Anti-proliferative and cytotoxic activity of rosuvastatin against melanoma cells

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Abstract

Introduction: Statins are considered potential candidate agents for melanoma chemoprevention. Statin-induced mevalonate pathway inhibition leads to reduction of cholesterol synthesis and also to decreased cellular levels of non-steroidal isoprenoids, geranylgeranyl pyrophosphate and farnesyl pyrophosphate. This results in the impairment of protein prenylation which affects carcinogenesis.

Aim: To analyze anti-proliferative and cytotoxic activity of rosuvastatin against melanoma cells.

Material and methods: Melanoma cell lines (A375 and WM1552C) and normal fibroblasts (BJ) were used as the primary research material. Cells were treated with rosuvastatin at concentrations ranging from 0.01 µM to 10 µM. Cell viability was analyzed with the use of an MTT assay. Expression of proliferation marker Ki67 was assessed on the basis of immunofluorescence staining.

Results: Rosuvastatin reduced A375 and BJ cell viability in a time- and dose-dependent manner. After 72 h incubation, the IC50, half maximal inhibitory concentration, was 2.3 µM for melanoma cells and 7.4 µM for normal fibroblasts. In turn, rosuvastatin exhibited relatively lower activity against WM1552C cells. A significant reduction of Ki67 expression was also noted for BJ fibroblasts after prolonged incubation with the tested drug.

Conclusions: The results indicate that the anti-melanoma properties of rosuvastatin are highly dependent on the tumor cell line assessed. However, the concentrations required to decrease melanoma cell viability in vitro exceed the plasma concentrations reached in patients treated with rosuvastatin at well-tolerated doses. What is more disturbing, reduction of proliferation and viability observed in BJ fibroblasts indicated that rosuvastatin at high doses may be toxic for normal cells.

Key words: rosuvastatin, melanoma, chemoprevention.
dose-dependent viability reduction has been observed in a number of cancer cells treated with different statins. In human melanoma cell lines, lovastatin has been shown to reduce viability/proliferation and induce caspase-dependent apoptosis through a geranylation-specific mechanism [6]. Similar results have been obtained using simvastatin. Viability reduction, DNA fragmentation, cell cycle arrest and subsequent increase in the mRNA levels of p21 and p27 have been observed after prolonged incubation with the tested drug. However, the level of sensitivity to simvastatin is different in various cell lines used in this study [7]. In turn, atorvastatin has been reported to inhibit rho geranyl-geranylation and thus reduce the metastatic potential of human melanoma cells in vitro [8].

To our knowledge, rosuvastatin activity against melanoma cells has not been assessed to date. Its anti-proliferative and cytotoxic activity has been demonstrated in the case of thyroid cancer cells in vitro. Rosuvastatin treatment caused an increase in caspase-3 activity and apoptosis confirmed by DNA fragmentation analysis [9]. Viability reduction has also been noted in hepatic, breast and cervical cancer cell lines [10]. Rosuvastatin has also been reported to reduce the cellular proliferation, colony formation and invasive potential of prostate cancer cells [11].

Material and methods

Cell culture

Human melanoma cell lines (A375 and WM1552C) and normal fibroblasts (BJ) were obtained from the American Type Culture Collection. Cells were routinely cultured in DMEM/Ham’s F-12 supplemented with 10% fetal bovine serum, 5 µg/ml amphotericin B, 100 U/ml penicillin, and 100 µg/ml streptomycin (Sigma-Aldrich, Germany). Cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂.

Viability measurement

Cells were seeded on 24-well plates (BD Biosciences, USA) at a density of 1 × 10⁴ per well for A375 cell line, 3 × 10⁴ for BJ cell line, and 5 × 10⁴ for WM1552C cell line. Differences in cell seeding density were due to the different growth characteristics of cultured cells that, at the time of use for a test, should be in a logarithmic growth phase. After 48 h of preincubation, cells were treated for 72 h with rosuvastatin at concentrations ranging from 0.01 µM to 10 µM. Cell viability was assessed with a tetrazolium-based colorimetric MTT assay. After 2 h of incubation with MTT solution (500 µg/ml, Sigma-Aldrich, Germany), formazan crystals were dissolved in dimethyl sulfoxide (POCH, Poland) and absorbance was measured at 570 nm using a UV-VIS spectrophotometer (Varian CARY 1E UV-Vis, Agilent Technologies, USA). Cell viability was expressed as a percentage of the untreated control.

