Na+-H+ EXCHANGER AND PROTON CHANNEL IN HEART FAILURE ASSOCIATED WITH BECKER AND DUCHENNE MUSCULAR DYSTROPHY

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Na⁺-H⁺ EXCHANGER AND PROTON CHANNEL IN HEART FAILURE
ASSOCIATED WITH BECKER AND DUCHENNE MUSCULAR DYSTROPHY

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Short title: NHE-1 and proton channel in heart failure

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Abstract

Cardiomyopathy is found in patients with Duchenne (DMD) and Becker (BMD) muscular dystrophies which are linked muscle diseases caused by mutations in the dystrophin gene. Dystrophin defects are not limited to DMD but are also present in mild BMD. The hereditary cardiomyopathic hamster of the UM-X7.1 strain is a particular experimental model of heart failure (HF) leading to early death in muscular dystrophy (dystrophin deficiency and sarcoglycan mutation) and heart disease (δ-sarcoglycan deficiency and dystrophin mutation) in human DMD. Using this model, our work showed a defect in intracellular sodium homeostasis before the appearance of any apparent biochemical and histological defects. This was attributed to the continual presence of the fetal slow sodium channel which was also found to be active in human DMD. Due to muscular intracellular acidosis, the intracellular sodium overload in DMD/BMD was also due to sodium influx through the sodium-hydrogen exchanger, NHE-1. Lifetime treatment with an NHE-1 inhibitor prevented intracellular Na$^+$ overload and early death due to HF. Our work also showed that another proton transporter, the voltage-gated proton channel (Hv1) exists in many cell types including heart cells and skeletal muscle fibers. The Hv1 could be indirectly implicated in the beneficial effect of blocking NHE-1.

Key words: Becker muscular dystrophy, Duchenne muscular dystrophy, heart failure, NHE-1, proton channel.
Introduction

Early death from heart failure (HF) is a major growing health problem in North America (Blair et al. 2013; Danielsen et al. 2017). There were nearly 5.7 million U.S. Americans ≥ 20 years of age (2.7% prevalence) living with HF, and 670,000 new cases ≥ 45 years of age per year in 2008 in a population of about 304 million (Blair et al. 2013; Roger et al. 2012). Similar statistics to those in the United States were reported in Canada and the number of cases is expected to increase with time (Blair et al. 2013; Ross et al. 2006). Although HF in general received a great deal of attention in the literature, less work has been dedicated to early death due to HF in hereditary cardiovascular diseases (CVD) such as in Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). Duchenne and Becker muscular dystrophies are the most common childhood forms of X-linked disorders affecting the synthesis of dystrophin, a large sarcomembral protein that is absent in DMD and reduced in amount or abnormal in size in BMD patients (Judge et al. 2011; Verhaert et al. 2011; Monaco et al. 1988). Several hypotheses have been proposed in order to explain the role of dystrophin and related glycoproteins such as δ-sarcoglycan in the cardiac defect and early death due to heart failure in DMD and BMD (Judge et al. 2011). However, the most commonly used model for dystrophin defects, the mdx mouse, as well as dystrophin knock-out mice do not have an early death from HF. This raises the possibility that the absence of dystrophin in DMD and BMD may have nothing to do with early death due to HF. Recent work from our group showed that in one of the best models for DMD and BMD, the UM-X7.1 hereditary cardiomyopathic hamster (HCMH) (Figure 1), intracellular sodium overload (Figure 2) is one of the earliest pathological signs (Bkaily et al. 2017, 1997; Chahine et al. 2005; Jacques et al. 2003). The latter was found to be, in part, a consequence of the development of intracellular acidosis and the resulting sodium influx via the Na⁺-H⁺ exchanger, NHE-1 (Figure
Treatment with an NHE31 blocker, EMD87580, not only prevented intracellular sodium overload, but also prevented the increase of circulating creatine kinase (CK) and early death due to HF (Bkaily et al. 2015). Consequently, a treatment that prevents intracellular sodium overload via NHE31 and/or prevents the development of acidosis, probably by increasing \( \text{H}^+ \) efflux via the proton channel may represent an excellent therapeutic approach to prevent early death due to HF (Figures 2 and 3) in DMD and BMD patients. A translational study for the use of an NHE31 inhibitor in DMD patients is gaining traction (Porte Thomé et al. 2016). However, the role of the voltage-gated proton channel in the development of HF leading to early death in DMD and BMD (Figure 2) awaits elucidation. For further information concerning sodium overload and NHE31 in DMD and BMD the reader should refer to key references (Bkaily et al. 2017, 2015, 1990; Al-Khoury et al. 2006; Chahine et al. 2005; Jacques et al. 2003, 1997; Honore et al. 2008).

