60

Begeleidingsformulier aanvraag dierproef DEC- UM Herziene versie	DECNR: 2011-155 Ontvangen: 15-06-2012

DEC datum goedkeuring#	Type aanvraag [2]
27-06-2012	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	**30973540E**

Titel van het onderzoek:

Ex vivo cystometry in guinea pig model concerning the autonomous bladder

[redacted] **01-07-2012** [redacted][9] **01-07-2013** *Duur van de proef[10]:* [redacted]

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mailadres	Bevoegd-heid[5]	Cap. groep /afdeling
1. Verantwoordelijk onderzoeker (VO)				Art. 9	
2. [redacted] VO				Art. 9	
3. Verantwoordelijk medewerker (VM) GGO[7]					
4. [redacted]				Art. 9	
5. [redacted]				Art. 9/12	

Diergroep	1	2	3	4	5	6	
ctrl/exp/sham	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Diersoort	04	04	04	04	04	04	
Stam	Hartley	Hartley	Hartley	Hartley	Hartley	Hartley	
Construct / mutatie ?	-	-	-	-	-	-	
Herkomst (leverancier) *	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Aantal	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Geslacht	M	M	M	M	M	M	
Dieren immuuncompetent ?	Ja	Ja	Ja	Ja	Ja	Ja	
Leeftijd/gewicht	250-350 gr	250-350 gr	250-350 gr	250-350 gr	250-350 gr	250-350 gr	
Doel van de proef *	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Belang van de proef *	01	01	01	01	01	01	
Toxicologisch onderzoek *	01	01	01	01	01	01	
Bijzondere technieken *	02	02	02	02	02	02	
Anesthesie *	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Pijnbestrijding *	01	01	01	01	01	01	
Mate ongerief *	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Toestand dier einde exp*	01	01	01	01	01	01	

* VHI-coderingen zie bijlage

1 Verantwoording

Aanvraag dierproef DEC-UM

Titel: Ex vivo cystometry in guinea pig model concerning the autonomous bladder.

1. Doel van de proef

The urinary bladder has the function to store urine and to expel it at a convenient time and place. Therefore, it is equipped with a sophisticated relay network to transfer information on bladder fullness to the brain. One of the proposed mechano-transduction systems is the stretch dependent release of mediator substances such as Acetylcholine (ACh), Nitric Oxide (NO), Adenosine Triphosphate (ATP) and Prostaglandins (PG). Intravesical prostaglandins are synthesized de novo rather than being released from an intracellular store. Cyclo-oxygenases I and II are essential enzymes for the production of prostaglandins. Prostaglandin E2 (PGE_2) is one of the main PGs synthesized in the human bladder. It exerts its effects by binding to the endoprostanoid (EP) receptors, of which 4 subtypes have been described: EP_1, EP_2, EP_3 and EP_4.
An isolated bladder shows micro-contractions, which are defined as non-micturition related autonomous contractions. In bladders of patients with overactive bladder syndrome, which is associated with urge complaints and incontinence, these micro-contractions are more outspoken. Also, higher levels of prostaglandin were measured in these subjects. How exactly the PG mediated signalling mechanisms for bladder sensation work, has not yet been fully understood. A crosslink between the muscarinic system and the prostanoid system has been hypothesised. The purpose of this research is to investigate the role of prostaglandin E_2 in muscarinic induced micro-contractions in the isolated urinary bladder of the guinea pig.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

The primary clinical symptom in the overactive bladder syndrome is an increased urge to pass urine, with or without incontinence. Remarkably, despite the prevalence and costs involved, the mechanisms underlying increased urge are not known. In order to simply describe the condition the term 'Over Active Bladder (OAB) Symptom Complex' has been introduced. We need more animal and translational research to understand the etiology and the pathophysiology of the bladder control to be able to develop targeted therapies.

