
<td>Risk group 3</td>
<td>29</td>
<td>18.2 (10.4–29.7)</td>
<td>0.18–0.69</td>
</tr>
<tr>
<td>Risk group 4</td>
<td>14</td>
<td>18.2 (7.8–28.8)</td>
<td>0.18–0.66</td>
</tr>
<tr>
<td>Reference group</td>
<td>94</td>
<td>20.1 (6.4–43.2)</td>
<td>0.11–1.35</td>
</tr>
</tbody>
</table>
these factors, however, unjustified conclusions may be drawn. In the present paper standardised ChE activities of employees in one risk group (group 3, low risk for exposure to organophosphorus insecticides) were significantly lower than in other groups and subgroups. To examine the question "Were workers in risk group 3 subjected to exposure to ChE inhibitors?" ChE specific catalytic activities (ChE activities relative to ChE substance concentrations) of all 410 subjects were compared. Table 6 shows ChE specific catalytic activities by ChE-1 phenotype, which is the only physiological factor known to influence this quantity. The specific catalytic activity of subjects in risk group 3 equals those of the other groups and subgroups. As inhibition of ChE activity by organophosphorus insecticides (phosphorylation of the catalytic active site) does not influence the plasma turnover rate, it was concluded that risk group 3 was not subjected to disproportionate exposure.

In health surveillance programmes, changes in plasma ChE activity are used to indicate occupational exposure of the person to organophosphorus insecticides. In accordance with earlier studies (each based on 22–24 subjects) the present study, including a more detailed analysis of the reference group shows substantial intraindividual variations of plasma ChE activity even in healthy unexposed persons. The magnitude of such normal variations are crucial in diagnostic decision making and for defining appropriate analytical goals. Analytical imprecisions of the actual ChE activity assay are immaterial compared with the biological intraindividual variations. Hence, analytical improvements will not significantly reduce the observed intraindividual variations, and strategies of health surveillance programmes are forced to accept such large variations in plasma ChE activity. According to the British Health and Safety Executive persons should be medically examined “if, during routine monitoring, plasma ChE activity has been shown to have fallen by more than 30% of pre-exposure levels.” This strategy, which corresponds with the intraindividual variation of ChE activity found in the present study (only four of the 318 employees showed intraindividual variations higher than 30%), might be replaced by a strategy based on variations of ChE specific activity that within healthy persons exhibit insignificant biological intraindividual variations. Unfortunately, the actual analytical imprecision of the ELISA assay for ChE substance concentrations (CV = 5–5.5%) so far eliminates most of the theoretical advantages of a strategy based on this quantity (table 7).

Until a real improvement in analytical precision of the ChE substance assay is achieved, ChE specific catalytic activity will be of limited value when dealing with minor intraindividual changes of ChE activity. When comparing groups and subgroups within a health surveillance programme and when unexpected large intraindividual variations in plasma ChE activity are to be interpreted, measurement of plasma ChE specific activity must be considered as a rational diagnostic tool.

I express my gratitude to Professor Rud Keiding of the University of Aarhus for inspiring discussions and encouraging support. All members of the participating staff at the Department of Clinical Chemistry, Randers Centralhospital, and the local Health Service, Cheminova, are acknowledged for their most professional and enthusiastic efforts. The Hormone Department, Statens Serum Institut, Copenhagen (head: B Nørgaard-Pedersen) is acknowledged for supplying the monoclonal antibody HAH 2–1 against human ChE. The study was financially supported by Aarhus University Research Foundation.

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Plasma cholinesterase activity and substance concentration in employees of an organophosphorus insecticide factory

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