

Scientific paper

Modulation of Hepatic Transcriptome in the Poloxamer P-407 Hyperlipidemia Mouse Model

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Abstract

The poloxamer P-407 hyperlipidemia mouse is a genetically unaltered, nondiet-induced model of atherosclerosis which is useful in studying effects of lipid lowering drugs. While the model is relatively well described on the biochemical level, the knowledge regarding the global gene expression was lacking so far. As expected, the P-407 elevates plasma triglycerides and cholesterol. Using microarray analysis and RT-PCR we show for the first time that poloxamer triggers a concerted modulation of hepatic genes involved in multiple steps of atherogenesis, such as lipid metabolism, initiation of atherogenesis, lesion formation, and atheroma progression. 106 hepatic genes were upregulated and only 40 downregulated, suggesting that upregulation of atherogenic genes precedes the atheroma formation. Modulated expression of circadian genes has also been observed in this type of hyperlipidemia but further studies are needed to elucidate whether this affects the rhythm of animals.

Keywords: Poloxamer, hyperlipidemia, atherogenesis, circadian

1. Introduction

Cardiovascular diseases are the leading cause of death in the developed world.^{1,2} Abundant data link hypercholesterolemia to atherogenesis. It has been appreciated recently that dyslipidemia and atheroma formation are coupled by inflammatory mechanisms.³ One of the important unsolved questions is how good are different animal hyperlipidemia models for reflecting the situation in humans. As alternative to knock-down and high fat diet, application of chemical substances can also lead to hyperlipidemia.² Poloxamer P-407 is a block copolymer comprised of repeated poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) units,⁴ that induces a dose-controlled dyslipidemia in mice.⁵ A single injection causes a dose-dependent hyperlipidemia in rats and mice, increasing both plasma triglyceride and cholesterol.⁶ The hypercholesterolemia seems to be associated with an up-regulation of both the protein expression and activity of the key regulatory enzyme of cholesterol synthesis, the HMG-CoA reductase, and a decrease in expression of the LDL-receptor, responsible for cholesterol uptake.⁵

The aim of our work was to evaluate by biochemical parameters and expression profiling the poloxamer P-407-induced chronic hyperlipidemia in the mouse liver. Liver has been chosen as a major metabolic organ and the large reservoir of macrophages in the body. In addition to disbalance of lipid metabolism, the poloxamer P-407 model reveals modulation of transcriptome markers of different stages of atherogenesis. It shows also for the first time that poloxamer-based hyperlipidemia modulates the expression of some circadian transcription factors. This knowledge can be translated to humans and applied in studying the effects of novel hypolipidemic drugs.

2. Material and Methods

2.2. Animal Treatment and Tissue Collection

The work on animals was performed in compliance with all relevant EU legislation, in particular with the Council Directive 86/609/EEC and the European Convention no. 123, the Slovenian Protection of Animals Act (98/99) and Instructions on the conditions for the authori-

zation of experiments on animals for scientific and research purposes (40/85)/Amsterdam protocol on animal welfare and approved by Veterinary Administration of the Republic of Slovenia.

Seven-week-old female mice (C57BL/6, Harlan, Italy) were housed under a 12:12h light:dark schedule with lights on at 7AM. They were fed the standard, low-fat chow diet (4.4% fat, diet 3430, Provimi Kliba AG, Kaiseraugst, Switzerland). They were randomly assigned into two groups. 24 mice were administered 0.5 g/kg poloxamer P-407 by intraperitoneal (i.p.) injection every third day (poloxamer P-407 group) and a control group (16 mice) was injected normal saline. Animals were sacrificed in the early light phase, between 7–9 AM. Blood was collected at regular intervals by retro-orbital sampling technique or from the heart. Livers were collected, weighted, frozen in liquid nitrogen and stored at -80°C or in RNeasy Lysis Buffer (Qiagen) for subsequent analyses.

2. 2. Determination of Plasma Cholesterol and Triglycerides and the Liver Cholesterol

Blood was transferred into EDTA-containing tubes (BD Biosciences-Pharmingen) and plasma collected by centrifugation. Total cholesterol and triglyceride analysis was determined on Cobas Mira analyzer by Roche Diagnostics kits. Results for each subgroup are presented as a median of subgroup measurements.

