Research project

Lipotoxicity in myocardial infarction and heart failure. Importance of endogenous lipoproteins for cardiac function, structure and arrhythmias.

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The heart is an organ heavily dependent on exogenous lipids for oxidative production of ATP, which is essential for maintenance of normal cellular energy homeostasis. During the last years surprising data have been reported showing unequivocally that the heart besides being dependent on exogenous lipids also synthesizes its own endogenous lipids. These lipids are produced and secreted in the form of apoB-containing lipoproteins (apoB) - which are structurally much alike plasma low-density lipoprotein particles. This phenomenon has been confirmed in several different species including humans. The fact that both humans and mice - two species parted by million years of evolution - have preserved the biochemical machinery for myocardial production of lipoproteins suggests important physiological and/or pathophysiological regulatory role.

What we know from experiments performed in transgenic animals supported by human data is that myocardial apoB production is probably not important for maintenance of normal cardiac function and structure. Both knock-out mice and humans with the rare genetic defect resulting in inability to express MTP (microsomal transfer protein – initial step in the synthesis of apoB) and consequently in abetalipoproteinemia, have normal cardiac structure and function. What we don’t know is the function of myocardial apoB in the heart under pathologic conditions. Our hypothesis is that myocardial apoB is a cardioprotective system mobilized during pathophysiologic conditions such as ischemia, pathologic remodeling and heart failure. These conditions are associated with accumulation of intracellular lipids (free fatty acids, triglycerides, ceramides, lysophospholipids etc.) in the heart. Excessive accumulation of lipids is damaging to cellular function and structure and results in development of lipotoxic heart disease. It has been demonstrated that excessive accumulation of lipids in the heart leads to:

1. reduction in mitochondrial electron transfer activity,
2. uncoupling of oxidative phosphorylation,
3. reduction in activity of ATP-ases,
4. induction of cardiac hypertrophy,
5. induction of mitochondrial death and apoptosis,
6. systolic and diastolic dysfunction.

Our hypothesis is that apoB isolates and exports toxic lipids from cardiomyocytes and therefore plays an important role in maintenance of normal membrane function of organelles such as mitochondria, sarcoplasmatic reticulum and sarcolemma. Functional disturbance of these cellular units results in development of cell death and/or electrophysiological instability. In clinical terms these events would translate into development of congestive heart failure (CHF), malignant ventricular arrhythmias and sudden death.

I. Are endogenous lipoproteins important for preservation of myocardial function, structure and survival during MI and CHF?

Intracellular lipotoxicity during MI and CHF causes cell dysfunction, cell necrosis and apoptosis.

**Hypothesis:** The heart has a protective system- apoB - which is mobilized during MI and CHF to counteract lipotoxicity by isolating and exporting toxic lipids (FFA, triglycerides, oxidized lipoproteins etc). Effective export of intracellular lipids accumulated during ischemia is essential for recovery of normal function of sarcolemma and other membrane-associated organelles. This improves myocardial function, attenuates pathologic remodeling and reduces malignant arrhythmias.

