| **Begeleidingsformulier aanvraag dierproef DEC-UM**<br>*Versie 2006* | **Herziene versie** | **DECNR: 2011-077**<br>**Ontvangen: 31-05-2011** |
|---|---|---|

| **DEC datum goedkeuring#** | Type aanvraag [2] |
|---|---|
| **21-06-2011** | Nieuw |

| **VROM/GGONR[3]** |
|---|
| **DGM/RB IG09-75** |

| **LNV/CBDNR[4]** |
|---|
| |

| Hoofdproject | CARIM<br>X | NUTRIM | Hersenen en gedrag | GROW | biomaterialen | Ander UM | Geen UM |
|---|---|---|---|---|---|---|---|
| Deelproject | 2. | 1. 2. 3. 4. | 1. 2. 3. | 1. 2. 3. | | | |

| Financieel beheerder | | Budgetnummer | 3098.2.298N |
|---|---|---|---|

Titel van het onderzoek:

Role of microRNAs during pathological cardiac remodeling following myocardial infarction.

startdatum Sept-2011 einddatum [9] Sept-2012 *Duur van de proef[10]:* *4 weeks*

| | 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] | | | | Art.9 | |
| 4. overige uitvoerenden | | | | Art.12 | |

| Diergroep | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| ctrl/exp/sham | sham | sham | MI | MI | sham | sham | MI | MI |
| Diersoort | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 |
| Stam | C57BL/6 | C57BL/6 | C57BL/6 | C57BL/6 | C57BL/6 | C57BL/6 | C57BL/6 | C57BL/6 |
| Construct / mutatie ? | WT | aMHC-miR199b | WT | aMHC-miR199b | vehicle | ant-199b | vehicle | ant-199b |
| Herkomst (leverancier) * | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 |
| Aantal | 41 | 11 | 61 | 16 | 11 | 11 | 16 | 16 |
| Geslacht | M/F | M/F | M/F | M/F | M/F | M/F | M/F | M/F |
| Dieren immuuncompetent ? | Ja | Ja | Ja | Ja | Ja | Ja | Ja | Ja |
| Leeftijd/gewicht | 8 wks | 8 wks | 8 wks | 8 wks | 8 wks | 8 wks | 8 wks | 8 wks |
| Doel van de proef * | 37 | 37 | 37 | 37 | 37 | 37 | 37 | 37 |
| Belang van de proef * | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 |
| Toxicologisch onderzoek * | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 |
| Bijzondere technieken * | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 |
| Anesthesie * | 04 | 04 | 04 | 04 | 04 | 04 | 04 | 04 |
| Pijnbestrijding * | 04 | 04 | 04 | 04 | 04 | 04 | 04 | 04 |
| Mate ongerief * | 05 | 05 | 05 | 05 | 05 | 05 | 05 | 05 |
| Toestand dier einde exp* | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 |

* VHI-coderingen zie bijlage

1 Verantwoording

*Aanvraag dierproef DEC-UM* (kaders zijn licht flexibel, maar het geheel is max. 5 pag. versie 2006)

**Titel: Role of microRNAs during pathological cardiac remodeling following myocardial infarction.**

**1. Doel van de proef.**

The general goal of this study is to better understand the role of microRNAs in the pathological cardiac remodeling that follows a myocardial infarction.

