Determination of plasma malondialdehyde-like material and its clinical application in stroke patients

MT SANTOS, J VALLES, J AZNAR, AND J VILCHES

From the Research Centre and Department of Clinical Pathology, Ciudad Sanitaria ‘La Fe’, Valencia, Spain

SUMMARY Plasma malondialdehyde-like material (MDA-LM) was evaluated in 138 normal subjects and in a group of 57 stroke patients using a modification of the method of Smith et al. (1976). The basal level of MDA-LM in the control group was 35 μmol/l with a range of 22-50 μmol/l. Values above 50 μmol/l were found in 80% of the patients suffering from subarachnoid haemorrhage, in 68% of those with cerebral thrombosis, and in 17% with transient ischaemic attacks. None of the patients with cerebral embolism, intracerebral haematoma, or lacunar infarct had values above 50 μmol/l. Significant statistical differences were found between the control group and all the patients except those with lacunar infarcts.

The laboratory techniques generally used in the diagnosis of thrombosis are based on changes in coagulation and fibrinolytic activity, and on the measurement of platelet-released products in plasma as an index of platelet activation. Although the fibrinopeptide A assay is more sensitive than evaluation of the platelet-released products in the detection of hypercoagulable states and intravascular thrombosis, in some conditions, for example, arterial thrombosis, its measurement could be less meaningful. Platelet factor 4 (PF4) and β-thromboglobulin (βTG) tests for the diagnosis of intravascular thrombosis require radioimmunoassay techniques which are not available in all laboratories. PF4 evaluation of antiheparin activity has low specificity. Therefore we decided to look for other simple techniques to detect platelet activation in vivo.

Thrombin and other inducers of platelet aggregation release arachidonic acid from the phospholipids of platelets. This acid is later metabolised by a cyclo-oxygenase, resulting in cyclic endoperoxides (PGG2, PGG2) which are then rapidly converted to stable prostaglandins (PGE2, PGF2, PGD2), thromboxanes, C15-hydroxyacid (HHT), and malondialdehyde (MDA). MDA determination has been suggested as a convenient assay for the evaluation of platelet function, as a method to monitor the action of new antiplatelet drugs, as a useful parameter for the control of aspirin therapy, and as a simple nonradioisotopic technique to determine the platelet life-span.

However, MDA-like material (MDA-LM) is not related only to platelets, since MDA is formed in the course of prostaglandin biosynthesis in various tissues and during the nonenzymatic autooxidation of polyunsaturated fatty acids. Lipid peroxidation is thought to be involved in various pathological conditions, such as damage to cells and lungs by air pollution, some phases of atherosclerosis, and some forms of liver injury. An increase in plasma MDA was found in both clinical and experimental chronic inflammatory processes as well as in β-thalassaemia major. Therefore, one cannot assume that an increase in plasma MDA-LM should necessarily be related to a platelet activation process, although an increase in plasma MDA-LM has been detected in experimental stroke and in patients with the sequelae of cerebrovascular disorders.

Owing to the clinical difficulties sometimes found not only in the detection of thromboembolism but also in the differential diagnosis of the various types of stroke patient, we decided to study plasma MDA-LM in those patients to see if there is a correlation between plasma MDA-LM concentration and the aetiology of stroke.

Material

The study comprised 138 normal subjects and 57 patients admitted to hospital with a diagnosis of
stroke. All the patients underwent clinical evaluation and the following routine tests: electroencephalogram, lumbar puncture, dynamic and static brain scintigraphy, and computerised tomography. Angiography was performed only in selected cases. The patients were classified according to previously established criteria\textsuperscript{25,26} into one of the following groups: transient ischaemic attack (12), brain infarct secondary to large artery thrombosis (22), lacunar infarct (6), cerebral embolism (6), intracerebral haematoma (6), and subarachnoid haemorrhage (5).

Venous blood was obtained within four days of the onset of the acute event, mixed with 3·8\% sodium citrate in a ratio of 9:1 v/v and centrifuged for 15 minutes at 1000 \( g \) to obtain platelet poor plasma (PPP). MDA-LM was determined by a modification of the method of Smith et al.\textsuperscript{9} 1 ml of 100\% w/v trichloroacetic acid in 0·6 M hydrochloric acid and 0·2 ml of thiobarbituric acid (TBA) reagent were added to duplicates of 0·1 ml PPP in 0·45 ml of isotonic saline. The TBA reagent was prepared as in Smith's method.\textsuperscript{8} After thorough agitation in a vortex mixer, the samples were heated for 30 minutes in a boiling water bath. After cooling to room temperature, the samples were diluted with 2 ml of distilled water, agitated, and centrifuged in order to obtain a clear solution. The optical density of the pink chromogen was read at 532 nm in a double-beam Beckman Acta III spectrophotometer.

The trichloroacetic acid concentration, the plasma volume as well as the time of the bath were chosen so that the optical density was between 0·1 and 0·2. With these conditions the chromogen was stable for at least 2 hours.

To quantify the plasma MDA-LM, calibration curves were made with MDA standard in isotonic saline using appropriate dilutions of an MDA stock solution. The molar extinction coefficient was 1·5 \( 10^5 \) (SD = 8·66, \( n = 66 \)) of that from saline solution; this was taken into account when evaluating the samples. The intra-assay coefficient of variation was 6\%. The day-to-day coefficient of variation was 11\%. The plasma values of MDA-LM from the normal subjects were arranged as a percentile cumulative frequency (Figure). Student's \( t \) test was used as the statistical method.