**Ad I. Experimental studies.**

We will use small animal models (mouse, rat) of myocardial infarction (MI) and ischemia-reperfusion injury. We will look at apoB response during early and chronic phase after myocardial injury. MI will be induced by coronary artery occlusion with and without reperfusion in normal and transgenic mice (apoB overexpression). First, we will determine how early apoB is activated,
how long this activation persists and possible maximal and minimal response points in time. We will therefore sacrifice animals after 0.5-3-6-24-48-120 hours and 8 weeks after initial injury for measurement of myocardial apoB using standard metabolic labeling procedure. To evaluate whether apoB contributes to functional recovery of injured myocardium we will use transgenic mice with overexpression of apoB. These animals have 60% more myocardial apoB compared to wild type. The experimental protocol will be as described above. Comparison to wild-type in regard to myocardial function at different time points will be performed by means of invasive hemodynamic assessment (Millar catheter) and echocardiography. To evaluate whether apoB may have impact on survival in acute MI we will study mortality rate in apoB transgenic mice and wild type after induction of MI. To evaluate whether apoB have impact on pathologic remodeling and progression of CHF we will evaluate apoB and wild type mice with echocardiography 2-4-6 weeks after induction of MI. Qualitative and quantitative assessment of myocardial energy metabolism during progression of CHF will be assessed in vivo with $^1$H and $^{31}$P MRS (Fig. 1 and 2) while myocardial tissue will be characterized metabolically. To determine whether apoB mediates antiarrhythmic protection, apoB and wild type mice will be investigated with telemetry after induction of MI for qualitative and quantitative analysis of malignant ventricular arrhythmias (Fig. 5). Furthermore, the animals will be investigated invasively before and after treatment with MTP inhibitor using programmed stimulation protocol (pacing) according to the same principles applied clinically in patients during risk stratification for malignant ventricular arrhythmias. Detailed electrophysiological studies during pharmacological challenge will be performed in cell cultures using multi-channel recording system (MEA) in neonatal cardiomyocytes from wild-type and apoB mice and in HL-1 cardiomyocytes (Fig. 3). To evaluate the possible role of apoB in neutralization of negative effects of oxidized lipoproteins (lysolecithin) and FFA we will perform $^1$H and $^3$P MRS on heart in vivo at 7 T in apoB mice. The same experiments will be performed during the presence of MTP inhibition. To investigate whether activation of myocardial apoB may rescue CHF phenotype, apoB transgenic mice will be crossed with cardiomyopathic mice (defective mitochondrial DNA). To evaluate possible gender differences in myocardial apoB we will include animals of both genders. This is important since it is known that women have more pronounced pathologic remodeling after MI and malignant arrhythmias are more frequent during morning time. To investigate whether myocardial apoB responds to pharmacological intervention we will use PPAR (peroxisome proliferator-activated receptors) - agonists (inducers of MTP), MTP inhibitors, statins, β-blockers, trimetazidine (β-oxidation inhibitor) as well as sympathomimetics and calcium sensitizer (levosimendan). It has been proposed that activation of myocardial phospholipases during acute cardiac ischemia results in the generation of cardiotoxic metabolites that precipitate lethal ventricular arrhythmias. Lyso phosphatidylcholine, a hydrolysis product of phospholipid degradation by phospholipase A₂, accumulates in the ischemic myocardium during the first minutes of cardiac ischemia, and this accumulation has been directly associated with the development of ventricular arrhythmias. The identity of the phospholipase responsible for the ischemia-induced lipolysis has recently been identified as a calcium-independent phospholipase A₂ (iPLA₂β). Our working hypothesis is that cardiac
lipoprotein biosynthesis removes the cardiotoxic phospholipids. To test this hypothesis, we have generated double transgenic mice overexpressing both cardiac iPLA₂β and cardiac apoB and backcrossed these to the FVB genetic background that is more susceptible to develop arrhythmias. Using these mice, we will determine whether overexpression of cardiac apoB protects against ventricular arrhythmias during ischemia.

**Ad I. Clinical study**

The specific polymorphism in the MTP gene (MTP-493 G/T) is associated with higher risk of cardiovascular mortality and morbidity⁸. This polymorphism, results in a functionally decreased apoB synthesis. To evaluate whether this polymorphism increases the risk for malignant ventricular arrhythmias post-MI and during heart failure, a study based on clinical material from two large randomized clinical studies (i.e. MERIT-HF and CORONA, n ~ 9000) in collaboration with AstraZeneca will be conducted. The data will be analysed with respect to the incidence of arrhythmias, mortality, sudden death, LV function and morphology. These variables will be related to the MTP genotype as well as to the genotype and the treatment with metoprolol and rosuvastatin. The study will continue with prospective MRI and ³¹P and ¹H MRS (Figure 4) analysis of the myocardial energy status and lipid content in a randomly sampled group of patients carrying the specific MTP polymorphism (n=25) and matched control subjects (n=25). These experiments will show in patients whether MTP polymorphism is associated with adverse postinfarct remodelling, impaired heart function and accumulation of intracellular lipids.

