142

Aanvraag dierproef DEC- UM Voorblad Herziene versie	DECNR: 2011-100 Ontvangen: 31-08-2011

DEC goedkeuring#	datum goedkeuring#	type aanvraag2	VROM/GGONR[3]	LNV/CBDNR[4]
31-08-2011		NEW		

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.~~			**Medtronic**

Financieel beheerder		budgetnummer	**Medtronic**

Titel van het onderzoek:

Does AV-node stimulation induce a decrease in cardiac inflammation?

startdatum	September, 2011	einddatum	September, 2013	25-days

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E_mail	Bevoegd-heid[5]	Cap. groep /afdeling
1.Verantwoordelijk onderzoeker (VO)				Art 9	
2. Vervangend VO				Art 9	
3. Biotechnicus					
4. overige uitvoerenden	CPV-team			Art 9/12	CPV
5. overige uitvoerenden				Art.12	
6. overige uitvoerenden				Art 12	
7. overige uitvoerenden				Art 12	

Diergroep	**1**	**2**	**3**	**4**	**5**
Bijzonderheid groep	**EXP**				
Diersoort	**Goat**				
Stam	**Milk goat**				
Construct/mutatie	**nvt**				
Herkomst (leverancier)	**03**				
Aantal	**10**				
Geslacht	**female**				
dieren imm	**YES**				
Leeftijd/gewicht	**~ 60 kg**				
Doel van de proef *	**31**				
Belang van de proef *	**01**				
Toxicologisch onderzoek *	**01**				
Bijzondere technieken *	**01**				
Anesthesie *	**04**				
Pijnbestrijding *	**04**				
Mate ongerief *	**03**				
Toestand dier einde exp*	**01**				
* VHI-coderingen zie bijlage					

Verantwoording

Aanvraag dierproef DEC-UM

Title: Does AV-node stimulation induces a decrease in cardiac inflammation?

1. Aim

Introduction.

We recently showed that we could stimulate the AV-node and reduce ventricular rate during AF both acutely and chronically in goats. Herefore a standard atrial lead was placed at a septal position, where parasympathetic nerves are situated innervating the AV-node. The hypothesis is that AV-Node stimulation induces afferent nerve trafficking from the heart in the direction of the brain, after which a signal is send to many organs to decrease inflammation marker release. This hypothesis is based on the work of Tracey ***(J (Clin Invest. 2007;117:289 –296)*** and Zhang (***Circ Heart Fail.*** **2009;2:692-699.**), who found a relation between systemic inflammation markers and vagal stimulation. Instead of electrical stimulation of the efferent vagus nerve to influence the reticulo-endothelial system (liver, spleen, intestines) directly, we suggest to stimulate afferent sensory vagal nerves to trick the system into thinking that there is an increased inflammatory response. A sensory signal will be send back from the stimulation site to the brain and next integrated in the nucleus tractus solitarus in the brain and be translated into an efferent vagal motor signal affecting the target organ like spleen liver, or intestines. In this way the sensory signal could be generated downstream like in the AV-node. We hypothesisize that such a sensory signal will lead to a decrease in general inflammation markers like TNF-alfa and IL-6 if they will be increased before. Patient groups in which inflammation markers are increased, which cover a large market, and might therefore benefit from vagal stimulation or AVNS are those with heart failure, atherosclerosis, atrial fibrillation, diabetis, shock, angina etc. The advantage of AVNS over vagal stimulation would be that in pacemaker patients only a certain placement of the atrial lead will be required, whereas with vagal stimulation this would require additional surgery in the neck.

Goal:

We would like to test in an AF inflammation goat model if endocardial or epicardial AVNS influences inflammation markers like for example TNF-alfa and IL-6. To get more insight in the mechanism, we will also sense on the vagal trunk in the neck if indeed during AVNS a signal is being sent via the afferent fibres going from the heart to the brain and/or pace sense from epicardial fatpads.

2. Relevance for society

Patient groups in which inflammation markers are increased, which cover a large market, and might therefore benefit from vagal stimulation or AVNS are those with heart failure, atherosclerosis, atrial fibrillation, diabetes, shock, angina, post-operative patients etc.

