Drug and Chemical Toxicology

http://informahealthcare.com/dct
ISSN: 0148-0545 (print), 1525-6014 (electronic)

Drug Chem Toxicol, Early Online: 1–6
 DOI: 10.3109/01480545.2013.851692

informa
healthcare

RESEARCH ARTICLE

Genotoxicity evaluation of HMG CoA reductase inhibitor rosuvastatin

Ahmet Ali Berber[1], Mustafa Çelik[2], and Hüseyin Aksoy[1]

[1]*Department of Biology, Faculty of Art and Sciences, Sakarya University, Sakarya, Turkey and* [2]*Department of Medical Biology, Faculty of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey*

Abstract

The genotoxic potential of rosuvastatin as one of the statin drugs was assessed by chromosomal aberrations (CAs), micronucleus (MN) and DNA damage by comet assay in the human peripheral blood lymphocytes. Rosuvastatin was used at concentrations of 0.0625, 0.125, 0.25, 0.5 and 1 μg/mL for these *in vitro* assays. In all assays, a negative and positive control were also included. CA frequencies were significantly increased in all concentrations at 24 hours and significantly increased in all concentrations except 0.0625 μg/mL at 48 hours, compared to the negative control. Rosuvastatin has a decreased mitotic index (MI) at 0.5- and 1-μg/mL concentrations at 24 hours and at 0.25, 0.5 and 1 μg/mL at 48 hours. A significant increase was observed for induction of MN in all treatments, compared to the negative control. Cytokinesis-block proliferation indices were not affected by treatments with rosuvastatin. In the comet assay, significant increases in comet tail length and tail moment were observed at 0.0625-, 0.5- and 1-μg/mL concentrations. Comet intensity was significantly increased in all concentrations except 0.0625 μg/mL. According to these results, rosuvastatin is cytotoxic and clastogenic/aneugenic in human peripheral lymphocytes. Further studies should be conducted in other test systems to evaluate the full genotoxic potential of rosuvastatin.

Keywords

Chromosomal aberrations, comet assay, genotoxicity, human blood lymphocytes, micronuclei, rosuvastatin, statin

History

Received 16 April 2013
Revised 3 August 2013
Accepted 25 August 2013
Published online 18 November 2013

Introduction

Statins are effective cholesterol-lowering agents that play a role by inhibiting the enzyme, 3-hydroxy 3-methylglutaryl coenzyme A (HMG CoA) reductase, which is the rate-controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids. Statins have proved to be remarkably successful as therapeutic agents. The throughputs from large-scale clinical trials has established the impressiveness of statin therapy in the primary and secondary protection of cardiovascular diseases (CVDs) associated with hypercholesterolemia (CTT, 2005, 2008; Gotto & Grundy, 1999; Schmidt et al., 2007; Stalker et al., 2001). Statins inhibit an enzyme that is essential not only for cholesterol biosynthesis, but also for the synthesis of several other nonsterol precursors essential for normal cellular function (MacDonald & Halleck, 2004). However, pleotropic and additional benefit shown with more intensive statin therapy (Cannon et al., 2004; LaRosa et al., 2005; Pedersen et al., 2005), there has been a trend toward using higher doses of statin. Further, cholesterol lowering is currently recommended for a wide range of people at cardiovascular risk, including those with average and below-average lipid levels (BRITISH, 2005; NCEP, 2001). This change is leading to increased statin use and the use of more-intensive regimens. Hence, the safety of this group of drugs is of considerable importance.

Rosuvastatin is also a class of medications in HMG-CoA reductase inhibitors. It is a synthetic lipid-lowering agent for oral administration. The chemical name for rosuvastatin calcium is bis[(E)-7-[4(4-fluorophenyl)-6-isopropyl-2[methyl (methylsulfonyl)amino] pyrimidin-5-yl](3R,5S)3,5-dihydroxyhept-6-enoic acid] calcium salt and it is sparingly soluble in water and methanol as well as slightly soluble in ethanol (Figure 1) (FDA, 2010).

Rosuvastatin produces its lipid-modifying effects in two ways. First, it increases the number of hepatic low-density lipoprotein (LDL) receptors on the cell surface to enhance uptake and catabolism of LDL. Second, rosuvastatin inhibits hepatic synthesis of very-low-density lipoprotein. Rosuvastatin has significantly greater efficacy in lowering LDL cholesterol and non-high-density lipoprotein cholesterol concentrations than other five statins. It has been shown to enable more patients to reach their LDL cholesterol goals than other statins and to do so with an acceptable safety profile (FDA, 2010; McKenney, 2005; McTaggart et al., 2001).

