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Continuous intracoronary versus standard intravenous infusion of adenosine for fractional flow reserve assessment: the HYPEREMIC trial

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KEYWORDS

- ACS/NSTE-ACS
- fractional flow reserve
- NSTEMI
- stable angina

Abstract

Aims: The aim of this study was to evaluate the accuracy of a continuous intracoronary (IC) adenosine infusion, administered through the novel HYPEREM™IC over-the-wire microcatheter, to measure fractional flow reserve (FFR).

Methods and results: The HYPEREMIC trial was a randomised, non-inferiority, crossover study in which patients with intermediate coronary lesions were enrolled for sequential pressure wire studies. FFR was measured using intravenous (IV) (140-180 mcg/kg/min) versus continuous non-weight-adjusted IC (360 mcg/min) adenosine. Patients were randomised and blinded to the order in which they received the adenosine, separated by a washout period. The primary endpoint was the mean hyperaemic FFR. Forty-one patients were enrolled at three UK sites between June and November 2016. The mean (standard deviation) FFR was 0.82 (±0.09) after IC versus 0.84 (±0.09) after IV adenosine. The difference of –0.02 (95% confidence interval [CI]: –0.03 to –0.01) confirmed the non-inferiority (margin <0.05) of IC to IV adenosine. Intracoronary adenosine was associated with a shorter mean time to maximal hyperaemia (difference –44 [95% CI: –59 to –29] seconds; p<0.0001). Chest discomfort was reported in 32/41 (78.0%) patients during IV adenosine versus 12/41 (29.3%) patients during IC adenosine.

Conclusions: Continuous IC adenosine was a reliable, faster and better tolerated method of achieving maximal hyperaemia compared to IV adenosine.

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Abbreviations

CI	confidence interval
FFR	fractional flow reserve
IC	intracoronary
IV	intravenous
PCI	percutaneous coronary intervention
SD	standard deviation

Introduction

Pressure wire-based fractional flow reserve (FFR) has become the reference standard index of haemodynamic significance to guide revascularisation of intermediate coronary artery lesions[1-3]. Establishing maximal hyperaemia is essential for accurate FFR assessment[4]. Given the guideline-mandated thresholds for decision making, it is crucial that maximal hyperaemia be achieved in a reliable and reproducible manner. FFR measurements have historically been based on an intravenous (IV) adenosine infusion administered via a central venous catheter[2]. Although this approach allows a stable steady state to be achieved rapidly in most patients, it has largely been superseded by peripheral IV adenosine infusion, which affords similar FFR values and aligns with the increasing use of radial artery access for coronary intervention[4,5].

Latterly, an intracoronary (IC) bolus of adenosine has gained widespread traction both for its simplicity and in response to the patient- and laboratory-level variability observed with IV administration. IC adenosine is better tolerated by the patient, obviates the need for extra venous access and allows repeat measurements to be conducted quickly[6]. An IC bolus, however, may require incremental doses of adenosine to achieve maximal hyperaemia, does not allow pullback measurements, and is inappropriate for the study of ostial lesions[4]. A continuous IC adenosine infusion can potentially overcome the drawbacks of bolus IC adenosine[7]. We therefore sought to determine whether hyperaemia induced by a continuous IC adenosine infusion, given through a novel microcatheter, would be non-inferior to the hyperaemia mediated by a standard IV protocol for the attainment of accurate FFR values.

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Methods

The HYPEREMIC trial (ClinicalTrials.gov Identifier: NCT02527616) was a randomised, non-inferiority, single-blinded, crossover study designed to evaluate the utility and accuracy of a novel over-the-wire microcatheter (HYPEREM™IC; Diasolve Diagnostic Solutions, Salisbury, United Kingdom) to administer a non-weight-adjusted continuous IC adenosine infusion to achieve maximal hyperaemia for FFR measurement. The study was granted approval by the research ethics committee in Cambridge, UK, and by the Medicines and Healthcare Products Regulatory Agency (MHRA). The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

DEVICE DESCRIPTION

The HYPEREMIC microcatheter is an over-the-wire microcatheter with a proximal assembly designed to allow both insertion of a pressure wire and infusion of a hyperaemic agent simultaneously through the same central lumen **(Figure 1)**. It has a 3 Fr outer diameter and is compatible with 6 Fr guide catheters and all commercially available pressure wires. The atraumatic radiopaque tip of the microcatheter can be placed a few millimetres into the artery under study without adversely influencing aortic or distal coronary pressure **(Supplementary Appendix 1, Supplementary Figure 1)**.

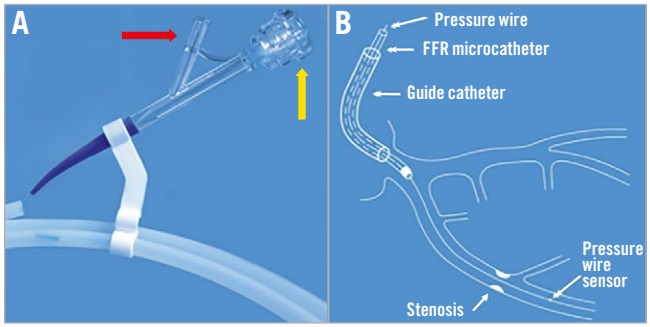

Figure 1. *Procedural use of the HYPEREMIC microcatheter. The microcatheter is flushed with heparinised saline via the bifurcation luer of the two-arm adaptor (A: red arrow). Using a guidewire introducer, the pressure wire is then inserted into the microcatheter via the specific pressure wire port valve (A: yellow arrow). The pressure wire is advanced to the tip of the microcatheter. The microcatheter (plus pressure wire) is then loaded into the guide catheter via the haemostatic valve of a standard Y connector. The microcatheter is then advanced until the proximal marker band 1 (Supplementary Appendix 1) aligns with the haemostatic valve on the Y connector. This indicates that the microcatheter tip is approaching the guiding catheter tip. Under fluoroscopy, the radiopaque tip of the microcatheter is then advanced until it reaches the tip of the guide catheter. Bleed-back should be seen freely from the bifurcation luer of the two-arm adaptor (A: red arrow) to ensure that no air bubbles are trapped within the lumen. The infusion line from a standard infusion pump is connected and purged with the vasodilator (1 ml) solution. The pressure wire is then advanced to exit the guide tip and the microcatheter to the desired sensor position for equalisation according to standard manufacturer instructions (B). The pressure wire can then be advanced distal to the lesion or diseased segment under investigation as per standard FFR measurement protocol (B). The radiopaque tip of the microcatheter can then be advanced under fluoroscopy to the desired position within the artery under investigation but should remain proximal to the lesion or diseased segment. Extensive bench and animal testing (unpublished) has demonstrated that the microcatheter does not cause a significant pressure effect within a 6 Fr guiding catheter system or when introducing it in the proximal segment of the diseased artery to be interrogated. Several preclinical measurements with and without the catheter in place have shown that pressure measurements have not been affected by the microcatheter (see highlighted sections in Supplementary Appendix 2). Infusion of the vasodilator solution can then be commenced.*

TRIAL DESIGN

Patients were enrolled at three centres in the United Kingdom (London, Glasgow and Brighton). All patients undergoing angiography, with a view to percutaneous coronary intervention (PCI) if mandated, were approached to enter the study. Eligible subjects had an angiographically identified intermediate coronary artery stenosis indicated for FFR assessment by standard criteria. Patients with acute ST-segment elevation myocardial infarction, haemodynamic instability, known hypersensitivity to adenosine or lesions deemed unsuitable for FFR measurement were excluded. Each trial participant gave written informed consent as approved by each institution.

Each patient received both IV and IC adenosine infusions, with the order determined by 1:1 randomisation in a blinded fashion. In effect, patients acted as their own control. The half-life of adenosine is <10 seconds; therefore, a three-minute washout period was instituted between each adenosine infusion to eliminate potential carryover effects from one treatment period to the next. Hyperaemia with IV adenosine was achieved with a standard infusion at 140-180 mcg/kg/min administered via a central or peripheral venous cannula according to operator discretion.

Hyperaemia with IC adenosine was achieved with a non-weight-adjusted infusion at 360 mcg/min administered via the microcatheter. The decision to use this dose for continuous IC infusion was derived primarily from an investigation by Yoon and colleagues, who used a different microcatheter and pressure wire assembly[7]. Animal testing with different IC infusion doses was also performed prior to this human study **(Supplementary Appendix 2)**. Randomisation was performed using a validated online tool (www.sealedenvelope.com).

In those lesions found to be haemodynamically significant, operators removed the complete microcatheter system, including pressure wire, to enable use of their preferred workhorse coronary guidewire to perform PCI. In practice, however, only the microcatheter need be removed using standard techniques, leaving the pressure wire in the coronary artery to perform PCI.

HYPOTHESIS AND ENDPOINTS

The study hypothesis was that the mean hyperaemic FFR value achieved with IC adenosine would be non-inferior (as low or lower) compared with that achieved by IV adenosine. The primary endpoint was the mean hyperaemic FFR measured in the distal coronary artery, beyond the intermediate coronary lesion under investigation, recorded after a steady state of maximal hyperaemia had been reached. Secondary endpoints included the incidence and severity of side effects, time to and dose of adenosine required to achieve maximal hyperaemia. Subjects were asked during and after each pressure wire sequence to grade verbally any symptoms of chest discomfort that they experienced. Other periprocedural and post-procedural adverse events were recorded.

STATISTICAL ANALYSIS

The non-inferiority margin was set at 0.05 based on previous acceptable limits[8]. The sample size calculation assumed an expected FFR of 0.77 after IV and an FFR of 0.74 after IC adenosine, with a common standard deviation of 0.10[7]. Within-subject correlation between the two modes of administration was estimated conservatively at 0.95. With a one-sided 2.5% alpha level, 90% power could be achieved with 30 subjects. To allow for some degree of deviation from these initial sample size assumptions and a potential 10% drop-out rate, we planned to recruit 44 subjects. Period effects were deemed unlikely due to the washout period and short half-life of adenosine but if a period by treatment interaction were to occur then this sample size would still have provided 79% power to test the trial hypothesis.

All statistical analyses were performed using SAS version 9.3 or higher (SAS Institute Inc., Cary, NC, USA) based on a one-sided 2.5% alpha level. Three analysis populations were defined – the intention-to-treat (ITT) population consisting of all subjects randomised, the full analysis set (FAS) consisting of all subjects satisfying eligibility criteria and with data for both adenosine administration groups, and the safety population consisting of all patients commencing any procedure with either protocol, analysed according to the route by which adenosine was received. The primary endpoint analysis was assessed in both the FAS and ITT populations. By excluding protocol violators, the FAS population may be the more conservative for a non-inferiority comparison but is susceptible to bias in either direction. The safety analysis population was used for the principal assessment of side effects and adverse events.

For each subject the difference in FFR (FFR_{DIFF}) was calculated as the FFR achieved during maximal steady-state hyperaemia with IC minus IV adenosine. This mean intra-patient difference with its standard deviation and two-sided 95% confidence interval was reported. The IC adenosine arm would be considered non-inferior to the IV adenosine arm if the upper limit of the two-sided 95% confidence interval for FFR_{DIFF} was <0.05. This analysis assumed no order or treatment by sequence interaction effects. These assumptions were tested using independent t-tests[9]. If there were evidence of an interaction effect, then only the first sequence would be used in a between-patient analysis. Safety endpoints relative to each infusion were summarised according to their occurrence during the IC versus IV infusions and expressed as the number and percentage of subjects in each category by way of a descriptive summary rather than a formal analysis.

Results

A total of 41 patients were enrolled in the HYPEREMIC trial between June and November 2016 at three sites **(Table 1)**. All subjects received their procedures in the correct randomised order, and all sequences were separated by the three-minute washout interval **(Figure 2)**. No patients withdrew prematurely from the study. Two subjects failed to reach maximal hyperaemia during one of their procedural runs (one in each of the randomised groups) and were therefore removed from the FAS population. These two subjects remained in the ITT and safety populations. Eleven patients received an IV dose of 180 mcg/kg/min and the

Table 1. Baseline characteristics.

		Order of adenosine administration	
		IC first (n=20)	IV first (n=21)
Age, years	mean (SD)	65.6 (12.8)	68.3 (9.2)
	median (IQR)	68.5 (42.0-92.0)	69.0 (47.0-81.0)
Male, n (%)		14 (70.0)	16 (76.2)
Body mass index, kg/m^2	mean (SD)	29.3 (5.6)	27.9 (5.0)
	median (IQR)	28.2 (22.8-46.0)	27.2 (22.8-47.2)
Diabetes, n (%)		2 (10)	1 (4.8)
Chronic obstructive airways disease, n (%)		1 (5)	0
Asthma, n (%)		0	1 (4.8)
Target coronary artery, n (%)			
Mid LAD artery		11 (26.83)	
Mid RCA		10 (24.39)	
Proximal LAD artery		5 (12.20)	
Proximal LCx artery		3 (7.32)	
Distal RCA		3 (7.32)	
Proximal RCA		2 (4.88)	
Obtuse marginal branch		2 (4.88)	
Diagonal branch		1 (2.44)	
Mid LCx artery		1 (2.44)	
Posterior left ventricular branch		1 (2.44)	
Posterior descending artery		1 (2.44)	
Left main stem artery		1 (2.44)	

IC: intracoronary; IQR: interquartile range; IV: intravenous; LAD: left anterior descending; LCx: left circumflex; RCA: right coronary artery; SD: standard deviation

remainder received a dose of 140 mcg/kg/min, based purely on operator preference. A total of 38 (93%) patients had a peripheral IV, whereas three (7%) subjects received a central venous infusion of adenosine. For the IC arm, all 41 patients received the 360 mcg/min adenosine infusion via the microcatheter[7]. Procedures were performed via the right radial artery in 28 (68.3%), the left radial artery in 3 (7.3%) and the right femoral artery in 10 (24.4%) subjects. All procedures were completed using 6 Fr guiding catheters.

