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ROSUVASTATIN IN THE CHEMOPREVENTION OF N-METHYL-N-NITROSOUREA – INDUCED MAMMARY CARCINOGENESIS IN FEMALE RATS

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The results of preclinical research have indicated anticarcinogenic effects of statins in diverse tumors including breast cancer. Lipophilic atorvastatin and simvastatin have demonstrated high anticarcinogenic effects in experimental breast cancer in our previous experiments. In this study, the chemopreventive potential of hydrophilic rosuvastatin in N-methyl-N-nitrosourea induced mammary carcinogenesis in female rats was evaluated. Chemoprevention started 7 days before carcinogen administration and subsequently continued 17 weeks – until the end of the experiment. Dietary administered rosuvastatin (250 mg/kg) decreased tumor frequency by 39% (p=0.146), average tumor volume by 64% (p=0.236), as well as lengthened the latency period by 11 days (p=0.143) compared to controls. Moreover, rosuvastatin (250 mg/kg) decreased average tumor volume by 85% (p=0.0082) compared to the group with rosuvastatin at lower dose in the diet (25 mg/kg). A histopathological analysis of mammary tumors has revealed a shift from poorly differentiated to well differentiated tumors after treatment with rosuvastatin (250 mg/kg). With the exception of HDL-cholesterol, the parameters of plasma lipid metabolism did not differ after rosuvastatin treatment. Rosuvastatin did not change the food intake and body weight in rats. This study is the first about rosuvastatin used in rat mammary carcinogenesis. Hydrophilic rosuvastatin have shown lower antineoplastic activity than lipophilic statins in this model of experimental breast cancer.

Key words: chemoprevention, mammary carcinogenesis, rat, rosuvastatin

INTRODUCTION

Statins – competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA), have become popular drugs because of their efficacy

and few side-effects in the treatment of high cholesterol patients. Within the last ten years, there has been a great influx of new information about possible anticancer effects of statins. Assumed mechanisms by which statins influence the carcinogenesis are induction of apoptosis, the inhibition of proliferation, angiogenesis, and consequently metastasis growth.

Statins, influencing mevalonate synthesis, inhibit dolichol-, farnesyl-, and geranylgeranyl pyrophosphate production, and block tumor cell proliferation (Rao *et al*., 1998; Denoyelle *et al*., 2001). In vitro studies on various cell lines have demonstrated the role of statins as growth inhibitors, either by induction of G1-arrest (Crick *et al*., 1998; Ghosh *et al*., 1999), G2/M arrest (Park *et al*., 2001) or cell death (Macaulay *et al*., 1999; van de Donk *et al*., 2002). It had been shown previously that statins can be devided into three groups with regard to antiproliferative effects: the inhibitory potency of simvastatin, lovastatin, fluvastatin and atorvastatin was in the same order of magnitude, whereas pravastatin was significantly less potent, and cerivastatin was more potent (Negre-Aminou *et al*., 1997). In the experiment of DeNoyelle *et al*. (2001), cerivastatin induced G1-arrest in breast cancer cells, but signs of apoptosis were not observed. The antiproliferative effects of cerivastatin on G1-S arrest in this study were related to an increase in $p21^{WAF1/CIP1}$ and $p27^{KIP1}$, two cyclin-dependent kinase inhibitors. This antiproliferative effect of statins is reversed by the addition of mevalonate. The mechanism of statin-induced apoptosis also appears to be mediated predominantly through depletion of geranylgeranylated proteins. In the study of Agarwal *et al*. (1999) it has been shown that addition of geranylgeranylated pyrophosphate prevented lovastatin induced apoptosis in colon cancer cells, whereas cotreatment with farnesyl pyrophosphate had no effect. This study also showed that lovastatin treatment resulted in decreased expression of the antiapoptotic protein Bcl-2 and increased expression of the proapoptotic protein Bax in cancer cells. In this context, pro-apoptotic shift of ratio in Bax/Bcl-2 mRNA expression in rat mammary gland tumors caused by atorvastatin in our study was confirmed (Kubatka *et al*., sent for publication). Proposed mechanisms for statin-mediated apoptosis include also activation of caspase-3, caspase-8, and caspase-9 (Cafforio *et al*., 2005). Wong *et al*. (2001) have observed that different statins were not equipotent in inducing apoptosis. In acute myeloid leukemic cell lines, cerivastatin was at least 10 times more potent than other statins in the induction of apoptosis. With regard to antiangiogenetic potential of statins, high-dose of cerivastatin decreased tumor vascularisation by 51% in a murine Lewis lung cancer model (Weis *et al*., 2002). In another experiments, statins have been shown to decrease production of vascular endothelial growth factor (Holash *et al.*, 1999) and to inhibit capillary tube formation (Vincent *et al*., 2001). In contrast, statins have also been shown to stimulate protein kinase B, which in turn activates endothelial nitric oxide synthase (eNOS) and increases proangiogenic activity (Kureishi *et al.*, 2000). And finally, statins have also been shown to inhibit cell signaling pathways associated with the invasive and metastatic properties of cancer. These effects on tumor cells are caused by inhibiting cell migration, attachment to the extracellular matrix, and invasion of the basement membrane (Nubel *et al*., 2004; Kusama *et al*., 2002).

