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Original Article

Preclinical study of the antimyotonic efficacy of safinamide in the myotonic mouse model

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ABSTRACT

Mexiletine is the first choice drug in the treatment of non-dystrophic myotonias. However, 30% of patients experience little benefit from mexiletine due to poor tolerability, contraindications and limited efficacy likely based on pharmacogenetic profile. Safinamide inhibits neuronal voltage-gated sodium and calcium channels and shows anticonvulsant activity, in addition to a reversible monoamine oxidase-B inhibition. We evaluated the preclinical effects of safinamide in an animal model of Myotonia Congenita, the ADR (arrested development of righting response) mouse. In vitro studies were performed using the two intracellular microelectrodes technique in current clamp mode. We analyzed sarcolemma excitability in skeletal muscle fibers isolated from male and female ADR (adr/adr) and from Wild-Type (wt/wt) mice, before and after the application of safinamide and the reference compound mexiletine. In ADR mice, the maximum number of action potentials (N-spikes) elicited by a fixed current is higher with respect to that of WT mice. Myotonic muscles show an involuntary firing of action potential called after-discharges. A more potent activity of safinamide compared to mexiletine has been demonstrated in reducing N-spikes and the after-discharges in myotonic muscle fibers. The time of righting reflex (TRR) before and after administration of safinamide and mexiletine was evaluated in vivo in ADR mice. Safinamide was able to reduce the TRR in ADR mice to a greater extent than mexiletine. In conclusion, safinamide counteracted the abnormal muscle hyperexcitability in myotonic mice both in vitro and in vivo suggesting it as an effective drug to be indicated in Myotonia Congenita.

Introduction

Non-dystrophic myotonias are genetic diseases with autosomal dominant or recessive transmission caused by mutations in the *CLCN1* or *SCN4A* genes coding for the ClC-1 chloride channel or Nav 1.4 sodium channel, respectively [1]. Enhanced activity of Nav 1.4 sodium channels or decreased activity of ClC-1 chloride channels, both increase sarcolemma excitability resulting in muscle stiffness after contraction. This is the most important symptom complained by patients, which compromises daily activities [2,3]. Muscle stiffness often worsens after a period of rest and, in some cases, may improve with physical exercise ("warm-up phenomenon"). On the other hand, paradoxical myotonia, which is worsening with exercise, is a cardinal feature of Paramyotonia Congenita. Weakness, pain, and tiredness are other symptoms commonly reported beside stiffness. Onset within the first year after birth results in severe neonatal episodic laryngospasm (SNEL), which causes potentially life-threatening respiratory distress [4,5].

The pharmacological treatment of non-dystrophic myotonias has been directed toward the use of drugs capable of blocking Nav1.4 sodium channels because drugs capable of increasing the activity of ClC-1 chloride channels are missing [6]. Mexiletine, a class 1B antiarrhythmic sodium channel blocker, has been identified as the drug of first choice in the treatment of non-dystrophic myotonias. The efficacy of mexiletine versus placebo in relieving the signs and symptoms of myotonia has been demonstrated in randomized clinical trials [7–9]. However, a subgroup of patients obtains little benefit from mexiletine, due to side effects (gastrointestinal disorders, dizziness),

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contraindications (cardiomyopathies), or limited efficacy possibly on a pharmacogenetic basis [10,11]. Mutations of sodium channel, especially around the pore region, may affect the apparent affinity of mexiletine, which may explain the lack or minor effect of the drug in patients carrying those mutations [12–15]. It is therefore necessary to identify alternative drugs to mexiletine for these patients. One possible strategy is to consider new mexiletine derivatives at the aim to obtain an improvement in the pharmacological profile [16–22]. Alternatively, the repositioning of already marketed drugs may represent a winning strategy, thanks to their well-known safety profile in the clinical setting, thereby accelerating drug development [23–25]. We recently have evaluated safinamide, already approved for Parkinson disease, as a potential anti-myotonic drug [26]. Safinamide was shown to inhibit Nav1.4 sodium channels in a voltage- and frequency-dependent manner, being about two-fold more potent than mexiletine. Safinamide also appeared effective in reducing hyperexcitability in isolated skeletal muscles induced by the chloride channel blocker, 9-anthracene carboxylic acid (9-AC). In vivo, safinamide reduced the time of righting reflex in the rat after i.p. injection of 9-AC, a pharmacologically induced model of myotonia [27].