In the scientific literature review that we made, we have not found any genotoxicity studies about rosuvastatin except for U.S. Food and Drug Administration (FDA) reports. In these reports, nonclinical toxicological throughputs about rosuvastatin were presented. In these reports, the incidence

Address for correspondence: Ahmet Ali Berber, Department of Biology, Faculty of Art and Sciences, Sakarya University, 54187 Sakarya, Turkey. Fax: +902642955950. E-mail: aberber@sakarya.edu.tr

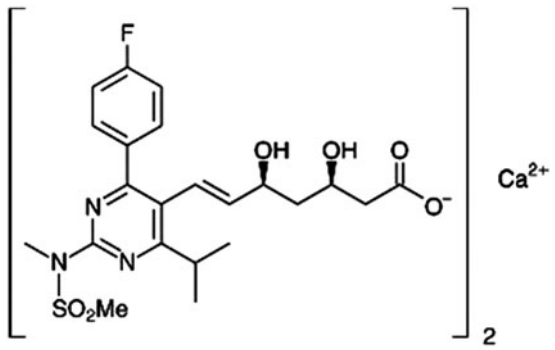

Figure 1. Molecular structure of rosuvastatin calcium.

of uterine stromal polyps in rats exposed to 2, 20, 60 or 80 mg/kg/day of rosuvastatin by oral gavage was significantly increased in females at 80 mg/kg/day. Increased incidence of polyps was not observed at lower doses (FDA, 2010). In a carcinogenicity study in mice given 10, 60 and 200 mg/kg/day by oral gavage, an increased incidence of hepatocellular adenoma (HCA) and carcinoma (HCC) was observed at 200 mg/kg/day. This mice carcinogenicity study is in agreement with rat carcinogenicity studies, and increased incidence of hepatocellular tumors was not observed at lower doses. The FDA also suggested that rosuvastatin was not mutagenic or clastogenic with or without metabolic activation in the Ames test with *Salmonella typhimurium* and *Escherichia coli*, the mouse lymphoma assay and the chromosomal aberration (CA) assay in Chinese hamster lung (CHL) cells. Rosuvastatin was negative in the *in vivo* mouse micronucleus (MN) test (FDA, 2010).

There are many test methods used to detect the potential genotoxicity of different chemicals. CAs, both *in vivo* and *in vitro*, and single-cell gel electrophoresis (SCGE) are very sensitive methods (Yilmaz et al., 2008). Hence, in the present study, we planned to determine the genotoxic potential of rosuvastatin *in vitro* in human lymphocyte cultures to increase knowledge on the genotoxic activity of this cholesterol drug with CAs, MN and DNA damage by comet assay.

Methods

Chemicals

Rosuvastatin was isolated from one of the commercial statin drugs. For this purpose, first, drugs were treated with 100 mL of methanol and put on a magnetic stirrer for 20 hours. Then, the solution was filtered with filter paper and was evaporated by Heidolph 4000. Purity of rosuvastatin was determined by ^{1}H NMR (nuclear magnetic resonance) spectrum and melting point. The results show that the purity of rosuvastatin is $\geq$98%. The ^{1}H NMR data and melting point (155–165 °C) of rosuvastatin corresponded to those in the literature (Dent, 2007; Fischer et al., 2010).

CA analysis in human lymphocytes

Heparinized peripheral blood samples of 4 healthy nonsmoking (2 male and 2 female) donors were cultured in chromosome medium B and treated with 0.0625-, 0.125-, 0.25-, 0.5- and 1-μg/mL concentrations of rosuvastatin. An untreated and a positive control mytomycin C (MMC; 0.2 μg/mL) were also maintained in all experiments. Cells in culture were exposed to test substance for 24 and 48 hours. Cultures were incubated for 72 hours at 37 °C, and colchicine (final concentration: 0.06 μg/mL) was added to each culture 2 hours before harvesting. Cells were then harvested by centrifugation (1200 rpm for 10 minutes), and the pellet was treated with 0.075 M of KCl for 30 minutes at 37 °C. Cells were centrifuged again and fixed in cold methanol/acetic acid (3:1). The fixation process was repeated three times. Slides were stained with 5% Giemsa (pH = 6.8) in Sorensen buffer for 20–25 minutes, washed in distilled water, dried at room temperature and mounted with Depex.