**II. Are endogenous lipoproteins important for maintenance of normal myocardial energy metabolism?**

**Hypothesis:** Creatine and PCr depletion inhibits mitochondrial β-oxidation and leads to intracellular lipid accumulation. This event will be reliably monitored and quantified in vivo by ¹H MRS. Lipid accumulation induces increased apoB synthesis which counteracts cellular lipotoxicity and reduces negative impact of creatine and PCr depletion on myocardial function, morphology and energy metabolism.

**Ad II.** Normal rats, wild-type and apoB transgenic mice will be exposed to β-GPA (β-guanidino propionic acid - a creatine analogue) introduced by subcutaneous osmotic minipumps over a period of 4 weeks. Myocardial function, morphology and energy metabolism will be evaluated non-invasively with echocardiography, MRI and ³¹P MRS. ¹H MRS will be used for quantitative in vivo measurements of intracellular lipids in the heart longitudinally. Invasive hemodynamic assessment of LV function will be performed after 4 weeks. Quantitative and qualitative lipid analysis using HPLC and gene-array of myocardial tissue will be performed. To study a possible reversibility-irreversibility in the LV remodeling, the mice will be examined 4 and 6 weeks after discontinuation β-GPA treatment. These experiments will show whether creatine depletion is sufficient to initiate myocardial dysfunction and pathologic biochemical remodelling with lipid accumulation and whether apoB may attenuate or prevent these negative events at subcellular and at organ level.

**III. Is the “broken heart syndrome” (takotsubo cardiomyopathy) a consequence of acute lipotoxicity and inadequate apoB response in the heart?**

We have recently (February 2005) discovered at our hospital what we believe is the first documented case of the “broken heart syndrome” in Sweden (Fig. 5). Since then we have accumulated 34 such cases at our clinic (32 females and 2 male, age 24-79). This syndrome was first described 1990 in Japanese women and has a strong association with severe emotional stress⁹,¹⁰. The epidemiology of the takotsubo is largely unknown but the first estimates suggest that it could be present in ~ 4 % of all patients with acute chest pain. The pathogenesis and the reason why it affects mostly women are unknown. It is generally believed that severe emotional stress paralleled by intense neurohormonal activation causes
derangement in myocardium resulting in progressive cell dysfunction and cell death. The patients develop characteristic LV apical “ballooning” (Fig. 4) representing severe LV dysfunction that may cause fulminate heart failure, cardiogenic shock and literally heart rupture. There is direct clinical evidence for disturbed myocardial lipid metabolism in these patients.\footnote{12}

**Hypothesis:** Takotsubo cardiomyopathy is a special case of acute myocardial lipotoxicity due to severe neurohormonal stress. Adequate apoB response may attenuate and/or prevent development of takotsubo cardiomyopathy.

**Ad III. Clinical studies:** An epidemiological study is ongoing with the aim to determine the incidence of takotsubo cardiomyopathy in Västra Götaland region (population of ~ 1 600 000). The work is in progress to establish a national registry for all patients with diagnosis takotsubo cardiomyopathy. This will be achieved in collaboration with SCAAR registry (Swedish Coronary Angiography and Angioplasty Registry). We have analyzed all coronary angiographies performed at Sahlgrenska Hospital since 2001 (18 000) and patient data using SCAAR and RIKS HIA (coronary care) registries. The patients with normal angiography and with evidence of transient left ventricular dysfunction will be scrutinized in detail in order to establish takotsubo diagnosis. The previously mentioned cohort of 34 patients with definite takotsubo diagnosis will be subjected to evaluation of myocardial metabolism using \(^1\)H and \(^{31}\)PMRS and positron emission tomography under metabolic and emotional stress and compared to matched controls. In addition, gene-array analysis and electron microscopy will be performed on myocardial biopsies from these patients and compared to matched controls. We will test the hypothesis that these patients have an overrepresentation of the specific MTP polymorphism resulting in decreased capacity to synthesize myocardial apoB. These experiments will show whether patients with takotsubo have distinct differences in myocardial lipid and energy metabolism at gene and at organ level.

