Begeleidingsformulier aanvraag dierproef DEC- UM	**DECNR: 2011-168**
Versie 2006 **Herziene versie**	**Ontvangen: 05-01-2012**

DEC datum goedkeuring#	Type aanvraag [2]
16-01-2012	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	30973540E

Titel van het onderzoek:

The Role of the Urothelial/Suburothelial Layer in Signal Transduction of the Guinea Pig Urinary Bladder.

startdatum Januari 2012 einddatum [9] December 2012 *Duur van de proef[10]:* *4 mnd*

	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.9)	
5. external reviewer					

Diergroep	**1**	**2**	.	.	.	.	.
ctrl/exp/sham	Exp						
Diersoort	04						
Stam	Hartley						
Construct / mutatie ?							
Herkomst (leverancier) *	02						
Aantal	12						
Geslacht	m						
Dieren immuuncompetent ?	ja	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]
Leeftijd/gewicht	250-350 g						
Doel van de proef *	33						
Belang van de proef *	01						
Toxicologisch onderzoek *	01						
Bijzondere technieken *	02						
Anesthesie *	02						
Pijnbestrijding *	01						
Mate ongerief *	01						

1 *Verantwoording*

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

Titel: *The Role of the Urothelial/Suburothelial Layer in Signal Transduction of the Guinea Pig Urinary Bladder.*

1. Doel van de proef.

The goal of this study is to investigate the pharmacology of signal transduction present in the urinary bladder. In earlier studies, the prostaglandin E2 receptors EP1 and EP2 as well as the producing enzyme COX-1 have been shown to be expressed by urothelial and suburothelial cellular structures *(DEC 2008-043)*. However, all three proteins showed different expression patterns regarding the location in the bladder wall, indicating that both, the urothelial and suburothelial layers react in different ways on prostaglandin E2 stimulation and that interaction of them results in proper signaling.

In this study we want to investigate whether instillation of PGE2 and noradrenaline to the urinary bladder will result in an altered expression of (1) the PGE2 specific receptors EP1 and EP2 and (2) the PGE2 producing enzyme COX-1. Furthermore, we hypothesize that both, urothelial and suburothelial signaling play a role in the respons2 to PGE2 and noradrenaline.

To support our hypothesis, urinary bladders will be isolated and tissue preparations will be made, which differ in tissue layer composition.

After stimulation with pharmacological substances (PGE2, noradrenaline), changes in expression levels and functionality will be determined.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

This study is fundamental and focuses on basic research to unravel mechanisms, which are involved in the regulation of micturition and possibly altered in patients suffering from the overactive bladder syndrome (OAB). The prevalence of OAB increases with advancing age and worldwide prevalence is expected to increase by 20.1% until 2018, compared to 2008 and based on the predicted aging of the worldwide population (1). Understanding the physiological and pathological processes involved in micturition and OAB could lead to the development of new, more specific treatment options and consequently reduced health care costs.

3. Alternatieven

Regarding this research question, **replacement** is not possible. This is a follow-up of an earlier study, in which guinea pigs were used. In this study it was important to use a whole-bladder preparation (DEC 2008-043). To compare data from both studies, it is important to use the same species.

In order to **reduce** the amount of used animals, we use internal controls instead of a control group. This reduces the amount of animals by 30%.

In our study the animals will be killed directly to isolate the urinary bladder, resulting in a low level of discomfort. Using anesthetics would **refine** the use of animals. However, anesthetics influence the cyclic GMP system, present in the urothelial and suburothelial layer, which in turn probably will influence our results. **In this study, animals will be killed by bleeding after knocking down with a spring operated captive bolt, which is suitable for small animals, like guinea pigs and rabbits. To refine this technique, the researcher will be trained on death animals.**

4. Ethische afweging

The overactive bladder syndrome (OAB) resembles a societal as well as medical problem, increasing in dimension (1). OAB has a high prevalence and a profound impact on quality of life. In our

opinion it is justified to make use of animal models in basal research, which contributes to a better understanding of OAB and the physiological mechanisms leading to micturition.

