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Malignant Hyperthermia: Clinical and Molecular Aspects

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Summary: Correia ACC, Silva PCB, Silva BA – Malignant Hyperthermia: Clinical and Molecular Aspects.

Content: Malignant hyperthermia (MH) is a potentially lethal pharmacogenetic disorder that affects genetically predisposed individuals. It manifests in susceptible individuals in response to exposure to Inhalant anesthetics, depolarizing muscle relaxants or extreme physical activity in hot environments. During exposure to these triggering agents, there is a rapid and sustained increase of myoplasmic calcium (Ca^{2+}) concentration induced by hyperactivation of ryanodine receptor of skeletal muscle (RyR1), causing a profound change in Ca^{2+} homeostasis, featuring a hypermetabolic state. RyR1, Ca^{2+} release channels of sarcoplasmic reticulum, is the primary locus for MH susceptibility. Several mutations in the gene encoding the protein RyR1 have been identified; however, other genes may be involved. Actually, the standard method for diagnosing MH susceptibility is the muscle contracture test for exposure to halothane-caffeine (CHCT) and the only treatment is the use of dantrolene. However, with advances in molecular genetics, a full understanding of the disease etiology may be provided, favoring the development of an accurate diagnosis, less invasive, with DNA test, and also will provide the development of new therapeutic strategies for treatment of MH. Thus, this brief review aims to integrate molecular and clinical aspects of MH, gathering input for a better understanding of this channelopathy.

Keywords: Anesthetics, Inhalation; Calcium; Malignant Hyperthermia; Neuromuscular Blocking Agents; Ryanodine.

INTRODUCTION

Malignant hyperthermia (MH) is a potentially fatal pharmacogenetic disorder. During a crisis of MH, inhalational anesthetics, muscle relaxants depolarizing (succinylcholine) or extreme physical activity in hot environments trigger a massive accumulation of calcium (Ca^{2+}) in myoplasm, which leads to an accelerated metabolism and skeletal muscle contractile activity. This hypermetabolic state generates heat and leads to hypoxemia, metabolic acidosis, rhabdomyolysis, and rapid increase in body temperature that can be fatal if not recognized and treated early [1,2].

This release of Ca^{2+} in myoplasm occurs due to a membrane depolarization that induces conformational changes in L-type calcium channels (CA_V-L) (or dihydropyridine receptors [DHPRs]), which lead to Ca^{2+} release channels activation from sarcoplasmic reticulum (or ryanodine receptor subtype-1 [RyR1] in skeletal muscle). This functional interaction between DHPRs and RyRs, which transforms the electrical impulse into chemical substance, is commonly referred to as excitation-contraction coupling (E-C) [3,4].

Several mutations in the RyR1 gene have been already identified and implicated in a wide range of channelopathies, and this defect is primarily responsible for susceptibility to MH; however, other genes may be involved [2]. This variation in genes related to susceptibility to MH is the major cause of the syndrome's different manifestations [5].

Thus, this paper aims to review the molecular and physiological bases of RyRs and outline the pathophysiological and genetic factors involved in malignant hyperthermia, in order to provide a condensed and updated source of scientific information for healthcare professionals and incorporate molecular and clinical aspects for a better understanding of this channelopathy.

RYANODINE RECEPTORS (RYRs)

Classification and location

Ryanodine receptors (RyRs) are high-conductance cation channels, which release Ca^{2+} from intracellular stores such as the endo/sarcoplasmic reticulum (ER/SR) [6]. RyRs are ubiquitous in all cell types and are involved in a variety of cellular processes (E-C coupling, neurotransmission, secretion etc.) [4]. There are three known isoforms of RYRs in mammals that have been classified according to the initially identified tissue: RyR1 is the dominant isoform in skeletal muscle, commonly referred to as skeletal ryanodine receptor; RyR2 is found in the heart muscle, also known as cardiac ryanodine receptor; and RyR3 is expressed at low levels in several tissues, but it is particularly associated with diaphragm and brain [4,7].