**Experimental studies:** Female and male mice will be exposed to immobilization stress during 30 min (a model for severe emotional stress). This protocol has been shown to induce takotsubo-like damage in animals.\footnote{13} To test whether apoB may protect against stress-induced cardiomyopathy we will use apoB transgenic and MTP knock-out mice to test for gain-of-the-function and loss-of-the-function in this model. The animals will be evaluated with echocardiography in vivo for assessment of cardiac function and morphology while in vivo \(^{31}\)P and \(^1\)H MRS will be used for evaluation of energy and lipid metabolism. Myocardial lipids, catecholamines will be analyzed quantitatively and qualitatively by HPLC while myocardial tissue will be examined with electron microscopy and gene-array. These experiments will elucidate whether myocardial apoB is involved in cardiac protection and pathogenesis of takotsubo cardiomyopathy. Analysis of the genome in these models will provide further pathophysiological insights. We will also learn whether myocardial apoB may exert different effects in the male and female animals.

**IV. Is myocardial lipotoxicity contributing to establishment of atrial fibrillation?**

Atrial fibrillation (AF) is a common arrhythmia which is prevalent in elderly population. AF can lead to serious adverse events related to systemic embolization (e.g. stroke) and to chronic tachycardia (e.g. heart failure).

**Hypothesis:** Atrial fibrillation (paroxysmal) leads to pathologic biochemical remodeling\footnote{14} of atrial myocardium and accumulation of lipids within cardiomyocytes. This lipid accumulation results in multiple cellular abnormalities resulting in electrophysiological instability that acts as a substrate for triggering, maintenance and establishment of permanent AF. Accumulation of lipids in atrial myocardium is associated with down-regulation of MTP (microsomal transport protein) and apoB lipoprotein.

**Ad IV. Clinical study:** Samples of atrial myocardium will be obtained from patients with permanent AF (n = 20), patients with paroxysmal AF (n = 20) and control patients without AF (n = 20). The samples will be obtained during MAZE operation as well as during routine cardiac surgery (CABG or valvular...
surgery). Quantitative and qualitative analysis of lipids (lipidomics), myocardial MTP, apoB and gene expression as well as electron microscopy will be performed on atrial biopsies.

**Importance**

Understanding mechanisms involved in development of disturbed production, transport and utilisation of chemical energy in the heart and lipotoxic heart disease would help to define future pharmacological targets to improve clinical outcomes in patients with MI, cardiomyopathy and CHF. Development and application of in vivo MRS techniques within basic sciences as well as clinical medicine should be an important goal for the national as well as regional research strategy.

**Preliminary results**

**Ad I.** In the pilot experiments we were able to show that myocardial apoB synthesis increases in different injury models. Induction of MI in normal mice increased apoB 4 weeks post-infarct. Surprisingly, 8 weeks post-infarct the level of apoB was down to 20% of the controls. The most obvious and pronounced activation (2-4 fold increase) of apoB synthesis was observed in the mouse model of ischemia-reperfusion injury after 24 and 48 hours. Acute and global myocardial damage induced by high dose of doxorubicin resulting in acute CHF (within 1-2 days) did not induce myocardial apoB production in mice and actually decreased in rats. Taken together, these data demonstrates that acute cardiac injury and CHF result in early upregulation and late depletion of myocardial apoB. However, the most promising result so far is the dramatically increased survival of apoB mice after MI compared to the wild-type. Six weeks after induction of MI, survival in the apoB group was ~70% while only ~30% of the wild type mice were alive (Fig. 6)\(^\text{15}\). This was associated with better LV function in the apoB mice. Interestingly, our results from in vitro electrophysiology on HL-1 cells suggest that VLDL particles (possibly as donor of exogenous apoB) may have anti-arrhythmic effects if the cells are preincubated for 24 hours with high dose of human VLDL prior to provocation with lysolcithin.

