DETECTION OF MALIGNANT HYPERTERMIA SUSCEPTIBILITY USING A SPIN LABEL TECHNIQUE ON RED BLOOD CELLS

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Malignant hyperthermia (MH) is a rare, but potentially lethal complication of general anaesthesia [1]. An individual is described as MH susceptible if s/he suffered a clinical malignant hyperthermic reaction under general anaesthesia or s/he has undergone special diagnostic tests which determine carrier status of the gene. Currently, the only widely accepted laboratory test for diagnosing MH susceptibility is the in vitro contracture test which is carried out on biopsied skeletal muscle [2].

Using spin probes and electron paramagnetic resonance spectroscopy (EPR), we have demonstrated that halothane fluidizes sarcoplasmic reticulum (SR) membranes isolated from MH susceptible swine [3,4]. Based on the hypothesis that there is a generalized membrane defect in MH [3—6], we have studied red blood cells from MH susceptible swine and humans using this same technique, looking for evidence that halothane might also fluidize red cell membranes. We were able to demonstrate that red blood cell membranes from both MH susceptible patients and swine became more fluid when exposed to halothane [7,8], a change similar to that noted in SR from MH swine. Subsequently we investigated if the technique might be useful as a diagnostic test in detecting MH susceptibility.

SUMMARY

Using spin labelled red blood cells and electron paramagnetic resonance spectroscopy (EPR), we observed that halothane 3 mmol litre⁻¹ produced a much greater decrease in the rotational correlation time of red blood cells from individuals who were at risk for malignant hyperthermia (MH), compared with normals. Subsequently we performed blind tests on 14 individuals whose MH susceptibility status had been determined on the basis of in vitro contracture studies. When compared with the results of the in vitro contracture studies, the EPR studies predicted correctly the patients' status in 13 of the 14 individuals.

PATIENTS AND METHODS

In vitro contracture tests. Patients were judged to be at risk for MH on the basis of in vitro contracture tests undertaken at Calgary on biopsied skeletal muscle using techniques described previously [9].

Blood. Venous blood was collected in Calgary using a 10-ml Vacutainer tube containing sodium heparin (blood volume 8—9 ml). Blood bank anticoagulant (citrate—phosphate—dextrose—adenine) (1.5 ml) was added to the collected blood and air allowed to enter the tube. The ratio of blood to anticoagulant was approximately 6:1. The tubes were packed in a styrofoam box which was kept cool by insertion of two previously frozen Cole-Paks (re-usable artificial ice manufactured by the Canadian Coleman Company, Toronto), and transferred to Philadelphia by Federal Express.

Spin labelling. EPR studies were performed at the University of Pennsylvania, School of Medicine. Upon arrival the following day, blood was
filtered through a small cotton column to remove leucocytes [10]. Red blood cells were wash-centrifuged with a buffer solution (mmol litre\(^{-1}\) composition: NaCl 108, KCl 5, MgCl\(_2\) 1.2, NaHCO\(_3\) 24, sodium phosphate 2.4 (pH 7.4), glucose 30, adenine 1, inosine 1) [11] containing 0.1% bovine serum albumin (BSA).

Washed red blood cells were re-suspended in the buffer solution containing 0.1% BSA to make the haematocrit value 20%. 16-Doxylstearic acid (16-DS) 6 μg ml\(^{-1}\) and ATP 1 mmol litre\(^{-1}\) were added and the mixture incubated at 4 °C for 15 h. The suspension was wash-centrifuged twice with the buffer solution (BSA omitted). The red blood cells were resuspended in the buffer solution, containing added 0.05% BSA, ATP 1 mmol litre\(^{-1}\) and CaCl\(_2\) 3 mmol litre\(^{-1}\), and the haematocrit value was readjusted to 30%.

**Halothane.** Halothane was distilled and dissolved in dimethyl sulphoxide to make stock solutions of 100, 300, 500 and 700 mmol litre\(^{-1}\). Solutions were stored at —20 °C.

**EPR measurement.** To a 100-μlitre red cell suspension (haematocrit 30%), 1 μlitre of a halothane stock solution (in dimethyl sulphoxide) was added and mixed thoroughly. To the control, only dimethyl sulphoxide was added. The mixture was introduced into a glass capillary (i.d. 0.08 mm, length 120 mm) and both ends were flame-sealed. The glass capillary was centrifuged (2000 g, 10 min) to pack the red cells into one end. The capillary was stored in ice water until measurement. Before measurements were made, the capillary was warmed to 37 °C for 10 min, and inserted into the cavity (kept at 37 (0.1) °C) of a Varian E-109 EPR spectrometer with packed cells at the bottom. The EPR spectrum was measured. Correlation time was calculated from the EPR spectrum by the method of Morse, Lusczakoski and Simpson [12].

**Blinded study.** Blood specimens from 14 individuals were transferred to Philadelphia, at random (seven packages of two specimens each during a 6-month period) without identification of MH susceptibility, for study by the spin label technique.

**Statistics.** The statistical significance of the difference between MH and normal red cells was estimated by Student's \(t\) test.

