Toxicology and Applied Pharmacology 360 (2018) 78–87

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Validation of a clinically-relevant rodent model of statin-associated muscle symptoms for use in pharmacological studies

Jordon Candice Irwin*, Andrew Stuart Fenning, Kimberly Rene Ryan, Rebecca Kate Vella

School of Health, Medical and Applied Sciences, Central Queensland University, Australia, Bruce Highway, North Rockhampton, Queensland 4702, Australia

ARTICLE INFO

Keywords:
Rodent model
Statin-associated muscle symptoms
Simvastatin

ABSTRACT

Various rodent models of statin-associated muscle symptoms (SAMS) have been used to investigate the aetiology of statin myotoxicity. Variability between these models, however, may be contributing to the ambiguity currently surrounding the pathogenesis of SAMS. Furthermore, few studies have assessed the reproducibility of these models. The aim of this study was to compare two established rodent models of statin myotoxicity, differing in treatment duration and dose, to determine which reproducibly caused changes characteristic of SAMS. Isolated skeletal muscle organ bath experiments, biochemical analyses, real-time quantitative-PCR and biometric assessments were used to compare changes in skeletal muscle and renal integrity in statin-treated animals and time-matched control groups. The SIM80 model (80 mg kg^{-1} day^{-1} simvastatin for 14 days) produced fibre-selective skeletal muscle damage characteristic of SAMS. Indeed, fast-twitch gastrocnemius muscles showed increased *Atrogin-1* expression, reduced peak force of contraction and decreased *Myh2* expression while slow-twitch soleus muscles were unaffected. Contrastingly, the SIM50 model (50 mg kg^{-1} day^{-1} simvastatin for 30 days) produced little evidence of significant skeletal muscle damage. Neither statin treatment protocol caused significant pathological changes to the kidney. The results of this study indicate that the SIM80 model induces a type of SAMS in rodents that resembles the presentation of statin-induced myalgia in humans. The findings support that the SIM80 model is reproducible and can thus be reliably used as a platform to assess the aetiology and treatment of this condition.

1. Introduction

Statin therapy is pivotal for the primary and secondary prevention of cardiovascular disease (CVD), particularly in individuals with coronary heart disease, diabetes or a history of stroke or myocardial infarction (Heller et al., 2017; Maningat et al., 2012). While generally well-tolerated, approximately 10–25% of individuals taking statins experience adverse statin-associated muscle symptoms (SAMS) (Khan et al., 2015). The clinical manifestation of SAMS varies considerably and may present as myalgia (muscle pain/cramps with normal serum creatine kinase, CK), myopathy (muscle weakness with normal or elevated CK), myositis (muscle inflammation with elevated CK) or myonecrosis/rhabdomyolysis (hyperCKemia with/without myoglobinuria or acute renal failure) (Rosenson et al., 2014; Muntean et al., 2017). The onset of SAMS is clinically significant as it can reduce quality of life in affected individuals (Parker and Thompson, 2012). Moreover, its development is one of the main contributors to statin discontinuation (Maningat et al., 2012), which in turn, is associated with a higher risk of mortality from CVD (Banach et al., 2015; Toth et al., 2018; Tziomalos et al., 2008).

The lack of consensus on the exact mechanism which underlies SAMS presents a considerable obstacle for effectively managing these events and thus improving cardiovascular health (Moßhammer et al., 2014; Taha et al., 2016; Irwin et al., 2018). Extensive rodent studies have been performed to elucidate the pathophysiology of statin myotoxicity using a range of statins including: simvastatin (Westwood et al., 2005; Simsek Ozek et al., 2014; Mallinson et al., 2009; Mallinson et al., 2012; Pierno et al., 1999; Goodman et al., 2015; Reijneveld et al., 1996; Sidaway et al., 2009), cerivastatin (Westwood et al., 2005; Sidaway et al., 2009; Obayashi et al., 2011; Schaefer et al., 2004), atorvastatin

Abbreviations: Ca^{2+}, calcium; CVD, cardiovascular disease; CK, creatine kinase; CON, control; FFCs, force-frequency curves; GAS, gastrocnemius; LDH, lactate dehydrogenase; MG, myoglobin; MTD, maximum tolerable dose; SAMS, statin-associated muscle symptoms; SIM, simvastatin-treated; SOL, soleus; TA, tibialis anterior

* Corresponding author at: School of Health, Medical and Applied Sciences, Central Queensland University, Building 7, Bruce Highway, North Rockhampton, Queensland 4702, Australia.

