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J Vet Res 62, 329-333, 2018
DOI:10.2478/jvetres-2018-0034

Simvastatin-induced changes in the leukocytic system of porcine bone marrow

Anna Snarska[1], Dominika Wysocka[1], Liliana Rytel[1], Sławomir Gonkowski[2], Hanna Pawelec[1], Przemysław Sobiech[1]

[1]Department and Clinic of Internal Diseases, [2]Department of Clinical Physiology, Faculty of Veterinary Medicine, University of Warmia and Mazury, 10-719 Olsztyn, Poland
anna.snarska@uwm.edu.pl

Received: May 14, 2018 *Accepted: August 28, 2018*

Abstract

Introduction: Simvastatin is a substance which is commonly used as a medicine to reduce cholesterol level. Unfortunately, it shows numerous side effects. Simvastatin affects various internal organs, and among other detriments to health may cause persistent muscle weakness, osteolytic processes, headaches, and rashes. Until now knowledge of the influence of simvastatin on bone marrow cells has been rather scant and fragmentary. **Material and Methods:** During this experiment the numbers of all types of cells in the leukocytic system of porcine bone marrow were evaluated after 28 and 56 days of oral administration of simvastatin at a dose of 40 mg/day/animal. **Results:** Simvastatin caused an increase in the number of all types of cells in the leukocytic system, and the most visible fluctuations concerned promyelocytes. **Conclusion:** Observations obtained during the present study indicated that the results of the action of simvastatin on porcine bone marrow differ from those observed in other mammal species, including human. This may be due to various metabolic pathways within the bone marrow in the particular species, but the exact mechanisms of these actions are unknown at the present time.

Keywords: pig, statins, bone marrow, leukocytic system.

Introduction

Simvastatin is an organic compound derivative of lovastatin, a natural metabolite of the *Aspergillus terreus* fungus (15). It is commonly used as a drug in human and veterinary medicine to reduce cholesterol levels (7). The action mechanisms of simvastatin rely on the reversible inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA) – the crucial enzyme in the mevalonate pathway (8). In turn, mevalonate plays an important role in cholesterol synthesis (5). Simvastatin also takes part in anti-inflammatory and anti-oxidative processes (2), thus demonstrating neuroprotective properties (22). Such activity finds this substance commonly used in the treatment of various metabolic disorders such as hypercholesterolaemia, dyslipidaemia, and arteriosclerosis (13), as well as prophylactically to prevent stroke and heart attacks (8).

Apart from its relatively well-known medicinal properties, simvastatin is unfortunately a substance which has also numerous adverse side effects. The most often observed of them are gastrointestinal disorders, headaches, and rashes (3), but some of them are a real threat to the life and health of patients, especially if occurring with liver diseases and during pregnancy and breastfeeding (14). One such effect is that treatment with statins often initiates osteolytic processes, which in turn increase the risk of fractures (9). Others are connected with their upregulation of monocyte and macrophage activities and the concomitant possibility of thrombosis (21), and effects on blood composition, which results in anaemia, thrombocytopaenia, and slight leukocytosis (23). Due to these side effects statins must not be administered in liver and muscular disease cases, nor during pregnancy or breastfeeding. In spite of many studies on the effects of statins on living organisms, some aspects of the activity of simvastatin still remain not fully elucidated. One of them is its influence on bone marrow. Therefore, the aim of the present study was to investigate the effects of simvastatin on the leukocytic system in porcine bone marrow. It should be pointed out that the selection of this species, as the experimental animal was not purely fortuitous because the pig seems to be an optimal animal model for studies on processes

in human due to similarities in anatomy, histology, and physiology between these two mammalian species (17).

Material and Methods

The present study was carried out on 32 clinically healthy sows of White Great Polish breed with a body weight of 35–40 kg. The animals were kept in standard laboratory conditions and fed commercial feed appropriate for the species. Animals were randomly divided into two equal groups: a control (C) group and an experimental (E) group. In the E group simvastatin was orally administered at a dose of 40 mg (as one tablet once a day) for 56 days. At the same time a placebo (in the form of empty gelatine capsules) was administered to the control animals.

Three bone marrow samples were taken from all animals on days 0 (a day before the beginning of simvastatin administration), 28, and 56 of the experiment. Bone marrow was sampled from the lateral condyle of the femur under local anaesthesia with xylazine hydrochloride (Rompun, Bayer, Germany, 1.5 mg/kg b.w., intramuscularly), and zolazepam and tiletamine (Zoletil, Virbac, France, 2.2 mg/kg b.w., intramuscularly), using Jamshidi bone marrow biopsy needles (Synthes, Austria).

