Multicentre evaluation of in vitro contracture testing with bolus administration of 4-chloro-m-cresol for diagnosis of malignant hyperthermia susceptibility

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Summary

Background and objective: The in vitro contracture test with halothane and caffeine is the gold standard for the diagnosis of susceptibility to malignant hyperthermia (MH). However, the sensitivity of the in vitro contracture test is between 97 and 99% and its specificity is 78–94% with the consequence that false-negative as well as false-positive test results are possible. 4-Chloro-m-cresol is potentially a more specific test drug for the in vitro contracture test than halothane or caffeine. This multicentre study was designed to investigate whether an in vitro contracture test with bolus administration of 4-chloro-m-cresol can improve the accuracy of the diagnosis of susceptibility to MH.

Methods: Three hundred and fifty-two patients from 11 European MH laboratories participated in the study. The patients were first classified as MH susceptible, MH normal or MH equivocal by the in vitro contracture test according to the European MH protocol. Muscle specimens surplus to diagnostic requirements were used in this study (MH susceptible = 103 viable samples; MH equivocal = 51; MH normal = 204). 4-Chloro-m-cresol was added to achieve a concentration of 75 µmol L⁻¹ in the tissue bath. The in vitro effects on contracture development and muscle twitch were observed for 60 min.

Results: After bolus administration of 4-chloro-m-cresol, 75 µmol L⁻¹, 99 of 103 MH-susceptible specimens developed marked muscle contractures. In contrast, only two of 204 MH-normal specimens showed an insignificant contracture development following 4-chloro-m-cresol. From these results, a sensitivity rate of 96.1% and a specificity rate of 99.0% can be calculated for the in vitro contracture test with bolus administration of 4-chloro-m-cresol 75 µmol L⁻¹. Forty-three patients were diagnosed as MH equivocal, but only specimens from 16 patients developed contractures in response to 4-chloro-m-cresol, indicating susceptibility to MH.
Conclusions: The in vitro contracture test with halothane and caffeine is well standardized in the European and North American test protocols. However, this conventional test method is associated with the risk of false test results. Therefore, an improvement in the diagnosis of MH is needed. Regarding the results from this multicentre study, the use of 4-chloro-m-cresol could increase the reliability of in vitro contracture testing.

Keywords: ANAESTHETICS, INHALATION, halothane; MALIGNANT HYPERTHERMIA; MUSCLE, muscle fibres; ORGANIC CHEMICALS, cresols, phenols; PURINES, caffeine, purinones, xanthines.

Malignant hyperthermia (MH) is a potentially lethal pharmacogenetic disease with an autosomal-dominant trait. The clinical syndrome presents with a hypermetabolic state with muscular rigidity, metabolic acidosis, hypercapnia, tachycardia, myoglobinuria and elevated body temperature as a response to certain anaesthetic agents, i.e. halogenated volatile anaesthetics and depolarizing muscle relaxants [1].

In many families the disorder is associated with a defect of the ryanodine receptor (RyR)1, the Ca^{2+} release channel of the skeletal muscle sarcoplasmic reticulum [2,3], which leads to an abnormally high release of Ca^{2+} from intracellular stores into the myoplasm [4]. The RyR1 contains binding sites for various agents and can be modulated by physiological agonists as well as volatile anaesthetics, caffeine and cresol [5]. Furthermore, cellular pathways that result in phosphorylation of the RyR1 may influence the gating and response of the Ca^{2+}-release channel. Whereas the normal skeletal muscle tolerates modest perturbations of Ca^{2+} homeostasis, the MH muscle cell presents an increased sensitivity against physiological ligands and drugs that mediate the Ca^{2+} release from the sarcoplasmic reticulum. The different behaviour of MH-susceptible and MH-normal muscles in response to various pharmacological triggers serves also as basis for the laboratory diagnosis of MH.

The first reliable methods for evaluation of susceptibility to MH became available in 1970 and 1971 when contracture tests with caffeine and halothane were introduced [6,7]. In the following years both were modified and standardized as in vitro contracture tests by the North American and the European Malignant Hyperthermia Groups [8,9]. A multicentre study ascertained the sensitivity of the in vitro contracture tests according to the European protocol to be 99.0% and the specificity 93.6% [10]. A more recent study presented pooled data from the North American Malignant Hyperthermia Registry [11]. In this evaluation, the sensitivity was 97% and specificity only 78% for the in vitro contracture tests. Owing to the lack of sensitivity and specificity of in vitro contracture tests with halothane and caffeine, associated especially with the clinical risks of false-negative results [12,13], it is important to improve the accuracy of in vitro contracture testing. New test agents for the in vitro contracture tests, such as ryanodine [14-18] and cresol [19-26], have the potential for improving diagnostic accuracy.