In this study, we go further in the evaluation of anti-myotonic activity of safinamide by testing the drug in vitro and in vivo in a genetic model of myotonia congenita, the myotonic ADR ("arrested development of righting response") mouse. The ADR mouse carries a spontaneous mutation in the ClC-1 channel, responsible for muscle stiffness [28–30]. The ADR mouse is a widely recognized model of human Myotonia Congenita in the recessive form (also called Becker's disease), which has already been used to test drugs [18,31,32]. Only the homozygous adr/adr mouse is symptomatic and shows myotonia, which can be evaluated as a slowing of the righting reflex (the ability to straighten up after being placed on its back) due to muscle stiffness. This translational study is aimed at better predicting clinical success.

Materials and Methods

Animal care

All the animal experiments complied with the ARRIVE guidelines [33]. Protocols were approved by the ethics committee of the University of Bari (OPBA) and by the Veterinary Authority of the Italian Ministry of Health (D.M. 514/2020-PR). The experiments were carried out in accordance with the Italian Guidelines for the Use and Care of the Laboratory Animals (D. Lgs. 2014 n. 26), which complies with the Guidelines of Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. The SWR/J–*Clcn1*$^{adr\text{-}mto}$/J mice (JAX stock #000939) were recovered from cryo-conserved embryos in the Jackson Laboratory (Bar Harbor, Maine, United States). The breeding colony was managed in our animal facility to generate affected homozygous adr/adr (ADR) mice. The mice were housed in a temperature-controlled room (20–24 °C) with a 12:12 h light-dark cycle. Mice had access to standard laboratory diet (7 g/day) and water ad libitum. The identification of Wild-Type (WT) mice (wt/wt) or heterozygous mice (wt/adr) was performed by genotyping. The genotyping protocol was conducted on DNA isolated from a 2-mm piece of the tail taken from the animal (at 30–40 days of life) anesthetized with ketamine/xylazine (100/10 mg/kg). The DNA was then amplified using primers specific for the transgene of interest in PCR reactions aimed at confirming the genotype of the animal. The mating and pregnancies of the mice were managed by qualified personnel using the guidelines reported in the Jackson Laboratory Resource Manual "Breeding Strategies for Maintaining Colonies of Laboratory Mice". Homozygous myotonic mice are recognizable by their smaller size and the presence of limb stiffness, therefore no genotyping was performed in these animals. A total of 15 ADR male or female mice were used for in-vivo and in-vitro experiments.

Parameters of excitability and action potential characteristics measured in mouse skeletal muscle fibers

The extensor digitorum longus (EDL) muscle was dissected under general anesthesia obtained with intraperitoneal injection of ketamine/xylazine (100/10 mg/kg body weight), in order to keep unchanged the functional characteristics of the muscle. The ADR mice were killed soon after the dissection by anesthetic overdose. The dissected muscle was transferred into a 25-ml recording chamber containing normal Ringer's saline solution at 30 °C [34–36]. Physiological solution contained (mM) 148 NaCl, 4.5 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 12.0 $NaHCO_3$, 0.44; NaH_2PO_4, and 5.5 glucose. The solution was constantly perfused with a mixture of 95% O_2 and 5% CO_2 to maintain pH = 7.2–7.3. For intracellular recordings, unwarranted contraction was prevented by loading muscles with 50 μM BTS (N-benzyl-p-toluenesulfonamide, Merck Life Science, Milan, Italy) dissolved in DMSO for 45 min before recordings. Experiments were performed by using the two-intracellular microelectrodes current-clamp technique in order to measure excitability parameters in EDL muscle [18,26,37]. Microelectrodes were made of borosilicate and filled with 3 mM KCl (for recording) or 2 mM K-citrate (for clamping). The two microelectrodes were inserted into the muscle fiber at about 50-μm distance, one for stimulation, capable of injecting depolarizing current, with an amplitude of about 15 nA and a duration of 100 or 200 ms and the other for the voltage recording. The membrane potential was held to −80 mV by injecting depolarizing current pulses of increasing amplitude to elicit first a single action potential and then a train of action potentials. Off-line analysis allowed calculation of the maximal number of elicitable action potentials (N spikes), as well as the after discharges (AfD) that rises in myotonic muscles by the end of stimulation. The occurrence of spontaneous discharges (in the absence of stimulation) was also recorded in myotonic muscles. The excitability parameters were measured in the absence and in the presence of increasing concentrations of safinamide (1-3-10 μM) or mexiletine (10–30 μM) applied in vitro on EDL muscle. The effects were measured 30 min after drug application.