Liver cholesterol was measured at days 96 and 111. For each time/treatment point samples of 4 animals have been pooled into 2 biological replicas, frozen, lyophilized and weighed. Sterols were extracted and analyzed by reverse phase HPLC as described previously.⁷ Quantity of sterols was normalized on tissue weight and is represented in ppm (mass of sterols in micrograms per dry mass of tissue sample in grams). The level of liver cholesterol is calculated as the average of two biological replicas.

2. 3. Microarray Experiment and Bioinformatic Analysis

Liver samples were homogenized in TRI reagent (Sigma, Taufkirchen, Germany), the total RNA isolated and subjected to RNeasy Cleanup (Qiagen, Valencia, CA). RNA concentration and quality was determined by Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). RNA from 12 poloxamer P-407 treated mice was pooled into 3 biological replicas on day 96. Reference total RNA has been obtained by pooling 4 mice from control group day 96. For cDNA arrays 10 g of total RNA was used for labelling. Synthesis of Cyanine 3- and Cyanine 5-labeled cDNA was performed by Fluorescent Direct label Kit Protocol (Agilent Technologies, CA, USA). For oligonucleotide arrays, 0.5 g of total RNA was labelled using the Low RNA input fluorescence linear amplification kit (Agilent

Technologies, CA, USA). After hybridization, the slides were scanned by TECAN LS200 scanner (Tecan Group Ltd., Maennedorf, Switzerland). Image data were analyzed by ArrayPro4.5 software (Media Cybernetics). Features where the Cy3 and Cy5 signals were less than 2.6 times higher than the local background, were filtered out. Remaining data were loess normalized (span 20%). Gene expression ratio (M) was calculated as the median of independently repeated microarray experiments. Statistical significance of differential gene expression was evaluated according to the Local Pooled Error test.⁸ Threshold for significance was set to $p < 0.01$ and to a minimum of 2.0 fold change. Genes were classified into functional categories based on Gene Ontology (GO) with modifications in Orange software⁹ and upgraded by expert-based biological classification. The data comply with the MIAME standard and are submitted to the National Centre for Biotechnology Information Gene Expression Omnibus (accession number GSE6305).

2. 4. Quantitative Real-time PCR (Q-RT-PCR)

Equal amounts of RNA from 4 mice of each group were pooled, treated with DNase I (Sigma) and converted to first strand cDNA. Q-RT-PCR was performed by standard procedures using either by TaqMan technology (Assay-on-demand for *Hmgr*, *Hmgs*, *Sc4mol*, *8,7-iso*, *Dhcr7*-Applied Biosystem, PE) or SybrGreen technology (*Vcam*, *Lpl*, *Srebp1a*, *Srebp1c*, *Srebp2*, *Saa3*, *Pon1*, *Fpps*, *Fabp5*, *Tim1*, *Clock*, *Cry1*, *Bmal1*, *Cyp51*, *Cyp7a1*, *Insig1*, *Cyp8b1*, *Insig2a*, *DBP*) on an Applied Biosystems Prism 7900 HT sequence detection system. Reactions were performed in triplicate. The relative amount of all mRNAs were calculated by the comparative Ct (cycle number at threshold) method (User Bulletin No. 2, Applied Biosystems, PE) using 18S RNA as the internal control. Relative mRNA levels were determined by expressing the mRNA amount of the poloxamer P-407 treated-animals relative to normal fed controls. Standard deviation and significance level of fold change comparison between the poloxamer P-407 treated and control group was evaluated as described.¹⁰ The treatment of RNA isolated from Hepal cells was the same as it is written for RNA isolated from mice liver.

3. Results

3. 1. Lipid Profiles of Poloxamer P-407 chronic Hyperlipidemia Mouse

The poloxamer P-407 chronic hyperlipidemia was assessed as described.¹¹ Figure 1 shows the time course of plasma total cholesterol (A) and triglycerides (B). Poloxamer increased plasma cholesterol above normal level