One specific aim of this study is to investigate the role of microRNA-199b (miR-199b) in the progression of cardiac hypertrophy and heart failure. MicroRNAs (miRs) are small, non-coding RNA molecules that post-transcriptionally regulate gene expression by base-pairing to target messenger RNAs (mRNAs). Recent work from our group (1) showed an increase in the expression of miR-199b in calcineurin transgenic mice, a genetic model of cardiac hypertrophy, and in murine hearts that were subjected to aortic constriction. To further understand the function of this miR in cardiac disease we have generated transgenic mouse lines overexpressing miR-199b in the postnatal myocardium, using the alpha-myosin heavy chain promoter. Overexpression of this miR did not result in an obvious cardiac phenotype in mice up to 6 months of age. However, cardiac stress in these animals either by cross breeding them with a calcineurin transgenic mouse model of heart failure (Calcineurin Tg) or after inducing pressure overload by transverse aortic constriction (TAC) did result in an exaggerated cardiac phenotype, with exaggerated cardiac hypertrophic growth. In the same study we showed the effect of knockingout miR-199b in vivo by using a novel class of chemically engineered oligonucleotides (termed 'antagomirs') to genetically block microRNA expression in vivo. We specifically silenced the activation of miR-199b in a Calcineurin tg mouse and in mouse models of cardiac pressure overload (TAC) by infusing a miR-199b specific antagomir. Histologic and molecular biologic analysis showed that in these two animal models, antagomir-199b treatment could prevent and even reverse geometric and functional pathological cardiac remodeling by modulation of NFAT activity. Analysis of cardiac function by Doppler/ echocardiography revealed that antagomir-199b treatment prevented and reversed left ventricular dilatation and normalized fractional shortening and systolic and diastolic contractile defects. Altogether, we have evidence that miR-199b plays an important role in Calcineurin/NFAT signaling. Furthermore, very recent data from our group (DEC #2010-085) indicate that Calcineurin/NFAT activity is necessary during cardiac pathological remodeling also following myocardial infarction.

**Objective 1a**. Because miR-199b is regulated by NFAT and NFAT activity seems to be necessary for cardiac remodeling following myocardial infarction, we plan to determine whether mice that overexpress miR-199b are sensitized to pathological remodeling following myocardial infarction. For that we will subject miR-199b transgenic animals to myocardial infarction and characterize their cardiac function by echocardiography. We expect that these animals will develop a exaggerated phenotype with worse cardiac function showing that miR-199b also has a crucial role in this cardiac condition.

**Objective 1b**. Because miR-199b is regulated by NFAT we also plan to subject wildtype animals to myocardial infarction and treat them with an antagomir-199b in order to antagonize the Calcineurin/NFAT signaling pathway. We expect that this strategy will reduce pathological cardiac remodeling and concomitant heart failure development

following myocardial infarction.

**Objective 2**. Define microRNA expression patterns during cardiac pathological remodeling following myocardial infarction. For this we will subject wildtype animals to myocardial infarction and collect cardiac tissue from all relevant areas (infarct, border zone and remote myocardium) at different time points post-infarct (6 hours, 3 days and 1 week). Total RNA will be isolated and RNA samples will be used for the profiling experiments (microRNA arrays).

**2. Maatschappelijke relevantie en/of wetenschappelijk belang**

In the Western world, the prevalence and incidence of heart failure are increasing steadily and heart failure is now the leading cause of hospitalization in the elderly. However, the progress in developing new heart failure therapies has been impaired by an incomplete comprehension of the signaling events underlying cardiac dysfunction.

**3. Alternatieven**

This study deals with pathological cardiac remodeling and the signaling pathways that are instigated during the process. There are up to date no cell lines in culture that can mimic the type of environment that we are interested in. One of our goals is to knockdown miR-199b with a pharmacological agent, antagomir-199b, which although very efficient, is very expensive. Furthermore, coronary artery ligation in rodents is widely used in medical research as an experimental model of heart failure induced by ischemia, and therefore, mimicking patients with coronary artery disease after experiencing a myocardial infarction episode. Altogether, we choose the mouse as animal model, a model with which our group has developed extensive expertise both in coronary artery ligation, as well as in administration of antagomirs. And since the mouse is the smallest mammal in which clinically relevant data can be produced which may help unfolding questions about the heart disease, we have chosen for this animal model to perform our in vivo experiments.

**4. Ethische afweging**

In the western world heart failure has the highest mortality and morbidity of all diseases. This brings great strain on our society, both from an economical, as well as a medical point of view. Therefore, it is of great importance to gain better insight in the molecular mechanisms that drive myocardial pathogenesis and which may help discovering novel therapeutic measures.

**5. Wetenschappelijke onderbouwing**

Heart failure, or the inability of the heart to meet hemodynamic demands, represents the end of stage of various forms of cardiac disease. In the Western world the prevalence and incidence of heart failure are increasing steadily, and heart failure is now the leading cause of hospitalization in the elderly (2). However, progress developing new therapeutic measures has been impaired by an incomplete comprehension of the signaling events underlying heart failure (3).

