USE OF THE CALCIUM AGONIST BAY K 8644 FOR IN VITRO DIAGNOSIS OF SUSCEPTIBILITY TO MALIGNANT HYPERTERMIA


SUMMARY

We have studied the effects of the calcium agonist BAY K 8644 on the in vitro halothane test in 10 malignant hyperthermia-susceptible (MHS), 12 MH “equivocal” to halothane (MHEh), 30 MH non-susceptible (MHN) and 10 control patients. BAY K 8644 potentiated the halothane-induced contracture in muscle strips from both MHS and MHEh patients. The drug produced a more obvious difference in contracture responses between the MHEh group compared with the MHN and control groups.

KEY WORDS

Hyperthermia: malignant, in vitro test. Pharmacology: calcium channel blocker, BAY K 8644

The halothane [1] and caffeine [2] contracture tests are the most reliable and specific indicators of malignant hyperthermia (MH) susceptibility [3, 4]. The European MH group [4] defined three categories of results for in vitro diagnosis: MH susceptible (MHS), normal (MHN) or MH equivocal (MHE) to either caffeine (MHEc) or halothane (MHEh). For the “equivocal” group, the in vitro results are inconclusive. In our experience, and that of others [3, 5–7], the halothane test appears to be the most reliable diagnostic test for MH susceptibility as more patients have an abnormal response to halothane than to caffeine. However, many of our MHEh patients do not develop a vigorous contracture to halothane and this causes difficulty in classification. The aim of this study was to examine the effects of BAY K 8644 in association with halothane on muscle bundles from the three categories of patients classified by the European MH Group. The agonist BAY K 8644 is structurally similar to dihydropyridine antagonists, but exerts opposite effects; it acts by stabilizing slow calcium channels in mode 2 (prolonged channel opening), thus greatly enhancing calcium influx [8, 9].

PATIENTS AND METHODS

We studied 52 patients presenting for diagnostic muscle biopsy as part of investigation for MH. None was taking drugs which might have influenced muscle contractility. Muscle biopsies were taken from the vastus lateralis muscle under combined block with lignocaine of the femoral nerve and lateral cutaneous nerve of the thigh. Biopsies were obtained also from 10 control patients with no personal or family history of MH, undergoing elective orthopaedic surgery. All muscle biopsies in the control patients were obtained from the quadriceps muscle. The control patients were apparently normal with regard to muscle function, in particular with respect to preoperative muscle atrophy induced by bed rest. The study was approved by the Lille University Studies Ethics Committee and informed consent was obtained from all patients.

For the in vitro test, muscle strips (approximately 15–20 mm in length with a diameter of
2–3 mm) were carefully dissected. One end was pinned to the silicone bottom of the tissue bath which was perfused continuously (4–5 ml min\(^{-1}\)) with Krebs–Ringer solution at 37 °C and bubbled with preheated 5% carbon dioxide in oxygen. The other end of the strip was attached by a thin silk thread to a force transducer (Bioscience dynamometer UFI and biological amplifier 120). Preparations were stimulated directly via silver electrodes by rectangular current pulses of 2 ms duration and at twice the threshold intensity delivered at a frequency of 0.2 Hz by a stimulator (CEA-DAM model GPI-GE2198). The preparation was stretched until the amplitude of muscle twitch could not be increased further and was then allowed to stabilize during 15 min of isometric relaxation. Baseline and twitch tension were recorded continuously at low speed on a C1013 Siemens polygraph. Halothane was mixed with carbon dioxide in oxygen by means of a calibrated vaporizer (Fluotec Mark III) in concentrations of 0.5, 1, 1.5, 2 and 3 vol%, as measured by gas chromatography corresponding to 0.097 ± 0.011, 0.204 ± 0.018, 0.364 ± 0.026, 0.534 ± 0.02 and 0.691 ± 0.072 mmol litre\(^{-1}\), respectively. Both static and dynamic halothane contracture tests were performed as described previously [7, 10]. Caffeine was added to the Krebs–Ringer solution in increasing concentrations of 0.5, 1, 1.5, 2, 3, 4, 8, 16 and 32 mmol litre\(^{-1}\). Two caffeine tests were performed also. For both halothane and caffeine procedures, the test giving the greatest contracture was used to make a diagnosis.

