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Begeleidingsformulier aanvraag dierproef DEC- UM

Herziene versie

DECNR: 2011-099

Ontvangen: 03-08-2011

DEC datum goedkeuring#	Type aanvraag [2]	VROM/GGONR[3]	LNV/CBDNR[4]
26-08-2011	Nieuw	IG 10-023	

Hoofdproject	CARIM						
Deelproject	2.						

Financieel beheerder		Budgetnummer	30983307 N

Titel van het onderzoek:

Follow up on DEC protocol 2010-126: Does long term NOX4 inhibition lead to an improved heart function after infarction or ischemia-reperfusion of the heart?

startdatum 22-7-2011 einddatum [9] 22-7-2014 *Duur van de proef [10]:* *28 dagen*

	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				Art. 12	
5. PI and hoofd Farmacologie					

Diergroep	1	2	3	4			
ctrl/exp/sham	Exp set 1 Group 1 (Exp)	Exp set 1 Group 2 (Control)	Exp set 2 Group 3 (exp)	Exp set 2 Group 4 (control)			
Diersoort	Mice	Mice	Mice	Mice			
Stam	C57Bl6	C57Bl6	C57Bl6	C57Bl6			
Construct / mutatie ?	NOX4 KO	Wildtype	NOX4 KO	Wildtype			
Herkomst (leverancier) *	01	01	01	01			
Aantal	46	46	56	56			
Geslacht	M	M	V	V			
Dieren immuuncompetent ?	ja	ja	ja	ja			
Leeftijd/gewicht	2-3maanden	2-3maanden	2-3maanden	2-3maanden			
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 *	5	5	5	5			
Toestand dier einde exp*	1	1	1	1			

Follow up on DEC protocol 2010-126: Does long term NOX4 inhibition lead to an improved heart function after infarction or ischemia-reperfusion of the heart?

1. Doel van de proef.

In earlier experiments (I refer to DEC 2010-126) we studied the effects of NOX4 gene abolition on ischemia/reperfusion in the acute phase (24 h after ischemia reperfusion) in the heart. Until now, we found a reduction of 30% in the infarct size in the NOX4 KO mice, but no difference in the functional parameters measured by ultrasound. These first results suggest that NOX4 KO mice may also be partly protected against cardiac damage in similar long-term studies when cardiac function will decrease in time due to detrimental remodeling processes. Evidence from mouse pressure overload models (Zhang et al 2010, Kurado et al.) mechanistically suggest that NOX4 may affect angiogenesis and cell growth and differentiation. However, up to now the effect of modulating NOX4 has never been studied in the setting of the long-term cardiac remodeling following ischemia-reperfusion of the heart.Therefore, we would like to investigate the long term effects of NOX4 gene abolition after ischemia/reperfusion, focusing on angiogenesis and the development of heart failure. In addition, we would like to extend our experiments by using a mouse model of permanent occlusion of the coronary artery (infarct mouse model), as this model represents an even more severe form of cardiac ischemia, with different long term remodeling.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

Ischemic heart disease and myocardial infarction present a heavy burden for society as they are major causes of death and disability in the growing elderly population. The proposed experiments will gain insights into the mechanisms behind these devastating diseases and help to open new doors to therapeutic strategies to treat or prevent them.

3. Alternatieven

We aim to establish the role of NOX4 in ischemia-reperfusion damage and permanent ischemia in the heart on the longer term. Since there is a complex interplay within an organism between organs, and regulatory systems (e.g.hormones and cytokines), it is not possible to study these effects in vitro. Animal experiments are the only option to study this complex disease. Since we aim to test the feasibility of a new approach that in future can be translated into a clinically applicable concept, pre-clinical experiments are desirable.