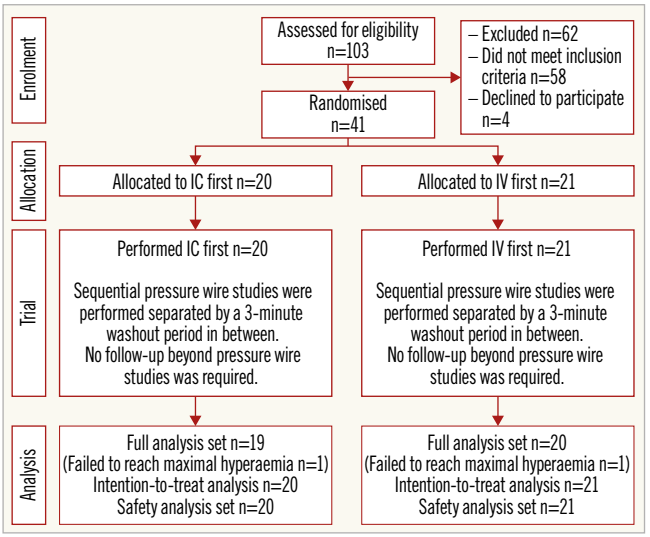

Figure 2. *HYPEREMIC trial CONSORT diagram.*

PRIMARY ENDPOINT

The mean (SD) FFR achieved was 0.82 (±0.09) by the continuous IC adenosine infusion and 0.84 (±0.09) via the IV adenosine infusion **(Table 2)**. There was no evidence of a period effect (p=0.90) or a period/procedure interaction (p=0.75). The mean FFR_{DIFF} was –0.02 (95% confidence interval [CI]: –0.03 to –0.01), thus confirming non-inferiority of continuous IC adenosine administered via the HYPEREMIC microcatheter versus a standard IV protocol for accurately measuring FFR **(Figure 3)**. Five of 39 patients (12.8%) were classified as non-ischaemic by IV adenosine (FFR >0.80) but were found to be ischaemic by IC adenosine (FFR ≤0.80). No patients with a functionally ischaemic lesion according to IV adenosine were classified as non-ischaemic by IC adenosine.

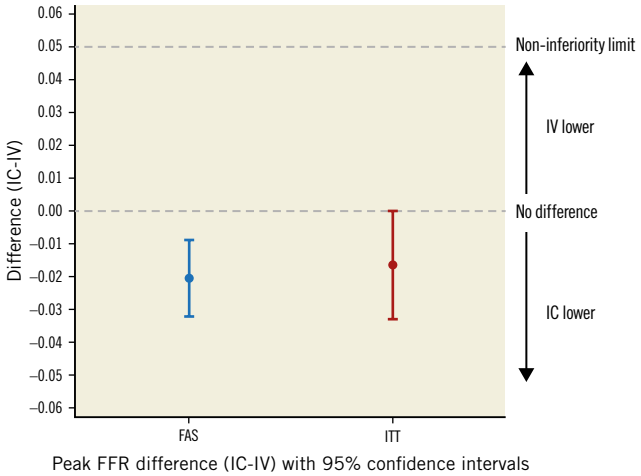

Figure 3. *Primary endpoint assessment in the full analysis set (FAS) and intention-to-treat (ITT) populations. The upper confidence interval was lower than the pre-specified non-inferiority margin of +0.05 in both populations, indicating that intracoronary (IC) infusion was non-inferior to intravenous (IV) infusion for achievement of maximal hyperaemia. In addition, the upper confidence interval was less than zero, indicating that hyperaemia achieved by IC adenosine infusion resulted in a lower mean FFR than with IV adenosine.*

TIME TO REACH MAXIMAL HYPERAEMIA AND DURATION OF ADENOSINE INFUSION

The IC infusion required a shorter mean time to achieve maximal hyperaemia (difference –44 [95% CI: –59 to –29] seconds; p<0.0001) **(Table 2, Figure 4)**, and a shorter mean total duration of infusion (difference –48 [95% CI: –61 to –34] seconds; p<0.0001) **(Figure 5)**.

Table 2. Primary and secondary endpoints.

		Mode of adenosine administration		
		IC	IV	*p*-value
FFR	Mean (SD)	0.82 (0.09)	0.84 (0.09)	<0.0001
	Median (IQR)	0.83 (0.57-1.00)	0.84 (0.60-0.98)	
	Difference (IC minus IV), mean (95% CI)	–0.02 (–0.03 to –0.01)		–
Time to FFR, sec	Mean (SD)	40 (24)	84 (37)	
	Median (IQR)	35 (5-140)	84 (16-181)	
	Difference (IC minus IV), mean (95% CI)	–44 (–59 to –29)		<0.0001
Total adenosine dose, mcg	Mean (SD)	345 (209)	21,676 (9,667)	
	Median (IQR)	297 (60-991)	19,491 (8,959-60,295)	
	Difference (IC minus IV), mean (95% CI)	–21,331 (–24,452 to –18,211)		<0.0001
Duration of adenosine infusion, sec	Mean (SD)	57 (35)	105 (33)	
	Median (IQR)	50 (10-165)	97 (50-203)	
	Difference (IC minus IV), mean (95% CI)	–48 (–61 to –34)		<0.0001

CI: confidence interval; FFR: fractional flow reserve; IC: intracoronary; IQR: interquartile range; IV: intravenous; SD: standard deviation

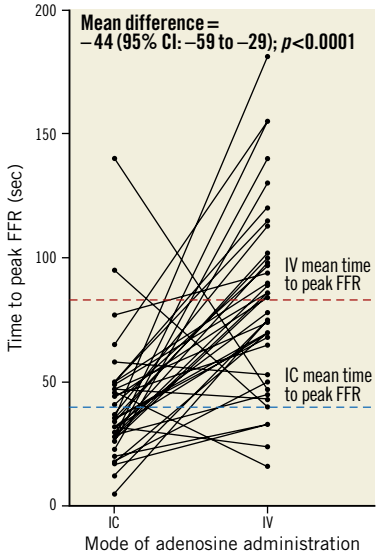

Figure 4. *Mean time in seconds to the nadir of the FFR. CI: confidence interval; FFR: fractional flow reserve; IC: intracoronary; IV: intravenous*

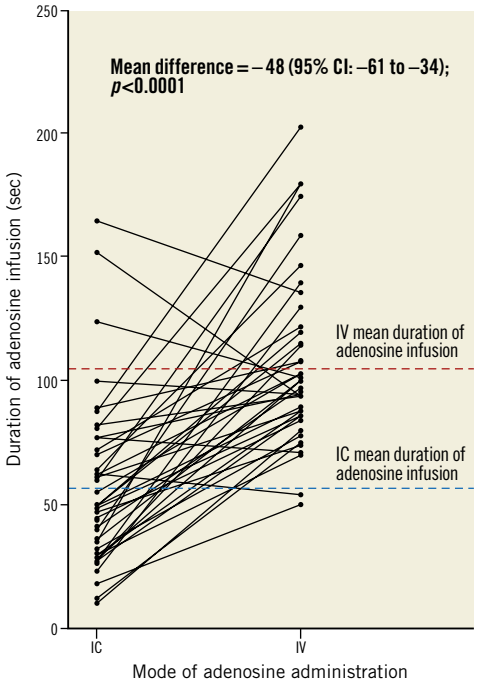

Figure 5. *Mean duration of adenosine infusion. CI: confidence interval; IC: intracoronary; IV: intravenous*

TOTAL ADENOSINE DOSE

IC infusion was associated with a lower total mean dose of adenosine required to induce maximal hyperaemia (difference –21,331 [95% CI: –24,452 to –18,211] mcg; p<0.0001) **(Table 2)**.

SIDE EFFECTS

Chest discomfort of any severity was reported in 32/41 (78.0%) patients during IV infusions compared with 12/41 (29.3%) patients during IC infusions **(Figure 6)**. No patients reported severe chest discomfort during IC infusion compared to 5/41 (12.2%) during IV infusion. The rate and severity of chest discomfort during and after IV adenosine was similar regardless of whether the IV infusion was the first or second procedure in the sequence.

Bradycardia was reported for one patient during their IV procedure (their second in the sequence). Four subjects had transient third-degree atrioventricular block reported (two during IC and two during IV procedures). Three of these were during the second procedure in the sequence. Haemodynamic indices recorded after intracoronary and intravenous administration of adenosine in the HYPEREMIC trial are shown in **Supplementary Table 1**.

