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18-01-2012	Nieuw

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

Financieel beheerder		Budgetnummer	3098 2250B

Titel van het onderzoek:

Modulation of the infarct cardiac rupture incidence in mice, by

startdatum **01-02-2012** einddatum [9] **30-4-2013** *Duur van de proef [10]: 10 dagen*

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mail adres	Bevoegd-heid[5]	Cap. groep /afdeling
1.Verantwoordelijk onderzoeker (VO)				Art 9	
2. Vervanger VO (VVO)				Art 9	
3. Niet deelnemend art. 9 bevoegde				Art 9	
4. Overige uitvoerenden				Art. 12	

Diergroep	1	2	3	4			
ctrl/exp/sham	Ctrl 1	Ctrl 2	Exp 1 (Ciprofloxacin)	Exp 2 (Donepezil)			
Diersoort	01	01	01	01			
Stam	Swiss	129S6	Swiss	129S6			
Construct / mutatie ?	n/a	n/a	n/a	n/a			
Herkomst (leverancier) *	01	01	01	01			
Aantal	49	55	64	55			
Geslacht	♂	♂	♂	♂			
Dieren immuuncompetent ?	ja	ja	ja	ja			
Leeftijd/gewicht	10-12 w	10-12 w	10-12 w	10-12 w			
Doel van de proef *	31	31	31	31			
Belang van de proef *	01	01	01	01			
Toxicologisch onderzoek *	01	01	01	01			
Bijzondere technieken *	01	01	01	01			
Anesthesie *	04	04	04	04			
Pijnbestrijding *	04	04	04	04			
Mate ongerief *	05	05	05	05			
Toestand dier einde exp*	01	01	01	01			

* VHI-coderingen zie bijlage

Wetenschap

5. Wetenschappelijke onderbouwing

Infarct Rupture is a life threatening complication of MI and cause of death that occurs within the first week after MI (Julian, 1991), (Pretre, et al., 2000). Infarct rupture accounts for 10% of the mortality from acute MI (Reeder, 1995) and is most frequent in the left ventricular wall, leading to cardiac tamponade and instant death (Antman & Braunwald, 1997). The matrix metalloproteinases (MMPs), a group of extracellular matrix (ECM) degrading proteins that contain 28 known proteins are of major importance in the process of the ECM degradation in a variety of cardiovascular diseases, including rupture and remodeling of the heart tissue post-MI (Creemers, et al., 2001). Several studies have investigated the role of MMPs in the development of infarct rupture following MI. The up-regulation of MMP-2, MMP-8 and MMP-9 is believed to be of major importance in cardiac rupture occurrence after myocardial infarction (Hayashidani, et al., 2003), (Matsumura, et al., 2005), (van den Borne, et al., 2009a). After MI occurs, inflammatory cells infiltrate the infarct area. The presence of neutrophils correlates to an increase in MMP-9 levels within 24 hours after MI. The MMP-2 levels are induced at day 4 and they are associated with macrophages, fibroblasts and myocytes (Vanhoutte, et al., 2006). Furthermore, the group of Heymans at al. demonstrated that mice lacking the MMP-9 plasminogen activator gene, had a significant lower incidence of infarct rupture during the first week after MI. Another important finding was also a diminished TIMP-1 protein expression. This suggests that MMP activation (direct but also via TIMP suppression) and thus collagen degradation, plays a crucial role in the pathogenesis of infarct rupture (Heymans, et al., 1999).

ınd are used for several conditions such as respiratory infections (Albertson, et al., 2010), (Gotfried & Grossman, 2010) or serious urinary tract infections (Carson & Naber, 2004) and others. Numerous studies have demonstrated that the use of leads to an increased risk for Achilles tendon rupture. *In vitro* data show that various can affect several MMPs and TIMPs (Corps, et al., 2002), (Tsai, et al., 2011), (Sendzik, et al., 2010), (Deng, et al., 2011) in tendon cells. Hence, mediate their ECM-degrading effects on tendon cells/tendons via MMPs and TIMPs.

On the other hand, drug agent used in the treatment of Alzheimer's Disease was shown to reduce the incidence of infarct rupture following MI (Arikawa, et al., 2011). The incidence of infarct rupture at days 3 and 4 was significantly lower in the group vs the untreated group (8.7% vs 30.6% respectively) and this beneficial effect of is mediated via a suppressive effect on MMP-9 expression.

The aim of this study is to expand our knowledge on the roles of the MMPs in the occurrence of infarct rupture and investigate whether can have a detrimental effect on its incidence. Moreover, another aim of this experiment is to ascertain whether other MMPs (other than MMP-9) are implicated in the actions of and whether this can serve a realistic protective role. Hence, valuable conclusions can be drawn about the molecular and cellular alterations occurring during infarct remodeling and rupturing, as well as important information about the actual role of MMPs and TIMPs in these complex processes.

6. **Wetenschappelijke beoordeling**
The scientific quality of this project has been approved by a non-participating article 9-qualified researcher of our research group (, University of Maastricht).

