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Pre-clinical study of 21 approved drugs in the *mdx* mouse

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Abstract

Duchenne muscular dystrophy, a genetic disease caused by the absence of functional dystrophin, remains without adequate treatment. Although great hopes are attached to gene and cell therapies, identification of active small molecules remains a valid option for new treatments.

We have studied the effect of 20 approved pharmaceutical compounds on the muscles of dystrophin-deficient *mdx5Cv* mice. These compounds were selected as the result of a prior screen of 800 approved molecules on a dystrophin mutant of the invertebrate animal model *Cænorhabditis elegans*. Drugs were administered to the mice through maternal feeding since 2 weeks of life and mixed in their food after the 3rd week of life. The effects of the drugs on mice were evaluated both at 6 weeks and 16 weeks. Each drug was tested at two concentrations. Prednisone was added to the molecule list as a positive control. To investigate treatment efficiency, more than 30 histological, biochemical and functional parameters were recorded. This extensive study reveals that tricyclics (Imipramine and Amitriptyline) are beneficial to the fast muscles of *mdx* mice. It also highlights a great variability of responses according to time, muscles and assays.

Keywords: Dystrophin; Duchenne muscular dystrophy; Nematode; *mdx* Mouse; Tricyclics; Antidepressants

1. Introduction

Duchenne muscular dystrophy (DMD) is a genetic disease caused by the absence or the malfunction of dystrophin, a large protein normally present under the sarcolemmal membrane of muscle fibers. The main symptom of DMD is a progressive muscle degeneration of striated muscles. Twenty-two years after the discovery of the dystrophin gene, the physiopathology of this neuromuscular disease is only partially understood [1–3]. Many pharmacological therapeutic options have been investigated [4], and Prednisone – an anti-inflammatory drug – is currently the standard treatment prescribed to DMD patients, but its benefits are modest and it has numerous side effects [5,6]. Gene therapy and cell therapy carry great hopes for the cure of genetic defects, but these techniques still need improvement before being routinely applied in human patients [7,8]. Thus, DMD remains without adequate treatment.

Several years ago, we initiated a strategy aimed at screening the existing pharmacopeia for molecules that might be beneficial to DMD patients. This strategy consists of two steps: in a first step, 800 approved drugs were randomly tested on the cheap and relatively fast invertebrate model *Cænorhabditis elegans* (*C. elegans*). In a second step, hits obtained in the first step were tested on the more costly and labor-intensive mouse model *mdx*.

The first step was validated by the fact that Prednisone reduces muscle degeneration in dystrophin-deficient

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C. elegans [9]. The results of this *C. elegans* screen have been partially reported elsewhere [10]. Serotonin and tricyclics – antidepressants – were able to diminish muscle loss in the *C. elegans* model of dystrophin-dependant degeneration. Moreover, serotonin and serotonin-related drugs were more beneficial to *C. elegans* than Prednisone.

In this paper, we report the result of the second step. Twenty molecules (plus Prednisone as a positive control) were tested on *mdx* mice at two time-points (6 and 16 weeks), and at a minimum of two concentrations each. Our results show that, among the molecules tested, tricyclics, and in particular Imipramine, are beneficial to *mdx* mice muscles. An unexpected result of this large study is the great variability of responses for each drug, according to time, muscle, and assay.

2. Materials and methods

2.1. Mice

C57BL/6J mice and C57BL/6Ros-*Dmd*$^{mdx\text{-}5Cv}$ mice (respectively referred to as wild type mice and *mdx* mice hereafter) were obtained from Transgene *via* Serge Braun and Ronald Rooke after approval from the Jackson Laboratory.

2.2. Treatments

2.2.1. Drugs

The following drugs were purchased from Sigma–Aldrich: Amitriptyline hydrochloride #A8404, Carisoprodol #C8759, Chlorprothixene hydrochloride #C1671, Cloxacillin sodium salt #27555, Cyclobenzaprine hydrochloride #C4542, Desipramine hydrochloride #D3900, Eburnamonine #194727, Fluoxetine hydrochloride #F132, Hydralazine hydrochloride #H1753, Imipramine hydrochloride #I0899, Prednisone #P9901, Mexiletine hydrochloride #M2727, Nifedipine #N7634, Nitrofurantoin #46502, Noscapine #363960, Orphenadrine hydrochloride #75517, Pentoxifylline #P1784, Tolazamide #T2408, Trimipramine maleate #T3146. Chlormezanone #0456 was purchased from Tocris.

2.2.2. Doses, preparation and administration

The drugs used in this study are all administered by oral route in humans. Their therapeutic groups and mechanisms of action (when known) are described in Table S1. Human to mice dose conversion is currently based on body surface area [11]. Accordingly, human doses have to be multiplied by approximately 10 in mice. The doses tested are expressed in fold of the oral human dose, *e.g.* 5× corresponds to five-fold the human dose in mg/kg but is the equivalent of half the human dose when considering the dose conversion rule stated above. Drugs were tested at two doses each: 5× and 20× (unless stated otherwise), this corresponds to half the equivalent human dose and twice the equivalent human dose. The number of animal tested, the doses and corresponding concentrations per drug are shown in Table S2. Drugs were mixed with mice food by Provimi Kliba (Kaiseraugst, Switzerland). Concentrations of drugs in food were calculated on the basis that *mdx* mice eat approximately 15% of their body weight per day (Carre-Pierrat and Ségalat, unpublished results). Young mice were treated as early as day 14. Between the second and third week of age, they feed partially on their mothers' milk and partially on drug-containing food thrown in the cages. Although most of the molecules used in this study are described to go to the maternal milk in humans or are not recommended during pregnancy because they might go into the milk, it is to be noted that during the first week we cannot guarantee that the young mice receive any molecule. From the third week of age they feed only on the food containing the drugs. Mice were treated from day 14 to day 42 after birth in the short treatments (ending at 6 weeks), and to day 112 in the long treatments (ending at 16 weeks). Food intake and body weight gain were monitored every 2 or 3 days. Group tests included at least eight mice (exceptionally down to five). Males and females were included for histological measurements after we checked that the readouts used were sex-independent (Carre-Pierrat and Ségalat, unpublished results). Functional tests were performed on males only.

2.3. Histological measurements at 6 weeks

2.3.1. Muscles preparation

Mice were sacrificed at day 42 by cervical dislocation. Right *extensor digitorum longus* muscles (EDL) and diaphragm muscle (DIA) were collected. DIA and right EDL were prepared for Hematoxylin–Eosin (H&E) staining (Anipath histological facility, Lyon). 3 μm-thick transverse sections were cut every 100 μm from the center of EDL muscles or from the internal edge of DIA muscles.

2.3.2. Centrally nucleated fibers and necrotic fibers in EDL

The percentage of centrally nucleated fibers (CNF) and necrotic fibers (NF) was determined in two transverse sections from the right EDL, 500–1000 fibers from the center of the sections were examined for each muscle.

2.3.3. Index of centrally nucleated fibers and necrotic fibers in DIA

The diaphragm section was divided in 15–20 adjacent microscopic fields. In each field, both CNF and NF were scored as follow: 0: area free from CNF or NF, 1: up to 5 CNF or NF in the area; 2: up to 20 CNF or NF, 3: more than 20 CNF or NF, 4: more than 50% of CNF or NF in the area. The average score of two sections was retained as the DIA index for CNF or NF.

Results of CNF and NF in EDL and DIA are presented as recovery scores, where the *mdx* measure = 0 and the wild type measure = 100, as recommended by J.-M. Gillis in the Standard Operating Procedure DMD_M 1.1.001 of the TREAT-NMD consortium.