3. Alternatives We will investigate the intricate relationship of different parts of the body. Namely, the neural and cardiac component. Therefore, isolated cell systems or organs cannot be used. Furthermore, the validity of the concept needs to be tested first. This can only be done in animals and cannot be studied in cells or organs. Furthermore, effectiveness, feasibility, and safety need to be tested first before going to human experiments. To resemble the human as much as possible a study in a larger animal should be performed. The goat will be selected on experience build up in the laboratory with the induction and treatment of AF and AV-node stimulation. Goat's atria are in size comparable to and show similar structural and electrical remodeling as in human atria.
The cardiovascular and neurological system of the goat are similar to that of the human. The reason for the number of animals is explained in 7c below.

4. Ethical considerations
Product safety and efficiency testing ensures that products are safe when used as directed and provides scientific data for physicians in the event a product is misused. Adequate testing of products is both a moral and legal obligation to the public. In the present study we will advance scientific understanding, develop solutions to medical problems and protect the safety of a large group of people, and possibly animals. Animals are used when there is a need to find out what happens in the whole living body, which is far more complex than the sum of its parts (excluding isolated cell systems/organs. It is very difficult, and in this case simply not yet possible, to develop non-animal methods to replace the use of living animals. As explained later we have made great progress on the refinement of our procedures. The experiments will be conducted minimally invasive and in accordance with the highest ethical standards and the maximal possible rate of success.

Patients suffering from diseases related to adverse inflammation (eg HF or AF) have symptoms which greatly impedes their quality of life (eg heart palpitations, dizziness, shortness of breath, emboli). Poor cardiac function can lead to serious complications like multi-organ failure. For instance, cerebro-vascular attacks not only decrease the life-expectancy but also increase the chances of a permanent physical handicap like paralysis. So, the incidence and severity of the complications of HF together with the likelihood of our success in inventing a new way to treat AF justifies the use of goats despite the associated discomfort.

5. Scientific support
The inflammatory process in heart failure patients can cause myocardial damage, while inflammatory agents contribute to the worsening and progression of HF (Levine B, et al. N Engl J Med. 1990; 323: 236-241. ; Niessner A, et al. Eur Heart J. 2009; 30: 789-796.) Our hypothesis is based on the work of Tracey ***(J (Clin Invest. 2007;117:289 –296)*** and Zhang (***Circ Heart Fail.*** **2009;2:692-699.**), who found a decrease in systemic inflammation markers during vagal stimulation. Instead of electrical stimulation of the efferent vagus nerve to influence the reticulo-endothelial system (liver, spleen, intestines) directly, we suggest to stimulate afferent sensory vagal nerves to trick the system into thinking that there is an increased inflammatory response. A sensory signal will be send back from the stimulation site to the brain and next integrated in the nucleus tractus solitarus in the brain and be translated into an efferent vagal motor signal affecting the target organ like spleen liver, or intestines. In this way the sensory signal could be generated downstream like at the nerves innervating the AV-node.

6. Scientific review

7. Animals

7a. Soort, stam / herkomst / eindbestemming

7.a. Species/breed: goat, dutch milk goat.

Former studies of (DEC 9728, 2002-93, 2003-103 and 2004-148) have shown that the Dutch white milk goat is a very suitable model for our purposes. They are bought from local farmers by the CPV. The goat has well defined electrophysiological properties which resemble those of human atria. We obtained experience in this model with stimulating the AV-node chronically in which we were successful (protocols (2007-075 and 2009-031)). With the experience of our team it is possible to do the instrumentation as well as the housing with least possible inconvenience for the animal. The animals will be sacrificed at the end of the experiment.

7.b. Sex: As in our former experiments we would like to continue using female goats because the handling and housing of male goats causes considerably more problems.

7.c. Numbers:

During this study the effectiveness of AVNS will be determined. When using data on the effectiveness of AVNS from previous experiments (DEC protocol 2007-75 and 2009-031) we determined the SD to be 20%. When we want to measure a difference of 30%, using a Power of 80% and α=0.05, Sachs calculations require a group size of 6.9 animals.

Based upon our previous experiments, we anticipate a drop out of 30%, mainly due to the technical challenges during the epicardial/endocardial placement of the different custom-built electrodes during the sacrifices or implantations. Group-size will therefore add up to 10 animals.

8. Experiment

This will be a non-randomized study in which each animal will serve as its own control.