**Ad II.** Myocardial depletion of creatine (by 40%) in rats and mice resulted in disturbed LV systolic and diastolic function, LV dilatation lower PCr/ATP ratio and intracellular lipid accumulation. When these animals underwent induction of acute MI, 96% died (mostly due to malignant arrhythmias, Fig. 7) within 60 min. post-MI compared to 50% of controls\(^\text{16}\). The results suggest that normal energy metabolism is essential for survival of acute MI. Using similar protocol in the mouse model we were able to demonstrated reversibility of functional, morphological and metabolic disturbances after normalisation of myocardial creatine content. These experiments suggest that intracellular creatine is probably not an inert intracellular compound as traditionally viewed. We are currently analysing myocardial lipidomics in material from previously described experiments. The BGP treatment of apoB transgenic mice did not induce pathologic alterations in cardiac function and morphology as seen in wild-type mice suggesting the possible protection of apoB genotype against creatine depletion in the heart.
**Ad III.** All patients with normal coronary angiogram examined at Sahlgrenska Hospital during the period 2001-2006 for indication acute coronary syndrome have been identified and listed from the SCAAR registry (385 patients). The work is ongoing to identify the patients with transitional left ventricular dysfunction and clinics compatible with diagnosis of takotsubo cardiomyopathy. Our preliminary results indicate that as much as ~ 10% of patients with normal coronary angiogram, chest pain and LV dysfunction may be caused by takotsubo cardiomyopathy. We have also established the small-animal model of takotsubo cardiomyopathy by means immobilization-stress in rats and mice.

**Ad IV.** The inclusion of suitable patient has begun and we have collected myocardial biopsies from ten patients so far.

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**Institutions and collaborators**

The group is based at The Wallenberg Laboratory and has at disposal one additional laboratory for animal experimentation at the University’s new facility for Experimental Biomedicine. This laboratory is located within the Center for Bioimaging and is close to the facilities for MR (state-of-the-art 7 T magnet for small animals), echocardiography and invasive hemodynamics. Three PhD students are currently engaged in the project (Malin Lindbom Phil. Mag., Truls Rämunddal MD and Sigfus Gizurarson MD). Prof. Lennart Bergfeld, as part-time tutor for Sigfus Gizurarson, will participate in experimental studies regarding mechanisms of arrhythmias. A MR physicist (Sharmila H.) has been recruited and is now working full-time on refinement and further development of MR methods described in this application. We have established close collaboration with Prof. Jan Boren and Prof. Sven-Olof Olofsson at The Wallenberg laboratory regarding molecular biology of lipoproteins in the heart. The clinical study on MTP polymorphism will be conducted in collaboration with AstraZeneca (Prof. Finn Waagstein and Prof. Åke Hjalmarsson). Through collaboration with Prof. Bassam Soussi we have unrestricted access to two additional magnets - 2.35 T and 11.7 T - for in vivo and in vitro experimentation. Gene-array analysis, lipidomics and proteomics of tissue samples will be performed in collaboration with AstraZeneca. Electron microscopy will be performed by Assoc. Prof. Ulf Nannmark at Institute of Anatomy and Cell Biology, Gothenburg. Clinical studies with $^{31}$P and $^{1}$H MRS will be carried out on 1.5 and 3 Tesla clinical magnets (Philips) at Sahlgrenska University Hospital in collaboration with one of the leading MRS groups headed by Prof. Kieran Klarke (Inst. for Biochemistry, Oxford, UK). Furthermore we will collaborate with Assoc. Prof. Håkan Arheden at University Hospital in Lund. Atrial fibrillation study will be conducted in collaboration with Assoc. Prof. Anders Jeppsson (Dpt. Thoracic Surgery, SU/Sahlgrenska).
References