5. Wetenschappelijke onderbouwing

Incontinence occurs with aging, but it can also be present in neurodegenerative diseases, eg. Alzheimer's disease. Incontinence can result in social and psychological problems. It is usually treated using anticholinergics. However, side effects such as decreased cognitive function and dryness of the mouth contribute to the fact, that 80% of the patients stop anticholinergic treatment.

Prostaglandin E2 levels have been shown to be increased in OAB patients (2, 3). Instillation of PGE2 results in an increase of non-voiding contractions, which play a role in the etiology of OAB (4). Functional studies have shown that the action of PGE2 on bladder contractions is possibly mediated via the PGE2 receptors EP1 and EP2.

Immunohistochemical studies have investigated the expression of several receptors, enzymes and substances not only of the prostaglandin system, but also mediators of the adrenergic, muscarinic, and cGMP/ nitric oxide (NO) systems. It is thought that noradrenaline stimulates production of NO, which inhibits the prostaglandin as well as the muscarinic system, all present in the urothelium (figure 1). Furthermore both, the prostaglandin and the muscarinic systems interact with ATP, also present in the urothelium.

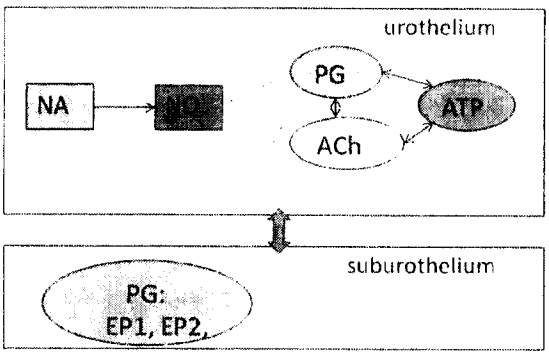


Figure 1 Urothelial signaling and its impact on the suburothelium

Furthermore, the prostaglandin E2 receptors type 1 and 2 (EP1 and EP2) as well as the producing enzyme COX-1 have been shown to be present in the guinea pig bladder. Both, urothelium and suburothelium, express EP1 and EP2 (5). Furthermore, COX-1 is expressed by the basal urothelial layer (6). This suggests some interaction of urothelium and suburothelium regarding the prostaglandin system. However, it is not known how this interaction takes place on a molecular level. Moreover, noradrenaline which is physiologically expressed by the urothelium is thought to inhibit the prostaglandin system in the urothelium. It has not yet been investigated if this also has an impact on the suburothelium.

6. Wetenschappelijke beoordeling

This DEC protocol has been reviewed and approved by the person, mentioned at No 5 on the front page.

7. Proefdier keuze
7a. Soort, stam / herkomst / eindbestemming

Species: Guinea pig. The structure of the guinea pig urinary bladder resembles the human situation in a better way than the urinary bladder of mice of rats.

Origin : Charles River

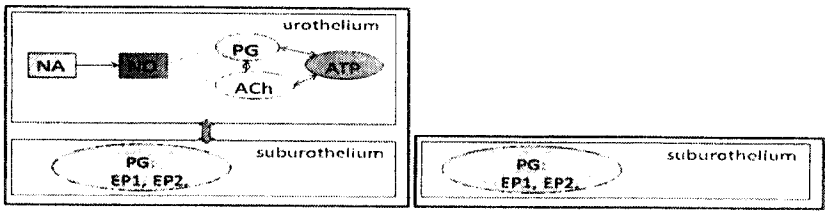
Ultimate goal: The guinea pigs will be killed before isolating the urinary bladder.

7b. Sexe
Male guinea pigs.
In earlier studies, obstruction models were used, which are only possible in male guinea pigs. To standardize as many parameters as possible and to compare our results to results from these studies, we have to use male animals again.