Chlorocresols were proposed as test agents for the in vitro contracture tests when 4-chloro-m-cresol was found to be a potent activator of Ca^{2+} release from skeletal muscle sarcoplasmic reticulum, an effect that was shown to be specific for the RyR1 [19]. Subsequent studies revealed that 4-chloro-m-cresol stimulated Ca^{2+}-activated {[H]ryanodine binding with an EC_{50} of approximately 100 μmol L^{-1}, which is about 10 times lower than that of caffeine [20]. In the same concentration range, 4-chloro-m-cresol directly activated the isolated Ca^{2+}-release channel from the luminal and the cytoplasmic site. Further experiments showed that the 4-chloro-m-cresol affinity of {[H]ryanodine binding to MH-susceptible vesicles from porcine sarcoplasmic reticulum was twofold higher compared with that in normal tissue [21].

In skeletal muscle specimens from MH-susceptible swine, it was demonstrated that 4-chloro-m-cresol induced in vitro marked contractures but no or insignificant contractures in specimens from control (MH-normal) animals [22]. These results were confirmed by in vitro contracture test studies in skeletal muscle specimens from patients [23-25]. Recently, a European multicentre investigation showed that an in vitro contracture test with cumulative administration 4-chloro-m-cresol might be able to distinguish between MH-susceptible and MH-normal patients [26]. In this study the optimal threshold concentration of 4-chloro-m-cresol was estimated to be 75 μmol L^{-1}. The purpose of the present study was therefore to determine whether bolus administration of 4-chloro-m-cresol at a concentration of 75 μmol L^{-1} might improve discrimination between MH-susceptible and MH-normal subjects.

Methods

Three hundred and fifty-two patients from 11 European Malignant Hyperthermia laboratories participated in this study. All investigations were
performed after obtaining written informed consent from the patients or their parents, as appropriate. Patients attending for diagnosis of MH because of a clinically suspected event in themselves or relatives were included. Patients with known neuromuscular diseases were excluded. The study protocol complies with the standards described in the Declaration of Helsinki and was approved by the Ethics Committees of the participating MH centres. In all individuals participating in this investigation, a single or double bolus test with 4-chloro-m-cresol, in addition to the routinely performed duplicate, cumulative halothane and caffeine tests, was performed.

Muscle biopsies
All investigations were performed according to the protocol of the European Malignant Hyperthermia Group [10]. In brief, muscle biopsies were taken from the m. vastus lateralis or medialis under regional or general anaesthesia without MH-triggering substances (i.e. volatile anaesthetics or succinylcholine, or both). The fresh specimens were suspended in Krebs–Ringer solution (mmol L−1: NaCl 118.1; KCl 3.4; CaCl2 2.5; MgSO4 0.8; KH2PO4 1.2; NaHCO3 25.0; glucose 11.1) equilibrated with carboxen (95% oxygen/5% carbon dioxide). The muscle bundles were then dissected into single strips (length 15–25 mm, width 2–3 mm, weight 100–300 mg).

Standard in vitro contracture tests
Only viable muscle samples (twitch amplitude to supramaximal stimulation >10 mN) were used for the in vitro contracture tests according to the protocol of the European Malignant Hyperthermia Group [10]. The specimens were suspended in a tissue bath perfused with Krebs–Ringer solution bubbled with carboxen continuously at 37°C and pH 7.4. The muscles were stimulated electrically with square-wave impulses to achieve a supramaximal response of duration 1 ms and frequency of 0.2 Hz. The resting length of the specimen was measured before testing, and the initial baseline tension before testing was achieved by stretching the samples slowly to 150 ± 10% of the resting length.

Patients were first classified by the in vitro contracture test with halothane and caffeine according to the procedure of the European Malignant Hyperthermia Group. In each patient, a minimum of two samples was tested with each drug. The halothane threshold was obtained using concentrations of 0.11, 0.22 and 0.44 mmol L−1, and each muscle sample was exposed to each halothane concentration for at least 5 min. The concentration of caffeine in the tissue bath was increased stepwise from 0.5 to 32.0 mmol L−1. Each successive concentration was administered as soon as the maximum contracture level has been reached, or after exposure of the muscle to the caffeine concentration for 3 min if no contracture occurred.