MN test in cultured human lymphocytes

Whole blood was added to 2.5 mL of chromosome medium B (Biochrom AG, Berlin, Germany). Human lymphocytes were incubated at 37 °C for 72 hours and treated with rosuvastatin at 0.0625, 0.125, 0.25, 0.5 and 1 μg/mL for 48 hours. Cytocalasin B (5.2 μg/mL) was added to arrest cytokinesis at 44 hours after the start of culture. Then, cells were harvested by centrifugation (1000 rpm for 10 minutes), and the pellet was treated with hypotonic solution (0.075 M of KCl) for 5 minutes at 4 °C. Cells were recentrifuged and fixed three times in cold methanol/acetic acid (3:1). In the last fixative, 1% formaldehyde was added to preserve the cytoplasm. Slides were prepared by dropping and air-drying. Slides were stained with 5% Giemsa (pH = 6.8) in Sorensen buffer for 13–15 minutes, washed in distilled water, dried at room temperature and mounted with Depex.

Comet assay in cultured human lymphocytes

The comet assay (SCGE) was conducted under alkaline conditions according to Singh et al. (1988). Heparinized peripheral blood was obtained before the performance of the test. Lymphocytes were isolated by Biocoll separating solution. To detect the viability of cells, the trypan blue exclusion test was used. Cell viability was >99%. Isolated human lymphocytes were incubated with 0.0625-, 0.125-, 0.25-, 0.5- and 1-μg/mL concentrations of rosuvastatin for 1 hour at 37 °C. Negative and positive controls (H_2O_2; 3.4 μg/mL) were also included at the same temperature and exposure time in parallel with rosuvastatin. The other procedure was applied according to Mamur et al. (2010).

Slide evaluation

In human lymphocytes, 100 well-spread metaphases per donor (total, 400 metaphases per concentration) were analyzed. The mitotic index (MI) was also determined by scoring 3000 cells from each donor (total, 12 000 cells per concentration). Micronuclei were scored from 1500 binucleated cells per donor (total, 6000 binucleated cells per concentration). Cell proliferation was evaluated using the cytokinesis-block proliferation index (CBPI), which indicates the average number of cell cycles. Five hundred lymphocytes (total, 2000 lymphocytes per concentration) were scored to evaluate the percentage of cells with one, two, three and four nuclei. The CBPI was calculated

according to Surrales et al. (1995) as follows: $[1 \times N1] + [2 \times N2] + [3 \times (N3 + N4)]/N$, where $N1$–$N4$ represent the number of cells with one to four nuclei, respectively, and N is the total number of cells scored. In the comet assay, slides were examined using a fluorescent microscope (BAB research microscope; BAB Imaging System, Ankara, Turkey). Slides were prepared for each concentration of rosuvastatin. The comet parameters used for the evaluation are tail length, tail DNA% and tail moment. For this reason, 100 comets on each slide were analyzed with a specialized image analyses system (BS 200 ProP; BAB Imaging System) to determine these parameters.

Statistical analysis

For the statistical analysis of the results, z-test for percentage of abnormal cell, CA/cell, CBPI, MI, MN was used. *t*-test was performed to determine the possible DNA damage induced by rosuvastatin. Concentration-response relationships were determined from the regression coefficients for the percentage of abnormal cell, CA/cell, MN, mean comet tail length, tail intensity and tail moment.

Results

To evaluate the effect of HMG-CoA reductase inhibitor rosuvastatin on human lymphocytes, three parameters (CAs, MN and comet) were analyzed. The results reported in Table 1 show the induction of CAs after treatment with increasing concentrations of rosuvastatin. Rosuvastatin induced six types of CAs: chromatid and chromosome breaks; fragments; sister-chromatid union; dicentric chromosomes and chromatid exchange. Chromatid breaks were observed as the most common aberrations, whereas chromosome breaks and fragments were also observed respectively. Sister-chromatid union and dicentric chromosome abnormalities were only observed in the 24-hour treatment period. The CA/cell ratio significantly increased in all concentrations in two treatments except 0.0625 μg/mL at 48 hours, when compared to the controls.

Rosuvastatin decreased the CBPI, but these results showed that the effect was statistically insignificant. Rosuvastatin reduced the percentage of MI significantly at 0.5- and 1-μg/mL concentrations at 24 hours. In addition to this, it

Table 1. Total chromosomal aberrations in human lymphocytes treated with rosuvastatin.