7.c. Aantallen
In our study two Groups will be designed. Group A will be stimulated with PGE2, group B will get noradrenaline stimulation. To reduce the amount of animals used in this study, we will use internal controls. Every bladder will be cut in two. One half will get the stimulation and one will serve as internal control. Because this is the first study using these design on urinary bladders, it is not possible to estimate the effect. Thus, we cannot perform a proper power calculation.
Every group will consists of 6 animals, resulting in 12 animals in total.

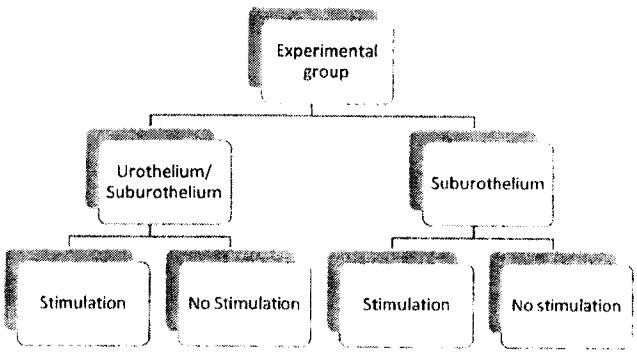
8. Experiment

Our study contains two different experimental groups; controls will be obtained internally from each bladder. Each bladder will be cut in two, one half, which will be stimulated and one which serves as control tissue. **Guinea pigs will be knocked out using a captive bolt and euthanized by bleeding.** Urinary bladders will be isolated and stored in ice-coid Krebs solution. Two different tissue preparations will be made, one containing both, urothelium and suburothelium and one, containing only the suburothelium.



In the PGE2 group, the tissue preparations of one half of each bladder are stimulated with PGE2. Tissue preparations of the other half serve as internal control. In the noradrenaline group, the same will be done, but stimulation will occur with noradrenaline instead of PGE2.

After that, expression levels of EP1, EP2 and cox-1 will be determined on mRNA and protein level. Furthermore altered functionality of these proteins will be determined.



9. Experimentele condities

9a. Anesthesie

In this study, guinea pigs will be knocked out using a spring operated captive bolt, which is suitable for knocking out small animals. Using anesthetics or other forms of sedation would interact with the cGMP/NO system present in the urothelial layer (7). We expect that the cGMP/NO system interacts with the prostaglandin system in the urothelial layer. Consequently, use of anesthetics would interfere with our results.

9b. Pijnbestrijding

N/A

9c. Euthanasie en Humane eindpunten

Guinea pigs will be directly knocked down using a spring operated captive bolt and euthanized by bleeding. When an animal shows symptoms of serious sickness of weakness, it will be euthanized.

Human endpoints:

- *The animal shows severe symptoms of general sickness*
- *Decrease in weight (15-20%)*
- *No adequate increase in weight dependet on the age of the animal*

Zorg

10a. Ongerief

Euthanasia without previous proceedings. Light/middling code 01.

10b. Welzijnsevaluatie

The animals will be euthanized directly without previous proceedings. Thus, wellbeeing of the animals will not be recorded after they became part of the experiment.

11. Verzorging en huisvesting

Standard CPV.

12. Deskundigheid

The animals are killed by the responsible researcher, who is experienced in animal research and supervised several studies, in which guinea pigs were used as the model of choice for bladder research. is conversant with animal research, too.