The in vitro contracture test gave the diagnostic thresholds for halothane and caffeine as follows: MHS = muscle contractures ≥2.0 mN at a caffeine concentration of ≤2.0 mmol L−1 and a halothane threshold concentration of ≤0.44 mmol L−1; MHN = muscle contractures ≥2.0 mN at a caffeine concentration of ≥3.0 mmol L−1 and a halothane threshold concentration >0.44 mmol L−1. All other results were deemed MH equivocal (MHE) – MHEh if reacting to halothane only or MHEc if reacting to caffeine only.

In vitro contracture test with 4-chloro-m-cresol
After investigation of susceptibility to MH, fresh muscle specimens were subjected to in vitro contracture tests with 4-chloro-m-cresol. Furthermore, muscle bundles not fulfilling the defined viability criteria were excluded from the evaluation.

The test set-up for the in vitro contracture test with 4-chloro-m-cresol was according to the procedure of the standard in vitro contracture test (Fig. 1). The initial baseline tension before testing was achieved by stretching the samples slowly to 150 ± 10% of the resting length. If viability criteria were achieved (muscle twitch response to supramaximal stimulation >10 mN) and the baseline tension was stable for at least 10 min, 4-chloro-m-cresol was administered as a bolus in order to obtain a concentration of 75 μmol L−1 in the tissue bath. The in vitro effects of 4-chloro-m-cresol were observed for at least 60 min and included measurement of contracture development and muscle twitch responses. The contracture course after administration of 4-chloro-m-cresol was defined by the attainment of different contracture levels (Fig. 1): (a) onset time of contracture (OT); (b) time to achieve a contracture level of 2 mN (TC2 mN); (c) time to reach a contracture level of 10 mN (TC10 mN); and (d) contracture maximum (Cmax).

The following chemicals were used: 4-chloro-m-cresol (Aldrich-Chemie, Steinheim or Fluka, Neu-Ulm, Germany; purity >99%), caffeine (Sigma, Deisenhofen, Germany) and halothane (Hoechst, Frankfurt, Germany; or Halocarbon, Hackensack, NJ, USA). Solutions were prepared freshly before each investigation in carboxygenated Krebs–Ringer solution at 37°C and administered directly to the tissue bath.

Statistical analysis
Statistical evaluation was performed using a computer-based statistical program (SPSS, Inc, Chicago, IL,
Figure 1. Method of data evaluation of the in vitro contracture test with 4-chloro-m-cresol. The schematic trace of an in vitro contracture test with 4-chloro-m-cresol illustrates the recorded parameters: the maximal preload tension, which is measured after stretching the muscle specimen to its optimal length and the twitch height before drug administration. The onset time (OT) is defined as the time following administration until the contracture increases, indicated by an increase in the baseline height exceeding the preload baseline height. Furthermore, times to achieve contractures of 2 mN (TC<sub>2mN</sub>) and of 10 mN (TC<sub>10mN</sub>) as well as the maximum contracture (C<sub>max</sub>) were measured.

Table 1. Participating malignant hyperthermia laboratories and in vitro contracture test results.

<table>
<thead>
<tr>
<th>MH laboratory</th>
<th>No. of specimens (no. of patients)</th>
<th>Malignant hyperthermia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Antwerp, Belgium</td>
<td>11 (11)</td>
<td>5</td>
</tr>
<tr>
<td>Basel, Switzerland</td>
<td>75 (38)</td>
<td>33 (16)</td>
</tr>
<tr>
<td>Cork, Ireland</td>
<td>11 (11)</td>
<td>4</td>
</tr>
<tr>
<td>Hamburg, Germany</td>
<td>63 (63)</td>
<td>26</td>
</tr>
<tr>
<td>Leeds, UK</td>
<td>13 (13)</td>
<td>9</td>
</tr>
<tr>
<td>Mainz, Germany</td>
<td>34 (34)</td>
<td>9</td>
</tr>
<tr>
<td>Padua, Italy</td>
<td>77 (77)</td>
<td>13</td>
</tr>
<tr>
<td>Paris, France</td>
<td>18 (18)</td>
<td>9</td>
</tr>
<tr>
<td>Ulm, Germany</td>
<td>44 (28)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Vienna, Austria</td>
<td>43 (26)</td>
<td>6 (3)</td>
</tr>
<tr>
<td>Würzburg, Germany</td>
<td>33 (33)</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>422 (352)</td>
<td>123 (101)</td>
</tr>
<tr>
<td>No. of muscle specimens excluded from the study*</td>
<td>64</td>
<td>20</td>
</tr>
<tr>
<td>Total number of investigated muscle specimens</td>
<td>358</td>
<td>103</td>
</tr>
</tbody>
</table>

*Exclusion criteria (see Methods).

