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

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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.1 Although the fibrinopeptide A assay2 is more sensitive than evaluation of the platelet-released products in the detection of hypercoagulable states and intravascular thrombosis,3 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 radiolunoassay techniques4 which are not available in all laboratories. PF4 evaluation of antiheparin activity has low specificity.5 Therefore we decided to look for other simple techniques to detect platelet activation in vivo.

Thrombin and other inductors of platelet aggregation release arachidonic acid from the phospholipids of platelets.6 This acid is later metabolised by a cyclo-oxygenase, resulting in cyclic endoperoxides (PGG2, PGH2) which are then rapidly converted to stable prostaglandins (PGE2, PGF2, PGD2), thromboxanes, C15-hydroxyacid (HHT), and malondialdehyde (MDA).7,8 MDA determination has been suggested as a convenient assay for the evaluation of platelet function,9-11 as a method to monitor the action of new antiplatelet drugs,7,12 as a useful parameter for the control of aspirin therapy,13 and as a simple nonradioisotopic technique to determine the platelet life-span.14

However, MDA-like material (MDA-LM) is not related only to platelets, since MDA is formed in the course of prostaglandin biosynthesis in various tissues15 and during the nonenzymatic autoxidation of polyunsaturated fatty acids.16 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.17,18 An increase in plasma MDA was found in both clinical19 and experimental20 chronic inflammatory processes as well as in β-thalassaemia major.21 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.22,23

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,24 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 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. 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. 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. The recovery of MDA from plasma was 77% (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 μmol/l (n = 138), range 22-50 μmol/l. The plasma MDA-LM was found to be above 50

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>Concentration of MDA-LM (μmol/l)</th>
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<tbody>
<tr>
<td></td>
<td>X</td>
<td>SD</td>
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<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>
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<td>Intracerebral haematoma</td>
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<td>43-83</td>
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<td>Subarachnoid haemorrhage</td>
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<td>49-31</td>
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<td>Cerebral embolism</td>
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<td>42-16</td>
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<tr>
<td>Lacunar infarct</td>
<td>6</td>
<td>35-5</td>
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<tr>
<td>Brain infarct</td>
<td>22</td>
<td>52-05</td>
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**Table 1 Plasma MDA-LM concentrations in patients and controls**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Concentration of MDA-LM (μmol/l)</th>
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<tr>
<td></td>
<td>44</td>
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<td>Controls</td>
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<tr>
<td>Transient ischaemic attack</td>
<td>58</td>
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<td>Intracerebral haematoma</td>
<td>50</td>
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<tr>
<td>Subarachnoid haemorrhage</td>
<td>80</td>
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<tr>
<td>Cerebral embolism</td>
<td>50</td>
</tr>
<tr>
<td>Lacunar infarct</td>
<td>0</td>
</tr>
<tr>
<td>Brain infarct</td>
<td>86</td>
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</tbody>
</table>

**Table 2 Percentage of subjects with MDA-LM values ≥ 44 μmol/l (P95%), ≥ 47 μmol/l (P97%), ≥ 50 μmol/l (P100%)**

*Figures [Graph not provided]*

*Plasma MDA-LM levels of stroke patients. The hatched area represents the cumulative frequency of the control group: p = percentile; DL = discriminatory limit.*
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It
also been shown recently that lipid peroxides
selectively inhibit PG12 synthesis,28 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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