Several *in vivo* experimental studies evaluated preventive effects of statins in various neoplasias. Results indicated preventive effects of statins in rodent colon (Narisawa *et al*., 1994) and hepatal (Tatsuta *et al*., 1998) carcinogenesis. Actual results of our group demonstrated substantial antineoplastic effect of dietary administered atorvastatin or simvastatin (Kubatka *et al*., sent for publication) in the chemoprevention of rat mammary carcinogenesis. Finally, several epidemiological (Poynter *et al*., 2005; Shannon *et al*., 2005; Blais *et al*., 2000; Cauley *et al*., 2003; Downs *et al*., 1998) and clinical studies (Kawata *et al*., 2001; Minden *et al*., 2001; Katz *et al*., 2005) have demonstrated the antitumor potential of statins in the prevention and treatment.

The main aim of this study was to evaluate of antineoplastic effects of rosuvastatin in the chemoprevention of N-methyl-N-nitrosourea-induced mammary carcinogenesis in female rats. The effects on plasma lipid metabolism and side effects of rosuvastatin in animals were observed. The results of this study are original as rosuvastatin has not been tested so far in rat mammary carcinogenesis.

MATERIALS AND METHODS

Animals

Female rats of Sprague-Dawley strain obtained from AnLab (Prague, Czech Republic) aged 32-36 days were used in the experiment. The animals were adapted to standard vivarium conditions with room temperature 23±2°C, relative humidity 40-60%, artificial light regimen (12 h : 12 h, lights on from 6 a.m., light intensity 150 lux per cage). During the experiment, animals drank tap water *ad libitum*. The chow containing rosuvastatin (Crestor) synthesized by Astra Zeneca (Astra Zeneca UK Ltd., Macclesfield, Great Britain) was prepared at SSNIFF Spezialdiäten GmbH (Soest, Germany). Rosuvastatin was administered in the chow at two concentrations of 25 mg/kg (0.0025%) and 250 mg/kg (0.025%).

Experiment

Mammary carcinogenesis was induced by N-methyl-N-nitrosourea (Sigma, Deisenhofen, Germany) (NMU) administered once intraperitoneally in a dose of 50 mg/kg body weight on average the 41th postnatal day. Carcinogen was freshly prepared and dissolved in isotonic saline solution. Chemoprevention with rosuvastatin began 7 days before carcinogen administration and lasted until the end of the experiment. Animals were randomly assigned to one of the three experimental groups: (1) control group without chemoprevention; (2) chemoprevention with rosuvastatin at a concentration of 25 mg/kg in the chow (ROSUVA 25); (3) chemoprevention with rosuvastatin at a concentration of 250 mg/kg in the chow (ROSUVA 250). Each group consisted of 20 animals. The animals were weekly weighted and palpated in order to register the presence, number, location and size of each palpable tumor.

In the last - 17th week of the experiment (dated from the NMU injection), the animals were quickly decapitated, mammary tumors were excised and tumor size recorded. At sacrifice, the blood was collected from each animal. Macroscopic

changes in the selected organs (liver, kidney, stomach, intestine, and lung) were evaluated on autopsy. Tissue samples of each mammary tumor were fixed in 10% formol and prepared for histological analysis. Specimens meant for histopathological examination were embedded in paraffin using conventional automated systems. The blocks were cut to obtain 4 to 5 μm-thick sections and were stained with hematoxylin-eosin. The tumors were classified according to the criteria for the classification of rat mammary tumors (Russo and Russo, 2000). The additional parameter – grading of malignat tumors was used. In the serum, the parameters of lipid metabolism – triacylglycerols, total cholesterol, cholesterol of low density-, very low density-, and high density lipoprotein (LDL, VLDL, HDL) fractions were evaluated by automatic biochemical analyser Olympus AU 640 (Olympus Optical, Tokyo, Japan).

