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The hypolipidemic effect of a new ACAT inhibitor, VULM 1457, in diabetic-hypercholesterolaemic rats

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The use of inhibitors of enzyme acyl-CoA: cholesterol acyltransferase (ACAT) seems to be a novel potential approach for a therapeutic treatment of dyslipidaemias and atherosclerosis. VULM 1457 is an ACAT inhibitor, which has expressed potent hypolipidemic and antiatherosclerotic effects in previous studies. In this study, we used streptozocin-induced diabetic rats, which were fed a fat-cholesterol diet to evaluate the affect of VULM 1457 on the atherogenic lipids levels in both plasma and liver. VULM 1457, with a slight influence on triglyceride levels, significantly reduced plasma and hepatic cholesterol concentrations ($p < 0.05$, $p < 0.001$; respectively) in the diabetic-hypercholesterolaemic rats.

At present, new hypolipidemic agents with various mechanisms of activity are being developed. MTP inhibitors, ACAT inhibitors, SREBP-SCAB ligands represent potential hypolipidemic drugs (Shah 2003). The substances with ACAT inhibition activity seem to represent a novel approach for treatment of both hypercholesterolaemia and atherosclerosis (Heinonen 2002). In experimental and clinical studies, ACAT inhibitors have exhibited favourable effects on lipid levels. The beneficial effect of ACAT inhibitors is principally (1) due to inhibition of cholesterol absorption via suppression of intestinal ACAT and (2) due to the changes in hepatic cholesterol metabolism via inhibition of ACAT in the liver. These effects, resulting in the reduction of both plasma and liver cholesterol levels, are a consequence of inhibition of isoform ACAT-2 (Chang et al. 1997; Joyce et al. 1999). VULM 1457 represents a new original ACAT inhibitor synthesized by the Drug Research Institute INC., Slovakia. To evaluate its effect on lipid metabolism many *in vitro* and *in vivo* studies have been designed. Animal models based on rats, rabbits, hamsters and human HepG 2 cells and macrophages have been used to determine its main mechanisms of hypolipidemic and antiatherosclerotic activities. It has been recognized that the hypolipidemic effect of VULM 1457 is associated with the inhibition of intestinal cholesterol absorption via ACAT-2. The antiatherosclerotic effect is due to the decrease of atherogenic lipids and/or the inhibition of cholesteryl ester macrophage accumulation within the arterial wall (Juranova et al. 2002; Faberova et al. 2002; Schmidtova et al. 1998). The results obtained with this agent are comparable with the other investigated ACAT inhibitors.

Our study was undertaken with the aim to evaluate the hypolipidemic activity of VULM 1457 in 5 and 10-day diabetic-hypercholesterolaemic rats. In this study we used the modified model of DM-HCH rat developed by Jiao et al. (1988). A single intraperitoneal dose (80 mg/kg) of streptozocin and a fat-cholesterol diet (20 g/day) which contained cholesterol and coconut oil, both at 1%, were used to simultaneously induce diabetes and hypercholesterolaemia. The experimental protocol of the study was performed in accordance with the Guide for the Care and Use of Laboratory Animals and with the approval of the Ethics Committee of the Faculty.

The study consisted of two separate 5 and 10-day experiments, in which Wistar rats (Anlab, Czech Republic) were

Table 1: Plasma glucose (GLU), cholesterol (CHOL), triglyceride (TAG) levels and hepatic total cholesterol (TCHOL) and triglyceride (TAG) content in 5-day control (C), non-treated diabetic-hypercholesterolaemic (DM-HCH) and treated diabetic-hypercholesterolaemic (DM-HCH + VULM 1457) rats

Group	PLASMA (mmol/l)			LIVER (mg/g)	
	GLU	CHOL	TAG	TCHOL	TAG
C (n = 6)	11.52 ± 0.92	1.79 ± 0.08	0.95 ± 0.12	2.99 ± 0.15	16.39 ± 0.91
DM-HCH (n = 7)	16.14 ± 1.48†	3.63 ± 0.70†	0.53 ± 0.05†	10.39 ± 1.01†††	14.74 ± 0.57
DM-HCH + VULM 1457 (n = 8)	17.58 ± 3.20	1.74 ± 0.12*	0.47 ± 0.05	3.88 ± 0.20***	16.39 ± 2.24

Table 2: Plasma glucose (GLU), cholesterol (CHOL), triglyceride (TAG) levels and hepatic total cholesterol (TCHOL) and triglyceride (TAG) content in 10-day control (C), non-treated diabetic-hypercholesterolaemic (DM-HCH) and treated diabetic-hypercholesterolaemic (DM-HCH + VULM 1457) rats

