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Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006

Herziene versie

DECNR: 2011-146

Ontvangen: 29-11-2011

DEC datum goedkeuring#	Type aanvraag [2]
30-11-2011	Nieuw / Herz.versie / Pilot

VROM/GGO NR[3]

LNV/CBDNR[4]

Hoofdproject	CARIM	NUTRIM	Hersenen en gedrag	GROW	biomaterialen	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:

The role of microRNA and microRNA- in two models of pressure overload-induced heart failure

startdatum 1 december 2011 einddatum [9] 1 december 2015 *Duur van de proef [10]: 31 days*

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mailadres	Bevoegdheid[5]	Cap. groep /afdeling
1.Verantwoordelijk onderzoeker (VO)				Art.9	
2. Vervanger VO (VVO)				Art.9	
3. Verantwoordelijk medewerker (VM) GGO[7]					
4. overige uitvoerenden	Artikel 12 functionaris			Art.12	
5.				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	34	38	24	30
Geslacht	m	m	m	m
Dieren immuuncompetent ?	ja	ja	ja	ja
Leeftijd/gewicht	6-12 wkn	6-12 wkn	6-12 wkn	6-12 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	5	5
Toestand dier einde exp*	1	1	1	1

* VHI-coderingen zie bijlage

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

Title: The role of microRNA and microRNA- in two models of pressure overload-induced 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 performed first studies with miRNA- and miRNA- in mice subjected to coxsackievirus B3 as a model of virus-induced heart failure and observed in the cardiac tissue, demonstrating the importance of miRNA and miRNA for the progression of the disease. To investigate the role of miRs and in hypertension-driven heart failure, we will study the role of miRNA and miRNA- in a mouse model of heart failure in which cardiac hypertrophy is induced by angiotensin II (Ang II) infusion and by transverse aortic constriction (TAC). There are important differences between these two models: while AngII is a pharmacological way of inducing pressure overload accompanied by both elevated blood pressure ánd AngII-mediated pro-inflammatory signaling, TAC is purely a mechanical form of pressure overload only to the heart.

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 (miRNAs), newly discovered RNA molecules that regulate gene expression, influence hypertrophy and fibrosis. In this study we will investigate whether manipulation of miRNA and miRNA affects the development of hypertrophy and heart failure in a mouse model of hypertension. An involvement of both miRNAs in this process is suggested by previous findings of our group. In an early study, we found that

To expand our comprehension on the role of these two miRNAs in hypertension-induced heart failure, we will use two mouse models of pressure overload-induced cardiac hypertrophy and heart failure: AngII and TAC. We will administer antagomiRs targeting miRNA and miRNA (or scrambled control) by tail vein injection on three consecutive days (and then subject mice to AngII- or TAC-induced cardiac hypertrophy. Antagomirs against miRNA and miRNA- are administered simultaneously, because both miRNAs

6. Scientific review

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

Experimental Animals

7. Choice of experimental animal

7a. Species, strain / origin / final destination

For the study we will use C57Bl/6J mice. 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.

7c. Number

We will study the manipulation of miRNA- and miRNA by administering an inhibitor (antagomir) to mice, followed by induction of heart failure by either angiotensin II (AngII) infusion or transverse aortic constriction (TAC). The antagomir groups will be compared to controls that receive a scrambled oligonucleotide.

Study end point for both AngII-infusion and TAC is echocardiographically assessed cardiac function as a measure of degree of heart failure.

Power calculation:

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

$\boxed{F_{0,80} = 15,7}$ for $\alpha = 0,05$ ($z_{\alpha/2} = 1.960$) and $\pi = 80\%$ ($z_{\pi} = -0.8416$)

1. AngII-infusion

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

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

AngII-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 5%, but is not associated with post-operative mortality. Therefore, each sham group will consist of (15.7/95) · 100 = 16.5 mice, or **17 mice.**

2. TAC

Based on our experience, the variation coefficient for TAC is 20% and the expected relative impact is 25%. The *P* value is 0.05 and power 80%.

$$\boxed{N = 15.7 \cdot (0.2/0.25)^2 = 10.05}$$

TAC leads to a peri-operative and post-operative mortality of 30%. If 10.05 animals represent 70% of the total group, we need (10.05/70) x 100 = 14.3 animals, or **15 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 (10.05/90) · 100 = 11.2 mice, or **12 mice.**

