Acta Veterinaria (Beograd), Vol. 63, No. 2-3, 137-143, 2013.

DOI: 10.2298/AVB1303137K UDK 577.171.7:611.018.54+577.125:618.19-006

DETRIMENTAL EFFECTS OF FLUVASTATIN ON PLASMA LIPID METABOLISM IN RAT BREAST CARCINOMA MODEL

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(Received 3rd November 2012)

From clinical practice, obvious positive effects of statins on plasma lipid metabolism are well known. On the other hand, there are several experimental rodent studies, where these beneficial effects were not confirmed. The effects of fluvastatin on selected serum lipid parameters in a rat model of experimental breast cancer were determined. The drug was dietary administered at two concentrations of 20 and 200 mg/kg. At the end of the study (experiment duration – 18 weeks) the blood from each animal was collected and serum lipid parameters were evaluated. Fluvastatin in both treated groups significantly increased parameters of serum lipids (mostly in a dose dependent manner). Fluvastatin in both treated groups of animals significantly increased serum levels of triacylglycerols, total cholesterol, and LDL-, HDL-, VLDL-cholesterol when compared to the control group. Our results pointed out to the apparent harmful effects of fluvastatin on plasma lipid metabolism in rat mammary carcinogenesis. Based on our previous results, it seems that rats commonly used in cancer model studies are generally unresponsive to the hypocholesterolemic effects of statins.

Key words: fluvastatin, mammary carcinogenesis, plasma, rat lipids

INTRODUCTION

Statins are the most commonly used drugs for treatment of hypercholesterolemia. They had become a first choice in current prescribing practice and are pivotal in the primary and secondary prevention of cardiovascular disease (Hindler *et al*., 2006; Vrecer *et al*., 2003). The discovery of statins significantly changed the approach to dislipidemic therapy and tremendously decreased morbidity and mortality from cardiovascular events by 50% (Fox *et al*., 2007). Statins are generally well tolerated drugs and this is one of

the reasons why they replaced previous drugs used to reduce cardiovascular events. However there are few side effects of statins that are monitored and reported. Statins can increase activity in hepatic transaminases in up to 5% of patients; this effect is dose-related. Adverse effects associated with treatment of statins are myotoxicity, including myopathy and rhabdomyolysis, what led to acute renal insufficiency, however with low incidence (0.1 %) (Bays, 2006).

Statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase. This enzyme is a rate limiting step in cholesterol biosynthesis in the liver. As an essential step in biosynthesis of mevalonate pathway statins affect levels of cholesterol and other downstream products (isoprenoids) important in many physiological processes such as cell signalling, apoptosis, protein synthesis, cell growth, posttranslational modifications and differentiation. Changes of these processes in neoplastic cells may therefore result in the control of tumor initiation, growth, and metastasis by statins (Chan *et al*., 2003). Based on preclinical (Kubatka *et al*., 2011 a,b; Kubatka *et al*., 2012) and clinical (Poynter *et al*., 2005; Shannon *et al*., 2005; Fagherazzi *et al*., 2010; Kawata *et al*., 2001) evidence, statins demonstrated a risk reduction for several types of neoplasia.

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 and rosuvastatin pointed to fact that the antineoplastic effect of these drugs in rat mammary carcinogenesis is independent from its effects on plasma lipid metabolism (Kubatka *et al*., 2011a,b). Rats are commonly used animals in cancer protective studies (Kubatka *et al*., 2011a,b; Popov *et al*., 1996; Radojicic *et al*., 2002) and also in other experimental approaches (Zorica *et al*., 2008; Djelic *et al*., 1999). On the other hand, some studies described unresponsiveness of rats to different statins within hypocholesterolemic treatment (Krause et Princen, 1998).

The aim of this study was to evaluate the effect of dietary administered fluvastatin on serum levels of triacylglycerols, total cholesterol, and LDL-, HDL-, VLDL-cholesterol in a well-established model of breast carcinoma in rats.

MATERIAL AND METHODS

Animals

Female rats of Sprague-Dawley strain (Charles River Laboratories, Sulzfeld, Germany) aged 32-36 days were used in the experiment. The animals were adapted to standard vivarium conditions with ambient temperature 23±2ºC, relative humidity 40-60 %, artificial regimen (L/D 12:12h). During the experiment the animals were fed the Ssniff diet (Soest, Germany) and drank tap water *ad libitum*. Fluvastatin (Novartis Pharma AG, Basel, Switzerland) was administered in the diet at two concentrations of 20 mg/kg (0.002 %) and 200 mg/kg (0.02 %). The lower concentration of fluvastatin was equivalent to clinical doses (Lescol XL). Because rats demonstrate different pharmacokinetics and pharmacodynamics of statins compared to humans, it was necessary to use also a 10 times higher dose of fluvastatin.

