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Short communication

Influence of simvastatin on red blood cell line in porcine bone marrow

A. Snarska[1], S. Gonkowski[2], L. Rytel[1], A. Pomianowski[1], I. Babińska[3], I. Otrocka-Domagała[4], K. Żarczyńska[1], D. Wysocka[1], P. Sobiech[1]

[1] Department and Clinic of Internal Diseases
[2] Department of Physiology
[3] Department of Pathophysiology, Forensic Veterinary Medicine and Administration
[4] Department of Pathological Anatomy, Faculty of Veterinary Medicine
University of Warmia and Mazury in Olsztyn, Oczapowskiego 13-14, 10-719 Olsztyn, Poland

Abstract

Simvastatin is a drug commonly used to reduce the cholesterol level. 32 clinically healthy female pigs with a bodyweight of 35-40 kg, kept in standard laboratory conditions were chosen for the experiment and divided into 2 groups (control and experimental) consisting of 16 animals. The experimental group received simvastatin orally at a dose of 40 mg (one tablet once a day) for 56 days, and at the same time the control group received placebo (empty gelatin capsules). Bone marrow smears and peripheral blood samples were evaluated. The obtained results indicate that simvastatin may inhibit erythropoiesis even after a relatively short period of administration, and observed changes can be the cause of some symptoms (for example anemia) occurring during statin therapy.

Key words: simvastatin, proerythroblast, blood, erythroblast, pig

Introduction

Simvastatin, a natural metabolite of the fungus *Aspergillus terreus*, is a relatively well known drug used in human and animals in order to reduce total cholesterol and LDL ("bad") cholesterol levels (Chello et al. 2007). Statins influence the increase of monocyte and macrophage activity, possibility of thrombosis (Nomura 1992) and effect blood composition, which results in anemia. The aim of this study was to investigate the effects of simvastatin on the red blood cell line in porcine bone marrow. This species was selected as an experimental animal since the pig is an optimal animal model for studies on processes in the human organism due to the similarity in anatomy, histology and physiology between these two species (Verma et al. 2011).

Materials and Methods

This study was performed on 32 clinically healthy female pigs with a bodyweight of 35-40 kg, kept in standard laboratory conditions. All experimental procedures were performed in compliance with the instructions of the Local Ethical Committee in Olsztyn (Poland), decision number 61/2010W of 17 June 2010. The animals were randomly divided into two groups:

