Organophosphates and monocyte esterase deficiency

E McClean, H Mackey, G M Markey, T C M Morris

Abstract

Aims—To examine the possibility that monocyte esterase deficiency (MED) could be caused by exposure to organophosphates.

Methods—Pseudocholinesterase, paraoxonase and arylesterase activities were measured in the serum and acetylcholinesterase activity was measured in the red cells of a group of monocyte esterase deficient subjects and compared with the enzyme activities of a control group of monocyte esterase positive subjects.

Results—No significant difference was found between the enzyme activities of the monocyte esterase deficient group and the control group for any of the esterases investigated.

Conclusion—Current or recent exposure to organophosphorus is not the cause of MED.

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Keywords: Monocyte esterase deficiency, organophosphates, neoplastic disease.

Monocyte esterase is an enzyme unique to cells of monocytic lineage.1 It is characterised in vitro by a positive reaction to the non-specific esterase cytochemical stain2 based on its ability to de-acylate naphthyl acetate or butyrate and also identified by its specific isoelectric focus on polyacrylamide gels.3 Using an automated cytochemistry analyser, it has been previously demonstrated that monocyte esterase deficiency (MED; defined as the consistent presence of >85% esterase negative monocytes in a subject’s peripheral blood) occurs in 0.8% of blood donors (four of 474) and significantly more frequently in patients with immunoproliferative (7.4%; 18 of 243) and gastrointestinal neoplastic disease (10%; 12 of 120).4,5 A 35% incidence of MED has also been shown in 15 families of patients with MED6,7 but a genetic basis for the familiality has not been found as yet. Linkage of MED to the chromosomal site (16q32.1)8 of a gene for monocyte esterase, HMSE1, has been excluded (manuscript in preparation; Dr K Ennis and Dr A Hughes, Department of Medical Genetics, Belfast City Hospital).

Lee and Waters,9 using the same make of cytochemistry analyser, showed that the activity of monocyte esterase was diminished in blood samples taken in the presence of traces of organophosphate insecticide and subsequently extreme exposure to organophosphates was demonstrated to diminish enzyme activity in vivo, also using the same analyser.10,11 Monocytes with either constitutional12 or acquired13 esterase deficiency manifest impaired tumour cell killing in vitro. Study of the occupational activity of subjects with MED has not revealed any evidence of exposure to organophosphates. Nevertheless, because of the association of the deficiency with malignant neoplastic disease and the suspicion that exposure to organophosphate might be a factor in lymphoma genesis,14 we felt it was important to prove/disprove any possibility of current exposure in our MED cohort, by examining other esterases, red cell cholinesterase and serum pseudocholinesterase, the activities of which are reduced in the presence of organophosphates and also to compare paraoxonase activities and phenotypes in normal subjects and in those with MED.

Methods

Blood was collected from 11 subjects with MED (identified in previous studies4,6,7 and who continued to manifest the deficiency) and from 11 subjects who had normal monocyte esterase activity. Serum was stored at −70°C and analysed for paraoxonase activity, with and without NaCl stimulation. The defrosted samples were then stored at 4°C and analysed for pseudocholinesterase and arylesterase activity within 48 hours. Two of the samples from the MED group were insufficient for pseudocholinesterase estimation. The paraoxonase phenotype was determined using the values obtained for paraoxonase and arylesterase activity. Washed red cells from the subjects with MED were stored at −20°C for analysis of red cell acetylcholinesterase activity.

MATERIALS

Paraaxon, phenylacetate, 4-nitrophenylacetate, acetyl-(methyl)thiocholine and 5,5'-dithio-bis(2-nitrobenzoic acid) were all supplied by Sigma Chemical Company Ltd. (Poole, Dorset, UK). The serum cholinesterase kit was supplied by Boehringer Mannheim UK (Lewes, Sussex, UK).

* Paraaxon is a very hazardous substance and must be handled with extreme care, use of a fume cupboard and protective clothing when making up the substrate is mandatory. However, it is completely inactivated in strong alkali (50% NaOH) which should be used for soaking all consumables used in the assay before disposal.
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Table 1  Comparison of cholinesterase and paraoxonase activities in subjects with MED and controls. Values expressed as range (median; quartiles)

<table>
<thead>
<tr>
<th></th>
<th>MED</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal paraoxonase activity (nmol/min/ml) (n=11, 11)</td>
<td>80-227</td>
<td>34-295</td>
<td>0.92</td>
</tr>
<tr>
<td>Salt stimulated paraoxonase activity (nmol/min/ml) (n=11, 11)</td>
<td>91-477</td>
<td>60-452</td>
<td>0.32</td>
</tr>
<tr>
<td>Pseudocholinesterase (KU/l) (n=9, 11)</td>
<td>4-4-6-9</td>
<td>3-4-7-0</td>
<td>0.13</td>
</tr>
<tr>
<td>Acetylcholinesterase (KU/l) (n=11, 90)</td>
<td>7-0-9-9</td>
<td>5-4-11-9</td>
<td>0.86</td>
</tr>
</tbody>
</table>

n = number of subjects in each group (MED, control).
* Contains two lipemic samples which had basal paraoxonase activities of 34 and 43 nmol/min/ml and salt stimulated activities of 60 and 76 nmol/min/ml.