Experiment

Mammary carcinogenesis was induced by N-methyl-N-nitrosourea (Sigma, Deisenhofen, Germany) administered intraperitoneally in one dose of 50 mg/kg body weight on average on the 41th postnatal day. Chemoprevention with fluvastatin began 1 week before carcinogen administration and lasted until the end of the experiment – 17 weeks after carcinogen administration. Animals were randomly assigned to one of three experimental groups: 1. control group without chemoprevention; 2. chemoprevention with fluvastatin at a concentration of 20 mg/kg (FLUVA 20); 3. chemoprevention with fluvastatin at a concentration of 200 mg/kg (FLUVA 200). Each group consisted of 20 animals. The animals were weekly weighted and palpated in order to register the presence and size of palpable tumors. Food and water intake was monitored in the 7th and 14th week of the experiment (four times in the given week).

In the last - 17th week of the experiment, the animals were decapitated, the blood from each animal was collected, mammary tumors were excised and tumor size recorded. Macroscopic changes in the selected organs (liver, spleen, kidney, stomach, intestine, and lung) were evaluated at autopsy. The tumors were classified according to the criteria for the classification of rat mammary tumors. Serum lipid parameters – 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 AU640 (Olympus Optical, Tokyo, Japan).

Statistical analysis

Kruskal-Wallis test and one-way analysis of variance were statistical methods used for data evaluation in this experiment.

RESULTS

This study is a follow-up of our previous paper where the significant tumor-preventive effect of fluvastatin in rat mammary carcinogenesis was recorded (Kubatka *et al*., in press). Fluvastatin in animals treated with both lower and higher doses apparently increased serum levels of triacylglycerols, total cholesterol, and LDL-, HDL-, VLDL-cholesterol when compared to the control group (Table 1). Triacylglycerols were increased by 9% in FLUVA 20 ($P=0.694$) and by 47% in FLUVA 200 ($P=0.006$). Total serum cholesterol was elevated by 40% ($P=0.00005$) in FLUVA 20 or by 57% ($P=0.0001$) in FLUVA 200, respectively. LDL-and VLDL-cholesterol were increased in a dose dependent manner by 25% ($P=0.0001$) and 83% ($P=0.00006$) or by 10% ($P=0.714$) and 52% ($P=0.803$), respectively. Moreover, HDL-cholesterol was elevated by 26% ($P=0.0004$) in FLUVA 20 and by 23% ($P=0.0007$) in FLUVA 200.

Highly significant body mass gain decrease in rats treated with fluvastatin was not accompanied by an apparent decrease in food intake in these animals. Slight decrease in food consumption when compared to control animals was observed only in FLUVA 20 group ($P=0.037$). Food and water intake by animals from all experimental groups is summarised in Table 2.

Table 1. Effects of fluvastatin on plasma lipid metabolism

Group	CONT	FLUVA 20	FLUVA 200
Triacylglycerols (mmol/L)	0.47±0.02	0.51±0.03 (+ 9%)	0.69±0.14[a] (+47%)
Total cholesterol (mmol/L)	1.67±0.06	2.34±0.12[b] (+40%)	2.63±0.19[b] (+57%)
LDL- cholesterol (mmol/L)	0.12±0.01	0.15±0.01[c] (+25%)	0.22±0.03[b] (+83%)
HDL- cholesterol (mmol/L)	0.39±0.01	0.49±0.02[c] (+26%)	0.48±0.02[c] (+23%)
VLDL- cholesterol (mmol/L)	0.21±0.01	0.23±0.02 (+10%)	0.32±0.06 (+52%)

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]$p<0.01$ vs CONT, [b]$p<0.0001$ vs CONT, [c]$p<0.001$ vs CONT

Table 2. Effects of fluvastatin on food and water intake

Group	Food intake (g)	Water intake (mL)
CONT	19.95±0.78	29.35±1.15
FLUVA	17.94±0.50 [a]	27.88±1.08
FLUVA 200	18.35±1.21	28.41±1.38