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Molecular structure

RyRs are homotetramers with a molecular mass of about 560 kDa, characterized by having a bell shape [8] (Figure 1). It shows ~ 70% homologous amino acid sequence and the higher level of similarity is at the C-terminal region. In all isoforms, the C-terminal portion of the protein contains the transmembrane domains. According to systematic analysis, it is suggested that there are between 4 and 12 transmembrane segments per RyR subunit [10] (Figure 2). There is also a large N-terminal cytoplasmic domain containing binding sites for protein and other channel modulators (e.g., Ca^{2+} channels) that control the RyR activity state [15]. Each RyR subunit is closely associated with a 12 kDa protein, FKBP12, which modulates the opening parameters (probability of the channel being open and average time of opening) [16].

Activators and blockers

The various cellular processes, physiological agents, pharmacological substances, and different associated proteins that regulate RyRs receptors are shown in Tables I and II.

Role of RyRs in excitation-contraction coupling (E-C)

There is clear evidence that RyRs interact with DHPRs near the T-tubule membrane. This functional interaction between DHPRs and RyRs is commonly referred to as E-C coupling, which is the transformation of an electrical signal into a chemical signal, and these receptors play an important role in this process [4]. The three genetically distinct isoforms of RyR (RyR1, RyR2, and RyR3) show release of Ca^{2+} induced by Ca^{2+} (ICRC), a process by which Ca^{2+} itself activates the channel to release Ca^{2+} [28]. DHFR is an L-type Ca^{2+} channel, also known as CA_V1, and the α-subunit of this channel is the pore-forming unit that functions as a voltage sensor and responds to changes in membrane potential. This α-subunit is the region in which there is binding of dihydropyridines. There are several isoforms of this channel classified according to their location. For example, subtype $Ca_V1.1$ is present in skeletal muscle and $Ca_V1.2$ in cardiac muscle [1].

In skeletal muscle, E-C coupling does not require the entry of extracellular Ca^{2+}. The release of Ca^{2+} by RyR1 (the predominant isoform in skeletal muscle) is triggered by conformational change in the voltage sensor of DHFR in T-tubule depolarization. This Ca^{2+} release is referred to as depolarization-induced Ca^{2+} release (DICR) [29]. Structurally, the DHFR-RyR1 complex organization is found in the ratio of 4:1, where RyR1 is physically coupled to four $Ca_V1.1$ [4] (Figure 3A). In cardiac muscle, however, plasma membrane depolarization activates DHFR ($Ca_V1.2$) to allow the entry of extracellular Ca^{2+} into cells. The entry of Ca^{2+}, in turn, triggers Ca^{2+} release by RyR2 (the predominant isoform in heart) through CICR mechanism (Ca^{2+} release induced by Ca^{2+} itself) [30]. Structurally, the DHFR-RyR2 complex organization is very different from that found in skeletal muscle, with about one $Ca_V1.2$ for every 5-10 RyR2 not aligned in a highly ordered manner [4] (Figure 3B).