3. Alternatieven

The research is about the anatomy of the bladder and the behaviour of the whole organ so we can only use whole bladder models. In order to do so we need ex vivo experiment with animal bladders. We can not use strips of human material because we are at the moment interested in the movement and anatomy of the whole bladder. The experiment will be done by means of standard operating procedures which we will follow very thoroughly. We will only perform surgical procedures after sacrifice.

4. Ethische afweging

We can not use strips of human material because we are at the moment interested in the movement and anatomy of the whole bladder.
Research using animal models is necessary for better interpretation and analysis of the neurobiological mechanisms in the bladder. We are confident that the proposed animal experiments and suffering of the animals will weight up against the new information that is going to be obtained from this study. That's why research with animals is justified in our opinion.

5. Wetenschappelijke onderbouwing

Overactive Bladder Syndrome (OAB) is defined by the International Urogynaecological Association (IUGA) and the International Continence Society (ICS) as: Urgency accompanied by frequency with or without urgency urinary incontinence (UUI) [4]. It is a highly prevalent disorder with a profound impact on quality of life. Several large-scale populations based and cohort studies have demonstrated that the prevalence of OAB increased with age in both sexes. In the seventh decade of life almost 20% of men and women are affected [5-9].

The costs of illness resulting from OAB form a substantial economic burden. The annual direct costs per patient vary from € 262 in Spain up to € 619 in Sweden. In total, the annual direct burden in six European countries together was estimated at € 3,9 billion. In addition, costs for nursing homes and OAB related work absenteeism added up to a total of € 4,7 billion and € 1,1 billion respectively [10]. Much is still to be unravelled about the details of mechanisms of bladder sensation, and consequently about the etiology of OAB.

The micturition reflex is controlled by the pontine micturition centre, which integrates afferent information from the lower urinary tract and other sites, to determine the transition from the urine storage phase to the voiding phase. The physiological basis of the generation of this sensory information concerning the bladder depends on direct stimulation of afferent fibres as a consequence of stretch in the bladder wall [11]. A more recent finding in the bladder sensation is the active role of the urothelium contributing in afferent signalling through stretch dependent urothelial release of various mediators as acetylcholine (ACh), nitric oxide (NO), adenosine triphosphate (ATP) and Prostaglandins (PG))[12].

Increased sensation of bladder fullness is associated with localized contractile activity in the bladder wall of human subjects, so called micro-motions [13]. These micro-motions are significantly more prevalent in patients with urgency than in healthy volunteers [13]. Also, Non Micturition Contractions (NMCs) can be detected in the anaesthetized and awake rat. The amplitude and frequency of this contractile activity varies during the filling phase of the micturition cycle [14]. NMCs are observed in many species, suggesting that they have a basic physiological role. Therefore, it has been hypothesised that urgency is associated with autonomous activity of the detrusor and altered micro-motions, and that these NMCs may be involved in the generation of phasic afferent nerve activity [14].

PGE_2 is the main prostaglandin synthesized by the human bladder [15]. In patients with OAB, urinary PG2 levels are elevated, compared to a control group of patients [16]. In male patients with OAB, increased levels of PGE_2 were measured, but there were no significant changes in different PG species as $PGF_{2\alpha}$ and PGI_2 [17]. Urinary PGE2 levels were also elevated in patients with suprapontine brain disease, showing symptoms of OAB [18]. Finally, in boys with lower urinary tract obstruction (LUTO), which is characterized by the same symptoms as OAB in adults, significantly higher PGE2 levels were measured, compared to control subjects without LUTO [19].

PGE_2 instillation of the bladder results in an increased number and amplitude of non voiding detrusor contractions detected in in-vivo mouse experiments [20]. Furthermore, experimental application of PGE2 in the isolated guinea pig bladder increased both frequency and amplitude of non voiding contractions, more than other prostanoids e.g. PGE1, PGF2a, and arachidionic acid [21]. Data above show that PGE_2 is the most important prostaglandin synthesized in the bladder and that it plays a crucial role in bladder sensation. It exerts its effect on the endoprostanoid receptors of which four subtypes (EP_1, EP_2, EP_3, EP_4) have been described [22].

