Begeleidingsformulier aanvraag dierproef DEC- UM *Versie 2006* **Nieuw**	**DECNR: 2011-004** **Ontvangen: 14-01-2011**

DEC datum goedkeuring#	Type aanvraag [2]
28-01-2011	Nieuw / Herz.versie / Pilot

VROM/GGONR[3]

LNV/CBDNR[4]

Hoofdproject	CARIM	NUTRIM	Hersenen en gedrag	GROW	biomaterialen	Ander UM	Geen UM
Deelproject	2.						

Financieel beheerder		Budgetnummer	3098.2228.N

Titel van het onderzoek:

Implication of the Purkinje network for CRT effectiveness.

startdatum 01—02-2011 einddatum [9] 01-02-2015 *Duur van de proef [10]:* *1 day*

	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[8]	
5.PI				Art.9/	

Diergroep	1	2	.	.	.	.	.
ctrl/exp/sham							
Diersoort	11						
Stam	NZW						
Construct / mutatie ?	-						
Herkomst (leverancier) *	01						
Aantal	42						
Geslacht	M/F						
Dieren immuuncompetent ?	Ja	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]
Leeftijd/gewicht	2.5-3.5						
Doel van de proef *	31						
Belang van de proef *	01						
Toxicologisch onderzoek *	01						
Bijzondere technieken *	01						
Anesthesie *	04						
Pijnbestrijding *	04						
Mate ongerief *	02						
Toestand dier einde exp*	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: . Implication of the Purkinje network for CRT effectiveness.

1. Doel van de proef.

The His-Purkinje system (HP) enables fast conduction of cardiac action potentials and is responsible for synchronous activation of the ventricles. Left bundle branch block (LBBB) is the most frequent and most studied pattern of conduction disturbance. However, the underlying mechanisms of the HP system remain largely unknown. Cardiac Resynchronization Therapy (CRT) has been validated as a treatment for heart failure with conduction disorders like LBBB. The basic principle is to apply biventricular stimulation to (partly) restore electrical synchrony. For this purpose, we previously demonstrated that endocardial left ventricular (LV) pacing is more efficient than LV epicardial pacing. It is not understood whether the HP network participates in the LV activation during CRT and if it influences the subsequent hemodynamic effect. In order to study the HP network we want to compare a model with an intact HP network with a model with a disabled HP network (using chemical ablation).We aim to assess the impact of a chemical ablation of the LV's HP network:

- on the LV activation compared to a proximal LBB ablation.
- on the effectiveness of CRT with LV epicardial pacing and LV endocardial pacing

2. Maatschappelijke relevantie en/of wetenschappelijk belang

Approximately one third of patients with heart failure present with conduction disturbances are most commonly depicted as a LBBB. However, it is not known whether this LBBB is due to a proximal or distal lesion in the HP system. CRT improves morbidity and mortality but at least 30% are 'non-responders'. If we demonstrate that different types of LBBB exist depending on the level and the extent of the HP network disease:

- It could be an explanation for the differences observed in clinic in response to CRT
- Future studies in CRT (animal models and computer modelling) can take this into account.
- Specific evaluation of the HP system is needed to better select new patients.

If we demonstrate that CRT response is in part influenced by the underlying HP function:

- Better results are expected with new pacing strategies (like endocardial pacing)

Improvement in the patient selection and the pacing technique may lead to improvement in efficacy and performance of the CRT.

3. Alternatieven

We want to investigate the properties of the entire Purkinje system in the heart, therefore an in vitro study would be not relevant. Computer model studies are currently limited by the lack of knowledge about contribution of the HP system in a paced heart.[2]

We need to excise a beating heart to perform the working heart model, so we need to take out the heart when the animal is still alive. Therefore we can't use slaughterhouse material. For training purposes, the working heart model and HP ablation (see 7C), we could use, if available, rabbit hearts from other experiments.

4. Ethische afweging

There are at least 15 million patients with heart failure in Europe. Overall, 50% of patients are deceased after 4 years. Because CRT improves the functional status and decreases hospitalizations and mortality, the search to improve this therapy is of major interest. The findings of these studies will be very helpful for the understanding of the physiological basis of dyssynchrony and CRT. In the present study the only discomfort

for the animal is induction of anesthesia. Therefore, the researchers are convinced that it is legitimate to use the animals for this study.

3 Wetenschap

5. Wetenschappelijke onderbouwing

The HP network consists of thin fibers, mainly just below the ventricular endocardium. It conducts the electrical activation approximately 4 times as fast as the normal myocardium, because of its dedicated architecture and fast conduction properties.

During sinus rhythm, this specialized conduction system stimulates the ventricles at multiple points termed "Purkinje-ventricular junctions" (PVJ). Conduction disturbance within the left ventricle is most often depicted as a LBBB on the surface ECG. LBBB deteriorates both diastolic and systolic left ventricular (LV) function and constitutes as a risk factor for the development and progression of cardiovascular diseases.[1] It is supposed, but not demonstrated, that several types of LBBB may exist depending on the level of block. The potential implication in the HP system in the ventricular myocardium excitation in case of LBBB (retrograde invasion) is also controversial.[2]

As with other conduction defects, LBBB appears to be irreversible despite pharmacological treatment, but can be mitigated by cardiac resynchronization therapy (CRT) in patients with moderate to severe HF and deteriorated LV systolic function. Resynchronization of atrio-ventricular, inter-ventricular, and left intra-ventricular dyssynchrony can be achieved by atrio-biventricular pacing, the LV being paced by a lead placed in a coronary sinus tributary draining the lateral or posterior LV wall.[3,4] When patients are selected according to electrocardiographic criteria consistent with ventricular dyssynchrony,(i.e. left bundle branch block and a QRS complex duration >120 ms), an immediate haemodynamic improvement is achieved with CRT. Moreover CRT improves quality of life and functional status, decreased hospitalizations and mortality. [5] Unfortunately, 30% of implanted patients have no significant clinical or echocardiographic improvement following CRT. Selection of patient to improve this response rate remains challenging because the pathophysiology of ventricular disturbance is not well understood. The mechanism of the CRT itself is still incompletely understood, an important question remaining being whether biventricular pacing restores a part of the HP network function.

In the rare instances of unsuccessful transvenous implantation, a few operators have implanted the LV lead transseptally to carry out endocardial LV pacing. The interest in this approach was recently renewed by 2 studies in an animal model (from Maastricht) and in humans (from Bordeaux), respectively, which both found a distinctly superior hemodynamic performance associated with endocardial compared with epicardial stimulation.[6,7] LV endocardial pacing provides a faster impulse propagation in the endocardial than in the epicardial ventricular layers, allowing, at least theoretically, a faster LV depolarization. Because of the proximity of the HP system to the endocardium, it has been suggested that the faster activation during endocardial CRT is caused by entering of paced impulses into the HP system. However, there is no data supporting this idea.

6. Wetenschappelijke beoordeling

This proposal has been read and approved by the PI of the research group.

4 Proefdier

7. Proefdier keuze
The Lugol's solution has been already used in an isolated rabbit heart model to assess the involvement of the Purkinje network for ventricular fibrillation genesis.[8,9,10]

7a. Soort, stam / herkomst / eindbestemming
Rabbit, White New Zealand.

7b. Sexe
Male and female.

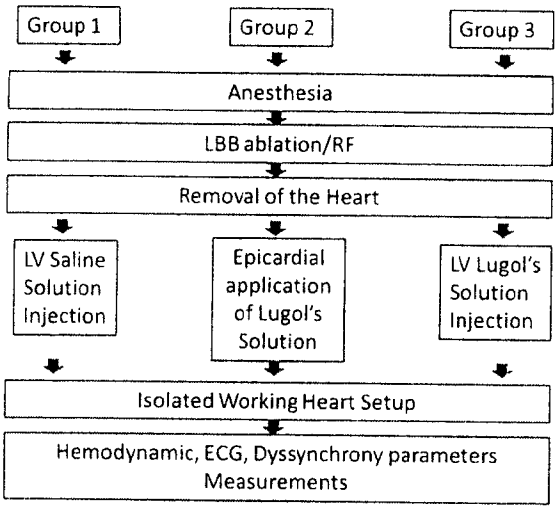
7.c. Aantallen
The isolated working heart setup has been used in several previous studies of this laboratory,[11-13] but we need some animals to develop a proper experimental preparation. 1) The setup is not currently used and we need some training to start it again. 2) The endocardial application of the Lugol's solution has been described in dogs and in rabbits, but we need to learn this technique. 3) We know how to create LBBB in dog with radiofrequency ablation, but it has never been done in the (smaller) rabbit heart. Therefore, for this first "training" part of the study we forecast to use **15** animals.
5 animals to learn the working heart setup
5 animals to develop a HP ablation protocol
5 animals to develop a technique to create LBBB in the rabbit.