Group	Number of animals
1. scrambled antagomiR + s.c. saline	17
2. antagomiR- + antagomiR- + s.c. saline	17
3. scrambled antagomiR + s.c. Ang II	19
4. antagomiR- + antagomiR- + s.c. Ang II	19
5. scrambled antagomiR + sham surgery	12
6. antagomiR- + antagomiR- + sham surgery	12
7. scrambled antagomiR + TAC	15
8. antagomiR- + antagomiR- + TAC	15
Total	**126**

Animal Experiments

8. Experiment
The following experiments will be conducted:

Tail vein injection of antagomirs (all groups). On the days -3 to -1, mice will be injected once a day with a volume of 0.2 ml antagomirs (scrambled or antagomiR- + antagomiR- dissolved in 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 (group 1-4). This procedure will be performed according to SOP Far-03-M for 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 Ang II treated animals.

Transverse aorta constriction (TAC) (group 5-8). TAC will be performed in 8-12 weeks old mice following SOP TAC (attached). In sham mice, the aorta will not be constricted.

Echocardiography will be performed following SOP Far-02-M.

Terminal hemodynamic characterization of the mouse (dP/dt) will be performed according to SOP Far-07-M.

Time schedule (all groups):

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

9. Experimental conditions
9a. Anesthesia
For subcutaneous minipump implantation, TAC and/or 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 Far-03-M and Far-02-M, respectively. The dP/dt measurement will be performed under urethane anesthesia.

9b. Pain relief
Prior to minipump implantation and TAC respectively, we will administer the pain killer carprofen (2.5mg/kg BW). Post-operative analgesia will be given up to three days after TAC using buprenorfine (0.05-0.1mg/kg BW SC each 8-12 hours).

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 200 mg/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 or TAC, 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 three tail vein injections on three consecutive days prior to the start of the experiment. At the beginning of the study (day 0) they will undergo echocardiography and minipump implantation or TAC (under anesthesia). One day before sacrifice (day 27), all mice will first be echoed under isoflurane anesthesia. At the day of sacrifice (day 28), urethane anesthesia will be administered for the hemodynamic measurements and sacrifice.
The overall duration of the study is 31 days. The discomfort is set to 04 (moderate-severe) for AngII (groups 1-4) because all animals will be put under anesthesia thrice, and 05 (severe) for TAC (groups 5-8) because of the severity of the surgery.

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
TAC	05	~30 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. 05 (severe)			

10b. Evaluation of well-being
AngII infusion and administration of scrambled antagomirs have no visible influence on the welfare of mice with this genetic background. However, we do not yet know the influence of miRNA and miRNA manipulation during pressure overload, which could promote heart failure. After echo, TAC and/or minipump implantation procedures on day 0, mice will be placed in a heated room (30° C) to recover, so that they do not need to spend energy on body temperature maintenance.

11. Care and housing
Groups 1-8: The mice will be housed within the CPV. On the day of sacrifice they will be transported to the
All animals will be housed in groups, and 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 or VVO 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: Far-02-M
Implantation of Alzet osmotic minipumps in the mouse: Far-03-M
Transverse aortic constriction: attached
Terminal hemodynamic characterization of the mouse: Far-07-M.

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

SOP Ongerief 05

Transverse aorta constrictie (TAC) bij de muis

BSc, MSc, Animal Welfare Offices. Art. 14 Art. 12)

Pre-operatief

Er wordt (~30 minuten tevoren) sub-cutaan 0.1ml Temgesic (0.03 mg/l) toegediend.
De muis wordt gewogen, waarna het dier pre-operatief steriele en verwarmde (37°C) NaCl 0.9% (0.5 ml Intra peritoneaal) krijgt toegediend.