In the bladder, PG release depends on de novo synthesis rather than release from pre-formed stores [23]. Cyclooxygenase type 1 and 2 (COX-I and COX-II) are the central enzymes in the production of PG [24]. COX-I is a constitutive form, whereas COX-II is described as an inducible form in the

bladder. Its expression is regulated by various stimuli, including pro-inflammatory cytokines and growth factors [24]. An increased expression of COX-II has been described immediately after experimentally induced bladder outlet obstruction [21].
There is an increasing amount of data available pointing to a role of PG in the regulation of non-voiding contractions and afferent activity [1, 21, 25, 26]. The autonomous small contractions in the isolated bladder, which resemble non-voiding contractions, increase in amplitude and frequency by an increase in bladder volume [27, 28], as well as by acetylcholine analogues [29, 30] and PG [21]. Similarly, intravesical PG administration in vivo increases non-voiding contractions during bladder filling and decreases the intermicturition interval [31]. EP_1 and EP_3 knockout mice show an increased micturition threshold. Moreover, in these animals, the PGE_2 induced hyperactivity is decreased [31].
The function of the prostaglandin motor-sensory mechanism in the urinary bladder is not yet understood. However, involvement of capsaicin sensitive afferents and autonomous ganglia has been suggested [26]. An interplay between the cholinergic and prostanoid pathway may be another mechanism of action, as acetylcholine analogues can induce production of PGI_2, PGE_2 and PGF_{2a} [32]. Acetylcholine can induce the production of ATP in urothelial cells [3]. It is known that ATP can cause a stretch independent release of PGE2 from the urothelium via the P_2X and P_2Y receptors, by stimulation of COX 1 [1]. Furthermore, PGE2 production was enhanced by ACh in a COX 1 dependent manner in the gastric mucosa of the guinea pig. This process was mediated by intracellular calcium concentration increase [33].
The current study aims to analyse the interplay between the muscarinic and prostanoid system in the isolated urinary bladder of the guinea pig. The effect of PGE_2 secretory inhibition by COX I and II inhibition, as well as selective EP-receptor inhibition on muscarinically induced micro contractions are analysed and discussed.

6. Wetenschappelijke beoordeling

[redacted]

5 Proefdier

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

We have chosen guinea pigs for this experiment because all our former experiments are with guinea pigs and this allows us to compare data from the underlying project with data collected earlier in the same animal. Also Guinea Pig bladders show a lot of similarities with human bladders (Mostwin et al. 1991).

Species: Guinea Pig
Stam: Hartley
Origin: Charles River
Age: Adults
End: All animals will be sacrificed by cervical dislocation

7b. Sexe

For guinea pigs we select only the male gender because of former experience and the anatomy of the urogenital tract (catheterisation possibilities)

7.c. Aantallen

Ex vivo Cystometry with guinea pigs

Power calculation:
We base the numbers of needed animals on a power calculation based on difference in amplitude obtained after using arecaidine and indometacin (ref Rahnamaí et al. yet unpublished data 2010) on ex vivo model of guinea pig bladders and recently reanalysed in 2012.
The significant effect is expected to be at [redacted] with a standard deviation of $\sigma = 25\%$.
The significance level is $P < 0.05$ and a power of $\pi = 0.8$.
Thus, we calculate the number of animals per group to be:

[redacted]

[redacted]