Experimental groups will be:
- Group 1: Endocardial saline solution application. Control
- Group 2: Epicardial Lugol's solution application. Sham
- Group 3: Endocardial Lugol's solution application. HP ablation

We anticipate to need 27 animals, as calculated according to Sach's formula:
The readout parameter is maximum increase of left ventricular pressure during contraction.
$F_{0,80} = 15,7 \quad n = 15,7 * (\sigma/\delta)^2$ We expect a variation of 10% and aim at an effect of 15%.
n=15.7*(10/15)= 6.98 animals per group.
Due to technical failure we expect a drop out of 15%. → 6.98/0.85=8.2
For all experimental groups we need **27** rabbits.

Total: 27+15=42 rabbits

8. Experiment



- LBB ablation/RF:

All rabbits will undergo left bundle branch ablation by radiofrequency under anaesthesia with the chest remaining closed. An ECG with LBBB is made.

- Lugol/Saline solution application

Under anesthesia the heart will be removed, immersed in ice-cold perfusion buffer for removal of non cardiac tissue and application of either saline or Lugol's solution. Both solution will be injected by transapical puncture and removed after 20s. Subsequently, the LV cavity will be flushed with saline solution to remove Lugol's solution.

- Isolated working heart setup:

Then the heart is attached to the perfusion apparatus. In this isolated working heart setup, the left atrium is cannulated to allow antegrade perfusion of the heart. The right ventricle will be also filled using a right atrium perfusion. In this working heart the left and right atrial filling pressure can be adjusted as well as the aortic and the pulmonary artery pressures. Epicardial electrodes will be attached to the right atrium and the RV apex. A third plunge electrode will provide epi and endocardial pacing of the LV free wall. Electrograms are recorded using pairs of electrodes attached to the right atrial appendage, the LV anterior and posterior wall. LV and RV pressures will be measured using cannulas introduced into the RV and LV apex. Aortic flow is assessed by means of a flow probe.

For each rabbit heart, ECG, hemodynamic measurement and dyssynchrony will be evaluated during RA pacing (AAI mode), Biv pacing using the epicardial (BIVepi), and the endocardial LV lead (BIVendo) (same heart rate).

- Comparisons will be made between the three groups in terms of ECG, hemodynamic parameters (cardiac output, LVP,RVP, LVdP/dt), dyssynchrony (assessed by echocardiography or sonomicrometry) for the three states: baseline (LBBB), BIVepi and BIVendo.

Comparisons between group 1 and 3 will allow us to understand:
Whether proximal LBBB (ablation) creates a different condition than distal LBBB (Lugol)
Whether the HP system contributes to impulse conduction during BiVepi and BiV endo pacing
Whether BIV endo superiority is related to HP system invasion.
Comparisons between group 1 and 2 (which are supposed to be similar):
To make sure than any differences between group 1 and 2 are due to the HP system and not to myocardial cell injury. (HP system and purkinje-cell junctions are distributed within the endocardium).

9. Experimentele condities

9a. Anesthesie

Induction: Ketamine 35 mg/kg IM and medetomidine 0.1-0.5 mg/kg IM.
Maintenance: Isoflurane 0.5-1.5 %

9b. Pijnbestrijding

Fentanyl 6µg/kg/h. I.V

9c. Euthanasie en Humane eindpunten

The animal will be killed by cervical dislocation under anesthesia.
We do not expect any diseases caused by the protocol, but if the animals encounter unexpected diseases the art 14 is contacted. In agreement with art 14 the animal can be treated/sacrificed.
Human end points are: severe weight loss (>15%) in the days before experiment or unexpected and uncontrollable diseases.