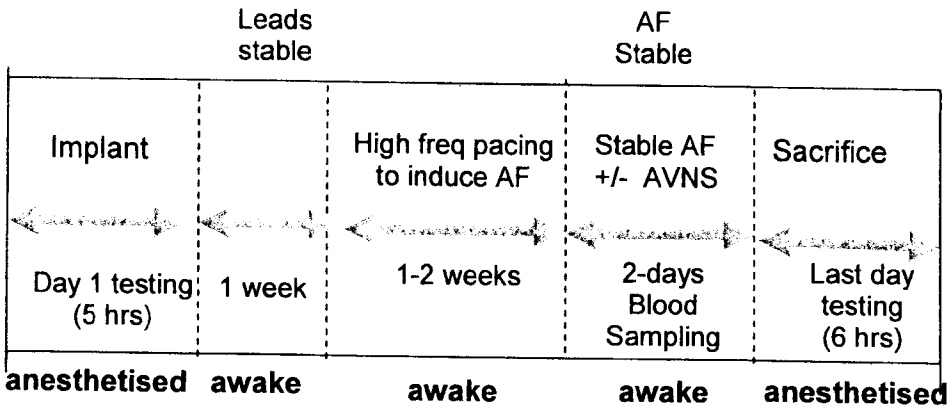
The protocol is shown in figure 1.
During the implant procedure and measurements at day 1 the goat will be aneasthetized. After the lead placements, blood samples will be determined in time (0, 10, 30 min, 1 hr and 2 hours after AF is induced. Subsequently AVNS (10V, 360mics, 40Hz) therapy is started and blood samples are taken at 0, 10, 30 min, 1 hr and 2 hours eafter AVNS is activated.

Next, the animal will be allowed to recover and during one week the leads will have the opportunity to become stable without any intervention. Next, stable AF will be induced by high frequency pacing using the atrial appendage lead for 1 or 2 weeks. If stable AF is induced the high frequency pacing will be stopped and blood will be collected in the awake state on two consecutive days. On these days, AVNS therapy will be turned On for 4 hrs. Blood samples will be collected just before AVNS-start at the end of the four hours AVNS-therapy and two hours after cessation of the AVNS-therapy.

During the sacrifice day (measurement day 2) the experiments will be repeated from day 1 and supplemented with the following: nerve sensing and stimulation protocols and pharmacological interventions studies (Atropine and Esmolol). Cardiac afferent nerve activity will be determined and compound action potentials will be generated and measured on the vagal nerve in the neck. The determination of nerve activity will be done to relate inflammation to nerve activity. The CAPs will provide additional information about the fibers involved and the direction and delay of the signal emanating from the heart.

TNF-alfa and IL-6 will be determined in the blood samples using an Elisa for goats.

Fig.1: Study design



At the implant and sacrifice days, blood samples, hemodynamics, and electrophysiological measurements are taken during AF with and without AVNS at different time-points.

During the week AF is stable AVNS therapy will be added temporarily and on two consecutive days blood samples will be taken (10 cc per time).

Elaborate protocol:

Instrumentation and Lead Placements.

Surface ECG will be derived from limb leads. After intubation and stabilization of the animal, an atrial lead will be positioned in the right atrial appendage for sensing, pacing and induction of AF. A ventricular lead will be placed in the right ventricular apex for sensing and pacing (40 bpm back-up pacing in case of AV-block). A dual sensor Millar® pressure catheter will be placed in this sheath to measure left ventricular and aortic pressures. To identify the correct position for AVNS, the postero-septal area of the right atrium will be mapped under continuous high frequency stimulation (40Hz, amplitude up to 10V, duration 180μs) by evaluating a reduction of RR interval during induced AF or a prolongation of A-V interval or AV-block during sinus rhythm. A-V time will be assessed as the difference between the atrial and the ventricular EGM-signal. Stimuli will be delivered at the distal electrode of an electrophysiology catheter which will be connected to an external high frequency stimulator). At the position with the largest negative dromotropic effect, a bipolar atrial 4F screw-in lead will be positioned, which will be entered via the jugular vein.

At this stage the experiments including hemodynamic, electrophysiological evaluation and blood draw will be conducted (see figure 1).