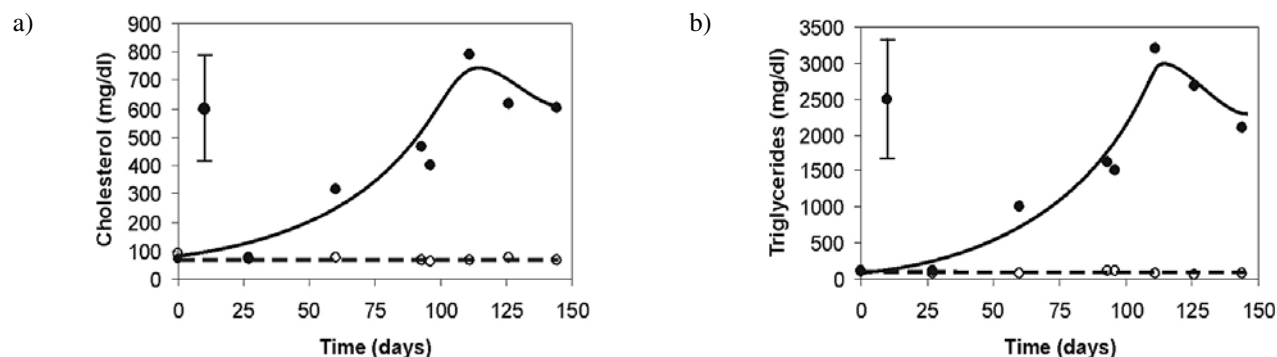


Figure 1: Time course of plasma cholesterol (A) and triglycerides (B) in the poloxamer P-407 treated (solid line) and control mice (dashed line).

Time represents duration of the poloxamer P-407 treatment. Medians of lipid levels of P-407 treated mice are shown by black circles, medians of lipid levels of control mice are designated with white circles. Number of animals per treated and control groups were $n = 8$ (days 0, 27, 60, 93), $n = 4$ (days 111, 124, 144) and $n = 12$ for treated group (day 96) and $n = 4$ for control group (day 96). Error bars represent an average of 1 standard deviation of measured lipid values for poloxamer P-407 treated mice which is more than 20 times larger as standard deviation of lipids measurements for saline treated mice (not shown).

nearly 8-times at day 111. Increase in cholesterol is accompanied by the increase in triglycerides, which is in accordance with previous reports.^{2,5,11} Surprisingly, the liver

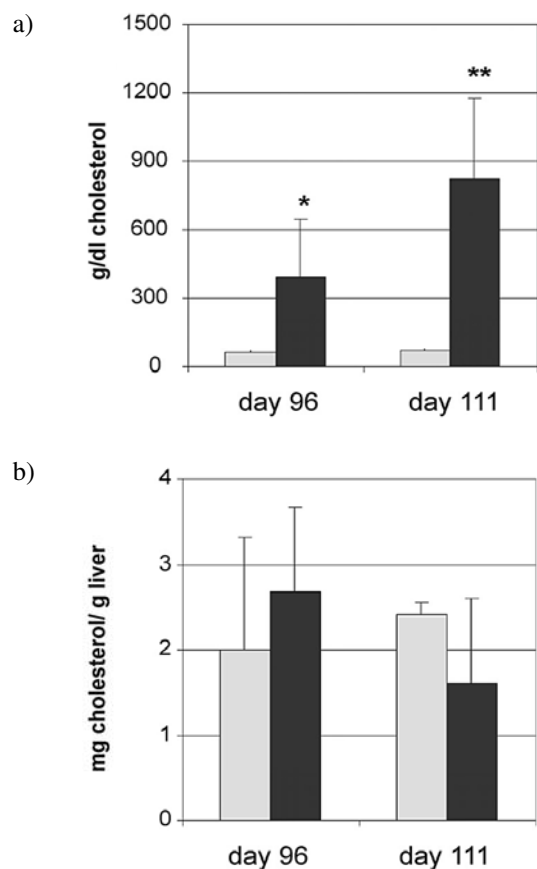


Figure 2: Plasma (A) and liver (B) cholesterol levels in poloxamer P-407 induced hyperlipidemia model.

Cholesterol levels are presented as mean \pm s.d. for poloxamer P-407 treated group (black bars) and for control group (grey bars); * $p < 0.05$, ** $p < 0.01$, $n = 4$ per group.

cholesterol at days 96 and 111 remains in the normal range (Fig. 2). Comparison to the published high fat diet models shows an inverse relation between plasma and liver cholesterol,^{12,13} suggesting that high fat diets result primarily in the increase of liver cholesterol while poloxamer P-407 application leads primarily to the increase of plasma cholesterol.

