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Perspective

Nitric oxide donor and non steroidal anti inflammatory drugs as a therapy for muscular dystrophies: Evidence from a safety study with pilot efficacy measures in adult dystrophic patients

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ABSTRACT

This open-label, single centre pilot study was designed to evaluate safety and tolerability of the combination of the drugs isosorbide dinitrate, a nitric oxide donor, and ibuprofen, a non steroid anti-inflammatory drug, in a cohort of adult dystrophic patients (Duchenne, Becker and Limb-Girdle Muscular Dystrophy). Seventy-one patients were recruited: 35, treated with the drug combination for 12 months, and 36 untreated. Safety and adverse events were assessed by reported signs and symptoms, physical examinations, blood tests, cardiac and respiratory function tests. Exploratory outcomes measure, such as the motor function measure scale, were also applied.

Good safety and tolerability profiles of the long-term co-administration of the drugs were demonstrated. Few and transient side effects (i.e. headache and low blood pressure) were reported. Additionally, exploratory outcomes measures were feasible in all the disease population studied and evidenced a trend towards amelioration that reached statistical significance in one dimension of the MFM scale. Systemic administration of ibuprofen and isosorbide dinitrate provides an adequate safety margin for clinical studies aimed at assessing efficacy.

1. Introduction

Muscular dystrophies have a complex pathogenesis since the original genetic defect leads to a host of concurrent pathogenic events. Despite substantial progress in understanding the pathophysiological bases of these diseases, no pharmacological therapies have been identified that increase muscle strength, other than corticosteroids. Several studies provide reliable data on the benefit of both prednisone/prednisolone and deflazacort [1–4].

The potential beneficial effects of corticosteroids include inhibition of muscle proteolysis, stimulation of myoblast proliferation, increase in myogenic repair, anti-inflammatory immunosuppressive effects, reduction of cytosolic calcium concentrations [5] and up regulation of utrophin [6].

Several side effects, however, limit steroids usefulness [1]. New therapies may not be able to substitute entirely the steroids but may complement them and thus limit their use and/or reduce their dosages. For muscular dystrophies in adulthood, there have been only few small clinical trials and none involving novel therapeutic drugs or drug combinations [7–13]. We recently carried out studies in the mdx and α-sarcoglycan-null mouse models of dystrophy combining nitric oxide (NO) release and non steroidal anti-inflammatory (NSAID) activity, using the NO-releasing NSAID compound HCT1026 (nitroflurbiprofen), a combination of the NO donor isosorbide dinitrate (ISDN) and the NSAID ibuprofen or a dual compound releasing NO and ibuprofen for up to 12 months [14–16]. In all studies the results show that a combination of NO and NSAID activities slows disease progression by reducing inflammation, enhancing activity of endogenous stem cells and preventing muscle wasting. The beneficial effects were persistent, while in animals treated with ISDN or ibuprofen alone

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beneficial effects were significantly less and transient. No toxic effects were registered. In addition, in terms of therapeutic outcome, combining NO release and NSAID activity was significantly more effective than the corticosteroid prednisolone. We also found that this pharmacological approach enhances fourfold homing and engrafting of arterially-delivered donor mesoangioblast stem cells, which is of importance in perspectives of combining stem cell and pharmacological approaches to yield synergic therapeutic effects [14].

Several mechanisms of action may synergise to the observed beneficial action of the combination of NO and NSAIDs. One is limiting local inflammation, which is now recognised to play a significant role in fibres destruction and in progression of muscular dystrophies [17,18]. Indeed, DNA microarray or biochemical data show that inflammatory mediators/effectors dominate the expression profile of muscles from the mdx mouse [19].

A second mechanism of action involves the beneficial effects of NO on skeletal muscle. NO stimulates muscle regeneration acting on survival, activation and differentiation of satellite cells, the mononuclear progenitors of myocytes, able to form new fibres [20–22]. Furthermore, NO enhances bioavailability of nutrients to muscle, as well as energy generation through both glycolysis and mitochondrial biogenesis [23–27]. Finally, NO enhances the ability of myogenic stem cells to engraft to the dystrophic muscle, their resistance to the damaging environment of the dystrophic muscle and their ability to differentiate into myogenic cells [22,28,29]. Given these preclinical evidence results, we decided to explore if the combination of NO release and NSAID activity is safe and useful in human dystrophy.

This article describes a clinical trial primarily aimed at assessing the tolerability and safety of the combination of NO donor ISDN and the NSAID ibuprofen. The biological activity of the drug combination through exploratory outcome measures, such as the motor function measure scale (MFM), was also explored.