SERUM STUDIES

Cholinesterase activity

The serum cholinesterase method was based on that of Eillman et al,16 using butyrylthiocholine iodide (7 mmol/l) as substrate. The reaction was monitored at 405 nm. The established reference range for this laboratory is 3-0-9-0 KU/l.

Arylesterase activity

Arylesterase activity (mol/min/ml) was measured using the fixed incubation method of Lorentz et al.17 The reaction was carried out at 25°C using a TRIS acetate buffer (100 mmol/l, pH 7.5) and phenyl acetate (10 mmol/l) as substrate. The reaction was monitored at 546 nm.

Paraoxonase activity

Serum paraoxonase activity (nmol/min/ml) was measured at pH 8-0 using the method described by Mackness and Walker,18 incorporating a 10 mM TRIS acetate buffer containing 2 mM calcium chloride. The rate of hydrolysis of paraoxon was assessed by measuring the liberation of p-nitrophenol at 25°C. The reaction was monitored at 412 nm without (basal activity) and with NaCl (salt stimulated activity) added to the substrate.

Paraoxenase phenotyping

The samples were phenotyped using the equations below.

1. Per cent stimulation of paraoxonase activity by 1 M NaCl calculated as follows:

   \[
   \text{Paraoxonase activity with 1 M NaCl - Basal paraoxonase activity} \times 100 \%
   \]

   Sixty per cent stimulation is used as the dividing point between the non-salt stimulated A phenotype (<60%) and the salt stimulated B phenotypes (>60%).19 This method cannot distinguish between B and AB phenotypes.

2. Paraoxonase to arylerase ratio:

   \[
   \text{Paraoxonase activity with 1 M NaCl} / \text{Arylerase activity}
   \]

   The A phenotype was taken as a P:A ratio of <2.5 x 10⁻¹, the AB phenotype as a ratio of 2.5-5 x 10⁻¹ and the B phenotype as >5 x 10⁻¹.20

Red cell cholinesterase (acetylcholinesterase)

Red cell cholinesterase was measured using a method based on that of Eillman et al16 and adapted for use on the COBAS FARA (Roche Products Ltd, Welwyn Garden City, UK). The washed packed cells were lysed with saponin (50 μl cells in 1 ml saponin (100 mg/ml)). Acetyl(-methyl)thiocholineiodide (7 mM/l) in a 0.1 M phosphate buffer (pH 7-2) was used as substrate21 and the thiocholine produced reacted with dithionitrobenzoate to give a yellow colour which was monitored at 412 nm. The reaction was carried out at 37°C. Ninety EDTA blood samples collected from a hospital and general practice population gave a range of 5.4-11.9 KU/l (median 8.1 KU/l, quartiles 7.5 KU/l and 8.8 KU/l).

STATISTICS

The Mann-Whitney U test was used to compare the results of MED samples with those of the controls.

Results

Basal paraoxonase, salt stimulated paraoxonase, serum cholinesterase, and red cell cholinesterase activities are presented in table 1. Subjects with MED were not significantly different from the control group for any of the enzymes measured. The phenotypes of the MED group were similar to the control group, as shown in tables 2a and 2b.

Discussion

Red cell cholinesterase, serum pseudo-cholinesterase and monocyte esterase are each diminished in vivo following exposure to organophosphate compounds. Monocyte esterase activity recovers rapidly when exposure ceases.10 22 This investigation has proved that MED in our subjects was not caused by either current or recent exposure to organophosphate compounds because we found no difference in the activities of serum or red cell cholinesterases between our MED cohort and the control group. The paraoxonase phenotype was the same for both the control and MED groups. We found more of the non-A phenotypes in both groups than has been previously reported,31 34 but the number of subjects included in the study is small; the discrepancy in the number of A phenotypes shown in the control group by the two methods was probably be-

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Table 2a  Paraoxonase phenotype as determined by per cent stimulation with NaCl

<table>
<thead>
<tr>
<th>Phenytype</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>MED</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 2b  Paraoxenase phenotype as determined by salt stimulated paraoxenase activity/arylerase activity