3. 2. Poloxamer P-407 Based Hyperlipidemia Activates Atherogenic Pathways of The Hepatic Transcriptome

The hepatic transcriptome has been evaluated at day 96 as a core data set for biological interpretation. A list of genes with statistical significance ($p < 0.01$ and minimal 2.0 fold change) was created and divided into physiologically relevant groups by (Table 1) the gene ontology widget of Orange.⁹ A representative set of genes has been confirmed by real time PCR (Fig. 3).

The poloxamer P-407 based chronic hyperlipidemia results in 106 upregulated and 40 downregulated genes. The vast majority of modulated genes is connected to various stages of atherogenesis (Fig. 4, Tab. 1), such as lipid metabolism, initiation of atherogenesis, lesion formation, and atheroma progression. Interestingly, major circadian oscillator genes are also modulated.

Disturbance of the lipid metabolism is reflected in modulated cholesterol and fatty acid homeostasis. This includes repression of genes involved in cholesterol synthesis, modulation of genes from bile acid synthesis, lipoprotein metabolism and fatty acid transport (Table 1, Fig. 3). Among 146 differentially expressed genes related to atherosclerosis (Table 1), 74 are linked to inflammation processes, initiation of atherosclerosis, lesion formation and atheroma progression. Nearly all genes, related to different stages of atherogenesis are overexpressed in the poloxamer model in comparison to control livers.

Table 1: Differential expression of genes in the 96-day poloxamer P-407 hyperlipidemia model.

Genes that were found differentially expressed also in the 84-day high fat diet model published by Recionos et al. [12] are printed in bold; genes that were previously linked to atherogenic processes are labelled by +.

Gene Symbol	Gene Bank ID	Gene name	Fold Change	P value	Athero-genesis
LIPID METABOLISM					
Lpl	NM_008509	lipoprotein lipase	2.01	>1.E-15	
Fabp5	NM_010634	fatty acid binding protein 5, adipocytes, macrophages	2.01		
Fabp7	NM_021272	fatty acid binding protein 7, brain	1.35	1.0E-09	
Apobec1	NM_031159	apolipoprotein B editing complex 1	1.15	5.0E-08	
Fabp4	NM_024406	fatty acid binding protein 4, adipocytes, macrophages	1.13	7.0E-07	
Stard4	NM_133774	StAR-related lipid transfer (START) domain containing 4	-1	1.0E-06	
Cyp51	NM_020010	cytochrome P450, 51	-1.05	4.0E-07	
Hmgcs1	AK045094	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	-1.11	3.0E-05	