Development of heart failure in Duchenne and Becker muscular dystrophies

Patients with Duchenne and Becker muscular dystrophies develop a cardiomyopathy that leads to early death due to heart failure. Such hereditary diseases are associated with mutations in the dystrophin gene (Oldfors et al. 1994; Toyo-oka et al. 2002). These mutations are present in different ways and situations of mild Becker muscular dystrophy (Oldfors et al. 1994). The latter is a slow developing form of DMD and is associated with severe cardiomyopathy (Oldfors et al. 1994). Female carriers of DMD can develop symptomatic skeletal myopathy alone or combined with dilated cardiomyopathy (DCM) (Oldfors et al. 1994). Furthermore, X-linked DCM has been found in association with dystrophin defects (Oldfors et al. 1994). However, the role of the
dystrophin defect in DCM development is still unsupported. Still, literature in the field claims that cardiomyopathy and HF are caused by dystrophin defects. Since the dystrophin defect is not yet proven to be the cause of cardiomyopathy and HF, it is wise to state that HF in DMD and BMD is associated with, and not necessarily directly caused by, a defect in dystrophin and its associated glycoproteins.

Cases of cardiomyopathy associated with dystrophin defects were reported in 14 day-old DMD patients and were diagnosed one month before death (Oldfors et al. 1994). This confirms that early death in DMD is largely due to HF. Among the entities that are associated with DCM in children are dystrophinopathies including DMD and BMD (Tsuda et al. 2014). Early and progressive DCM occurred in 70% of BMD patients (Tsuda et al. 2014). The severity of DCM in BMD patients depends on how early is the onset of the disease. However, in DMD, severe DCM occurred rapidly and was reported to lead to early death of patients (Oldfors et al. 1994).

It is also worth mentioning that symptoms including CM leading to HF and early death develop slowly in BMD when compared to DMD (Toyo-oka et al. 2002; Oldfors et al. 1994; Tsuda et al. 2014; Ueda et al. 1995). Therefore, a better prognosis can be made for BMD patients than DMD (Toyo-oka et al. 2002; Oldfors et al. 1994; Tsuda et al. 2014; Ueda et al. 1995; Saito et al. 1994; Finsterer and Stollberger 2003). In both DMD and BMD, the heart is affected to various degrees by DCM, depending on the stage of the disease (Figure 1) and the type of mutation (Toyo-oka et al. 2002; Oldfors et al. 1994; Tsuda et al. 2014; Ueda et al. 1995; Saito et al. 1994; Finsterer and Stollberger 2003). In both DMD and BMD, CM remains subclinical in the early stages of the disease (Finsterer and Stollberger 2003) with apparent spreading of fibrosis (Finsterer and Stollberger 2003) and is present in 90% of DMD/BMD patients as was reported in the UM-X7.1 hamster model (Figure 1). Furthermore, early death occurred often within two years following
DMD diagnosis (Finsterer and Stollberger 2003). Early death in DMD/BMD patients is most commonly attributed to CM (Darras et al. 2014). In addition, this cardiac involvement has also been documented in several muscular dystrophies including DMD (Toyo-oka et al. 2002; Oldfors et al. 1994; Tsuda et al. 2014; Ueda et al. 1995; Saito et al. 1994; Finsterer and Stollberger 2003; Darras et al. 2014; Politano et al. 2008).

As mentioned earlier, due to the importance and high incidence of DCM in muscular diseases, early assessment and treatment of HF in DMD and BMD were recommended (Romfh and McNally 2010). It has therefore recently been highly recommended to monitor cardiac disease early in childhood in DMD patients and treat them as soon as possible (Romfh and McNally 2010). Finally, without any doubt, the life expectancy of DMD/BMD patients is largely dependent on the severity of DCM (Blain and Straub 2011).

Animal models for heart failure in DMD and BMD

The hereditary CM of the cardiomyopathic Syrian hamster is a particular experimental model for muscular dystrophy (dystrophin deficiency and sarcoglycan mutation) and heart disease (δ-sarcoglycan deficiency and dystrophin mutation) in human DMD and XLDC. Figure 1 shows the different phases of the development of hereditary CM and HF leading to early death.

