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INFLUENCE OF SOME STATINS ON BACTERIA AND ACTINOMYCES

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INTRODUCTION

Arteriosclerotic plaque formation is connected with accumulation of cholesterol deposits in the subendothelial part of vascular walls accompanied by inflammatory process of varied degree of intensity. The cause of the onset of inflammatory process and its further persistence in vessel walls is still a matter of debate. The discovery of bacterial antigens from species of *Chlamydia pneumoniae* and *Helicobacter pylori* in the vascular wall and epidemiological observations have lead to the formulation of infectious theory of arteriosclerosis (SAIKKU 2000). SAIKKU (2000) suggested also existence of the relationship between the presence of increased titer of serologic markers for *Chlamydia pneumonie* infection and higher cardiovascular risk.

Epidemiological studies carried out in the United Kingdom showed that acute infections predisposed to more frequent cardiovascular events (SMEETH et al. 2004) . Risk of a stroke and myocardial infarction was particularly increased in the first three days of infection. These studies confirm the existence of a link between infection, inflammatory response and vascular damage. Numerous clinical trials with prophylactic antibiotic therapy aimed at decreasing cardiovascular risk produced equivocal results. SINISALO et al. (2002) and STONE et al. (2002) found significant reduction of cardiac events after completing an antibiotic treatment whereas in more recent trials O'CONNOR et al. (2003) and CERCEK et al. (2003) did not confirm these salutary effects.

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Many randomised studies confirmed beneficial influence of statin therapy on survival patients with coronary artery disease or on those with other cardiovascular arteriosclerotic involvement (KINLAY et al. 2003, ROULEAU 2005, SIPAHI et al. 2006). This benefit cannot be explained simply by statins lowering lipids but needs to be attributed to other, not entirely known, multifactorial mechanisms including their antioxidative, antiinflammatory, antiproliferative and immunomodulative properties (BONETTI et al. 2003, DAVIGNON, GANZ 2004). According to RIDKER et al. (2005) statin therapy results in decreasing C-reactive protein in plasma, well known inflammatory marker, which correlates with better long-term prognosis for patients.

The role of statins in this process is not entirely elucidated and their direct influence on bacterial multiplication, to our knowledge, has never been examined. The importance of recognition of different factors influencing on microorganisms was also considered by BARABASZ (2002).

The purpose of this study was to verify lack of direct antimicrobial properties of few commonly used statins on the *in vitro* growth of bacterial and actinomycetes strains.

MATERIALS AND METHODS

The effect of three statins: atorvastatin (Sortis 20), simvastatin (Zocor 40), fluvastatin (Lescol XL) on the growth of the bacteria: *Bacillus subtilis, Bacillus cereus, Proteus vulgaris, Pseudomonas fluorescens* and *Escherichia coli*, and Actinomyces: *Streptomyces longisporoflavus, Streptomyces intermedius, Streptomyces odoriver i Streptomyces viridis* was tested. The experiments were conducted on five strains of each species in six replications.

The microorganisms originated from a collection maintained at the Chair of Microbiology of the University of Warmia and Mazury in Olsztyn. Prior to using them for the tests, the bacteria and *Actinomyces* were cultured for 144 h on agar slabs. After that time, the cultures were washed with 5 cm^3 of aqueous solution of 0.85% NaCl, and placed in 500 cm^3 flasks containing appropriate agar nutrient media. *Bacillus subtilis* and *Escherichia coli* were cultured on the following substrate: 15 g nutrient broth and 1 dm^3 H_2O, the substrate pH was 7.0 ; *Pseudomonas fluorescens*, *Bacillus cereus*, *Proteus vulgaris*: 15 g nutrient broth, 10 g glucose and 1 dm^3 H_2O, 7.0 pH ; *Streptomyces longisporoflavius*, *Streptomyces intermedius*, *Streptomyces odoriver*, *Streptomyces viridis* : 10 g soluble starch, 0.3 g casein, 2.0 g KNO_3, 2.0 g NaCl, 2.0 g K_2HPO_4, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.02 g $CaCO_3$, 0.01 g $FeSO_4$, 20.0 g agar, 1 dm^3 H_2O, 7.0 pH (Kuster and Williams' substrate, according to PARKINSON et al. 1971). The media used for culturing these microorganisms were identical to those in which pure microbial cultures were maintained. In each medium used for culturing the bacteria on slabs and plates, 15 g agar and 1 dm^3 substrate was used.