The following basic parameters of mammary carcinogenesis in each experimental group were evaluated: tumor incidence, tumor frequency, tumor latency, average tumor volume, and cumulative tumor volume. The effect of rosuvastatin on food, water intake, and animal body weight was observed. Food and water intake during 24 hours in the 7^{th} and 14^{th} week after carcinogen administration were monitored, overall in 4 measurements (twice in a given week).

Statistical analysis

Results are expressed as means ± SEM. Tumor incidence was evaluated by Mann-Whitney test, other parameters by one-way analysis of variance or Kruskal-Wallis test. Tumor volume was calculated according to formula:
$V = \pi \cdot (S_1)^2 \, , \, S_2 / \, 12$; S_1 and S_2 are tumor diameters ($S_1 < S_2$).

RESULTS

With the exception of tumor incidence, rosuvastatin in the group ROSUVA 250 has shown apparent tumor-suppressive effects in the prevention of mammary carcinogenesis in female Sprague-Dawley rats (Table 1). In Table 2, a histopathological classification of mammary tumors has revealed a shift in the rate of poorly differentiated (high grade, HG) and well differentiated (low grade, LG) malignant tumors to higher representation LG malignant tumors after treatment with rosuvastatin (control group: 9/17 (HG/LG); ROSUVA 25: 8/11; ROSUVA 250: 3/14) (Fig. 1). Compared to controls, rosuvastatin in the group ROSUVA 250 decreased tumor frequency by 39% ($P=0.146$) (Fig. 2), average tumor volume by 64% ($P=0.236$), cumulative tumor volume by 79%, as well as lengthened the latency period by 11 days ($P=0.143$). Rosuvastatin in the group ROSUVA 250 markedly decreased average tumor volume by 85% ($P=0.0082$) compared to group ROSUVA 25. Rosuvastatin in both treated groups did not significantly change the levels of triacylglycerols, total cholesterol, and LDL- and VLDL-cholesterol compared to control group (Table 3). Compared to control animals, HDL-cholesterol was decreased by 15% ($P=0.0149$) in the group ROSUVA 250.

Table 1. Effects of rosuvastatin in N-methyl-N-nitrosourea induced mammary carcinogenesis in female Sprague-Dawley rats at the end of experiment

Group	Cont	Rousava 25	Rousava 250
All animals / tumor bearing animals	19 / 14	18 / 12	18 / 13
Tumor incidence (%)	73.68	66.67 (-10%)	72.22 (-2%)
Tumor frequency per group*	1.63±0.36	1.33±0.34 (-18%)	1.00±0.21 (-39 %)
Tumor latency* (days)	95.50±5.94	96.83±6.44 (+1 day)	106.62±4.13 (+11 days)
Average tumor volume* (cm^3)	0.42±0.12	0.98±0.33 (+133%)	0.15±0.02[a] (-64 %)
Cumulative tumor volume** (cm^3)	13.11	22.43 (+71%)	2.73 (-79%)

Cont – control group, ROSUVA 25 – group with administered rosuvastatin at a concentration of 25 mg/kg in the diet, ROSUVA 250 – group with administered rosuvastatin at a concentration of 250 mg/kg in the diet.
*Data are expressed as means±SEM. **Data are expressed as a sum of volumes per group. Values in brackets are calculated as %-ual deviation from the 100% of non-influenced control group (with exception of latency). Significantly different, [a]$p<0.01$ vs ROSUVA 25.

Table 2. Histopathological classification of mammary tumors

Animal, Nr.	Type	Grade	SOLID	ATYP	MAI	NECR
Control group, 31 lesions, 14 tumor bearing animals						
1	P (Fig. 1a)	HG	+	+	+	-
2	P-C	LG	-	-	-	-
2	DCIS					
4	P-T	LG	-	-	-	-
4	DCIS+PPS					
5	P-C	HG	+	+/-	+/-	-
5	C	HG	+	+	+	-
5	DCIS					
5	P	LG	-	-	-	-
5	P	LG	-	-	-	-
5	P-C	LG	-	-	-	-
6	P-C-T (Fig. 1b)	HG	+	+	+	+
6	C	HG	+	+	+	-

cont Table 2.