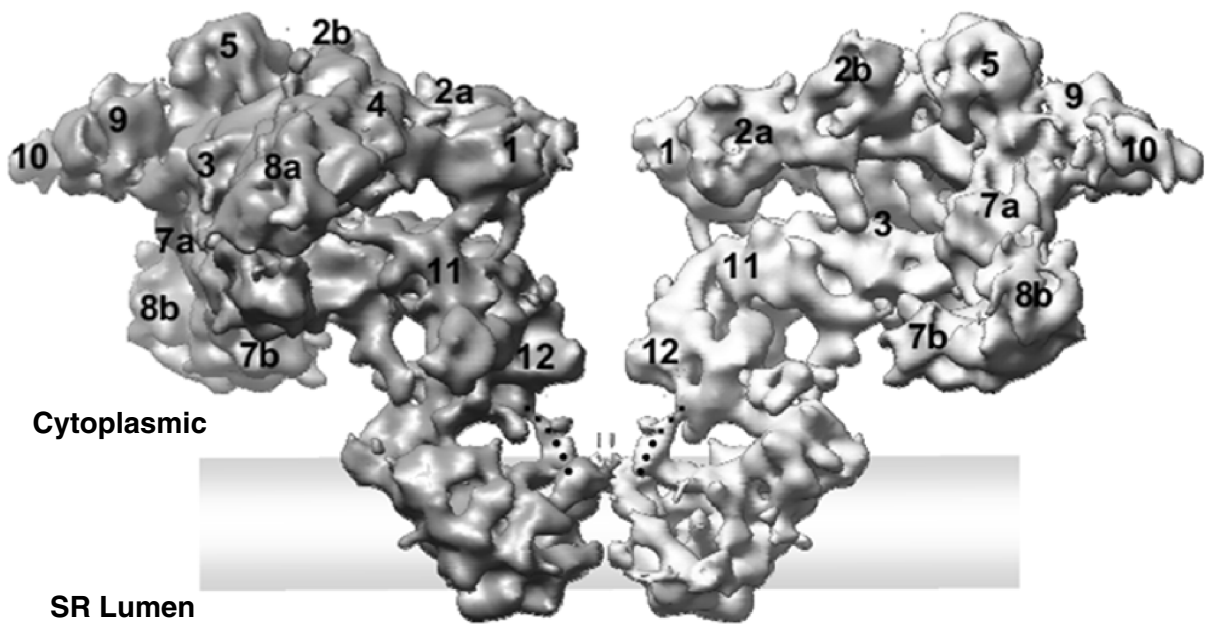

Figure 1 Two Opposing Subunits of the RyR1 Tetramer are Displayed in a Side View.
Figure adapted from Serysheva et al. [9]

Table I – Exogenous Substances Regulating RyRs

Common name	Chemical Nature	Effect on activity of RYRs	Concentration range	Pharmacological or clinical use	References
Ryanodine	Alkaloid	+/-	nM-mM	Inappropriate	Fill and Copello [4]
4-cloro-meta-cresol (4-CmC)	Chlorinated phenol	+	μM-mM	Fungicide	Mackrill [6], Fessenden et al. [17]
Caffeine	Methylxanthine	+	mM	Stimulant	Mackrill [6]
Dantrolene	Hydantoin derivatives	-	μM	Treatment of malignant hyperthermia, muscle spasticity	Mackrill [6], Paul-Pletzer et al. [18]
Procaine and tetracaine	Amino ester	-	μM-mM	Local anesthetic	Mackrill [6], Brum et al. [19]
Ruthenium red	Polycationic dye	-	nM-μM	-	Mackrill [6]

Table II – Physiological Agents Regulating RyRs

Common name	**Chemical nature**	**Effect on activity of RYRs**	**Concentration range**	**Comments**	**References**
Cytosolic Ca^{2+}	Ion	+/-	μM/mM	Inhibits or blocks, according to concentration	Fill and Copello [4]
ATP	Nucleotide	+	mM	RyR1 is more sensitive to ATP than other RYR subtypes	Copello et al. [20]
Mg^{2+}	Ion	-	mM	Mg^{2+} competes with Ca^{2+} for its activation site on RyR1	Copello et al. [20], Steele and Duke [21]
REDOX state	Oxidizing or reducing state	+/-	-	Oxidizing state increases and reducing state decreases channel activity	Voss et al. [22]
Cyclic ADP-ribose	Metabolite of nicotinamide adenine dinucleotide phosphate (NADP)	+	-	May activate Ca^{2+}-ATPase, indirectly activating the RYR receptor	Copello et al. [20], Lukyanenko et al. [23]
Phosphorylation	Addition of a phosphate group	+/-	-	Protein kinase A (PKA) activates and protein phosphatase 1 (PP1) blocks RYR	Reiken et al. [24]
Calmodulin	Accessory protein	+/-	nM	Activates with low levels of Ca^{2+} or blocks with high levels of Ca^{2+}	Balshaw et al. [7], Hamilton et al. [25]
Calsequestrin	Accessory protein	+/-	nM	Needs further studies	Fill and Copello [4], Beard et al. [26]
FKPB12	Accessory protein	-	-	Decreases the likelihood of the open state and frequency of RYR	Mackrill [6]
Calstabin1	Accessory protein	-	-	Stabilizes the closed state of RYR	Bellinger et al. [27]

