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Protective effect of simvastatin and VULM 1457 in ischaemic-reperfused myocardium of the diabetic-hypercholesterolemic rats

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This study examined the effects of simvastatin (10 mg/kg) and VULM 1457 (50 mg/kg), an ACAT inhibitor, in the heart model of 6 min ischemia followed by 10 min reperfusion injury in the diabetic-hypercholesterolaemic (DM-HCH) rats. In the DM-HCH rats, the incidence of ventricular tachycardia (VT) had a tendency to be increased, while ventricular fibrillation (VF) occurred in all diseased rats ($p < 0.01$). Simvastatin and VULM 1457 with the shown hypolipidemic effect, significantly ($p < 0.01$) suppressed a formation of VF (38% and 29%; respectively).

Cholesterol (CHOL) metabolism disorders are a recognized risk factor of cardiovascular diseases and participate in a growth of population mortality and morbidity. Two enzymes, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) and acyl-CoA: cholesterol acyltransferase (ACAT) have been shown to play a critical role in the altered metabolism of CHOL. HMG-CoA reductase is the rate-limiting enzyme in the biosynthesis of CHOL and the ACAT enzyme (Endo 1992), which exists in two different isoforms (ACAT-1 and ACAT-2), is responsible for esterification of CHOL. In addition, it is involved in the absorption of chylomicrones, secretion of VLDL and formation of cholesterol-enriched monocytes/macrophages (Burnett et al. 1999). Both enzymes, HMG-CoA reductase and ACAT, are thus involved in the processes of hypercholesterolaemia and atherosclerosis. The inhibitors of these enzymes, statins and ACAT inhibitors, have been used and/or continue to be studied for the therapy of the above-mentioned pathological conditions. Furthermore, it has been indicated, that statins may also progressively improve the outcome of cardiovascular diseases, particularly ischaemic heart disease and myocardial infarction, by both dependent and independent mechanisms of their cholesterol-lowering effects (Laws et al. 2004; Sposito and Chapman 2002).

A benefit of statins on the cardiovascular system has been also shown in experimental studies using a model of myocardial ischaemia-reperfusion injury (MIRI), which allows to study episodes occurring during ischaemic heart disease. Statins were able to attenuate a cardiac dysfunction after MIRI in healthy (Ikeda et al. 2003; Birnbaum et al. 2003; Lefer et al. 1999), diabetic (Lefer et al. 2001) and hypercholesterolaemic (Scalia et al. 2001) animals. To our knowledge, no attention has been focused on how these drugs influence the outcome of MIRI in the hearts of diabetic-hypercholesterolaemic animals. Likewise, any influence of the other hypolipidemics, such as ACAT inhibitors, on a course of MIRI, has not yet been studied.

The objective of our study was to find out any effect of statin (simvastatin) and the ACAT inhibitor (VULM 1457) on MIRI-induced arrhythmias in acute (5-day) chemically-induced diabetic-hypercholesterolaemic rats. After an adaptation period and following the induction of pathological conditions, the left coronary-descending arteries of male Wistar control (C), diabetic-hypercholesterolaemic (DM-HCH), simvastatin-treated diabetic-hypercholesterolaemic (DM-HCH + simvastatin) and VULM 1457-treated diabetic-hypercholesterolaemic rats (DM-HCH + VULM 1457) were subjected to 6 min ischaemia followed by 10 min reperfusion (Lepran and Szekeres 1992). The induced heart rhythm disorders were recorded by an electrocardiograph (Seiva, Czech Republic) and the arrhythmias were analysed according to guidelines known as The Lambeth Conventions (Walker et al. 1988).

To induce DM and HCH, a single intraperitoneal dose (80 mg/kg) of streptozocin and a fat-cholesterol diet (1% cholesterol and 1% coconut oil; 20 g/day) were used. This induction of DM and HCH allowed the above-mentioned pathological conditions to develop for a short time (Jiao et al. 1988). We limited our study to 5 days. Both simvastatin (10 mg/kg) and VULM 1457 (50 mg/kg), were administered to the treated DM-HCH rats in the fat-cholesterol diet for the same 5 days. To detect the hypolipidemic efficacy of the drugs, plasma and liver samples were collected after the 10 min reperfusion phase. Plasma GLU, CHOL and liver total cholesterol (TCHOL) levels were enzymatically measured using an assay kit (Spinreact, USA) and the bioanalyzer, ELISA 200 (USA).