**Diagnosis of susceptibility to MH.** All patients were investigated according to the European MH protocol [4]. The criterion for MH susceptibility (MHS) was an increase in resting tension of at least 0.20 g at 2 vol% of halothane or less and at caffeine 2 mmol litre\(^{-1}\) or less. Patients were classified MH equivocal (MHE) if one test was negative and the other was positive, and MH negative (MHN), if both tests were negative. All patients were tested against both halothane and caffeine, using separate muscle strips.

**Experimental procedure.** Using muscle strips obtained from the same biopsies, BAY K 8644 10 \(\mu\)mol litre\(^{-1}\) was added to the Krebs solution 10 min before administration of halothane according to the same procedure as the static contracture test. BAY K 8644 solution was protected from light during the experiments.

Differences between the means of the groups for maximum contracture response to each concentration of halothane were analysed using Student’s \(t\) test for independent samples. A \(P\) value < 0.05 was taken as significant.

**RESULTS**

Ten patients were diagnosed as MHS, 12 as MHEh and 30 as MHN. All the control patients had normal contracture responses to halothane and to caffeine. None of the patients was found to be equivocal to caffeine (MHEc). Indications for investigation of the 62 patients and their classification based on the results of the in vitro tests (fig. 1) are shown in table I.

**TABLE I. Reasons for investigation and results of in vitro tests in 52 patients investigated for susceptibility to malignant hyperthermia and 10 control patients**

<table>
<thead>
<tr>
<th>Reason for Investigation</th>
<th>MHS</th>
<th>MHN</th>
<th>MHE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative of MHS proband</td>
<td>10</td>
<td>19</td>
<td>7</td>
<td>36</td>
</tr>
<tr>
<td>Possible MH reaction</td>
<td>2</td>
<td>2</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroleptic malignant syndrome</td>
<td>2</td>
<td>2</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Heat stroke</td>
<td>1</td>
<td>1</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Duchenne muscular dystrophy</td>
<td>1</td>
<td>1</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Non-specific myopathy</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Totals</td>
<td>10</td>
<td>40</td>
<td>12</td>
<td>62</td>
</tr>
</tbody>
</table>

**FIG. 1. Diagram by the European MH group. Threshold concentration for each patient to halothane and caffeine.**

\(O = \) Patients investigated for MH; \(\nabla = \) controls.
The muscle responses (means (SEM)) of the MHS, MHEh, MHN and control groups to increasing concentrations of halothane are expressed graphically in figure 2. No increase in resting tension was observed in the MHN and the control group, whereas concentration-dependent contractures developed in the MHS group ($P > 0.05$) with 1, 1.5, 2 and 3% halothane (fig. 2A). In the MHEh group, the halothane contracture was significant only with 2 and 3% halothane. In 11 of the 12 MHEh patients, the halothane threshold concentration (the minimal concentration of halothane eliciting a sustained increase in tension of 0.2 g) was 2%. With 2% halothane, the contracture was 0.23 (0.03) g in the MHEh group and 0.77 (0.17) g in the MHS group.

Preincubation for 10 min with BAY K 8644 10 μmol litre$^{-1}$ significantly enhanced the halothane contracture in both the MHS and MHEh groups (fig. 2B). The contracture was significant at all halothane concentrations in the two groups and the effect was marked particularly with the smallest concentration of halothane. In the MHS group, the muscle strips developed a contracture of 1.01 (0.17) g as soon as (0.5%) halothane was added to the carbon dioxide in oxygen. This was observed in 10 muscle bundles from the 10 MHS patients. In this MHS group, the addition of BAY K 8644 was associated with a decrease in tension as the halothane concentration increased. When 2% halothane was given, there was no difference between the tension developed in the presence or absence of BAY K 8644. In the MHEh group, the muscle strips developed a contracture of 0.44 (0.15) g with 0.5% halothane.