USA). The in vitro data are presented as medians and ranges. The effects of 4-chloro-m-cresol on contraction development were assessed using the Kruskal-Wallis and U-non-parametric comparative tests and a t-test to determine differences among the groups. Results were considered as significant if \( P < 0.05 \).

Results

Eleven MH laboratories from seven European countries participated in this multicentre evaluation (Table 1). From 352 patients, 101 (28.7%) were diagnosed as MH susceptible, 43 (12.2%) showed pathological contracture responses only for halothane.
(MHEh) and the remaining 208 (59.1%) were diagnosed as normal for MH. None of the patients from this study was classified as MHEh.

A total of 422 muscle specimens were used for the in vitro contracture test with 4-chloro-m-cresol. Sixty-four specimens were excluded from this study because they did not meet the viability criteria ($n = 57$, 89.1%) or a previously unrecognized neuromuscular disease was found in the proband ($n = 7$, 10.9% — six patients with central core disease and one with myositis). Therefore, 103 MH susceptible, 51 MHEh and 204 MH-normal specimens were used for the in vitro contracture test with 4-chloro-m-cresol.

In the MH-susceptible group, contractures following administration of 4-chloro-m-cresol 75 μmol L$^{-1}$ started after a median (range) time of 0.5 (0.0—7.0) min (Fig. 2a). In the MHEh group only 16 of 51 specimens developed contractures with an OT of 2.8 (0.0—25.0). Two MH-normal specimens developed contractures of 1.0 or 1.8 mN, respectively. The onset times for contracture in these specimens were 1.2 and 1.5 min.

The time to reach a contracture level of 2 mN was 1.1 (0.0—13.3) min in the MH-susceptible group. Only 12 of 51 MHEh specimens developed contractures $\geq 2$ mN; $\text{TC}_{2\text{ mN}}$ was 4.2 (0.0—28.0) min. None of the MH-normal specimens developed contractures $\geq 2$ mN. The contracture level of 10 mN was reached after 2.5 (0.0—18.0) min in the MH-susceptible group. In the MHEh group only five of 51 specimens developed contractures $\geq 10$ mN; $\text{TC}_{10\text{ mN}}$ in these muscle samples was 4.0 (0.0—51.0) min.

The contracture maximum was significantly higher in the MH-susceptible than in the other groups (Fig. 3). $C_{\text{max}}$ following administration of 4-chloro-m-cresol 75 μmol L$^{-1}$ in the MH-susceptible group was 14.0 (1.0—79.0) mN. In the MHEh specimens, $C_{\text{max}}$ was 5.5 (1.0—19.8) mN. Only two of 204 MH-normal samples showed contractures (1.0 and 1.8 mN).

Four of 103 MH-susceptible specimens developed no ($n = 2$) or insignificant ($n = 2$; 1.0 and 1.7 mN, respectively) contractures, whereas two of 204 MH-normal showed contracture development (Table 2). From these results, a sensitivity rate of 96.1% and a specificity rate of 99.0% can be calculated for the in vitro contracture test with bolus administration of 4-chloro-m-cresol 75 μmol L$^{-1}$ based on the results of the halothane/caffeine contracture test.

**Discussion**

MH presents as a syndrome with a large diversity of clinical symptoms, molecular and genetic findings, rendering susceptible individuals difficult to identify. It is widely believed that MH susceptibility