**Objective 1.**

Over the last several years, calcineurin has been the focus of intense research interest based on the discovery that it plays a central role in the main hypertrophic signaling cascade (4). Elevated intracellular Ca2+ levels result in the activation of the Ca2+/calmodulin-dependent phosphatase calcineurin. In turn, once activated, calcineurin will dephosphorylate members of the nuclear factor of activated T-cells (NFAT) transcription factor family (5-7). This results in translocation of NFAT into the nucleus where in cooperation with other transcription factors it will regulate the expression of calcineurin-sensitive target genes. Earlier studies from our group have demonstrated that the pro-hypertrophic properties of calcineurin are contingent upon NFAT activation, leading us to believe that calcineurin-NFAT activation represents a nodal point in the control of cardiac pathologocal remodeling and eventual failure.
Recently we have identified one microRNA, miR-199b (1), as a direct calcineurin/NFAT target gene. MiR-199b increases in expression in mouse and human heart failure, and targets the nuclear NFAT kinase, dual specificity tyrosine-(Y)-phosphorylation regulated kinase 1a (Dyrk1a), constituting a pathogenic feed forward mechanism that affects calcineurin-responsive gene expression. Because very recent data from our group (DEC #2010-085) also indicate that NFAT activity is necessary during cardiac pathological remodeling following myocardial infarction and because miR-199b is regulated by NFAT we plan a) to determine the sensitivity of animals that overexpress miR-199b to myocardial infarction and b) to subject wildtype animals to myocardial infarction and treat them with an antagomir-199b in order to antagonize the Calcineurin/NFAT signaling pathway. We expect that a) animals with high expression of miR-199b are more susceptible to myocardial infarction and therefore will develop a stronger remodeling response with impaired cardiac function and b) treatment of animals with an antagomir will reduce pathological cardiac remodeling and concomitant heart failure development following myocardial infarction.

**Objective 2.**

Despite the significant impact of reperfusion strategies in ameliorating the mortality and morbidity of myocardial infarction, there is still significant room for increasing the quantum of myocardial salvage. Resultantly, the maintenance of cell numbers is critical to the overall preservation of both structural integrity and function of the heart. It is possibly for this reason that cardiac myocytes are extremely resistant to activation of cell death programs compared to other tissues in the body (8, 9). However, given the appropriate stimulus, cardiac myocytes do die. Studies indicate that cardiac myocytes may undergo all three forms of programmed cell death, i.e., apoptosis, necrosis and autophagic cell death. While the core of the infarct with anoxia and near-total energy depletion displays necrotic changes, the periphery with impaired but somewhat intact oxygen supply display changes suggestive of varying amounts of autophagy, apoptosis and necrosis. (10). Programmed cell death is an important concept. Cell death is "programmed" if it is genetically controlled.

Apoptosis and autophagy-associated cell death are the two fundamental types of programmed cell death (8, 11) The recognition that cell death can occur by genetically controlled processes has enabled advances in unraveling the mechanisms of many diseases, and this new knowledge has facilitated the development of pharmacologic agents that initiate or inhibit programmed cell death (10, 12-14)Moreover, there is also evidence that necrosis, traditionally considered an accidental form of cell death, can in certain instances be initiated or modulated by programmed control mechanisms (15-18). Preliminary data in our lab generated from cardiac profiling of miR expression levels in different mouse models of cardiac disease and also biopsies of human subjects with ischemic heart disease identified a few miRs that seem to be regulating genes involved in apoptosis and autophagy. In order to better understand the role of miRs and also the specific role of the three different types of cell death in and around the infarct area it is necessary to define miR expression patterns during cardiac pathological remodeling following myocardial infarction. For this we will subject wildtype animals to myocardial infarction and collect cardiac tissue from all relevant areas (infarct, border zone and remote myocardium) at different time points post-infarct (6 hours, 3 days and 1 week). Total RNA will be isolated and RNA samples will be used for the profiling experiments (microRNA arrays).

**6. Wetenschappelijke beoordeling**

This project application has been read and approved by
UM).