4. Ethische afweging

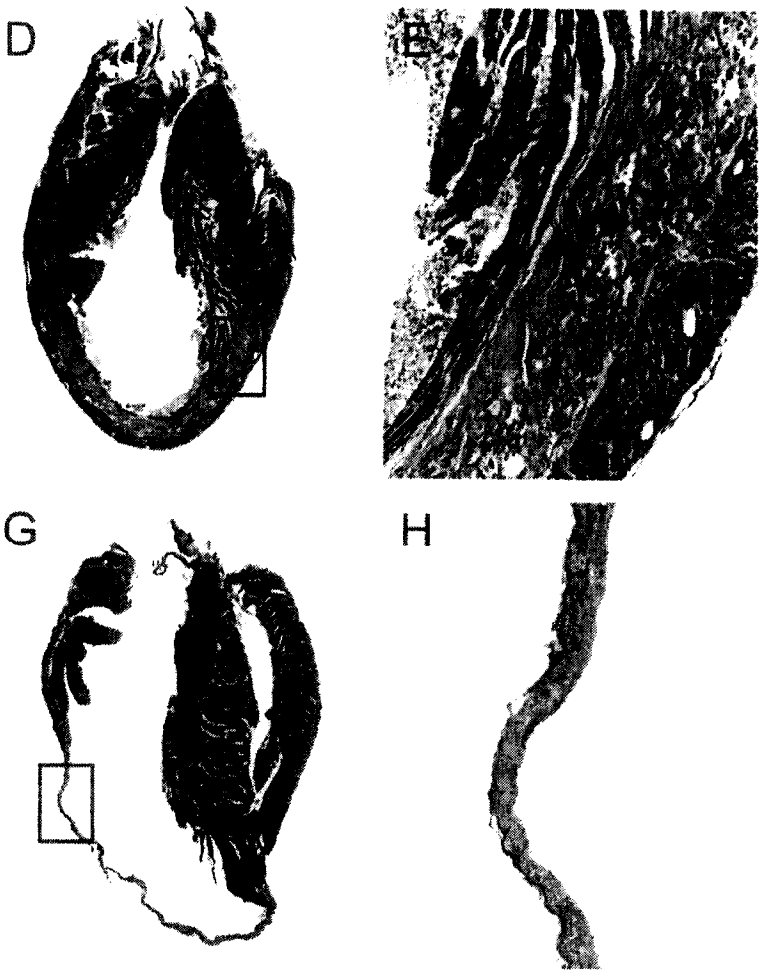
While the experiments on the one hand will help to find potential new therapeutic strategies to tackle cardiac ischemia-reperfusion damage and thus improve the life expectancy and quality of life in man, they on the other hand will inevitably lead to serious discomfort in animals. In our view we consider that the serious discomfort of the animals is outweighed by the scientific importance.

5. Wetenschappelijke onderbouwing

If the blood supply to a tissue is temporarily reduced by transient occlusion of the main feeding blood vessels, followed by a reestablishment of blood flow during reperfusion, oxidative stress i.e. excessive reactive oxygen species (ROS) formation occurs , resulting in extensive tissue damage. Nevertheless, despite the well established role of ROS in the pathophysiology of major cardiovascular (ischemic) diseases, so far the therapeutic success of tackling the produced ROS by using antioxidants like vitamin C and E has been disappointing [1]. Whereas the antioxidant approach relies on inactivating ROS once they are formed, a different - and in our opinion more meritorious - approach to tackle ROS-mediated tissue damage is to prevent their formation by blocking the trigger of the chain of events that results in excessive ROS production in the target issue. NADPH oxidase (NOX) is a family of enzymes known to produce ROS and to be involved in a number of important pathological and physiological events [2,3]. The different types of NOX enzymes display distinct tissue distribution patterns and can be activated in a highly localized manner *e.g.* by ischemia. It is believed that once activated, NOX derived ROS initiate the local activation of a battery of other enzymes that subsequently generate ROS in bulk amounts [4]. Thus, with NOX being the initiator of a cascade resulting in excessive pathologic ROS formation, inhibiting NOX seems a promising approach to reduce tissue damage in ischemia-reperfusion. Indeed, the research group of (f UM), recently discovered that in mice in which the NOX4 gene was abolished (NOX 4 knockout mice) ischemic stroke produced far less brain tissue damage (smaller infarcts) than in normal (wildtype) mice. In addition, applying a NOX inhibitor to mice also reduced stroke induced brain damage, even when the inhibitor was applied 2 hours after the ischemia. This indicates that NOX4 plays a crucial role in ischemia-reperfusion damage in the brain and that NOX inhibition opens challenging new therapeutic perspectives. However, it is currently unknown whether these stroke findings also apply in a more general fashion to ischemia reperfusion. Myocardial infarction - even if perfusion is restored - leads to extensive cardiac tissue damage, hence increased risk for heart failure, reduced quality of life and death. Therefore, we aim to elucidate the role of NOX4 in ischemia-reperfusion injury in the heart.