Collected bone marrow samples were immediately used to prepare smears. The smears were stained with the May–Grünwald–Giemsa method (May–Grünwald stain for 2 min and Giemsa stain diluted 9 times with phosphate buffer (pH 7.2) for 4 min). Then, the smears were evaluated under light microscope (Nikon, Eclipse 80i, Japan) using an SH-96/24D haematological counter (Alchem, Poland).

The number of particular forms of cells in the leukocytic system of the bone marrow was defined by count per 1,000 bone marrow cells of all types. Cell forms of interest were myeloblasts, promyelocytes, neutrophilic myelocytes, neutrophilic metamyelocytes, band neutrophils, neutrophilic granulocytes, eosinophilic myelocytes, eosinophilic metamyelocytes, band eosinophils, eosinophilic granulocytes, basophilic myelocytes, basophilic metamyelocytes, band basophils, and basophilic granulocytes.

Statistical analysis was performed with a nonparametric Anova test using Statistica 10 software (StatSoft, USA). The differences were considered statistically significant at $P \leq 0.05$.

Results

The differences in the numbers of all types of cells belonging to the leukocytic system between control and experimental groups on day 0 of the experiment were not statistically significant. This situation changed radically on the 28th and 56th days of the study. The influence of simvastatin on the majority of types of cells in the white cell line of the porcine bone marrow was noted. Generally, these changes consisted in an increase in the number of a particular cell type but the degree of these changes clearly depended on the type of cells studied (Table 1).

The most visible changes were observed in the case of promyelocytes, the number of which in the experimental animals was about 15 times higher than in control pigs. Slightly less visible changes were observed in myeloblasts, eosinophilic myelocytes, and eosinophilic metamyelocytes (Fig. 1). In the case of all these types of cells, simvastatin caused about a tenfold increase in their count per 1,000 bone marrow cells of all types.

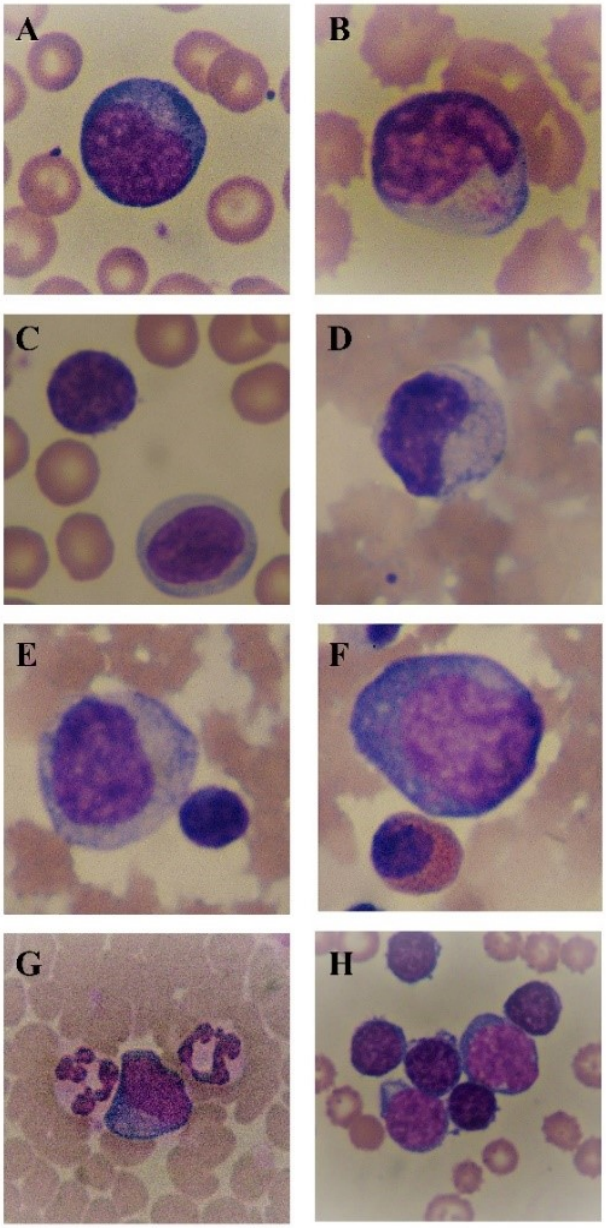

Fig. 1. Myeloid cells in bone marrow smears
A – prolymphocyte, B – eosinophilic myelocyte, C – lymphocytes, D – metamyelocyte, E – promyelocyte and lymphocyte, F – lymphoblast, eosinophilic myelocyte, G – neutrophilic granulocytes and monocyte, H – lymphocytes and prolymphocytes

Table 1. The average number (mean ±SEM) of leukocytic system cells as count per 1,000 bone marrow cells of all types under physiological conditions (control group) and after simvastatin administration (experimental group)