The incidence of potentially lethal ventricular arrhythmias, such as ventricular tachycardia (VT) and ventricular fibrillation (VF), was expressed as a percentage, and compared by the 2×2 chi-square test. The plasma and liver biochemical parameters were statistically analysed using first a one-way ANOVA and in cases of significance, a two-tailed unpaired Student's t-test was applied. A p value of ≤ 0.05 was considered significant. The protocol of study was performed in accordance with the Guide for the Care and Use of Laboratory Animals and the work was approved by the Ethics Committee of the Faculty.

Table: Plasma glucose (GLU), cholesterol (CHOL) and liver total cholesterol (TCHOL) levels in control (C), diabetic-hypercholesterolaemic (DM-HCH), simvastatin-treated diabetic-hypercholesterolaemic (DM-HCH + simvastatin) and VULM 1457-treated diabetic-hypercholesterolaemic (DM-HCH + VULM 1457) rats

Group	Plasma		Liver
	GLU (mmol/l)	(CHOL mmol/l)	(TCHOL mg/g)
C	11.20 ± 0.65	1.54 ± 0.10	2.62 ± 0.17
DM-HCH	$18.03 \pm 2.43^{\dagger}$	$2.93 \pm 0.46^{\dagger}$	$7.41 \pm 1.03^{\dagger\dagger}$
DM-HCH + Simvastatin	13.25 ± 1.13	2.15 ± 0.14	$4.82 \pm 0{,}3^{*}$
DM-HCH + VULM 1457	17.58 ± 3.20	$1.74 \pm 0.12^{*}$	$3.88 \pm 0.20^{**}$

The data is expressed as means ± SEM for 7–10 measurements.
† $p < 0.05$; †† $p < 0.01$ significant difference from the control group
* $p < 0.05$; ** $p < 0.01$ significant difference from the non-treated group

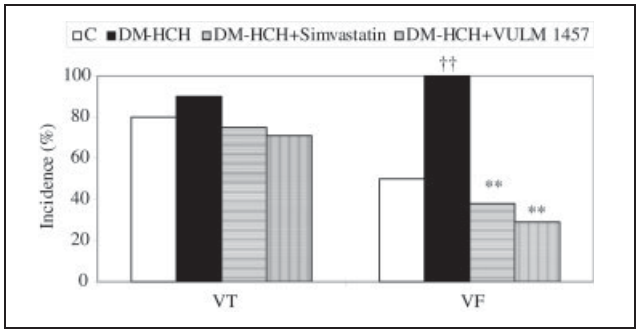

Fig. 1: Incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) in control (C), diabetic-hypercholesterolaemic (DM-HCH), simvastatin-treated diabetic-hypercholesterolaemic (DM-HCH + simvastatin) and VULM 1457-treated diabetic-hypercholesterolaemic (DM-HCH + VULM 1457) rats.
†† $p < 0.01$ significant difference from the control group
** $p < 0.01$ significant difference from the untreated DM-HCH group

In the DM-HCH rats with the significant increased plasma GLU and CHOL ($p < 0.05$) and TCHOL levels ($p < 0.01$), the incidence of VT had a tendency to be increased as compared to controls (90% vs. 80%). The most life-threatening ventricular arrhythmia, VF, occurred in all DM-HCH rats, while only 50% of controls expressed this type of arrhythmia ($p < 0.01$). Based on this data, the animals with acute simultaneously occurring DM and HCH are more susceptible to MIRI. These results represent an original finding. Previous studies examined MIRI-induced vulnerability of animals, which had either DM or HCH. These earlier studies showed a paradoxically protective influence of DM (Feuvray and Lopaschuk 1997) and a supposed deleterious effect of HCH during MIRI (Ferdinandy 2003).

Simvastatin administered at a dose of 10 mg/kg for 5 days to the DM-HCH rats significantly ($p < 0.05$) decreased the liver TCHOL levels and caused a reduction of incidence of both life-threatening MIRI-induced ventricular arrhythmias. VT occurred in 75% and VF in 38% ($p < 0.01$) of such treated DM-HCH rats. A similar cardioprotection of simvastatin on MIRI was detected in the studies of Lefer et al. (2001) and Scalia et al. (2001) who used either diabetic or hypercholesterolaemic apo E-deficient mice. The affect on CHOL levels and/or increase of NO production, anti-inflammatory effects, reduction of neutrophils adhesion and infiltration may be the main features accounting for the ameliorated outcome of MIRI (Lefer et al. 2001; Birnbaum et al. 2003; Lefer et al. 1999; Scalia et al. 2001).