Zorg

10a. Ongerief
Intramuscular injection & anesthesia: Gering/Matig ongerief (code 2)
Sacrifice: Gering/Matig ongerief (code 2)
Totaal: Gering/Matig code 2

10b. Welzijnsevaluatie
-

11. Verzorging en huisvesting
Standard CPV group housing. Water and food ab libitum.

12. Deskundigheid
Most of the experimental work will be performed by k and . Both are used to perform animal experiments. With the help of a former co worker ( ), who has much experience in the working heart model, both technicians and part of our research group) will be trained to learn the working heart model.

13. Standard Operation Procedures (SOP)
Not applicable.

Relevante literatuur

1. Zannad F, Huvelle E, Dickstein K, van Veldhuisen DJ, Stellbrink C, Køber L, Cazeau S, Ritter P, Maggioni AP, Ferrari R, Lechat P. Left bundle branch block as a risk factor for progression to heart failure. Eur J Heart Fail. 2007Jan;9(1):7-14

2. Romero D, Sebastian R, Bijnens BH, Zimmerman V, Boyle PM, Vigmond EJ, Frangi AF. Effects of the purkinje system and cardiac geometry on biventricular pacing: a model study. Ann Biomed Eng. 2010 Apr;38(4):1388-98

3. Vernooy K, Cornelussen RNM, Verbeek XAAM, Vanagt WYR, Van Hunnik A, Kuiper M et al. Cardiac resynchronization therapy restores dyssynchronopathyin canine LBBB hearts. Eur Heart J 2007;28:2148–55.

4. Auricchio A, Prinzen FW. Update on the pathophysiological basics of cardiac resynchronization therapy. Europace. 2008 Jul;10(7):797-800.

5. *Cleland JGF, Daubert JC, Erdmann E, et al. Longer-term effects of cardiac resynchronization therapy on mortality in heart failure (the CArdiac REsynchronization-Heart Failure [CARE-HF] trial extension phase).* Eur Heart J. *2006;27:1928-1932*

6. Van Deursen C, van Geldorp IE, Rademakers LM, et al. Left ventricular endocardial pacing improves resynchronization therapy in canine left bundle-branch hearts. Circ Arrhythm Electrophysiol 2009; 2:580-587

7. Derval N, Steendijk P, Gula LJ, et al. Optimizing hemodynamics in heart failure patients by systematic screening of left ventricular pacing sites the lateral left ventricular wall and the coronary sinus are rarely the best sites. J Am Coll Cardiol 2010; 55:566-575

8. Wennemark Jr, Blake DF, Kezdi P, Lev Extensive intraventricular conduction defect. Experimental production with destruction of total Purkinje-net system of *the canine right ventricle. Circ Res. 1962 Dec;11:904-9*

9. Wu TJ, Lin SF, Hsieh YC, Chiu YT, Ting CT. Repetitive endocardial focal discharges during ventricular fibrillation with prolonged global ischemia in isolated rabbit hearts. Circ J. 2009 Oct;73(10):1803-11.

10. Chen PS, Wolf PL, Cha YM, Peters BB, Topham SL. Effects of subendocardial ablation on anodal supernormal excitation and ventricular vulnerability in open-chest dogs. Circulation. 1993 Jan;87(1):216-29

11. Vanagt WY, Cornelussen RN, Poulina QP, Blaauw E, Vernooy K, Cleutjens JP, van Bilsen M, Delhaas T, Prinzen FW. Pacing-induced dys-synchrony preconditions rabbit myocardium against ischemia/reperfusion injury. Circulation. 2006 Jul 4;114(1 Suppl):I264-9

12. Vanagt WY, Cornelussen RN, Baynham TC, Van Hunnik A, Poulina QP, Babiker F, Spinelli J, Delhaas T, Prinzen FW. Pacing-induced dyssynchrony during early reperfusion reduces infarct size. J Am Coll Cardiol. 2007 May 1;49(17):1813-9

13. Babiker FA, Lorenzen-Schmidt I, Mokelke E, Vanagt WY, Delhaas T, Waltenberger J,Cleutjens JP, Prinzen FW. Long-term protection and mechanism of pacing-induced

postconditioning in the heart. Basic Res Cardiol. 2010 Jul;105(4):523-33.

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan.

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
1	8	03-02-2011

Project: *Implication of the Purkinje network for CRT effectiveness.*

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:	**2011-004**
Diersoort:	**konijn**
Aantal dieren:	42
Einddatum:	28-01-2015

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

Vice-Voorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (04

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