Discussion

Contemporary surveys indicate that a significant proportion of interventional cardiologists do not routinely perform FFR to

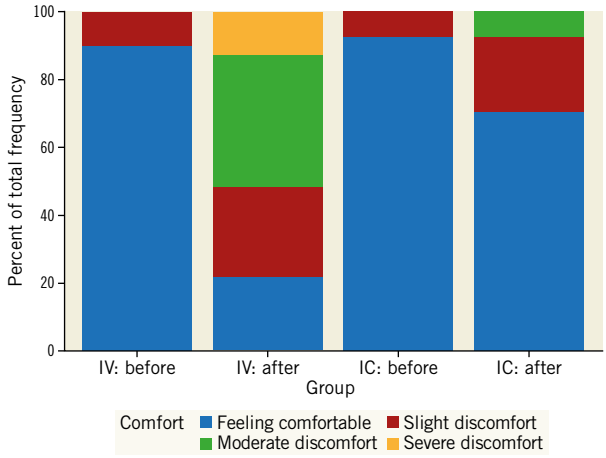

Figure 6. *Chest discomfort recorded before and after each administration of adenosine. No patient experienced severe chest discomfort after an intracoronary (IC) infusion. The majority of patients (76%) felt comfortable after IC adenosine, whereas the majority of patients (78%) felt some degree of chest discomfort after intravenous adenosine. Chest discomfort was set as an exploratory categorical variable in the original statistical analysis plan.*

support their decision-making process[10]. The reasons for this are multifactorial: increased costs and lack of reimbursement predominate, alongside the extra time required for and side effects associated with central venous administration. For these reasons, operators moved to peripheral IV infusions[4,5]. Whilst faster and safer, patient- and laboratory-level variability persisted[4,11]. IC bolus adenosine requires a lower total dose of drug and is associated with fewer side effects, minimal haemodynamic disturbance, improved test/retest concordance, and general equivalence to FFR measurements acquired by an IV infusion[4,8]. Coronary artery-specific IC bolus dosing regimens that induce maximal hyperaemia with a minimal side-effect profile have been confirmed[6]. Steady-state hyperaemia, however, is short-lived with IC bolus adenosine, precluding pullback assessments of serial stenoses or diffuse disease. Evaluating ostial lesions may not be possible with IC bolus adenosine given the risk of plaque disruption or pressure damping during guide catheter engagement to ensure adequate distal drug delivery[8].

A continuous IC infusion of adenosine may maintain the advantages of IC bolus adenosine while overcoming most if its drawbacks. Yoon and colleagues first investigated this concept by using a microcatheter that was introduced into the coronary ostium via a guide catheter. A separate externally calibrated pressure wire, sitting adjacent to the microcatheter within the same guide catheter, was then passed distal to the coronary lesion of interest[7]. Despite this burdensome set-up, a continuous IC adenosine infusion was shown to be safe, effective and quicker at achieving maximal hyperaemia than with IV adenosine, although the mean FFR values achieved were slightly lower.

Building on from this, the HYPEREMIC trial employed a dedicated over-the-wire microcatheter to administer a non-weight-adjusted continuous IC infusion of adenosine to achieve maximal hyperaemia. This device allows the pressure wire to be housed within the microcatheter rather than outside it. A continuous IC infusion using this microcatheter resulted in a slightly lower mean FFR compared with a standard IV adenosine protocol. The physical presence of the catheter itself could possibly explain this. However, the difference was just 0.02, and in 5/41 cases (12.2%) the ≤0.80 decision threshold was reached with IC but not with IV adenosine, and in four of these the FFR by IV adenosine assessment was <0.83, a nearly abnormal result within the error of the test. No subjects with a functionally ischaemic lesion (FFR ≤0.80) were classified incorrectly by IC infusion. This could have wide-ranging implications with regard to subsequent revascularisation decision making and raise important questions as to whether standard IV adenosine may be failing to achieve sufficient maximal hyperaemia in a proportion of subjects undergoing functional assessment of intermediate coronary artery stenoses.

IC compared with IV adenosine infusion also resulted in a significantly shorter time to attain maximal hyperaemia (more than twice as fast), and a shorter total duration of drug delivery. These outcomes could potentially result in significant time savings for operating staff and patients, thereby maximising workflow efficiency through the catheterisation laboratory.

The total average dose of adenosine administered to achieve maximal hyperaemia was significantly lower when using the IC protocol. In concert with the much lower baseline IC dosage, this resulted in the total hyperaemic drug delivery being ~1.5% of that given intravenously, a marked reduction which could translate into meaningful fiscal savings.

IC adenosine infusion was notably better tolerated by patients, with less chest pain compared with IV infusion. Furthermore, no subjects reported severe chest discomfort during and after IC adenosine infusion. Chest discomfort, however, was used as an exploratory categorical endpoint, and thus only a descriptive summary has been provided. Due to the crossover nature of the trial and the categorical nature of the endpoints, it was felt this would be the method providing most clarity. Interpretation should thus be based on clinical judgement of the expected/acceptable levels of chest discomfort.

The trial should be placed into context given the evolving landscape of physiological lesion assessment. The use of contrast medium to induce hyperaemia may be an option for centres in which adenosine is not easily available or very expensive, or in cases of adenosine hypersensitivity. Contrast FFR (cFFR) has demonstrated approximately 85% diagnostic performance against adenosine-based FFR[12]. The accuracy of cFFR in left main or proximal left anterior descending artery lesions may not, however, be as good as a standard hyperaemic adenosine protocol, presumably due to the large area of myocardium subtended by such lesions[13]. The evolution of non-hyperaemic methods of assessing functional impact should also be borne in mind. Resting Pd/Pa suffers from some of the same drawbacks as cFFR[12,13]. Instantaneous wave-free ratio (iFR), however, has been shown to be non-inferior to hyperaemia-induced FFR, whilst avoiding

all of the potential adverse effects associated with adenosine[14]. Nonetheless, correlation of iFR with hyperaemic FFR is not perfect, and both still play an important role in assessing the borderline coronary artery lesion in accordance with site-specific protocols and operator preference.

Limitations

The number of lesions studied in the HYPEREMIC trial was small. Ostial and serial stenoses were not specifically studied, although a continuous IC infusion via the HYPEREMIC microcatheter can theoretically evaluate these lesion subsets. In ostial lesions, for example, positioning the microcatheter at the tip of the guide produced a similar hyperaemic effect without significant lag in hyperaemia compared to positioning the microcatheter in the proximal segment of the artery. This has been demonstrated in our own animal and bench testing **(Supplementary Appendix 2)**. Beat-to-beat tracing analysis showing the transition to hyperaemia was not available for analysis.

Total costs were not collected, and therefore the trade-off for IC adenosine in reducing drug costs versus an additional cost for the microcatheter and performance of five extra PCI procedures were not ascertained. Finally, hard clinical endpoints were not assessed in the present study. Results from the HYPEREMIC trial warrant a larger clinical outcomes study comparing continuous IC adenosine infusion versus IV adenosine, bolus IC adenosine and/or iFR lesion assessment, inclusive of both ostial and serial coronary artery stenoses.

Conclusions

The utility of FFR to aid decision making for the management of intermediate coronary artery lesions is well established. The HYPEREMIC trial has demonstrated that a continuous infusion of a fixed dose of adenosine through a dedicated microcatheter effectively establishes maximal hyperaemia, with mean FFR values slightly lower than those achieved by a standard IV adenosine infusion, and results in a substantially faster and better tolerated procedure (for the patient) with a markedly lower total dose of adenosine required.