5 Proefdier

**7. Proefdier keuze**

**7a. Soort, stam / herkomst / eindbestemming**

Adult (8 weeks of age) C57BL/6J and α-MHC-miR199 (miR-199b transgenic mice), of both sexes (males and females) will be obtained from our mouse colonies at laboratories. At the end of the study, the animals will be submitted to euthanasia in order to collect tissues for further biomolecular analysis. Re-use of animals is therefore not possible.

**7b. Sexe**

Mice of both sexes (males and females) will be used to prevent unnecessary extra breeding and disposal of animals.

**7.c. Aantallen**

The relative effect (difference in experimental parameters) that we can expect in our study is known. Different parameters will be measured or assessed in this study. The parameter with the most inter-individual variability is the contraction capability of the heart (measured by echocardiography). For the sample size calculation we adopted the equation proposed by Sachs ($N=2[(Z_{\alpha/2}-Z_{\pi})2/(\delta/\sigma)2]$, in "Principles of Laboratory Animal Science" (editors: LFM van Zutphen, V Baumans, AC Beynen, ISBN:0444506128). We will start from a minimal relative effect ($\delta$) of 30%. We know from previous experience that the variation coefficient ($\sigma$) is 25% (variability between the animals that undergo the same experimental procedures and are genetically related). We also adopted a power value of 0.80 ($\pi$) and a 0.05 for alpha ($\alpha$). Finally, we calculated the minimal number of animals: 10.9 (or 11 animals per group).

The mortality after MI surgery is maximal 30% (spanning a period of 4 weeks, intra operative and as a consequence of heart failure development). 30% will be added to the group size of 11, which will result in 16 animals per group to correct for the expected mortality (30% of 16 = 11.2 animals). Because all animals stay alive after the sham surgeries, we decided to take a much smaller group sizes in the sham-operated groups.

For objective 2 we need to isolate 7-10μg of total RNA from every region of the infarct and therefore have to collect cardiac tissue from all relevant areas (infarct, border zone and remote myocardium) at different time points post-infarct (6 hours, 3 days and 1 week). From previous experience and to be able to isolate such amounts of RNA from such small pieces of tissue we estimate that we will need tissue from at least 10 hearts per each condition. Taking into account a mortality rate after MI surgery of 30% we will need 15 animals per condition (30% of 15 = 10.5). Because all animals stay alive after the sham surgeries, we decided to take a much smaller group sizes in the sham-operated groups (10 animals per group).

| Objective 1a | genotype | procedure | animals | total |
|---|---|---|---|---|
| | WT | sham | 11 | |
| | WT | MI | 16 | |
| | α-MHC-199b | sham | 11 | |
| | α-MHC-199b | MI | 16 | 54 |
| **Objective 1b** | **procedure** | **treatment** | **animals** | **total** |
| | Sham | Vehicle | 11 | |
| | Sham | Antagomir-199b | 11 | |
| | MI | Vehicle | 16 | |
| | MI | Antagomir-199b | 16 | 54 |
| **Objective 2** | **procedure** | **Time post-MI** | **animals** | **total** |
| | Sham | 6 hrs | 10 | |
| | Sham | 3 days | 10 | |
| | Sham | 1 week | 10 | |
| | MI | 6 hrs | 15 | |
| | MI | 3 days | 15 | |
| | MI | 1 week | 15 | 75 |
| **Total** | | | | 183 |

Dierproef

**8. Experiment**

General experimental setup:
For all three objectives, animals will be divided in two subgroups: Sham and MI. The mice will be housed in special cages (4-5 per cage), in a soundproof room under conditions of controlled humidity, temperature and a 12h light-dark cycle (lights on from 7am to 7pm). Mice will receive surgery at the Maastricht University. The day of the MI surgery all animals that will be operated will receive 0.05-0.1 mg/kg/BW of buprenorfine s.c. (minimally 30 minutes before surgery). This will be repeated every 6-12 hours during the 24-48 hours postoperative. Animals will also receive NSAID (Carprofen 2.5-5mg/kg/BW s.c.) once a day, for three consecutive days (including the day of surgery). For more details see "9b. Pijnbestrijding".

