Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006 **Herziene versie**

DECNR: 2011-120

Ontvangen: 03-10-2011

DEC datum goedkeuring#	Type aanvraag [2]	VROM/GGO NR[3]	LNV/CBDNR[4]
	Nieuw / Herz.versie / Pilot		

Hoofdproject	**CARIM**	NUTRIM	Hersenen en gedrag	GROW	biomateri alen	Ander UM	Geen UM
Deelproject	1. **2**. 3.	1. 2. 3. 4.	1. 2. 3.	1. 2. 3.			

Financieel beheerder		Budgetnummer	3098.2246N

Titel van het onderzoek:

Unraveling the role of micr[illegible] in a model of heart failure

startdatum 1 oktober 2011 einddatum [9] 1 oktober 2015 *Duur van de proef [10]: 49 days*

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mailadres	Bevoegd-heid[5]	Cap. groep /afdeling
1.Verantwoordelijk onderzoeker (VO)				Art.9	
2. Vervanger VO (VVO)				Art.9	
3. Verantwoorde-lijk medewerker (VM) GGO[7]					
4. overige uitvoerenden	Artikel 12 functionaris			Art.12	
5.	(PI)			Art.9	
6.				Art.9	
7.					

Diergroep	1-2	3-4	5-6	7-8
ctrl/exp/sham	sham	exp	sham	exp
Diersoort	muis	muis	muis	muis
Stam	C57Bl/6	C57Bl/6	C57Bl/6	C57Bl/6
Construct / mutatie ?	wildtype	wildtype	wildtype	wildtype
Herkomst (leverancier) *	1	1	1	1
Aantal	36	38	36	38
Geslacht	m	m	m	m
Dieren immuuncompetent ?	ja	ja	ja	ja
Leeftijd/gewicht	6-12 wkn	6-12 wkn	4-9 wkn	4-9 wkn
Doel van de proef *	31	31	31	31
Belang van de proef *	1	1	1	1
Toxicologisch onderzoek *	1	1	1	1
Bijzondere technieken *	1	1	1	1
Anesthesie *	4	4	4	4
Pijnbestrijding *	4	4	4	4
Mate ongerief *	4	4	4	4
Toestand dier einde exp*	1	1	1	1

* VHI-coderingen zie bijlage

Application for the conduction of animal experiments (DEC-UM)

Title: Unraveling the role of micro in a model of heart failure

Accountability

1. Aim of the experiment.

Arterial hypertension is an important risk factor for heart failure (HF), a significant cause of mortality worldwide. But still our knowledge of the mechanisms involved in development of heart failure is incomplete.

We identified miR- differentially regulated in human and mouse heart failure. We suggest that miF has a positive impact on the development of heart failure by repressing the expression of deleterious gene products, one of which is the pro-hypertrophic a known target of mi

We hypothesize that inhibition of mil will aggravate the development of hypertrophy and heart failure, while overexpressing pre-m will protect against heart failure. We will study these hypotheses in a mouse model of heart failure in which cardiac hypertrophy is induced by angiotensin II infusion. We will administer inhibitors of mil (antagomiRs) or overexpressors of pre-miR (encoded by adeno-associated viral vector type 9, AAV9) via tail vein injection and examine cardiac function and histology.

2. Social relevance and/or scientific importance

Heart failure is a progressive and severely invalidating disease with a bad prognosis and no treatment available other than symptomatic therapy. Heart failure is the number 2 cause-of-death in Western society, directly after cancer. Most patients suffering from heart failure die within 5 years after diagnosis. The current study will improve our understanding of the mechanisms that lead to heart failure, and of clinically important actors in this process. This is highly relevant on the social as well as economical level.

3. Alternatives

The heart is a complex organ composed of different cell types. One cell type interacts with its surrounding environment. Above that, other cell types (infiltrate during heart failure and interact with cells that reside in the heart. This creates a highly complex *in vivo* situation, and no *in vitro* model is able to simulate these complex processes. Hence, it is essential that we examine the heart in the *in vivo* setting.

4. Ethical consideration

Heart failure is the number 2 cause of death in The Netherlands and other Western countries; yearly more than 6000 people die of this disease in The Netherlands. Current knowledge about the crucial contributing factors is flawed. This animal experiment will contribute to a better understanding of the causes of heart failure, and eventually to better treatment and diagnosis of patients. Given the complexity of the heart and the benefit offered by this study, we consider it essential to conduct an animal study without underestimating the serious inconvenience of using laboratory animals.

