Increased Membrane Fluidity of Erythrocytes in a Case with Malignant Hyperthermia

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In a case with malignant hyperthermia (MH), membrane fluidity of erythrocytes was studied by electron spin resonance (ESR)–spin labeling technique. The measurement was made with samples obtained during and after pyrexic crisis until full recovery. The results indicated that membrane fluidity increased during pyrexic crisis and that these changes were serially restored. As the physicochemical characteristics of the erythrocyte membrane resemble those of cellular and cell organella membrane, it was concluded that membrane composition and configuration might have been altered pathophysiologically during the crisis of MH in our case. (Key words: malignant hyperthermia, erythrocyte membrane)

(Kawamoto M et al.: Increased membrane fluidity of erythrocytes in a case with malignant hyperthermia. J Anesth 1:88–92, 1987)

Malignant hyperthermia (MH) is a systemic disease resulting from muscle disorders, usually induced by anesthetic drugs. Specific changes are postulated on the cell membrane in a case with malignant hyperthermia (MH)[1].

Since the morphology of erythrocyte membrane resembles that of cell organella, including sarcoplasmic reticulum, the erythrocyte has been studied with many techniques for the investigation of membrane physiology, such as functional and structural abnormalities in some disorders[2-6]. In this case report, fluidity of erythrocyte membrane was studied with electron spin resonance (ESR) spectroscopy in a case with MH[7] and it was revealed that membrane fluidity was altered during the crisis of MH.

Case Report

A 39-year-old male (165 cm in height and 65 kg in weight) underwent surgery of a herniated lumbar intervertebral disc. He had been in good health and had no history of neuromuscular disorders. After premedication with atropine 0.5 mg and hydroxyzine 50 mg im, general anaesthesia was induced with thiopental 5.0 mg kg^{-1} and succinylcholine 1.0 mg kg^{-1}, and maintained with halothane and N_2O. Hyperthermic crisis developed during the surgery with symptoms due to abnormal hypermetabolism and rectal temperature up to 41°C. After all the anesthetics were discontinued, arterial gas analysis revealed mixed type acidosis: pHa 7.05, $Paco_2$ 58.8 mmHg, base excess -14.3 $mEql^{-1}$ and Pao_2 176.4 mmHg. The patient responded well to treatment with dantrolene 2.0 mg kg^{-1} iv, methylprednisolone 35 mg kg^{-1} iv, fluid therapy and aggressive surface cooling. Neither masseter spasms nor skeletal muscle rigidity was observed throughout the incident. After vital signs and temperature returned to normal, he was transfered to the intensive care ward. He had an uneventful postoperative course and was discharged 2 weeks later.

Serum creatine phosphokinase (CPK, 27–118 IU) and lactate dehydrogenase (LDH, 50–400 U) were determined every day after the episode. Extremely high values of CPK (59,000 IU) and

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LDH (2,700 U) were obtained on the day of crisis. Isozyme levels over 98% of CPK and 59% of LDH were CPK-MM and LDH_5, respectively. Myoglobinuria persisted a week after the crisis. No myocardial damages were observed by echocardiography and muscle scintigraphy[7]. Muscle specimens were obtained after a week to investigate in vitro calcium-induced calcium= release rate using skinned fibers[8] and abnormally increased calcium-release rate was observed. This case was determined to be MH because of clinical signs and abnormal findings in vitro.

In order to investigate membrane fluidity of erythrocytes, venous samples were obtained during the crisis and several times within two weeks after the crisis. Blood sample was again obtained five months later to determine his normal values and drug effects on membrane fluidity.

All the procedures described herein were conducted after obtaining informed consent from the patient.

Methods

In order to investigate membrane fluidity of erythrocytes, ESR-spin labeling method was applied by using 16-nitroxide stearic acid (Aldrich Chemical, USA) as a labeling reagent. The blood specimens were prepared for measurement of ESR spectra according to Sato et al.[5] The ratio, h_0/h_{-1} (see appendix), was obtained at every 4°C from 4 to 56°C, using a X-band spectrometer equipped with a thermal controller (JES - FE1X and NM-7700, JEOL, Japan), and plotted against the reciprocal of the absolute temperature. Normal values of the ratio with confidence interval (mean ± 2SD) were obtained from 10 healthy adults.

For the study of drug effects on membrane fluidity, two ml of the erythrocyte suspension was incubated for one hour with 1 ml of dantrolene solution (0.4 mg ml^{-1}) and 1 ml of methylpredonisolone solution (25 mg ml^{-1}). The other part of the blood was incubated for two hours at 40°C to investigate pyrexic effects on membrane fluidity. After these procedures, the erythrocyte was prepared for ESR measurement and the ESR spectra were analyzed as described above.