	Treatment		Aberrations (structural)								
Test substance	Period (hours)	Doses (μg/mL)	ctb	csb	f	cte	scu	dc	Abnormal cell ± SE (%)	CAs/cell ± SE	MI ± SE (%)
Control	24	0.00	1	–	–	–	–	–	0.25 ± 0.25	0.003 ± 0.003	5.81 ± 0.21
Positive control	24	0.20	118	31	12	22	–	5	36.50 ± 2.41	0.470 ± 0.025	3.21 ± 0.16
Rosuvastatin	24	0.0625	9	4	–	–	2	–	3.75 ± 0.95[c]	0.038 ± 0.010[c]	5.38 ± 0.21
		0.125	23	3	–	–	–	–	6.50 ± 1.23[c]	0.065 ± 0.012[c]	5.83 ± 0.21
		0.25	17	2	4	–	–	–	5.75 ± 1.16[c]	0.058 ± 0.012[c]	5.30 ± 0.21
		0.50	32	8	1	1	–	–	10.25 ± 1.52[c]	0.105 ± 0.015[c]	4.95 ± 0.20[b]
		1.00	18	5	4	1	–	1	7.25 ± 1.30[c]	0.073 ± 0.013[c]	4.48 ± 0.19[c]
Control	48	0.00	8	–	–	–	–	–	2.00 ± 0.70	0.020 ± 0.007	6.1 ± 0.12
Positive control	48	0.20	529	138	35	49	–	–	75.25 ± 2.16	1.878 ± 0.680	1.73 ± 0.12
Rosuvastatin	48	0.0625	12	1	–	–	–	–	3.25 ± 0.89	0.033 ± 0.009	5.82 ± 0.21
		0.125	19	–	–	–	–	–	4.50 ± 1.04[a]	0.048 ± 0.011[a]	4.78 ± 0.19[c]
		0.25	24	2	2	1	–	–	7.00 ± 1.28[c]	0.073 ± 0.013[c]	3.60 ± 0.17[c]
		0.50	41	6	3	–	–	–	11.00 ± 1.57[c]	0.125 ± 0.017[c]	2.21 ± 0.13[c]
		1.00	49	4	1	–	–	–	11.75 ± 1.61[c]	0.135 ± 0.017[c]	1.82 ± 0.12[c]

Four hundred metaphases were scored for each treatment for CAs, and 12 000 metaphases were scored for each dose level for the MI.
[a]Significantly different from the negative control; $p < 0.05$ (*z*-test). [b]Significantly different from the negative control; $p < 0.01$ (*z*-test). [c]Significantly different from the negative control; $p < 0.001$ (*z*-test).
ctb, chromatid break; csb, chromosome break; f, fragment; cte, chromatid exchange; scu, sister chromatid union; dc, dicentric; SE, standard error.

Table 2. The MN frequency and CBPI in human lymphocytes treated with rosuvastatin.

	Treatment			Distribution of BN cells according to the no. of MN				
Test substance	Period (hours)	Dose (μg/mL)	BN cells scored	(1)	(2)	(3)	MN/cell (%) ± SE	CBPI ± SE
Negative control	48	0.00	6000	7	–	–	0.12 ± 0.05	1.41 ± 0.26
Positive control	48	0.20	6000	844	93	6	17.47 ± 0.49	1.19 ± 0.24
Rosuvastatin	48	0.0625	6000	25	–	–	0.42 ± 0.08[c]	1.26 ± 0.25
		0.125	6000	37	–	1[a]	0.85 ± 0.12[d]	1.34 ± 0.26
		0.25	6000	48	3	1	0.95 ± 0.13[d]	1.31 ± 0.25
		0.50	6000	52	2	1[b]	1.03 ± 0.13[d]	1.22 ± 0.25
		1.00	6000	72	2	–	1.27 ± 0.15[d]	1.13 ± 0.24

[a]Cell with 14 micronuclei.
[b]Cell with six micronuclei.
[c]Significantly different from the control; $p < 0.01$ (*z*-test). [d]Significantly different from the control; $p < 0.001$ (*z*-test).
BN, binucleated; SE, standard error.

also reduced MI at all concentrations except 0.0625 μg/mL at 48 hours, compared to negative control.

To evaluate possible clastogenic and/or aneugenic effects, the cytokinesis-block MN assay was conducted. Rosuvastatin increased the frequency of lymphocytes with micronuclei. Micronuclei frequency significantly increased in all concentrations (Table 2).

The rise in MN frequency nearly reached 11-fold, compared to the negative control, at 1 μg/mL. Most cells showed just one micronucleus, seven cells with two micronuclei, one cell with three micronuclei, one cell with six micronuclei (Figure 2) and one cell with 14 micronuclei (Figure 3). However, in this assay, we observed an apoptotic cell.