**RESULTS**

**Effects of halothane on the rotational correlation time**

Although the absolute values of the rotational correlation time in the absence of halothane vary from individual to individual, the effect of halothane was clearly different in MH patients and normal subjects (fig. 1). In the case of MH patients, halothane 3 mmol litre\(^{-1}\) decreased the rotational correlation time remarkably. Between 3 and 7 mmol litre\(^{-1}\) the additional decrease was

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**Fig. 1.** Effect of halothane on rotational correlation time of red blood cell membranes. A: Normal subjects; B: MH-susceptible patients. EPR measurements were performed using a Varian E-109 spectrometer at 37 (0.1) °C. Red blood cells were labelled with 16-Doxylstearic acid for 15 h at 4 °C.
TABLE I. Blind screening test for 14 biopsied individuals by EPR method (change in rotational correlation time: $-\Delta t_r$)

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>$-\Delta t_r$ (10$^{-11}$ s)</th>
<th>EPR</th>
<th>Biopsy</th>
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<tbody>
<tr>
<td>1</td>
<td>5.0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>0.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>5.4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>0.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
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<td>8</td>
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</tr>
<tr>
<td>9</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>0.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>7.7</td>
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<td>2.3</td>
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</tr>
<tr>
<td>14</td>
<td>7.0</td>
<td>+</td>
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</tr>
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</table>

less. On the other hand, the decreases of rotational correlation time in normal subjects were small with halothane 3 mmol litre$^{-1}$, and greater between halothane 3 and 7 mmol litre$^{-1}$. From these results, we established the following criteria: an individual would be considered MH positive if the decrease of the rotational correlation time caused by the addition of 3 mmol litre$^{-1}$ was larger than 4.0 x 10$^{-11}$ s, and MH negative if the decrease was less than this value.

**Blind study.** In this study, the decrease in the rotational correlation time caused by the addition of halothane 3 mmol litre$^{-1}$ was measured from 14 patients, and the patients were classified according to the above criteria. Six specimens had values greater than 4.0 x 10$^{-11}$ s and thus were considered to be MH positive (table I). Specimens from eight individuals exhibited values lower than this and were assessed as normal. **In vitro** contracture testing results suggested that seven individuals were MH and seven were normal. Thus with the exception of one MH patient (specimens Nos 13), the result of EPR measurement agreed with the diagnosis made by **in vitro** contracture testing.

After completion of these experiments, the EPR data from the seven biopsied MH positive subjects and the seven biopsied MH negative subjects were plotted. The mean values for the changes in rotational correlation times induced by halothane 3 mmol litre$^{-1}$ in MH susceptible and normal individuals were 5.39 (1.7) ($n = 7$) and 0.37 (0.33) ($n = 7$), respectively (fig. 2)—a statistically significant difference ($P < 0.001$).

**DISCUSSION**

Data shown in figure 1 of this report, in addition to data from earlier studies [7, 8] suggest that the membranes of red blood cells of MH susceptible patients may have a structural abnormality, similar to that of MH pig SR. Both red blood cells and SR membranes were fluidized remarkably with halothane. As halothane also produces an increase in calcium permeability of SR from MH pig [3-6], it is tempting to speculate that this increase in fluidity may be the mechanism underlying the pathogenesis of MH in susceptible subjects. In SR, a halothane concentration of the order of 200 μmol litre$^{-1}$ induced the fluidity change, whereas in red blood cells, the value was 3 mmol litre$^{-1}$. As the anaesthetic concentration of halothane is of the order of 1 mmol litre$^{-1}$, halothane could enhance calcium release in vivo from the SR of skeletal muscle, which might be important in triggering MH. However, at the same concentration, there would be little effect on red blood cells.

Based on these observations, we studied an additional 14 patients prospectively in a blinded fashion. The patients' MH susceptibility status was revealed only when the results of the rotational correlation studies were known. To date, of seven MH susceptible patients, the spin...
probe studies have identified six correctly. All seven normal subjects were categorized correctly as normal.

Many more patients will require testing by this method and the results compared with clinical data and contracture tests before a role can be firmly established for red blood cell/EPR spin probe studies as a diagnostic method for determining MH susceptibility. Caution in interpreting the potential diagnostic usefulness of this type of study is particularly important as, over the years, several reports have appeared suggesting that laboratory tests other than in vitro contracture test were useful for determining MH susceptibility status of patients [13–15], only to be later proven of little or no value [16, 17]. Although we observed that most of the MH patients had abnormal red cell membranes, there may be instances where an individual has abnormal red cell membranes, but is not susceptible to MH; for example, other blood diseases are known also to affect membrane fluidity [18, 19]. Further studies will be required to answer these questions.

Studies by Klip and co-workers [20] have suggested that white blood cells may be useful for the diagnosis of MH susceptibility. It is exciting to speculate that both red and white blood cells may have a role in the identification of individuals who are carriers of the MH gene. If these studies are validated, we may be on the threshold of providing a screen for MH susceptibility without resort to muscle biopsy.

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REFERENCES