<table>
<thead>
<tr>
<th>Phenytype</th>
<th>A</th>
<th>AB</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>MED</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Controls</td>
<td>6</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>
cause of the presence of two lipaemic serum samples giving a low basal paraoxonase activity. Newcombe has suggested that exposure to organophosphates might add to lymphoma-genic stress in subjects with MED. Certainly, chromosomal changes have been described in workers who are exposed to high levels of organophosphates and there is suggestive epide-miological evidence of an excess of lymphoma amongst these subjects. As it is possible to deduce from the reduction in the activities of plasma and red cell cholinesterases, and monochrome and monochrome esterases on exposure to organophosphates that these enzymes have a role in the detoxification of organophosphates, then it is reasonable to suggest that a constitutional lack of one of them might diminish the detoxification capacity of affected individuals and expose them to the deleterious effects of these compounds. However, an increase in the other normally occurring esterases might compensate for the effect of a single deficiency. Amplification of an esterase gene leading to organophosphate resistance has been described in insects and it has been suggested that omnivorous and herbivorous birds have developed a wider range of esterases than carnivorous birds in order to detoxify organophosphates which can contaminate plant foods. In this context, we have often noted unusually large and frequent esterase positive "dots" in lymphocytes of subjects with MED under standard staining conditions (unpublished observation). Alternatively, mutation may alter enzyme sensitivity to inhibitors. Pseudocholinesterase genes are subject to frequent point mutations and it has been suggested that this may represent a host selection response to ecological danger from choline esterase inhibitors. One small study, however, has shown that variant pseudocholinesterases do not appear to have altered sensitivity to organophosphates. The molecular basis for MED is not known as yet, but it is probable that a lack of esterase activity is because of the presence of variant enzyme(s) which may in fact be equally sensitive/resistant to organophosphates. The suggestion that subjects with MED might be more susceptible to the ill effects of organophosphates may not be tenable. It is also possible that the major bodily function of monochrome esterase is unrelated to the properties of the active site which are recog-nised by the cytochemical esterase stain, but the accumulating evidence suggests that monochrome esterase function is at least in-fluenced by the activity of this site. This study has shown, however, that exposure to organophosphates is not the cause of the inactivity of the site in our subjects.

The authors would like to thank Dr Michael Mackness for his help with the paper and for allowing us to use his laboratory facilities to measure the paraoxonase activity of the samples. We also thank Miss C Shiel and Mrs M Ferris for their expertise in preparing the manuscripts.

18 Eckerson HW, White CM, La Du BN. The human serum paraoxonase/esterase polymorphism. Am J Hum Genet 1985;33:1122-30. The molecular basis for MED is not known as yet, but it is probable that a lack of esterase activity is because of the presence of variant enzyme(s) which may in fact be equally sensitive/resistant to organophosphates. The suggestion that subjects with MED might be more susceptible to the ill effects of organophosphates may not be tenable. It is also possible that the major bodily function of monochrome esterase is unrelated to the properties of the active site which are recog-nised by the cytochemical esterase stain, but the accumulating evidence suggests that monochrome esterase function is at least in-fluenced by the activity of this site. This study has shown, however, that exposure to organophosphates is not the cause of the inactivity of the site in our subjects.

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Job description of MLSOs
Histopathologists and cytologists as a group tend to be obsessive individuals which in our work can be a useful trait but when this is taken to extreme degrees it becomes a disease (common sense is ignored and everyday life is interfered with). This neurotic state is made much more serious when it is encouraged by bodies responsible for maintaining standards, The Royal College and the CPA. When Dr Tim Ashworth wrote to the Io to say that MLSOs should do all or most trimming and even report many histological specimens he caused a furor. When, in my view, the just as extreme idea that they should do nothing apart from reporting branches of medicine there is ever increasing endoscopic biopsy specimens into cassettes, is proposed by the College and policed by the CPA, there is silence. As a grass roots pathologist who (to comply with accreditation requirements) has just waded through a lake of negative urine cytology and sputa without a hint of a malignant cell I feel this silence should be broken.

A competent MLSO should be able to report a negative urine or spatum and pass on a doubtful case to a pathologist; this is surely easier than the screening of cervical smears. The overall responsibility is still the pathologist’s for ensuring that this is done safely but in a service which is consultant based in most non-teaching hospitals it is a waste of consultant time to report this kind of material. I wrote to the CPA recently to confirm their position on this. My/her rank to learn, I quote, that these are “mainly diagnostic specimens” and that the “responsibility for reporting them lies with the pathologist”. This was based on the Authoritative Guidelines for Histopathology Laboratories published by The Royal College of Pathologists in 1989! Are they really mainly diagnostic specimens or are they much more the equivalent of a full blood count carried out when a patient has a particular symptom complex? I think the latter. I know that quite a few of my colleagues at other hospitals are paying lip service to this accreditation requirement. This is dangerous medically, if I put my name to a report it implies that I have looked at the slides carefully.