To perform the chronic AF study, this AVNS lead will be connected to an implantable neurostimulator and the effect obtained with the external

neurostimulator during the implant procedure will be reconfirmed. The lead in the right atrial appendage and the ventricular lead will be connected to a pacemaker (Insync with custom software to induce AF for the chronic study). Both devices will be positioned in individual subcutaneous pockets in the neck, at least 10 cm from each other. Next the animal will recover from aneasthesia and wake up. After a one week recovery period to allow lead stabilization, high-frequency pacing from the right atrial appendage lead will be delivered for one or two weeks to induce stable AF.

The animal will be considered to be in stable AF when AF will be still present 24 hours after the algorithm to induce AF is de-activated. From there on the pacemaker will be turned off for the remaining of the study. AVNS will be turned On for four hours during the two consecutive days before the termination experiment. Blood samples will be collected just before AVNS-start at the end of the four hours AVNS-therapy and two hours after cessation of the AVNS-therapy.
Thereafter, the animal will be aneasthetized again and electrophysiological, hemodynamic measurements will be conducted (see figure 1). These measurements may include also vagal nerve trunk stimulation, CAP measurements and drug administration (atropine and esmolol) combined with AVNS therapy. During drug administration blood samples will be taken.

Vagal trunk nerve stimulation and sensing.
The left vagal nerve will be carefully dissected free from the surrounding tissue at the cervical level and a self-coiling cylindrical cuff electrode configurations will be placed around the nerve. One cuff will be 15 mm long and have three circular Pt/Ir electrode contacts with an inter-electrode distance of 4 mm. The inner electrode will serve as cathode and the two outer as anodes. The cuffs will be made in various diameters (2, 3.5 and 5 mm), to ensure a proper fit around the nerve. Variations in nerve diameter can also be accommodated by the flexibility in tightness of the cuff's coiling.

Compound action potentials (CAPs) will be generated at the AVNS lead and measured at the vagal cuff. The stimulation settings of the CAP will be chosen in that way that the heart is not affected. Recordings of neural activity, CAPs and ECG will be made with a duration of five minutes. We will have a recovery time in between measurements.

9. Experimental conditions
Anesthesia and General Peri-Operative Measures

9a. Anesthesia (both experimental days)

After prescreening of the animals by CPV-personnel the animals will be withheld from "krachtvoer" for 12 hours before the experiments, not from water.

During the implantation day and the termination day:
After, induction of anesthesia with Nesdonal® (thiopental, ~15mg/kg intravenously) the animals will be intubated.

Anesthesia will be maintained by infusion with SuFentanyl (6 ug/kg/h), propofol (2.5-10mg/kg/h) and Dormicum (0.8 mg/kg/h) and a 1:2 mixture of O_2 and air. Body temperature will be kept at 36-38°C. The ventilator will be adjusted to maintain arterial pCO_2 in a range of 40-45 mmHg and arterial pH in a range of 7.35-7.45. Lactated Ringer's solution will be infused through a peripheral vein for euvolemia. Prophylaxis will be provided by administration of intravenous Ampicillin (1g) before the experiment.

In addition, HEPARIN (5000 IE/h) i.v. will be given. During the experiment animals will monitored by ECG, peripheral oxygen saturation and expiratory CO_2 content. To prevent dehydration Ringers Lactate (500cc/h) will be given. An external defibrillator will be available for cardioversion of spontaneous AF and as (backup) for treatment of VF.

9b. Pain medication
Implantation day:
Sufentanyl will provide perioperative analgesia.
Buprenorfine will be administered postoperatively (twice per day 10 ug/kg intramuscular) for the first 24 hours. If necessary, buprenorfine will be continued for the 2 following days.

Pain and health will be evaluated using clinical signs (heart rate, breathing pattern, behavior and looks) and documented in the "welzijnsdagboek".
Pre-operative: 1 g Ampicilin i.v., 3 mg/kg Gentamycine i.v.
Post-operative: 1 g Ampicilin i.m. until 3 days post-operative.

9c. Euthanasia and reason for premature euthanasia.
Euthanesia during the last experiment will be induced by extirpation of the heart

Premature Euthanasia: In case the animals display uncontrollable infections (despite antibiotic treatment, fever above 40 degrees centigrade), serious discomfort (heavy breathing, high heart rate, visible restlessness). Humanitarian endpoints will also be weightloss (>20%).

Zorg

***10a.* Discomfort**

Procedure	Duration	Frequency	Discomfort
Pacemaker implantation incl. Waking up	5 hours	1 X	03
AF maintenance	~2 weeks	1 X	02
AVNS therapy and 3 times blood sampling	~ 4 hours	2 X	02
Terminal experiment	~ 8 hours	1 X	02
Total			***03***

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10b. Animal well being/welfare evaluation.

Discomfort for the animals will be caused by food restriction (12 hours before each experiment), induction (venapunction). Post-operative infection rate after electrode implantation is low. However, there is a slight chance for infection also when the blood is withdrawn for the inflammation markers. During the acute experiment the animal will be anesthetized and therefore the level of discomfort will be kept to a minimum.
Sacrifice experiment: Code 02. Experience from earlier experiments are confirmatory with this welfare evaluation.

11. Verzorging en huisvesting

Goats will be kept in groups (2 or more). Daily care of the animals is in hands of CPV. In need of an emergency (eg in case of infection or illness) see the list of people at the beginning of the protocol.

12. Expertise

Within our group a large experience is at hand for proper execution of the proposed experiments. The CPV-team is licensed and experienced/capable of performing the animal procedures/operations as well as handling the laboratory equipment needed for the present study. or collageue from the University Twente has experience with the sensing equipment and large animal experiments and will perform the measurements.

13. Standard Operation Procedures (SOP)
Instrumentation and Lead Placements.
Cardiac Lead Placements.

SOP 1: Anesthesia

After prescreening of the animals by CPV-personnel the animals will be withheld from "krachtvoer" for 12 hours before the experiments. After induction of anesthesia with Nesdonal® (thiopental, ~15mg/kg intravenously) the animals will be intubated. Anesthesia will be maintained by ventilation with Fentanyl (6 ug/kg/h), Dormicum (0.8 mg/kg/h), propofol (2.5-10mg/kg/h), Pavulon (0.3 mg/kg/h) and a 1:2 mixture of O_2 and air. The addition of pavulon is optional. In addition, HEPARIN (5000 IE/h) i.v. will be given.

During the experiment animals will monitored by ECG, peripheral oxygen saturation and expiratory CO_2 content. To prevent dehydration Ringers Lactate (500ml/h) will be given.

SOP 2: Instrumentation and Lead Placements. Implantation day. (for elaborate protocol see Appendices)

After intubation and acclimatization of the animals, leads will be introduced via the right and/or left jugular veins. Veins will be punctured directly with standard catheterization sheaths. A standard 7F atrial (screw-in) pacemaker lead will be positioned in the right atrial appendage for sensing, pacing and induction of AF. A standard 7F ventricular (screw-in) lead will be positioned in the right ventricular apex for sensing and pacing. In addition, a bipolar costum-made lead will be positioned on the right atrial septum and right atrial appendage. The actual lead positions are determined by the observed effect of stimulation on R-R (or alternatively pressure; thereto an arterial entrance at the a. Carotis or a. Femoralis will be used for the pressure catheter). Leads will be connected to a pacemaker or neurostimulator.

Additions during the Termination day:

For vagal nerve stimulation and sensing the left and if feasible also right vagal nerve will be carefully dissected free from the surrounding tissue at the cervical level and two custom-made self-coiling cylindrical cuff electrode configurations will be placed around the nerve. Optional will be the placements of epicardial leads on the fat-pads of the heart. Thereto, the thorax will be opened from the left side. The 5th rib will be removed for visualization of the heart where after the mapping electrodes can be placed. Mapping and entrainment protocols are initiated. The feasibility of the different entrainment protocols, including the administration of Class 1 and 3 anti-arrhythmic drugs, will be tested.

At the end of the termination experimental protocol the heart will be extirpated or put into fibrillation and the animal will bleed to death or die of pump failure.

Relevant literature

1) The inflammatory reflex. Tracey KJ. Nature. 2002 Dec 19-26;420(6917):853-9.

2) Chronic vagus nerve stimulation improves autonomic control and attenuates systemic inflammation and heart failure progression in a canine high-rate pacing model.
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J Neural Eng. 2010 Dec;7(6):066001. Epub 2010 Oct 22.

4) Vagal tone augmentation to the atrioventricular node in humans: efficacy and safety of burst endocardial stimulation.
Rossi P, Bianchi S, Monari G, Della Scala A, Porcelli D, Valsecchi S, Canonaco S, Kornet L, Azzolini P. Heart Rhythm. 2010 May;7(5):683-9. Epub 2010 Feb 1.

