Changed fatty acid composition in platelets from workers with long term exposure to organic solvents

H BEVING, R MALMGREN, P OLSSON

From the Department of Experimental Surgery, Thoracic Clinics, Karolinska Hospital, 104 01 Stockholm, Sweden

There are several indications that long term occupational exposure to organic solvents is harmful to health. Organic solvents have haematotoxic effects and may cause structural changes in platelet membranes. On the other hand, symptoms such as depression, forgetfulness, headache, and nausea suggest a damage at the neuronal level in the central nervous system. Direct evidence for a lesion within the brain can only be obtained in animal studies. In man platelets have been shown to serve as models for the serotonergic neurons. The analogy is founded on a proposed origin from the embryonic ectoderm and the similarities in uptake and storage of serotonin.

We have reported that occupational exposure to organic solvents is associated with an increase in the maximal uptake capacity [Vmax] of serotonin in platelets. Since alterations in platelet membrane lipids may influence membrane related functions, the aim of the present study was to investigate whether exposure to solvent vapours from paints had any impact on the fatty acid composition of platelet membranes.

Materials and methods

We studied nine men aged 22-47 and three women aged 23-64 who were exposed to solvents in a paint manufacturing plant. The solvent mixture in the paint consisted of 70% white spirit, 30% aromatic hydrocarbons and various acid esters. The exposure level of solvents was estimated as moderate. Five men aged 20-32 and 14 women aged 19-50 with clerical occupations acted as controls. The exposed group was similar to the control group regarding general health and background variables such as alcohol and tobacco consumption.

Blood sampling and platelet preparation

Blood (100 ml) was drawn from an antecubital vein using a Wassermann cannula (1.2 mm inner diameter) and anticoagulated with disodium ethylenediamine tetra-acetate (0.077 mol/l; 1 ml/9 ml blood) in polyethylene test tubes. Platelet rich plasma (PRP) was obtained by centrifuging the blood at 190 x g for 15 minutes at room temperature (Beckman TJ-6, Beckman Instrument Inc, Irvine, CA). Aliquots of PRP (10 ml) were centrifuged at 6700 x g for 20 minutes at room temperature. The platelet pellet was washed three times with 1 ml of 0.9% saline solution and frozen at −20°C. Analyses of the frozen platelet pellets were performed within a month of storage.

Extraction of lipids and preparation of fatty acid esters

The total lipids in the pellets were extracted with 15 ml chloroform-methanol 2:1 for one hour at 30°C. Nitrogen was blown into the test tubes during the extraction to prevent oxidation. The chloroform phase was washed twice with 10 ml of water and the chloroform was thereafter evaporated to dryness at 30°C under a stream of nitrogen.

The phospholipids were separated from the neutral lipids by thin layer chromatography on silica gel coated glass plates (Silica Gel 60 F 254 S, 20 x 20 cm, Merck, Darmstadt, FRG). The plates were processed at room temperature. The mobile phase was chloroform-methanol-water 65:25:4. The position of the zones, containing neutral lipids and phospholipids, on the plates were identified by exposing the edges of the plates to iodine vapours. The zones were scraped off the plates and treated with hydrochloric acid (3 ml; 3 mol/l) in methanol at 80°C for two hours. After methylation, the phospholipids were extracted with 3 ml of hexane and the neutral lipids were extracted with 3 ml of chloroform. The organic phases were separated from the silica gel by centrifugation and washed consecutively with water (1 ml), sodium carbonate (1 ml; 0.1%), and water (1 ml). The samples were evaporated to dryness under a stream of nitrogen and redissolved in 5 μl of hexane.

Gas chromatographic analysis of fatty acid esters

The fatty acid methyl esters were separated on a fused
silica capillary column (Cyanosiloxane 60) with hydrogen as carrier gas. A temperature programme of 180–240°C and 2.5°C/min was set. The injection temperature of the system (Shimadzu GC-Mini 3) was set to 300°C and the temperature of the flame ionisation detector was 300°C. The injected sample volume was 2 µl. Each sample was analysed in duplicate and the mean was taken as the sample value. An electronic integrator (Hewlett-Packard 3390 A) was used for evaluating the peak area. The proportion in per cent of fatty acids was calculated as the ratio between the peak area of the acid and the total peak area for the five fatty acids analysed in the phospholipid fraction or the six fatty acids in the neutral lipid fraction.

