Deelproject aanvraag

Projectnummer -299
Ontvangst: 09-08-2010

I Project

Onderzoek

- *Aanvrager*

Naam	
Organisatie/Eenheid	
Onderafdeling	
Adres	
Postcode	
Plaats	
Telefoon	
E-mail	
Vergunninghouder	: [1] RUG

- *Algemeen*

Titel basisproject	: In vitro models for cadiac hypertrophy and fibrosis
Aanvang basisproject	: 01-11-2010
Gewenste duur	: 4 jaar
Voorgaande (deel)project(en)	:
Geldstroom	: [1] 1e geldstroom
Subsidiegever	: 2de gelstroom. Universiteit, Guide, ICIN, Veni

- *Eind- of medeverantwoordelijke*

Titel en naam	:

- *Andere personen betrokken bij de handelingen aan proefdieren*

Naam	Functie

Deelproject

- *Algemeen*

Titel deelproject	: In vitro models for cadiac hypertrophy and fibrosis
Aanvang deelproject	: 01-10-2010
Gewenste duur	: 2 jaar

II Onderzoeksopzet

- *Behaalde resultaten*

Isolated neonatal cardiomyocytes,are commonly used to investigate hypertrophy and we have recently started to use this system, to explore amongst others drug specific effects on cardiomyocytes. In addition we have used this system to identify cardiomyocyte specif genes that are differentially expressed during hypertrophy inducing conditions. In particular we identified, amongst others, two genes Dhrs7c and AKIP that are differentially expressed during hypertrophy induction in vitro. We have been able to confirm this also in vivo and importantly, recent results have confirmed that Dhrs7c protein expression is also altered in human heart failure (Abtract ESC, 2010, manuscript in preparation). The function of these proteins and their particular contributions to hypertrophy development are still unknown and requires further investigations. In addition, we study several other genes in the lab implicated in heart failure, including Gal3 (fibrosis) and PRR (heart failure)and will also use the in vitro system to further delineate the functions of these proteins.
Furthermore, we will use this cell system to analyse the effects of particular treatments or drugs. We have recently shown that such in vitro investigation can be very fruitful, also when combined with other in vivo studies. In vitro we can stimulate these cells with different hormones to generate hypertropy or induce fibrosis and moreover we have a unique setting with a tachopacing and stretch device.

Finally, we have observed differences in gene and protein expression between neonatal and adult heart tissues. Since, hypertrophy also induces a set of fetal genes in the heart, we like to further investigate these particular differences.

- *Vraagstelling van het deelproject*

The research questions are outlined below:
1. What is the role of particular genes, including Dhrs7c, PRR, AKIP1 in cardiomyocyte hypertrophy and cardiac fibroblast proliferation and fibrosis (Gal3, AKIP1). These investigations will include a large number of techniques including overexpression and siRNA using adenoviral vectors, real-time PCR, Western blot analysis, immunofluorescence microscopy to determine the spatial and temparal localization of these proteins, 3H-leucine labelling, enzymatic assays, etc.
2. How do hypertrophy inducing agents and strech and pacing effect atrial and ventrical cardiomyocytes and can we identify specific treatments that improve cardiomyocyte function under these conditions. Similarly for fibroblasts.
3. What are the differences between neonatal and adult cardiomyocytes and what are the effects of forced expression or depletion of these specific genes (1) in the respective cell?

- *Onderzoeksopzet*

Pregnant mother rats will be purchased and after birth the neonatal rats will be terminated and their hearts used to isolate cardiomyocytes and cardiac fibroblasts for in vitro experiments.
Similarly adult rat hearts (whenever possible from the mother rats) will be used to isolate adult cardiomyocytes and adult cardiac fibroblasts from the hearts for in vitro experiments

- *Beschrijving van de uitvoering van de experimenten*

0-5 days after birth neonatal rats will be terminated by decapitation and their heads will be directly transfered into liquid nitrogen to stop any brain activity. Subsequently the hearts will be removed for isolation of the particular cell types and these will be used in in vitro experiments. Generally 18-24 pups are used from two mother rats, every other two weeks.