7. Proefdier keuze

7a. Soort, stam / herkomst / eindbestemming

The use of laboratory animals is mandatory, since an *in vivo* experiment is necessary in order to prove the hypothesis. The development of infarct rupture is necessary in order to answer our research question. Infarct rupture is the result of a complex interplay of protein-degrading enzymes produced by inflammatory cells that cannot be monitored otherwise than by its result, i.e. infarct rupture. Hence, without the development of infarct rupture our hypothesis cannot be answered.

The choice of the two specific strains of mice (Swiss and 129S6) can be justified by a previous study from our group (van den Borne, et al., 2009b). Swiss mice show a myocardial rupture incidence of 23%, while 129S6 show a high incidence of 62%. The choice of Swiss mice is the optimal in order to study the possible increase in the incidence of rupture with , while the choice of 129S6 would be more convenient in order to study the protective effect of

Male Swiss and 129S6 mice will be used (aged 10-12 weeks). These animals are bred in Jackson Laboratories (Bar Harbor. ME, USA) and housed in the University of Maastricht Animal Facility.

After the completion of the experiment, the animals will be sacrificed by bleeding under urethane anesthesia. Their hearts will be removed, in order to conduct immunohistochemical and biochemical (zymography, western blotting, qPCR) measurements.

7b. Sexe

Only male mice will be used for the experiment. The reason for this is that there is a significant difference in the myocardial healing after an MI, between male and female mice. The incidence of infarct rupture in males is considerably higher than in females and the cardiac function of males is poorer compared to females. Hence, the mixing of populations of both sexes is not reasonable (Cavasin, et al., 2004). Moreover, The sex-related variable in tendinopathy in humans is 4:1, with male predominance (Childs, 2007).

7.c. Aantallen

Hearts of Swiss and 129S6 mice are expected to rupture around the 3rd-4th day post-MI. Readout parameter for the animals sacrificed on day 3 is presence of MMPs and the parameter for the animals sacrificed on days 6-7 is rupture vs not-rupture.

In order to calculate the numbers needed for the day 3 MMP investigation, the equation of Sachs was used. **Our estimation of the variance is based on mouse data from a previous study in our group. In this study, the variation in the MMP-9 activity was found to be approximately 45% for the Swiss mice and 49% for the 129S6 mice (van den Borne, et al., 2009b).**

As regards to the numbers needed for the days 6-7 investigation, the Kaplan-Meier survival curve was used to calculate the group size for the animals.

Overall mortality (combination of peri-operative plus early HF mortality) for both strains is estimated to be **20%**, based on several previous experiments in our lab. It includes the acute mortality (<24 hours post-MI), the result of acute heart failure, arrhythmias and animals that cannot

be weaned from the ventilator. By having the surgery performed by skilled technicians, we have minimized the peri-operative mortality. Depending on its effects (see hypothesis), might be expected to show increased mortality due to infarct rupture (approx. 2x) while should show decreased infarct rupture incidence and mortality due to this reason (approx. half).

Formula of L. Sachs for day 3 sub-group:

α=0.05; Discrimination π=80%. Hence, $F_{0,80}$ = 15.7

$n=15.7 \times (\sigma/\delta)^2$

Swiss mice: Variation σ=45%; Difference δ=50%, so $n=15.7 \times (0.45/0.5)^2 = 12.7$

129S6 mice: Variation σ=49%; Difference δ=50%, so $n=15.7 \times (0.49/0.5)^2 = 15.1$

Swiss (Ctrl 1) treatment group size: $(\alpha-0.2*\alpha)$=12.7

Hence, n=16

129S6 (Ctrl 2) treatment group size: $(\alpha-0.2*\alpha)$= 15.1

Hence, n=19

Swiss (n) treatment group size: $(\alpha-0.2*\alpha)$=12.7

Hence, n=16

129S6 (treatment group size: $(\alpha-0.2*\alpha)$= 15.1

Hence, n=19

Calculation of animal numbers for days 6-7 sub-group:

A statistician was contacted in order to make this calculation. The Fisher's Exact Test was recommended, in order to estimate the correct number of animals needed. The results of the tests are attached in the end of the document. The usual rupture incidence of previous experiments conducted in our group was also taken into consideration (20% for Swiss mice and 60% for 129S6 mice). So, the numbers needed for the days 6-7 sub-group are:

Control group Swiss: Needed 44 animals, but taking into consideration the 20% drop-out, this is 53. However, we have data from 20 historical controls from our previous study (van den Borne, et al., 2009b) which can be added in the current study, in order: i) to confirm our past results and ii) to drastically reduce the amount of animals needed for the current study. Hence, only 33 Swiss mice (instead of 53 will be needed for the control group.

group Swiss: Infarct rupture incidence is expected to double. The number of animals needed should be 40, according to the Fisher's Exact Test, but taking into consideration the 20% drop-out, this is 48.

Control group 129S6: Needed 30 animals, but taking into consideration the 20% drop-out, this is 36.

group 129S6: Infarct rupture incidence is expected to be halved. The number of animals needed (according to the Fisher's Exact Test) should be 30, but taking into consideration the 20% drop-out, this is 36.