The use of hamsters as laboratory specimens is generally associated with the introduction of the Syrian hamster by Adler in 1931 (Adler and Theodor 1931; Adler 1948). Accidental observation of muscle dystrophy in the Syrian hamster by the Jackson laboratory led to its use as a model for human muscular dystrophy (Homburger et al. 1962; Homburger 1972). The most commonly used model to-date, the Bio 14.6 hamster strain, was derived by Brink and Lochner in 1967 from the Bio 1.5 line. Later, the UM-X7.1 (Bajusz 1969; Jasmin and Bajusz 1973) and the Bio TO2
(Sole 1986; Trippodo et al. 1993) lines were derived from the Bio 14.6 line. Thus, the Bio 14.6 hamster line was used as a model for human DMD. However, with time, this animal model was used mostly for its heart disease leaving behind its muscular dystrophy aspect. This gave, with time, the name that we know for this animal model which is hereditary cardiomyopathic instead of a muscular dystrophy animal model. Thus, even though biochemical and anatomical muscle dystrophy begins before the dilated cardiomyopathy, the latter aspect was the center of attention till today. It is worth noting that, contrary to UM-X7.1 hamsters, the Bio 14.6 and Bio TO2 hamster models showed only the first two phases of the disease (Figure 1) but did not show the final stage of HF and early death (Figure 1).

In fact, hereditary muscular dystrophy and its associated CM in the UM-X7.1 hamsters is characterized by a slow progression towards cardiac failure and early death following well defined phases, and by its uniform and predicted nature with respect to morphological, biochemical, and electrophysiological anomalies (Bajusz 1969; Jasmin et al. 1991; Hatem et al. 1994; Honore et al. 2008; Chahine et al. 2005, Bkaily et al. 2015) (Figure 1). One of the weaknesses of these hereditary cardiomyopathic hamster models, with the exception of the UM-X7.1 strain, is the lack of heterogeneity between animals of the same population. Cardiomyopathy in the three strains of hamsters, and in particular the UMX-7.1 strain, is a hereditary autosomal recessive disease having a 100 % incidence in the offspring (Bkaily et al. 1996) and could be caused, in part, by a mutation of the δ-sarcoglycan (δ-SG) (Sakamoto et al. 1997) and dystrophin (Oldfors et al. 1994; Tsuda et al. 2014; Kawada et al. 2005) genes.

The hereditary CM of the UM-X7.1 hamsters strongly resembles the disease in humans at the morphological and functional levels, in the response to treatments with drugs, in the concomitant
presence of a muscular dystrophy and in the progress of the disease following three phases (Figure 1 and Bkaily et al. 2015):

1. A necrosis phase characterized by the formation of focal lesions in the cardiac muscles especially at the level of the left ventricle. This phase appears between 30 and 120 days after birth (Bajusz 1969; Jasmin et al. 1991; Hatem et al. 1994).

2. A fibrosis or a healing phase characterized by sequestration of the calcified fragments of cardiomyocytes in the fibrous tissue leading to an increase in heart weight and volume. The atria and the ventricles show varying degrees of hypertrophy and dilatation. This phase lasts until 200 days after birth (Bajusz 1969; Jasmin et al. 1991; Hatem et al. 1994).

3. A terminal phase typical of cardiac failure which eventually leads to a premature death between 250 and 300 days after birth (Bajusz 1969; Jasmin et al. 1991; Hatem et al. 1994).

It is worth noting that the three hamster models share several features. Among the similarities is the development of necrosis and hypertrophy as well as a reduction in blood pressure which accompanies the HF stage in humans (Patterson and Adams Jr 1996) as observed in both the Bio 14.6 (Yamauchi-Kohno et al. 1999; Honore et al. 2002, 2008) and the UM-X 7.1 hamsters (Cachofeiro et al. 1990). Among the differences, is the fact that after repeated inbreeding, the Bio 14.6 animals showed a decrease in the severity of symptoms with modulation of the signs of cardiac failure and consequently an increase in longevity. However, as in humans (Mestroni et al. 1999), the UM-X 7.1 hamsters die early (between the ages of 250 and 300 days) due to HF
(Jasmin and Proschek 1982). Thus, it is highly recommended to use the UM-X 7.1 strain as an animal model of HF leading to early death. In addition, this naturally occurring genetic model is an excellent model for muscular dystrophy, particularly DMD and BMD. This is evidenced by the skeletal muscle fiber remodeling where nuclei re-localized to the center of the fibers as shown in figure 3. We have to also mention that this model develops an increase of weight due to overall subcutaneous edema (see Figure 4) and could be used as a model for acute edema.