First the animal is weighed. The animals will be anesthetized with isoflurane (induction: 3-4%, maintenance: 1.5-2.5%) in an induction box. When the mouse is under complete anesthesia, the anterior thorax is shaved at the top of the sternum. After cleaning, the mouse is fixed to a heating pad (set at 37°C) with self-made loops or tape. The mouse is then intubated with a canulla (blunt 20-gauge needle). The canulla is connected to a volume cycled rodent respirator (Hugo Sachs Elektronik model 845) on oxygen, mixed with isoflurane in a closed system with a tidal volume of 0.25 ml and a respiratory rate of 150-210 breaths/min. Further procedures are performed with visual help of a micro-

dissecting microscope.

The actual surgery is performed under sterile conditions and is in accordance with
- cm incision in the left parasternal in the skin is made between the 4th and 5th ribs. After large and small muscle dissection, the thorax is entered through the 3rd intercostal space (±2mm lateral to the sternum) and pulled with two microretractors. Sterile gauze, moistened with sterile saline, is used to keep the lungs aside to avoid contact during the surgical procedure. The pericardium is opened and, with minimal manipulation of the fat pad surrounding the heart, the left anterior descending coronary artery can be easily visualized. A 6-0 prolene suture is then passed under the left coronary artery at 1mm distal to the left atrial appendage, immediately after the bifurcation of major left coronary artery. The chest wall is closed with two single knots of an absorbent suture (5.0) between the 4th and 5th ribs. The skin is also closed with two single knots of a non-absorbent suture (5.0). At the end of the surgical procedure the animals will receive 0.5ml warm sterile saline 0.9% by s.c. injection (animals still under anesthesia) to prevent dehydration caused by the anesthesia. The animals will be let to recover in a heated room with a temperature of 30°C. The sham-operated group is submitted to a similar procedure but without the coronary artery ligation.
Two days after surgery all operated animals will receive two injections, one at 7am and one at 7pm to completely cover these days with sufficient analgesic treatment.

Specific setup for objectives 1a and 1b
Two days after surgical procedures both sham and MI groups will be submitted to a high-resolution transthoracic echocardiographic exam (Vevo 2100, VisualSonics, Toronto, Canada), using a single-element mechanical transducer with a center frequency of 30 MHz, under anesthesia (2% isoflurane and 98% oxygen), in order to evaluate myocardial infarction area. In addition, echocardiography will be again performed in the middle (2wks) and at the end of the protocol (4 weeks) to evaluate cardiac ventricular dimensions (M-mode) and function.
At the end of the protocol (4 wks), animals will be submitted to a terminal hemodynamic evaluation When the mouse is under general anesthesia (urethane, 2.5mg/kg i.p.), the neck is shaved and disinfected with iodine. Mouse is positioned in a hotplate with a temperature probe put into the rectum to keep body temperature controlled at 37°C. Both right carotid artery and jugular vein are dissected. A catheter (PE10) is placed in the jugular vein for intravenous injections. A Millar catheter is positioned into the left ventricle (via right carotid artery) enabling minimally invasive and continuous intracardiac pressure measurements. Left ventricle end-diastolic pressure, and max and min dP/dt (as indexes of cardiac contractility) are measured at basal conditions and after dobutamine infusions (1-8μg/min),
After the terminal hemodynamic measurements (4 wks), when the animals are still under anesthesia, mice will be sacrificed by cervical dislocation. Tissue will be collected to determine body, heart, lung and liver weights and tibial length (morphometrical indications of cardiac hypertrophy and heart failure) and to perform histological and molecular biological analysis.