Total number of animals:

Animal group 1 (Control Swiss): 16+ 33 = 49
Animal group 2 (Control 129S6): 19 + 36= 55
Animal group 3 (s): 16 + 48 = 64
Animal group 4 129S6): 19 + 36 = 55

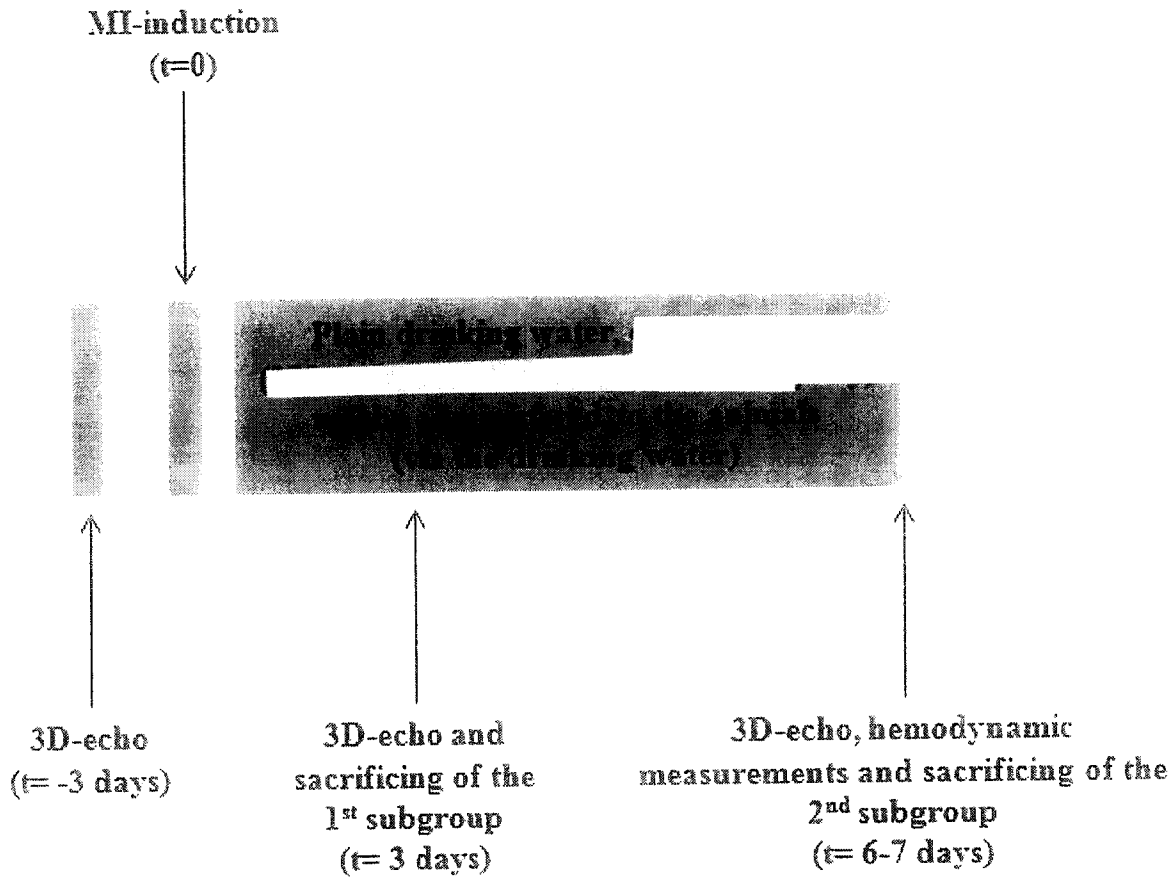
Dierproef

8. Experiment

Three days before the initiation of the experiment, a 3D-echocardiography will be performed (SOP), in order to get a baseline reading. On day "zero" (t=0) of the experiment, a myocardial infarction will be induced to the animals, by occlusion of the coronary arteries (SOP
These two interventions cannot be combined, because the duration of the isoflurane anesthesia would be around 1.5 hours (3D-echo 30 min, followed by Temgesic injection which takes 30 min to become active and MI procedure 30 min) which will cause a significant increase in peri-operative mortality.

Treatment with /day) (Brunner & Zeiler, 1988), (Shasha, et al., 1992), (Piercy, et al., 2005), (Toda, et al., 2009) which is the most commonly used , or mg/kg/day) (Arikawa, et al., 2011) via the drinking water will be initiated immediately after MI, while control animals will receive just water (without any additives). Both groups will be subdivided into two further sub-groups: one sub-group sacrificed on day 3 (3D-echocardiography will be performed before the animals are sacrificed). The second subgroup will be sacrificed on days 6-7 post-MI (animals will be subjected to 3D-echocardiography and hemodynamic measurements before the sacrifice).

The diagnosis of infarct rupture will be confirmed based on the presence of a wall perforation at the left ventricular infarct area.


MI-induction
(t=0)
Plain drinking water,
3D-echo
(t= -3 days)
3D-echo and
sacrificing of the
1st subgroup
(t= 3 days)
3D-echo, hemodynamic
measurements and sacrificing of the
2nd subgroup
(t= 6-7 days)

9. Experimentele condities

9a. Anesthesie

Anesthesia will be introduced by 4% isoflurane and maintained by 1.5-2% isoflurane.

9b. Pijnbestrijding

At 30 min before the operation, 0.1 mg/kg of the opioid temgesic (buprenorphine) will be administered by s.c. injection. By the end of the day of operation another dose will be given and then, for the following 2 days, temgesic (0.1 mg/kg) will be administered twice daily to provide adequate pain-relief for the animals.

9c. Euthanasie en Humane eindpunten

The sacrificing of an animal without any means of anesthesia of analgesia is ethically unacceptable. When specific criteria are met, an animal should be sacrificed, in order to prevent it from feeling excessive discomfort or unnecessary pain. The criteria are the following:

- General cachexia, severe lack of activity when handled
- Severe lack of exploration of the surrounding area
- Breathing problems (the animal's respiratory function will be monitored in order for a diagnosis of a probable Pulmonary Oedema to be made) or cyanosis
- Extreme loss of weight (>20% of original weight before the initiation of the experiment)

The euthanizing of the animal in these cases will be done by isoflurane anesthesia followed by cervical dislocation.

Zorg

10a. Ongerief

intervention	duration	frequency	discomfort
3D-echocardiography	10 min	3x	02
MI induction	30 min	1x	05
Living with MI *	6-7 days	1x	03
Final hemodynamics	45 min	1x	02
Myocardial rupture development **	seconds	1x	02

Total discomfort for the animal: **05.**
In case the animals develop severe heart failure during the cause of the experiment, the animal will be sacrificed when the humane endpoints are reached as listed under 9c.

* Following the induction of MI, and due to the remodeling of the myocardium that is initiated shortly after MI, the animals might experience moderate discomfort (03), which may last for 6-7 days.

** The development of myocardial rupture is a phenomenon that occurs instantaneously. During the development of infarct rupture, the ventricular wall ruptures, the blood pressure falls very rapidly, the cardiac output falls to zero within seconds and the animal loses consciousness within seconds, since the brain is not receiving adequate amounts of blood. Hence, it would be expected that the development of myocardial rupture would cause a discomfort level, similar to the one that is experienced when an animal is put into anesthesia (discomfort level 02).

10b. Welzijnsevaluatie
The Verantwoordelijk onderzoeker will be responsible for the well-being of the animals throughout the experiment. He will be in close liaison with the Animal Facility Veterinary Doctors, in order to spot any unusual deterioration to the animal's health and act immediately as required. The distress caused to the animals will not be greater compared to the one caused during the experiments of our group in the past.

11. Verzorging en huisvesting
Animals will be socially housed in the Animal Facility of the University of Maastricht. No special conditions are needed for these laboratory animals. In a case of an emergency an "Article 14 accredited" person (or his representative) will be called. All experiments will be executed in the . Animal Facility.

12. Deskundigheid
All experiments will be under the responsibility of the verantwoordelijk onderzoeker (or his representative) and will be conducted in the Animal Facility. All persons that will be involved in the experiments and the handling of the animals are "Article 9 certified" (technicians assisting in the operations are all "Article 12" certified).

13. **Standard Operation Procedures (SOP)**
- **Echocardiografie bij de muis** (SOP Far-02-M)
- **Terminale hemodynamische karakterisatie** van de muis (SOP Far-07-M)
- **Myocardinfarct bij de muis** (SOP Far-05-M)

Relevante literatuur

ALBERTSON, T.E., DEAN, N.C., EL SOLH, A.A., GOTFRIED, M.H., KAPLAN, C. & NIEDERMAN, M.S. (2010). Fluoroquinolones in the management of community-acquired pneumonia. *Int J Clin Pract* **64**(3), 378-388.

ANTMAN, E. & BRAUNWALD, E. (1997). Acute myocardial infarction. Heart disease, a textbook of cardiovascular medicine. E Braunwald, Editor, WB Saunders & Co, Philadelphia, pp. 1184–1268. .

ARIKAWA, M., KAKINUMA, Y., HANDA, T., YAMASAKI, F. & SATO, T. (2011). Donepezil, anti-Alzheimer's disease drug, prevents cardiac rupture during acute phase of myocardial infarction in mice. *PLoS One* **6**(7), e20629.

BRUNNER, H. & ZEILER, H.J. (1988). Oral ciprofloxacin treatment for Salmonella typhimurium infection of normal and immunocompromised mice. *Antimicrob Agents Chemother* **32**(1), 57-62.

CARSON, C. & NABER, K.G. (2004). Role of fluoroquinolones in the treatment of serious bacterial urinary tract infections. *Drugs* **64**(12), 1359-1373.

CASPARIAN, J.M., LUCHI, M., MOFFAT, R.E. & HINTHORN, D. (2000). Quinolones and tendon ruptures. *South Med J* **93**(5), 488-491.

CAVASIN, M.A., TAO, Z., MENON, S. & YANG, X.P. (2004). Gender differences in cardiac function during early remodeling after acute myocardial infarction in mice. *Life Sci* **75**(18), 2181-2192.

CHILDS, S.G. (2007). Pathogenesis of tendon rupture secondary to fluoroquinolone therapy. *Orthop Nurs* **26**(3), 175-182; quiz 183-174.