There is no cure for DMD and current treatment options focus on alleviation of symptoms and management of complications (Ryder et al. 2017 and references within). Patients seem to live longer which is attributed to the widespread prescribing of corticosteroids, improved access to ventilation and the publication of more thorough and specific guidelines of care (Ryder et al. 2017 and references within). In cardiomyopathic hamsters, there is an increase in the levels of plasma and cardiac AngII (Nakamura et al. 1994). The activation of the renin-angiotensin system (RAS) in HF (including DMD and BMD patients) is well established (Schrier and Abraham 1999; Sudano and Noll 2009). It was also reported that the activation of cardiac RAS contributes to the development of cardiac necrosis (Gavras et al. 1971, 1975; Giacomelli et al. 1976; Tan et al. 1991; Kabour et al. 1994) and hypertrophy (Baker and Aceto 1989; Sadoshima and Izumo 1993; Schorb et al. 1993; Miyata and Haneda 1994). In addition, treatments with different angiotensin-converting enzyme (ACE) inhibitors decreased cardiac remodeling (Davison et al. 1994; Mansoor et al. 1996; Masutomo et al. 1996), preserved contractile function (Hirakata et al. 1990; Chemla et al. 1991; Haleen et al. 1991; Nakamura et al. 1994), prevented left ventricular failure and increased the probability of survival (Chemla et al. 1991; Haleen et al. 1991; Narita et al. 1996) of cardiomyopathic hamsters as well as in DMD patients. Based on the treatment of HF with ACE inhibitors, this category of drugs was used to treat HF in DMD patients. As for
corticosteroids (Ryder et al. 2017 and references within), the treatment with ACE inhibitors seems to delay the development of DCM and more particularly in the group of patients that received the drug early in life (for review see Judge et al. 2011 and references within). Although the different presently used treatments increase the quality of life and survival of DMD patients, none of these treatments prevents early death. The contribution of the RAS to HF was further supported by evidence that the density of AT$_1$ receptors increased in the ventricles of cardiomyopathic hamsters early during the development of the disease before apparent myocardial damage (Lambert et al. 1995). In fact, it was shown that blockade of the AT$_1$ receptor has beneficial effects on the cardiac structure and performance in UM-X7.1 hamsters (Nakamura et al. 1994) and decreased cardiac remodeling in Bio TO-2 cardiomyopathic hamsters (De Mello and Specht 2006; Shimizu et al. 2006). This may again justify therapeutic targeting of the AngII system in the treatment of DMD. Even though these justifications are accurate, one may question why all the treatments used in DMD, including those controlling the AngII system, did not prevent early death in these patients.

**Calcium overload in heart failure of DMD and BMD**

The muscular dystrophy and XLDCM of the hereditary cardiomyopathic hamster, like other forms of muscular dystrophy and HF, is characterized by an intracellular calcium overload at the level of skeletal muscle fibers and cardiomyocytes (Jasmin and Proschek 1984; Bkaily and Jacques 1994; Jasmin and Proschek 1994; Minamisawa et al. 2004; Chahine et al. 2005; Bkaily et al. 2015).

Early and recent work in the literature still support the contention that the first damage in several forms of congestive HF including in DMD and BMD is related to intracellular calcium overload.
in heart muscles (Bkaily and Jacques 1994; Minamisawa et al. 2004; Jasmin and Proschek 1984, 1994). In the hereditary cardiomyopathic hamster, UM-X7.1, it was proposed that the calcium overload gives rise to an energy depletion and that a decrease in mitochondrial oxidative phosphorylation is the primary defect (Proschek and Jasmin 1982). This leads to the assumption that intracellular calcium disturbance is the main cause of the development of necrosis, hypertrophy and early death due to HF. Several attempts were made using calcium blockers in order to prevent the development of necrosis. In some cases, necrosis was decreased by pretreatment with the L-type calcium channel blocker, verapamil (Jasmin et al. 1991) or the R-type calcium channel blocker, isradipine (Jacques et al. 2003). However, other calcium channel blockers such as galopamil, diltiazem, nifedipine, and bepridil had no effect (Jasmin et al. 1991).