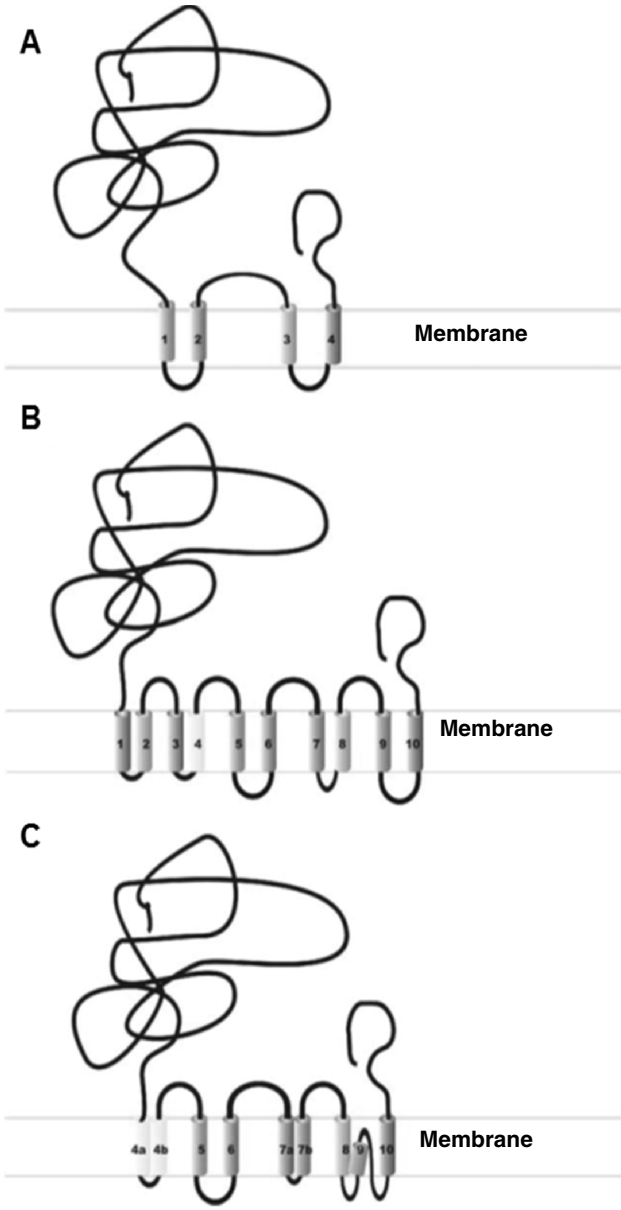

Figure 2 Models of Transmembrane regions of RyR1. (**A**) Model of Takeshima et al. [11]; (**B**) Model of Zorzato et al. [12]; (**C**) Model of Du et al. [13]. Figure adapted from Hamilton [14].