CLEUTJENS, J.P., BLANKESTEIJN, W.M., DAEMEN, M.J. & SMITS, J.F. (1999). The infarcted myocardium: simply dead tissue, or a lively target for therapeutic interventions. *Cardiovasc Res* **44**(2), 232-241.

CORPS, A.N., HARRALL, R.L., CURRY, V.A., FENWICK, S.A., HAZLEMAN, B.L. & RILEY, G.P. (2002). Ciprofloxacin enhances the stimulation of matrix metalloproteinase 3 expression by interleukin-1beta in human tendon-derived cells. A potential mechanism of fluoroquinolone-induced tendinopathy. *Arthritis Rheum* **46**(11), 3034-3040.

CREEMERS, E.E., CLEUTJENS, J.P., SMITS, J.F. & DAEMEN, M.J. (2001). Matrix metalloproteinase inhibition after myocardial infarction: a new approach to prevent heart failure? *Circ Res* **89**(3), 201-210.

DENG, Y., CHEN, B., QI, Y., MAGDALOU, J., WANG, H. & CHEN, L. (2011). The effects of levofloxacin on rabbit anterior cruciate ligament cells in vitro. *Toxicol Appl Pharmacol.*

GOTFRIED, M.H. & GROSSMAN, R.F. (2010). Short-course fluoroquinolones in acute exacerbations of chronic bronchitis. *Expert Rev Respir Med* **4**(5), 661-672.

HAYASHIDANI, S., TSUTSUI, H., IKEUCHI, M., SHIOMI, T., MATSUSAKA, H., KUBOTA, T., IMANAKA-YOSHIDA, K., ITOH, T. & TAKESHITA, A. (2003). Targeted deletion of MMP-2 attenuates early LV rupture and late remodeling after experimental myocardial infarction. *Am J Physiol Heart Circ Physiol* **285**(3), H1229-1235.

HEYMANS, S., LUTTUN, A., NUYENS, D., THEILMEIER, G., CREEMERS, E., MOONS, L.,

DYSPERSIN, G.D., CLEUTJENS, J.P., SHIPLEY, M., ANGELLILO, A., LEVI, M., NUBE, O., BAKER, A., KESHET, E., LUPU, F., HERBERT, J.M., SMITS, J.F., SHAPIRO, S.D., BAES, M., BORGERS, M., COLLEN, D., DAEMEN, M.J. & CARMELIET, P. (1999). Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure. *Nat Med* **5**(10), 1135-1142.

JULIAN, D.G. (1991). Why do patients die after myocardial infarction? *J Cardiovasc Pharmacol* **18 Suppl 2**, S80-82.

KAROUSOU, E., RONGA, M., VIGETTI, D., PASSI, A. & MAFFULLI, N. (2008). Collagens, proteoglycans, MMP-2, MMP-9 and TIMPs in human achilles tendon rupture. *Clin Orthop Relat Res* **466**(7), 1577-1582.

KING, D.E., MALONE, R. & LILLEY, S.H. (2000). New classification and update on the quinolone antibiotics. *Am Fam Physician* **61**(9), 2741-2748.

MATSUMURA, S., IWANAGA, S., MOCHIZUKI, S., OKAMOTO, H., OGAWA, S. & OKADA, Y. (2005). Targeted deletion or pharmacological inhibition of MMP-2 prevents cardiac rupture after myocardial infarction in mice. *J Clin Invest* **115**(3), 599-609.

PIERCY, T., STEWARD, J., LEVER, M.S. & BROOKS, T.J. (2005). In vivo efficacy of fluoroquinolones against systemic tularaemia infection in mice. *J Antimicrob Chemother* **56**(6), 1069-1073.

PRETRE, R., BENEDIKT, P. & TURINA, M.I. (2000). Experience with postinfarction left ventricular free wall rupture. *Ann Thorac Surg* **69**(5), 1342-1345.

REEDER, G.S. (1995). Identification and treatment of complications of myocardial infarction. *Mayo Clin Proc* **70**(9), 880-884.

SENDZIK, J., SHAKIBAEI, M., SCHAFER-KORTING, M., LODE, H. & STAHLMANN, R. (2010). Synergistic effects of dexamethasone and quinolones on human-derived tendon cells. *Int J Antimicrob Agents* **35**(4), 366-374.

SHASHA, B., LANG, R. & RUBINSTEIN, E. (1992). Therapy of experimental murine brucellosis with streptomycin, co-trimoxazole, ciprofloxacin, ofloxacin, pefloxacin, doxycycline, and rifampin. *Antimicrob Agents Chemother* **36**(5), 973-976.

TODA, T., OHI, K., KUDO, T., YOSHIDA, T., IKARASHI, N., ITO, K. & SUGIYAMA, K. (2009). Ciprofloxacin suppresses Cyp3a in mouse liver by reducing lithocholic acid-producing intestinal flora. *Drug Metab Pharmacokinet* **24**(3), 201-208.

TSAI, W.C., HSU, C.C., CHEN, C.P., CHANG, H.N., WONG, A.M., LIN, M.S. & PANG, J.H. (2011). Ciprofloxacin up-regulates tendon cells to express matrix metalloproteinase-2 with degradation of type I collagen. *J Orthop Res* **29**(1), 67-73.

