Food and Chemical Toxicology 90 (2016) 10–17

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Modulating effect of simvastatin on the DNA damage induced by doxorubicin in somatic cells of *Drosophila melanogaster*

P.C. Orsolin [a], R.G. Silva-Oliveira [a], J.C. Nepomuceno [a, b, *]

[a] Universidade Federal de Uberlândia, Instituto de Genética e Bioquímica, Bloco 2E, Campus Umuarama, Uberlândia, Minas Gerais, Brazil
[b] Centro Universitário de Patos de Minas, Laboratório de Citogenética e Mutagênese, Patos de Minas, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 3 November 2015
Received in revised form
15 January 2016
Accepted 26 January 2016
Available online 29 January 2016

Keywords:
Simvastatin
Doxorubicin
Drosophila melanogaster
Antigenotoxicity
SMART
Wing spot assay

ABSTRACT

Simvastatin is an antilipemic drug that promotes inhibition of HMG-CoA reductase. Simvastatin can also inhibit the formation of other products, such as isoprenoids, conferring additional benefits to this drug, which include antiproliferative, anti-invasive and pro-apoptotic effects. This study was carried out with the aim of evaluating the mutagenic/recombinogenic effect of simvastatin as well as the possible modulatory effects of this statin on the DNA damage induced by doxorubicin (DXR). This analysis was performed using the somatic mutation and recombination test (SMART) in *Drosophila melanogaster*. To study these effects, larvae descendants of both crosses (ST and HB) were chronically treated with five concentrations of simvastatin, separately and in association with DXR. The results revealed no mutagenic/recombinogenic effect of simvastatin for any of the concentrations tested. A modulating effect of simvastatin was also observed on DNA damage induced by DXR. The reduction of total mutant frequency was observed for spots from descendants of both crosses, but the inhibition was more effective in descendants from the standard cross (ST). It is believed that this modulating effect is mainly associated with the antioxidant activity of this class of drugs, although this parameter has not been directly assessed in this study.

1. Introduction

Simvastatin is an antilipemic drug of the statin class, which acts to reduce plasma cholesterol levels and, consequently, lowers the risk of atherosclerosis and myocardial infarction (Sparrow et al., 2001; Tang et al., 2006; Ishikawa et al., 2014). Statins were first derived from the fungi *Penicillium citrinum* and its mechanism of action is inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA reductase), an enzyme present in the cell's endoplasmic reticulum, responsible for the conversion of HMG-CoA to mevalonate in the cholesterol biosynthetic pathway (Hindler et al., 2006; Saito et al., 2008). The main point of limitation and control in intracellular cholesterol metabolism occurs precisely at this stage, and is largely influenced by the degree of activity of this enzyme (Marie et al., 2008). Inhibition of HMG-CoA reductase promotes reduction of LDL cholesterol through two principal mechanisms: a decrease in the endogenous synthesis of cholesterol and, simultaneously, an increase in receptor synthesis for LDL cholesterol in hepatic cells, which increases the clearance thereof (Fonseca, 2005).

There are striking pharmacokinetic differences between the statins currently available on the market, including variations in the coefficient of hydrophilicity, hepatic pathway, plasma half-life and efficacy in lowering lipid profiles. Statins also differ regarding interactions with other drugs that use the same pathway (Fonseca, 2005; Schachter, 2005). In this context, although all statins have the same mechanism of action, there are differences in the effectiveness of responses observed for each statin individually.

With respect to the chemical structure of statins, there are open or closed lactone ring forms (which makes them structural similar to HMG-CoA), constituting the active portion of this class of drugs. Simvastatin, specifically, has a closed lactone ring, and is considered a pro-drug activated *in vivo* by chemical or enzymatic reaction (Campo and Carvalho, 2007; Banzato, 2013). In the process of metabolism, the lactone form is converted to the active form hydroxy acid (Chan et al., 2003; Marinho et al., 2012.). Simvastatin is derived from 2'-methylated lovastatin, a compound isolated from

* Corresponding author. Universidade Federal de Uberlândia, Instituto de Genética e Bioquímica, Bloco 2E, Campus Umuarama, Uberlândia, Minas Gerais, Brazil. Tel.: +55 34 3823 0169; fax: + 55 34 3821 0300.
E-mail address: nepomuceno@ufu.br (J.C. Nepomuceno).

http://dx.doi.org/10.1016/j.fct.2016.01.022
0278-6915/

the first fungi product, compactin, and is therefore considered a semisynthetic statin (Levinski and Brown, 2007).

Besides the main action of inhibiting the synthesis of mevalonate, with a consequent reduction in cholesterol levels, simvastatin can also act by inhibiting the formation of other products, such as isoprenoids (Demierre et al., 2005), including farnesyl and geranylgeranyl groups, which bind to various proteins (superfamily Ras/Rho) via prenylation, during the post-translational process (Edwards and Ericsson, 1999; Yamashita et al., 2008), which gives the drug additional benefits (pleiotropic). Such benefits include antiproliferative, anti-invasive, pro-apoptotic and antitumor effects (Collisson et al., 2002; Liao, 2002; Chan et al., 2003; Sleijfer et al., 2005; Borahay et al., 2014). According to Ishikawa et al. (2014) the antiproliferative effect of simvastatin is one of the most significant, being greater than the effect of other statins such as fluvastatin and atorvastatin. The antioxidant action of statins has also been reported in previous studies (Shishehbor et al., 2003; Mennickent et al., 2008; Gamaleldin et al., 2015).