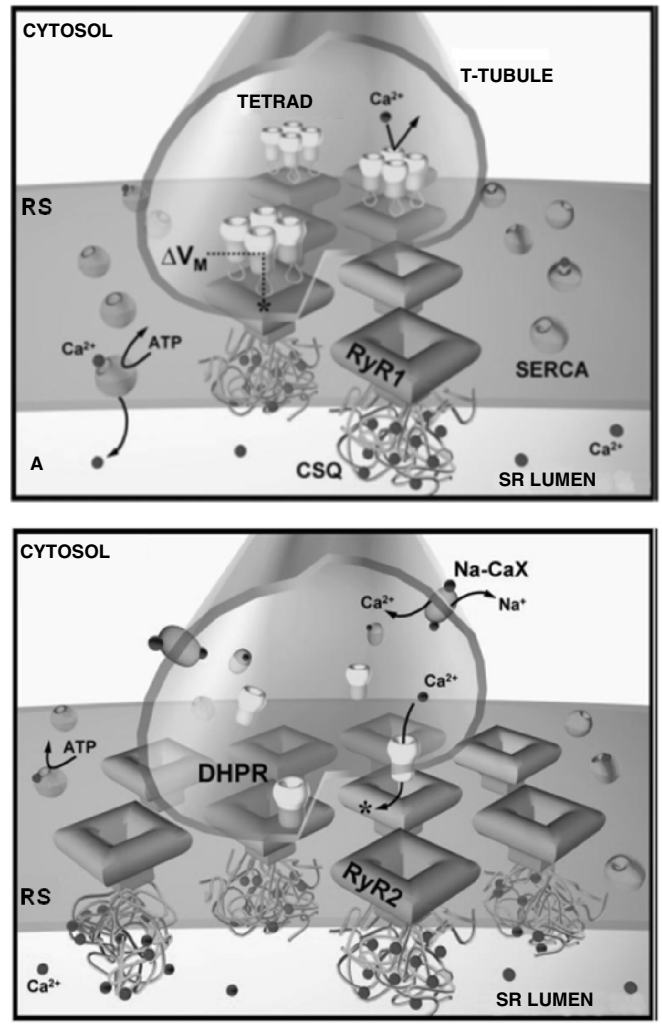

Figure 3 Organization of DHPR-RyR Complex in Skeletal (**A**) and Cardiac (**B**) Muscles.
Figure adapted from Fill et al. [4]

Correlated channelopathies

RyRs are encoded by three distinct genes located on human chromosomes 19q13.1 (RyR1), 1q42.1-1q43 (RyR2), and 15q14-q15 (RyR3) [31]. Mutations in both RyR1 and RyR2 are correlated with disease [14]. To date, over 100 mutations in RyR1 have been identified and grouped into three regions of the protein: N-terminal, Central, and C-terminal [6]. These mutations have been implicated in a wide range of conditions, among them the susceptibility to malignant hyperthermia and various congenital myopathies, including central core disease, multiminicore myopathy with external ophthalmoplegia and, rarely, centronuclear myopathy. Although malignant hyperthermia is predominantly inherited, the central core disease involves both autosomal dominant and recessive inheritance. Multiminicore myopathy with external ophthalmoplegia is associated with recessive inheritance and quantitative defects of RyR1 protein expression [32].

RyR2 mutations are associated with two forms of arrhythmia induced by stress, called catecholaminergic polymorphic ventricular tachycardia type 1, and a form of arrhythmogenic right ventricular dysplasia type 2. There are more than 80 mutations related to the RyR2 gene and they are clustered in three regions of the protein, similar to the distribution of changes in RyR1 [6].

MALIGNANT HYPERTHERMIA

Concept

Malignant hyperthermia (MH), also known as malignant hyperpyrexia, is a potentially lethal pharmacogenetic disorder which affects genetically predisposed individuals [2,33].

Etiology

There is clear evidence that individuals susceptible to MH have a skeletal muscle disorder associated with uncontrolled release of Ca^{2+} from sarcoplasmic reticulum [33]. Two genes related to susceptibility to MH have been identified and at least four genes are in the process of positive identification [5] (Table III). Individuals susceptible to MH respond abnormally when exposed to inhalational anesthetics (halothane, enflurane, isoflurane, desflurane, sevoflurane), depolarizing muscle relaxants (e.g. succinylcholine) or extreme physical activity in hot environments [1]. During exposure to these triggering agents, there is a rapid and sustained growth of myoplasmic Ca^{2+} concentration due to RyR1 hyperactivation, which causes a profound change in $Ca2^{+}$ homeostasis and characterizes a hypermetabolic state [27].

Epidemiology

MH was described in all ethnic groups and its susceptibility occurs equally in both sexes, although seizures are more common in men. The incidence of anesthetic MH in adult patients is 1/50,000 and 1/15,000 in pediatric patients, although cases have been reported in extreme ages. Its true prevalence is difficult to define because some individuals present mild or no reactions and the variable penetrance of the inherited trait [34,35]. The incomplete penetrance indicates that, although the individual has the genetic mutation for MH susceptibility, it does not mean that this dysfunction will be expressed during the first or even after the exposure to a triggering agent [35].

Pathophysiology

Under normal conditions, the myoplasmatic levels of Ca^{2+} are controlled by RyR1, DHFR, and Ca^{2+}-adenosine triphosphatase (Ca^{2+}-ATPase) system [35]. In MH crisis, there is intense change in Ca^{2+} homeostasis in which RyR1 hyperactivation causes an increase in cytoplasmic Ca^{2+} concentration, which results in sustained activation of muscle contraction [18]. Sometimes, the first symptom may be the presence of a masseter muscle spasm. This signal is considered by many authors as a sign of suspected syndrome[5].