Data are expressed as means±SEM. Significantly different, [a]$p<0.05$ vs CONT

DISCUSSION

Although the favourable effects of statins in the prevention of cardiovascular diseases resulting from hypercholesterolemia are generally known, the increasing evidence suggests that these drugs exert pleiotropic effects, independent of cholesterol reduction. Fluvastatin in our experiment revealed apparent tumor-suppressive effects in rat mammary carcinogenesis (Kubatka *et al*., in press), however significant detrimental effects on plasma lipids were recorded. Our previous study with dietary administered atorvastatin (10 and 100 mg/kg) also pointed to the fact that strong 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*., 2011a). Similarly in our study with rosuvastatin (25 and 250 mg/kg), changes of serum concentrations of total, LDL- and VLDL-cholesterol and triacylglycerols after rosuvastatin treatment did not significantly differ as compared to control animals (Kubatka *et al*., 2011b). 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*., 2011c). Interestingly, atorvastatin (10 mg/kg), simvastatin (180 mg/kg), and rosuvastatin (250 mg/kg) significantly decreased serum HDL-cholesterol in our experiments what 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; PROSPER study group, 2002).

Statins as cholesterol-lowering drugs are effective in guinea pigs or rabbits. The hypocholesterolemic effects of statins do not occur in rats because these animals have little or no LDL (Huff et Burnett, 1997). Statins in rats affect VLDL assembly and secretion, and this is evidenced by a reduction in triacylglycerols rather than cholesterol because VLDL is rich of triacylglycerols. This mechanism of action is in accordance with our results in experiments where rats were treated by simvastatin or rosuvastatin, respectively. Simvastatin and rosuvastatin in higher doses decreased serum triacylglycerols by 30.5% or by 11% when compared to untreated controls (Kubatka *et al*., 2011a,b). On the other hand, simvastatin and rosuvastatin did not change total cholesterol significantly in comparison with control animals. With the advent of new mouse models expressing specific human genes it is now possible to re-examine the effects of established hypolipidemic drugs and also new compounds with respect to site and mechanism of action. These model mice are now being screened for substances that lower plasma Lp (a) and cholesterol in the absence of LDL receptors (Krause and Princen, 1998).

Several studies in rodents have shown protective effects of statins in experimental carcinogenesis. In our study, fluvastatin with significant antineoplastic effects on mammary carcinomas demonstrated evident adverse effects on plasma cholesterol and triacylglycerols in rats. Based on our results and results of other authors, we can conclude that rats are generally unresponsive to the hypocholesterolemic effects of statins observed in humans. It is proposed that new genetic mouse models may afford a more focused examination of new drugs and provide better prediction of the human response.

ACKNOWLEDGEMENTS:
The experiment was approved by Ethical Commission of Jessenius Faculty of Medicine of Comenius University (Protocol No. EK320/2007) and by State Veterinary and Food Administration of the Slovak Republic (accreditation No. Ro-2061/08-221). This work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic under contract no. VEGA 1/0029/08.

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ŠTETNI EFEKTI FLUVISTATINA NA METABOLIZAM LIPIDA U MODELU KARCINOMA MLEČNE ŽLEZDE PACOVA

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SADRŽAJ

Iz kliničke prakse su poznati pozitivni efekti statina na metabolizam lipida krvne plazme. Istovremeno, postoji nekoliko eksperimentalnih studija na glodarima u kojima ovi efekti nisu potvrđeni. U ovom radu su prikazani rezultati ispitivanja uticaja fluvistatina na vrednosti odabranih lipidnih parametara u krvnoj plazmi pacova na modelu karcinoma mlečne zlezde. Fluvistatin je aplikovan preko hrane u koncentracijama od 20 i 200 mg/kg a ogled je trajao 18 nedelja. Po isteku ovog vremena, od svih životinja su prikupljeni uzorci krvi i određivane su vrednosti serumskih lipida. Fluvistatin je u obe ogledne grupe značajno povećavao koncentraciju serumskih lipida pri čemu je zapažena dozna zavisnost. U oglednim grupama, fluvistatin je signifikantno povećavao koncentraciju triglicerida, ukupnog holesterola, LDL, HDL i VLDL holesterola u odnosu na vrednosti istih parametara u kontrolnoj grupi. Naši rezultati ukazuju da postoje štetni efekti fluvistatina na metabolizam lipida tokom karcinogeneze mlečne žlezde pacova. Na osnovu naših prethodnih rezultata se čini da su pacovi koji se koriste u eksperimentalnim modelima kancera generalno rezistentni na hipoholesterolemijski efekat statina.