We already showed that after 24 hours of reperfusion, the infarct size is reduced in NOX4 KO mice.(DEC 2010-126). There is also evidence that NOX4 plays a role in angiogenesis and cell differentiation/growth in the chronic phase after damage to the heart.[5,6] At a time-point of 4 weeks after ischemia/reperfusion or infarction, the heart tissue will have had enough time to regenerate and the remodeling should thus be final at that point. It is thus an ideal time point to study the effects of NOX 4 on remodeling and angiogenesis.

Figures to depict the difference in long term cardiac remodeling between I/R (D and E) en myocardial infarction (G H) as studied in our department before: see DeCelle et al, 2004.



Since the cardiac remodeling after I/R and infarction is different, as shown by earlier studies in our department [7], we would like to test both the I/R model and the more severe myocardial infarction model. We will divide our experiments into 2 phases:
During phase 1 we will investigate the effects of Nox4 gene abolition after I/R and MI only in male mice. We will start with the infarct model, since a difference there might be easier found, when positive results are found, we will also do the I/R model. If positive results are found in the male mice, we will also extend our experiments to female mice (phase 2).

6. Wetenschappelijke beoordeling
This specific project has been approved by

Proefdier

7. Proefdier keuze

7a. Soort, stam / herkomst / eindbestemming

For all experiments, homozygous NOX 4 knockout mice (developed at Monash University and now transferred and bred at Maastricht University, (GGO approved: IG 10-023) will be used along with wildtype mice (C57Bl6 mice). The reason for using mice is the fact that genetically modified strains are available (NOX4 KO mice, but also NOX 4 flox/flox mice and other NOX isoenzyme KO/floxed that are planned for later experiments) and that we would like to be able to compare the experimental results. In addition, the Pharmacology lab is well equipped for conducting surgery, hemodynamic measurements, histology and other techniques using mice.
At the end of the experiments all animals will be sacrificied.

7b. Sexe

In phase 1, we will only use male mice, since it is known that the infarct healing is more pronounced in males and thus a difference might be easier to find. If results are positive we will also use females in phase 2,since we are testing the feasibility of a potential treatment option for cardiac ischemia-reperfusion damage, that may be translated to a clinical application in the future. Furthermore, we do have evidence that NOX abolition results in subtle sex dependent differences in metabolic processes. Therefore, it is not unlikely that sex differences in the effect on cardiac damage may be observed.

7.c. Aantallen

In experiment set 1, we will be assessing functional parameters, angiogenesis, infarct size and the development of heart failure after myocardial infarction or ischemia/reperfusion. Our primary read-out will be histologic assessment angiogenesis and functional measurements (dp/dt and ultrasound). We would like to be able to find a difference in these parameters of 50%. **We will start with only males doing the infarct model and if positive results are found also test the ischemia/reperfusion model and also look at females.**

Using a power analysis according to the formula $n=2x\ s^2x\ (Z\alpha/2+Z\beta)^2/D^2$(L. Sachs, Angewandte Statistik, Springer, 1983, Berlijn, Springer Verlag), with an α 0,05 and a power of 80%, a minimal statistically assessable treatment effect (D) of 50% **for the males and 40% for the females** and a variance of 50% (s) **(based on the long experience at out own animal lab with this model and results from our earlier study in these mice)**, this implies that at a power of 80 % the minimum number of animals **for the males** is $n= 15.7\ s^2/D^2 = 15.7*(0.5/0.5)= 15.7$ per group. Taking into account an animal loss (due to acute mortality of the infarction and possible infarct rupture afterwards) of 30 %, this implies a total animal number of 15.7/0.7 = 22.4 = 23 per group. Since we will test 2 methods, we need to double the amount of animals, resulting in 46 per group.

For the females we need $n=15.7\ s^2/D^2 = 15.7*(0.5/0.4)=19.6$ per group. Again, taking into account the animal loss of 30%, the total number of animals will be 19.6/0.7= 28 per group. Doubling this because of the 2 different models gives a total of 56.
Thus the total number of animals is
Experiment set 1: 46 male NOX4 KO mice + 46 male wildtype mice = 92 mice
Experiment set 2: 56 female NOX4 KO mice + 56 female wildtype mice = 112 mice
Total= 204 mice