The process of muscle contraction and re-absorption of excess Ca^{2+} consume large amounts of ATP and generate excessive heat (hyperthermia), which is the hallmark of disease [18]. Depletion of ATP stocks results in disruption of the skeletal muscle membrane and there is leakage of cellular constituents, including potassium, creatine, phosphate, and myoglobin. Loss of potassium from muscle cells results in metabolic acidosis and cardiac arrhythmias [37]. Decreased concentration of ATP causes muscular rigidity, as the presence of ATP is normally required to allow muscle relaxation, in addition to the combination of actin and myosin to allow muscle rigidity and inextensibility [18].

A potential increase in oxygen consumption through uncontrolled glycolysis and aerobic metabolism leads to cell hypoxia, progressive lactic acidosis, and excessive carbon dioxide generation [34]. Thus, the most common initial signal of acute malignant hyperthermia is an unexplained increase in values of capnography ($EtCO_2$), a method that evaluates the gradient of CO_2 present during expiration, in which excess expired CO_2 does not easily decreases with increased minute ventila-

Table III – Classification Summary of Genetic Mutations Associated with Susceptibility to Malignant Hyperthermia

Mutation	Localization	Comments
MSH1	Mutation associated with RyR1gene in chromosomal locus 19q13.1.	Most frequently reported mutation (> 50%).
MSH2	Mutation associated with chromosomal locus 17q11.2-q24, related to voltage-dependent sodium channel of skeletal muscle. Possible gene: SCN4A	Reported in American and South African families.
MSH3	Mutation associated with chromosomal locus 7q21-q22, corresponding to the site encoding the dihydropyridine receptor $\alpha 2/\Delta$ subunit, voltage sensor of the T-tubule for RyR. Possible gene: CACNL2A	Causative genes have not been located, yet.
MSH4	Mutation associated with chromosomal locus 3q13.1.	Causative genes have not been located, yet.
MSH5	Mutation associated with the gene encoding the dihydropyridine receptor $\alpha 1$ subunit in chromosomal locus 1q32. Gene: CACLN1A3P	Present in 1% of malignant hyperthermia cases.
MSH6	Mutation associated with chromosomal locus 5p.	Validity for MSH6 mutation needs confirmation.

Adapted from Gómez [5], Litman et al. [34]

tion. This increased $EtCO_2$ is associated with the presence of tachycardia (due to sympathetic stimulation by acidosis) [5].

This hypermetabolic state generates heat and leads to hypoxemia, metabolic acidosis, rhabdomyolysis (breakdown and lysis of muscle cells) and a rapid increase in body temperature, which can be fatal if not recognized and treated early [2].

Signs and symptoms

The onset of acute malignant hyperthermia is characterized by one or more signals of systemic hypermetabolism during or immediately after administration of a triggering agent [34]. The first symptoms are tachycardia, hyperventilation, localized muscle stiffness, cyanosis, arrhythmias, excessive sweating and hyperthermia. The crisis of MH can manifest itself later on a recurring basis in up to 20% of cases, even after discontinuation of the triggering agent, and fever above 40°C, cyanosis, poor cutaneous perfusion, pressure instability, and generalized muscle rigidity may occur [35].

Additional and potentially fatal complications include disseminated intravascular coagulation, congestive heart failure, intestinal ischemia, and limb compartment syndrome with a deep muscle edema [34].

Diagnosis

Clinical

MH diagnosis is based on clinical and laboratory findings. MH manifestation may be immediately after exposure to the triggering agents or even a few hours after its discontinuation. Without this prior exposure, it is usually impossible to identify a susceptible patient, which makes the clinical diagnosis very difficult [33,35,38].

Crises are classified according to clinical presentation and symptoms may vary from fulminant to abortive conditions, according to its intensity [35] (Table IV).

The most common initial symptoms are listed in Table V. Although nonspecific, these initial symptoms associated with exposure to triggering agents in the absence of other apparent cause will be sufficient to establish a preliminary diagnosis of MH and immediately refer the patient to treatment. MH may evolve rapidly, presenting additional clinical and laboratory manifestations (Table V). Between 12 and 24 hours after the crisis onset, the peak plasma levels of creatine kinase (CPK) can be observed. Susceptibility confirmation will depend on caffeine-halothane contracture test (CHCT) outcome, indicated only three months after the crisis' onset [35].