As population averages are of little value in deciding if an individual patient belongs to the MHN group, data are presented in graphic form (fig. 3), which may clarify the range of values separating obvious normal from abnormal results. MHS muscle strips developed consistently a contracture of at least 0.20 g with 0.5% halothane and MHEh muscle strips developed the same contracture.

**Fig. 2.** Effects of BAY K 8644 on the response to halothane in diagnostic muscle biopsies from MHS, MHEh, MHN and control patients. A: Halothane dose–response curves in the absence of BAY K 8644. O = MHS (n = 10); □ = MHEh (n = 12); △ = MHN (n = 30); ▽ = control (n = 10). B: Halothane dose–response curves in the presence of BAY K 8644. ◦ = MHS (n = 10); ■ = MHEh (n = 12); ▲ = MHN (n = 30); ▼ = control (n = 10). $P < 0.05$: *MHS compared with MHN and control; † MHEh compared with MHN and control.

**Fig. 3.** Relationship between halothane test in the presence or in the absence of BAY K 8644 in 62 patients. See figure 2 for means, SEM and statistical analysis. ◦ = MH susceptible (MHS); ■ = MH equivocal to halothane (MHEh); ▲ = MH non-susceptible (MHN); ▽ = control.
level of contracture with 1% halothane or less. No contracture was seen for the control patients and for all but one of the MHN patients with 1% halothane.

**DISCUSSION**

This study indicates that BAY K 8644 potentiated the halothane-induced contracture in skeletal muscle from both MHS and MHEh patients. This action was marked particularly with the smallest concentration of halothane for the MHS group. The very high sensitivity (contracture) of these MHS muscle bundles rapidly induced muscle fatigue which could explain the paradoxical decrease in tension observed when halothane concentration increased. Furthermore, in the presence of BAY K 8644, the halothane test induced a greater difference in contracture responses between the MHEh group on one hand and the MHN and the control group on the other.

In our study, five of the 12 MHEh patients were patients with neuromuscular disease. Muscle strips from these patients behaved in the same manner as MHEh muscle from relatives of MHS probands. Our results with myopathic muscle may not necessarily be equated with clinical susceptibility to MH. However, other studies performed with the halothane contracture test [11] or the caffeine skinned fibre test [12] have also shown an augmented response in several neuromuscular disorders. Hence, these previous reports, together with the present findings, may suggest that this population is at higher risk for MH than the general population.

Some authors have suggested that the use of other tests in addition to the classical halothane-caffeine test improves the reliability of diagnosis. Tests involving simultaneous exposure to both halothane and caffeine produce data which overlap between normal and known MHS patients [3]. Takagi and colleagues [12] have suggested the use of a single skinned muscle fibre test which seems to correlate with the muscle bundle test [13]. However, this method is difficult to perform and time consuming. Furthermore, the slightest contamination of distilled water with calcium invalidates the test.

The action of BAY K 8644 on MH susceptible muscle in the presence of halothane is not unexpected. In MH muscle, intramyoplastic calcium increases with the application of halothane and this change correlates well with the magnitude of developed contracture. The source of this increased intramyoplastic calcium is thought to be either sarcoplasmic reticulum [14, 15] or sarcolemma [16]. The addition of BAY K 8644 to MH muscle provides an extracellular source of calcium and enhances the effects of halothane.

The fact that the tests in the MHEh group became more similar to those in the MHS group with the combination of BAY K 8644 and halothane suggests that the combined test may allow more accurate diagnosis in equivocal patients. The MHE population was not affected by the combined test. We conclude that the use of BAY K 8644 may help to establish more definite end-points for normal and abnormal responses to halothane tests.

**ACKNOWLEDGEMENTS**

This study was supported in part by grants from l'Association Francaise Contre Les Myopathies. The authors are grateful to B. A. Britt for help in preparing the manuscript.

**REFERENCES**