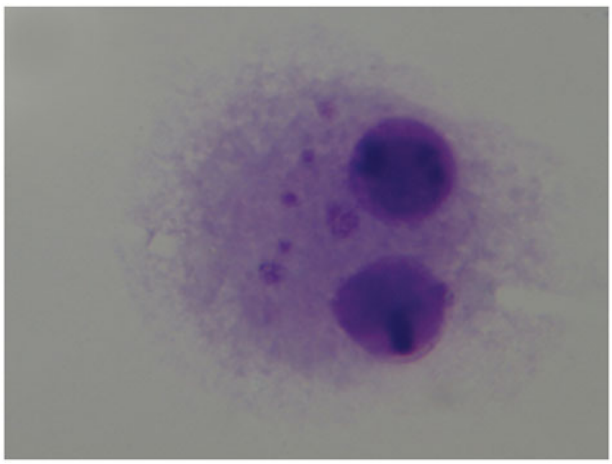

Figure 2. Binucleated cell with six micronuclei at 0.125 μg/mL in 48-hour treatment.

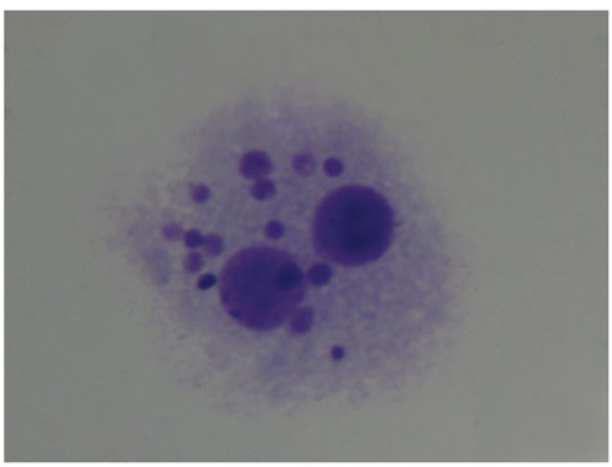

Figure 3. Binucleated cell with 14 micronuclei at 0.5 μg/mL in 48-hour treatment.

In the comet assay, tail length and tail moment showed significant increases at 0.0625-, 0.5- and 1-μg/mL concentrations, when we applied rosuvastatin to isolated human lymphocytes. These increases were dose dependent (both $r = 0.52$), and in tail intensity, a significant increase was observed at all concentrations except 0.0625 μg/mL. This increase was also dose dependent ($r = 0.81$; Table 3).

Discussion

Rosuvastatin has joined other statins that are used in lowering LDL cholesterol, treating dyslipidemia and reducing the risk of coronary heart disease (CHD). The importance of cholesterol lowering in CHD risk reduction has been established in numerous randomized, clinical trials (SSSSG, 1994; Tonkin et al., 1998). Statins may also avail in the therapy of other disorders, including Alzheimer's disease, osteoporosis and, especially, cancer (Chan et al., 2003; Crisby et al., 2002; Waldman & Kritharides, 2003). Ertugrul et al. (2011) demonstrated that 25-hydroxyvitamin D level (a protector against CVD) was increased with rosuvastatin treatment.

Verreth et al. (2007) pointed out that *in vitro* administration of rosuvastatin restored superoxide dismutase 1 expression in THP-1 macrophages and foam cells. The latter was associated with less-oxidized LDL accumulation within atherosclerotic plaques and inhibition of plaque progression.

Rosuvastatin is not only a lipid lowering drug. It could significantly decrease oxidative stress (OS) and has immunomodulatory properties in a dose- and LDL-independent manner (Resch et al., 2006). Ajith et al. (2008) suggest that rosuvastatin (1.5 or 2 mg/mL) is able to protect against lipid peroxidation (LPO). Further, the investigators reported that H_2O_2-induced changes in pBR322 plasmid DNA and fragmentation of hepatic DNA were alleviated by rosuvastatin. However, rosuvastatin did not show any superoxide anion-scavenging activity. Kamigaki et al. (2011) suggest that statins may be a new cholangiocarcinoma treatment option that could potentially enhance the anticancer effect of preexisting anticancer drugs. These studies show that rosuvastatin has many beneficial pleotropic effects. Compared to our study, lowering of OS and LPO, reducing H_2O_2-induced DNA fragmentation and anticancer effect by rosuvastatin are not in agreement with our results. In our study, rosuvastatin has genotoxic potential in *in vitro* human lymphocytes.

Table 3. Assessment of DNA damage by comet assay after *in vitro* exposure of human lymphocytes to rosuvastatin.