Laboratory – Susceptibility to MH

Creatine kinase (CPK) at rest

Increased CPK is found in 50% of relatives of patients with anesthetic malignant hyperthermia. The presence of increased CPK at rest, other than in strenuous exercise or muscle trauma, has relative value only in relatives of susceptible patients. Without additional explanation, high levels of CPK at rest raise the suspicion of myopathy. These changes are common and do not justify plasma CPK measurement in the general population [39].

Contraction test for exposure to caffeine-halothane (CHCT)

Even in classic cases, diagnosis confirmation is mandatory because it will be from the confirmed cases that the investigation planning for relatives of those affected will be made. The standard test adopted for MH diagnosis is the contraction test of exposure to caffeine-halothane (CHCT) [35]. Through analysis of the contractile response to caffeine-halothane exposure, it is possible to discriminate patients as susceptible (MHS) when the answer to both caffeine and halothane is abnormal;

Table IV – Classification of Malignant Hyperthermia Crisis

Classical fulminant: potentially fatal, multiple metabolic and muscular manifestations.	**Fulminant**
Moderate: metabolic and muscular manifestations without the severity of a classical fulminant.	**Abortive**
Mild: mild metabolic changes without muscle involvement.	**Masseter spasm**
Masseter muscle rigidity with evidence of muscle injury (e.g., increased serum creatine kinase and myoglobinuria).	
Masseter muscle rigidity associated with metabolic changes (e.g., increased temperature, cardiac arrhythmias).	
Masseter muscle rigidity alone.	
Sudden death or unexplained cardiac arrest during anesthesia.	**Atypical**
Other: postoperative fever, rhabdomyolysis, renal failure, suspected family history	

Adapted from Amaral et al. [35].

Table V – Clinical Manifestations of Malignant Hyperthermia crisis

Clinical	Laboratory
Early	
Tachycardia	Hypercapnia (respiratory acidosis)
Progressive increase of exhaled CO_2	Metabolic acidosis
Tachypnea	Hyperlactacidemia
Localized muscle stiffness (including masseter rigidity)	Hyperkalemia
Cyanosis	Central venous desaturation
Arrhythmias	
Hyperthermia	
Profuse sweating	
Late	
Fever above 40°C	Myoglobinemia
Cyanosis	Increased serum creatine kinase
Poor skin perfusion	Increased serum creatinine
Pressure instability	Disseminated intravascular coagulation
Generalized muscle rigidity	

Adapted from Amaral et al. [35].

negative (MHN) when the response to caffeine and halothane is normal; and equivocal (MHE) when the response to caffeine or halothane is abnormal. All patients diagnosed as MHE are treated as MHS due to their susceptibility. Clinical diagnosis is considered positive when there is a contracture ≥ 0.5 g for 3% halothane and ≥ 0.3 g at 2 mM of caffeine [40].

The procedure for muscle biopsy preparation varies depending on the laboratory. Some of them follow the United States' protocol (97% sensitivity, but low specificity, with 22% false positives) while others follow the European protocol - also known as in vitro contracture test - which differs from the American protocol only by including the use of ryanodine or 4-chloro-m-cresol (99% sensitivity, 94% specificity) [5].

In Brazil, both the Muscle Biopsy Center of the Universidade Federal do Rio de Janeiro and the Study, Diagnosis and Malignant Hyperthermia Research Center (Cedhima) of the Escola Paulista de Medicina, Universidade Federal de São Paulo use the American protocol for the diagnosis of MH [41].

Genetic testing

From the first reported case of MH, it was suspected to be a family inherited disorder [40]. With the demonstration that a mutation in the gene encoding RyR1 in pig muscles was the basis of MH, a simple DNA test in humans to diagnose it increased the expectation. However, this expectation has not yet been achieved because there are many changes in skeletal muscle that may be the cause for the different forms of the syndrome [42] (Table III).

The mutations associated with the six listed genes correspond to approximately 50% of families surveyed. In other families, the gene involved is still unknown [39]. Furthermore, despite the MHS1 mutation being the only direct genetic cause for MHS, the additional presence of MHS3, MHS4, or MHS6 mutations may interact and increase the phenotype expression in some individuals [40].

However, with time, an accuracy test based on DNA and applicable to most patients will be available and, once identified the mutation in a case of MH, all family members may be tested for that specific mutation through a blood sample. A major international effort is underway to clarify the molecular genetic basis of MH [42].