E-mail address: j.irwin@cqu.edu.au (J.C. Irwin).

https://doi.org/10.1016/j.taap.2018.09.040
Received 22 June 2018; Received in revised form 8 September 2018; Accepted 26 September 2018
Available online 28 September 2018
0041-008X/

(Camerino et al., 2011; D'Antona et al., 2013; Muraki et al., 2012; El-Ganainy et al., 2016a; El-Ganainy et al., 2016b), lovastatin (Reijneveld et al., 1996; Smith et al., 1991), fluvastatin (Camerino et al., 2011; Sugatani et al., 2010), pravastatin (Reijneveld et al., 1996; Muraki et al., 2012; Smith et al., 1991; Naba et al., 2004) and rosuvastatin (Sidaway et al., 2009; El-Ganainy et al., 2016b; Westwood et al., 2008). In addition to differences in statin type, the age and gender of rats, as well as drug doses, used in these investigations have also varied considerably and each of these variables can influence the severity of SAMS (Irwin et al., 2018; Buettner and Lecker, 2008; Shear et al., 1992). Indeed, male rodents have been used previously in studies of SAMS despite evidence that, in both humans and rodents, females are more sensitive to statin myotoxicity (Schaefer et al., 2004; Sathasivam and Lecky, 2008; Katz et al., 2014; Seachrist et al., 2005). Furthermore, as rodents develop pathological alterations in skeletal muscle with age (Caccia et al., 1979; Altun et al., 2010), it is more appropriate for young rats to be used in models of SAMS to ensure that only statin-associated skeletal muscle damage is assessed.

The differences between rodent models of SAMS are likely to be introducing inconsistencies and/or ambiguity about the exact pathogenesis of this condition. Therefore, in order to produce more consistent findings, a standardised and reproducible rodent model of SAMS is required. This model should induce changes characteristic of SAMS including reduced power output (Mallinson et al., 2015), increased mitochondrial oxidative stress (Bouitbir et al., 2016) and enhanced expression of atrophy-related genes (Hanai et al., 2007). Furthermore, these effects should be fibre-selective whereby type II fast-twitch glycolytic fibres are susceptible to myotoxicity while type I slow-twitch oxidative fibres are resistant to any statin-induced damage (Seachrist et al., 2005; London et al., 1991).

Since the discontinuation of cerivastatin, simvastatin has become the formulation most frequently associated with adverse muscle effects in the clinical setting (Keltz et al., 2013). Simvastatin is also the most commonly used statin in rodent models of SAMS, even though the changes in muscle physiology exerted by statins are similar between the different formulations (Thompson et al., 2016; Gluba-Brzozka et al., 2016). Of the simvastatin treatment schedules employed in rodent studies of SAMS, the most frequently used is 80 mg kg^{-1} day^{-1} for approximately two weeks (Westwood et al., 2005; Mallinson et al., 2009; Goodman et al., 2015; Sidaway et al., 2009). While this high-dose protocol produces a rapid onset of myotoxicity (i.e. within 14 days), it does reach the maximum tolerable dose (MTD) of statins in rodents (Westwood et al., 2005). Hence, there is a risk when using this model that any physiological changes associated with statin myotoxicity may be masked by potential toxicological consequences caused by statin overdose. In light of this, alternative models which administer a lower (mid-range) dose of statin for a longer period, such as 50 mg kg^{-1} day^{-1} for 30 days (Simsek Ozek et al., 2014), may be more preferable for studying SAMS. However, the ability of simvastatin when administered at doses lower than 80 mg kg^{-1} to reproducibly induce noteworthy changes in skeletal muscle functionality has been questioned (Westwood et al., 2005). Moreover, studies in *mdx* dystrophic mice have shown that long-term, low-dose simvastatin treatment (5–10 mg kg^{-1} day^{-1} for 8 months) can actually improve muscle health (Whitehead et al., 2015).