Animal, Nr.	Type	Grade	SOLID	ATYP	MAI	NECR
6	P-C	LG	-	-	-	-
7	P	LG	-	-	-	-
8	P	LG	-	-	-	-
9	P-C	LG	-/+	-	-	-
9	P-C	LG	-	-	-	-
9	C	HG	+	+	+	-
9	P	LG				
10	P-C	LG	-	-	-	-
10	C	HG	+/-	+	+	+
12	C	HG	+	+	+	-
16	C-CO-DCIS	HG	+	+	+	+
16	C	LG	-	-	-	-
16	C-CO	LG	-	-	-	+
17	C	LG	-	-	-	-
17	P	LG	-	-	-	-
18	P-C	LG	-	-	-	-
19	DCIS					
19	IDP					
ROSUVA 25 group, 24 lesions, 12 tumor bearing animals						
3	C	LG	-	-	-	-
5	P	LG	-	-	-	-
6	C	HG	+	+	+	+
8	SA					
8	P	LG	-	-	-	-
8	P-C-DCIS	LG	-	-	-	-
10	P-C	HG	+	+	+	-
10	FA-C	HG	+	+	+	+
11	DCIS					
13	IDP					
15	P-C	HG	-	+	+	-
15	P-C-DCIS					
17	C (Fig. 1c,d)	HG	+	+	+	-
17	C	LG	-	-	-	-
17	C-P	LG	-	-	-	-
17	C	LG	-	-	-	-

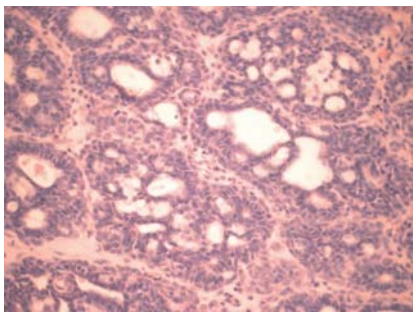
cont Table 2.

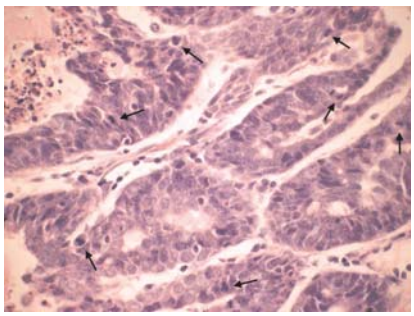
Animal, Nr.	Type	Grade	SOLID	ATYP	MAI	NECR
18	MTS - LN					
18	C	HG	+	+	+	-
19	C-T (Fig. 1e,f)	LG	-	-	-	-
19	P-C	LG	-	-	-	-
19	DCIS-C	LG				
19	C-P (Fig. 1g)	HG	+	+	+	+
19	P	HG	+	+	+	-
20	P-C	LG	-	-	-	-
ROSUVA 250 group – 18 lesions, 13 tumor bearing animals						
1	DCIS	LG				
2	P-C	LG	-	-	+	-
3	C	HG	+	+	+	+
4	C	HG	+	+	+	+
5	P-C	HG	+	+	+	-
5	P-C	LG	-	-	-	-
5	C	LG	-	-	-	-
6	P	LG	-	-	-	-
7	IDP					
8	P-C	LG	-	-	-	-
9	P	LG	-	-	-	-
9	P+DCIS	LG	-	-	-	-
9	C	LG	-	-/+	-/+	-
11	P-C (Fig. 1h,i)	LG	-	-	-	-
13	P-C	LG	-	-	-	-
13	C	LG	-	-	-	-
14	P-C-DCIS	LG	-	-	-	-
18	P-C	LG	-	-	-	-

Type: invasive carcinoma (C – cribriform, P – papillary, CO – comedo, T – tubular), DCIS – ductal carcinoma in situ, IDP – intraductal proliferation, FA – fibroadenoma, SA – sarcoma, LN – lymph node, MTS – metastasis. Dominant type in mixed tumors is the first in order.

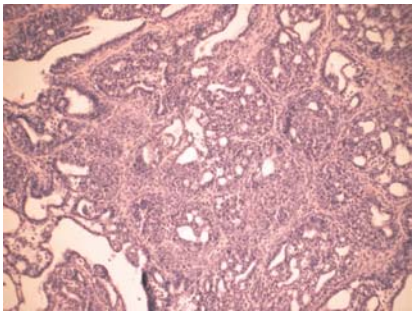
Grade: Grading is evaluated only in invasive carcinoma, LG (low-grade) – well differentiated carcinoma, HG (high-grade) – poorly differentiated carcinoma; SOLID – solidization; ATYP – cell atypia; MAI – mitotic activity index, NECR – necrosis.