Test substances	Period (hours)	Doses (μg/mL)	Tail length (μm)	Tail moment	Tail intensity (%)
Control	1	0	8.012 ± 0.45	6.262 ± 0.44	234.771 ± 0.23
Rosuvastatin	1	0.0625	9.923 ± 0.70[a]	8.203 ± 0.68[a]	234.602 ± 0.27
		0.125	9.446 ± 0.57	7.520 ± 0.55	236.375 ± 0.25[a]
		0.250	7.222 ± 0.46	5.488 ± 0.44	236.096 ± 0.18[a]
		0.50	11.430 ± 0.83[a]	9.732 ± 0.82[a]	236.113 ± 0.26[a]
		1	10.438 ± 0.69[a]	8.628 ± 0.68[a]	237.187 ± 0.25[a]
H_2O_2	1	3.4	61.931 ± 2.13	59.599 ± 2.13	244.239 ± 0.23

[a]Significantly different from the negative control; $p < 0.05$ (*t*-test).

As we pointed out, there are no studies about the genotoxic profile of rosuvastatin except premarketing studies that are reported on by the FDA (2010). In this report, nonclinical toxicology (carcinogenesis, mutagenesis and fertility) and animal toxicology studies were performed. In one of the nonclinical toxicology trials, a 104-week carcinogenicity study in rats (2, 20, 60 or 80 mg/kg/day by oral gavage) was performed. The frequency of uterine stromal polyps was significantly increased in females at 80 mg/kg/day in this trial. In a 107-week carcinogenicity study in mice given 10, 60 and 200 mg/kg/day by oral gavage, an increased incidence of HCA and HCC was observed at 200 mg/kg/day. In these studies, increased incidence of carcinogenicity with administration of rosuvastatin was shown at a higher dose or long time exposure, but in our study, we established a genotoxic and cytotoxic effect of rosuvastatin with a daily usage dose on human lymphocytes *in vitro*.

In the mevalonate pathway, rosuvastatin not only inhibits cholesterol synthesis, but also inhibits many molecules, such as coenzyme Q10 (CoQ10), which is one of the electron transport chain components known as ubiquinones. CoQ10 contributes to aerobic cellular respiration, producing adenosine triphosphate (ATP) energy. It is also a important molecule in coronary artery disease (Yalcin et al., 2004). Further, CoQ10 levels were significantly lower in melanoma patients than in control subjects and in patients who developed metastases than in the metastasis-free subgroup. Reduced concentrations of CoQ10 are particularly a problem in the regulation of existing mitochondrial dysfunction, because ample CoQ10 can bypass a range of respiratory chain defects (Rusciani et al., 2005). McMurray et al. (2010) also showed that rosuvastatin reduced CoQ10. Because of this reduced CoQ10 level, rosuvastatin may lead to different adverse effects. It is known that this molecule produces ATP energy. When CoQ10 breaks with rosuvastatin, the production of ATP decreases. This effect may lead to cytotoxic effects. In the same way, in our study, rosuvastatin showed significant cytotoxic effect in 24- (0.5 and 1 μg/mL) and 48-hour treatment (0.125, 0.25, 0.5 and 1 μg/mL).

The FDA (2010) suggests that rosuvastatin is not mutagenic or clastogenic with or without metabolic activation in the Ames test with *S. typhimurium* and *E. coli*, the mouse lymphoma assay and the CA assay in CHL cells. In the same reports, rosuvastatin is negative in the *in vivo* mouse MN test. On the contrary, our results demonstrated that rosuvastatin significantly induced CAs and MN.

Gajski & Garaj-Vrhovac (2008) pointed out atorvastatin showed significant differences on sister chromatic exchange at 30 and 21 ng/mL, both in 20- and 48-hour treatment on human lymphocytes, compared to the negative control. Researchers also showed that atorvastatin significantly increased tail length at 30-ng/mL concentrations at 24, 48 and 72 hours and tail moment at 48 hours, compared to the negative control. In the present study, DNA damage was observed in rosuvastatin-treated lymphocytes. Tail length and tail moment showed significant increases at 0.0625-, 0.5- and 1-μg/mL concentrations. In tail intensity, significant increase was observed at all concentrations except 0.0625 μg/mL. Our results are in agreement with those of Gajski & Garaj-Vrhovac (2008).

The comet assay detects strand breaks. Generation of DNA damage is considered to be an important initial event in carcinogenesis (Møller, 2005). The alkaline version of the method is widespread and frequently used for detecting genetic damage induced by different genotoxic agents, such as radiation, pesticides and chemical compounds.