In vivo studies in the genetic model of myotonia congenita and measure of TRR

In vivo, myotonia was evaluated by measuring the time of righting reflex (TRR), which is the time the mouse needs to turn back on its four legs from the supine position [18,23,26,27]. The experiments were performed in mice aged 4 weeks or more. The experimental protocol is shown in Fig. 1. The TRR was measured by an operator with a manual stopwatch, 15 min before and 20, 60, 90, and 120 min after intraperitoneal (i.p.) injection of drug or saline (time 0). At each time point, the TRR was measured five times at 1-min interval to calculate an average value. In control conditions, the TRR is usually less than 0.5 s in the wt/wt or wt/adr mouse. In adr/adr mouse, the TRR is higher. An anti-myotonic drug is expected to counteract the TRR increase. Safinamide was tested at three doses (10 mg/kg, 3 mg/kg and 1 mg/kg) and compared to mexiletine at the same doses or saline solution (drug vehicle). Each dose was tested in five mice. Safinamide and mexiletine effects were fully reversible in a few hours, and no side effects were observed. The animals were allowed to recover for several days between two experiments. No more than three experiments were performed in each mouse.

Chemicals and drugs

Safinamide methanesulphonate salt was supplied by Zambon SpA (batch 14A03C0483). Mexiletine hydrochloride was purchased from Sigma Aldrich (Milan, Italy). Both compounds were dissolved in Ringer's saline solution. For the in vivo experiments, safinamide and mexiletine were dissolved in 0.9% NaCl solution, the final volume for intraperitoneal injection did not exceed 200 μL. An equal volume of 0.9% NaCl solution was used as vehicle.

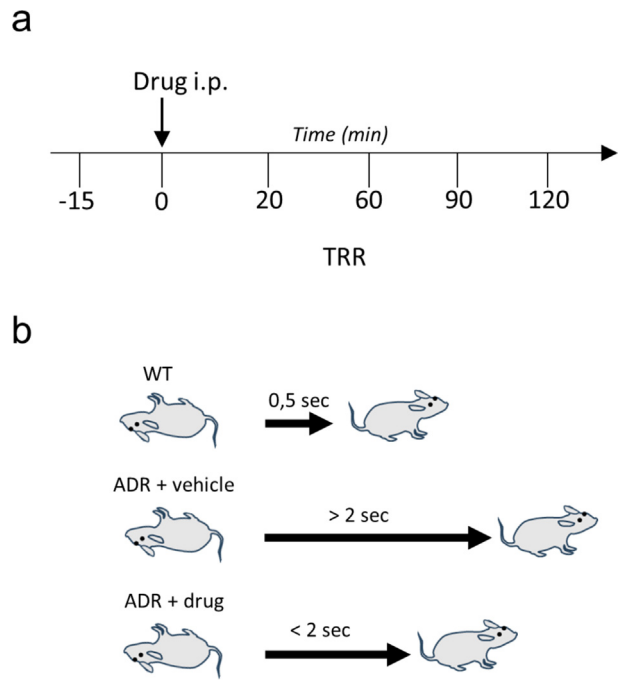

Fig. 1. Scheme reporting the experimental protocol. **a.** The time of righting reflex (TRR) was measured 15 min before and 20 min, 60 min, 90 min and 120 min after drug administration through i.p. injection. **b.** Wild-Type (WT) mice placed in supine position take less than 0.5 s to straighten themselves on their four legs. In contrast, the ADR myotonic mice need much more time, typically greater than 2 s. The drug is expected to reduce the TRR vs. the WT value.

Data analysis and statistics

Data were shown as mean ± SEM (standard error of the mean) from n experiments and statistical analysis was performed using Student's *t*-test or two-way ANOVA followed by Bonferroni's *t*-test, as reported in the Results section. Supplementary Tables 3 to 6 showed statistical analysis to compare TRR values in all drug conditions at each time point (vehicle and three doses of safinamide and mexiletine) by using analysis of variance (ANOVA) followed by Bonferroni's *t*-test. In Supplementary Table 7 mexiletine and safinamide effect was compared at the same dose using unpaired Student's *t*-test.

Results

Effects of safinamide and mexiletine on sarcolemma excitability in ADR mouse skeletal muscle

The in vitro electrophysiological experiments, using the two intracellular microelectrode technique, allowed evaluating the anti-myotonic effects of safinamide compared to mexiletine on sarcolemma excitability in skeletal muscle fibers isolated from 9 ADR mice. As expected from chloride channel dysfunction, the typical signs of sarcolemma hyperexcitability were observed, including spontaneous discharges (SpD) in absence of stimulation, the abnormal repetitive firing during the entire stimulation period and the presence of AfD recorded after cessation of stimulus (Fig. 2 a, b). These excitability alterations are responsible for the main clinical manifestation of myotonia, which is the delay in muscle relaxation after contraction. The effects of safinamide and mexiletine were shown in Figs. 2 and 3 and Table 1.