Science

5. Scientific background

The pathogenesis of heart failure is complex, and is characterized by enlargement of cardiomyocytes (hypertrophy), cardiac tissue fibrosis and inflammation. It is known that microRNAs, newly discovered RNA molecules that regulate gene expression, influence hypertrophy and fibrosis. An involvement of mi in this process is suggested by downregulation of this microRNA during human and mouse cardiac hypertrophy and failure. We established the m is predominantly expressed by cardiomyocytes in the heart (see arrows in inset; m tained blue; nuclei in pink).

In this study we will investigate whether manipulation of mi affects the development of hypertrophy and heart failure in a mouse model of hypertension.

First, we will administer antagomiRs targeting mi (or scrambled control) by tail vein injection on three consecutive days , and then subject mice to angiotensin II-induced cardiac hypertrophy by implantation of osmotic minipumps that deliver AngII subcutaneously. Sham mice will receive an osmotic minipump for continuous subcutaneous saline injection.

Second, we will treat mice with AAV9 overexpressing pre-mi (or scrambled control), which is administered by tail vein injection. AAV9 specifically infects cardiomyocytes. After 3 weeks, to allow pr expression in the heart, mice are treated with AngII as above.

6. Scientific review

The current project is independently evaluated and approved by the PI of the department.

Experimental Animals

7. Choice experimental animal

7a. Species, strain / origin / final destination

For the study we will use C57Bl/6J mice from Harlan. The mice will be sacrificed at the end of the experiment to assess heart function, histology and RNA/protein levels in the hearts.

7b. Sex

The sex differences between males and females with heart failure are well-known but insufficiently understood. Therefore, to exclude possible hormonal differences, we will use male mice for the study.

7.c. Number

First, we will study the manipulation of miR y administering an inhibitor (antagomiR) to mice, followed by induction of heart failure by angiotensin II infusion. The antagomiR will be compared to controls that receive a scrambled oligonucleotide. Second, we will study the manipulation of mi by administering AAV9 overexpressing pre-mi or pre-scrambled.

Study end point is echocardiographically assessed cardiac function.

Power calculation:

Formula of L. Sachs: $n = 2(z_{\alpha}/2 - z_{\pi})^2 \cdot (\sigma/\delta)^2 = F \cdot (\sigma/\delta)^2$

$F_{0,80} = 15,7$ for $\alpha = 0,05$ and $\pi = 80\%$

Based on our experience, the variation coefficient for angiotensin II infusion is 20% and the expected relative impact is 20%. The *P* value is <0.05 and power 80%.

$$N = 15.7 \cdot (0.2/0.2)^2 = 15.7$$

Angiotensin II-treatment leads to a peri-operative and post-operative mortality of about 15%.
If 15.7 mice represent 85% of the total group, we would need (15.7/85) · 100 = 18.5 or **19 animals** per group.

We would like the same number of surviving mice in the sham groups. Saline-treatment leads to a peri-operative mortality of 10%, but is not associated with post-operative mortality. Therefore, each sham group will consist of (15.7/90)·100 = 17.4 mice, or **18 mice.**

Group	**Number of animals**
1. scrambled antagomiR + s.c. saline	18
2. antagomi + s.c. saline	18
3. scrambled antagomiR + s.c. Ang II	19
4. antagomiI + s.c. Ang II	19
5. AAV9-pre-scrambled + s.c. saline	18
6. AAV9-pre-mi + s.c. saline	18
7. AAV9-pre-scrambled + s.c. Ang II	19
8. AAV9-pre-m + s.c. Ang II	19
Total	**148**

Animal Experiments

8. Experiment

The following experiments will be conducted:

Tail vein injection of antagomiRs (groups 1-4). On day -3 to -1, mice will be injected once a day with a volume of 0.2 ml antagomiRs (scrambled or antagom issolved in saline, or saline alone), by tail vein injection. For the injections, the mice will briefly be placed in a heating cage and then in a restrainer.

Tail vein injection of AAV9 (groups 5-8). On day -21, mice will be injected once with a volume of 0.2 ml AAV9 (scrambled or n saline), by tail vein injection. For the injections, the mice will briefly be placed in a heating cage and then in a restrainer.