VAN DEN BORNE, S.W., CLEUTJENS, J.P., HANEMAAIJER, R., CREEMERS, E.E., SMITS, J.F., DAEMEN, M.J. & BLANKESTEIJN, W.M. (2009a). Increased matrix metalloproteinase-8 and -9 activity in patients with infarct rupture after myocardial infarction. *Cardiovasc Pathol* **18**(1), 37-43.

VAN DEN BORNE, S.W., VAN DE SCHANS, V.A., STRZELECKA, A.E., VERVOORT-PETERS, H.T., LIJNEN, P.M., CLEUTJENS, J.P., SMITS, J.F., DAEMEN, M.J., JANSSEN, B.J. & BLANKESTEIJN, W.M. (2009b). Mouse strain determines the outcome of wound healing after myocardial infarction. *Cardiovasc Res* **84**(2), 273-282.

VANHOUTTE, D., SCHELLINGS, M., PINTO, Y. & HEYMANS, S. (2006). Relevance of matrix metalloproteinases and their inhibitors after myocardial infarction: a temporal and spatial window. *Cardiovasc Res* **69**(3), 604-613.

An interactive calculation tool for Fisher's exact probability test for 2 x 2 tables

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8	2	7	3	6	4	5	5	4	6	3	7

with corresponding probabilities:

.007	.093	.326	.392	.163	.019

Those tables outlined in yellow constitute the configurations more extreme than the observed configuration in the same direction. More extreme configurations in the same direction are identified by locating the smallest frequency in the table, subtracting 1, and then computing the remaining items given the observed marginal frequencies. Those tables outlined in green are the configurations more extreme in the *opposite* direction. Extremity is defined in terms of probability, so the probability of any configuration to the right of the table of observed frequencies with probability less than or equal to that of the observed configuration are added to the total probability of more extreme configurations.

Thus, the one-tailed probability for this table would be:

.326 + .093 + .007 = .426

...whereas the two-tailed probability would be:

.326 + .093 + .007 + .163 + .019 = .608

The probability for the fourth configuration is not included because it is less extreme (more probable) than the observed frequency configuration.

How to use this page

Type your observed frequencies into the four upper left cells of the table below, then click on "Calculate." The status bar at the bottom will tell you if there is an error of some sort.

Warning

If you get a "Stack overflow" error, see "NaN" in the output boxes, or get no results at all when you click on "Calculate," your frequencies are probably too large for the program to handle. Consider instead using the chi-square approximation with Yates' correction for continuity.

	Gp 1	Gp 2		
Class 1:	18	12	30	
Class 2:	9	21	30	
	27	33	60	

p-values:

Calculate	this tail:	0.0184962
Reset	other tail:	0.99558621
	both tails:	0.0369924
Status:		Status okay

Acknowledgments

Original version posted May, 2001. We wish to thank Frank Tola for extensive and thorough testing. Free JavaScripts provided by Free JavaScript Source and John C. Pezzullo.

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8 | 2	7 | 3	6 | 4	5 | 5	4 | 6	3 | 7

with corresponding probabilities:

.007	.093	.326	.392	.163	.019

Those tables outlined in yellow constitute the configurations more extreme than the observed configuration in the same direction. More extreme configurations in the same direction are identified by locating the smallest frequency in the table, subtracting 1, and then computing the remaining items given the observed marginal frequencies. Those tables outlined in green are the configurations more extreme in the *opposite* direction. Extremity is defined in terms of probability, so the probability of any configuration to the right of the table of observed frequencies with probability less than or equal to that of the observed configuration are added to the total probability of more extreme configurations.

Thus, the one-tailed probability for this table would be:

```
326 + 093 + .007 = .426
```

...whereas the two-tailed probability would be:

```
326 + 093 + .007 + .163 + .019 = .608
```

The probability for the fourth configuration is not included because it is less extreme (more probable) than the observed frequency configuration.

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	Gp 1	Gp 2	
Class 1:	8	36	44
Class 2:	16	24	40
	24	60	84

p-values:

Calculate	this tail:	0.02415006
Reset	other tail:	0.99320236
	both tails:	0.03203428

Status: Status okay

Acknowledgments

Original version posted May, 2001. We wish to thank Frank Tola for extensive and thorough testing. Free JavaScripts provided by The JavaScript Source and John C. Pezzullo.

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

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

Geachte Onderzoeker,

Uw projectaanvraag: "*Modulation of the cardiac rupture incidence following myocardial infarction, by and* ", is op de DEC vergadering van 18 november 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

1) De DEC verzoekt het privételefoonnummer van de vervangend verantwoordelijke onderzoeker nog toe te voegen op het voorblad.
2) Op het voorblad en bij punt 6, verzoekt de DEC het woord "onafhankelijk", te verwijderen (evt vervangen door 'niet-deelnemend').
3) Bij punt 7c ontbreekt de onderbouwing voor de (hoge) variantie van 50% en eveneens het verschil van 50%.
4) Bij het 2e deel van punt 7c- de Kaplan Meier curves, die de basis vormen voor de groepsgroottes, dienen te worden toegevoegd. In deze powerberekening kan niet reeds worden uitgegaan dat de behandelingen inderdaad het verwachte effect hebben. De DEC adviseert contact op te nemen met een statisticus teneinde de groepsgrootte te bepalen van deze studies.
5) Punt 9c- Humane eindpunten. De DEC merkt op dat de dood geen uitleesparameter kan zijn. Deze dient gebaseerd te zijn op humane eindpunten. Gaarne onderbouwen als dat niet mogelijk is.