A lot has changed since 1989. The present policy is bizarre and should be reviewed urgently. Is it right that at a time when in other branches of medicine there is ever increasing delegation of responsibility, even the possibility that nurses will one day carry out endoscopies, we are heading in the opposite direction. I am very worried that pathology in its current state will not be able to cope with the pace of new discoveries, which are after all inevitable even if all urines and sputa were reported by professors of pathology, is losing its way and is increasingly out of step with the rest of medicine. A profession afraid to delegate sensibly will eventually be forced to do so by others.

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Book review

Human Tissue: Ethical and Legal Issues.
Working party on human tissue chaired by Professor Dame Rosalinde Hurley. (Pp 153; £10.00.) Published by and available from The Nuffield Council on Bioethics, 28 Bedford Square, London WC1B 3EG. ISBN 0 9522701 1 0.

Advances in medical treatment, scientific research and biotechnology have highlighted the need for public concern over ethical issues raised by the use of human tissue. For instance, questions have been raised regarding the sale of organs, the patenting of life forms and the commercial exploitation of products derived from the tissues of patients or research projects.

Quite rightly, society expects the human body and its parts to be treated with respect and that human tissue should not be used at will or abused, but in general has welcomed advances resulting in the use of human tissue in therapy, such as transplant surgery, and some of the developments resulting from genetic research. The potential of scientific advances for improvements in patient care is considerable, but raises many ethical and legal questions that affect us all. Some of the ethical challenges will be difficult to handle and there will be different opinions as to how they should be handled within different cultures.

The Nuffield Council on Bioethics decided to establish a working party, under the chairmanship of Professor Dame Rosalinde Hurley, to define the ethical and associated legal questions raised by the medical and scientific uses of human tissue. The terms of reference included current and prospective medical and scientific uses of sub-cellular structures, cells and their products, tissues and organs; to give some account of developments in research and exploitation of tissue, identifying current and potential benefits and difficulties; to identify and define ethical issues and questions of public policy and current practices arising from the use and exploitation of human tissue.

The report clarifies the current provisions of the law and highlights many areas requiring further development of statutory provisions or regulation or may be required. It is clearly written and follows a logical sequence, including introduction, areas of public concern, definition, sources and uses of human tissue, ethical principles and legal matters, and concludes with a list of recommendations. The conclusions and recommendations include advice on the ethical principles in the use of human tissue, legal barriers arising from the use of human tissue, guidance for consent procedures, guidance relating to constraints on commercial transactions, the responsibilities of medical intermediaries such as tissue banks that supply human tissue, the need for the government, together with other member states, to seek the adoption of a protocol to the European Patent Convention relating to patents in the area of human and animal tissue, and advice on safety and quality.

The Working Party concludes that organisation the removal and supply of human tissue along commercial lines is unethical that more should be done to encourage the concern of donors for others in the hope that more will come forward; that when tissue is removed in the course of medical treatment, consent to the treatment should cover any further uses of the tissue; including the registering of tissue banks and monitoring of their activity. Complex issues regarding the removal of tissue from living persons who are deemed legally incompetent and from children are highlighted, the present legality of which is uncertain. The authors recommend that any removal should be exceptional and limited to procedures that pose negligible risk and minimal burden, and that the Law Commission's proposals, which would permit non-therapeutic research on incompetent adults, subject to strict safeguards, should be enacted.

This report is timely and, in addition to its importance to all those involved in the provision of health care, medical research and teaching, should generate widespread interest and debate within the general public. The Working Party’s advice, that relevant professional bodies should now ensure that their professional guidelines clearly establish the responsibilities of the increasing number of their members who will find themselves acting as medical intermediaries involved in the acquisition and supply of human tissue, must be accepted. I hope that this document stimulates worldwide debate involving the general public and, in particular, educational institutions. I strongly recommend it.

W R TIMPERLEY

Notice

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June 16–19 1996
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Organiser: Singapore Association of Clinical Biochemists
Topics for scientific sessions include: enzymology related to malignancy, clinical application of tumour markers, hormones and cancer, new instrumentation/methodology, and new developments in onco-genes and tumour biology.
The Conference will also feature free oral/poster presentation sessions and an industrial exhibition.
Organising Committee Co-Chairmen: Dr It-Koon Tan/Dr Edward Jacob, Department of Pathology, Singapore General Hospital, Singapore. (Tel: (65) 321 4914; fax: (65) 222 6826.)
Secretary: Academy of Medicine, Singapore, 16 College Road #01-01, Singapore 0316. (Tel: (65) 223 8968; fax: (65) 225 5155.)

Correction
Organophosphates and monocytes esterase defficiency (J Clin Pathol 1995;48:768–79). An editorial error occurred in the Arylesterase activity subsection of the Methods section. The units for arylesterase activity are µmol/min/ml and not µmol/mg/min.
On p 768, introduction, column 1, line 21, 16q3:22.1 should read 16q3:22.1.