8. Experiment

Guinea Pig experiments

The urinary bladder and proximal urethra of the guinea pig will be excised immediately after cervical dislocation, and placed into Krebs' solution (mM: NaCl 121.1; KCl 1.87; $CaCl_2$ 1.2; $MgSO_4$ 1.15; $NaHCO_3$ 25; KH_2PO_4 1.17; glucose 11.0), bubbled with 5% CO_2 and 95% O_2 (pH 7.4, 30-37°C). within 15 minutes. The urethra will be cannulated with a solid plastic cannula, diameter 2 mm, and secured with a fine ligature. The bladder is allowed to empty before transfering it to a 40 mL organ bath, containing 37°C constantly gassed Krebs' solution. The cannula is connected through a fluid filled tube, and a three-way connector to a pressure transducer (DTX Plus, Becton Dickinson, Franklin Lakes, NJ, USA). The transducer output will be amplified, digitized at 20 Hz and recorded using a data capture system (MP100 with AcqKnowledge 3.7.3 software, BIOPAC systems inc, California). The pressure range of this device is 0.02–180 cmH2O. The transducer will be calibrated before each experiment. Recordings start directly after applying the bladder to the organ bath.

Protocol:

A 50 minutes resting period is kept before filling the bladder to 1000 µl. To our knowledge, no data are available on physiological urine output in the guinea pig. Therefore the bladder filling rate was based on studies in the rat [34, 35]. It will be filled with a rate of 25 µl/min (1000 µl/30 minutes = 33,3 µl/min) (we aim for a filling time of 30 minutes), using a Treonic IP4 syringe infusion pump (Vinckers Medical, Basingstoke, United Kingdom). These conditions are implemented to minimize physical irritation and to approach a physiological situation [34]. The trials start by performing a wash step directly after completion of bladder filling. This wash step is performed by emptying the organ bath, after which the organ bath is instantaneously re-filled with 100 mL of fresh Krebs' solution from below. Superfluous 60 mL Krebs' solution will be able to drain at the top of the organ bath.

The different bladders will be treated with different pharmaca like indicated in the scheme below:

Research Design:

Action (T)	Control Group	Aspirin	Naproxen	[illegible]	[illegible]	[illegible]
0.30	Calibration Bladder (30 m)	Calibration Bladder (30 m)	Calibration Bladder (30 m)	Calibration Bladder (30 m)	Calibration Bladder (30 m)	Calibration Bladder (30 m)
0.45	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)
1.00	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)
1.15	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)
1.30	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)

1.45	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)	Arecaidin Stimulation (15 m)
2.00	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)
2.15	PGE2 Stimulation (15 m)	Arecaidin + Aspirin (15 m)	Arecaidin + Naproxen (15 m)	Arecaidin + EP Blocker 1 (15 m)	Arecaidin + EP Blocker 2 (15 m)	Arecaidin + EP Blocker 3 (15m)
2.30	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)
2.45	PGE2 Stimulation (15 m)	Arecaidin + Aspirin (15 m)	Arecaidin + Naproxen (15 m)	Arecaidin + EP1 Blocker 1 (15 m)	Arecaidin + EP1 Blocker 2 (15 m)	Arecaidin + EP1 Blocker 3 (15 m)
3.00	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)
3.15	PGE2 Stimulation (15 m)	Arecaidin + Aspirin (15 m)	Arecaidin + Naproxen (15 m)	Arecaidin + EP1 Blocker 1 (15 m)	Arecaidin + EP1 Blocker 2 (15 m)	Arecaidin + EP1 Blocker 3 (15 m)
3.30	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)
3.45	Arecaidin + PGE2 Stimulation (15 m)	Arecaidin+ PGE2 + Aspirin Stimulation (15 m)	Arecaidin + PGE2 + Naproxen Stimulation (15 m)	Arecaidin + PGE2 + EP1 Blocker 1 Stimulation (15 m)	Arecaidin + PGE2 + EP1 Blocker 2 Stimulation (15 m)	Arecaidin + PGE2 + EP1 Blocker 3 Stimulation (15 m)
4.00	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)
4.15	Arecaidin + PGE2 Stimulation (15 m)	Arecaidin+ PGE2 + Aspirin Stimulation (15 m)	Arecaidin + PGE2 + Naproxen Stimulation (15 m)	Arecaidin + PGE 2 + EP1 Blocker 1 Stimulation (15 m)	Arecaidin + PGE 2 + EP1 Blocker 2 Stimulation (15 m)	Arecaidin + PGE 2 + EP1 Blocker 3 Stimulation (15 m)
4.30	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)
4.45	Arecaidin + PGE2 Stimulation (15 m)	Arecaidin+ PGE2 + Aspirin Stimulation (15 m)	Arecaidin + PGE2 + Naproxen Stimulation (15 m)	Arecaidin + PGE 2 + EP1 Blocker 1 Stimulation (15 m)	Arecaidin + PGE 2 + EP1 Blocker 2 Stimulation (15 m)	Arecaidin + PGE 2 + EP1 Blocker 3 Stimulation (15 m)
5.00	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)	Wash Step (15 m)