Results

Cells viability assessed on the basis of MTT assay

Rosuvastatin reduced the viability of A375 melanoma cells and BJ fibroblasts in a dose-dependent manner (Figure 1). After 72 h treatment with rosuvastatin at a concentration of 5 µM, the metabolic activity of A375 cancer cells was reduced by 79.2% and the calculated half max-
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In recent years, growing interest has been focused on cancer chemoprevention that is defined as the use of natural or synthetic compounds to prevent, inhibit or reverse the multi-step process of carcinogenesis and its secondary prevention (early diagnosis of skin melanoma) [12, 13]. The use of statins as melanoma chemopreventive agents has been based on epidemiological data suggesting their effect on melanoma incidence [14]. Numerous reports from preclinical studies have confirmed the cytotoxic and anti-proliferative effects of statins on melanoma cell lines [6, 7, 15] and animal models [16, 17]. Meta-analysis of clinical trials has not, however, been able to demonstrate any correlation between statin use and melanoma incidence [18–20]. The most recent literature report showed that, instead of preventing melanoma incidence, statins at well-tolerated doses might reduce the growth and metastatic spread of melanoma cells and improve survival [21].

Inhibition of the mevalonate pathway depresses the synthesis of non-steroidal isoprenoids that, through post-translational modification, activate e.g. small G proteins involved in various cellular processes such as proliferation, differentiation and apoptosis. Thus, by influencing protein prenylation statins may alter the biology of cancer cells [22]. Studies on melanoma cell lines by Shellmann et al. demonstrated that the inhibition of protein prenylation mediated by geranylgeranyl pyrophosphate reduces cell viability and induces apoptosis via a geranylpyrophosphate specific mechanism. After 72 h treatment with lovastatin at a concentration of 4 µM, cell viability was reduced by 30% to 80%, depending on the melanoma cell line. Similar to our observations, lovastatin-treated cells were rounded in shape and detached from the growth surface [6].

The varying sensitivity of melanoma cell lines to simvastatin was demonstrated by Saito et al. Simvastatin showed anti-proliferative activity against melanoma cells through induction of apoptosis and cell cycle arrest. Time- and dose-dependent cytotoxicity was also observed in normal human fibroblasts, which is consistent with our results. The addition of geranylgeranyl pyrophosphate to the culture medium completely reversed simvastatin-induced inhibition of cell growth, indicating
Figure 2. Cells morphology after 72 h treatment with rosuvastatin. Images were taken on a Nikon phase-contrast microscope (magnification 10×).
that protein prenylation is implicated in the decrease in cell viability [7]. The inhibitory effects of statins on melanoma cell proliferation and viability have been confirmed by other research groups [14, 23, 24].

Studies on a murine B16F10 melanoma cell line confirmed the cytotoxic activity of statins in vitro [25]. However, in an animal model fluvastatin failed to reduce tumor growth [26]. In turn, atorvastatin and fluvastatin significantly inhibited lung metastasis. The observed inhibitory effect was due to reduced expression of matrix metalloproteinases, integrin α2, integrin α4, integrin α5 and reduced adhesion to extracellular matrix proteins, i.e. type I collagen, type IV collagen, fibronectin, and laminin [15]. These results indicate a prophylactic potential of statins against metastasis that should be further explored.

**Figure 3.** Cells proliferation after 72 h treatment with rosvastatin assessed on the basis of Ki67 expression. A – Proliferation expressed as a percentage of the untreated control. B – BJ cells stained for Ki67. Images were taken on a Nikon fluorescence microscope (magnification 100×). Arrow indicates Ki67-negative cell. Scale bar, 200 µm

**Conclusions**

The results of our study showed significant differences in the sensitivity of melanoma cell lines to rosvastatin. What is more concerning, rosvastatin used at the same concentration range exhibited cytotoxic and anti-proliferative activity against normal human fibroblasts. Investigation of both mechanisms involved in the anti-proliferative and anti-metastatic activity of rosvastatin and whether these effects are reversible with the addition of geranylgeranyl pyrophosphate is necessary to evaluate the anti-melanoma activity of rosvastatin.

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Conflict of interest

The authors declare no conflict of interest.

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