Impact on daily practice

Continuous intracoronary adenosine given via a dedicated over-the-wire microcatheter is non-inferior to standard intravenous adenosine for the accurate assessment of FFR. Continuous intracoronary adenosine achieved maximal hyperaemia significantly faster, caused less chest pain and used approximately 1.5% of the dose of a standard intravenous adenosine infusion, which could translate into meaningful time and cost savings for catheterisation laboratories that continue to use adenosine-based pressure wire studies. A standardised intracoronary adenosine infusion given via a dedicated microcatheter could increase the uptake of guideline-mandated pressure wire studies to guide revascularisation of intermediate coronary artery lesions.

Funding

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Conflict of interest statement

A. Elghamaz is a medical advisor for the sponsor. The other authors have no conflicts of interest to declare.

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Supplementary data

Supplementary Appendix 1. HYPEREMIC microcatheter – directions for use.

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Supplementary Figure 1. HYPEREMIC microcatheter.

Supplementary Table 1. Haemodynamic indices recorded after intracoronary and intravenous administration of adenosine in the HYPEREMIC trial.

The supplementary data are published online at: https://eurointervention.pcronline.com/doi/10.4244/EIJ-D-18-01067

Supplementary data

Supplementary Appendix 1. HYPEREMIC microcatheter – directions for use.

Preparation

- Examine the microcatheter for bends, kinks or other damage. Do not use if a bend, kink or other damage is seen.
- Soak the product in a heparinised saline solution bath.
- Seal the pressure wire port valve of the two-arm adaptor, ready for flushing.
- Using a syringe, prime the product with heparinised saline solution through the bifurcation luer of the two-arm adaptor to remove air, and then place cap on bifurcation luer.

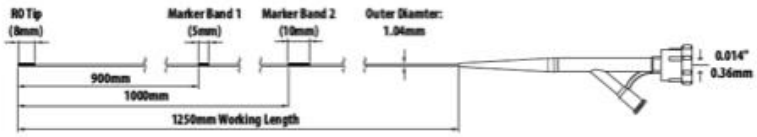
Insertion of the microcatheter

- Prepare and calibrate a standard 0.014" (0.36 mm) pressure wire according to manufacturer's instructions.
- Insert the pressure wire carefully into and through the lumen of the microcatheter via the pressure wire port valve of the two-arm adaptor using a guidewire introducer. Advance the pressure wire to the tip of the catheter.
- Withdraw the needle introducer, close the pressure wire port valve and create a seal around the pressure wire. **Note - use sufficient pressure to create a seal without damaging the pressure wire.**
- Open the haemostasis valve of the Y connector attached to the guiding catheter and insert the product, loaded with a pressure wire, through the haemostasis valve into the guiding catheter. **Note - make sure that the haemostasis valve is open enough for insertion of the product. If not, the valve may cause resistance.**
- Tighten the haemostasis valve to create a seal around the product without inhibiting movement of the product. This will allow continuous recording of the proximal coronary artery pressure.
- Shaft diameter differences should be taken into consideration when opening and tightening the haemostatic valve and upon withdrawal of the catheter. It is important that the haemostatic valve be closed tightly enough to prevent blood leakage around the catheter shaft, yet not so tight that it restricts the flow of hyperaemic agent along the pressure wire lumen or restricts pressure wire movement.

- Advance the product and pressure wire until the appropriate proximal marker aligns with the haemostasis valve on the guiding catheter. This indicates that the microcatheter tip is approaching the guiding catheter tip.
- Under fluoroscopy, advance the pressure wire until the sensor exits the product. Remove the introducer needle and close the pressure wire port valve around the pressure wire.
- Under fluoroscopy, advance the radiopaque tip of the product so that it reaches the tip of the guide catheter. If the guiding catheter is not fully engaged in the coronary ostium, the product can be advanced to the ostium so that the hyperaemic agent is delivered into the coronary arteries.
- The product tip should not protrude from the tip of the guiding catheter by more than 8 mm (the length of the radiopaque tip).
- The tip of the pressure wire should always be beyond the tip of the product.
- Perform equalisation of the pressure wire according to the manufacturer's instructions.
- Reintroduce the guidewire introducer and advance the pressure wire to the desired lesion.

Vasodilator infusion over pressure wire

- Prepare the vasodilator solution for infusion and load it into the infusion pump according to the manufacturer's instructions. Prime the infusion line with the pump before connecting it to the product.
- If the infusion line is not primed, blood pressure from the patient may cause the drug to be pushed back up the infusion line.
- Remove cap from bifurcated luer port and connect an infusion line from the infusion pump to the bifurcation luer of the two-arm adaptor, ensuring no air bubbles are present.
- If blood clots or air bubbles are seen, use a syringe to withdraw them.
- Start infusion of the vasodilator solution using the infusion pump. Wait until a steady state of hyperaemia is reached before taking pressure readings as per the manufacturer's instructions. **Note - there will be a delay as the drug fills the product before it is released into the coronary arteries. This delay is dependent on the flow rate from the infusion pump.**



Supplementary Figure 1. HYPEREMIC microcatheter.

The HYPEREMIC microcatheter has a 3 Fr outer diameter and is compatible with a 6 Fr guide catheter. It has an atraumatic radiopaque tip and a proximal assembly that allows simultaneous insertion of a pressure wire and infusion of a hyperaemic agent.

RO: radiopaque

Supplementary Table 1. Haemodynamic indices recorded after intracoronary and intravenous administration of adenosine in the HYPEREMIC trial.

		Procedure		Sequence order	
		IV	**IC**	**1**	**2**
Systolic (mmHg)	Mean	129.93	133.51	131.90	131.54
	Median	127.00	130.00	127.00	131.00
	Standard deviation	25.01	28.06	27.64	25.60
	Standard error	3.91	4.38	4.32	4.00
	Minimum	80.00	80.00	80.00	80.00
	Maximum	193.00	222.00	222.00	193.00
	N	41	41	41	41
Diastolic (mmHg)	Mean	69.15	72.12	70.00	71.27
	Median	68.00	70.00	69.00	70.00
	Standard deviation	11.42	11.07	11.18	11.47
	Standard error	1.78	1.73	1.75	1.79
	Minimum	49.00	55.00	50.00	49.00
	Maximum	95.00	95.00	95.00	95.00
	N	41	41	41	41
Heart rate (beats per minute)	Mean	65.61	65.46	66.00	65.07
	Median	63.00	65.00	65.00	63.00
	Standard deviation	12.46	12.48	12.31	12.61
	Standard error	1.95	1.95	1.92	1.97
	Minimum	43.00	43.00	43.00	43.00
	Maximum	102.00	107.00	107.00	102.00
	N	41	41	41	41
FFR	Mean	0.94	0.94	0.93	0.95
	Median	0.95	0.95	0.94	0.95
	Standard deviation	0.05	0.05	0.05	0.05
	Standard error	0.01	0.01	0.01	0.01
	Minimum	0.82	0.81	0.81	0.84
	Maximum	1.00	1.02	1.00	1.02
	N	41	41	41	41

Supplementary Appendix 2. Safety and performance of the Diasolve FFR micro-catheter in a porcine model.