9. Experimentele condities
9a. Anesthesie

Not applicable

9b. Pijnbestrijding

Not applicable

9c. Euthanasie en Humane eindpunten

All animals will be sacrificed by cervical dislocation. They will be sedated with urethane or CO2 before hand.
When we see an animal is in pain or suffers during the experiment time we will euthanatize it.

If an animal will become sick we will ask the veterinary doctor of the CPV for help.
Human endpoints can be classified by the following things:
1 Behaviour
2 Attitude
3 Mobility
4 Welfare state
5 Weight
6 Other problems

The endpoints can be scored by the following score: 0=normal, 1=minimally disabled 2= averagely disabled 3= severely disabled
When an animal scores 3 points on one of the above mentioned endpoints or when the animal scores over 4 points in general it will be euthanized.

Zorg

10a. Ongerief

Due to the fact that the animals will only be handled and sacrificed immediately so the degree of discomfort will be 01.

10b. Welzijnsevaluatie
In former studies there were no problems with animals as far as we know.

11. Verzorging en huisvesting

During the entire experiment, all animals will be housed in groups. The cages and water will be renewed daily by the employees of the CPV.

12. Deskundigheid

The persons involved in this experiment (see above) possess a strong background of laboratory animal welfare experiences with valid licenses issued by CPV (WOD art. 9)

13. Standard Operation Procedures (SOP)

SOP1 guinea pig/mouse bladder preparation & ex-vivo cystometry (see attachment)

Relevante literatuur

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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:

Uw referentie: Onze referentie : Maastricht, 24-11-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Ex-vivo cystometry in guinea pig model concerning the autonomous bladder*", is op de DEC vergadering van 18 november 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

1) 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.
2) De DEC verzoekt op het voorblad het telefoonnummer op het werk van de vervangend verantwoordelijke onderzoeker toe te voegen
3) Bij punt 6 verzoekt de DEC te verwijderen. Je kunt als verantwoordelijke onderzoeker en uitvoerende van een project, niet de aanvraag zelf wetenschappelijk beoordelen. De DEC verzoekt aan te geven door wie of welke commissie **dit DEC protocol** wetenschappelijk is beoordeeld en goedgekeurd.
4) Bij punt 7c wenst de DEC een navolgbare powerberekening, met de juiste uitleesparameter (dit is de uitleesparameter met de grootste variantie) en opgave van variantie, de sigma en delta en het beoogt verschil.

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 2011-155, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

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

Uw referentie: Onze referentie : Maastricht, 19-12-2011

Geachte Onderzoeker,

De **herziene versie** van uw projectaanvraag: "*Ex-vivo cystometry in guinea pig model concerning the autonomous bladder*", is op de DEC vergadering van 16 december 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

1) 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. Dit werd niet aangepast.
2) De DEC mist de brief met de beantwoording van de vragen. Hier wordt 2x om gevraagd, (1x in de mail en 1x in de brief).
3) De DEC verzoekt op het voorblad het telefoonnummer op het werk van de vervangend verantwoordelijke onderzoeker toe te voegen. Dit werd niet aangepast.
4) Punt 7c: in een powerberekening wordt de delta uitgedrukt als percentage verschil tussen 2 groepen (en niet als absoluut getal), de sigma wordt ook uitgedrukt als een percentage. De DEC verzoekt de powerberekening op deze wijze aan te passen.
5) De DEC verzoekt de vragen van de DEC in een brief te beantwoorden.