Group	PLASMA (mmol/l)			LIVER (mg/g)	
	GLU	CHOL	TAG	TCHOL	TAG
C (n = 8)	10.39 ± 1.17	2.20 ± 0.12	0.95 ± 0.12	2.86 ± 0.13	16.32 ± 0.74
DM-HCH (n = 8)	13.59 ± 1.13	2.29 ± 0.12	0.54 ± 0.06†	10.79 ± 0.93†††	19.60 ± 1.62
DM-HCH + VULM 1457 (n = 7)	13.29 ± 2.11	2.01 ± 0.17	0.63 ± 0.10	3.71 ± 0.20***	14.76 ± 1.03*

The data are expressed as means ± SEM.
† $p < 0.05$, ††† $p < 0.001$ significant difference from the control group
* $p < 0.05$, *** $p < 0.001$ significant difference from the non-treated group (unpaired Student's t-test)

used. After an adaptation period, the rats were randomly divided into the following experimental groups: control rats (C), STZ-induced diabetic rats fed on the fat-cholesterol diet for 5 days and 10 days (DM-HCH), STZ-induced diabetic rats fed on the fat-cholesterol diet which included an orally administered dosage of VULM 1457 (50 mg/kg) for 5 and 10 days (DM-HCH + VULM 1457). Then plasma and liver samples were collected and glucose (GLU), cholesterol (CHOL) and triglyceride (TAG) levels were enzymatically measured using a commercial assay kit (Spinreact, USA) and the bioanalyzer ELISA 200 (USA). Data was statistically analyzed using first a one-way ANOVA and in cases of significance, a two-tailed unpaired Student's t-test was applied.

Administration of a single-dose of streptozocin and a fat-cholesterol diet for 5 days to Wistar rats caused the significant ($p < 0.05$) increase of plasma GLU and CHOL levels, as well as the significant increase ($p < 0.001$) of the hepatic total CHOL content (Table 1). Decrease of the plasma TAG levels was detected in 5-day DM-HCH rats as compared with the values of the control rats. VULM 1457 application for 5 days to the DM-HCH rats significantly changed both plasma and hepatic CHOL concentrations ($p < 0.05$, $p < 0.001$; respectively), but TAG levels were not significantly changed in the plasma and in the liver.

The 10-day feeding of the fat-cholesterol diet to the streptozocin-induced diabetic rats had a tendency to increase the plasma GLU and CHOL levels. The hepatic content of total CHOL in 10-day DM-HCH rats was approximately four times higher than that in the controls ($p < 0.001$). The hepatic TAG levels were not significantly affected by the fat-cholesterol diet given to the diabetic rats. As shown in Table 2, VULM 1457 did not significantly influence the plasma GLU and lipids levels in 10-day DM-HCH rats. However, significant reductions of the hepatic total CHOL and TAG levels were demonstrated ($p < 0.001$, $p < 0.05$; respectively).

VULM 1457, like other ACAT inhibitors (CL-277082, FR 0145237) in the studies done by Maechler et al. (1993) and Sakuma et al. (1997) using DM-HCH rats, showed the beneficial effects on the plasma CHOL levels. In VULM 1457 treated DM-HCH rats, a decrease of cholesterol content in the liver was also demonstrated. These findings of hypolipidemic effect of VULM 1457 in the DM-HCH rats confirm the previous results of Faberova et al. (2002), who demonstrated the decline of atherogenic lipids levels in both treated hypercholesterolemic rats and hamsters. In this study VULM 1457 did not show a clear effect on TAG levels.

The apparent differences in efficacy of VULM 1457 on TAG levels may be differently interpreted. There is a hypothesis that agents with ACAT inhibitory activity may also influence microsomal triglyceride transfer protein (MTP), which is involved in the formation of apoB VLDL particles (Burnett et al. 1999). As the primary core of VLDL is TAG rather than the cholesteryl ester, the inhibition of hepatic ACAT with ACAT inhibitors in consequence of an interference with MTP may lead to decrease of hepatic TAG secretion and plasma TAG concentrations. These findings were observed in the livers of 10-day treated DM-HCH rats, however a similar effect of VULM 1457 in 5-day treated DM-HCH rats was not shown.

We conclude that the original ACAT inhibitor, VULM 1457, with a slight effect on triglyceride levels has normalized the increased cholesterol levels achieved in the diabetic-hypercholesterolaemic rats.

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