NPIMR *Northwick Park Institute for Medical Research*

STUDY REPORT

STUDY TITLE
SAFETY AND PERFORMANCE OF THE DIASOLVE FFR MICRO-CATHETER IN A PORCINE MODEL

TEST FACILITY:
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STUDY NUMBER: DIA 276/15

EXPERIMENTAL PHASE START DATE: 5th March 2015

EXPERIMENTAL PHASE COMPLETION DATE: 5th March 2015

Northwick Park Institute for Medical Research

STUDY TITLE

SAFETY AND PERFORMANCE OF THE DIASOLVE FFR MICRO-CATHETER IN A PORCINE MODEL

STUDY NUMBER: DIA 276/15

STUDY DIRECTOR AUTHENTICATION

The study was performed in compliance with the Good Laboratory Practice Regulations 1999 (S.I. No. 3106) as amended by the 2004 regulations (S.I. 994) which are based on the principles of Good Laboratory Practice as adopted by the Organisation for Economic Co-operation and Development (OECD), ENV/MC/CHEM (98) 17. They are in conformity with, and implement the requirements of, Directives 2004/09/EC and 2004/10/EC.

This report represents the methods used and the results generated during the study.

There are 30 pages in this report.

Study Director:

Date:

STUDY TITLE
SAFETY AND PERFORMANCE OF THE DIASOLVE FFR MICRO-CATHETER IN A PORCINE MODEL

STUDY NUMBER: DIA 276/15

QUALITY ASSURANCE STATEMENT

This study has been inspected and the report audited by the Quality Assurance Unit of NPIMR in compliance with the Good Laboratory Practice Regulations 1999 (S.I. No. 3106) as amended by the 2004 regulations (S.I. 994) which are based on the principles of Good Laboratory Practice as adopted by the Organisation for Economic Co-operation and Development (OECD), ENV/MC/CHEM (98) 17. They are in conformity with, and implement the requirements of, Directives 2004/09/EC and 2004/10/EC. As far as can be reasonably established, the methods described and the results provided in this report accurately represent the Protocol and Standard Operating Procedures and the results obtained accurately reflect the raw data generated during the course of the study.

DATE	INSPECTION/AUDIT	DATE REPORTED TO STUDY DIRECTOR AND MANAGEMENT
27.01.15	Draft Protocol	27.01.15
05.03.15	Surgical Procedures and Termination	10.03.15
10.03.15	Data Review	10.03.15
08.04.15	Histological Procedures	14.04.15
29.04.15	Draft Report	29.04.15
06.05.15	Final Report	06.05.15

During the period, or around the time of, the study, facility inspections were performed in those areas not covered by study-specific inspection and included the Archives (7th October 2014) and the Large Animal Accommodation (5th November 2014).

Quality Assurance Unit:

Print name:

Date:

STUDY TITLE
SAFETY AND PERFORMANCE OF THE DIASOLVE FFR MICRO-CATHETER IN A PORCINE MODEL

STUDY NUMBER: DIA 276/15

MANAGEMENT APPROVAL

Sign:

Prof PD Sibbons:
For and behalf of NPIMR

Date:

The report was distributed to:

Sponsor
Prof PD Sibbons
Study File

CONTENTS

CONTENTS continued

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Surgical Advisor:	A Elghamaz
Operating Theatre Personnel:	C Gray L Fletcher
CT Fluoroscopy Operator	C Shurey
Histology Procedures Personnel:	F Surti K Sachdev
Pathologist:	P Sibbons
Animal Husbandry Personnel:	D Shepherd (NACWO) M Harrison
Laboratory Manager:	J Knight
Named Veterinary Surgeon:	M Gardiner
Quality Assurance Unit:	J & K Mallard, Lea View House, Higham Cross Road, Hanslope, Milton Keynes, Bucks MK19 7DB

1.0 INTRODUCTION

The FFR micro-Catheter is a pressure wire and hyperaemic agent delivery catheter. The FFR micro-catheter will allow clinicians to infuse a hyperaemic agent (e.g. adenosine) by constant infusion into the coronary circulation at the same time as they perform Fractional Flow Reserve measurements using currently available FFR systems (e.g. PressureWire systems from St Jude Medical).

Fractional Flow Reserve (FFR) using the pressure wire is a widely validated and well-recognised tool for assessing the physiological significance of angiographically intermediate coronary artery stenosis. This is currently done by advancing the pressure wire sensor into a coronary artery and then beyond the stenosis. The resting FFR reflects the ratio between the pressure sensed by the pressure wire sensor (distal to the stenosis) and the guiding catheter tip. The wire sensor is then advanced beyond the stenosis or the diseased segment to be assessed. A hyperaemic agent, normally adenosine, is then infused via a large peripheral vein or a central venous catheter to obtain a steady state of hyperaemia often referred to as peak or stress FFR (pressure differential between the guiding catheter tip and pressure sensor). A reading at or below 0.80 correlates well with myocardial ischaemia and favours coronary intervention as the best treatment strategy.

In current procedures, adenosine is most commonly given either by continuous intravenous infusion via a large central vein (femoral) or peripheral cannula, or by intra-coronary bolus injection. The FFR catheter could offer the following benefits to clinicians and patients:

- It will allow the physician to use a single access point, making the procedure more time efficient with no need for large bore central venous access. This will also reduce the vascular risk related to obtaining a central venous access.
- The dose of adenosine required to achieve maximal hyperaemia should be lower as it is being administered directly into the coronary circulation, helping to reduce the peripheral side effects such as chest tightness, difficulty breathing and flushing.
- It may provide a more rapid and reliable hyperaemic state and steady state compared to the peripheral or central intra-venous infusion method.

The FFR catheter has now reached design freeze and this study represents a key part of the design validation prior to a clinical study.

2.0 STUDY OBJECTIVES

The objectives of the study were:

1. To demonstrate that the micro-catheter has an atraumatic tip, easily seen under fluoroscopy and can be used without causing damage to the patient's vasculature in an air tight system (to avoid air embolization)
2. To demonstrate that it can deliver a hyperaemic agent via continuous infusion at the desired infusion rate without suffering or causing damage or triggering an alarm on the infusion pump.
3. To show that all components (tip, shaft and valve) function appropriately, with convenient handling and without any failures or unexpected malfunction
4. To show that the FFR catheter does not dampen aortic pressure measurements when *in situ.*

3.0 REGULATORY GUIDELINES

The UK Home Office controls scientific procedures on animals in the UK and does so by the issue of licences under the Animal (Scientific Procedures) Act 1986. The regulations conform to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, Council of Europe).

The Home Office Licence governing this study directly specifies the limits of severity of effects on the animals.

The study will be performed in compliance with the Good Laboratory Practice Regulations 1999 (S.I. No. 3106) as amended by the 2004 regulations (S.I. No. 994) which are based on the principles of good laboratory practice as adopted by the Organisation for Economic Co-operation and Development (OECD), ENV/MC/CHEM (98) 17. They are in conformity with, and implement the requirements of, EU Directives 2004/09/EC and 2004/10/EC.

4.0 JUSTIFICATION FOR CHOICE OF SPECIES

The pig's heart is recognised to be similar to the human heart and a single-subject, pilot study in the porcine model run previously at Northwick Park Institute for Medical Research (ref: Diasolve Test Report TR-001 'Report of 2nd Pilot Study') indicated that it is a suitable model for testing of the catheter and also that a limited hyperaemic effect of adenosine may be observed in the model.

5.0 QUALITY ASSURANCE

5.1 Quality Assurance Inspections

Various stages of the study, including the protocol, critical animal phases at the test facility and this final report were audited/inspected by the NPIMR Quality Assurance Unit (QAU) in compliance with facility quality assurance standard operating procedures.

5.2 Final Report

The final report was audited to ensure that the methods described and the results reported accurately reflect the raw data generated in the study.

5.3 Standard Operating Procedures

Unless specified, all procedures described in the protocol were the subject of detailed standard operating procedures which are contained in the SOP manuals of the operating departments of the test facility.