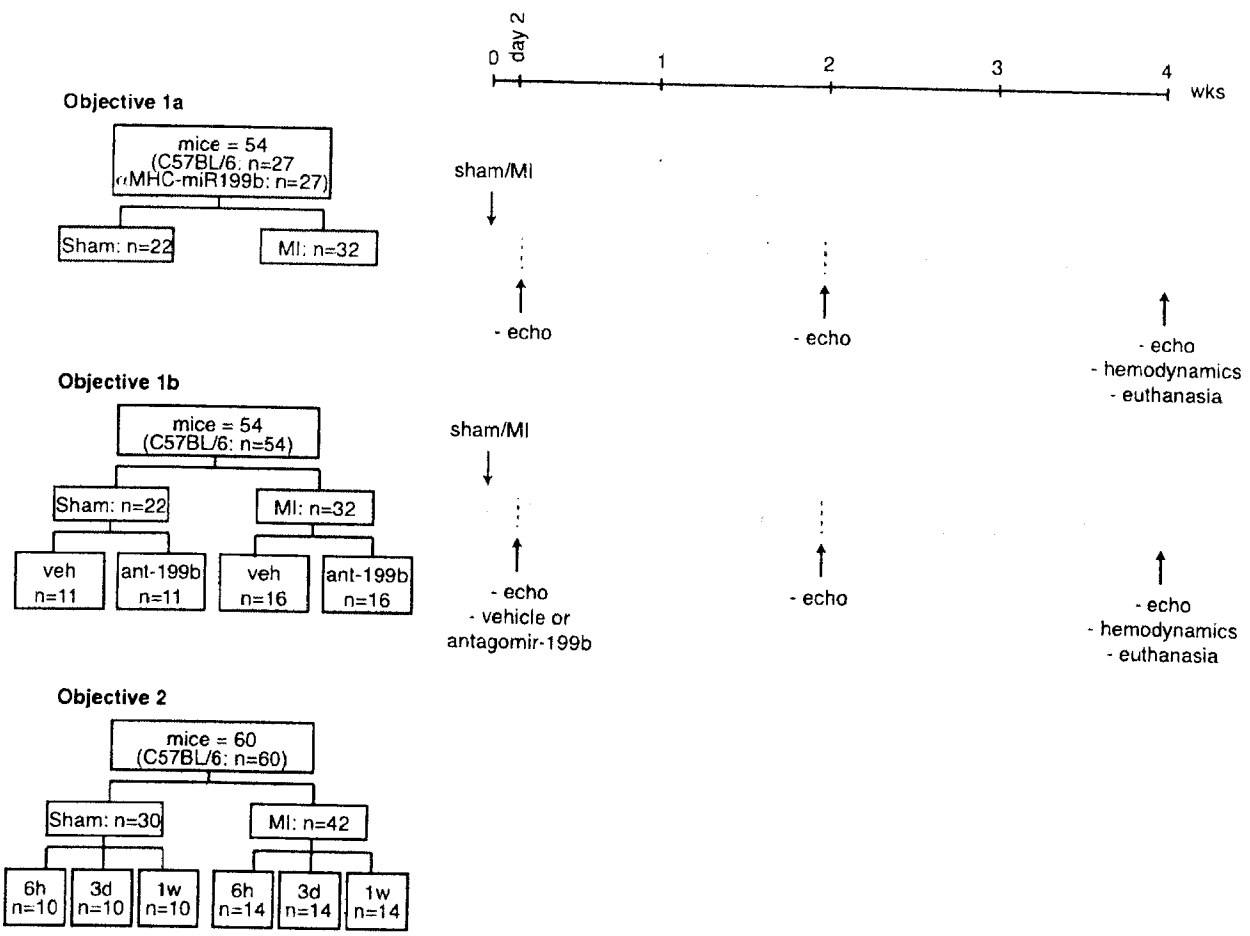
Specific setup for objective 1b
Sham and MI groups will be divided in two subgroups: vehicle (saline) and antagomir-199b. Two days after surgery, the animals will receive vehicle (PBS, 0.1 ml/day) or

antagomir-199b (80mg/kg/BW/day) by i.p. injection on three consecutive days. Antagomir administration will take place once the high-resolution transthoracic echocardiographic exam has been performed and initial cardiac function has been assessed.

Specific experimental setup for objective 2

For this objective the animals will also be subjected to either sham or MI surgery according to the general procedure mentioned above. However, in this part of the study we will not follow the animals for longer then 1 wk and we are not assessing cardiac function by echcardiography. The main goal is to obtain cardiac tissue at different time points post-MI (6 hrs, 3 days and 1 wk). For that, the animals will be sacrificed at the respective time points post-MI, by cervical dislocation and tissue from the different infarct areas will be collected for total RNA isolation and consecutive microRNA expression profiling.