Treatment

The internationally recommended protocol for treatment of malignant hyperthermia is based on discontinuation of exposure to the triggering agents, administration of specific drug (dantrolene), and support measures or measures aimed at preventing associated complications, such as:

1. Replacing the anesthesia circuit by another circuit uncontaminated by anesthetic agent;
2. Hyperventilating the patient with 100% oxygen;
3. External cooling and, if necessary, internal;
4. Correction of metabolic acidosis;
5. Reduction of hyperkalemia;
6. Correction of cardiac arrhythmias;
7. Maintenance of diuresis [33,35].

Dantrolene

Dantrolene was originally synthesized by Snyder et al. in 1967. It was found to have muscle relaxing properties after intravenous administration in animals. The studies demon-

strated that these relaxing properties are due to the depression of excitation-contraction coupling (EC). It was initially used as a muscle relaxant in long-term treatment of skeletal muscle spasticity [43]. Dantrolene has been used since 1975, but currently its clinical use is restricted to malignant hyperthermia [44,45].

Dantrolene blocks the RyRs, acts directly on RyR1 and RyR3 isoforms, reduces the channel activation by calmodulin, and reduces the channel sensitivity to Ca^{2+}. RyR2 is not blocked by dantrolene, which explains its lack of negative inotropic effect on the heart [7,10,46].

The molecular structure of dantrolene, a hydantoin derivative, is planar. It is highly lipophilic and, therefore, poorly water-soluble. This created problems for its introduction into clinical practice until the 1980s. Its widespread use had to wait for a suitable intravenous preparation [47]. Currently, dantrolene is available for intravenous use in vials containing 20 mg of lyophilized sodium dantrolene added to 3 g of mannitol to enhance water solubility. The vial contents should be dissolved in 60 mL of water, which yields a final dantrolene concentration of 0.33 $mg.mL^{-1}$ at pH 9.5. The resulting alkaline solution is highly irritating to peripheral veins and must be injected into a large vein or be rapidly infused [43].

Rapid preparation and administration of dantrolene are essential. Therapy begins with the administration of 2.5 $mg.kg^{-1}$ and must be repeated every five minutes until the hypermetabolic state normalization and disappearance of all MH symptoms [48]. Continuous intravenous infusion of dantrolene at 10 $mg.kg^{-1}$ should be given at least 24 hours after successful initial therapy. Support therapy includes body cooling; administration of sodium bicarbonate to treat acidosis; beta-blockers or lidocaine in case of cardiac arrhythmias persistency; and furosemide and glucose-insulin infusion in case of hyperkalemia, hypercalcemia, and myoglobinuria. Thus, early diagnosis generates a successful treatment in most patients [43].

Azumolene

Azumolene is 30 times more soluble in water than its analog, dantrolene. This is due to the replacement of the para-nitrophenyl group in dantrolene by the para-bromophenyl group. Compared to dantrolene, azumolene is equipotent for treatment and prevention of MH clinical manifestations during a crisis induced by halothane or succinylcholine. *In vitro* studies showed azumolene equipotential for relaxing porcine skeletal muscle and, *in vivo*, it was more potent for inhibiting the gastrocnemius muscle contractions. Therefore, this product may be useful for treating MH in the future. However, for economic reasons, it has not been introduced into clinical practice yet [43].

PERSPECTIVES

The elucidation of the molecular genetic basis of MH has the perspective of making a pre-symptomatic diagnosis, without the need for biopsies, in addition to having a full understanding of the disease etiology. With the advancement in human genome mapping, there is a promising future for characterizing new mutations related to this syndrome and unveiling the genetic heterogeneity of MH, as phenotypic variations may be caused by interactions of several genes, yet unknown, such as the RYR1 gene.

In recent years, a major breakthrough has occurred in understanding the dynamics of Ca^{2+} release via RyR1 from the several modulating proteins of this receptor. Therefore, further studies on these numerous modulating proteins represent potential therapeutic targets. Therefore, studies related to the properties of RyR1 may yield results applicable to clinical practice, such as drugs that increase or disrupt the interaction of these modulating proteins of RyR1, and, thus, provide the development of new therapeutic strategies for treating MH.

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