Toxicology 329 (2015) 40–48

Contents lists available at ScienceDirect

Toxicology

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

Inhibition of prenyltransferase activity by statins in both liver and muscle cell lines is not causative of cytotoxicity

Rowena H. Gee, Jenny N. Spinks, Jason M. Malia, Jonathan D. Johnston, Nick J. Plant *, Kathryn E. Plant

Department of Biochemistry and Physiology, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK

ARTICLE INFO

Article history:
Received 19 November 2014
Received in revised form 7 January 2015
Accepted 7 January 2015
Available online 8 January 2015

Keywords:
Statin toxicity
Prenylation
Cholesterol
HMG COA

ABSTRACT

As inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase, statins are an important first-line treatment for hypercholesterolemia. However, a recognized side-effect of statin therapy is myopathy, which in severe cases can present as potentially fatal rhabdomyolysis. This represents an important impediment to successful statin therapy, and despite decades of research the molecular mechanisms underlying this side-effect remain unclear. Current evidence supports a role for reduced levels of mevalonate pathway intermediates, with the most accepted hypothesis being a reduction in isoprenoids formation, leading to faulty post-translational modifications of membrane-associated proteins. We have undertaken a comprehensive analysis of the impact of nine statins on two human cell lines; Huh7 hepatoma and RD rhabdomyosarcoma. In both cell lines, concentration-dependent inhibition of prenylation was observed for cerivastatin and simvastatin, which could be rescued with the pathway intermediate mevalonate; in general, muscle cells were more sensitive to this effect, as measured by the levels of unprenylated Rap1A, a marker for prenylation by geranylgeranyl transferase I. Concentration-dependent toxicity was observed in both cell lines, with muscle cells again being more sensitive. Importantly, there was no correlation between inhibition of prenylation and cell toxicity, suggesting they are not causally linked. The lack of a causal relationship was confirmed by the absence of cytotoxicity in all cell lines following exposure to specific inhibitors of geranylgeranyl transferases I and II, and farnesyl transferase. As such, we provide strong evidence against the commonly accepted hypothesis linking inhibition of prenylation and statin-mediated toxicity, with the two processes likely to be simultaneous but independent.

1. Introduction

Statins remain a front line treatment for the prevention of cardiovascular disease. As inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), the rate limiting enzyme of cholesterol synthesis, they significantly reduce cholesterol production in the liver, and circulating cholesterol levels (Goldstein and Brown, 1990; Istvan and Deisenhofer, 2001). However, significant adverse-effects associated with statin usage either limit or preclude their utility in some individuals. The major statin-mediated adverse effects are myopathies, muscle related side-effects that can range from mild (muscle aches and cramps) to severe (rhabdomyolysis). While in some patients these myopathies are tolerable, in many cases they necessitate the withdrawal of treatment, and in some cases rhabdomyolysis can be fatal (Arora et al., 2006; Graham et al., 2004).

Cholesterol biosynthesis is one endpoint within the mevalonate pathway, which is responsible for the production of a number of biologically-important molecules, including cholesterol, ubiquinone, phosphodolichol and the isoprenoids farnesyl and geranylgeranyl pyrophosphate (FPP and GGPP; Fig. 1). As HMGCR sits within the shared portion of this pathway, its inhibition by statins potentially impacts upon all these biosynthetic endpoints (Takemoto and Liao, 2001).

The mechanism that underlies statin toxicity is not fully understood, but is likely to be a direct consequence of the inhibition of the mevalonate pathway, rather than an indirect transcription-mediated effect (Howe et al., 2011), since mevalonate supplementation prevents toxicity both in vitro (Johnson et al., 2004) and in vivo (Westwood et al., 2008). Depletion of cholesterol is not thought to be a primary cause of myopathy as squalene synthase inhibitors, which block the first step in the cholesterol branch of the mevalonate

Abbreviations: HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; FTase, farnesyl transferase; GGTase, geranylgeranyl transferase.

* Corresponding author. Tel.: +44 0 1483 689160; fax: +44 0 1483 686401.
E-mail address: n.plant@surrey.ac.uk (N.J. Plant).