Flow charts for all objectives:



**9. Experimentele condities**

**9a. Anesthesie**

For the MI surgical procedure mice will be initially anesthetized with 3-4% isoflurane and maintained with 1.5-2.5% of isoflurane during the procedure via an endotracheal tube, according to During echocardiographic evaluation mice will be placed under general anesthesia through isoflurane inhalation (induction 3-4%, maintenance 1.5-2.5%). The anesthesia is mixed with medical air and is administrated through facemask and testing of eye and toe reflex constantly to control the depth of anesthesia. For the terminal hemodynamic evaluation mice will be anesthetized with uretane (2.5mg/kg i.p.).

**9b. Pijnbestrijding**

Pre-operative: 0.05-0.1mg/kg BW of buprenorfine s.c. (minimum 30 min before operation) with a duration of pain relief of 6-12 hours. Post-operative: 0.05-0.1mg/kg BW buprenorfine s.c. repeated every 6-12 hours (after previous dose) with an effect for 24-48 hours in combination with NSAID (carprofen 2.5-5mg/kg/BW s.c.), once a day for 3 consecutive days (including the day of operation). If necessary additional pain relief will be given.

**9c. Euthanasie en Humane eindpunten**

With all types of surgery, especially with complex microsurgery like this, there is always a chance for unforeseen inconvenience. If an animal shows any signs of complications, the animal will be immediately euthanized to prevent any further pain. From our experience, we expect 20% of the animals to die during the procedure, but these animals will be under complete anesthesia and therefore will suffer mild inconvenience. Postoperatively, we expect 10% of the animals to die of the surgical procedure, due to lethal arrythmias and recovery from surgery. However, we have experienced that these animals die in the first two days after procedure and we attempt to shield this with buprenorphine at twelve hour intervals for 48 hours. At the end of the study the animals will be sacrificed by cervical dislocation under anesthesia. Organs will be collected for further analysis so that re-use of animals in not possible. A humane end-point will be carried out when animals display: changes in physical appearance (e.g. coat texture: hair soiled with urine or faeces), changes in clinical signs (e.g. dyspnea; posture, >15-20% body weight loss; changes in food and water consumption; presence of persistent and non-treatable infection at the site of surgery; severe discomfort after surgery), changes in unprovoked behaviour (e.g. self mutilation; compulsive behaviour; posture; grooming patterns; activity levels); behavioral changes in response to external stimuli (e.g. excitability; righting reflex). If euthanasia is necessary, a person with WOD art.12 and/or a person with WOD art.14 will be consulted for advise.

Zorg

***10a. Ongerief***

Estimated discomfort levels:
- s.c. injection of warm saline (under anesthesia): level 2
- s.c. injection of temgesic: level 2
- echocardiography (including induction and recovery from anesthesia): level 2
- MI (including induction and recovery of anesthesia, recovery after surgery at day 1): level 3
- anesthesia procedure for hemodynamic measurements and euthanasia: level 2
- i.p. injection of urethane: level 2
- i.p. injection of saline: level 2
- i.p. injection of antagomir-199b: level 2
- development of heart failure (during the 4wk period): level 5

In total, the discomfort level is classified as: 5.

| Groups | Genotype/ treatment | | | s.c. saline | s.c. temgesic | | Euthanasia (cervical dislocation) | i.p. saline | i.p. ant-199b |
|---|---|---|---|---|---|---|---|---|---|
| sham | WT | 3x | 1x | 1x | 4x | 1x | 1x | | |
| | aMHC-199b | | | | | | | 3x | |
| | vehicle | | | | | | | | 3x |
| | antagomir | | | | | | | | |
| | Objective2 | | 1x | | | | 1x | | |
| MI | WT | 3x | 1x | 1x | 4x | 1x | 1x | | |
| | aMHC-199b | | | | | | | 3x | |
| | vehicle | | | | | | | | 3x |
| | antagomir | | | | | | | | |
| | Objective2 | | 1x | | | | 1x | | |

Echocardiography in the mouse
: Myocardial infarction (MI) in the mouse
Terminal hemodynamic characterization of the mouse

The animals will experience severe discomfort due to the surgery. In addition, animals will experience moderate discomfort during the echocardiographic exam. The combined discomfort is therefore qualified as severe (score 5). From previous experience and literature it is expected that mice will totally recover from MI surgery after 1 week.