Results

The fatty acids quantified in the phospholipid fraction were: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and arachidonic (20:4) acids (table 1). In the phospholipid fraction of platelets from the workers exposed to solvents the proportion of oleic acid was higher (p < 0.01) and that of arachidonic acid was lower (p < 0.05) compared with the control group.

In the neutral lipid fraction the quantified fatty acids were: myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), and linoleic (18:2) acids (table 2). The exposed group had a higher proportion of myristic acid (p < 0.01) and oleic acid (p < 0.001) and a lower proportion of palmitoleic acid (p < 0.05) in their platelets compared with the controls.

Table 1  Fatty acid composition (%) in the phospholipid fraction of platelets from workers exposed to organic solvents and from unexposed controls. (Mean and SD are shown)

<table>
<thead>
<tr>
<th></th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 19)</td>
<td>25-2</td>
<td>22-1</td>
<td>19-4</td>
<td>8-0</td>
<td>25-4</td>
</tr>
<tr>
<td>Exposed (n = 12)</td>
<td>2-8</td>
<td>2-8</td>
<td>2-8</td>
<td>9-0</td>
<td>3-8</td>
</tr>
<tr>
<td>Two tailed t test</td>
<td>NS</td>
<td>NS</td>
<td>p &lt; 0.01</td>
<td>NS</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

The quotients of saturated fatty acids in platelets in relation to unsaturated fatty acids were increased in both the phospholipid and the neutral fractions in the solvent exposed group compared with the proportions of saturated and unsaturated fatty acids in the two fractions respectively in the platelets from the control group.

Discussion

The platelet lipid composition is basically determined by the megakaryocyte. Platelets are nevertheless capable of synthesising fatty acids de novo or by chain elongation and also to integrating fatty acids from blood plasma.

Dietary changes in fatty acid intake are rapidly reflected by changes in the fatty acid composition of the platelet membrane. Essential fatty acids that cannot be synthesised in vivo such as arachidonic acid (20:4) particularly reflect the dietary intake. The present study indicates that occupational exposure to organic solvents alters the fatty acid composition of the membrane lipids in the platelet. Whether this alteration is caused by an impact on the megakaryocyte, or by a direct effect on fatty acid synthesis and incorporation in the circulating platelet is uncertain.

The phospholipid matrix is the backbone of the cell membrane. The activity of membrane bound enzymes modulates the movements of uptake receptors, and the passive permeability of solutes across the membrane are affected by the membrane fluidity. Hence, any alteration in the membrane composition of the lipid fatty acids affecting membrane fluidity may change basic cellular functions.

Platelets are good models for presynaptic re-uptake of serotonin, and a lowered Vmax for the platelet serotonin uptake in affective disorders is probably associated with a lowered serotonin turnover in the brain. We have found that occupational exposure to organic solvents results in an increase in Vmax for the platelet serotonin uptake. This may indicate that exposure to organic solvents causes an increased turnover of serotonin, in the brain, by analogy with the effects of toluene exposure in rats, where an enhancement of the serotonin concentrations in the brain was shown.

The alteration in lipid fatty acid composition found in the present study in platelets from solvent workers may reflect a similar change taking place in serotonergic neurons in the central nervous system. The finding that the content of saturated fatty acids was increased in relation to the unsaturated fatty acids in the phospholipid fraction may reflect the change in phospholipid composition of the platelet membranes of exposed workers.

Table 2  Fatty acid composition in the neutral lipid fraction in platelets from exposed workers and unexposed controls. (Mean and SD are shown)

<table>
<thead>
<tr>
<th></th>
<th>14:0</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 14)</td>
<td>4-6</td>
<td>23-8</td>
<td>33-8</td>
<td>12-6</td>
<td>14-2</td>
<td>10-0</td>
</tr>
<tr>
<td>Exposed (n = 5)</td>
<td>4-2</td>
<td>26-4</td>
<td>27-1</td>
<td>11-0</td>
<td>19-1</td>
<td>10-2</td>
</tr>
<tr>
<td>Two tailed t test</td>
<td>p &lt; 0.01</td>
<td>NS</td>
<td>p &lt; 0.05</td>
<td>NS</td>
<td>p &lt; 0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>
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References


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