The ACAT inhibitor VULM 1457 (Faberova et al. 2002) caused significant ($p < 0.01$) reduction of TCHOL in the liver of the DM-HCH rats. During MIRI, VULM 1457, significantly ($p < 0.01$) decreased the incidence of VF (29% vs. 100%). Because similar studies with ACAT inhibitors are not available, our results can not be compared to others. The shown benefits of VULM 1457 might be mediated by similar effects like those observed with statins. In the future, it will be necessary to perform molecular studies to define exactly the cardioprotective efficacy of VULM 1457 during MIRI.

In conclusion, statins and ACAT inhibitors are able to attenuate the exacerbated outcome of MIRI in the DM-HCH rats.

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References

Birnbaum Y, Ashitkov T, Uretsky BF, Ballinger S, Motamedi M (2003) Reduction of infarct size by short-term pretreatment with atorvastatin. Cardiovasc Drugs Ther 17: 25–30.

Burnett JR, Wilcox LJ, Huff MW (1999) Acyl coenzyme A: cholesterol acyltransferase inhibition and hepatic apolipoprotein B secretion. Clin Chem Acta 286: 231–242.

Endo A (1992) The discovery and development of HMG-CoA reductase inhibitors. J Lipid Res 33: 1569–1582.

Faberova V, Kakalik I, Juranova D, Schmidtova L, Sadlonova I, Zigova J, Zemanek M, Bezek S (2002) VULM 1457-A novel hypocholesterolemic and atiatherosclerotic agent. Bratisl Lek Listy 103: 312.

Ferdinandy P (2003) Myocardial ischaemia/reperfusion injury and preconditioning: effects of hypercholesterolaemia/hyperlipidaemia. Br J Pharmacol 138: 283–285.

Feuvray D, Lopaschuk GD (1997) Controversies on the sensitivity of the diabetic heart to ischemic injury: the sensitivity of the diabetic heart to ischemic injury is decreased. Cardiovasc Res 34: 113–120.

Ikeda Y, Young LH, Lefer AM (2003) Rosuvastatin, a new HMG-CoA reductase inhibitor, protects ischemic reperfused myocardium in normocholesterolemic rats. J Cardiovasc Pharmacol 41: 649–656.

Jiao S, Matsuzawa Y, Matsubara K, Kihara S, Nakamura T, Tokunaga K, Kubo M, Tarui S (1988) Increased activity of intestinal acyl-CoA: cholesterol acyltransferase in rats with streptozocin-induced diabetes and restoration by insulin supplementation. Diabetes 37: 342–346.

Laws PE, Spark JI, Cowled PA, Fitridge RA (2004) The role of statins in vascular disease. Eur J Vasc Endovasc Surg 27: 6–16.

Lefer AM, Campbell B, Shin YK, Scalia R, Hayward R, Lefer DJ (1999) Simvastatin preserves the ischemic-reperfused myocardium in normocholesterolemic rat hearts. Circulation 100: 178–184.

Lefer DJ, Scalia R, Jones ST, Sharo BR, Hoffmeyer MR, Farvid AR, Gibson MF, Lefer AM (2001) HMG-CoA reductase inhibition protects the diabetic myocardium from ischemia-reperfusion injury. FASEB 15: 1454–1456.

Lepran I, Szekeres L (1992) Effect of dietary sunflower seed oil on the severity of reperfusion-induced arrhythmias in anesthetized rats. J Cardiovasc Pharmacol 19: 40–44.

Scalia R, Gooszen ME, Jones SP, Hoffmeyer M, Rimmer DM 3rd, Trocha SD, Huang PL, Smith MB, Lefer AM, Lefer DJ (2001) Simvastatin exerts both anti-inflammatory and cardioprotective effects in apolipoprotein E-deficient mice. Circulation 103: 2598–2603.

Sposito AC, Chapman MJ (2002) Statin therapy in acute coronary syndromes: mechanistic insight into clinical benefit. Arterioscler Thromb Vasc Biol 22: 1524–1534.

Walker MJ, Curtis MJ, Hearse DJ, Campbell RW, Janse MJ, Yellon D M, Cobbe SM, Coker SJ, Harness JB, Harron DW, Higgins AJ, Julian DG, Lab MJ, Manning AS, Northover BJ, Parratt JR, Riemersma RA, Riva E, Russell DC, Sheridan DJ, Winslow E, Woodward B (1988) The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia infarction, and reperfusion. Cardiovasc Res 22: 447–455.