Cyp39a1	NM_018887	cytochrome P450, family 39, subfamily a, polypep. 1	-1.12	3.0E-07	
Acas2	NM_019811	acetyl-Coenzyme A synthetase 2 (ADP forming)	-1.16	3.0E-07	
Sc4mol	NM_025436	sterol-C4-methyl oxidase-like	-1.22	3.0E-06	
Idi1	AK029302	isopentenyl-diphosphate delta isomerase	-1.29	2.0E-07	
Hmgcr	M62766	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	-1.37	3.0E-10	
Fdps	NM_134469	farnesyl diphosphate synthetase	-1.39	9.0E-08	
Sqle	NM_009270	squalene epoxidase	-1.39	6.0E-10	
INITIATION OF ATHEROSCLEROSIS					
Monocyte adhesion					
Vcam1	NM_011693	vascular cell adhesion mol 1 1	2.07	>1.E-15	+
Itgax	NM_021334	integrin alpha X	1.26	3.0E-10	
Iqgap1	NM_016721	IQ motif containing GTPase activating protein 1	1.26	5.0E-09	
Sdcbp	NM_016807	syndecan binding protein	1	1.0E-05	
Chemoattractions and monocyte migration					
Anxa2	NM_007585	annexin A2	1.42	5.0E-10	
Anxa3	NM_013470	annexin A3	1.34	1.0E-09	
Ccl6	NM_009139	chemokine (C-C motif) ligand 6	1.84	2.0E-16	
Capg	NM_007599	capping protein (actin filament), gelsolin-like	1.76	2.0E-16	
Ccl4	NM_013652	chemokine (C-C motif) ligand 4	1.35	3.0E-11	
Cotl1	NM_028071	coactosin-like 1 (Dictyostelium)	1.31	2.0E-08	
Pkib	NM_008863	protein kinase inhibitor beta, cAMP dependent	1.26	1.7E-10	
Tmsb4x	NM_021278	thymosin, beta 4, X chromosome	1.21	6.0E-07	
Evl	NM_007965	Ena-vasodilator stimulated phosphoprotein	1.2	5.4E-08	
Ccl2	NM_011333	chemokine (C-C motif) ligand 2	1.14	8.0E-08	+
Ptpns1	AI835480	protein tyrosine phosphatase, non-recept. type substrate 1	1.04	3.5E-07	
LESION FORMATION					
Macrophage activation					
Ms4a7	AF237917	membrane-spanning 4-domains, subfamily A, member 7	2.05	>1.E-15	
Lyzs	NM_017372	lysozyme	1.98	>1.E-15	
Cd68	NM_009853	CD68 antigen	1.63	7.0E-14	
Cd63	NM_007653	Cd63 antigen	1.61	7.0E-13	
Mpeg1	L20315	macrophage expressed gene 1	1.57	1.1E-11	
Csf2rb2	NM_007781	colony stimulating factor 2 receptor, beta 2, low-affinity (granulocyte-macrophage)	1.55	2.0E-13	+
Icb-1	BC013712	cDNA sequence BC013712	1.39	2.6E-10	
Cd51	NM_009690	antigen-like	1.38	3.0E-07	+
Csf2rb1	BF134814	colony stimulating factor 2 receptor, beta 1, low-affinity (granulocyte-macrophage)	1.26	2.5E-09	+
Glipr1	BC025083	GLI pathogenesis-related 1 (glioma)	1.21	4.8E-10	
Cd9	NM_007657	CD9 antigen	1.14	5.0E-07	
Gdf15	NM_011819	growth differentiation factor 15	1.1	2.0E-07	+
Csf2ra	NM_009970	colony stimulating factor 2 receptor, alpha, low-affinity	1.08	1.0E-06	+
Cd53	NM_007651	CD53 antigen	1.06	5.0E-07	