Angiotensin II or saline infusion with subcutaneous minipump (all groups). This procedure will be performed according to SOP r 28 days. We know that a dose of 2.5 mg/(kg·day) angiotensin II causes elevation of blood pressure, cardiac hypertrophy and failure in C57Bl/6J mice. Implantation of (saline-filled) minipumps in control animals is necessary, because control animals need to undergo similar stress and surgery to be compared to the AngII treated animals.

Echocardiography will be performed following SOP

Terminal hemodynamic characterization of the mouse (dP/dt) will be performed according to SOP

Time schedule groups 1-4:

Day				
-3	-2	-1	0	27-28
Daily i.v. injection of antagomiR or scrambled control			Echo and implantation of minipump with Ang II or saline	Echo, dP/dt and sacrifice

Time schedule groups 5-8:

Day		
-21	0	27-28
i.v. injection of AAV9	Echo and implantation of minipump with Ang II or saline	Echo, dP/dt and sacrifice

9. Experimental conditions

9a. Anesthesia

For subcutaneous minipump implantation and echocardiography, mice will be put under anesthesia with 3-4% isoflurane, after which anesthesia is kept at 1.5-2.5% isoflurane, as also described in SOPs nd respectively. The dP/dt measurement will be performed under urethane anesthesia.

9b. Pain relief

Prior to minipump implantation we will administer the pain killer carprofen (2.5mg/kg).

9c. Euthanasia and humane end points

After the dP/dt measurement, the mouse will be sacrificed for perfusion-fixation under urethane-anesthesia. In case of premature sacrifice of a mouse based on the criteria below (humane end points), the mouse will be euthanized with an i.p. overdose of 200mg/kg pentobarbital.

Humane end points

A mouse will be prematurely euthanized in case of:

- swelling and inflammation of the wound after minipump implantation, open wound with exiting of the pump and abscesses.
- Overt heart failure as a consequence of Ang II infusion, with difficulty of breathing and apathy.
- Other illnesses or conditions that induce severe pain, discomfort or suffering.

The Animal Welfare will be informed in case of calamities.

Care

10a. Discomfort

All animals will receive at least one tail vein injection (and max. 3) prior to the start of the experiment. At the beginning of the study (day 0) they will undergo echocardiography and minipump implantation (one anesthetization). At day of sacrifice (day 28) or the day before (day 27), all mice will first be echoed under isoflurane anesthesia. Urethane anesthesia will be administered to awake animals on day 28 for the hemodynamic measurements and sacrifice. Waking-up before urethane administration is essential for the animals to recover from the isoflurane anesthesia. Without prior recovery the drop-out numbers during the hemodynamic measurement would increase dramatically.

The overall duration of the study is 31 days for groups 1-4 and 49 days for groups 5-8. The discomfort is set to 04 (moderate-severe) because all animals will be put under anesthesia thrice.

Group 1-4

type	severity	duration	frequency
i.v. injection in restrainer	02	< 1 minute	daily for 3 days
echo (isoflurane)	02	~15 min	twice in 4 weeks
minipump implantation (isoflurane)	03	~15 min	once
hemodynamic measurements (urethane)	02	~15 min	once
possible heart failure	(04)	unforeseeable	
possible other illnesses	(02)	unforeseeable	
The discomfort is estimated to sum up to max. 04 (moderate-severe)			

Group 5-8

type	severity	duration	frequency
i.v. injection in restrainer	02	< 1 minute	daily for 3 days
echo (isoflurane)	02	~15 min	twice in 4 weeks
minipump implantation (isoflurane)	03	~15 min	once
hemodynamic measurements (urethane)	02	~15 min	once
possible heart failure	(04)	unforeseeable	
possible other illnesses	(02)	unforeseeable	
The discomfort is estimated to sum up to max. 04 (moderate-severe)			

10b. Evaluation of well-being

Angiotensin II infusion, scrambled antagomiR and AAV9 have no visible influence on the welfare of mice with this genetic background. However, we do not yet know the influence of manipulation, which could promote heart failure. After echo and/or minipump implantation procedures on day 0, mice will be placed in a heated room (30°C) to recover, so that they don't need to spend energy on body temperature maintenance.

11. Care and housing

Groups 1-4: The mice will be housed within the CPV. On the day of sacrifice they will be transported to the laboratories on the 4th floor.