Adult rats (mothers) will be terminated by heart removal under anesthesia and these hearts might be used for isolation of adult cardiomyocytes and cardiac fibroblasts for in vitro experiments.

- *Ongerief voor dieren*

Handeling	Ongerief
Termination	[1] gering

Pregnant females will be housed separately. Baby rats are terminated within 1-5 days by decapitation. The heads of the pups will be tranfered to liquid nitrogen to eliminate residual brain activity.
The mother rats will be terminated within 7 days after birth of the baby rats, except when they will be used for adult cardiomyocyte isolation in which case we might keep them for up to 20 days. Other rats will be used 7-14 days after arrival at the animal house. All adult animals will be terminated by heart excision under anesthesia.

Totaal ongerief deelproject

[1] gering

Toelichting

Is limited to termination

Verfijning van uitvoering

Only termination is involved. No further improvements possible.

- *Dieren: soort(en) en aantal(len) voor de periode van dit deelproject*

Diersoort	Stam	Inteelt	Aantal
Ratten	pups Sprague Dawley	Geen inteelt	1000
Ratten	moeders Sprague Dawley	Geen inteelt	100

Motivering aantal dieren

Of 20 rat pups we isolate approximately 60 million cardiomyocytes. Depending on the analysis we will need different numbers of cells per condition (Microscopy, 0.5 million; RNA, 1 million; WB 2-5 million; IP and enzyme assays 5-10 million). Because ofthe basic character of the experiments we cannot perform a calculation of the number of experiments required to obtain significant differences. However, it is typical that in vitro experiments an n=3 to 6 is required to generate significant differences. Based on the number of experiments we plan to perform we will need to isolate cells almost every other week.

So we will isolate cells almost every other week (except holidays)and use pups (about 20) of two mother rats. In addition we will use some mothers to isolate adult cardiomyocytes. As a control we will also need to use some female animals, which have not been pregnant or alternatively some males. In total this will result in the use of about 22 animals x 25 weeks x 2 years is about 1100 animals.

Vermindering van aantallen

We have used several cardiomyocyte cell lines (HL-1, H9C2, P19CL6), but none of these cells lines show the particular cardiomyocyte characteristicssince these cells are proliferating (in contrast to normal cardiomyocytes) it is not possible to study hypertrophy in these cells.
Nevertheless, we use them sometimes for setting-up experiments or methods and for initial investigations.

III Proefdierkundige gegevens

- *Dieren: herkomst en huisvesting*

Herkomst van dieren

[1] aankoop

Toelichting herkomst van dieren

Purchase from standard commercial suppliers

Dieren uit ander onderzoek

For this research project predominantly healthy pregnant animals are required. If these animals are available from other research
projects, these could also be used, as long as the conditions do not affect the neonatal animals.

Afwijkende huisvesting

Pregnant females will be housed separately

Locatie van de experimenten

[7] PDU Ant.Deusinglaan 1

Elders

Alternatively, [5] CDL (when the PDU closes).

- *Anesthesie en pijnbestrijding*

Anesthesie

[4] wordt toegepast

Welke of waarom niet

The adults animals will be terminated under anesthesia with isofluraan.

Pijnbestrijding (per- en) postoperatief

[1] wordt niet toegepast (geen aanleiding)

Zo ja: Welke

Diersoort	Medicament	Dosering (mg/kg)	Route	Interval (uur)

Eventuele andere pijnbestrijding

not applicable

Toedieningsschema: frequentie en periode

not applicable

- *Einde experiment*

Toestand dier na proef

[1] dood in kader van de proef

Indicaties humane eindpunten

Note that only healthy animals will be used and no opperative or medical treatments are involved. Therefore, general endpoints could be used, like severe illness or extensive body weight loss.

Wijze van termineren

Babies: decapitation
Adults: removal of heart after aneasthetizing the animal.

IV Biotechnologie

Type	Nummer en specificatie
	: