Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006

Herziene versie

DECNR: 2011-011

Ontvangen: 08-02-2011

DEC datum goedkeuring#	Type aanvraag [2]
09-02-2011	Nieuw ~~/ Herz.versie / Pilot~~

VROM/GGONR[3]

LNV/CBDNR[4]

Hoofdproject	CARIM	~~NUTRIM~~	~~Hersenen en gedrag~~	~~GROW~~	~~biomaterialen~~	~~Ander UM~~	~~Geen UM~~
Deelproject	~~1. 2.~~ 3.	~~1. 2. 3. 4.~~	~~1. 2. 3.~~	~~1. 2. 3.~~			

Financieel beheerder		Budgetnummer	30983262N

Titel van het onderzoek:

Imaging of thrombi using SPIO contrast agents with molecular MRI

startdatum 01-maart-2011 einddatum [9] 01-maart-2015 *Duur van de proef [10]: 1 dag*

	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. Verantwoordelijk medewerker (VM) GGO[7]					
4. overige uitvoerenden				Art.12	
5.				Art.12	

Diergroep	1a/b	2a/b					
ctrl/exp/sham	Exp mol MRI	Biodist					
Diersoort	01	01					
Stam	Swiss	Swiss					
Construct / mutatie ?	nee	nee					
Herkomst (leverancier) *	01	01					
Aantal	18	8					
Geslacht	m/f	m/f					
Dieren immuuncompetent ?	ja	ja					
Leeftijd/gewicht	30-35 g	30-35 g					
Doel van de proef *	31	31					
Belang van de proef *	01	01					
Toxicologisch onderzoek *	01	01					
Bijzondere technieken *	01	01					
Anesthesie *	04	04					
Pijnbestrijding *	04	01					
Mate ongerief *	03	03					
Toestand dier einde exp*	01	01					

* VHI-coderingen zie bijlage

Verantwoording

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

Titel: Imaging of thrombi using a SPIO contrast agent with molecular MRI

1. Aim of the study

Rupture of atherosclerotic plaques followed by thrombus formation, often leads to heart and brain infarction and is one of the most important causes of death in the Western world. Therefore it is necessary to diagnose vulnerable atherosclerotic plaques and thrombus formation.

In this study proposal our aim is to start a study to improve molecular imaging of thrombi in mice with the use of Magnetic Resonance Imaging (MRI). The formation of the thrombus will be induced artificially by exposing the carotid artery to a ferric chloride solution. For the imaging of the induced thrombi a Superparamagnetic iron oxide (SPIO) will be used as a labeled contrast agent. These SPIOs particles with a size of approximately 100 nm give a larger contrast on MR images than conventional small contrast agents based on Gadolinium because of its superparamagnetic properties. Unlabeled iron oxide particles have been used in human research. For example, SPIOs (Feridex®) for liver-research [1] or Ultra Small Particles of Iron Oxide (USPIO, Sinerem®) for imaging of atherosclerotic plaques [2] or imaging of lymph nodes by rectum cancer in patients [3]. These contrast agents are approved by the FDA.

... Until now it is unclear if labeled SPIOs are able to bind to specific biomarkers and proteins in large vessels.

In this study the SPIO contrast agent will be labeled · With this contrast agent we will investigate whether it is possible to visualize thrombi in vivo using the contrast agent.

Aim of the research

The main aim of this research is to investigate whether non-invasive imaging of thrombi in mice is possible using an acute thrombus model and a SPIO contrast agent labeled with a fibrin specific antibody.

2. Social Relevance and/or Scientific Importance

Thrombus formation due to rupture of atherosclerotic plaques is the most important cause of acute infarction. Thrombi can be treated by fibrinolytic therapy. In the current study, we aim to use molecular MRI to improve imaging of thrombi in a murine model for acute thrombus formation. It is expected that by using the newly proposed contrast agent, thrombi can be imaged with a greater sensitivity resulting in an improved treatment of thrombi. It is necessary to demonstrate the feasibility of and validate this modern imaging technique first in small animals and it is expected that in a later phase this technique can be used in patients.

3. Alternatives

Molecular MRI uses a contrast agent which is not (yet) approved for clinical application, therefore it is legally not allowed to perform a study in humans. The SPIO contrast agent was used before in animals. For future clinical application of the contrast agent it is necessary that the contrast agent is injected in the bloodstream and can circulate. Therefore a bloodstream is of essential importance in this study.

Replacement

It is known that the experimental contrast agent binds in vitro to thrombi. However, it is not known if the labeled contrast agent also binds sufficiently to thrombi in vivo, for example due to the high wall shear stress in large vessels, a quick clearance of the contrast agent or by an interaction between the contrast agent and the proteins (factors) present in the blood. For these reasons it is necessary to use living animals to investigate the functioning of the contrast agent.

Reduction

Reduction is achieved by performing the contrast injection through a cannulation of the arteria femoralis instead of injection in the tail vein. **Experience has shown that contrast injection during the MRI experiment in the tail vein has a lower success rate in contrast to intra-arterial injection, due to transport and positioning of the animal from the operating room to the MRI scanner.**

Refinement

During all phases of the experiment, the discomfort for the mice is kept as low and as short as possible. All the procedures will be done under one single anesthesia procedure (including the thrombus formation, the cannulation of the arteria femoralis and the MRI measurement). The discomfort for the animals during these handlings is scored as moderate (code 03). The animals will also receive pre-operative analgesia (beprenorphine 0.1 mg/kg).

4. Ethical Consideration

It is impossible to develop new therapies without reliable non-invasive imaging techniques. For the future clinical application of fibrinolytic therapy it is important to be able to quickly diagnose thrombi using a non invasive imaging technique. It is expected that with the proposed contrast agent thrombi can be diagnosed more accurate due to the higher sensitivity of the contrast agent.
During the study, an artificial thrombus will be induced in mice. Previous experiments (DEC 2007-097) have shown that the mice only experience some minor suffering from this operation. For the MRI measurements the animals will be placed under sedation using isoflurane and a cannulation of the arteria femoralis will be made, resulting in moderate discomfort.

In our view the purpose and social importance from this study compared to the discomfort justifies the use of laboratory animals in this clinically relevant research study.

Wetenschap

5. Scientific Motivation

In modern industrialized society cardiovascular diseases are one of the major reasons for mortality and morbidity. Most clinical manifestations are caused by organ complications, which are due to fragmentation of ruptured atherosclerotic plaques. When an atherosclerotic plaque ruptures, necrotic material from the plaque, will enter the blood circulation resulting in the formation of blood cloths (thrombi). These thrombi may cause locally, or by emboli formation in more distant locations, an occlusion that possibly results in a heart or brain infarction. An accurate diagnosis of these thrombi would be of great influence on the treatment of patients. For this accurate diagnose a sensitive non invasive imaging technique is required. Advancement in thrombotic research and therapy depend therefore not only on knowledge of the molecular processes, but also on the availability of effective techniques to image the thrombus formation in vivo.

In this study the possibility to detect thrombus formation in an acute thrombus model with the use of molecular MRI will be investigated. MRI is a clinically relevant technique, which can reveal information about morphological, functional and molecular properties. A clinical widely used contrast agent for contrast enhancement in MRI is Gd-DTPA. On MR images made using traditional acquisition techniques this contrast agent will produce an enhanced signal, resulting in a positive contrast. Because of the fact that a single Gd-DTPA will not give a sufficient signal enhancement for molecular MRI, large constructs (e.g. liposomes and quantum dots) containing several hundreds to thousands Gd-DTPA units, are developed [4] [5]. However, these large constructs accumulate in the body (especially in the spleen and liver) and are therefore difficult to translate clinically.

Another clinically used contrast agent are small iron oxide particles (SPIOs) [1] [2] [3]. These contrast agents give a larger contrast compared to Gd-DTPA, resulting in a lower detection limit, but this is a negative contrast instead of a positive contrast. In recent years new MRI acquisition techniques were developed making it possible to convert the negative contrast from SPIOs into a positive contrast, so called positive contrast imaging techniques [6] [7]. Currently these techniques are still not tested extensively in vivo using vascularly injected labeled SPIO contrast agents.

The results from the MRI study have to be validated using a second method. In this study rhodamine is coupled to the SPIOs in order to visualize the used contrast agent with two photon laser scanning microscopy (TPLSM) post mortem.

In this study the following research questions will be investigated:

1. Is the contrast agent suitable for in vivo detection of thrombus?
2. What is the added value of new positive contrast imaging techniques for in vivo detection of iron oxides compared to traditional negative contrast imaging techniques?
3. What is the biodistribution and blood half-life time of) contrast agent?

6. Scientific Evaluation

The principal investigators ( ), and ') of the MUMC+ have analyzed and approved this study on its scientific value.

Proefdier

7. Animal Choice

7a. Species, strain / origin / destination

In our department there is already experience with mice from a previous experiment (DEC 2007-097). In this study Swiss mice will be obtained from a recognized supplier (Charles River).

7b. Sex

In this experiment, the sex of the animal is not of importance.

7c. Numbers

Group 1: MRI experiment

$$n \geq 2\frac{\left(z_\alpha + z_\beta\right)^2}{\delta^2}\sigma^2 = 2\frac{\left(z_\alpha + z_\beta\right)^2}{\left(\delta/\mu\right)^2}\left(\frac{\sigma}{\mu}\right)^2$$

In this formula δ/μ is the relative signal difference and μ/σ is the signal-to-noise relation (SNR). The smallest, significant difference is set at 5%. The SNR of the 7.0 T small animal MRI scanner is estimated to be 30. With a power β of 80% and a significance level α of 0.05 this leads to:

$$n \geq \frac{15.7}{(0.05)^2}\frac{1}{30^2} = 7$$

The drop out for this experiment is estimated (based on previous research) to be 15 % (taking into account the success rate in thrombus formation). This means that 9 $\left(\frac{*}{0.85} = 8.2\right)$ mice are needed. In this experiment, two animal groups (see part 8. Experiment) are needed. This means a total of 18 mice are needed.

Group 2: Biodistribution

Because we will use an experimental contrast agent, the biodistribution of this contrast agent is a priori unknown. For a good biodistribution study, the number of successful measurements (based on previous experience, DEC 2008-170) is estimated to be three for every subgroup. Taking into account that there will be a drop out of 10% caused by unexpected complications (e.g. complications during the MRI examination, early death of the mouse or an unsuccessful contrast injection). For every subgroup the number of animals is 4, The total number of animals for the biodistribution group will be then 8.

Total number of mice: 18 (Group 1) + 8 (Group 2) = 26.

Mice will be randomly distributed over the different animal groups.

Dierproef

8. Experiment

Animal Group 1: Experiment

The mice in group 1 will undergo an operation to induce endothelial damage and subsequently induce thrombus formation in the right carotid artery. Active warming of the animal will take place with a heating plate. Under general anesthesia and in sterile circumstances, the neck will be shaved and the skin will be disinfected (using betadine). A medial longitudinal incision of circa 1 cm will be made. By separating the m. sternocleidomasteoideus and the trachea the carotid artery on the right side of the mouse will become visible

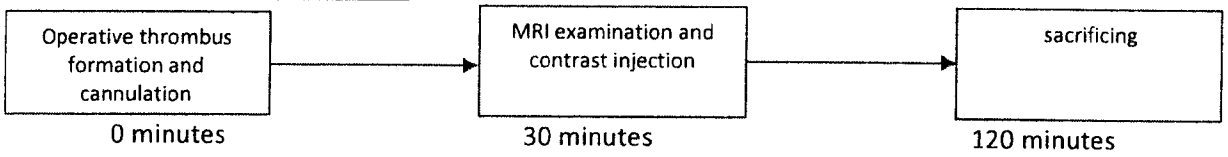
After this a cannulation of the arteria femoralis will be made. The cannulation will be used during the experiment for injection of the contrast agent to make pre and post contrast images. **The arteria femoralis cannulation for contrast agent injection is preferred above a tail vene cannulation. During transportation from the operating room to the MRI scanner and positioning of the mouse, the more fragile tail vene cannule can easily be damaged. This can result in an unsuccessful contrast injection, with an arteria femoralis cannule this risk is less.**

Directly after thrombus formation and cannulation of the arteria femoralis, the mouse will be placed in the MRI system and both pre- and post-contrast MRI of the carotid artery will be performed. Upon completion of the MRI acquisitions euthanasia will be performed by an overdose of anesthetics.

1. **Group 1a (9 mice)**
   In these mice the contrast agent will be used.
2. **Group 1b (9 mice)**
   In these mice the contrast agent will be used.
   This group is the control group,

After euthanasia the carotid artery will be removed and fixated in formalin for depiction with Two Photon Laser Scanning Microscopy (TPLSM) and histology.

Flowchart animal groups 1a and 1b



Animal Group 2: Biodistribution

For each contrast agent the dynamic biodistribution will be measured. After the experiment the mice will be sacrificed using an overdose of anesthetics. Post mortem another MRI scan will be acquired to obtain very high spatial resolution images. Finally, one kidney, the spleen, a muscle of the upper leg and the liver will be removed for TPLSM measurements.

In preparation of the experiment, a cannulation of the arteria femoralis will be made for administration of the contrast agent. The contrast agent will be administered during the MRI examination in order to acquire pre- and post-contrast images.

Blinding

All mice will be randomly assigned to group 1 and 2 and the respective subgroups. The researcher will be blinded for the type of contrast agent.

9. Experimental Conditions

9a. Anesthesia

Since the mice need to be calm and not moving, during the MRI measurements sedation is needed. Induction of the anesthesia is done using 3.0 – 4.0 % isoflurane in an isobox. Maintenance of the

anesthetic state is accomplished by 1.5-2.0% isoflurane via a snout cap.

9b. Analgesia

In animal groups 1a and 1b pre-operative pain relief is done using beprenorphine (0.1 mg/kg). During the MRI experiment no further pain relief will be given.

When a sign of pain in the animals is observed, consultation of the article 14 functionary will take place and an appropriate action will be taken.

9c. Euthanasia and humane endpoints

The mice will be sacrificed immediately after the MRI experiment by an overdose of the anesthetic. Based on previous studies with this murine model no complications are expected. All the mice will go under anesthesia before the operation, anesthesia will remain during the MRI experiment immediately after operation. If the animals show abnormal behavior or experience discomfort during the experiment (e.g. 10 % weight loss, signs of bad self care or complications during the experiment) that cannot be treated, euthanasia will be performed.

When the animals, during the whole experiment, show a sign of pain and/or discomfort, there will be direct contact with the principle investigator and article 14 functionary to determine the policy. The welfare of the animal has the priority in all cases!

Zorg

10a. Discomfort

Animal groups 1a and 1b

Total discomfort: moderate (code 03). The surgery (under complete anesthesia) applied to these groups will cause little/moderate discomfort (code 02). During the experiment, a cannulation of the arteria femoralis will be made (moderate discomfort code 03). The anesthesia and the MRI measurements, followed by euthanasia will cause little/moderate discomfort (code 02).

Animal group 2a and 2b

Total discomfort: moderate (code 03). During the experiment, a cannulation of the arteria femoralis will be made (moderate discomfort, code 03). The anesthesia and the MRI measurements, followed by euthanasia will cause little/moderate discomfort (code 02)

10b. Welfare Evaluation

Previous experiments involving an artificially created thrombus in the carotid artery are performed several times. During the MRI measurements, the welfare of the animals will be checked by ECG, temperature and respiratory monitoring. This will result in early detection of discomfort and subsequently adequate handling can be done.

11. Care and Housing

During the study, the mice will be socially housed in the experimental room of the CPV. Daily care of the animals will be done by the CPV personnel according to general CPV guidelines. The MRI measurements will be performed using the 7.0 Tesla small animal experimental MRI scanner, which is situated on floor 0 of the academic hospital Maastricht (azM), department of Radiology.
The 7.0 T animal experimental MRI scanner is localized on floor 0 of the academic hospital. However, the animals will be transported in a special trolley, so that the animals cannot be seen, meeting the hospital hygiene requirements.
In case of any calamities the principal investigator should be warned.

12. Expertise

(DEC 2009-097). Therefore we do not expect problems during this surgery.

All handlings with or concerning the mice will be done by certified (art. 9 WOD or art. 12 WOD) personnel.

13. Standard Operation Procedures (SOP)

Mice from animal groups 1a and 1b will undergo the following procedures:

1. The animals will undergo sedation using inhalation of isoflurane. The sedation will be induced with use of an isobox (3.0 – 4.0 % isoflurane) and maintenance with a snout cap (1.5 – 2.0 % isoflurane). The anesthetic is applied using pressure air as a carrier gas. Depth of anesthesia is checked via the eyelid and toe reflex tests.
2. Pre-operative pain relief is accomplished by application of beprenorphine (0.1 mg/kg).
3. During surgery, body temperature will be retained using a heat plate.
4. A medial longitudinal incision of approximately 1 centimeter will be made. By separating the m. sternocleidomasteoideus from the trachea, the carotid artery will become visible at the right side of the mouse.
5.

6. After this, the wound will be closed with a 5-0 suture (non resorbable).
7. A cannula will be placed in the arteria femoralis according to FAR-01-M.
8. The mouse will be positioned in the special animal container for MRI. The mouse will be connected to the monitoring equipment (ECG, respiration and temperature) and heat plate.
9. Directly after this procedure the mouse will undergo the MRI examination. During the MRI examination the contrast agent will be administered through the cannula in the arteria femoralis to acquire pre and post contrast images.
10. The mouse will be removed from the MRI system and sacrificed with an overdose of anesthetics.

Mice from animal group 2 will undergo the following procedures

1. The animals will undergo sedation using inhalation of isoflurane. The sedation will be induced with use of an isobox (3.0 – 4.0 % isoflurane) and maintenance with a snout cap (1.5 – 2.0 % isoflurane). The anesthetic is applied using pressure air as a carrier gas. Depth of anesthesia is checked via the eyelid and the toe reflex tests.
2. A cannulation of the arteria femoralis will be made according to FAR-01-M.
3. Positioning of the mouse in the special animal container for MRI.
4. Connection of the mouse to the monitoring equipment (ECG, respiration and temperature). Also connection to the heat plate will be done to keep the body temperature constant during the experiments.
5. Directly after this the mouse will undergo the MRI examination. Injection of the MRI contrast agent is done during the MRI examination to acquire pre and post contrast images.
6. The mouse will be sacrificed with an overdose of anesthetics and high resolution post mortem MRI examination is performed to determine the biodistribution.

Literature

1. Superparamagnetic iron oxide (SPIO)-enhanced liver MRI with ferucarbotran: efficacy for characterization of focal liver lesions. Namkung, S., J Magn Reson Imaging, 2007, Vol. 25, pp. 755-765.
2. Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by in vivo magnetic resonance imaging. Kooi, M.E., Circulation, 2003, Vol. 107, pp. 2453-2458.
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6. Susceptibility Gradient Mapping (SGM): A new postprocessing method for positive contrast generation applied to superparamagnetic iron oxide particle (SPIO)-labeled cells. Dahnke, H., 2008, Magnetic Resonance in Medicine, Vol. 60, pp. 595-603.
7. Positive Contrast Visualization of Iron Oxide-Labeled Stem Cells using Inversion-Recovery With ON-Resonant Water Suppression (IRON). Stuber, M. 2007, Magnetic Resonance in Medicine, Vol. 58, pp. 1072-1077.
8. Murine Thrombosis Models. Day, S.M. 2004, Thromb Haemost, Vol. 92, pp. 486-494.

University Maastricht

Faculty of Health, Medicine
and Life Sciences

Dierexperimenten Commissie

DEC

Aan:

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

Uw referentie: Onze referentie : Maastricht, 02-02-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Imaging of thrombi using SPIO contrast agents with molecular MRI*", is op de DEC vergadering van 28 januari 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt de eerste twee zinnen, tot en met "organization", te verwijderen.
- De DEC neemt kennis van de opmerking bij punt 8 (Previous experiments, enzovoort) en stelt vast dat het ongewenst is dat de invloed van de vaardigheid van de biotechnicus het succes van de IV injectie in de staartvene bepaalt.

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 **2011-011**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

academisch ziekenhuis
Maastricht

telefoon
telefax

azM

, voorzitter
p/a Secretariaat DEC-UM
Postbus 616
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uw kenmerk
ons kenmerk
doorkiesnummer
datum 8 februari 2011

Betreft: Wijzigingen projectaanvraag 2011-011

Hoofd

Wnc hoofd

Geachte heer ,

Bijgaand treft u de gewijzigde projectaanvraag 2011-011 aan waarin vragen en opmerkingen naar aanleiding van de DEC vergadering van 28 januari 2011 zijn beantwoord. In deze versie zijn de volgende vragen beantwoord:

- De eerste twee regels van punt 6 (Scientific Evaluation) zijn verwijderd.
- In punt 8 (Experiment) en punt 3 (Alternatives) is de argumentatie om een arteriële femorale canulatie te preveren boven een staart vene canulatie op basis van de ervaring van de uit te voeren ingreep verwijderd.

Met dit schrijven hoop ik de DEC naar tevredenheid te hebben geïnformeerd.

Hoogachtend,

Promovendus

Universiteit Maastricht

The Netherlands

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan: [redacted]

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
		09-02-2011

Project: *Imaging of thrombi using SPIO contrast agents with molecular MRI.*

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:	**2011-011**
Diersoort:	**muis**
Aantal dieren:	26
Einddatum:	07-02-2015

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
[redacted]
p/a secretariaat DEC-UM

Secretariaat DEC-UM

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