The extensive knowledge about the genetics of *Drosophila melanogaster* and the long trial experience with this organism have made it the primary tool for research on genetic mutation and toxicology (Sarıkaya and Memmi, 2013). The SMART (Somatic mutation and recombination test) assay conducted using *D. melanogaster*, is a quick somatic test system, used to detect substances that can cause changes in DNA and is also widely used to detect antimutagenic/antirecombinogenic substances (Graf and Singer, 1992; Graf et al., 1998). This bioassay is based on the premise that, during the embryonic development of *D. melanogaster*, groups of cells proliferate mitotically to differentiate into the adult fly body structures. If there are genetic changes in the imaginal disc cells, a mutant cell clone will be formed and detected as a smear of mutants by the wings of the adult fly (Guzman-Ricón and Graf, 1995). The analysis of these genetic alterations determines the phenotypic expression of the marker genes *mwh* or flr^3 responsible for changes in the trichomes (Graf et al., 1984).

Considering the above, the present work was carried out with the main objective of assessing, through the SMART assay, the mutagenic/recombinogenic effect of simvastatin alone, as well as the possible modulatory effects of statins on DNA damage induced by doxorubicin (DXR). DXR is an antitumor drug of the anthracycline class, which promotes intercalation with the DNA molecule and inhibition of topoisomerase II (Islaih et al., 2005), and the production of free radicals (Nascimento and Martins, 2005; Kaiserová et al., 2006). Antineoplastic DXR is a powerful and effective chemotherapeutic agent in the treatment of several types of tumors, but its use is limited due to its cardiotoxicity (Ewer and Ewer, 2015).

2. Material and methods

2.1. Chemical agents

The substance tested was simvastatin (Sinvastacor®), CAS 79902-63-9, batch CV6653, with a molecular formula of $C_{25}H_{38}O_5$ and a molecular weight of 418.57 g/mol, produced by Sandoz do Brasil Indústria Farmacêutica, Cambé, Paraná. Five concentrations of simvastatin were prepared for use in the experiment: 12.5; 25; 50; 100 and 200 μM. The chemical structure of the drug tested is shown in Fig. 1.

Doxorubicin (Adriblastina®, CAS 25316-40-9, batch 3PL0341, registered, imported and distributed by the laboratory Pfizer, Guarulhos, São Paulo) was used as a positive control. Doxorubicin, with a molecular formula of $C_{27}H_{29}NO_{11}$-HCl and a molecular weight of 580 g/mol, was used at a concentration of 0.125 mg/mL. The negative control used as a reference and for dilution of the other substances was 5% ethanol. All of the dilutions were prepared immediately before use. The use of DXR as a positive control in our work, as well as the concentrations used, was based on studies done previously, which demonstrated generation of reactive oxygen species and induction of homologous recombination in *D. melanogaster*.

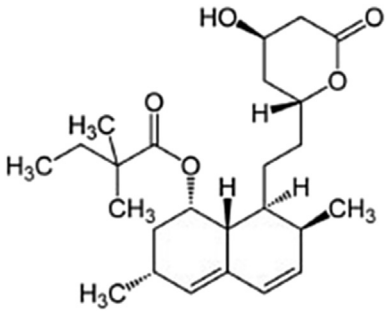

Fig. 1. Chemical structure of simvastatin.

2.2. Somatic mutation and recombination test (SMART) in *Drosophila melanogaster*

2.2.1. Stock lineages and crosses

Three mutant lineages of *D. melanogaster* were used: *mwh*, flr^3 and *ORR*, which possess the genetic markers *multiple wing hairs* (*mwh*, 3–0.3) and *flare*^{3} (flr^3, 3–38.8). More detailed information about the genetic symbols and descriptions of these linkages can be found in Lindsley and Zimm (1992).

These linkages were submitted to two crossing schemes:

a) Standard (ST) Cross, in which virgin females flr^3/*In(3 LR)TM3*, *ri* p^p *sep I(3)89Aa* bx^{34e} *and* Bd^s were crossed with males *mwh/mwh* (Graf et al., 1989);
b) High Bioactivation (HB) Cross, in which virgin females *ORR/ORR*; flr^3/*In(3 LR)TM3*, *ri* p^p *sep I(3)89Aa* bx^{34e} *and* Bd^s were crossed with males *mwh/mwh* (Graf and Van Schaik, 1992).

The use of these two types of crosses enables an evaluation of the mutagenic and anti-mutagenic agents, of direct and indirect action, according to the basal and elevated levels of the metabolizing enzymes of cytochrome P450. This is possible because the lineage *ORR* (employed in the HB cross) was created with the aim of increasing the performance of the referred to test in the case of activation of promutagens dependent on activation via cytochrome P450 (Andrade and Lehmann, 2003).

From these crosses two types of descendents were obtained: marked trans-heterozygous (MH, *mwh* +/+ flr^3), which have wings with smooth edges, and balancer heterozygous (BH, *mwh* +/+ *TM3*, Bd^s), which have serrated wings (Guzmán-Rincón and Graf, 1995).

2.2.2. Treatments

The collection of eggs of the descendents of the two crosses described previously, ST and HB, was conducted over a period of 8 h, in vials containing solid agar base and a layer of biological yeast supplemented with sucrose. After 72 ± 4 h, third stage larvae were washed with reverse osmosis water, collected, and placed in glass vials containing 1.5 g of instantaneous mashed potato (HIKARI®). To each tube, 5 mL of simvastatin (in the concentrations 12.5; 25; 50; 100 and 200 μM) was added, diluted in 5% ethanol, alone and in association with DXR. The association between DXR and simvastatin occurred in a co-treatment system.

These larvae were submitted to a chronic treatment, over a