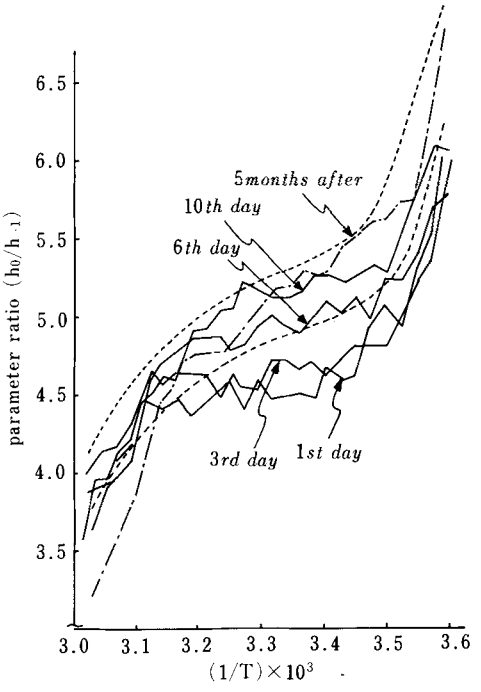

Fig. 1. Serial changes of the parameter ratio. The parameter ratio was plotted against the reciprocal of the absolute temperature (See appendix). Decreased values were serially restored day by day.

Results

a) Fluidity alteration of the erythrocyte membrane of the patient

Normal ranges of thermal dependence of the ratio are shown as an area within two dotted lines in fig. 1, 2 and 3. Serial changes of thermal dependance of the ratio obtained in our case are presented in fig. 1. Decreased values from the normal ranges were obtained between 3.22 to 3.53 ($(1/T) \times 10^3$), i.e. 10.3°C to 37.6°C, on the first and third day after the pyrexic crisis, and they were restored serially with clinical improvement. Data obtained five months later were within normal ranges.

b) Effects of agents and pyrexia on fluidity of erythrocyte membrane

Neither drugs nor pyrexia had any significant effect compared with their normal values determined five months after the crisis in vitro (fig. 2 and 3).

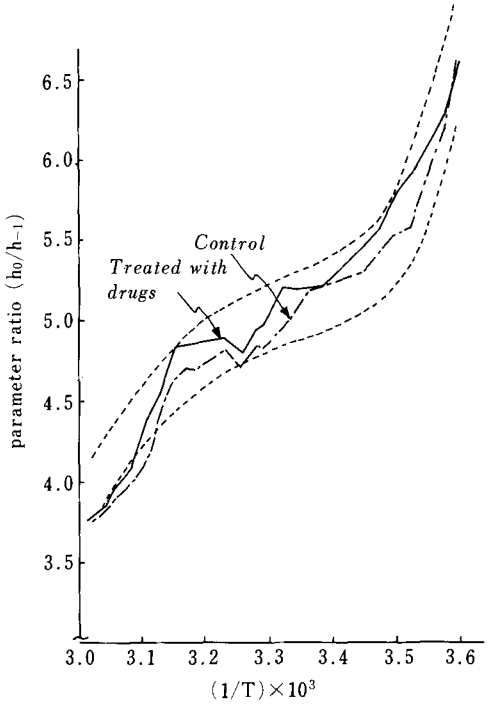

Fig. 2. Drugs effects on the parameter ratio
The parameter ratio was obtained with the blood samples incubated in the solution of dantrolene and methylprednisolone in vitro (See text). No drug effects were obtained.

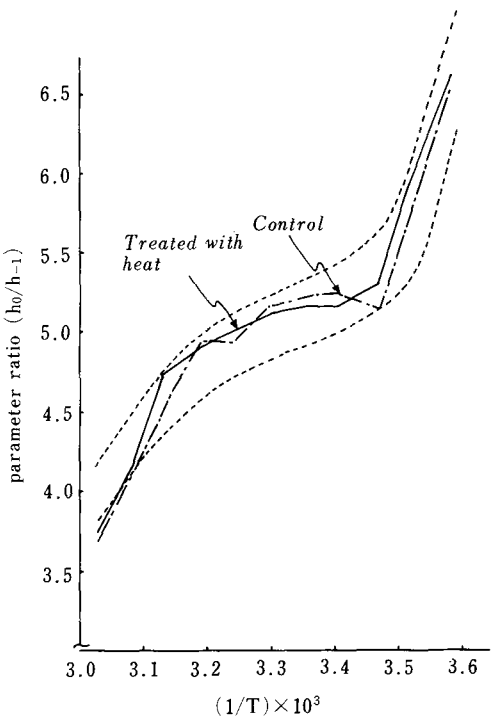

Fig. 3. Pyrexic effects on the parameter ratio
The parameter ratio was obtained with blood samples treated with heat in vitro (See text). No pyrexic effects were obtained.