Safinamide showed a potent anti-myotonic activity in reducing N spikes at all the concentrations tested. Safinamide at 10 μM significantly

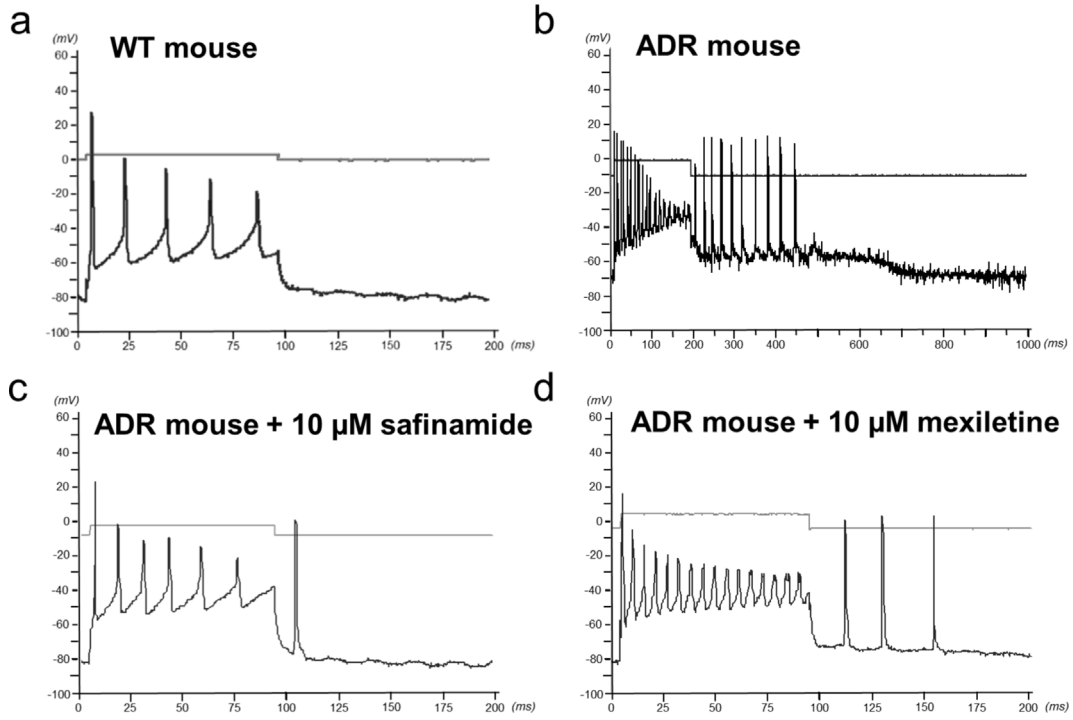

Fig. 2. Sarcolemma excitability in skeletal muscle fibers of myotonic ADR and Wild-Type (WT) mice. Representative picture of action potential trains elicited by threshold current in extensor digitorum longus (EDL) muscles of WT mice (**a**), in myotonic muscle in the absence of drug (**b**) and after application of 10 μM Safinamide (**c**) or 10 μM Mexiletine (**d**). Action potential firing was recorded in response to 100 ms-long depolarizing currents and 200 ms acquisition time. Due to the long train of myotonic discharges the recordings from ADR mice were done at 200 ms stimulation and 1 s acquisition time. After-discharges (AD) were observed in 100% of the fibers recorded in ADR mice but their frequency was reduced in the presence of the different drugs, as detailed in the Results section.