It is worth noting that all pharmacological therapies done in hereditary cardiomyopathy and more specifically using the hereditary cardiomyopathic hamster model were to prevent necrosis that takes place during the early development of HF and no studies were done to verify the effect of all the drugs used on HF leading to early death. Nevertheless, all of these early studies raised the issue that calcium channels do not necessarily contribute to the development of HF in the animal model of DMD and BMD (Bkaily et al. 1996, 2017). This question was partially answered by the discovery of the early fetal TTX and Mn$^{++}$-insensitive slow sodium channel in both fetal human and chick embryo heart cells (Bkaily et al. 1990, 2017). This channel was found to have, to some extent, a pharmacology similar to that of the L-type calcium channel since it was blocked by verapamil (Bkaily et al. 1990, 2017) which was shown to prevent necrosis and hypertrophy in UM-X7.1 hamsters (Jasmin et al. 1991). This discovery led to the hypothesis that this channel may continue to function during differentiation of muscle cells and may lead to an intracellular sodium overload (Bkaily et al. 1990, 2017). This latter hypothesis was thought to be the primary
defect in hereditary cardiac diseases such as in heart failure leading to early death in DMD and BMD (Bkaily et al. 1990, 2017).

**Intracellular sodium overload as the primary defect in heart failure of DMD and BMD**

The intracellular sodium overload in heart muscle of the DMD and BMD animal model was first reported in 1997 (Bkaily et al. 1997). In this study, it was shown that an intracellular sodium overload takes place early in life of the UM-X7.1 DMD/BMD hamster model and in the mdx mouse DMD model (Bkaily et al. 1997). It was also shown that the reported intracellular calcium overload follows the intracellular sodium overload. In addition, cardiac necrosis was associated with both sodification and calcification (Chahine et al. 2005). Furthermore, pre-necrotic heart cells in UM-X7.1 hamsters showed only an intracellular sodium overload (Chahine et al. 2005) which demonstrates that sodium overload precedes calcium overload. The intracellular sodium overload was also reported (Bkaily and Jacques 1994; Karmazyn et al. 2003; Karmazyn 2001; Meng and Pierce 1991; Cingolani and Ennis 2007) to be due, in part, to an increase in Na⁺ influx caused by upregulation of cardiac NHE-1 activity (Figure 2) (Karmazyn et al. 2003; Meng and Pierce 1991; Cingolani and Ennis 2007). This supports the hypothesis that the sodium overload taking place during the development of HF in DMD and BMD cardiomyopathic hamsters consequently leads to a sustained calcium accumulation due to the increased activity of the sodium-calcium exchanger (NCX) (Figure 2) (Chahine et al. 2005; Baartscheer et al. 2008). Studies using animal models of HF in general and in isolated heart cells have demonstrated the beneficial effect of NHE-1 blockade in preventing cardiac hypertrophy (Baartscheer et al. 2008; Loennechen et al. 2002; Engelhardt et al. 2002; Yoshida and Karmazyn 2000; Chen et al. 2004,
Karmazyn et al. 2003, 2008). Such sodium and calcium overloads were found to be associated with an increase in the density and activity of NHE-1 in the DMD and BMD hereditary HF hamster model.

**Contribution of NHE-1 to HF and early death**

As previously mentioned, it was suggested that the impairment of the intracellular calcium level in muscular dystrophy and HF could be associated with intracellular sodium overload in DMD skeletal muscle myotubes (Bkaily et al. 1990) as well as in cardiomyocytes of MDX mice (Bkaily et al. 1997). This intracellular sodium overload was found to be brought about by sodium influx via NHE-1 in the UM-X7.1 cardiomyopathic hamster (Chahine et al. 2005; Bkaily et al. 2015) (Figure 2). In fact, it was demonstrated that during the development of cardiac necrosis and hypertrophy in the UM-X7.1 hamsters, there was an increase in the cardiac NHE-1 level associated with calcium and sodium overloads (Chahine et al. 2005). Furthermore, treatments with the NHE-1 inhibitor EMD87580 not only prevented the increase in the cardiac NHE-1 density, and in intracellular sodium as well as calcium, but also strongly attenuated the development of both necrosis and dilation of the left ventricle (Chahine et al. 2005) and muscular dystrophy. Furthermore, as Figure 5 shows, lifetime treatment with the NHE-1 inhibitor also prevented the development of edema. This was accompanied by a decrease in CK level and prevention of early death (Bkaily et al. 2015). This suggests that NHE-1 contributes to the pathogenesis of muscular dystrophy and heart failure of the hereditary cardiomyopathic hamster. Thus, the cardiomyopathic hamster, particularly the UM-X7.1 line, constitutes an excellent
model to be used in preclinical studies of treatment of DMD/BMD muscular and cardiac disorders with an NHE-1 blocker and particularly EMD87580.