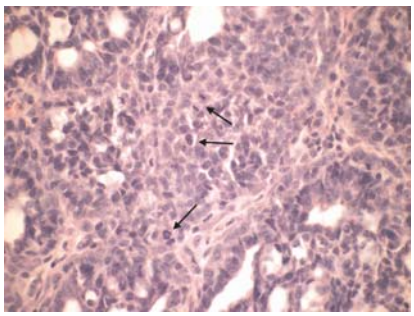
Explanatory note: SOLID – if >30% of tumor sample displays solid growth; MAI – if ≥10 mitosis is observed in 10 high power fields; NECR – occurrence of comedo (not infarct) necrosis. HG – tumor with ≥2 positive criteria, LG – tumor with ≤1 positive criteria.

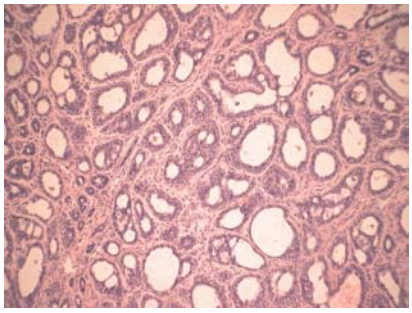
a) 

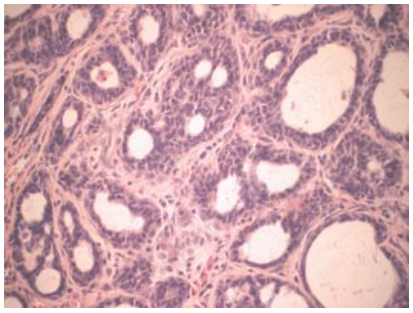
b) 

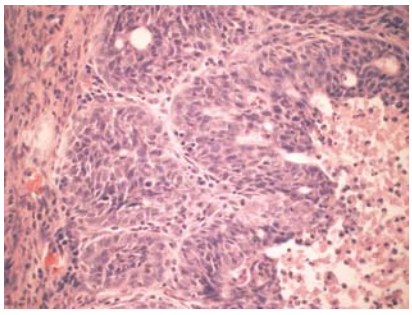
Tumors in the control group: (a) HG carcinoma with indicated solidization and higher cell atypia (magnification 400x) and (b) HG carcinoma with high cell atypia coupled with frequent mitosis (marked by arrows) and necrosis (top left) (600x)

c) 

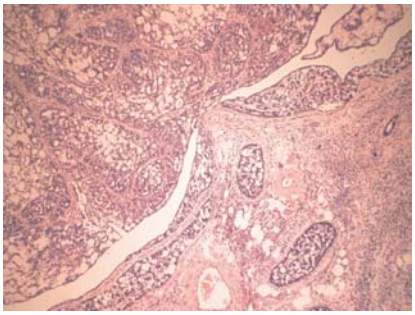
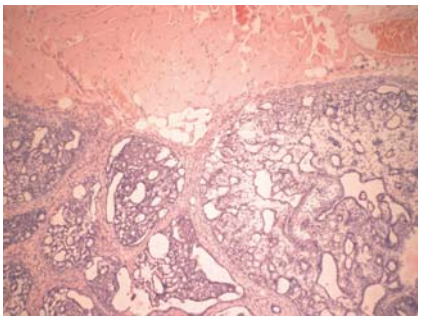
d) 

e) 

f) 

g) 

Tumors in the group ROSUVA 25: (c) HG carcinoma with indicated solidization (200x), (d) detail on cell atypia and elevated mitotic activity (arrows) (400x), (e) LG carcinoma with cribriform and tubular architecture (200x), (f) detail on relatively uniform cell population ordered predominantly in tubular formations (400x), (g) HG carcinoma with solid growth design and comedo necrosis (400x)

h)  i) 

Tumors in the group ROSUVA 250: (h) LG carcinoma with cribriform a papillary growth (100x) and (i) invasion into striated muscle (on the top) (200x)

Figure 1. The histomorphological characteristics of mammary tumors in the control group and the groups ROSUVA 25 and ROSUVA 250