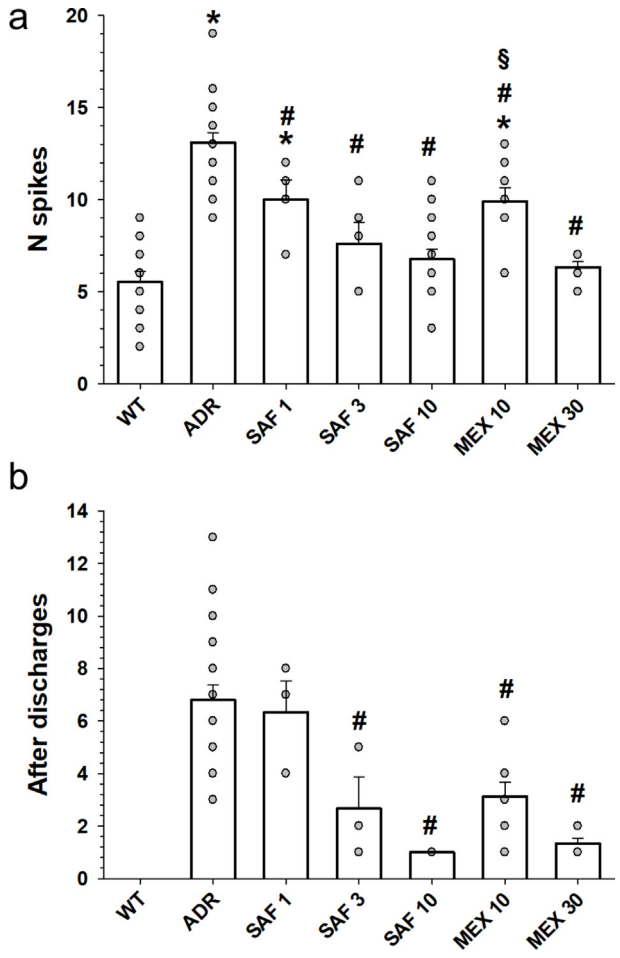

Fig. 3. Excitability parameters measured in EDL muscle of Wild-Type (WT) and myotonic ADR mice. **a.** Maximum number of action potentials (N Spikes) elicited by a fixed depolarizing current pulse. **b.** Maximum number of after-discharges (AD) measured in muscle fibers. In WT, this number is zero. Columns represent the mean ± SEM of 4–20 muscle fibers from nine ADR mice and five WT mice. Statistical differences were calculated using one-way ANOVA followed by Bonferroni's *t*-test. *Significantly different with respect to WT ($P < 0.05$ or less); #Significantly different with respect to ADR ($P < 0.05$ or less); §Significantly different with respect to safinamide 10 μM ($P < 0.05$).

Table 1
Effect of safinamide and mexiletine on excitability parameters of skeletal muscle fibers of WT and myotonic ADR mouse.

Experimental conditions	n fibers/N mice	RP (mV)	AfD (% of fibers)	SpD (% of fibers)
WT	15/5	−75.2 ± 1.2	0%	0%
ADR	20/9	−72.8 ± 2.2	100%	100%
ADR + Saf 1 μM	5/2	−72.9 ± 1.2	75.0%	100%
ADR + Saf 3 μM	6/2	−73.1 ± 2.3	37.5%	98%
ADR + Saf 10 μM	17/4	−74.1 ± 2.5	11.7%	90%
ADR + Mex 10 μM	11/2	−72.3 ± 1.8	72.7%	98%
ADR + Mex 30 μM	9/2	−73.5 ± 1.9	66.7%	92%

n fibers: number of fibers; N mice: occasionally the measure of different concentrations has been done in the same muscle; RP: resting potential; AfD: after discharges; SpD: spontaneous discharges.

reduced the N spikes by 48.1 ± 3.5 % compared to the value measured in untreated ADR muscles (Fig. 3a). Notably, at 10 μM safinamide, the EDL muscle sarcolemma excitability of ADR mice was very similar to that of WT mice. The same concentration of mexiletine (10 μM) was less effective, reducing N spikes by 24.4 ± 4.6 % as compared to untreated EDL muscles (Fig. 3a). We also found a significant difference between these two values (mexiletine 10 μM vs. safinamide 10 μM). The dose-dependent effects are shown in Fig. 3. At 3 μM, safinamide significantly reduced the maximum number of spikes by 41 ± 8.3%, as compared to the value measured in untreated ADR mouse muscle. Also the dose of 1 μM produced a significant effect (Fig. 3a). The application of 30 μM mexiletine induced a reduction of the N spikes comparable to that of safinamide 10 μM and to the condition of healthy WT muscles (Fig. 3a). Thus, at this concentration mexiletine was about 3-times less potent than safinamide.