Having mixed a suspension of microorganisms with its medium, 17 cm^3 of the mixture was poured onto Petri's plates. Solidified substrata with microorganisms were covered with filter paper rings (6 mm in diameter) soaked with 5 mm^3 of an aqueous solution containing the following concentrations of the statins (in ěg of activity substance per ring): fulvastatin (Lescol XL): 44.4, 53.3 and 61.5; simvastatin (Zocor 40): 40.0, 66.0, 100.0; atorvastatin (Sortis 20): 20.0, 33.0 and 50.0. After that the plates were incubated in a thermostat at 28°C. Diameters of the zones of inhibited microbial growth were measured after 48 h of incubation.

The results underwent statistical analysis using single variant analysis of variance (ANOVA). Duncan's multiple range test was also used for statistical elaboration. All statistical analyses were made with the aid of the software package Statistica (Statsoft, Inc. 2003).

RESULTS AND DISCUSSION

The response of the microorganisms tested to the statins was varied. The strongest influence was exerted by fluvastatin (Table 1). This preparation inhibited the growth of all species of *Actinomyces* analysed. It produced the strongest effect on *Streptomyces longiporoflavus*, and the weakest – on *Streptomyces viridis*. The effect produced by fluvastatin on the bacteria was somewhat different

Table 1
Tabela 1

Effect of fluvastatin on diameter zones of retarded of microorganisms grown on solid media
Wpływ fluwastatyny na średnicę strefy organiczonego rozwoju mikroorganizmów na stałych pożywkach (mm)

Microorganisms Mikroorganizmy	Fluvastatin dose (µg·disc^{-1}) Dawka fluwastatyny (mg·krążek^{-1})			$LSD_{p=0.01}$ $NIR_{p=0,01}$
	44.4	53.3	61.5	
Diameter zones of retarded – Średnica strefy ograniczonego wzrostu (mm)				
Proteus vulgaris	14.67	15.60	15.87	0.51
Bacillus subtilis	9.37	10.53	11.37	0.47
Bacillus cereus	23.83	29.43	29.83	1.03
Escherichia coli	0	0	0	0
Pseudomonas fluorescens	0	0	0	0
Streptomyces longisporoflavus	14.60	14.47	21.10	1.39
Streptomyces viridis	8.63	8.80	9.50	0.60
Streptomyces intermedius	8.23	10.37	11.50	1.12
Streptomyces odoriver	9.33	9.60	9.90	n.s.

– the preparation was neutral to *Escherichia coli* and *Pseudomonas fluorescens*, but it strongly inhibited the growth of *Bacillus cereus*. It also had an inhibitory effect on *Proteus vulgaris* and *Bacillus subtilis*. In most experimental objects, the extent of the inhibitory effect produced by fluvastatin was positively correlated with its dose.

The inhibitory effects obtained by simvastatin (Figure 1) and atorvastatin (Figure 2) were much weaker. While neither of these preparations produced any influence on *Actinomyces*, simvastatin was able to inhibit the growth of *Bacillus cereus* and atorvastatin inhibited the multiplication of *Proteus vulgaris*. The remaining bacteria were unaffected by any of the applied doses of either of the two statins. Although these statins produced very weak effects, it cannot be excluded that they may have adverse effect on growth of other microorganisms, which were not tested in our study, and this could mean that the range of possible applications of these three statins might be broader than it would appear

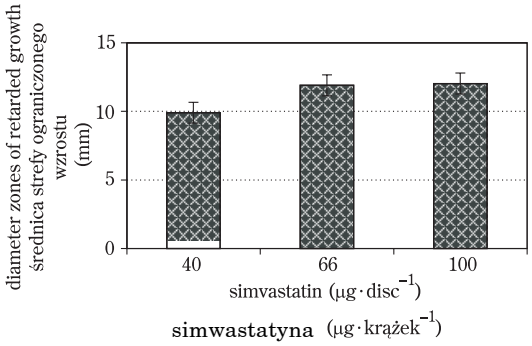

Fig. 1. Effect of simvastatin on diameter zones of retarded of *Bacillus cereus*

Rys. 1. Wpływ simwastatyny na średnicę strefy ograniczonego wzrostu *Bacillus cereus*