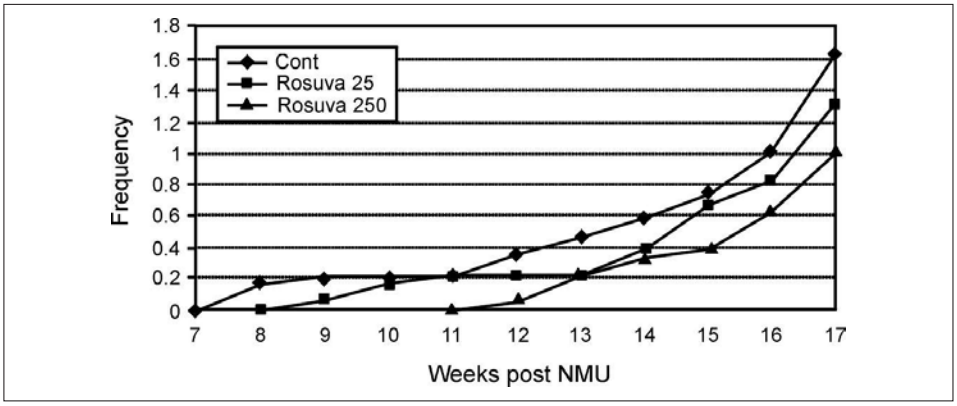


Figure 2. Development of mammary tumors per group after chemoprevention with rosuvastatin. Values are expressed as means

Table 3. Effects of rosuvastatin on plasma lipid metabolism

Group	Cont	Rosuva 25	Rosuva 250
Triacylglycerols (mmol/L)	0.54±0.03	0.58±0.04 (+7%)	0.48±0.02[a] (–11%)
Total cholesterol (mmol/L)	2.43±0.10	2.32±0.13 (–5%)	2.24±0.13 (–8%)
LDL- cholesterol (mmol/L)	0.19±0.01	0.19±0.02 (0%)	0.18±0.01 (–5%)
HDL- cholesterol (mmol/L)	0.71±0.02	0.63±0.03 (–11%)	0.60±0.03[b] (–15%)
VLDL- cholesterol (mmol/L)	0.25±0.02	0.26±0.02 (+4%)	0.22±0.01[a] (–12%)

Data are expressed as means±SEM. Values in brackets are calculated as %-ual deviation from the 100% of non-influenced control group. Significantly different, [a]$p<0.05$ vs Rosuva 25, [b]$p<0.05$ vs Cont.

The drug was well tolerated by the animals, no macroscopic changes due to rosuvastatin administration in the selected organs (liver, kidney, stomach, intestine, and lung) were observed. In both groups with rosuvastatin, the evaluation of final body weight gain and food and water intake in rats did not reveal significant changes in comparison to control animals. Average daily food intake per rat in all experimental groups was between 18.5-19.5 g of chow. The rosuvastatin doses per rat were calculated in accordance with the amount of chow consumed. An average daily dose of rosuvastatin per rat was 0.48 mg in the group ROSUVA 25 and 4.87 mg in group ROSUVA 250. The lower average dose of rosuvastatin in this experiment was equivalent to the double of maximal daily clinical dose of Crestor (40 mg/day) administered to patients with hypercholesterolemia. Based on our previous experience with use of statins in rat mammary carcinogenesis (statins in rats demonstrate different pharmacokinetics and pharmacodynamics than in humans), it was necessary to use high doses of rosuvastatin to prove its antineoplastic effect in this experiment.