5) Atrioventricular (AV) node vagal stimulation by transvenous permanent lead implantation to modulate AV node function: safety and feasibility in humans.
Bianchi S, Rossi P, Della Scala A, Kornet L, Pulvirenti R, Monari G, Di Renzi P, Schauerte P, Azzolini

P. Heart Rhythm. 2009 Sep;6(9):1282-6. Epub 2009 May 9.

6) Reduction of atrial fibrillation burden by atrial overdrive pacing: experience with an improved algorithm to reduce early recurrences of atrial fibrillation.

Pürerfellner H, Urban L, de Weerd G, Ruiter J, Brandt J, Havlicek A, Hügl B, Widdershoven J, Kornet L, Kessels R. Europace. 2009 Jan;11(1):62-9. Epub 2008 Nov 12.

7) Post-operative atrial fibrillation management by selective epicardial vagal fat pad stimulation.

Rossi P, Bianchi S, Barretta A, Della Scala A, Kornet L, De Paulis R, Bellisario A, D'Addio V, Pavaci H, Miraldi F.J Interv Card Electrophysiol. 2009 Jan;24(1):37-45. Epub 2008 Aug 30.

8) Endocardial transcatheter stimulation of the AV nodal fat pad: stabilization of rapid ventricular rate response during atrial fibrillation in left ventricular failure.

Bianchi S, Rossi P, Della Scala A, Kornet L.

J Cardiovasc Electrophysiol. 2009 Jan;20(1):103-5.

Appendices

SOP Atrial lead implantation in the Goat

12 hours prior to the experiment feeding is withdrawn from the goat, water is given ad libitum. The awake goat is transported in the standard carriage without food/water pot. In the OR the goat is removed from the carriage and fixated (still awake and standing) by one person.

The neck is shaved and cleaned with alcohol. Both sides of the neck are shaved so that the operation field is shaved already as well.
Thiopenthalnatrium (20mg/kg) is injected in one of the jugular veins. Take care NOT to inject the Thiopenthalnatrium in the vein where the lead placed!!
When the goat is sedated, she will be lifted onto the operating table. The goat is placed on the back and held by one person. The neck is placed straight and lifted a bit to help intubation. An endotrachealtube (9/10 mm) is placed in the trachea.

Ventilation is set at physiological settings (10-15/min, 400-600 ml/cycle). Ventilation is controlled and the settings are adjusted to reach the following values; end tidal CO_2 (4.0-4.5 %), O_2 saturation (90-100%) and ventilation pressure (pressure between 15 and 25 cm H_2O) as markers.
The hind leg is shaved and cleaned with alcohol. An intra venous catheter (braunule 16/17G) is placed in the vena ceaphalica. Through this braunule prophylactic 1 gram Ampicilline is given.
Anesthesia will be maintained by Fentanyl (6 ug/kg/h), Dormicum (0.8mg/kg/h). Volume lost is replaced by a continue infusion with Ringers lactaat. Up to 500 ml/h
Depth of anesthesia will be monitored by checking reflexes to sound and pain.

The operation field is shaved already. Loose fur is removed by vacuum cleaner and the skin is washed with aseptic soap. The skin is then disinfected with *jodium tinctuur 2%.*

The jugular vein is prepared, depending on the follow up experiments, the left or the right is chosen. With a Surgipro 4-0 a purse string stitch is made in the vessel. Through this purse string the lead is inserted. Medtronic (Type 5076, 4076 & 5568 (j-lead)) screw-in leads are used. Preferably J-leads because they are easier to place but straight leads are possible as well. Under fluoroscopy the lead is placed in the atrial appendage. Signal is checked for an injury current which, when present, represents successful insertion of the screw in the atrial wall. This fixation is also checked by gently pulling on the lead. Pacing threshold is determined just above normal sinus rate. An external pacemaker used and the pacing threshold is sufficient if the threshold is lower than 1,5 mA. For a short period a high output is administered to check if there is any diaphragm stimulation. In case of a diaphragm contraction the lead is placed in another location. The same procedure is also used for the implantation of the AVNS-lead. This lead will be connected to a second pacemaker (see below).