Correspondence to: S. Gonkowski, e-mail: slawekg@uwm.edu.pl

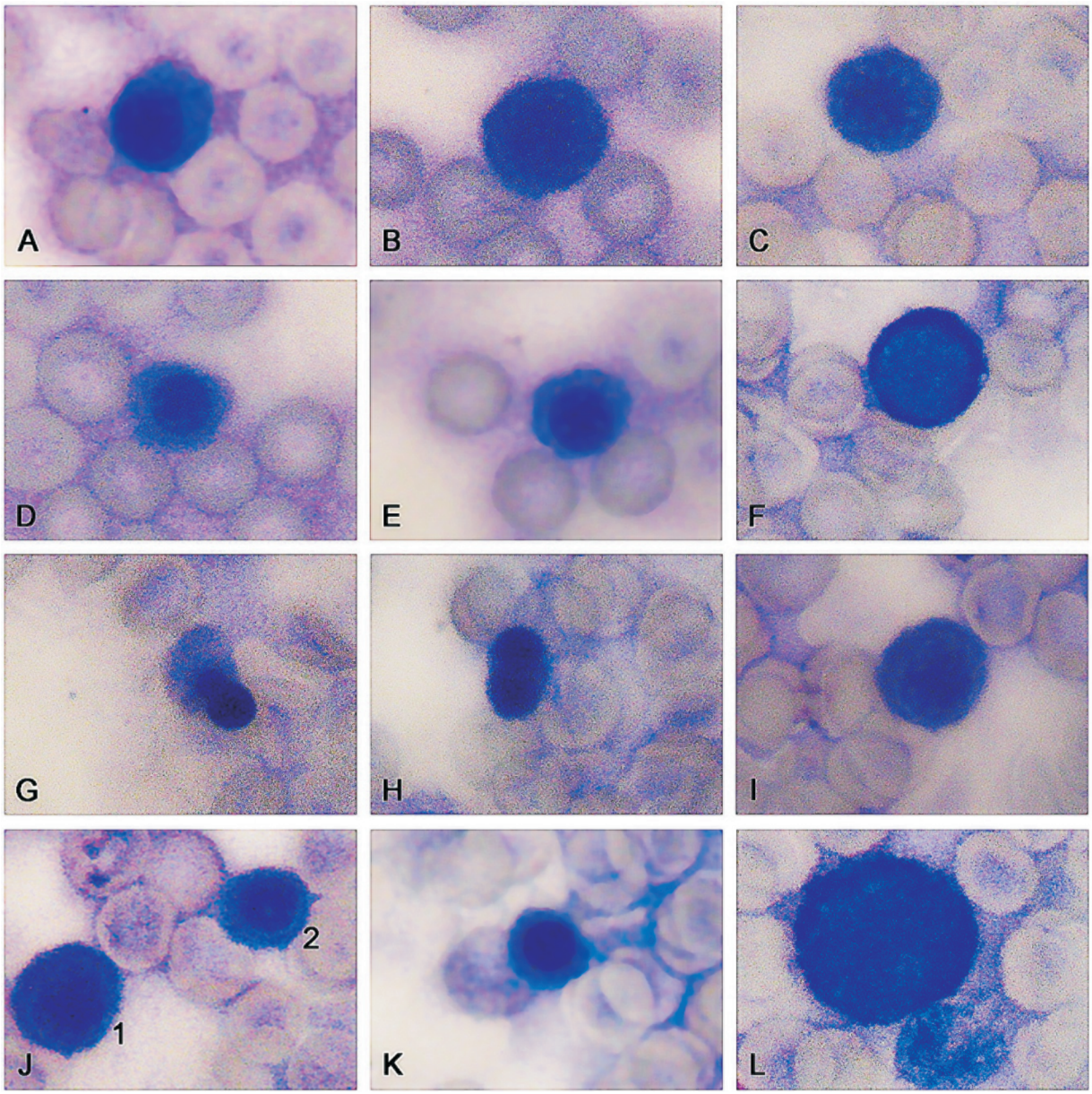

Fig. 1. A-F: control group; A, D – orthochromatic erythroblast, B – basophilic erythroblast, C, E – polychromatic erythroblast, F – proerythroblast; G-L: simvastatin group; G -orthochromatic erythroblast, H – erythroblast in telophase stage, I – polychromatic erythroblast, J – 1-polychromatic erythroblast, 2-orthochromatic erythroblast, K – basophilic erythroblast, L – proerythroblast. MGG stain, x 1000.

control (C group) and experimental (E group). Each group consisted of 16 animals. Simvastatin (Polpharma) at a dose of 40 mg (one tablet once a day) was orally administered to pigs of the experimental group for 56 days. At the same time a placebo (empty gelatin capsules) was administered to the control animals. Three bone marrow samples were taken from all animals: in 0 (a day before beginning simvastatin administration) 28 and 56 days of the experiment. Bone marrow was sampled from the lateral condyle of the femur under local anesthesia with xylazine hydrochloride (Rompun, Bayer, Leverkusen, Germany, 1.5 mg/kg of body weight, I.M.), and zolazepam and tiletamine (Zoletil, Virbac, France, 2.2 mg/kg of body weight, I.M.), using Jamshidi bone marrow biopsy needles (Synthes, Austria). Bone marrow was collected into 2 ml tubes without anticoagulant and used to prepare bone marrow smears. The smears were stained using Pappenheim method and evaluated under a light microscope (Nikon, Eclipse 80i) using a SH-96/24D haematological counter (Alchem, Polska). The numbers of particular forms of erythropoietic cells were defined in relation to 1000 all bone marrow cells. For haematological analysis,

Table 1. The average number (mean ± SEM) of red blood cell line cells in relation to 1000 all porcine bone marrow cells.