**Presence of the voltage-gated H⁺ channel (Hv1) in cardiovascular cells**

Intracellular acidosis was reported to take place early during the development of hypertrophy and heart failure. This intracellular acidosis concomitantly takes place with the early development of the intracellular sodium overload in heart cells of the DMD and BMD hereditary hamster model. Escaping intracellular acidosis is mainly taken care of by at least two major proton transporters, the NHE-1 and the voltage-gated H⁺ channel (Hv1) (for more information about Hv1 please refer to DeCoursey 2012, 2013, 2015). Although there is no shortage of information about NHE-1 and its contribution to heart failure, there is no information concerning proton channels in the heart and whether these channels could be indirectly implicated with NHE-1 in the development of sodium overload leading to the development of heart failure and early death. This type of channel was reported to be mostly present in immune cells (DeCoursey 2012, 2013, 2015). As can be seen in figure 5, and using quantitative 3D confocal microscopy (Bkaily et al. 1997, 2017), this type of channel is present in all cardiovascular cells that constitute the heart such as human cardiomyocytes, endocardial endothelial cells, vascular endothelial cells and vascular smooth muscle cells. Furthermore, this type of proton channel was also found in intact heart and skeletal muscles of normal hamsters (Figure 6). One of our hypotheses is that, in addition to NHE-1, voltage-gated H⁺ channels may contribute to the development of intracellular acidosis and targeting both NHE-1 and/or Hv1 (Figure 2) would present a good rationale for the treatment of HF in general and more particularly preventing early death in DMD and BMD. This should be verified in the future.
Discussion and conclusion

Hereditary cardiomyopathy and its associated early death due to HF such as in DMD and BMD are difficult to study due to limited access to an appropriate animal model. An appropriate animal model of HF should reach the end point of early death. The only animal model that fulfills this requirement is the hereditary cardiomyopathic hamster of the UM-X7.1 strain. Furthermore, amongst all of the animal models for naturally occurring DMD and BMD, the UM-X7.1 hamster model is the best, if not the only, model in which the development of the disease mimics that in humans. In addition, the development of HF and early death is similar to BMD and slower than that occurring in DMD patients. This gives the time to better study the different phases of the disease.

As in UM-X7.1 hereditary cardiomyopathic hamsters, in DMD and BMD patients intracellular acidosis was reported in skeletal muscles (Lo Cascio et al. 2014 and references within) indicating impaired energy metabolism. During the development of muscle disease and particularly in DMD and BMD, myocyte metabolism is impaired. Such a deregulation of cell metabolism would explain, in part, the low intracellular pH (Sharma et al. 2003). Such a defect also increases free radical generation. Generation of an elevated level of intracellular protons due to the deregulation of intracellular metabolism and mitochondrial dysfunction is a characteristic of DMD/BMD and the UM-X7.1 animal model. Such a generation of a high level of protons will for sure be compensated by proton efflux via at least both NHE-1 and Hv1 channels (Figure 2). The limited capacity of the Hv1 channels to evacuate intracellular protons would promote a maintained intracellular acidosis which cannot be compensated by NHE-1 unless there is a change in the activity as well as the density of both proton extruders. These latter conditions are not sufficient to bring intracellular pH back to a normal level. This has at least one consequence
which is an increase of sodium influx through NHE-1 which promotes an intracellular sodium accumulation (Figure 2). The latter effect would, in turn, promote calcium influx through sodium-calcium exchange and lead to the well-known intracellular calcium overload. Intracellular calcium overload would activate many enzymes and proteins that are calcium dependent which promotes remodeling of cardiomyocytes and skeletal muscle fibers as shown in figure 6 by the re-localization of the nuclei to the center of skeletal muscle fibers. It is clear from the literature in the field of DMD and BMD that dystrophin or δ-SG deficiencies cannot be the initiators of the defect in excitation-contraction coupling of heart and skeletal muscles; they may contribute but we believe that the genetic defect implicates an enzyme that is yet to be discovered. Some of the literature in the field suggests that this enzyme could be calcium-dependent. This may or may not be the case. In waiting to discover the real product of the genetic defect behind HF and early death, several treatments for DMD are already in use (Judge et al. 2011 and references within). Among these are ACE inhibitors, beta blockers, glucocorticoids, as well as diuretics and fluid management. Other emerging pharmacological treatments were also proposed recently such as phosphodiesterase-5 inhibition with Sildenafil (Judge et al. 2011 and references within) and the NHE-1 inhibitor rimeporide (Porte Thome et al. 2016). All of these drugs, with the exception of the NHE-1 inhibitor, were mostly used in DMD leading to HF and early death because of their vasodilator properties. However, none of these drugs, with the exception of the NHE-1 inhibitor, prevented early death in animal models. Our work with the NHE-1 inhibitor showed that this type of drug prevents the development of necrosis, hypertrophy, heart failure and early death in the DMD/BMD animal model, UM-X7.1. These findings support the concept that intracellular sodium overload could be one of the most important targets for the treatment of HF leading to early death in hereditary muscular diseases.
(Chahine et al. 2005; Baartscheer et al. 2008; Engelhardt et al. 2002; Karmazyn et al. 2008; Darmellah et al. 2007). Similar results using another model for HF demonstrated that treatment with an NHE-1 inhibitor, cariporide, for two months following the development of cardiac hypertrophy, prevented cardiomyocyte intracellular Na\(^+\) and Ca\(^{2+}\) overloads (Baartscheer et al. 2008). These results demonstrate the causal relation between NHE-1 activity and cardiac remodeling during the development of HF.