Dierproef

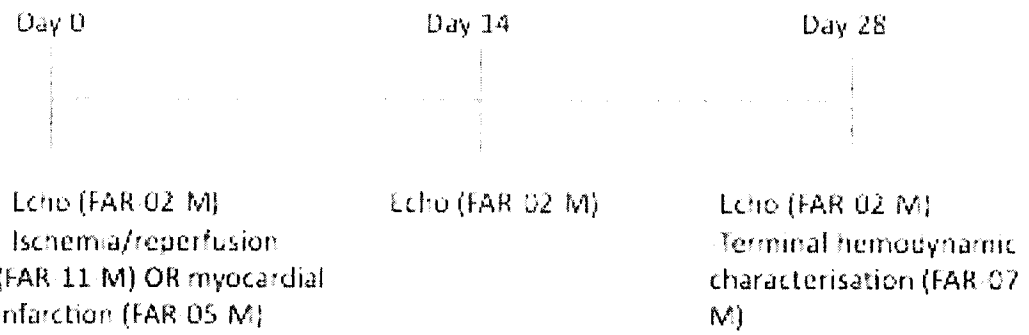
8. Experiment

Aim experiment set 1: To-investigate the role of NOX4 in the chronic phase after ischemia-reperfusion injury and myocardial infarction in male mice. **We will start with using 10 mice of each genotype testing the infarct model, when positive results we will also use the other mice of this group. For the ischemia/reperfusion model we will also start with 10 mice and use the full set of animals if positive results are found.**

Aim experiment set 2: To investigate the role of NOX4 in the chronic phase after ischemia-reperfusion injury and myocardial infarction in female mice.

At day 0, 14 and 28 an echocardiography will be made. On day 0, a myocardial infarction or ischemia/reperfusion will be conducted. At day 28, a terminal hemodynamic measurement will be conducted (exp set 2 and 3) or animals will be sacrificed by terminal bleeding (exp set 1). Organs will be collected for further analysis.

Timeline:



9. Experimentele condities

9a. Anesthesie

In all experiments anesthesia (and analgesia) will be applied according to the approved SOPs. At day 0, day 14 and day 28, echocardiography (FAR-02-M). On day 0, a myocardial infarction (FAR-05-M) or ischemia/reperfusion (FAR-11-M) will be conducted under isoflurane anesthesia (initiating at 3-4%, maintenance at 1.5-2.5 %). Finally, at day 28, immediately after the echocardiography under isoflurane anesthesia:

- exp set 1 and 2: terminal hemodynamic measurement (FAR-07-M), harvesting of tissue for further analysis

9b. Pijnbestrijding
Analgesia will be performed according to the approved SOPs. Buprenorphine will be used as analgesic instead of NSAID's. NSAID's not only have an analgesic effect but also an anti-inflammatory effect. Myocardial infarction and ischemia/reperfusion injury cause oxidative stress, a process intertwined with inflammation. NOX4 is involved in this oxidative stress response, moreover remodeling and angiogenesis (which are the points we want to study) are also affected by the inflammation response. Thus NSAID's would interfere with our measurements. Buprenorphine will be applied s.c. at a dose of 0.05 mg/kg **once before** surgery, once 8-12 hours later and once another 8-12 hours later.

9c. Euthanasie en Humane eindpunten
Animals will be inspected once daily and monitored for signs of illness and discomfort (e.g. passive behaviour also at approaching, lack of grooming, > 15 % weight loss, skin/fur condition). At the first 48 hours after surgery, animals will suffer from discomfort because of the surgery and the acute response to myocardial infarction. By giving analgesics (Buprenorphine) this discomfort will be kept to a minimum. Should severe discomfort signs (**e.g >15% weight loss, lack of grooming, passive behaviour also at approaching, signs of heart failure: edematous increase in body weight, respiratory distress**) be observed after the first 48h after surgery, the animals will be sacrificed by applying a pentobarbitone overdose (200 mg/kg i.p, diluted 1:10). Should other signs of discomfort or signs of inflammation and disease be observed, the Article 14 animal welfare officer will be consulted and if necessary the animals will be sacrificed.

10a. Ongerief

Nature of intervention	Approximate Duration	Frequency	Discomfort
Exp set 1 (group 1 and 2)			
Echo (under isoflurane anesthesia)	10-15min	3	Code 02
Myocardial infarction/ischemia-reperfusion (mouse is kept under anesthesia after first echo)	45-60 min	1	Code 05
Living after MI/IR surgery	4 weeks	1	Code 03
Terminal haemodynamic measurement (after waking up after the last echo)	40-45min	1	Code 02
Experiment set 2 (group 3 and 4)			
Echo (under isoflurane anesthesia)	10-15min	3	Code 02
Myocardial infarction/ischemia-reperfusion (mouse is kept under anesthesia after first echo)	45-60 min	1	Code 05
Living after MI/IR surgery	4 weeks	1	Code 03
Terminal haemodynamic measurement (after waking up after the last echo)	40-45min	1	Code 02