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 2011-155, 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

University Maastricht

Faculty of Health, Medicine
and Life Sciences

Dierexperimenten Commissie

DEC

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

From:

Your reference: Our reference: Maastricht, 05-01-2012

Dear

hereby the letter according to your remarks and questions of the project "*Ex-vivo cystometry in guinea pig model concerning the autonomous bladder*":

1) The "duur van de proef", the duration of one experiment is now adjusted to one day.
2) The letter according to your remarks and questions is made.
3) The telephone numbers are adjusted on the front page.
4) Chapter 7c: Both delta and sigma are expressed in percentage.
5) See 2

We are sorry for the omission of the explenatory letter.
Thank you very much for your consideration and we hope for a quick favourable outcome.

With best regards,

on behalf of

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

Uw referentie: Onze referentie : Maastricht, 01-02-2012

Geachte Onderzoeker,

De herziene versie van uw projectaanvraag: "*Ex-vivo cystometry in guinea pig model concerning the autonomous bladder*", is op de DEC vergadering van 27 januari 2012 besproken.

De DEC heeft **één enkele vraag/opmerking:**

1) De DEC merkt op dat de powerberekening nog onjuist is. De gegeven verschillen voor controle en interventie groep zijn dermate klein dat (een correct uitgevoerde) powerberekening (Sachs) leidt tot een groepsgrootte van n=214. Het is ondoenlijk en onverantwoord om met zo'n groot aantal dieren te gaan werken. De veronderstelling dat gestart kan worden met n=15 is onjuist. Blijkbaar is de functie van de eerder uitgevoerde powerberekening niet duidelijk. Aangezien er bij gebrek aan vaststelling van de correcte benodigde groepsgroottes een grote kans bestaat dat de voorgestelde dierexperimenten geen bruikbare (statistisch toetsbare) resultaten oplevert, verzoekt de DEC een en ander te bespreken met een statisticus en een kloppende berekening op te nemen.

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 2011-155, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter 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

From:

Your reference: Our reference:

Dear Mr Hoenderken,

hereby the letter according to your remarks and questions of the project "*Ex-vivo cystometry in guinea pig model concerning the autonomous bladder*":

1) The power calculation is adjusted.
When we base the numbers of needed animals on a power calculation based on difference in amplitude and frequency after using several pharmaca in the ex vivo model of the guinea pig we can use the data we have from former experiment done with guinea pig bladders and arecaidine and indometacin (ref Rahnamaí et al. yet unpublished data 2010)

[redacted]

When we use a power calculation with a paired t test with the assumption of alpha=0.05 and the power=0.80 and we assume a correlation coefficient of 0.8 we can calculate the needed animals in both groups with the above indicated statistics. So that we can find a difference in amplitude of the contractions between the micro contractions with after using and cox-inhibitor.
With this calculation and **based on the difference in frequency** we assume according to the experiments described above we need 34 experiments.
Based on the amplitude difference we assume according to the experiments described above we will need 46 experiments to prove statistical significant difference in amplitude with a COX inhibitor.
We would suggest to include 31 animals at first and when needed we can adjust the DEC protocol in a later stage and include more experiments.

Thank you very much for your consideration and we hope for a quick favourable outcome.

With best regards,

on behalf of

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

Uw referentie: Onze referentie : Maastricht, 03-04-2012

Geachte Onderzoeker,

De **herziene versie** van uw projectaanvraag: "*Ex vivo cystometry in guinea pig model concerning the autonomous bladder*", is in de DEC vergadering van 30 maart 2012 besproken.