Safinamide was also able to strongly reduce the occurrence of AfD as well as their number. Safinamide at 10 μM reduced the number of AfD by 85.3 ± 1.5%, while mexiletine at 30 μM reduced it by 80.9 ± 2.4%. Thus, the effect of 10 μM safinamide and the effect of 30 μM mexiletine were comparable (Fig. 3b). As it can be seen in Fig. 3b also the effects of 3 μM safinamide and 10 μM mexiletine were similar. In Table 1, was reported the occurrence of AfD. Safinamide was more potent than mexiletine in reducing the occurrence of AfD, calculated as the number of times in which the AfD appeared with respect to the total number of fibers recorded. In addition, the occurrence of the SpD was slightly reduced with 10 μM safinamide as compared to the same concentration of mexiletine (Table 1).

In vivo anti-myotonic effects of safinamide and mexiletine in the ADR mouse model of myotonia congenita

In vivo, the time of righting reflex (TRR), as the time the mouse takes to stand up on four legs from the supine position, was evaluated. The TRR was longer in ADR mice (2.59 ± 0.08 s, n = 35) compared to WT (<0.5 s). The TRR values at each time point were reported in the Supplementary Table 1 as means ± SEM from 5 ADR mice.

For the analysis, in each mouse, the TRR value at each time point was normalized to the TRR value measured 15 min before drug administration (T-15). Fig. 4 a shows the normalized TRR values (mean ± SEM, n = 5 mice) at different time intervals. The effects of safinamide and mexiletine were time- and dose-dependent, showing safinamide more potent than mexiletine. Indeed, at 3 mg/kg and 10 mg/kg, safinamide exerted a strong anti-myotonic activity (in terms of significant reduction of TRR) at 20, 60, 90 min after administration (Fig. 4 a). The effect was long lasting up to 120 min after drug injection. At 1 mg/kg, safinamide exerted a significant anti-myotonic activity 20 and 60 min after injection. The anti-myotonic effect of 10 mg/kg mexiletine was significant at all time points, although the effect was always less potent than safinamide. At 3 mg/kg, mexiletine effect was significant at 60 and 90 min, but not significant at 20 and 120 min. Mexiletine was ineffective at 1 mg/kg. Supplementary Table 2 showed the TRR variation calculated as the percentage reduction of TRR in treated ADR mice and graphically reported in Fig. 4 b.

Supplementary Tables 3 to 6 showed the comparison of efficacy (as statistical significance calculated by ANOVA test followed by Bonferroni *t*-test) between the two drugs and with respect to the vehicle 20 min, 60 min, 90 min and 120 min after drug administration. P values are shown. In the Supplementary Table 7 mexiletine and safinamide effect were compared at the same dose using unpaired Student's *t*-test.

This analysis confirmed the higher potency of safinamide. Of note, mexiletine at 1 mg/kg was completely ineffective, whereas safinamide at the same dose showed a significant effect at 20 and 60 min suggesting the possible use of low doses in therapy when needed.

Discussion

Activity of the voltage-gated chloride channel type 1 (ClC-1) is important to maintain skeletal muscle function. This channel is the main contributor to the resting membrane conductance of sarcolemma, indeed it carries about 85% of the total conductance. Its dysfunction results in the muscle fiber unable to compensate the depolarization generated by