Cumulatively the total discomfort is **code 05**

10b. Welzijnsevaluatie

As said, the mice will be regularly inspected for signs of illness or discomfort. In our experience, ischemia of the heart may lead to an acute (within 12-24 hours) drop-out of 30% of the animals due to sudden death (cardiac arrest, arrhythmias and sometimes infarct ruptures). During the first few days , animals are in the acute phase of inflammation and healing after a myocardial infarction/ischemia-reperfusion. This could result in discomfort which will be kept to a minimum by applying analgesia as desribed in point 9b. After recovery of this acute wound healing phase no extra discomfort is expected. It is possible that cardiac function is very much depressed and animals will be inspected for clinical signs of heart failure (edematic increase in body weight and respiratory distress). . Should this occur, the animal will be sacrificed as specified under 9. Should other signs of illness, discomfort or disease occur, the Article 14 animal welfare officer will be contacted.

11. Verzorging en huisvesting

Surgery and 1 day of recovery (in a special heated room) of the animals will take place in the animal lab of . Thereafter animals will be housed socially at the CPV with food and water ad libitum and cage enrichment. In case of questions or problems (e.g. the observation by the CPV that an animal's condition is getting worse), an article 12 person of the animal lab of may be contacted or as the responsible article 9 researcher.

12. Deskundigheid

Myocardial infarction and ischemia-reperfusion experiments will only be conducted by article 12 staff. All other animal handling procedures will be conducted by experienced article 12 staff or by article 9 staff (under supervision of article 12 staff). The involved article 12 staff has previously conducted similar experiments on numerous occasions.

13. Standard Operation Procedures (SOP)

For the procedures/animal handlings (including anesthesia and analgesia) we would like to refer to the approved SOPs on the CPV website, i.e., SOP FAR-02-M (echocardiography), SOP FAR-07-M (terminal hemodynamics; dP/dt) , SOP FAR-11-M (Ischemia/reperfusion) and SOP FAR-05-M (myocardial infarction)

Relevante literatuur

1. Dotan Y, Pinchuk I, Lichtenberg D, Leshno M. Decision analysis supports the paradigm that indiscriminate supplementation of vitamin E does more harm than good. *Arterioscler Thromb Vasc Biol*, 29(9), 1304-1309 (2009).
2. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev*, 87(1), 245-313 (2007).
3. Lambeth JD. Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy. *Free Radic Biol Med*, 43(3), 332-347 (2007).
4. McNally JS, Davis ME, Giddens DP *et al.* Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. *Am J Physiol Heart Circ Physiol*, 285(6), H2290-2297 (2003).
5. Kuroda et al. NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. *PNAS* 107 (35) p. 15565-15570
6. Zhang et al; NADPH oxidase-4 mediates protection against chronic load-induced stress in mouse hearts and by enhancing angiogenesis. *PNAS* 107 (42) p.18121-18126
7. de Celle et al; Long-term structural and functional consequences of cardiac ischaemia-reperfusion injury in vivo in mice. *Exp biol* 2004; 89(5): 605-615
8. vd Borne et al; Mouse srain determines the outcome of wound healing after myocardial infarction. *Cardiovascular research* 2009; 84: 273-282

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 [illegible]

Aan:

Uw referentie: Onze referentie Maastricht, 19-07-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Follow up on DEC protocol 2010-126: Does long term NOX4 inhibition lead to an improved heart function after infarction or ischemia-reperfusion of the heart?*", is op de DEC vergadering van 15 juli 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- Bij punt 4 verzoekt de DEC het woord "serious" toe te voegen bij discomfort (serious discomfort) en de zin "In addition the discomfort, enzovoort" te verwijderen.
- De DEC constateert bij punt 7c dat er waarschijnlijk een typefout is gemaakt, te weten 64 moet 46 zijn (2 x 23). Er moet dan ook verder gerekend worden met 46.
- De DEC vraagt zich of de aantallen bij experiment 3 hetzelfde zijn als bij experiment 2, aangezien de onderzoekers kleinere verschillen verwachten bij experiment 3.
- De DEC wil graag weten waarop de 50% variatie is gebaseerd.
- Punt 8- Het is ethisch onverantwoord dieren in experiment te nemen waar niet volledige data over worden verkregen. De DEC begrijpt dat de informatie verkregen uit experiment 1 ook verkregen wordt uit experiment 2. Daarom ziet de DEC niet in dat hiervoor 40 extra dieren, met ongerief code 5 moeten worden gebruikt. Tijd en beschikbaarheid mogen hiervoor geen rol spelen. Dit is voor de DEC geen argument.
- Bij punt 9c verzoekt de DEC "severe discomfort signs" toe te lichten.