Notably, both treatment protocols use high doses of statins when compared to the amounts prescribed in humans. Indeed, while the average statin dose for humans ranges between 0.1 and 1 mg kg^{-1}, most rodent studies employ concentrations between 1 and 100 mg kg^{-1} (Björkhem-Bergman et al., 2011). The discrepancy in dosages is the result of the pharmacodynamic-resistance to statins displayed by rodents (Westwood et al., 2005; Björkhem-Bergman et al., 2011). Hence, higher doses need to be given to rodents in order to induce the same physiological changes observed in humans at comparatively lower doses (Westwood et al., 2005).

The aim of this investigation was to clarify which of the two aforementioned treatment protocols (two weeks of high-dose treatment or four weeks of mid-range therapy) reproducibly caused changes characteristic of statin myotoxicity in humans when administered to young, female rodents. It is anticipated that the selected model will provide a stable platform against which factors which are postulated to influence the severity or likelihood of SAMS (such as lipophilicity, dose, polypharmacy, gender and age) (Banach et al., 2015; Gluba-Brzozka et al., 2016) can be assessed in order to verify their effects on statin myotoxicity. Likewise, the model may also be used to determine which molecular/metabolic pathways should be targeted to provide novel therapeutic interventions to more effectively manage SAMS.

2. Materials and methods

2.1. Animals and treatment protocols

Young (12-week old) female Wistar rats (250–350 g) were randomised to one of four treatment groups: no intervention for 14 days (CON80), 80 mg kg^{-1} day^{-1} simvastatin for 14 days (SIM80), no interventions for 30 days (CON50) or 50 mg kg^{-1} day^{-1} simvastatin for 30 days (SIM50). Simvastatin was dissolved in solution comprised of 10% v/v Tween 20 in milliQ water as this has been identified as a suitable vehicle to use in pharmacological studies for administering drugs (AL-Wajeeh et al., 2017; Hajrezaie et al., 2012; Porwal et al., 2017; Saeed Al-Wajeeh et al., 2016), including statins (Loch et al., 2006). An a priori power analysis was performed to determine the minimum number of animals required to achieve statistically valid results (alpha level 0.05, power beta level of 0.8, effect size 0.980). Rodents were housed in a constant 12-h light/darkness cycle at a temperature of 22 ± 2 °C and permitted access to water and food (standard rat chow) ad libitum. Experimental procedures were approved by the Animal Ethics Committee of Central Queensland University (CQU AEC 0000019911) under guidelines from the National Medical Research Council of Australia.

2.2. Biometric assessments

Water consumption and changes in body mass was assessed every two or three days during the treatment period, respectively. Upon completion of the dosing protocol, rats were euthanised via a 1.0 mL intraperitoneal injection of sodium pentobarbitone (187.5 mg mL^{-1}) and death was confirmed by a lack of pedal reflex and corneal reflexes. The wet mass of the gastrocnemius (GAS) muscle (predominate fibre type IIB), soleus (SOL) muscle (fibre type I) and tibialis anterior (TA) muscle (fibre type I, IIA and IIB), as normalised to body mass, were recorded. As skeletal muscle injury can result in kidney damage through release of toxic concentrations of myoglobin (Keltz et al., 2013), kidney mass was measured as one indicator of renal health.

2.3. Ex vivo skeletal muscle functional assessment

Isolated skeletal muscle tissue bath experiments were completed using a modified protocol (Simsek Ozek et al., 2014). GAS, SOL and TA muscles were transferred to 25 mL warmed (37 °C) organ baths containing gassed (carbon dioxide (CO_2) 5% / oxygen (O_2) 95%) Krebs-Henseleit buffer (KHB) (all in mM concentrations: sodium chloride 135, potassium chloride 5, magnesium chloride 1, disodium hydrogen phosphate 1, sodium bicarbonate 15, calcium chloride 2 and glucose 1; pH ~ 7.4). Muscles were suspended between two platinum zigzag electrodes and loaded with 2 g tension. Following a 10-min equilibration period, electrical field simulation trains were applied at 100 V at an increasing frequency from 1 to 100 Hz for 5 s every 135 s. Stimulations were induced twice at each frequency; any tissue responses were detected using Grass FT03 transducers and recorded using Lab Chart software and PowerLab® data acquisition units (ADInstruments, Bella Vista, Australia). For SOL and TA muscles, the protocol was halted after