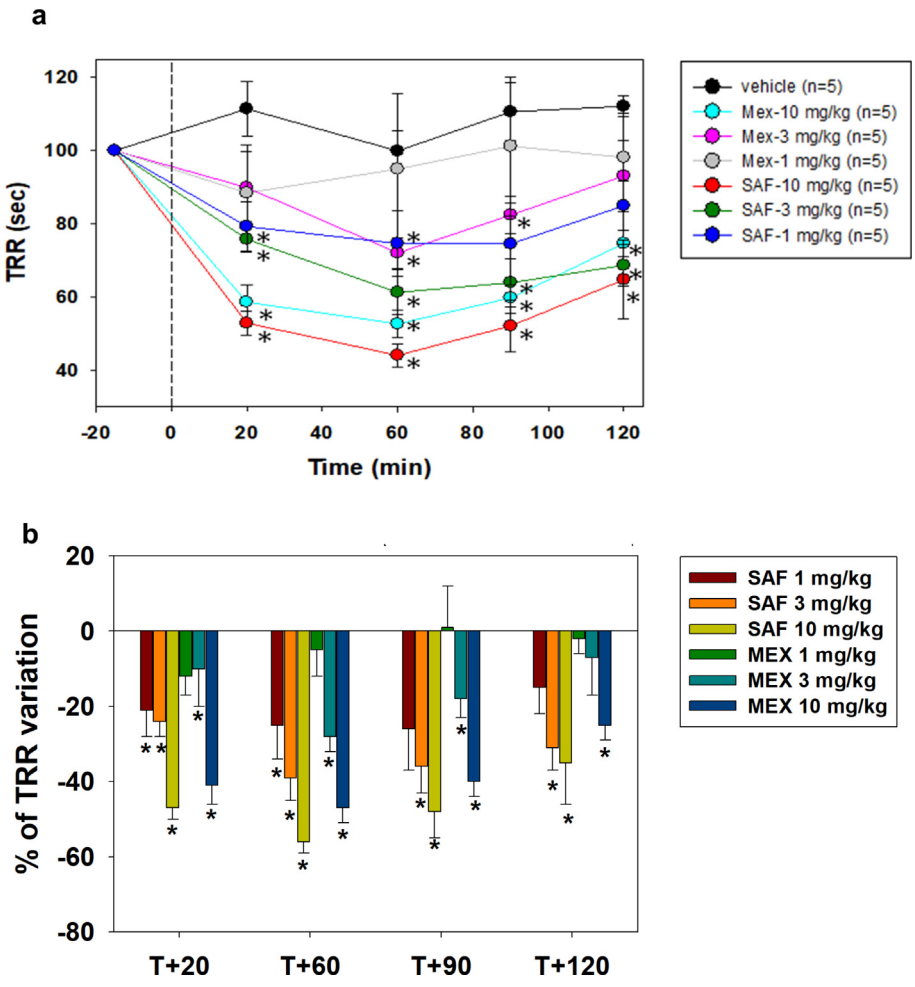

Fig. 4. Normalized time of righting reflex (TRR) measured in myotonic ADR mice in the absence of drugs (vehicle) and after treatment with safinamide at three different doses (1–3–10 mg/kg) in comparison with the same doses of mexiletine. **a**. Each point represents the TRR (±SEM) value from 5 mice normalized to the value measured in the absence of drug (T-15 min before drug application). *Significantly different (by Student's t-test) with respect to TRR at T-15 min. Details on the statistical analysis are also reported in Supplementary Table 2. **b**. Percent of TRR variation measured before and after drug administration in ADR mice at different time points. *Significantly different with respect to the value measured in the absence of drug ($p < 0.05$ or less by Student's t-test).

potassium accumulation in T-tubules during contraction, which induces abnormal bursts of action potentials persistent after cessation of stimulus (the so-called after-discharges), responsible for the muscle stiffness typical of myotonia [38,39]. The increase in K^+ concentration causes an initial depolarization and may consequently activate a sodium persistent inward current (NaPIC) that leads to the further depolarization and generation of myotonic action potentials [40]. To date, there is no definitive cure for myotonic syndromes generated by a reduced activity of mutated ClC-1 channels in skeletal muscle. The treatment is symptomatic and directed toward reduction of sarcolemma hyperexcitability by acting on Na channels. Mexiletine is the first-choice drug indicated for Myotonia Congenita and other myotonic syndromes, reducing both stiffness and transient weakness [7–9,25,41–43]. However, the drug may result unsatisfactory in 30% of patients, depending on drug tolerability, pharmacogenetics, or contraindications [5,10,11,44,45]. Thus, alternative drugs are needed to improve the therapy of myotonia.

Safinamide was found to be a potent voltage- and frequency-dependent blocker of voltage-gated sodium channels in neurons [46–48] exerting anticonvulsant activity in animal models of epilepsy and in pilot studies in epileptic patients [49]. It also inhibits N-type calcium channels involved in neurotransmitter release [46,48]. Since safinamide is an inhibitor of MAO-B enzyme, it is currently indicated in Parkinson Disease (PD) as an add-on to levodopa. Inhibition of abnormal glutamate release may also contribute to efficacy in PD [50–52]. We previously showed that safinamide inhibited sodium current in HEK293T cells transfected with human Nav1.4, being more potent than mexiletine [26]. Moreover, in vitro safinamide counteracted skeletal muscle fiber hyperexcitability pharmacologically induced by application of the chloride channel blocker, 9-anthracene carboxylic acid (9-AC). Safinamide was also tested in vivo in a rat model of myotonia induced by the intraperitoneal administration of 9-AC, which can increase the TRR due to muscle stiffness. In this condition, safinamide reduced the TRR more potently than mexiletine.

Here, we showed that safinamide exerts a potent anti-myotonic effect in vitro and in vivo in the myotonic ADR mouse, a model more closely related to human myotonia. When applied in vitro on ADR mouse muscle fibers, the effects of safinamide were very similar to those measured on 9-AC-treated rat muscle fibers [26], with a full recovery to normal firing with 10 μM safinamide. In muscle fibers of ADR mouse, we also observed a reduction of occurrence of after-discharges, of the number of spikes during after-discharges, and a slight reduction of the spontaneous discharges, observed after impalement of the fiber with the recording microelectrode. These results are in line with a reduction of stiffness and pain.