Conclusie:
Het project wordt aangehouden.

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 2011-099, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

[illegible]/Voorzitter DEC-UM

University Maastricht

Faculty of Health, Medicine and Life Sciences

Aan: DEC UM

02/08/2011, Maastricht

Betreft: DEC aanvraag 2011-099

Geachte DEC-leden,

Mijn projectaanvraag: *Follow up on DEC protocol 2010-126: Does long term NOX4 inhibition lead to an improved heart function after infarction or ischemia-reperfusion of the heart?*", is op de DEC vergadering van 15 juli 2011 besproken en als project aangehouden.

Onderstaande opmerkingen zijn aangepast in de nieuwe versie:

- Bij punt 4 verzoekt de DEC het woord "serious" toe te voegen bij discomfort (serious discomfort) en de zin "In addition the discomfort, enzovoort" te verwijderen.
- De DEC constateert bij punt 7c dat er waarschijnlijk een typefout is gemaakt, te weten 64 moet 46 zijn (2 x 23). Er moet dan ook verder gerekend worden met 46.
- Bij punt 9c verzoekt de DEC "severe discomfort signs" toe te lichten.

Verder had de DEC de volgende opmerkingen/vragen:

- De DEC vraagt zich of de aantallen bij experiment 3 hetzelfde zijn als bij experiment 2, aangezien de onderzoekers kleinere verschillen verwachten bij experiment 3: **Als we inderdaad een verschil gaan vinden in de groep vrouwtjes, zal dit verschil waarschijnlijk kleiner zijn dan bij de mannen. We willen dan ook het aantal gevraagde dieren verhogen, maar daarbij opmerken dat we, indien we geen verschil zien bij de eerste dieren, we deze 'extra' dieren niet zullen gebruiken.**
- De DEC wil graag weten waarop de 50% variatie is gebaseerd: **deze 50% variatie in met name de dp/dt metingen en echografische metingen is gebaseerd op de jarenlange ervaring die met dit model is opgedaan op het dierenlab van de farmacologie. Daarnaast hebben we resultaten van onze eerdere NOX4 muis studie die laten zien dat de spreiding in de basiswaarden bij de dp/dt metingen behoorlijk groot is. Zie hiervoor onderstaande tabel met de dp/dt basiswaarden van respectievelijk de NOX4KO en NOX4 WT muizen:**

	Dp/Dt uitgangswaarde	
Animal	KO	WT
1	2436.00	3604.00
2	3994.00	4020.00
3	2725.00	5777.00
4	5797.00	7054.00
5	6607.00	5417.00
6	6070.00	4384.00
mean	**4604.83**	**5042.67**
SD	1799.298465	1286.412324
variatiecoefficient	39.07412788	25.51055639

Bij de echografische data laten een aantal waarden een soortgelijke spreiding zien. Om er dus zeker van te zijn dat we valide resultaten verkrijgen, waarbij ondanks de spreiding, verschillen tussen de groepen aan te tonen zijn, gaan we daarom uit van een spreiding van 50%.

- Punt 8- Het is ethisch onverantwoord dieren in experiment te nemen waar niet volledige data over worden verkregen. De DEC begrijpt dat de informatie verkregen uit experiment 1 ook verkregen wordt uit experiment 2. Daarom ziet de DEC niet in dat hiervoor 40 extra dieren, met ongerief code 5 moeten worden gebruikt. Tijd en beschikbaarheid mogen hiervoor geen rol spelen. Dit is voor de DEC geen argument: **de pilot is geheel weggelaten in de nieuwe versie, we zullen eerst starten met het infarctmodel (inclusief alle metingen) en daarna bij positieve resultaten uitbreiden naar het ischemie/reperfusie model en naar het vrouwelijk geslacht.**

Hopende hiermee te hebben voldaan aan de gevraagde wijzingingen van de DEC, verblijf ik,

Met vriendelijke groet,

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	30-08-2011

Project: *Follow up on DEC protocol 2010-126: Does long term NOX4 inhibition lead to an improved heart function after infarction or ischemia-reperfusion of the heart?*

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:	**2011-099**
Diersoort:	muis
Aantal dieren:	204
Einddatum:	26-08-2015

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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