DISCUSSION

Statins have become a widely prescribed family of lipid lowering agents because of their high efficacy and relatively few adverse effects after long-term administration. Within the last ten years, the results of preclinical research have proven the pleiotropic effects of statins in cell systems, which are due to their influence on several regulation mechanisms. These processes, such as cell cycle, differentiation, vascularisation, invasiveness, and apoptosis, play a crucial role in carcinogenesis. In our previous experiments, an apparent antineoplastic effect of dietary administered atorvastatin and simvastatin in the chemoprevetion of rat mammary carcinogenesis was observed. Atorvastatin (100 mg/kg) suppressed tumor frequency by 80.5% and tumor incidence by 49.5%, as well as lengthened the latency period by 14 days in comparison with control animals. A proapoptotic shift of the ratio Bax/Bcl-2 mRNA expression caused by atorvastatin in our experiment was confirmed (Kubatka *et al.*, sent for publication). In another study, simvastatin revealed similar antineoplastic effects as atorvastatin in rat mammary carcinogenesis. The agent administered in a higher dose (180 mg/kg) decreased tumor frequency by 80.5% and tumor incidence by 58.5%, as well as lengthened the latency period by 14.5 days compared to control animals (Kubatka *et al*., sent for publication). The most sensitive parameter in the drug's antineoplastic evaluation in rat mammary carcinogenesis is tumor frequency (Mehta, 2000). In this study, rosuvastatin distinctly decreased tumor frequency by 39% in the group ROSUVA 250 and a decrease by 18% was observed in the group ROSUVA 25 compared to controls. A slight antineoplastic effect of ROSUVA 250 was also observed in the lengthening of tumor latency and decreasing of average tumor volume compared to the control group or ROSUVA 25 group, respectively. In this experiment with rosuvastatin and similarly to our previous experiments with atorvastatin and simvastatin, tumors in the untreated control groups have shown higher cellular pleiomorphism and poorer grade of differentiation. On the other hand, tumors in effectively treated groups of these experiments generally

demonstrated milder cellular atypia and higher grade of differentiation. Used doses of rosuvastatin were well tolerated by animals without macroscopic changes in the observed organs (liver, kidney, stomach, intestine, and lung).

The lower efficacy of rosuvastatin (compared to atorvastatin and simvastatin) in our model of breast cancer could be explained by the different hydrophatic profile of the drug. Lipophilic statins, such as atorvastatin, fluvastatin, lovastatin, and simvastatin, readily dissolve in lipids and can therefore cross lipid layers in cell membranes in both liver and nonliver tissues by passive diffusion. On the other hand, hydrophilic statins, such as pravastatin and rosuvastatin, are unable to cross cell membrane lipid layers and therefore require active carrier-mediated processes to enter into the hepatocytes. Hydrophilic statins have also a reduced potential for uptake by extrahepatic cells (Campbell *et al*., 2006). There is epidemiologic evidence (PROSPER study group, 2002; Sacks *et al*., 1996) that hydrophilic pravastatin increases the incidence of some extrahepatic cancers. Duncan *et al*. (2005) hypothesized that pravastatin is able to promote carcinogenesis by a compensatory increase of HMG-CoA reductase activity and consequently mevalonate synthesis in extrahepatic tissues what elevates the proliferation of breast cancer cells. In the experiment of the above mentioned authors, no uptake of pravastatin by most extrahepatic cells was observed and the agent was unable to mitigate the increase in mevalonate synthesis in extrahepatic tissues that accompanies the decrease in circulating cholesterol caused by its inhibition of hepatic HMG-CoA reductase (Duncan *et al*., 2004). In contrary, lipophilic statins with diffusion-mediated extrahepatic cell uptake mitigates the increase in mevalonate synthesis in extrahepatic tissues that accompanies the decrease in serum cholesterol that they induce (Stone *et al*., 1989). Several studies in rodent models (Inano *et al*., 1997; Kikuchi *et al*., 1997; Matar *et al*., 1998), breast cancer cell lines (Mueck *et al*., 2003; Seeger *et al*., 2003) and our results with atorvastatin and simvastatin have clearly demonstrated a protective effect of lipophilic statins on the growth of diverse tumors.

It is known that statins differs in their proapoptotic effects in a variety of cancers. Lipophilic statins have been shown to induce apoptosis in various cell types, including vascular smooth muscle cells (Guijarro *et al*., 1998) cardiac myocytes (Demyantes *et al*., 2006), hepatocytes (Kubota *et al*., 2004) and glioma cells (Koyuturk *et al.*, 2004), whereas hydrophilic statins (rosuvastatin and pravastatin) do not (Katsiki *et al*., 2010). In the recent study of Kato *et al*. (2010), the apoptotic potential of two lipophilic statins – lovastatin and simvastatin and one hydrophilic statin – pravastatin was assessed in ovarian, endometrial and cervical cancer cell lines. Lovastatin and simvastatin, but not pravastatin induced cell death through activation of extrinsic and intrinsic apoptotic cascades (caspase-8 and -9; BID cleavage, cytochrome C release and PARP cleavage) in cancerous cells, which expressed high levels of HMG-CoA reductase. In our experiment, weaker proapoptotic potential of hydrophilic rosuvastatin could be one of the reasons for its lower antineoplastic activity in rat mammary carcinogenesis.