http://dx.doi.org/10.1016/j.tox.2015.01.005
0300-483X/

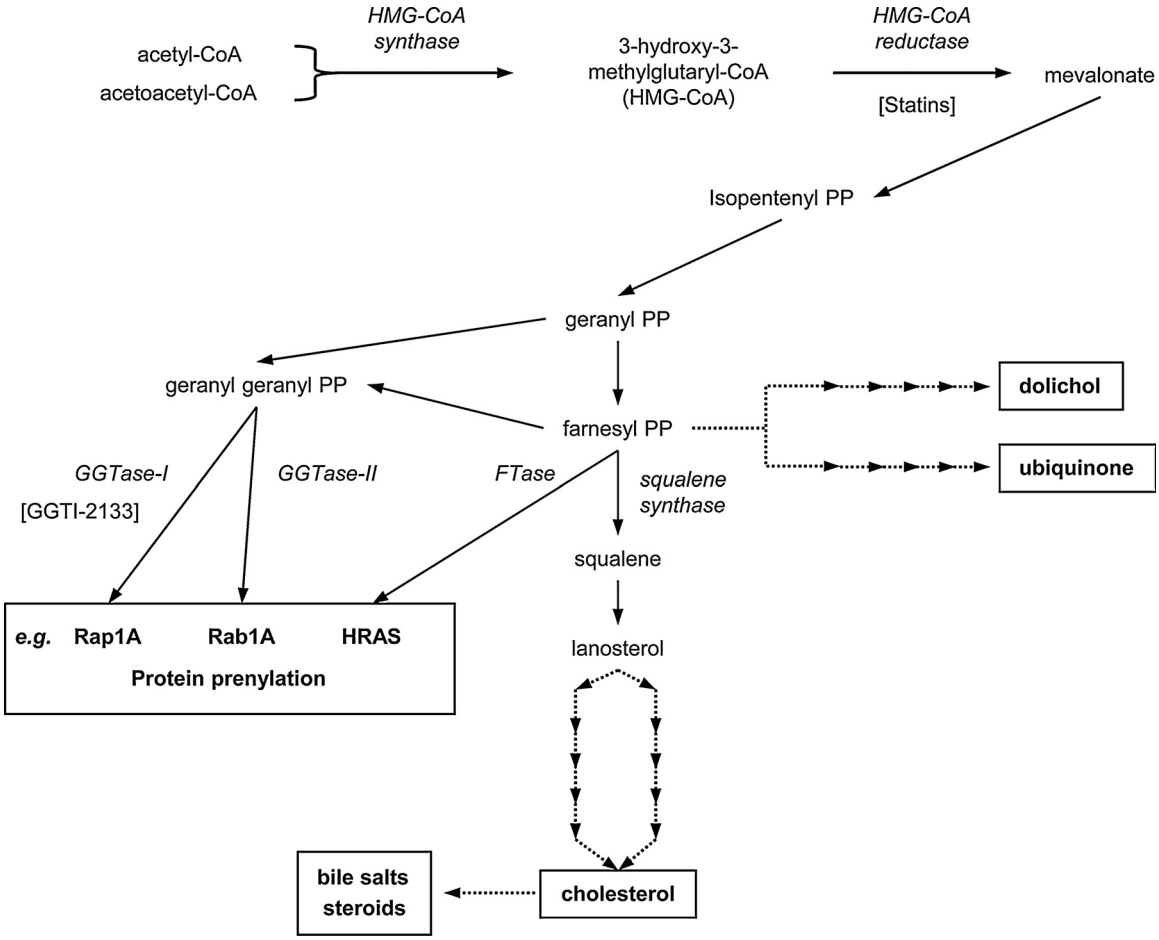

Fig. 1. The mevalonate pathway. Principle products are in boxes. Key enzymes are shown in italics, with relevant inhibitors in square brackets. Multistep processes (for instance in the production of cholesterol from lanosterol) are indicated with dotted lines and arrows.

pathway are not myotoxic (Nishimoto et al., 2007, 2003). In addition, insects and nematodes both lack the cholesterol biosynthetic arm of the mevalonate pathway, but faithfully reproduce the other biosynthetic endpoints seen in mammals; they thus represent ideal models to examine non-cholesterol-dependent effects of statins. Experiments in *Drosophila melanogaster* and *Caenorhabditis elegans* are able to replicate both some of the beneficial effects (for example, cardioprotection) and the adverse effects of statins, demonstrating that these endpoints are not reliant on the cholesterol-lowering effects of these drugs (Morck et al., 2009; Rauthan et al., 2013; Spindler et al., 2012). Taken together, this evidence is consistent with myopathic adverse endpoints being mediated through inhibition of one (or more) of the alternate biosynthetic endpoints of the mevalonate pathway. Evidence exists to support disruption of ubiquinone (Marcoff and Thompson, 2007), dolichol-mediated N-linked glycosylation (Mullen et al., 2010; Siddals et al., 2004) and prenylation (Blanco-Colio et al., 2002; Guijarro et al., 1998; Itagaki et al., 2009; Matzno et al., 2005; Sakamoto et al., 2011; Satoh et al., 2001) following statin treatment, but it is unclear as to which, if any, is the primary determinant of the observed human myopathies.

In the current work, we have used secondary cell lines as a tool to delineate the molecular mechanisms underlying statin-induced myopathy, and in particular the potential role of inhibition of prenylation. We have demonstrated that while liver and muscle cell lines differ in their sensitivity to statins both in terms of cell death and reduction in prenylation, these are not causally linked, since cells inhibited for prenylation do not show a reduced viability or morphological defects. As such, we provide strong evidence that statin-induced myopathy is not mediated via inhibition of prenylation, as commonly assumed.

2. Materials and methods

2.1. Materials

Statins were obtained from the following sources: simvastatin (lactone), lovastatin, and fluvastatin from Calbiochem (Merck KGaA, Darmstadt, Germany); atorvastatin and rosuvastatin from Molekula (Dorset, UK); and cerivastatin, simvastatin (sodium salt) and pravastatin from Sequoia Research Products Limited (Pangbourne, UK). Mevalonate (lithium salt), and the prenyltransferase inhibitors GGTI-2133, FT-277 and perillyl alcohol were purchased from Sigma–Aldrich (Dorset, UK).

Primary antibodies were purchased from Santa Cruz Biotechnology (TX, USA) for Rap1A (C17), Rap1 (I21), HMGCR (H-300), GGTase-I (XX-12), GGTase-II (17-Q), FTase (H-300), from the Developmental Studies Hybridoma Bank (IA, USA) for MyoG (clone F5D) and MYH3 (F1-652) or from Sigma–Aldrich for β-actin (A5441). Appropriate secondary antibodies were purchased from Santa Cruz Biotechnology.

2.2. Cell culture

The human hepatoma cell line Huh7 (Nakabayashi et al., 1982) was a kind gift from Steve Hood (GlaxoSmithKline, Ware, UK) whereas the human rhabdomyeloma cells RD (McAllister et al.,