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**Figure 2.** Contracture development following bolus administration of 4-chloro-m-cresol 75 μmol L$^{-1}$ in skeletal muscle preparations of malignant hyperthermia (MH) susceptible (MHS), MH equivocal (MHE; b: halothane; c: caffeine) and MH-normal patients (MHN). The contracture course is characterized by the attainment of three different contracture levels (min): onset time of contracture development (OT; a) and the times to achieve contractures of 2 mN (TC$_{2\text{ mN}}$; b) and of 10 mN (TC$_{10\text{ mN}}$ c). Data are medians (——) and ranges. $^*P < 0.05$ versus MH normal. n.c.: no contracture.
it has been demonstrated that $[^1]H$ryanodine has a greater affinity to the Ca$^{2+}$-release channel protein from MH-susceptible than MH-normal pigs [2]. From a consideration of these effects, it has been proposed that ryanodine can be used as a contracture test in order to distinguish between MH susceptible and MH normal. The first report of ryanodine-induced contractures in skeletal muscle specimens was published in 1991 [15]. In that investigation, six muscles from MH-susceptible patients developed contractures during incremental administration of at least ryanodine 10 μmol L$^{-1}$; skeletal muscles of two MH-equivocal patients behaved in a similar way. In contrast, no MH-normal muscle developed contractures following administration of ryanodine. Although ryanodine was shown to differentiate in principle between the diagnostic groups, an overlap in the range of times between MH susceptible and MH normal was observed. To improve discrimination between MH susceptible and MH normal, the in vitro effects of different preparations, concentrations and techniques of administration were investigated [16,17]. These studies led the European Malignant Hyperthermia Group to decide to use only highly purified ryanodine at a concentration of 1 μmol L$^{-1}$ when performing the in vitro contracture tests. A European multicentre study was subsequently performed in a large series of patients to assess the validity of common diagnostic end-points of a ryanodine contracture test [18]. In this study, it could be demonstrated that susceptible and normal individuals could be distinguished within a single laboratory. However, owing to a high level of variability between testing centres, the use of common cut-off values for the ryanodine contracture test was not possible.

Another promising agent for in vitro contracture testing is 4-chloro-m-cresol, which was found to be a potent activator of Ca$^{2+}$ release from skeletal muscle sarcoplasmic reticulum by a specific interaction with the ryanodine receptor [19]. Furthermore, 4-chloro-m-cresol directly activates the isolated Ca$^{2+}$-release channel from the luminal and cytoplasmic site in a concentration range between 50 and 200 μmol L$^{-1}$, and it stimulates Ca$^{2+}$-activated $[^1]H$ryanodine binding with an EC$_{50}$ of approximately 100 μmol L$^{-1}$ — which is about 10 times lower than that of caffeine [20]. The same authors showed that the 4-chloro-m-cresol affinity of $[^1]H$ryanodine binding to MH-susceptible vesicles from porcine sarcoplasmic reticulum was twofold higher compared with that in normal tissue [21]. 4-Chloro-m-cresol was therefore suggested to be a specific tool to distinguish between MH susceptible and MH normal.

In 1986, a commercial preparation of succinylcholine containing 4-chloro-m-cresol as a preservative
was shown to induce contractures in skeletal muscle specimens from MH-susceptible swine whereas pure succinylcholine produced no contractures [22]. Consequently, 4-chloro-m-cresol was used in a concentration of 56 μmol L⁻¹ as the test agent for an in vitro contracture test and demonstrated in vitro contractures in MH-susceptible but not in specimens from control (MH-normal) animals. These results were confirmed by numerous in vitro contracture studies in skeletal muscle preparations from MH-susceptible and MH-normal patients [21,23–25,27]. Following cumulative administration, 4-chloro-m-cresol induced dose-dependent contractures in MH-susceptible but only small contractures in MH-normal skeletal muscles. The contracture course after 4-chloro-m-cresol was comparable with caffeine-induced contractures, but with greater potency.

As a result of these preliminary studies, the European Malignant Hyperthermia Group agreed upon a common protocol for an in vitro contracture test with cumulative administration of 4-chloro-m-cresol, and conducted a multicentre evaluation to prove the value of an in vitro contracture test using it for diagnosis of susceptibility to MH in a large series of patients [26]. In this investigation, 4-chloro-m-cresol was administered cumulatively in concentrations between 25 and 200 μmol L⁻¹, and it was shown that contractures started at significantly lower concentrations in the MH-susceptible than in the MH-normal muscles. Furthermore, contracture tension was greater in the MH-susceptible group than in the MH-normal group. The threshold concentration of 4-chloro-m-cresol for the MH-susceptible group was ≤75 μmol L⁻¹, whereas contractures in the MH-normal group started at concentrations ≥100 μmol L⁻¹, which was consistent with previous investigations from single MH laboratories.