When a correct position is achieved the purse string suture is closed. Around the lead is a white anchoring sleeve witch at this time is sutured with R-knots to muscle. This is all to fixate the lead in its position. A pacemaker (Itrell, Enpulse, Insync or Kappa from Medtronic or Insignia from Guidant) is connected to the lead and placed in a pocket below the musculus sternocleidomastoideus. During all this the lead had an opportunity to move, so under fluoroscopy is checked of it is still in position. To make sure the pacemaker can't move around under the muscle the pocket is closed with Polysorb 2-0. Then the other wound layers are closed as well with Polysorb 2-0. This is a resorbable suture so the sutures don't need to be removed. During closing of the wound the anesthesia is gradually turned down to help a quick recovery.

After surgery is completed the threshold is checked again using the implanted pacemaker. Also another dose of Ampicilline (1 gram) is administered, this time intra muscular in the hind leg.

The goat then can wake up and is placed back in the carriage. At CPV a specially prepared cage is waiting. This has padded mats so the goat can't hurt herself when she's trying to stand up.

After 1 week of recovery the pacemaker can be turned on.

SOP Implantation of a ventricular lead in the goat

Under general anesthesia the jugular vein is prepared, depending on the follow up experiments, the left or the right is chosen. With a Surgipro 4-0 a purse string stitch is made in the vessel. Through this purse string the lead is inserted. Medtronic (Type 5076, 4076) straight screw-in leads are used. Under fluoroscopy the lead is placed in the right ventricular apex. Signal is checked for an injury current which, when present, represents successful insertion of the screw in the ventricular wall. This fixation is also checked by gently pulling on the lead. Pacing threshold is determined just above normal sinus rate. An external pacemaker used and the pacing threshold is sufficient if the threshold is lower than 1,5 mA.

When a correct position is achieved the purse string suture is closed. Around the lead is a white anchoring sleeve witch at this time is sutured with R-knots to muscle. This is all to fixate the lead in its position. A pacemaker (Enpulse, Insync or Kappa from Medtronic) is connected to the lead and placed in a pocket below the musculus sternocleidomastoideus. During all this the lead had an opportunity to move, so under fluoroscopy is checked of it is still in position. To make sure the pacemaker can't move around under the muscle the pocket is closed with Polysorb 2-0. Then the other wound layers are closed as well with Polysorb 2-0. This is a resorbable suture so the sutures don't need to be removed. During closing of the wound the anesthesia is gradually turned down to help a quick recovery.

After surgery is completed the threshold is checked again using the implanted pacemaker. Also another dose of Ampicilline (1 gram) is administered, this time intra muscular in the hind leg.

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

Aan:

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

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

Geachte Onderzoeker,

Uw projectaanvraag: "*Does AV-node stimulation induces a decrease in cardiac inflammation?*", is op de DEC vergadering van 15 juli 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- Punt 7c- De DEC is van mening dat er een fout is geslopen in de berekening van de aantallen. Uitval gaarne als percentage berekenen over de niet afgeronde aantallen. Inschatting proberen te maken over de totale uitval. Na de goede berekening is het niet nodig om nog 1 of 2 dieren extra te vragen.
- Bij punt 8 wil de DEC graag weten wat wordt bedoeld met de zin "the other 3 experiments"?
- Punt 8- De beschrijving van het experiment komt niet overeen met de tijd, de aantallen, het ongerief en het voorblad. Het lijken nu 2 groepen te zijn, 1 groep die onder anesthesie geeuthanaseerd wordt en 1 groep die nog een aantal weken leeft na de operatieve ingreep. Dat zou betekenen dat er op het voorblad 2 kolommen moeten staan. Gaarne de gehele aanvraag herzien en in overeenstemming brengen.
- Bij punt 9a constateert de DEC dat de beschreven dosering niet van Fentanyl maar van Sufentanyl is. Volgens de DEC interfereert Pavulon met het onderzoek.
- De DEC verzoekt bij punt 9b te beschrijven welke postoperatieve pijnbestrijding wordt toegediend. De beschreven medicatie is geen pijnstilling.
- Bij punt 9c verzoekt de DEC "serious pain" uitvoeriger te beschrijven.
- Bij punt 10a verzoekt de DEC in een tabel, de aard, ernst, duur en frequentie beter te definiëren per handeling en groep, en het totale ongerief aan te geven.