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 2011-156, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Faculty of Health, Medicine & Life Science

To:

Dier Experimenten Commissie (DEC)

Faculty of Health, Medicine & Life Science

Reference number:

Maastricht, 02-12-2011

Dear

Thank you very much for your comments regarding our protocol application with the title: "*Modulation of the cardiac rupture incidence following myocardial infarction, by and* " which has the DEC protocol number *2011-056*. Our research team has taken all your points into serious consideration and we are in a position to send the revised DEC protocol for approval.

Regarding your points/comments:

1) The private (mobile) telephone number of the "Vervanger VO" has been added in the protocol (Part I).

2) The title of the person indicated in Point 6 of the protocol, is now indicated as "niet-deelnemend", instead of "onafhankelijk".

3) The selection of the 50% variation (Point 7c of the protocol) has been based on data from human samples acquired in previous experiments of our group that have been published in a scientific journal. These experiments compared infarct rupture vs non-infarct rupture in patients that had suffered MI. Two MMPs (MMP-8 and MMP-9) were investigated in these experiments and the variation was found to be approximately 50% (please see DEC protocol revision for publication details).

4) Regarding the comment for Point 7c, a statistician was contacted as it was recommended by DEC. The statistician recommended the use of the "Fisher's Exact Test", in order to make an estimation of the number of animals needed. The calculation was done online (please see attached files in the end of the DEC protocol) and revealed that the numbers of the Swiss and 129S6 mice required for the experiment are almost equal to the numbers indicated by the Kaplan Meier's curve. We have adapted the numbers accordingly in the DEC protocols.

5) Regarding the comment on Point 9c of the protocol, about the end-point of the study, we would like to clarify that we use infarct rupture as an end-point rather than death. Infarct rupture is a phenomenon that occurs instantaneously. During the development of infarct rupture, the ventricular wall ruptures, the blood pressure falls very rapidly, the cardiac output falls to zero within seconds and the animal loses consciousness within seconds, since the brain is not receiving adequate amounts of blood. Hence, the development of infarct rupture is a phenomenon that is not regarded as one that is causing excessive distress to the animal. The purpose of our proposal is to study the development of infarct rupture following MI and investigate whether specific factors (MMPs, TIMPs) play a role in its development and whether their levels can explain or predict the occurrence of the rupture. Hence, the development of the infarct rupture is absolutely crucial, in order for the study to answer its scientific questions.

We are looking forward to the verdict of the DEC, regarding the revisions of our protocol.

Kind Regards,

(on behalf of the research-team)

Calculation for Fisher's Exact Test

An interactive calculation tool for Fisher's exact probability test for 2 x 2 tables

Curriculum vitae
Selected publications
Supplemental material for publications
Introduction to Statistical Analysis
Friends and colleagues
Organizations
Online utilities
Mediation & moderation material
Vanderbilt Dept. of Psychology & Human Development
Vanderbilt Quantitative Methods (QM) program
Contact me

8 2	7 3	6 4	5 5	[illegible]	[illegible]

with corresponding probabilities:

.007	.093	.326	.392	.163	.019

Those tables outlined in yellow constitute the configurations more extreme than the observed configuration in the same direction. More extreme configurations in the same direction are identified by locating the smallest frequency in the table, subtracting 1, and then computing the remaining items given the observed marginal frequencies. Those tables outlined in green are the configurations more extreme in the *opposite* direction. Extremity is defined in terms of probability, so the probability of any configuration to the right of the table of observed frequencies with probability less than or equal to that of the observed configuration are added to the total probability of more extreme configurations.

Thus, the one-tailed probability for this table would be:

.326 + .093 + .007 = .426

whereas the two-tailed probability would be:

.326 + .093 + .007 + .163 + .019 = .608

The probability for the fourth configuration is not included because it is less extreme (more probable) than the observed frequency configuration.

How to use this page

Type your observed frequencies into the four upper left cells of the table below, then click on "Calculate." The status bar at the bottom will tell you if there is an error of some sort.

Warning

If you get a "Stack overflow" error, see "NaN" in the output boxes, or get no results at all when you click on "Calculate," your frequencies are probably too large for the program to handle. Consider instead using the chi-square approximation with Yates' correction for continuity.

	Gp 1	Gp 2	
Class 1:	8	36	44
Class 2:	16	24	40
	24	60	84

p-values:

Calculate	this tail:	0.02415006
Reset	other tail:	0.99320236
	both tails:	0.03203428

Status: Status okay

Acknowledgments

Original version posted May, 2001. We wish to thank Frank Tola for extensive and thorough testing. Free JavaScripts provided by The JavaScript Source and John C. Pezzullo.