Gene Symbol	Gene Bank ID	Gene name	Fold Change	P value	Atherogenesis
Emr1	X93328	EGF-like module containing, mucin-like, hormone receptor-like sequence 1	1.03	5.8E-06	
Laptm5	NM_010686	lysosomal-associated protein transmembrane 5	1.03	2.0E-06	
Spic	NM_011461	Spi-C transcription factor (Spi-1/PU.1 related)	1	1.5E-06	
Fut2	NM_018876	fucosyltransferase 2	-1.07	3.0E-06	
Cd163	NM_053094	CD163 antigen, SRCR family class B	-1.72	2.0E-16	+
Inflammatory response					
Clecsf12	NM_020008	Ca ²⁺ -dependent lectin-like receptor gene, DECTIN-1	2.66	>1.E-15	
Bcl2a1a	NM_007534	B-cell leukemia/lymphoma 2 related protein A1a	2.17	0.0E+00	
Egr1	NM_007913	early growth response 1	2.13	0.0E+00	+
Blnk	Y17159	B-cell linker	2	>1.E-15	
Tnfaip2	NM_009396	tumor necrosis factor, alpha-induced protein 2	1.72	2.0E-14	
Tyrobp	NM_011662	TYRO protein tyrosine kinase binding protein	1.7	4.0E-14	
Ear11	BC027557	eosinophil-associated, ribonuclease A family, member 11	1.66	7.0E-14	
Ear1	NM_007895	eosinophil-associated, ribonuclease A family, member 1	1.6	9.0E-14	
Pld3	NM_011116	phospholipase D3	1.52	1.5E-11	
Vim	NM_011701	vimentin	1.38	5.0E-10	
Fcerg	NM_010185	Fc receptor, IgE, high affinity I, gamma polypeptide	1.37	2.0E-10	
Nfil3	NM_017373	nuclear factor, interleukin 3, regulated	1.35	5.0E-10	
Clec2	NM_019985	C-type lectin-like receptor 2	1.24	5.0E-08	
Igsf7	AY048685	immunoglobulin superfamily, member 7	1.18	1.1E-07	
Cd48	X53526	CD48 antigen	1.14	5.0E-08	
C1qg	NM_007574	complement component 1, q subcomponent, gamma polypeptide	1.12	1.0E-06	
Tbxas1	NM_011539	thromboxane A synthase 1, platelet	1.11	2.0E-07	+
Cyba	AK021200	cytochrome b-245, alpha polypeptide	1.08	8.0E-07	
Phlda1	NM_009344	pleckstrin homology-like domain, family A, member 1	1.04	4.0E-06	+
Tslpr	NM_016715	thymic stromal-derived lymphopoietin, receptor	1.01	8.0E-06	
Lgmn	NM_011175	legumain	1.01	9.0E-06	+
Ear6	NM_053111	eosinophil-associated, ribonuclease A family, member 6	1.01	6.0E-07	
Pim3	BC017621	proviral integration site 3	-1.18	7.0E-07	
Atheroprotection					
Gp49b	NM_013532	glycoprotein 49 B	1.59	8.0E-13	
Serpinb6c	AF425084	serine (or cysteine) proteinase inhibitor, clade B, member 6c	1.23	5.2E-08	
Il18bp	NM_010531	interleukin 18 binding protein	1.1	6.0E-07	+
Il10rb	NM_008349	interleukin 10 receptor, beta	1.02	6.0E-06	+
ATHEROMA PROGRESSION AND COMPLICATION					
Ctss	NM_021281	cathepsin S	1.96	1.0E-14	+
Ctsb	AK083393	cathepsin B	1.31	3.0E-06	+
Clpx	NM_011802	caseinolytic protease X (E.coli)	1.31	3.0E-08	
Crip1	BC030406	cysteine-rich protein 1 (intestinal)	1.3	9.6E-09	
Rgs2	NM_009061	regulator of G-protein signaling 2	1.2	6.0E-08	+
Oas1f	AK037025	2'-5' oligoadenylate synthetase 1F	1.09	6.5E-07	+
Ctsd	NM_009983	cathepsin D	1.01	4.0E-05	+
Plk3	U21392	polo-like kinase 3 (Drosophila)	-1.48	2.0E-11	
Calcification					
Gpmb	NM_053110	glycoprotein (transmembrane) mb	4.62	0.0E+00	
Trem2	NM_031253	triggering receptor expressed on myeloid cells 2	4.42	>1.E-15	
S100a9	NM_009114	S100 calcium binding protein A9 (calgranulin B)	2.04	>1.E-15	+
Adam8	NM_007403	a disintegrin and metalloprotease domain 8	1.66	1.0E-15	
S100a8	NM_013650	S100 calcium binding protein A8 (calgranulin A)	1.48	2.0E-13	+
CIRCADIAN REGULATION					
Bmal1	NM_007489	aryl hydrocarbon receptor nuclear translocator-like	1.21	4.0E-10	
Per2	NM_011066	period homolog 2 (Drosophila)	-1.47	2.0E-11	
Ccrn4	U70139	CCR4 carbon catabolite repression 4-like (S. cerevisiae)	-2.37	>1.E-15	
Dbp	NM_016974	D site albumin promoter binding protein	-2.81	>1.E-15	