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 **2011-100**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

From:
Sent: vrijdag 12 augustus 2011 11:28
To:
Cc:
Subject: RE: Project 2011-100-w
Attachments: 2011-10Aug- na commentaar DEC_DEC aanvraag formulier-11aug.doc

Beste leden van de DEC,

Hierbij de aangepaste DEC-aanvraag. Allereerst onze excuses voor de slordigheden die in de originele DEC-aanvraag zijn geslopen.
De commentaren van de DEC-leden zijn verwerkt en in het grijs aangegeven in de nieuwe versie zoals verzocht. Met name paragraaf 8 is in zijn geheel herzien. Additioneel is er nu een bloedafname in wakkere dieren, vlak voor het opofferingsexperiment.

Wij hopen dat deze DEC-aanvraag goedgekeurd zal worden.

Met vriendelijke groet,

Senior Scientist - Research & Technology
Tel:

Endepolsdomein 5, 6229 GW MAASTRICHT, The Netherlands
www.medtronic.com

From:
Sent: Tuesday, July 19, 2011 10:23 AM
To:
Subject: Project 2011-100-w

Geachte onderzoeker,
Uw projectaanvraag is in de DEC-UM – vergadering van **15 juli 2011** besproken.
De uitslag treft u aan in bijgaand attachment.
Voortaan zult u uit efficiency overweging geen schriftelijke bevestiging meer ontvangen per post wanneer het een wijzigingsbrief betreft.
De DEC verzoekt U in een brief de vragen van de DEC te beantwoorden en de wijzigingen in het protocol duidelijk grijs te markeren, zodat het bij het kopiëren ook zichtbaar is.
Wannneer uw project is aangehouden (dit staat altijd in de brief) moet u er rekening mee houden dat de herziene terug moet naar de gehele commissie. Uw herziene versie dient uiterlijk 12 augustus 2011 binnen te zijn voor de vergadering van 26 augustus 2011 op het secretariaat.dec.
De eerstvolgende vergadering is 26 augustus 2011.
Met vriendelijke groet namens DEC-UM:

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, 31-08-2011

Geachte Onderzoeker,

De **herziene versie** van uw projectaanvraag: "*Does AV-node stimulation induces a decrease in cardiac inflammation?*", is op de DEC vergadering van 26 augustus 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC merkt op dat het ongerief op voorblad niet is aangepast.
- De duur van de proef op het voorblad is niet juist (dit is de langste periode binnen één project dat één dier in proef is). De DEC verzoekt dit aan te passen.
- De DEC mist de brief met de beantwoording van de vragen van de DEC. De DEC verzoekt in de toekomst een brief bij de herziene versie mee te sturen.

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 **2011-100**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

From:
Sent: woensdag 31 augustus 2011 12:29
To:
Cc:
Subject: RE: Project 2011-100-w
Attachments: 2011-10Aug- na commentaar DEC_DEC aanvraag formulier-11aug.doc

Geachte DEC-UM,

In het attachment de herzien versie van DEC 2011-100.
De veranderingen zijn doorgevoerd;
te weten: Mate ongerief van 2 naar 3, en het aantal dagen in experiment van 1-dag naar 21-dagen.

Hopelijk kan de herziene versie de goedkeuring van de DEC krijgen.

Met vriendelijke groet,

, PhD
Senior Scientist - Research & Technology

From:
Sent: Wednesday, August 31, 2011 11:23 AM
To:
Subject: Project 2011-100-w

Geachte onderzoeker,
De **herziene versie** van uw projectaanvraag "", is in de DEC-UM – vergadering van **26 augustus 2011** besproken.
De uitslag treft u aan in bijgaand attachment.
Voortaan zult u uit efficiency overweging geen schriftelijke bevestiging meer ontvangen per post wanneer het een wijzigingsbrief betreft.
De DEC verzoekt U in een brief de vragen van de DEC te beantwoorden en de wijzigingen in het protocol duidelijk grijs te markeren, zodat het bij het kopiëren ook zichtbaar is.
Met vriendelijke groet namens DEC-UM:

Ambtelijk Secretaris Dierexperimentencommissie

Postbus 616, , 6200 MD Maastricht
T 043
E-mail:

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	05-09-2011

Project: *Does AV-node stimulation induces a decrease in cardiac inflammation?*

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:	**2011-100**
Diersoort:	**geit**
Aantal dieren:	**10**
Einddatum:	**31-08-2015**

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

U

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (0431)

Bezoekadres

Postadres
Postbus 616
6200 MD Maastricht