2. Materials and methods

We performed an open-label single-centre clinical trial with an historical control group, with a 12 months follow up.

The study was approved by the local Ethics Committee and all patients (and parents) gave written informed consent before participation in the study. The patients were informed of the preliminary published results (preclinical data), the objectives, the study design, risks and benefits of participation. The study was conducted in agreement with the Declaration of Helsinki guidelines.

2.1. Dose selection and treatment

To choose the appropriate dose for the study we relied on both preclinical/clinical evidence and the known pharmacokinetic profile of the two drugs. According to the National Italian Drug Agency (AIFA) and to international drug regulatory agencies (European Medicines Agency. EMA, Food and Drug Administration, FDA) patients should be treated with 200–300 mg of ibuprofen 2–3 times per day. As more conservative approach, we decided to apply the most stringent recommendation: 200 mg of ibuprofen BID. This dose was chosen because it is the one approved for OTC in Europe [30] and far below the doses (800–1200 mg/day) approved in many European Countries for non-prescription in adults [31].

The same conservative approach was applied for ISDN. According to drug regulatory agencies (AIFA, EMA, FDA) adults patients should be treated with 20–120 mg of ISDN daily, eventually divided in different subfractions [32,33]. Accordingly, our patients were given ISDN at 20 mg/day during the first month, eventually up-titrated to 40 mg thereafter.

2.2. Patients

Patients were recruited from the ones referring to the E. Medea Scientific Institute for periodic clinical assessments.

All patients fulfilling the inclusion/exclusion criteria (see below) were recruited from April 2007 to April 2008.

Inclusion criteria were a minimum age of 16 years; certainty of diagnosis (clinical, histological and immunohistochemical, biochemical and molecular diagnosis of DMD, BMD, or one of the following forms of LGMD: 2A, 2B, 2C, 2D, 2E, or 2I) [2,3,34,35]; adequate comprehension of the purpose of the study; signing of the informed consent; presence of at least two "baseline" clinical evaluations.

Main exclusion criteria were: Ejection fraction <40% at the Echocardiogram; forced vital capacity <40% of predicted; concomitant pathologies: gastrointestinal disorders/diseases, hepatic and renal dysfunctions, psychiatric symptoms, allergies, migraine; inability or unwillingness of the patient to give written informed consent; inability to comply with evaluation procedures as assessed by investigator; inability to take capsules.

2.3. Study design

At screening evaluation, the patients were seen by one experienced neurologist (MG D'A, S G, S B). All relevant demographic, clinical and laboratory data were reported in a dedicated case record form. Patients fulfilling the inclusion criteria started treatment with ibuprofen (200 mg BID) and ISDN (20 mg/per day). Four weeks after starting treatment, ISDN was up-titrated to 40 mg/per day. Gastric protection was guaranteed in the treated arm by pantoprazole 20 mg/per day. Patients were maintained on ibuprofen/ISDN for 12 months. For each patient treated with ibuprofen and ISDN (case), one patient treated conservatively (control) who satisfied the same inclusion/exclusion criteria was identified among the same population of patients with muscular dystrophy. Reference cases and controls were matched with cases for gender, age and specific muscular dystrophy (DMD, BMD, LGMD).

Cases and controls were monitored periodically up to the study end (12 months after the screening visit) as specified below.

2.4. Assessments

All patients had at least one preliminary assessment, 6 months before baseline, as well as a baseline assessment immediately before beginning treatment (T0). Subsequently, they were evaluated 1 (T1), 3 (T3), 6 (T6) and 12 months (T12) after the start of the treatment.

The protocol evaluation which was applied consisted of full physical examination (including the measurements of vital signs), neurological examination, manual muscle testing (a total of 18 muscle groups were examined on both sides, testing limb movement around the neck, shoulders, elbows, wrists, hips, knees, and ankles) with the application of the Medical Research Council score (MRC), application of the motor function measure (MFM scale) [36–38], evaluation of cardiac function via Echocardiogram, 24 h electrocardiogram (ECG) registration and blood pressure measurements, and evaluation of the respiratory function via spirometry and oxyhaemoglobin saturation measurements. Plasma and urine were obtained to determine renal and liver function, electrolytes levels, complete cell counts, activated partial-thromboplastin time, creatine phosphokinase (CPK). In addition, serum pro-inflammatory cytokines (Transforming Growth Factor β, TGF-β, and Interleukin 6, IL-6) were measured in patients on combined ibuprofen plus ISDN at T0, T3, T6 and T12 by ELISA kits.