De DEC heeft een aantal vragen en opmerkingen:

1) De DEC merkt op dat de aantallen op het voorblad en bij punt 7c niet overeenstemmen. Dit moet in overeenstemming zijn.
2) Punt 6- De DEC verzoekt aan te geven door wie of welke commissie **dit DEC protocol** wetenschappelijk is beoordeeld en goedgekeurd. Je kunt als verantwoordelijke onderzoeker en uitvoerende van een project, niet de aanvraag zelf wetenschappelijk beoordelen. Het is niet de bedoeling dat verwezen wordt naar personen in de aanvraag, in verband met de Wet Openbaarheid van Bestuur. De DEC heeft hier eerder een opmerking over gemaakt.
3) De DEC merkt op dat de powerberekening nog steeds onnavolgbaar is. De DEC ziet zich genoodzaakt de aanvraag af te keuren, indien in de volgende herziene versie de statistische berekening nog steeds onnavolgbaar is.

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 2012-155, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

From:

Sent: donderdag 5 april 2012 14:58

To:

Cc:

Subject: RE: Project 2011-155-herziene versie-w

Attachments: Front page DEC guinea pigs 2011-155_april 2012.doc; Dec guinea Pig 2011-155_april 2012.doc

Beste

Gisteren is overleg geweerst met over het bovengenoemde DECprotocol.
Van hem kreeg ik de toestemming om het volgens zijn aanwijzingen geadapteerde protocol samen met de frontpage tussentijds in te dienen:

hereby the letter according to your remarks and questions of the project "*Ex-vivo cystometry in guinea pig model concerning the autonomous bladder*":

1) According to your remarks ,the front page and the point 7 c express now the same number of animals.
2) In chapter 6 the given names of the researcher are deleted and we refer to the front page and the independent scientific reviewer.
3) The power calculation is adjusted according to the telephone conversation with . Now we have a drop-out of animals of 5% and therefore we need a total number of 33 guinea-pigs.

Bij voorbaat dank voor deze mogelijkheid voor tussentijdse indiening en we hopen dat we snel de experimenten kunnen starten.

Met vriendelijke groet,

coordinator fundamental

Maastricht University Medical Centre
P. Debeyelaan 25
P.O.Box 5800
6202 AZ Maastricht

Phone:
Mobile:
Fax:
Email:

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

Uw referentie:

Onze referentie :

Maastricht, 02-05-2012

Geachte Onderzoeker,

De 4e herziene versie van uw projectaanvraag: "*Ex vivo cystometry in guinea pig model concerning the autonomous bladder*", is in de DEC vergadering van 27 april 2012 besproken.

De DEC constateert dat de statistische poweranalyse nog altijd niet congruent is met de proefopzet, ondanks het overleg dat u heeft gehad met één van de DEC-leden.

Om dit probleem op te lossen, stellen we voor dat we een afspraak maken met u en het DEC-lid
De ambtelijk secretaris , zal contact met u opnemen om een afspraak in te plannen in de week van 7 mei 2012.

De Voorzitter van de DEC-UM, , zou graag met u een gesprek hebben op donderdag 31 mei 2012 om 12.30 uur (UNS 50-kamer 1.155), indien dit voor u mogelijk is.

Uw project staat bij de DEC geregistreerd onder nummer 2011-155, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend.

Voorzitter DEC-UM

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
		27-06-2012

Project: *Ex-vivo cystometry in guinea pig model concerning the autonomous bladder.*

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:	**2011-155**
Diersoort:	**cavia's**
Aantal dieren:	72
Einddatum:	27-06-2016

Uw project staat bij de DEC en CFV geregistreerd onder bovenstaand nummer. Gelieve dieren, die voor dit project bestemd zijn, ook onder dit nummer aan te vragen.

Voorzitter DEC-UM

Vicevoorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM

Bezoekadres

Postadres
Postbus 616
6200 MD Maastricht