Another example supporting the hypothesis that deregulation of intracellular sodium homeostasis is one important component contributing to the development of HF and early death in hereditary muscular diseases is the continual presence of the slow sodium channel (Jacques et al. 1997) and decrease in the expression of cardiac Na\(^+\)-K\(^+\) ATPase (Watanabe et al. 1998). These two phenomena contribute to the development of intracellular sodium overload in the DMD/BMD UM-X7.1 hereditary cardiomyopathic hamster model. Blocking of the slow sodium channel by beta blockers could be the reason why these blockers have some beneficial effects in the treatment of DMD patients. On the other hand, the use of ACE inhibitors in DMD patients seem to have beneficial effects, however, the mechanism responsible for such effects is controversial and could be due, in part, to the effect of this type of drugs on vascular endothelial remodeling (Pitt 1996) that was reported to take place during the development of HF leading to early death (Al-Khoury et al. 2006).

There is no doubt that the NHE-1 inhibitor is the most promising treatment for HF and early death due to a genetic defect such as in DMD and BMD (Bkaily et al. 2015). Our recent results showed that early treatment with an NHE-1 inhibitor prevents the development of necrosis during the first phase of the development of HF as well as the second phase characterized by the development of hypertrophy (Bkaily et al. 2015). Finally, this treatment succeeded in preventing
the development of HF leading to early death (Bkaily et al. 2015). All of these effects were found to be associated with a decrease of intracellular sodium and calcium overloads as well as a decrease in the activity and density of NHE-1 (Bkaily et al. 2015). The latter effect is a good indication that the prevention of early death was due largely to normalization of NHE-1 density and activity. This highly suggests that acidosis is responsible, at least in part, for the deregulation of intracellular ionic homeostasis and consequently the damage that leads to heart failure and early death. It is worth mentioning that the beneficial effect of the treatment with the NHE-1 inhibitor is accompanied with a high decrease in circulating CK levels which indicates that pretreatment prevents both cardiomyocyte and skeletal muscle damage (Bkaily et al. 2015). The fact that NHE-1 inhibition did not completely prevent the increase in CK suggests that mechanisms other than NHE-1 could be implicated in cardiomyocyte and skeletal muscle damage during the development of hereditary cardiomyopathy leading to HF and early death. This mechanism could be due, in part, to an abnormal functioning of the proton channel. The latter hypothesis should be verified.

In summary, intracellular acidosis associated with intracellular sodium overload could be considered as the first indication for the development of hereditary muscular diseases leading to HF and early death such as in DMD and BMD. Early prevention of acidosis could be one of the excellent targets for the prevention of HF leading to early death. The defect leading to intracellular acidosis in DMD and BMD is still to be elucidated. Literature in the field as well as our own work show no direct relation between dystrophin and δ-sarcoglycan deficiencies and the development of acidosis and its associated intracellular sodium overload. Whatever the genetic defect is, this should not deter us from developing an accurate treatment based on scientifically accepted facts that are related to the disease such as acidosis and intracellular sodium overload.
Further work in this direction will for sure shed new light on the development of a new bi-drug therapy that will not only improve the quality of life of DMD and BMD patients, but also prevent their early death. Finally, since according to our work NHE-1 function and density seem to be critical to cardiac and skeletal muscular defects, it is possible to use the overexpression of this exchanger as a marker for the development of HF and early death in DMD/BMD.
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References


Figure Legends

Figure 1: Heart muscle changes occurring during the development of hereditary cardiomyopathy of the UM-X7.1 hamster strain. The starting age and the duration of the treatments administered are also shown. (Modified from Bkaily et al. 2015).