Chromosomal breaks or interference with the mitotic process, resulting in lagging of the chromosomal material during cell division, leads to the MN (Gudi et al., 1990). In this study, to evaluate possible clastogenic and/or aneugenic effects, the cytokinesis-block MN assay was conducted. In our results, rosuvastatin significantly increased micronuclei in all concentrations. After these results, we suggested that rosuvastatin could have resulted in clastogenic and/or aneugenic effects *in vitro*. On the other hand, in this study, we did not observe any polyploidy in CA analyses. For this reason, to understand whether rosuvastatin has an aneugenic effect, the MN/fluorescence *in situ* hybridization assay should be conducted. However, in the present study, we did not observe any nucleoplasmic bridges and nuclear buds.

Link et al. (2011) showed that rosuvastatin treatment in patients with acute coronary syndrome resulted in the expression of B-cell lymphoma 2 (antiapoptotic protein) being down-regulated within 24–72 hours in $CD4^+$ T cells. The number of apoptotic $CD4^+$ $CD28^{null}$ T cells remained low on placebo, but on statins, these apoptotic T cells increased. Compared to placebo, this difference was statically significant. Already, after 72 hours, $CD4^+$ $CD28^{null}$ T cells of patients treated with rosuvastatin entered an apoptotic pathway. Likewise, in our study, with administration of rosuvastatin, we detected apoptotic cells on human lymphocytes with the MN assay.

Conclusions

Rosuvastatin induces cytotoxicity, clastogenicity and DNA effects in human lymphocytes in *in vitro*. However, it should be investigated in other mammalian test system(s) for its genotoxic effects. Further, biomonitoring studies should also be conducted with patients receiving therapy with this drug.

Declaration of interest

This study was supported by the Sakarya University Research Fund (grant no.: 2012-50-02-023).

References

Ajith TA, Riji, T, Anu, V. (2008). In vitro anti-oxidant and DNA protective effects of the novel 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor rosuvastatin. Clin Exp Pharmacol Physiol 35: 625–629.

BRITISH (British Cardiac Society, British Hypertension Society, Diabetes UK, HEART UK, Primary Care Cardiovascular Society, Stroke Association). (2005). JBS 2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. Heart 91: 1–52.

Cannon CP, Braunwald E, McCabe CH, et al. (2004). Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med 350:1495–1504.

Chan KKW, Oza AM, Siu LL. (2003). The statins as anticancer agents. Clin Cancer Res 9:10–19.

Crisby M, Carlson LA, Winblad B. (2002). Statins in the prevention and treatment of Alzheimer disease. Alzheimer Dis Assoc Disord 16: 131–136.

CTT (The Cholesterol Treatment Trialists' Collaborators). (2005). Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. Lancet 366:1267–1278.

CTT (The Cholesterol Treatment Trialists' Collaborators). (2008). Efficacy of cholesterol-lowering therapy in 18 686 people with diabetes in 14 randomized trials of statins: a meta-analysis. Lancet 371:117–125.

Dent BR. (2007). Certificate of analysis rosuvastatin-d3 calcium salt. BDG synthesis. Available from: www.bdg.co.nz/coa/coa_130404_6732.4_20120314_V1_Rosuvastatin-d3_Calcium_Salt.pdf. Accessed on October 7, 2013.

Ertugrul DT, Yavuz B, Cil H, et al. (2011). STATIN-D Study: comparison of the influences of rosuvastatin and fluvastatin treatment on the levels of 25 hydroxyvitamin D. Cardiovasc Ther 29:146–152.

FDA (U.S. Food and Drug Administration). (2010). Full prescribing information. Available from: www.accessdata.fda.gov/drugsatfda_docs/label/2009/021366s015lbl.pdf. Accessed on August 12, 2012.

Fıscher J, Szemzö A, Vukıcs K, et al. (2010). Process for the preparation of rosuvastatin and intermediates. Paris, France. Patent WO 2006/126035.

Gajski G, Garaj-Vrhovac V. (2008). Application of cytogenetic endpoints and comet assay on human lymphocytes treated with atorvastatin in vitro. J Environ Sci Heal 43:78–85.

Gotto AM, Grundy SM. (1999). Lowering LDL cholesterol: questions from recent meta-analyses and subset analyses of clinical trial data: issues from the Interdisciplinary Council on Reducing the Risk for Coronary Heart Disease, ninth Council meeting. Circulation 99:E1–7.