Calculation for Fisher's Exact Test

quantpsy.org

An interactive calculation tool for Fisher's exact probability test for 2 x 2 tables

8	2		7	3		6	4		5	5		4	6		3	7

with corresponding probabilities

.007	.093	.326	.392	.163	.019

Those tables outlined in yellow constitute the configurations more extreme than the observed configuration in the same direction. More extreme configurations in the same direction are identified by locating the smallest frequency in the table, subtracting 1, and then computing the remaining items given the observed marginal frequencies. Those tables outlined in green are the configurations more extreme in the *opposite* direction. Extremity is defined in terms of probability, so the probability of any configuration to the right of the table of observed frequencies with probability less than or equal to that of the observed configuration are added to the total probability of more extreme configurations.

Thus, the one-tailed probability for this table would be

.007 + .093 + .326 = .426

...whereas the two-tailed probability would be

.007 + .093 + .326 + .163 + .019 = .608

The probability for the fourth configuration is not included because it is less extreme (more probable) than the observed frequency configuration.

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Warning

If you get a "Stack overflow" error, see "NaN" in the output boxes, or get no results at all when you click on "Calculate," your frequencies are probably too large for the program to handle. Consider instead using the chi-square approximation with Yates' correction for continuity.

	Gp 1	Gp 2	
Class 1:	18	12	30
Class 2:	9	21	30
	27	33	60

p-values:

Calculate — this tail: 0.0184962

Reset — other tail: 0.99656521

both tails: 0.0369924

Status: Status okay

Acknowledgments

Original version posted May, 2001. We wish to thank Frank Tola for extensive and thorough testing. Free JavaScripts provided by The Javascript Source and John C. Pezzullo.

From: Dec Secretariaat
Sent: dinsdag 3 januari 2012 20:31
To:
Subject: FW: DEC protocol 2011-056

Attachments: DEC Part I (2011-156) rev.doc; DEC Part II (2011-156) rev.doc; Letter to DEC (
) rev.doc; t Test (calculation of (treated-
untreated).JPG; Test (calculation of treated-untreated).JPG


DEC Part I
)11-156) rev.doc

DEC Part II
011-156) rev.doc

Letter to DEC
Or

Exact
) . Test (calculati...

Exact
Test (calculati...

Geachte onderzoeker, beste

De beste wensen voor 2012!

Allereerst mijn excuses dat dit het wat langer heeft geduurd dan normaal gebruikelijk is. Dit heeft te maken met het overlijden van mijn moeder voor de kerst en de drukte van de laatste commissievergadering meteen erna.

De DEC heeft wederom de herziene versie besproken en heeft nog de volgende vragen en/of opmerkingen:
Ad 1. (met betrekking tot de groepsgroottes): aangezien de onderzoekers voornemens zijn de vraagstelling mbv proefdieren te onderzoeken, is het raadzaam om de sigma en delta te schatten op basis van bestaande literatuur van MI-infaction/rupture modellen in muizen in plaats van de humane data, omdat daarmee wellicht een kleinere spreiding (delta) voor de te bepalen primaire uitleesmaat gewerkt kan worden

Ad 2. (ongerief/dood): de alinea waarin wordt gesteld dat de dieren geen ongerief ondervinden (aan ruptuur) dient te worden verwijderd. Tevens verzoekt de DEC om het leven met de gevolgen van MI en het toch mogelijke (zeer tijdelijke) ongerief ten gevolge van ruptuur beter te omschrijven, gezien het ongerief code 05

Graag je reactie.

Met vriendelijke groet namens DEC-UM:

Ambtelijk secretaris

Postbus 616- , 6200 MD Maastricht T
E-mail:
Werktijden: M

-----Original Message-----
From:
Sent: vrijdag 2 december 2011 20:36
To: Dec Secretariaat
Subject: DEC protocol 2011-056

Dear

I am sending you the revised DEC protocols, the 2 extra documents about the test utilized for the statistics

Department of

Faculty of Health, Medicine & Life Science

To:

Dier Experimenten Commissie (DEC)

Faculty of Health, Medicine & Life Science

Reference number: I

Maastricht, 11-01-2012

Dear

Thank you very much for the latest comments regarding our protocol application with the title: "*Modulation of the cardiac rupture incidence following myocardial infarction, by s and* " which has the DEC protocol number *2011-056*. Our research team has taken the extra points into serious consideration and we are sending the revised DEC protocol for approval.

Regarding the points/comments:

1) We have removed the human data where we had originally based our lab-animal number estimation on. Instead, we have used data from a mouse study that was conducted by our group and was published in a scientific journal in 2009. The numbers of the animals needed, according to the new calculation, are reduced slightly.

2) Regarding to the 2nd comment, we have revised the text accordingly, with a motivation on the possible discomfort of the animals in the days following MI induction, as well as a motivation of the discomfort experienced during the myocardial rupture development.

We hope that these adaptations are sufficient for the DEC to approve our proposal. Please contact me in case there are any questions that remain.

Kind Regards,

(on behalf of the research-team)

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

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

Project: *Modulation of the cardiac rupture incidence following myocardial infarction, by* , *and* .

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:	2011-156
Diersoort:	muis
Aantal dieren:	223
Einddatum:	18-01-2016

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

Viceyoorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM

Bezoekadres

Postadres
Postbus 616
6200 MD Maastricht