Figure 2: Schematic representation showing the contribution of two major mechanisms of $H^+$ efflux during intracellular acidosis. This acidosis, together with increased circulating factors such as ET-1 and AngII contribute to the activation of NHE-1 leading to intracellular sodium and calcium overloads. NCE: sodium calcium exchanger, ET-1: endothelin-1, AngII: angiotensin II, NE: norepinephrine, Hv1: voltage gated proton channel, CaM: calmodulin, PI: phosphatidylinositol, PIP: phosphatidylinositol phosphate, DAG: diacylglycerol, PKC: protein kinase C, IP$_3$: inositol-3-phosphate (Modified from Karmazyn et al. 1999).

Figure 3: Presence of central nuclei in skeletal muscle cross-sections of UM-X7.1 hereditary cardiomyopathic hamsters. Syto-11 staining of nuclei (green color) of skeletal muscle cross-sections from hereditary cardiomyopathic hamsters of the UM-X7.1 strain during the different phases of the development of hereditary cardiomyopathy: cardiac necrosis phase (A), cardiac hypertrophy phase (B) and heart failure phase (C). The arrows point to some of the central nuclei in the muscle fibers. The white scale bar is in $\mu$m.

Figure 4: Dorsal photographs of hereditary cardiomyopathic hamsters (228 days old) of the UM-X7.1 strain (A) and their corresponding hearts (B and C) showing the effect of a preventive treatment (starting at the age of 30 days) for 198 days with an NHE-1 inhibitor, EMD87580, on
the development of body edema as well as cardiac necrosis and hypertrophy. The black scale bar is 1mm.

**Figure 5:** Presence of Hv1 in human vascular smooth muscle cells (hVSMCs), human vascular endothelial cells (hVECs), human cardiomyocytes (hCARD), and human endocardial endothelial cells (hEECs). (A-D) 3-D fishnet plots of fluorescence intensity of Hv1 in (A) hVSMCs, (B) hVECs, (C) hCARD, and (D) hEECs. The images are presented with a tilt of -80°. (E-G) Statistical compilation of the results of 3-D fluorescence intensity measurements in whole cell, PM+cytosol, and NEMs+nucleoplasm in hVSMCs, hVECs, hCARD, and hEECs. Values for fluorescence intensity are expressed per volume (µm3) according to arbitrary units from 0 to 255, where the value for black is zero, and for white is 255, and is represented by the pseudocolor bar. The white scalebar is in micrometers. The inserted panels show the labeling of nuclei of cells with the syto31 probe. The arrows show the position of the nuclei inside their respective cells. N is the number of different experiments and n is the number of different cells. Values are expressed as mean ± SEM; *, p < 0.05, ** p < 0.01, ***, p < 0.001 and ****, p < 0.0001.

**Figure 6:** Presence of Hv1 in normal hamster skeletal (A) and cardiac (B) muscles. The images are presented as 3-D fishnet plots of Hv1 fluorescence intensity with a tilt of -56° and a rotation of 22°. The pseudocolor scale represents the fluorescence intensity levels from 0 (black) to 255 (white). The white scalebar is in µm.
Figure 1: Heart muscle changes occurring during the development of hereditary cardiomyopathy of the UM-X7.1 hamster strain. The starting age and the duration of the treatments administered are also shown. (Modified from Bkaily et al. 2015).

253x240mm (300 x 300 DPI)
Figure 2: Schematic representation showing the contribution of two major mechanisms of H+ efflux during intracellular acidosis. This acidosis, together with increased circulating factors such as ET-1 and AngII contribute to the activation of NHE-1 leading to intracellular sodium and calcium overloads. NCE: sodium calcium exchanger, ET-1: endothelin-1, AngII: angiotensin II, NE: norepinephrine, Hv1: voltage gated proton channel, CaM: calmodulin, PI: phosphatidylinositol, PIP: phosphatidylinositol phosphate, DAG: diacylglycerol, PKC: protein kinase C, IP3: inositol-3-phosphate (Modified from Karmazyn et al., 1999).
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293x252mm (300 x 300 DPI)
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Figure 6: Presence of Hv1 in normal hamster skeletal (A) and cardiac (B) muscles. The images are presented as 3-D fishnet plots of Hv1 fluorescence intensity with a tilt of 45° and a rotation of 22°. The pseudocolor scale represents the fluorescence intensity levels from 0 (black) to 255 (white). The white scalebar is in µm.