During the experimental study the laboratory technicians were unaware of the patients' diagnoses, and clinicians who made the diagnoses were unaware of the MDA-LM results when grouping the patients.

Results

The mean value of plasma MDA-LM in the control group was 35 \( \mu \text{mol/l} \) (\( n = 138 \)), range 22-50 \( \mu \text{mol/l} \). The plasma MDA-LM was found to be above 50

**Table 1 Plasma MDA-LM concentrations in patients and controls**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>Concentration of MDA-LM (( \mu \text{mol/l} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \bar{X} )</td>
</tr>
<tr>
<td>Controls</td>
<td>138</td>
<td>35·11</td>
</tr>
<tr>
<td>Transient ischaemic attack</td>
<td>12</td>
<td>42·75</td>
</tr>
<tr>
<td>Intracerebral haematoma</td>
<td>6</td>
<td>43·83</td>
</tr>
<tr>
<td>Subarachnoid haemorrhage</td>
<td>5</td>
<td>49·31</td>
</tr>
<tr>
<td>Cerebral embolism</td>
<td>6</td>
<td>42·16</td>
</tr>
<tr>
<td>Lacunar infarct</td>
<td>6</td>
<td>35·5</td>
</tr>
<tr>
<td>Brain infarct</td>
<td>22</td>
<td>52·05</td>
</tr>
</tbody>
</table>

**Table 2 Percentage of subjects with MDA-LM values \( \geq 44 \) \( \mu \text{mol/l} \) (\( P_{95\%} \)), \( \geq 47 \) \( \mu \text{mol/l} \) (\( P_{97\%} \)), \( \geq 50 \) \( \mu \text{mol/l} \) (\( P_{100\%} \))**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Concentration of MDA-LM (( \mu \text{mol/l} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>44</td>
</tr>
<tr>
<td>Controls</td>
<td>6</td>
</tr>
<tr>
<td>Transient ischaemic attack</td>
<td>28</td>
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<tr>
<td>Intracerebral haematoma</td>
<td>20</td>
</tr>
<tr>
<td>Subarachnoid haemorrhage</td>
<td>30</td>
</tr>
<tr>
<td>Cerebral embolism</td>
<td>50</td>
</tr>
<tr>
<td>Lacunar infarct</td>
<td>86</td>
</tr>
</tbody>
</table>

\( \text{MDA-LM} \) = 5,4-dihydroxy-\( \text{2,3-dimethyl-3,4-dihydrophthalic acid} \) 5,4-DHMDA.
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µmol/l in 80% of the subarachnoid haemorrhage and in 68% of the large artery thrombosis patients. On the other hand, only 17% of the patients with transient ischaemic attacks and none of those suffering from embolism or lacunar infarct exceeded the 50 µmol/l limit (Figure; Tables 1 and 2). All the patients, except for the lacunar infarct group, had a moderate but significant increase in MDA-LM in plasma when compared to the control group.

Discussion

The thiobarbiturate assay gave colour not only in the supernatant but also in the protein precipitate obtained by the addition of trichloroacetic acid. Therefore, in our method, we evaluated the MDA-LM in both fractions, unlike other authors who determined the MDA-LM only in the supernatant or in the precipitate. The higher plasma MDA-LM values obtained in our method, as compared to those of other authors, may be due to this.

In the control group, none of the subjects had a plasma MDA-LM above 50 µmol/l. We found a plasma MDA-LM greater than 50 µmol/l in 80% of patients with subarachnoid haemorrhage, in 68% of those with cerebral thrombosis, and in 17% of those suffering from transient ischaemic attacks. On the other hand, all the patients with intracerebral haematoma, lacunar infarct, or embolism had values below that limit. Therefore, an increase in the plasma MDA-LM above 50 µmol/l, excluding subarachnoid haemorrhage patients, strongly suggests a cerebral thrombosis in stroke patients, although a lower value does not rule out this possibility.

The difference in the plasma MDA-LM values found in patients with subarachnoid haemorrhage and intracerebral haematoma is interesting and could help in the differential diagnosis of both phenomena, which is sometimes difficult to establish by clinical means. The plasma MDA-LM level and the cerebral damage seem to be unrelated since a positive correlation could not be found between the plasma MDA-LM and the extent of the damage diagnosed by computerised tomography. Also, in some patients in whom cerebral damage was to be expected, normal plasma MDA-LM values were found.

The fact that MDA-LM was elevated several days after the onset of the thrombotic event is difficult to explain at this time since the metabolism of plasma TBA-reactive products is not completely understood. On the other hand, it is known that TBA-reactive materials may alter some cell structures, especially proteins, and they may modify the function of some organs as well as play a role in some chronic pathological conditions such as atherosclerosis. It has also been shown recently that lipid peroxides selectively inhibit PG12 synthesis, a substance with antiaggregant and vasodilator action in vivo. Hence increased levels of plasma MDA-LM could indicate a tendency to intravascular thrombosis.

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Requests for reprints to: Dr Justo Aznar Lucea, Ciudad Sanitaria de la Seguridad Social 'La Fe', Research Center and Department of Clinical Pathology, Valencia, Spain.
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M T Santos, J Valles, J Aznar and J Vilches

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