5.4 Protocol Amendments

An amendment to this protocol was documented and approved by the Study Director and Sponsor prior to implementation as defined in SOP 1:1:26.

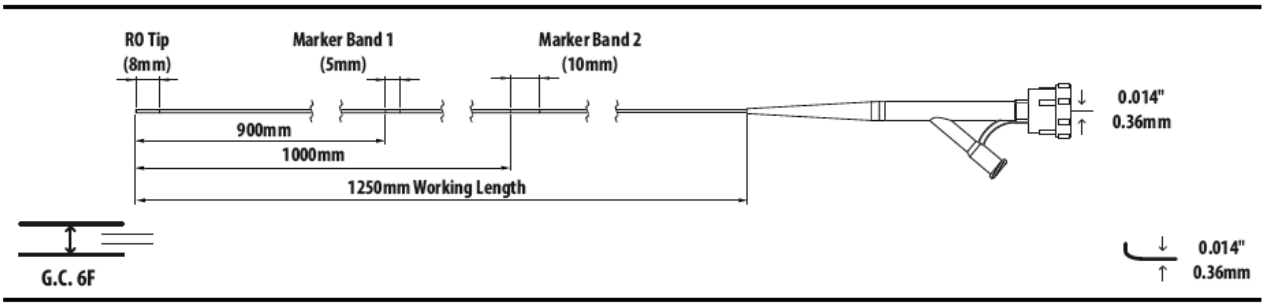
5.5 Protocol Deviations

There were no deviations to this protocol.

6.0 TEST ITEM

6.1 Test Item Description

The FFR catheters are approximately 3F outer diameter with an atraumatic, radiopaque tip to avoid damage to vessels and allow adequate visibility under fluoroscopy. They have a proximal assembly, which allows insertion of a pressure wire and infusion of a hyperaemic agent, such as Adenosine, simultaneously without leaking.



6.2 Transport, Storage and Handling of Test Item

The FFR catheters were supplied fully packaged in a pouch and carton and sterilised by ethylene oxide. They were stored in ambient conditions and handled with appropriate care for a medical device to avoid damage.

7.0 ANIMALS

7.1 Animal and Husbandry Details

Number:	3
Species:	Pig
Breed:	Large White/Landrace crossbreed
Sex:	Female
Body Weight:	65-70kg on day of surgery
Identification:	Unique identification number marked with marker pen on the outer aspect of one ear
Supplier:	D.J. & J Witherick and Son, Winsley Farm Park, Sharnbrook, Bedford MK4 1NA. The animals arrived 25th February, 8 days prior to surgery, when they were examined by the Named Animal Care and Welfare Officer (NACWO) shortly after their arrival and thereafter, by the person in day-to-day charge of them.
Housing:	Kept as a group of three until two days before surgery, when they were individually housed, in a concrete pen with a steel barred gate
Bedding:	Wood shavings and straw
Diet:	Super Grow Pig Pellets (Manufacturer: Attlee Diets; Supplier: William Lillico & Son)
Water:	Available ad libitum supplied by Three Valleys Water

Environmental monitoring:
The light cycles were controlled automatically to supply 12 hours light and 12 hours dark and ambient temperature (target range: 20°C ±3°C) and relative humidity (target range: 55% ±10%) were recorded. The temperature range over the acclimatisation period remained within the target range whilst the relative humidity fell outside by 5%, dropping to less than 45% on 6 of the nine days. This is considered to have had insignificant effects on the animals' welfare and does not compromise the integrity of the study.

Table 1 Environmental Conditions

Temperature range over the acclimatisation period (25th Feb-5th Mar)	17.5-21.1°C
Relative humidity range over the acclimatisation period (25th Feb-5th Mar)	40-58%

No known substances were expected in the diet at levels which might adversely affect the results of the study. A sample of the diet was stored refrigerated for analysis in the event of a problem related to the diet being suspected. On completion of the study and acceptance of the report by the Sponsor, the sample was discarded.

No known substances were expected in the drinking water at levels which might adversely affect the results of the study. A sample of water was collected and stored refrigerated as detailed in SOP 8:1:17 for analysis in the event of a problem related to the water being suspected. On completion of the study and acceptance of the report by the Sponsor, the sample was discarded.

7.2 Body Weights

The animals were weighed on the day of the initial procedure prior to commencement and body weights were recorded on form PU 92a.

Table 2 Animal Body Weights

Animal No.	**Body Weight (kg)**
474	*65*
475	*70*
476	*70*

8.0 EXPERIMENTAL PROCEDURE

8.1 Instruments

8.1.1 GE Healthcare Innova 4100 Cardiology CT Scanner (System No. 00074VAS02)

A computerised tomography (CT) scanner which was used to obtain computerised x-ray images during perioperative real-time manipulations (fluoroscopy) and post-operative image analysis (SOP 1:2:32)

8.1.2 Radi Xpress Analyser (SN: XP2469)

Apparatus which was used to display blood pressures and fractional flow reserve (FFR) enabling assessment during the operative procedure. This equipment was supplied by the Sponsor.

8.1.3 Graseby 3400 Infusion Pump (SN: 24250) supplied by Smiths Medical

Equipment which was used to deliver adenosine intravenously and into the coronary vessels. This apparatus was also supplied by the Sponsor.

8.2 Study Design

This study comprised three animals that underwent several procedures to measure intra-articular blood pressure.

Two or three coronary vessels were accessed in each animal providing 7 vessels to the study. Each vessel underwent up to 5 repeat FFR measurements providing 39 measurements.

Aortic pressure measurements were taken firstly without the FFR micro-catheter and then with it to show no difference in pressure wave or reading. Then FFR comparison measurements were taken using the micro-catheter with the pressure wire in place.

Safety tests were also performed replicating clinical misuse scenarios i.e. over-advancement of the catheter and infusing saline solution at the highest infusion rate achievable with the infusion pump.

Damage caused by the catheter in the main part of the study and during the safety phase was assessed macroscopically and recorded as a Study Note.

8.3 Surgery Dates

Table 3 Surgery Dates

Animal Number	Surgery Date	Termination Date
474	*05.03.15*	*05.03.15*
475		
476		

8.4 Anaesthesia and Preparation

8.4.1 Animals were pre-medicated with ketamine (5mg/kg)/ xylazine (1mg/kg) intramuscularly (SOP 8:3:11).
8.4.2 General anaesthesia (GA) was induced with oxygen over isoflurane and nitrous oxide delivered via a close fitting face mask.
8.4.3 The animals were transferred to the CT Fluoroscopy suite on a trolley and intubated with a cuffed endotracheal tube.
8.4.4 After intubation, anaesthesia was maintained with oxygen over isoflurane and nitrous oxide with the respiration controlled via ventilator (SOP 8:3:13).
8.4.5 Oxygen saturation, pulse rate, rectal temperature, expired CO_2 and respiratory rate were measured and recorded on Form PU 92a.
8.4.6 The outer aspect of one ear was marked with the animal's unique number with a marker pen.
8.4.7 Hartmann's fluid was administered intravenously via a slow drip throughout the procedure and the volume recorded on Form PU 92a.