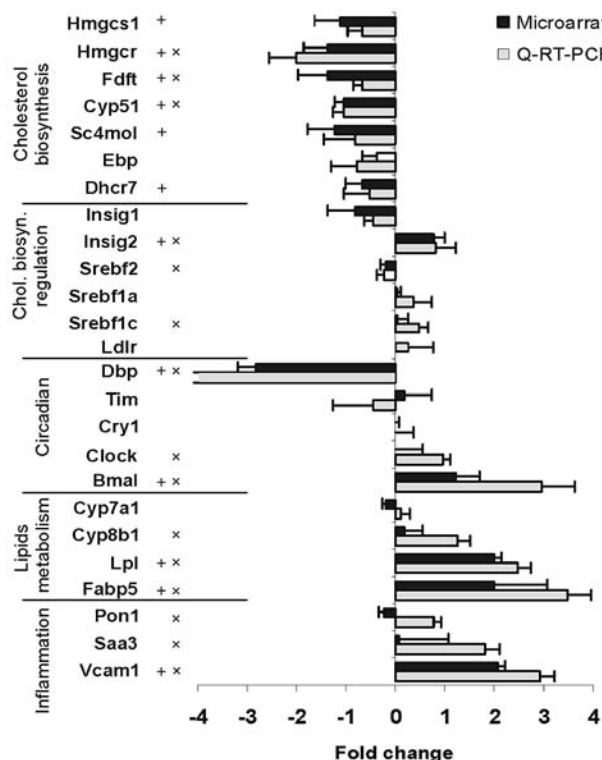


Figure 3: Comparison of real time PCR and microarray gene expression assessments

Relative gene expression of poloxamer P-407 treated animals (day 96) vs control group is presented as mean \pm s.d. of biological replicates ($n = 3$ per group). In microarray results both oligo and cDNA microarray measurements are considered. Significantly modulated genes ($p < 0.01$) are designated by the symbols \times (measurement obtained by Q-RT-PCR) and $+$ (measurement obtained by DNA microarray). Gene encoding DBP enzyme was downregulated over the presented graph range (DBP fold change: -8.07 ± 3.72).

3. 3. Poloxamer P-407 Based Hyperlipidemia Modulates Circadian Genes

Major circadian oscillator genes and clock-controlled downstream genes are among the most modulated genes detected by microarray analysis (Table 1, Fig. 3). While *Bmal* is overexpressed in the early light phase in hyperlipidemia compared to normolipidemic controls, *Ccm4*, *Dbp* and *Per2* are at the same time downregulated. This opposite type of regulation is in accordance to the circadian pattern of expression of these genes. While *Ccm4* (nocturin), *Dbp* and *Per2* peak close to the time of light offset (zeitgeber time ZT 12), *Bmal* is highest at the start of the light phase (ZT 0).^{14,15}

4. Discussion

The poloxamer P-407 chronic hyperlipidemia mouse model is a genetically unaltered, nondiet-induced mouse model of atherosclerosis that was studied regarding kinetics of hyperlipidemia, atherosclerosis, treatment with

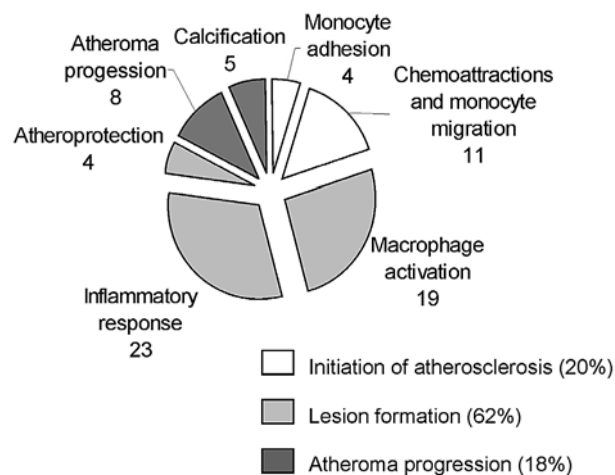


Figure 4: Modulated genes involved in different atherogenic processes.

Classified 93 genes are listed in Table 1.

statins and the lipid metabolism related enzyme activities.^{5,16} The model shows an intense modulation of the atherosclerosis-related transcriptome, which was similar to the atherosclerosis-prone genetically compromised LDLR $-/-$ mice,¹⁷ but changes are more significant as detected in the high fat diet models.^{12,13,17}

Our study shows for the first time that poloxamer P-407 triggers a concerted modulation of a variety of hepatic genes involved in multiple steps of atherogenesis (Table 1). Some of them have already been described in connection to the development of atherosclerosis either at the level of transcriptome^{12,13,17,18} or as protein markers.^{19–21} It is important to note that the gene modulation that was measured at day 96 of the poloxamer P-407 treatment seems to precede the observable atheroma formation since observable atherosclerotic lesions have been described at day 120.¹¹