The in vivo results assume a translational importance because the effect of the two drugs were tested in the whole body, which reproduces the same defect observed in the human pathology. The preclinical studies were designed and conducted at the aim to confirm drug effects prior to possible trials in humans. The effects of safinamide in ADR mice were impressive, reaching a 60% TRR reduction with 10 mg/kg dose. The mice treated with safinamide 10 mg/kg were able to move normally into the cage and did not show any myotonic sign. These results strongly suggest that safinamide may be able to reduce the number and severity of myotonic episodes in humans suffering from Myotonia Congenita or other myotonic conditions. Although the higher potency of safinamide with respect to mexiletine, it should be underlined that in vivo the

potency ratio between safinamide and mexiletine is less evident with respect to the effect showed in vitro. Perhaps in this case other factors that depend on the animal (i.e. drug pharmacokinetics, the collaboration of the animal during the test) are involved.

The anti-myotonic effect of safinamide is likely the consequence of the inhibition of skeletal muscle sodium channels, since the drug was shown to inhibit hNav1.4 channels in a dose and frequency dependent manner, reminiscent of mexiletine effects [26]. Safinamide also inhibits neuronal sodium channels [46,47], and the selectivity of action would rely on frequency-dependent behavior, allowing the drug to act more specifically on the over-excited neurons and/or myotonic muscle. Indeed, glutamate release inhibition in the brain by safinamide was observed only in condition of hyper-excitability induced by veratridine [48]. According to previous studies, the free plasma concentration of safinamide reached with the dose of 10 mg/kg, which allowed significant reduction of myotonia symptoms in the ADR mouse for at least 2 h, should be safe and well tolerated in humans [49,53]. In addition, the clinical safinamide dose used in Parkinson disease was shown to reduce the abnormal glutamate neurotransmission in humans, which is thought to depend on sodium channel inhibition by the drug [50–52].

Thus, safinamide may represent a valid alternative to mexiletine based on its efficacy and specificity. Indeed, the possibility to rely on promising new drugs is critical for patients that cannot assume mexiletine. Safinamide is a marketed drug and its proposal for a new potential therapeutic indication may have many advantages, such as the knowledge of the pharmacokinetic profile and clinical safety, shorter development timelines and lower costs [24]. Phase 2 clinical trials are warranted to verify the antimyotonic potential of safinamide. Since myotonic disorders are rare diseases, randomized, double-blind, cross-over or N-of-1 clinical trials of safinamide versus mexiletine should be favored [7–9]. Noteworthy, it should be taken into account that no information so far is available regarding the use of safinamide in the pediatric setting.

Author Contributions

Ileana Canfora: Writing - original draft, Methodology, Data curation, Formal analysis, Investigation. **Concetta Altamura**: Methodology, Data curation, Formal analysis, Investigation. **Jean-Francois Desaphy**: Conceptualization, Supervision, Writing - original draft, Writing - review and editing, Methodology, Data curation, Formal analysis. **Brigida Boccanegra**: Writing - review and editing, Methodology, Data curation, Formal analysis. **Silvia Vailati**: Writing - review and editing, Methodology, Data curation. **Carla Caccia**: Conceptualization, Writing - review and editing, Methodology, Data curation. **Elsa Melloni**: Writing - review and editing Methodology, Data curation. **Gloria Padoani**: Writing - review and editing, Methodology, Data curation. **Annamaria De Luca**: Writing - review and editing, Data curation, Formal analysis, Supervision, Funding acquisition. **Sabata Pierno**: Conceptualization, Supervision, Writing - original draft, Writing - review and editing, Methodology, Data curation, Formal analysis, Investigation.

Data availability

All data presented in this manuscript and supplementary materials are available from the corresponding author upon reasonable request.

Declaration of competing interest

Silvia Vailati, Elsa Melloni, Gloria Padoani are employees of Zambon SpA, and Carla Caccia is a consultant of Zambon SpA. The International patent application PCT/EP2019/063733 entitled “Safinamide for treating myotonia” was filed on May 28, 2019 in the name of Zambon S.p.A. Designated inventors are Desaphy J.F., Pierno S., Conte D., Melloni E., Vailati S., Padoani G., and Caccia C.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurot.2024.e00455.

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