Cell type	Day of experiment	Control group	Experimental group
Myeloblasts	0	0.67 ±0.4^{A}	0.53 ±1.8^{A}
	28	0.59 ±0.31^{A}	5.32 ±1.6^{B}
	56	0.48 ±0.29^{A}	4.37 ±1.59^{B}
Promyelocytes	0	0.66 ±0.23^{A}	0.64 ±2.8^{A}
	28	0.69 ±0.23^{A}	9.62 ±4.2^{B}
	56	0.54 ±0.34^{A}	9.31 ±3.0^{B}
Neutrophilic myelocytes	0	2.18 ±0.69^{A}	2.31 ±5.1^{A}
	28	2.13 ±0.75^{A}	14.82 ±4.61^{B}
	56	2.11 ±0.76^{A}	15.18 ±5.23^{B}
Neutrophilic metamyelocytes	0	7.76 ±3.28^{A}	8.62 ±8.4^{A}
	28	8.41 ±2.36^{A}	32.54 ±6.21^{B}
	56	8.28 ±2.27^{A}	38.68 ±7.38^{B}
Band neutrophils	0	56.95 ±11.39^{A}	66.87 ±20.7^{A}
	28	54.75 ±16.40^{A}	109.81 ±21.77^{B}
	56	68.1 ±15.84^{A}	112.81 ±2.48^{B}
Neutrophilic granulocytes	0	156.31 ±48.91^{A}	143.25 ±40.65^{A}
	28	167.0 ±36.34^{A}	235.38 ±56.1^{B}
	56	139.43 ±31.62^{A}	235.27 ±46.09^{B}
Eosinophilic myelocytes	0	0.45 ±0.35^{A}	0.39 ±1.2^{A}
	28	0.36 ±0.48^{A}	4.21 ±1.9^{B}
	56	0.32 ±0.21^{A}	4.43 ±1.86^{B}
Eosinophilic metamyelocytes	0	0.76 ±0.34^{A}	0.62 ±1.74^{A}
	28	0.85 ±0.86^{A}	6.24 ±2.34^{B}
	56	0.53 ±0.28^{A}	6.56 ±1.90^{B}
Band eosinophils	0	5.46 ±2.75^{A}	5.43 ±4.0^{A}
	28	6.50 ±2.66^{A}	8.42 ±2.40^{B}
	56	5.43 ±1.0^{A}	8.52.4 ±4.03^{B}
Eosinophilic granulocytes	0	16.95 ±6.67^{A}	15.18 ±8.5^{A}
	28	16.25 ±6.59^{A}	27.18 ±9.6^{B}
	56	15.74 ±6.66^{A}	27.18 ±9.57^{B}
Basophilic myelocytes	0	0.51 ±0.09^{A}	0.51 ±1.6^{A}
	28	0.45 ±0.1^{A}	1.65 ±2.3^{B}
	56	0.75 ±0.96^{A}	1.04 ±1.92^{B}
Basophilic metamyelocytes	0	1.4 ±0.23^{A}	1.57 ±1.5^{A}
	28	1.56 ±0.24^{A}	2.68 ±2.8^{B}
	56	1.43 ±1.23^{A}	2.34 ±1.82^{B}
Band basophiles	0	1.96 ±0.34^{A}	1.62 ±1.6^{A}
	28	1.24 ±0.31^{A}	1.68 ±1.5^{B}
	56	1.43 ±1.31^{A}	1.56 ±1.59^{B}
Basophilic granulocytes	0	1.49 ±3.24^{A}	1.54 ±1.4^{A}
	28	2.22 ±5.21^{A}	1.75 ±1.3^{B}
	56	1.26 ± 3.69^{A}	1.75 ±1.29^{B}

$^{A, B}$ – Statistically significant data ($P \leq 0.05$) in particular cell types are marked by different letters in the same row and unsignificant data are marked by the same letters in the same row

The most numerous types of cells within the leukocytic system of the porcine bone marrow both in control and experimental animals were neutrophilic granulocytes, the number of which in control animals amounted to about 150 cells, and in experimental animals increased from about 150 cells (on day 0 of the experiment) to about 230 cells (on days 28 and 56 of the investigation) (Table 1). Interestingly, the duration of simvastatin administration did not affect the number of cells within the leukocytic system. During the present study, statistically significant differences in the number of all types of cells studied between the 28th and 56th days of the experiment were not observed in Student's *t*-paired test.

Discussion

The results obtained during the present study confirm that statins not only reduce the lipid levels, but also may act on bone marrow. These observations are in agreement with previous investigations where the influence of statins on various physiological processes were described (4). This is due to the fact that HMG-CoA, inhibited by statins, catalyses other very diverse processes (13) as well as co-synthesises cholesterol (Fig. 2).