Gudi RD, Sandhu SS, Athwal SR. (1990). Kinetochore identification in micronuclei in mouse bone marrow erythrocytes: an assay for the detection of aneuploidy-inducing agents. Mutat Res 234:263–268.

Kamigaki M, Sasaki T, Serikawa M, et al. (2011). Statins induce apoptosis and inhibit proliferation in cholangiocarcinoma cells. Int J Oncol 39:561–568.

LaRosa JC, Grundy SM, Waters DD, et al. (2005). Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med 352:1425–1435.

Link A, Selejan S, Hewera L, et al. (2011). Rosuvastatin induces apoptosis in $CD4^{+}CD28^{null}$ T cells in patients with acute coronary syndromes. Clin Res Cardiol 100:147–158.

MacDonald JS, Halleck MM. (2004). The toxicology of HMG–CoA reductase inhibitors: prediction of human risk. Toxicol Pathol 32: 26–41.

Mamur S, Yüzbaşıoğlu D, Ünal F, Yılmaz S. (2010). Does potassium sorbate induce genotoxic or mutagenic effects in lymphocytes? Toxicol In Vitro 24:790–794.

McKenney JM. (2005). Efficacy and safety of rosuvastatin in treatment of dyslipidemia. Am J Health Syst Pharm 62:1033–1047.

McMurray JJV, Dunselman P, Wedel H, et al. (2010). Coenzyme Q10, rosuvastatin, and clinical outcomes in heart failure. J Am Coll Cardiol 56:1196–1204.

McTaggart F, Buckett L, Davidson R, et al. (2001). Preclinical and clinical pharmacology of rosuvastatin, a new 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. Am J Cardiol 87: 28B–32B.

Møller P. (2005). Genotoxicity of environmental agents assessed by the alkaline comet assay. Basic Clin Pharmacol 6:1–42.

NCEP (Executive summary of The Third Report of The National Cholesterol Education Program). (2001). Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). JAMA 285:2486–2497.

Pedersen TR, Faergeman O, Kastelein JJP, et al. (2005). High-dose atorvastatin vs usual-dose simvastatin for secondary prevention after myocardial infarction: the IDEAL study: a randomized controlled trial. JAMA 294:2437–2445.

Resch U, Tatzber F, Budinsky A, et al. (2006). Reduction of oxidative stress and modulation of autoantibodies against modified low-density lipoprotein after rosuvastatin therapy. Br J Clin Pharmacol 61: 262–274.

Rusciani L, Proietti I, Rusciani A, et al. (2005). Low plasma coenzyme Q10 levels as an independent prognostic factor for melanoma progression. J Am Acad Dermatol 54:234–241.

Schmidt WM, Spiel AO, Jilma B, et al. (2007). In-vivo effects of simvastatin and rosuvastatin on global gene expression in peripheral blood leucocytes in a human inflammation model. Pharmacogenet Genom 18:109–120.

Singh NP, McCoy MT, Tice RR, Schneider ELA. (1988). simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191.

SSSSG (The Scandinavian Simvastatin Survival Study Group). (1994). Randomised trial of cholesterol lowering in 4444 patients with coronary artery disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 344:1383–1389.

Stalker TJ, Lefer AM, Scalia R. (2001). A new HMG-CoA reductase inhibitor, rosuvastatin, exerts anti-inflammatory effects on the microvascular endothelium: the role of mevalonic acid. Br J Pharmacol 133: 406–412.

Surrales J, Xamena N, Creus A, et al. (1995). Induction of micronuclei by five pyrethroid insecticides in whole blood and isolated human lymphocytes cultures. Mutat Res 341:169–184.

Tonkin A, Aylward P, Colquhoun D, et al. (1998). Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. N Engl J Med 339:1349–1357.

Verreth W, De Keyzer D, Davey PC, et al. (2007). Rosuvastatin restores superoxide dismutase expression and inhibits accumulation of oxidized LDL in the aortic arch of obese dyslipidemic mice. Br J Clin Pharmacol 151:347–355.

Waldman A, Kritharides L. (2003). The pleiotropic effects of HMG-CoA reductase inhibitors: their role in osteoporosis and dementia. Drugs 63:139–152.

Yalcin A, Kilinc E, Sagcan A, Kultursay H. (2004). Coenzyme Q10 concentrations in coronary artery disease. Clin Biochem 37:706–709.

Yilmaz S, Aksoy H, Ünal F, et al. (2008). Genotoxic action of fungicide conan 5FL (hexaconazole) on mammalian cells in vivo and in vitro. Russ J Genet 44:273–278.