The poloxamer P-407 treated mice reach 8 times higher plasma lipid levels compared to the diet induced models while liver cholesterol is comparable to controls. Hypercholesterolemia seems to be associated with upregulation of both the protein expression and the activity of HMG-CoA reductase, the key regulatory enzyme of cholesterol synthesis.⁵ The downregulation of *Hmgcr* and other genes of cholesterol synthesis might reflect an adaptation mechanism aimed to protect cells from synthesizing cholesterol. Repression of genes of cholesterol synthesis with no significant repression of SREBP-2 is expected since “cholesterol sensing” as a part of cholesterol feedback regulation works at the post-transcriptional level.^{22,12} A significant decrease in the activity of *Cyp7a1* is combined with no change in the *Cyp7a1* mRNA and a reduced expression of *Cyp39* from the alternative pathway.² Reduced cholesterol elimination seems to represent an important factor leading to hyperlipidemia. Additionally, the poloxamer P-407 inhibits the lipo-

protein lipase protein,²⁴ while an upregulated expression is observed in the liver (Table 1). It seems that for some proteins that are affected by poloxamer P-407, adaptive mechanisms lead to expression aimed to minimize the effect of this pathological stimulus. The overlap between transcriptomes of the chronic poloxamer and cholesterol diet models¹² is low. Only *Lpl* from the lipid metabolism, *S100a9*, *Cd53* and *Spic* from lesion formation, *Iqgapq* and *Crip1* from initiation of atherosclerosis and *Chka* from cell stress/defence response mechanisms are over 2-fold upregulated in both models.

Studies describing the proteome biomarkers of atherogenesis show fatty acid binding proteins FABP 4 and 5, cathepsins D and B, S100 calcium binding protein A14 and annexin 5 proteins¹⁹ significantly upregulated in macrophages after treatment with oxidized LDL. We observed upregulation of genes from the same families (Table 1), indicating that they represent true markers of atherogenesis. All proteome studies^{19–21} underline the importance of CCL family proteins, especially the chemokine Ccl2 (*Mcp1*) from initiation of atherogenesis, that are overexpressed in our model. Interestingly, while the downregulated genes in diet and poloxamer P-407 models represent genes of cholesterol synthesis,^{12,13,17} no similar observation has been reported at the proteome level.

An unexpected observation was the modulation of circadian oscillators (*Clock*, *Bmal1*, *Per2*) and circadian output genes (*Dbp*, *CCr4*). Circadian rhythms are daily cycles of physiology and behaviour that are driven by an endogenous oscillator with a period of about one day. The suprachiasmatic nucleus represents the master circadian synchronizer but most peripheral cells circadian oscillators that are based on interconnected transcription-translation feedback loops.²⁵ CLOCK and BMAL1 transcription factors activate genes encoding negative components of the major feedback loop, such as genes of the period (*Per*) or cryptochrome (*Cry*) families.²⁶ Recent data on the conditionally knock out clock *-/-* mice challenge a central feature of the current mammalian circadian clock model regarding the necessity of CLOCK:BMAL1 heterodimers for clock function.²⁷ Our study indicates that in the early light phase *Clock* and *Bmal1/Arntl1* are upregulated in livers of poloxamer P-407 treated mice while *Per2*, *Dbp* and *CCr4* are among the few downregulated genes. A broader investigation is needed to determine through which mediators hyperlipidemia affects the circadian gene expression and whether this affects also the circadian behaviour of these hyperlipidemic animals.

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Povzetek

Mišji model hiperlipidemije povzročene s poloksamero P-407 je primeren za proučevanje ateroskleroze in vpliva zdravil za zniževanje lipidov. Zanj je značilno, da hiperlipidemija ni povzročena z genskimi spremembami živali, oziroma z energetsko bogato dieto. Model je relativno dobro poznan na nivoju biokemije, medtem ko še ni bil obravnavan z globalno analizo transkriptoma. Po pričakovanjih P-407 povzroči dvig trigliceridov in holesterola v plazmi miši. Z uporabo DNA biočipov in RT-PCR prvič pokažemo, da P-407 povzroči usklajene spremembe v izražanju jetrnih genov, ki so vpleteni v različne stopnje aterogeneze, kot so lipidni metabolizem, iniciacija aterogeneze, formiranje lezij in napredovanje aterome. V jetrih se je izražanje povečalo 106-krat, zmanjšalo pa 40-krat genom, kar napeljuje na domnevo, da povečanje izražanja aterogenih genov napoveduje formiranje aterome. Zaznali smo tudi spremembo izražanja cirkadičnih genov, vendar bodo potrebne dodatne analize za potrditev povezave z časovnim ritmom živali.