13. Standard Operation Procedures (SOP)

N/A

Relevante literatuur

1. Irwin DE, Kopp ZS, Agatep B, Milsom I, Abrams P. Worldwide prevalence estimates of lower urinary tract symptoms, overactive bladder, urinary incontinence and bladder outlet obstruction. BJU Int Oct;108(7):1132-8.
2. Kim JC, Park EY, Hong SH, Seo SI, Park YH, Hwang TK. Changes of urinary nerve growth factor and prostaglandins in male patients with overactive bladder symptom. Int J Urol2005 Oct;12(10):875-80.
3. Kim JC, Park EY, Seo SI, Park YH, Hwang TK. Nerve growth factor and prostaglandins in the urine of female patients with overactive bladder. J Urol2006 May;175(5):1773-6; discussion 6.
4. Schroder A, Newgreen D, Andersson KE. Detrusor responses to prostaglandin E2 and bladder outlet obstruction in wild-type and Ep1 receptor knockout mice. J Urol2004 Sep;172(3):1166-70.
5. Rahnama'i MS, van Koeveringe GA, Essers PB, de Wachter SG, de Vente J, van Kerrebroeck PE, et al. Prostaglandin receptor EP1 and EP2 site in guinea pig bladder urothelium and lamina propria. J Urol Mar;183(3):1241-7.
6. Rahnama'i MS, de Wachter SG, van Koeveringe GA, van Kerrebroeck PE, de Vente J, Gillespie JI. The relationship between prostaglandin E receptor 1 and cyclooxygenase I expression in guinea pig bladder interstitial cells: proposition of a signal propagation system. J Urol Jan;185(1):315-22.
7. Toda N, Toda H, Hatano Y. Nitric oxide: involvement in the effects of anesthetic agents. Anesthesiology2007 Nov;107(5):822-42.

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 : MQ- 688-11 Maastricht, 19-12-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*The role of the Urothelia/Suburothelial Layer in Signal Transduction of the Guinea Pig Urinary Bladder*", is op de DEC vergadering van 16 december 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

1) De DEC verzoekt de coderingen op het voorblad na te kijken en aan te passen. Wanneer de code voor bijzondere technieken 02 is, dienen de coderingen eronder code 01 te zijn, echter deze moeten wel overeenstemmen met hetgeen in de aanvraag beschreven staat bij 9a en 9b.
2) De DEC verzoekt de naam van de PI bij punt 6 te verwijderen en op het voorblad te vermelden. Het is niet de bedoeling dat verwezen wordt naar personen in de aanvraag, in verband met de Wet Openbaarheid van Bestuur.
3) Punt 9c- De DEC wenst voorafgaande aan de decapitatie, een sedatie van de dieren, in overleg met de proefdierdeskundige.

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 2011-168, gelieve dit nummer in verdere correspondentie te vermelden.

De DEC-UM wenst u en uw familie fijne feestdagen en een voorspoedig en vooral gezond 2012!

Hoogachtend,

Voorzitter DEC-UM

Dierexperimenten commissie

, voorzitter

p/a Secretariaat DEC-UM

Postbus 2616

NL-6200 MD Maastricht

Tel: 043-

Postbus 5800

NL-6202 AZ Maastricht

Tel: 043-

Subject: Project 2011-168 ' The role of the Urothelial/ Suburothelial Layer in Signal Transduction of the Guinea Pig Urinary Bladder'

Dear members of the Animal experiment committie,

Recently, I recieved your questions regarding the project 2011-168 (The role of the Urothelial/ Suburothelial Layer in Signal Transduction of the Guinea Pig Urinary Bladder).

- The DEC asked to change the codes on the front page at the points special techniques and anaesthesia. They asked to change the code for anaesthesia to 01 instead of 02. However, anaesthetics could not be used in our study, because they will interfere with our results. Due to this, I changed the code of special techniques to 13, because euthanazia without previous proceedings, includes usage of anaesthesia.
- The DEC asked to remove the name of the reviewer on point 6. This has been done. The name is now stated on the front page.
- The DEC asked for sedation of the animals previous to the decapitation. In accordance with the animal wellfare officer, we decided to include a pilot study, which will investigate the effect of anaesthetics or sedation on bladder signaling (see 'wijzigingsbrief'). However, we designed another possibility to kill the animals without using chemical sedation. The animals will be knocked out using a spring operated captive bolt, which is suitable for small animals, like rabbits and guinea pigs. The researcher will be trained previous on dead guinea pigs, how to use this device. Afterwards, the animals will be killed by bleeding.

Go

All changes in the DEC protocol and on the front page are marked in grey.Hopefully, I answered all your questions.