Although the favourable effects of statins in the prevention of cardiovascular diseases resulting from hypercholesterolemia are well established, the increasing

evidence suggests that these drugs exert pleiotropic effects, independent of cholesterol reduction. Our previous study with atorvastatin pointed to the fact that antineoplastic effects of this drug in rat mammary carcinogenesis are independent from its effects on plasma lipid metabolism: atorvastatin in both concentrations in the diet did not change the serum levels of total cholesterol, and LDL- cholesterol, and triacylglycerols (Kubatka *et al.*, sent for publication). Similarly, in this study changes of serum concentrations of total, LDL- and VLDL-cholesterol and triacylglycerols after rosuvastatin treatment did not differ as compared to control animals. In another study, inhibitory effect of pravastatin against colon carcinogenesis in rats was not related to the cholesterol-lowering effect of this agent (Narisawa *et al*., 1996). In the experiment of Lubet *et al*. (2009), atorvastatin and lovastatin did not change serum triglyceride levels in rat mammary carcinogenesis. Contrary to these results, simvastatin (18 and 180 mg/kg) significantly decreased the levels of triacylglycerols and VLDL-cholesterol in comparison with the controls in rat mammary carcinogenesis (Kubatka *et al*., in press). Interestingly, atorvastatin (10 mg/kg), simvastatin (180 mg/kg) and rosuvastatin (250 mg/kg) significantly decreased HDL-cholesterol in our experiments which is in contrast with the results in clinical trials, where the increase of this lipoprotein fraction ranged from 5 to 15% (Scandinavian simvastatin survival study, 1994; Waters *et al.*, 1994; PROSPER study group, 2002).

In the last years, the role of statins as anticarcinogenic agents is seriously discussed in clinical and experimental oncology. In the future, important issues to be addressed in cancer risk reduction studies include: a) the role of specific classes of statins, e.g. lipophilic vs. hydrophilic; b) therapeutic regimens, e.g. dose, frequency, duration; and c) types of cancer, e.g. some data suggest greater effects in colorectal cancer prevention. Our results pointed out to high anticarcinogenic effects of lipophilic atorvastatin and simvastatin and lower antineoplastic effects of hydrophilic rosuvastatin in the chemoprevention of rat mammary carcinogenesis.

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ROSUVASTATIN U HEMO-PREVENCIJI KARCINOGENEZE IZAZVANE N-METIL-N-NITROZOUREOM U MLEČNOJ ŽLEZDI PACOVA

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SADRŽAJ

Rezultati pretkliničkog istraživanja su ukazali na antikarcinogene efekte statina kod različitih tumora, uključujući i tumor dojke. U prethodnim eksperimentima, lipofilni atorvastatin i simvastatin su ispoljili visok antikarcinogeni efekat kod eksperimentalnog raka dojke. U ovom ispitivanju je procenjivan potencijal hidrofilnog rosuvastatina u hemo-prevenciji karcinogeneze u mlečnoj žlezdi pacova izazvanoj N-metil-N-nitrozoureom. Hemo-prevencija je započeta 7 dana pre aplikovanja karcinogena i trajala je 17 nedelja – do kraja eksperimenta. U poređenju sa kontrolnim rezultatima, rosuvastatin aplikovan u ishrani (250 mg/kg) smanjio je učestalost tumora za 39% ($p=0,146$), prosečnu veličinu tumora za 64% ($p=0,236$), a takođe je produžio latentni period za 11 dana ($P=0,143$). Štaviše, primenom rosuvastatina u količini od 250 mg/kg hrane, smanjena je prosečna veličina

tumora za 85% ($p=0,0082$) u poređenju sa grupom u kojoj je primenjena manja doza rosuvastatina (25 mg/kg). Histopatološke analize tumora mlečne žlezde su ukazale na pomak od slabo diferenciranih ka dobro diferenciranim tumorima posle tretmana sa rosuvastatinom (250 mg/kg). Sa izuzetkom HDL-holesterola, parametri u krvnoj plazmi koji odražavaju metabolizam lipida nisu bili promenjeni nakon tretmana rosuvastatinom. Tretman rosuvastatinom nije uticao na unos hrane, niti na telesnu masu pacova. Ovo je prvo ispitivanje ove vrste u vezi sa karcinogenezom u mlečnoj žlezdi pacova. U ovom modelu eksperimentalnog raka dojke hidrofilni rosuvastatin je pokazao slabiju antineoplastičnu aktivnost od lipofilnih statina