Narcose:

- Inleiding met isofluraan (3-5% in zuurstof)
- Onder anesthesie gehouden met isofluraan (1.5-2.5% in zuurstof)

Als de muis goed onder narcose is:

- Hals en borstkas scheren
- Ogen behandelen met Kunsttraan (Duodrops).
- De muis wordt op de rug gelegd.
- Intuberen met een afgestompte naald (20 Gauge).
- De muis wordt geventileerd met een slagvolume van 250ml en een slagritme van 150-180 slagen per minuut.
- De muis fixeren.
- Ontharen van de operatie-plaats.
- Desinfecteren met jodium en afdekken met operatiefolie.
- De muis onder anesthesie houden met isofluraan 1.5-2.5%.
- De huid aan de bovenkant van het sternum wordt ingeknipt om een helder beeld te krijgen van de thorax.
- Let vooral op de kleine bloedvaatjes die in het gebied lopen.
- Het sternum wordt van onder af gedeeltelijk ingeknipt tot aan de 2^{e} rib.
- De twee borstdelen worden aan de kant gehouden met haakjes om een helder beeld van de thymus te krijgen.
- Deze wordt gesplitst in zijn twee lobjes, waaronder de aortaboog zich bevindt.
- Prepareer de aortaboog vrij van weefsels bijv: vlies, vet tussen de de trunces brachiocep brachiocephaicus en de linker carotis arterie (1^{e} en 2 ^{e} aftakking) wordt afgebonden.
- Tijdens het afbinden wordt er een afgestompte 27(Gauge) naald tussen de aorta en de hechting gelaten die direct na het afknopen wordt verwijderd (7-0 proleen).
- Dit afbinden mag niet langer dan 12 seconden duren, om ernstige hartproblemen te verkomen.
- Deze procedure garandeert een standaardvolume van de aorta na constrictie.
- De thymus lobjes worden terug op hun plaats gelegd.
- Het sternum wordt gesloten.
- De borstkast wordt met een enkelvoudig hechtingen dichtgemaakt en de huid gesloten.
- Nadat de huid is gesloten wordt de isofluraan toevoer gesloten en kan het dier langzaam aan de ventilator bijkomen.

- Als het dier redelijk bij is gekomen (als het reageert op een lichte prikkel), wordt de intubatienaald verwijderd.
- Geopereerde muizen worden teruggezet in een schone kooi en op een verwarmingsmat + verwarmings lamp geplaatst en op het einde van de dag in de verkouverkamer (30graden).
- Het herstel van de operatiewond wordt gevolgd en vastgelegd in het welzijnsdagboek en operatieverslag.

Pijnbestrijding:

Tijdens de operatiedag krijgen de dieren aan het einde van de dag een subcutane injectie van 0.1ml temgesic (0.03 mg/l). Ook de twee volgende dagen worden de dieren 's morgens en 's avonds worden de dieren met Temgesic behandeld om een adequate pijnbestrijding te kunnen garanderen.

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

, voorzitter
p/a Secretariaat DEC-UM
Postbus 616
NL-6200 MD Maastricht
Telefoon: 043

Aan:

Uw referentie: Onze referentie : Maastricht, 24-11-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*The role of microRNA and microRNA- in two models of pressure overload-induced heart failure*", is op de DEC vergadering van 18 november 2011 besproken.

De DEC heeft één enkele vraag/opmerking:

1) Volgens de DEC staat er een fout bij punt 8. (All groups) moet volgens de DEC groep 1 t/m 4 zijn. De DEC verzoekt dit nog aan te passen.

Opmerking:
De DEC hoopt dat er gewerkt wordt aan verfijning van het model.

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 **2011-146**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Maastricht, 29 november 2011

Betreft: DEC 2011-146

Geachte DEC,

Middels deze brief wil ik graag Uw vragen beantwoorden met betrekking tot project 2011-120, "*The role of microRNA- and microRNA- in two models of pressure overload-induced heart failure*". De aanpassingen zijn grijs gemarkeerd in de aanvraag.

- In punt 8 van de aanvraag zijn de correcte groepen tussen haakjes aangegeven bij de beschreven procedures.

Ik hoop hiermee Uw vraag naar voldoening te hebben beantwoord.

Met vriendelijke groet,

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	30-11-2011

Project: *The role of microRNA and microRNA in two models of pressure overload-induced 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:	**2011-146**
Diersoort:	**muis**
Aantal dieren:	**126**
Einddatum:	**30-11-2015**

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
T (043)

Bezoekadres

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