Discussion

It was assumed that our case was a nonrigid MH[7] in view the elevated temperature of 41°C with such associated clinical findings as mixed type severe acidosis, myoglobinuria, increased CPK and LDH, but no rigidity of skeletal muscle. In support of our diagnosis, positive results were obtained with skinned muscle fibers[8].

As MH is regarded to be a generalized disorder of abnormal membrane, affecting calcium movements in the sarcoplasmic reticulum, abnormalities may be expected in metabolism. Increased osmotic fragility of porcine erythrocyte has been reported[4,9] and membrane abnormalities are also assumed to be present in the sarcoplasmic reticulum. Abnormal findings of human blood cells have also been reported in fragility[10,11].

In this report, serial changes of membrane fluidity obtained by ESR spectroscopy are presented. In order to investigate the biological membrane structure, ESR spectroscopy has been applied to obtain information as to membrane fluidity about the environment of spin labels - - - stable free radicals attached to a specific site of a molecule. In some myotonic disorders such as Duchenne muscular dystrophy, membrane fluidity has been well investigated with this technique and increased fluidity of erythrocytes has been reported[3,5]. Since alterations of protein-lipid and lipid-lipid interaction for membrane organization are presumed to affect membrane fluidity[2,7], abnormal fluidity may be involved in these diseases and they are assumed to be membrane disorders.

In our case, neither history nor clinical symptoms of myotonic disorders were observed. Therefore, increased fluidity indicated structural changes in membrane environment, because the

decreased values of the ratio, h_0/h_{-1}, reflect increased membrane fluidity. Although our findings were obtained from a single patient, these data were assumed to be induced by pathophysiology of pyrexic crisis in MH, since the membrane fluidity was restored to the normal level after complete recovery. Drugs had no effects on the erythrocyte membrane in vitro, though these concentrations were much higher than clinical conditions. No effects of pyrexia were observed in vitro. Therefore, it was postulated that disturbed organization of cellular and cell organella membrane was involved during MH crisis.

It was concluded that membrane composition and configuration might have been altered pathophysiologically during the crisis of MH. However, it could not be clearly defined that increased membrane fluidity was specific to hyperthermic shock or MH itself. Further studies on ESR-spin labeling of cell membrane are required to resolve this problem.

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Appendix

A typical ESR spectra obtained by spin labeling method using 16-nitroxide stearic acid in erythrocyte membrane are presented here. Spectrometer settings employed were: modulation width, 1.6 G at 100 Hz; scan range, 3260 ± 50 G; time constant, 1.0 sec; scan speed, 12.5 G min^{-1}; and field modulation, 100 KHz. Three peak heights are seen along the magnetic field. The ratio of the peak height of the midfield line to the peak height of the high field line, h_0/h_{-1}, could be determined as a conventional empirical motion parameter, which assumes correlation time for isotropic rotation of spin label (nitroxide radicals) in the nonpolar region in double layer membrane environment (See fig.).

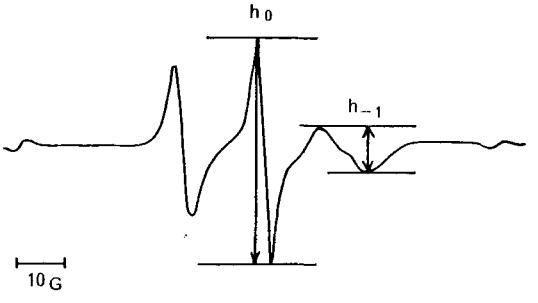

Fig. The parameter ratio as the empirical motion parameter
The ESR spectra were analyzed in terms of the ratio of the peak height of the midfield line to the peak height of the high field line (h_0/h_{-1}).

Correlation time for rotation reflects liability of free movement of spin label. Therefore, the ratio reflects membrane fluidity and the lower values of the ratio means increased membrane fluidity. As presented in Figures in the text, plotted values of the ratio against the reciprocal of the absolute temperature indicate definite thermal dependence of membrane fluidity and increased fluidity is generally observed at high temperature.