**10b. Welzijnsevaluatie**

The body weight (measured weekly) will be used to assess the well being of the mice. Animals will also be checked for any discomfort (any hunching). The first 24 hours of recovery after surgery may be critical for the animals but most of the animals will show complete recovery. However, after a period of 4 weeks after surgery the animals may develop heart failure, characterized by cachexia, increased respiratory rate, lack of physical activity, edema in the legs and poor fur. A person with WOD art.12 and/or a person with WOD art.14 will be consulted for advise in case the animals display any of the signs mentioned above and euthanasia may be necessary.

**11. Verzorging en huisvesting**

Both C57BL/6 and αMHC-miR-199b animals will be bred at . Laboratories. The animal facility (CPV) of the Maastricht University will be responsible for the housing and caring of the animals during the protocol. The mice will be housed in special cages (4-5 per cage), in a soundproof room under conditions of controlled humidity, temperature and a 12h light-dark cycle (lights on from 7am to 7pm). Mice will be

allowed free access to tap water and chow. The surgical procedures and the echocardiographic exams will be performed at the , and the molecular analysis will be done at the

**12. Deskundigheid**

The myocardial infarction surgery, the echocardiography and hemodynamic exam and other procedures (cardiac perfusion for histological analysis and euthanasia) will be performed by a zoological technician, (art.12), (art. 9, postoctoral fellow) and (art. 9, assistant professor), from the Department of and with extended experience in the surgical techniques mentioned. All surgeries will be executed at the Department of . In case of emergency/questions on the animal condition, we will contact a person with WOD art.12 and/or a person with WOD art.14.

**13. Standard Operation Procedures**

All procedures described here will be in accordance to the established by the Maastricht University in the mouse); Far-05-M (myocardial infarction in mice); (terminal hemodynamic characterization of the mouse).

**Relevante literatuur**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University Maastricht

Faculty of Health, Medicine
and Life Sciences

Dierexperimenten Commissie

DEC

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Postbus 616
NL-6200 MD Maastricht
Telefoon:

Aan:

Uw referentie:

Onze referentie :

Maastricht, 31-05-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Role of microRNAs during pathological cardiac remodeling following myocardial infarction*", is op de DEC vergadering van 27 mei 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt de diersoort van groep 7 op het voorblad te vermelden.
- De DEC wijst erop dat de uitval bij punt 7c niet correct is berekend (zie aanvraagformulier-website CPV, voor de berekening). Dit heeft gevolgen voor de groepsgrootte. De DEC verzoekt dit aan te passen en het voorblad in overeenstemming te brengen.
- Bij punt 9b constateert de DEC een typefout; dosis buprenorfine moet zijn: 0.05-0.1 mg/kg (en niet 0.05-01 mg/kg) BW iedere 6-12 uur.

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 **2011-077**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Maastricht, 31-05-2011

**Project 2011-077**

Dear

Thank you for the evaluation of my DEC proposal.

Regarding the questions/comments, I have corrected the "voorblad" and also the proposal. Changes are marked in grey.

- we have mentioned the "diersoort" van group 7 in the front page (01);

- the calculations were done has followed: 1) for assessment of cardiac function post-MI we need 11 animals per MI group; taking into account the expected mortality rate of 30%, we need 16 animals per group (30% of 16 = 11.2); these calculations were correct in the original proposal; 2) for RNA isolation from cardiac tissue (MI) we need 10 hearts per group; taking into account the expected mortality rate of 30%, we need 15 animals per group (30% of 15 = 10.5), these calculations were wrong in the original proposal and we have now changed the numbers of animals requested both in the proposal and the front page

- the typing error on the buprenorfine concentration was corrected in the proposal.

I hope I have informed you enough and that the changes made will be sufficient to get the project approved.

With kind regards.

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

| *Ons kenmerk* | *Doorkiesnummer* | *Maastricht* |
|---|---|---|
| | 043- | 23-06-2011 |

**Project:** *Role of microRNAs during pathological cardiac remodeling following myocardial infarction.*

**Verantwoordelijk onderzoeker (VO):**

Hierbij 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:** | **2011-077** |
| **Diersoort:** | **muis** |
| **Aantal dieren:** | **183** |
| **Einddatum:** | **21-06-2015** |

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

Voorzitter DEC-UM

Vice-Voorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

*Secretariaat DEC-UM*
T (043)

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