Previous studies described the pleiotropic effects of simvastatin, including, among others, the improvement of endothelial function, increase in nitric oxide activity, diminution of oxidative stress, prevention of chronic pulmonary hypertension, and acceleration of wound healing (16, 28, 29, 31). Moreover, it is known that statins have beneficial effects if used to treat inflammatory, renal and cancerous diseases (10, 24).

The study by Snarska *et al.* (27) has shown that simvastatin also affects bone marrow erythropoiesis at each stage. This results in the development of anaemia with a decrease in RBC count and all RBC parameters.

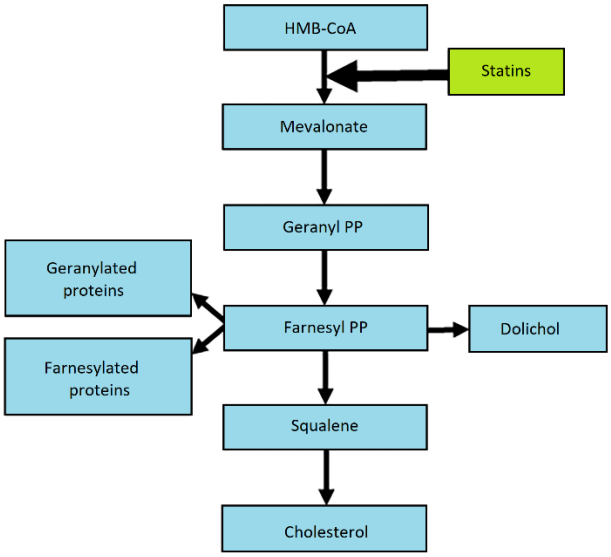

Fig. 2. Mevalonate pathway and the place of action of statins

Actions of statins on mature white blood cells are relatively well known. Previous studies have shown that these substances are potent modulators of immune responses. It is known that they may reduce immune responses during bacterial infection and reduce levels of total serum immunoglobulins (1). Statins affect also the lipid metabolism within human basophils by the upregulation of low-density lipoprotein receptors (LDLR) on these cells (12). Other studies have presented the influence of statins on IL-5-induced chemotaxis of human eosinophils as well as their co-induction of eosinophil apoptosis. This activity of statins suits them to being anti-inflammatory medicines in some diseases, including asthmatic lung inflammation. However, such therapies seem to be controversial (18).

Knowledge of the influence of statins on bone marrow is less advanced. It is known that statins may increase erythropoiesis by mechanisms independent of erythropoietin, namely by influence on hepcidin and iron regulatory pathways (6). These mechanisms may be connected with the effects of simvastatin on cholesterol level, which may have an important regulatory function during erythropoiesis, and intracellular redistribution of cholesterol leads to nuclear membrane rigidity and nucleus expulsion in maturing erythrocytes (11). Other substances also participate in this process, including dolichol, geranyl, and farnesyl pyrophosphates. Their synthesis is suppressed by statins.

Dolichol, taking part in protein-N-glycosylation and intracellular transport of oligosaccharides, is known as a substance which enhances colony formation of haematopoietic progenitors (including colony-forming unit– erythroid (CFU-e), burst-forming unit–erythroid (BFU-e), and colony-forming unit–granulocyte, monocyte (CFU-gm) in bone marrow (25). Geranyl and farnesyl pyrophosphates are involved in post-translational modification of a wide range of proteins including G proteins, the RAS proteins superfamily, and centromere proteins (26). Moreover, it is known that statins may affect the leukocytic system of porcine bone marrow. The majority of studies on various mammal species including human have described that statins show anti-leukaemic properties. It is connected with inhibitory effects on geranyl pyrophosphate, farnesyl pyrophosphate, and cholesterol synthesis and consequently with inhibition of adhesion, migration, and proliferation of leukaemic cells (30). Due to these properties, statins seem to be substances which can be applied in the treatment of various types of leukaemia (19, 20).

Contrary to other mammal species, results obtained during the present study show that simvastatin in the domestic pig causes an increase in the number of all types of cells in the leukocytic bone marrow system. Differences observed between other species and pigs may result from various metabolic pathways within the bone marrow, but the exact mechanisms of these actions are unknown at the time of writing. Nevertheless, in the light of the obtained results it is quite certain that the domestic pig, which seems to be an optimal animal model for a lot of processes taking place in the human organism, cannot be used to investigate simvastatin activity in human.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The present study was self-funded.

Animal Rights Statement: The Local Animal Ethics Committee in Olsztyn approved the present study (Decision no. 61/2010W).

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