In previous studies, it has been demonstrated that an in vitro contracture test with bolus administration of 4-chloro-m-cresol also enables a clear discrimination between the diagnostic MH groups [24,28]. In humans, an in vitro contracture test with single bolus administration of 4-chloro-m-cresol 50, 75 and 100 μmol L⁻¹ was used to discriminate between MH susceptible and MH normal, and in this study a clear cut differentiation between the groups was achieved using 75 μmol L⁻¹ [24]. Therefore, it was concluded that an in vitro contracture test with bolus administration of 4-chloro-m-cresol in a concentration of 75 μmol L⁻¹ might provide an improvement in diagnosing susceptibility to MH, and consequently this hypothesis should be tested in a second European multicentre evaluation. Hence, from the results of the present study, the sensitivity rate of the 4-chloro-m-cresol in vitro contracture test was calculated with 96.1% and the specificity rate was 99.0%. However, calculation of the sensitivity in this investigation was based on the comparison of the standard in vitro contracture test using halothane and caffeine with the 4-chloro-m-cresol in vitro contracture test, consequently leading to a systematic error. It remains unclear whether the discrepancies between both test methods are due to either inaccuracies in the standard test method or the 4-chloro-m-cresol in vitro contracture test. To achieve more reliable results for the catimation of the sensitivity and specificity of a diagnostic method, this test must be performed in a large series of so-called probands, i.e. patients with an MH event in their own history, and unrelated control patients without such event in their own history.

In the European protocol, ryanodine as well as 4-chloro-m-cresol were introduced as optional test agents. Whereas the members of the European Malignant Hyperthermia Group have agreed on a bolus administration technique for the ryanodine test, no final decision concerning a 4-chloro-m-cresol test has been made until now. Compared with the 4-chloro-m-cresol test – using a cumulative administration technique and considering the results from this study — it can be concluded that the bolus test is easier to perform (one 4-chloro-m-cresol administration compared with six to eight administrations for the cumulative technique); moreover, the duration of the bolus test is shorter (after 20 min the bolus test could be stopped because in the responding MH-susceptible muscles the maximum time to reach 10 mN was 18.0 min).

Furthermore, patients showing an equivocal result (MHE; contracture response only to halothane or caffeine) need clarification. In European MH test centres, between 8 and 20% of the patients were diagnosed as MH equivocal; in the present study 12.2% of the patients showed pathological contractures only in response to halothane. From a clinical point of view these patients are regarded as susceptible for MH; however, owing to the low specificity of the in vitro contracture test with halothane these individuals are presumably tested as false-positive. Thus, it has been proposed to use additional test agents in order to assign MH-equivocal patients to either the MH-susceptible or the MH-normal groups by contractures induced by 4-chloro-m-cresol. Using this approach, 16 of 51 MH equivocal specimens (31.4%) would be assigned to the MH-susceptible group, whereas the remaining samples would indicate a normal condition. The main problem for the European Malignant Hyperthermia Group, as well as for the North American Malignant Hyperthermia Group, is to decide how to assign patients with a pathological contracture response in only one of the three tests (e.g. with halothane, caffeine and 4-chloro-m-cresol) to the MH-normal group with potentially dangerous
consequences for the patient. Therefore, further investigations and discussions are needed to solve this important problem.

Regarding the results of the 4-chloro-m-cresol in vitro contracture test in porcine specimens from previous studies, and from contracture testing using human muscle preparations from the present investigation, a greater variability in contracture response in the latter was observed [23–29]. It is speculated that these results are due to genetic differences in human and porcine MH (for a review, see [30,31]). Porcine genetic linkage studies showed that a single amino acid mutation, Arg615 to cysteine, in the skeletal muscle ryanodine receptor gene on chromosome 6 is tightly linked to the MH phenotype. The corresponding mutation in the human ryanodine receptor gene is on chromosome 19q13.1–13.2. Up to now, more than 20 different point mutations have been identified in the human skeletal muscle RyR1 gene in families with a hereditary predisposition to MH. The incidence rate of the various mutations ranges from 2 to 10%, though a combination of different mutations within one pedigree has not been ascertained yet. Nonetheless, in about 50% of the susceptible families a linkage of MH to chromosome 19 cannot be demonstrated. On top of that, several studies demonstrate linkage of MH susceptible to DNA markers from chromosomes 1, 3, 5, 7 and 17. Furthermore, two disease-causing mutations were identified in the candidate gene of the region encoding the dihydropyridine receptor α1-s- subunit [31]. To estimate the influence of different mutations on in vitro contracture test results, further investigations need to determine the genetic effects on the variability of contracture test responses with 4-chloro-m-cresol because this has already been performed for the standard test agents [32] as well as for ryanodine [33].

The results from this study confirm that the inclusion of a 4-chloro-m-cresol in vitro contracture test as an additional test method into the standard test protocols might be a useful approach to improve the reliability of diagnosis of MH. Further studies should determine the sensitivity and specificity of this new method more precisely, using bolus as well as cumulative administration techniques in specimens from probands with a history of fulminant MH and control subjects, and whether the contracture responses following administration of 4-chloro-m-cresol are influenced by different MH-specific mutations.

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References