Groups 5-8: The mice will be housed within the CPV in a DM-III room and on the day of experiments mice will be transported to the laboratories on the 4th floor (as approved in GGO).

For all animals, water and food will be provided *ad libitum*. During the experiment the researcher will take care of the animals in consultation with veterinarians from the CPV department. In case of emergencies, the VO should be contacted.

12. Expertise

All interventions and procedures are performed by authorized and qualified persons (art. 9 or art. 12).

13 Standard Operation Procedures (SOP)

Echocardiography mouse:
Implantation of Alzet osmotic minipumps in the mouse
Terminal hemodynamic characterization of the mouse:

Relevant literature

Sayed D, Hong C, Chen IY, Lypowy J, Abdellatif M. *MicroRNAs play an essential role in the development of cardiac hypertrophy.* Circ Res. 100 (2007). 416-424.

Schroen B, Heymans S. *MicroRNAs and beyond: the heart reveals its treasures.* Hypertension 54 (2009). 1189-1894

University Maastricht

Faculty of Health, Medicine
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Dierexperimenten Commissie

DEC

Aan:

, voorzitter
p/a Secretariaat DEC-UM
Postbus 616
NL-6200 MD Maastricht
Telefoon:

Uw referentie:

Onze referentie : M

Maastricht, 28-09-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Unraveling the role of micro* *in a model of heart failure*", is op de DEC vergadering van 23 september 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De duur van de proef op het voorblad is niet juist (dit is de langste periode binnen één project dat één dier in proef is). De DEC verzoekt dit aan te passen. *(49 dg)*
- De DEC verzoekt het GGO nummer van het voorblad te verwijderen. GGO is niet van toepassing op dit protocol (er wordt niet gewerkt met genetisch gemodificeerde dieren). *MM laten weten.*
- Bij punt 10a verzoekt de DEC de tekst om te zetten in een tabel waarin de aard, ernst, duur en frequentie van de individuele handelingen **per** groep weergegeven wordt, te sommeren tot een totaal ongerief **per** groep en tevens de laatste 2 punten van punt 9c (humane eindpunten) en het leven met "heartfailure" ook te vermelden.
- De DEC verbaast zich erover dat er geen relevante literatuur bestaat over dit onderwerp.

Gelieve eventuele vragen te beantwoorden in **een brief en indien noodzakelijk Uw project aan te passen en duidelijk de aanpassingen grijs te markeren.**

Uw project staat bij de DEC geregistreerd onder nummer **2011-120**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Maastricht, 3 oktober 2011

Betreft: DEC 2011-120

Geachte DEC,

Middels deze brief wil ik graag Uw vragen beantwoorden met betrekking tot project 2011-120, "*Unraveling the role of microR[illegible] in a model of heart failure*". De aanpassingen zijn grijs gemarkeerd op het voorblad en in de aanvraag zelf.

- De duur van de proef op het voorblad is aangepast (49 dagen).
- Het GGO nummer is van het voorblad verwijderd.
- Bij punt 10a is de tekst omgezet in een tabel waarin de aard, ernst, duur en frequentie van de individuele handelingen per groep weergegeven wordt, alsook het opgetelde totaal ongerief per groep. Het leven met "heart failure" en mogelijke andere optredende ziekten (punt 9c, humane eindpunten) zijn ook in de tabel vermeld.
- Relevante literatuur is toegevoegd.

Ik hoop hiermee Uw vragen naar voldoening te hebben beantwoord.

Met vriendelijke groet,

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan

Ons kenmerk 1 *Doorkiesnummer* *Maastricht* 04-10-2011

Project: *Unraveling the role of microR p in a model of heart failure.*

Verantwoordelijk onderzoeker (VO):

Namens de Vergunninghouder van de DEC-UM, delen wij u mede dat voornoemd project aan de ethische toetsingscriteria voor proefdiergebruik voldoet.
De DEC maakt geen bezwaar tegen uitvoering van dit project zoals aangevraagd en geeft een **positief advies**.

Projectnummer:	**2011-120**
Diersoort:	**muis**
Aantal dieren:	**148**
Einddatum:	**04-10-2015**

Uw project staat bij de DEC en CPV geregistreerd onder bovenstaand nummer. Gelieve dieren, die voor dit project bestemd zijn, ook onder dit nummer aan te vragen.

Voorzitter DEC-UM

Vicevoorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM

Bezoekadres

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