Group	Day	Proerythroblasts ($\bar{x}$ ± SEM) number/1000 cells	Basophilic erythroblasts ($\bar{x}$ ± SEM) number/1000 cells	Polychromatic erythroblasts ($\bar{x}$ ± SEM) number/1000 cells	Orthochromatic erythroblast ($\bar{x}$ ± SEM) number/1000 cells
Control (n=16)	0	9.25 ± 1.20	62.06 ± 8.00	118.94 ± 9.41	160.50 ± 8.62
	28	9.69 ± 1.12	62.19 ± 5.74	119.75 ± 7.33	159.25 ± 8.71
	56	10.06 ± 1.01	66.38 ± 3.16	116.56 ± 6.76	160.44 ± 811
Experimental (n=16)	0	8.56 ± 0.43	59.00 ± 4.31	108.50 ± 4.54	157.56 ± 7.04
	28	7.62 ± 0.46*	45.75 ± 4.75*	83.50 ± 5.80*	97.31 ± 4.22*
	56	7.81 ± 0.48*	45.87 ± 4.73*	81.25 ± 4.89*	97.25 ± 4.26*

Statistically significant data ($p \leq 0.05$) in particular animal groups are marked by *

Table 2. Selected parameters of whole blood.

Group	Day	RBC x 10^6/µl ($\bar{x}$ ± SEM)	HGB g/dl ($\bar{x}$ ± SEM)	HCT l/l ($\bar{x}$ ± SEM)	MCV f/l ($\bar{x}$ ± SEM)	MCH pg ($\bar{x}$ ± SEM)	MCHC g/dl ($\bar{x}$ ± SEM)	% RETIC (± SEM)	RETIC x10^9/l ($\bar{x}$ ± SEM)
Control (n=16)	0	6.31 ± 0.31	10.64 ± 0.81	0.365 ± 0.022	58.41 ± 1.91	17.03 ± 1.09	28.58 ± 2.08	1.01 ± 0.30	36.4 ± 0.54
	28	6.24 ± 0.43	10.72 ± 0.88	0.366 ± 0.024	52.98 ± 2.32	16.81 ± 1.11	29.02 ± 1.37	0.98 ± 0.21	36.1 ± 1.54
	56	6.29 ± 0.30	10.67 ± 0.82	0.367± 0.023	58.54 ± 1.98	17.06 ± 1.12	29.02 ± 1.26	0.89 ± 0.20	35.7 ± 1.76
Experimental (n=16)	0	5.60 ± 0.42	8.71 ± 0.86	0.291 ± 0.022	51.73 ± 1.90	15.47 ± 0.96	30.30 ± 1.16	0.87 ± 0.13	35.3 ± 1.41
	28	5.58 ± 0.39	8.81 ± 0.64	0.289 ± 0.020	51.79 ± 1.70	15.66 ± 0.78	30.49 ± 1.21	0.88 ± 0.22	34.3 ± 1.21
	56	5.67 ± 0.32	8.96 ± 0.69	0.293 ± 0.020	51.71 ± 1.72	15.79 ± 0.76	30.61 ± 0.70	0.78 ± 0.13	33.1 ± 1.12

full-blood was taken under standard conditions from the external jugular vein to a tube containing K_2EDTA. Peripheral blood was analyzed using the Siemens ADVIA 2120i diagnostic hematology analyzer for count of red blood cells (RBC), level of hemoglobin (Hb), hematocrit (HCT), mean cell volume (MCV), mean hemoglobin in red blood cell count (MCH), mean hemoglobin concentration in red blood cell (MCHC), reticulocyte percentage (RETIC%) and absolute reticulocyte count (RETIC). Statistical analysis was performed using Anova test using Statistica 10 software (StatSoft Inc., Tulsa, OK, USA). The differences were considered statistically significant at $p \leq 0.05$.

Results and Discussion

During the current study simvastatin induced a decrease in the number of all types of cells in the red blood cell line (Table 1), clearly showing that simvastatin suppresses erythropoiesis. Research concerning the influence of statins on proerythroblasts (Fig. 1F) and erythroblasts is rather scanty (Newman et al. 1994, Namazi et al. 2005). The most numerous populations in the red blood cell line in control were polychromatic (Fig. 1C,E) and orthochromatic erythroblasts (Fig. 1A,D). The most visible changes after simvastatin administration were observed in the case of orthochromatic erythroblasts (Fig. 1G,J). The decrease in the number of this type of cell reached 40% (Table 1). Simvastatin caused less pronounced fluctuations in the populations of basophilic (Fig. 1K) and polychromatic (Fig. 1I,J) erythroblasts, where a decline of about 30% was noted. These observations are in agreement with studies where the influence of statins on various physiological processes in the living organism have been described (Fan et al. 2009, Granger Vallee and Canaud 2011). Results of selected peripheral blood parameters are presented in tabular form (Table 2). Selected peripheral blood parameters clearly indicate a significant decrease in assessed haematological parameters in the experimental group.

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