Kind regards,

1/5/2012

From:
Sent: vrijdag 13 januari 2012 14:29
To:
Cc:
Subject: FW: DEC 2011-168

Importance: High

Attachments: Antwoord aan de Dierexperimenten commissie.docx; dec senior stage 120112.docx; Voorblad aanvraag dierproef DEC senior stage.doc; wijzigingsbrief DEC 2011-168.doc




Antwoord aan Dierexperimente... | dec senior 120112.docx | Voorblad raag dierproef | wijzigingsbrief IEC 2011-168.d...

Hallo
Ik ga contact opnemen met de inspecteur van VWA omdat er door de nieuwe Europese wetgeving enige onduidelijkheid is ontstaan of de beschreven wijze van euthanasia (gebruik schietmasker) wel of niet is toegestaan en of het mag beschouwd worden als doden zonder voorafgaande handelingen (code 02 bijzondere technieken) of als trauma moet gezien worden (code 13 bijzondere technieken). De juiste coderingen kunnen we dan achteraf nog toevoegen. Mochten de DEC leden er behoefte aan hebben kan ik een foto meebrengen van de procedure met het schietmasker.
MVG

www.maastrichtuniversity.nl

POB616, 6200MD Maastricht, The Netherlands
P.O. Box 616, 6200 MD Maastricht, The Netherlands

Note: the email addresses have changed!

 -----Original Message-----
From: [mailto:]
Sent: donderdag 12 januari 2012 10:20
To:
Cc:
Subject: DEC 2011-168
Importance: High

Beste

Donderdag, 5 januari hebben we al een verbeterde versie van het DEC

From:
Sent: maandag 16 januari 2012 15:32
To:)
Subject: FW: cavia's

Zie hier het antwoord van de inspectie.

Maastricht University

www.maastrichtuniversity.nl

POB616, 6200MD Maastricht, The Netherlands
P.O. Box 616, 6200 MD Maastricht, The Netherlands

Note: the email addresses have changed.

From:
Sent: maandag 16 januari 2012 11:09
To:
Subject: RE: cavia's

Geachte proefdierdeskundige, beste

Zoals telefonisch besproken mijn ervaringen zijn beperkt bij cavia'tot gebruik van schietmaskers aan de achterzijde van het hoofd.
De tabel verbiedt inderdaad het gebruik van schietmaskers, maar Article 6 EU/2010/63

"Competent authorities may grant exemptions from the requirement in paragraph 3: (a)...(b) when, on the basis of scientific justification, the purpose of the procedure cannot be achieved by the use of a method of killing set out in Annex IV "

Ik veronderstel dat de competent authorities o.a. de DEC's / Animal Welfare Bodies of de Minister/ Inspectie zullen zijn.

Daarnaast is het merkwaardig dat een percussive blow to the head bij een dier met een korte gespierde nek wel mag en "killing must be completed" o.a. toegestaan door destructie van het brein aan te tonen. Ik kan dat niet geheel passen bij verbod op gebruik van schietmaskers bij cavia's. Ik veronderstel dat de tabel te weinig onderscheid maakt tussen de diverse knaagdiersoorten! Gelukkig is er dus de " ontheffingsmogelijkheid"

Mijn voorstel is daarom de gebruik van schietmaskers bij cavia's wel toe te staan indien het voor het onderzoek nodig is! En nu al vast voor de invoering van de nieuwe Wod goed te onderbouwen waarom de euthanasie zo moet gebeuren en dat dit ook niet meer ongerief veroorzaakt dan de in de richtlijn toegestane methodes (conform art 6. lid 4 onder a).

met vriendelijke groet

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	19-01-2012

Project: *The role of the Urothelia/Suburothelial Layer in Signal Transduction of the Guinea Pig Urinary Bladder.*

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (043

Bezoekadres

Postadres
Postbus 616
6200 MD Maastricht

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 met de opmerking dat de proefdierdeskundige aanwezig wenst te zijn bij de euthanasie.**

Projectnummer:	**2011-168**
Diersoort:	**cavia**
Aantal dieren:	**12**
Einddatum:	**16-01-2016**

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