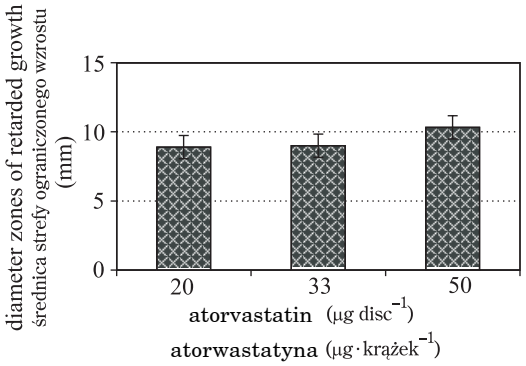

Fig. 2. Effect of atorvastatin on diameter zones of retarded of *Proteus vulgaris*

Rys. 2. Wpływ atorwastatyny na średnicę strefy ograniczonego wzrostu *Proteus vulgaris*

from the recommended use. ALMOG et al. (2004) stated that prior statin therapy may have protective effect against developing severe sepsis and reduce the rate of infection-related mortality. Contrary to this, FERNANDEZ et al. (2006) observed only non-significantly lower infection rate and delayed ICU acquired infection in a statin–treated patient group with higher mortality rate. In a recent large population-based cohort study, HAKAM et al. (2006) pointed out that the use of statin in patients with arteriosclerosis is associated with reduced risk of subsequent sepsis. One of the possible explanation is direct statin inhibition of bacterial growth. PROVE-IT TIMI 22 study failed to confirm the reduction of cardiovascular events in the gatifoxacin arm in comparison with placebo (CANNON et al. 2005). Both arms were treated with either atrovastatin or pravastatin.

In the light of our results an inhibitory effect of statins on multiplication of certain microbial strains could play a role in decreasing the frequency of infections in the arm not receiving gatifloxacine, so potential predisposition to higher risk of cardiovascular events may be considerably reduced.

CONCLUSIONS

1. Some popular statins, such as atorvastatin (Sortis 20), simvastatin (Zocor 40), fluvastatin (Lescol XL), can inhibit the growth of certain microorganisms.

2. Lescol XL rather than Zocor 40 or Sortis produces a stronger effect on bacteria and *Actinomyces*.

3. This experiment showed the importance of further statin testing to determine their influence on morbidiform bacterial strains.

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Key words: atorvastatin, simvastatin, fluvastatin, bacteria, actinomyces.

Abstract

In an *in vitro* experiment the effect of three statins (atorvastatin [Sortis], simvastatin [Zocor], fluvastatin [Lescol]) on the growth of 5 bacterial strains *Bacillus subtilis, Bacillus cereus, Proteus vulgaris, Pseudomonas fluorescens, Escherichia coli* and 4 saprophytic actinomycetes *Streptomyces longisporoflavus, Streptomyces intermedius, Streptomyces odoriver, Streptomyces viridis* was tested. The experiments were carried out on solidified substrata with microorganisms covered with filter paper rings soaked with aqueous solution of every tested statin used in different concentrations.

It was found that examined substances can inhibit the growth of certain saprophytic microorganisms. Fluvastatin rather than simvastatin or atorvastatin produces stronger effect on bacteria and actinomycetes.

WPŁYW NIEKTÓRYCH STATYN NA BAKTERIE I PROMIENIOWCE

Słowa kluczowe: atorwastatyna, simwastatyna, fluwastatyna, bakterie, promieniowce.

Abstrakt

W doświadczeniu in vitro badano wpływ trzech statyn (atorwastatyny [Sortis], simwastatyny [Zocor], fluwastatyny [Lescol]) na rozwój 5 szczepów bakterii: *Bacillus subtilis, Bacillus cereus, Proteus vulgaris, Pseudomonas fluorescens, Escherichia coli* oraz 4 saprofitycznych promieniowców: *Streptomyces longisporoflavus, Streptomyces intermedius, Streptomyces odoriver, Streptomyces viridis*. Doświadczenia prowadzono na zestalonych podłożach, przy czym mikroorganizmy przykrywano krążkami papieru filtrującego, nasączonymi wodnym roztworem poszczególnych statyn w różnych stężeniach.

Stwierdzono, że badane substancje mogą ograniczać rozwój pewnych mikroorganizmów saprofitycznych. Fluwastatyna miała silniejszy wpływ hamujący na bakterie i promienowce niż simwastatyna lub atorwastatyna.