8.5 Surgical Procedure

8.5.1 The animals were administered heparin intravenously at the human dose of 100U/kg.
8.5.2 A blood sample of 100µL was collected from a peripheral ear vein approximately every 30 minutes and used to measure Activated Clotting Time (ACT) (SOP 8:2:37) – the heparin dose was modified as necessary (on the advice of Dr Ahmed Elghamaz) to maintain an ACT of at least 250 seconds – ACT was recorded as a file note.
8.5.3 The left jugular vein was surgically exposed.
8.5.4 A central line catheter was inserted and secured.
8.5.5 A femoral artery was surgically exposed.
8.5.6 A 6F sheath (Merit Medical, lot no. H644706) was inserted.
8.5.7 A 0.035 J guide wire (Merit Medical, lot no. K703704 used in animals 474 and 475; lot no. K706989 used in 476) was inserted into the sheath and advanced to the aortic root using CT fluoroscopy.
8.5.8 A guiding catheter (Merit Medical lot nos. E683564 and E663278 used in 474 whilst only E663278 used in 475 and 476) was then advanced over the wire to intubate the aorta.
8.5.9 The catheter was attached to blood pressure measuring apparatus (Radi Xpress analyser) via a Tuoby Borst adaptor and manifold (Argon) and the pressure at the aortic root (base blood pressure) recorded on the Data Capture Form.
8.5.10 A FFR micro-catheter (HYPERAEM™ IC lot no. DS13461) was then prepared according to the Instructions for Use including soaking in a bowl of heparinised saline (1U/mL), loading of the pressure wire, flushing with heparinised saline and connection to infusion pump (Graseby 3400).
8.5.11 The micro-catheter was then advanced into the guiding catheter until it reached the tip according to the Instructions for Use.
8.5.12 The aortic pressure (pressure transduced following insertion of micro-catheter) wave reading was reviewed on screen, recorded on Data Capture Form and compared to the value recorded in 8.5.9 to ensure the signal had not been damped. Once an equivalent reading was established (recorded on the Data Capture Form as the pressure at point of equalisation), the study was continued.
8.5.13 The pressure wire was then advanced only far enough so that the pressure sensor (radio-opaque segment of the wire) exited the guide into a coronary artery. The specific artery under study that the pressure wire was advanced into was recorded on the Data Capture Form. The introducer needle was then withdrawn to allow for equalisation (step 8.5.14).
8.5.14 The pressure wire was equalised with aortic pressure measured at the transducer.
8.5.15 The wire was then advanced to the desired location into the coronary artery and the introducer needle withdrawn again. The live pressure trace on the Radi screen constituted the resting FFR measurement which was taken and recorded on the Data Capture Form.
8.5.16 Adenosine was then administered either intravenously *140mcg/kg/min (IVADN as undiluted *Adenoscan for solution*) or by intracoronary infusion 360mcg/min (ICADN as a 20-fold sodium chloride 0.9% dilution *Adenoscan for solution*, infused at a rate of 2.4ml/min). Recording on the Radi analyser was started at the same time that the infusion was started.

*This dose rate was true of animal 474 for which the dose was specifically calculated but, due to an oversight, no new calculation

was made for 475 and 476. This resulted in these animals which were the same body weight as each other, receiving a dose rate of 130mcg/kg/min which is 93% of the protocol dose. Because of the minimal difference between the responses between animals (see Data Capture Forms Appendix 1), the consequence of the lower dose was considered insignificant and does not compromise the integrity of the study.

8.5.17 Blood pressures and FFR were measured and recorded on the Radi during the infusion until the FFR reached a steady state of hyperaemia (see Appendix 1). The recording was stopped and the lowest FFR value captured from the analyser on the Data Capture Form.

8.5.18 The adenosine infusion was stopped and the FFR allowed to return to the resting value/baseline (2 to 3 minutes). This stress FFR was then recorded on the Data Capture Form.

8.5.19 The wire was then withdrawn to the tip of the guide catheter and the FFR recorded on the Data Capture Form.

8.5.20 The pressure was allowed to equalise and this was also recorded on the Data Capture Form along with the duration of the delay before starting the next run.

8.5.21 Steps 8.5.18 to 8.5.19 were repeated up to 5 times.

8.5.22 The FFR micro-catheter was then withdrawn and the guiding catheter was repositioned to provide access to a different coronary artery.

8.5.23 A new FFR micro-catheter was then used to repeat the steps 8.5.10 to 8.5.20 in the new artery.

8.5.24 For each new pressure wire used or vessel investigated, a repeat IV dose as per 8.5.16 was measured to provide a comparison.

8.5.25 No damage to the coronary arteries was observed during the procedure.

8.5.26 After all FFR readings were completed in each animal, an anticipated misuse scenario was enacted by infusing saline solution at the highest infusion rate achievable with the infusion pump without triggering the occlusion alarm, for five minutes (or until a 50mL syringe had been emptied if shorter).

8.5.27 Finally, a further anticipated misuse scenario was enacted by deliberately over-advancing the catheter by the length of the tip plus 5mm. This was repeated up to 10 times. Also, in run no. 39, the micro-catheter was advanced 8cm into the coronary artery with and without the wire (see Appendix 1)

8.5.28 The catheter and wire was withdrawn and the animal killed by an intravenous overdose of sodium pentobarbitone (Lethobarb).

8.5.29 The animal was then transferred to the Post-mortem room and the heart harvested and placed for temporary storage into 0.9% sodium chloride.

8.5.30 When all three hearts were harvested, the coronary arteries were examined macroscopically for signs of damage.

8.5.31 Observations were recorded as a study note and photographs were taken.

9.0 OBSERVATIONS

9.1 Comparison of aortic pressure measurement reading and waveform with and without catheter which was recorded on the Data Capture Form.

9.2 Aortic pressure, coronary pressure and lowest FFR (maximal hyperaemia) measurements displayed on the RADI analyser using the recording system:

- 9.2.1 At rest
- 9.2.2 During IV adenosine infusion
- 9.2.3 During IC adenosine infusion

9.3 Visibility and handling of devices was recorded on the Questionnaire for each FFR micro-catheter as it was removed at end of study.
9.4 Macroscopic examination of coronary arteries post-mortem which was recorded on the Post-mortem Examination Form PU 1a. Microscopic examination of the coronary vessels post-mortem was reported, a transcription of which is presented in Appendix 4.

10.0 TERMINATION

Animals were killed by an intravenous overdose of sodium pentobarbitone (Lethobarb) at the end of the procedure.

The hearts were collected and placed into sodium chloride 0.9% and then stored at approximately 4°C until examined macroscopically later the same day. The coronary vessels were opened surgically and the intima examined by A Elghamaz, S Parsapour and S Shurey. No damage was observed in any artery by any of the examiners.

The heart was then halved in the coronal plane and placed into 10% buffered formal saline.

The hearts will be stored in 10% buffered formal saline at NPIMR for possible later examination for up to 5 years, during which period, the Sponsor will be contacted regarding their disposal.

11.0 HISTOLOGY

The results of the macroscopic examination indicated that histology would not be required. Nevertheless, a decision was taken to perform histology to provide further satisfaction of safety. Samples were prepared as follows:

11.1 Specimens of heart tissue including cannulated coronary arteries were processed by routine automated procedures to wax embedding as described in SOPs 6:1:3 and 6:1:4.
11.2 Sections of 5 microns were cut from each block and stained with haematoxylin & eosin.
11.3 The sections were examined for evidence of physical damage; it was anticipated that damage from the catheter tip will be differentiable from damage caused by the wire.
11.4 Details, such as the number of sections, were recorded on PU 1a.
11.5 The histopathology report is presented as Appendix 5.

12.0 EVALUATION

Blood pressure and FFR values at each time-point recorded were dictated from the Radi Analyser by the Surgical Advisor and transcribed in the data collection forms on behalf the Sponsor by Mr Paul Weinberger. They are summarised in Appendix 1. A usability questionnaire was completed for each catheter tested by the Surgical Advisor, in consultation with the Surgeon. Macroscopic and histological examination of coronary arteries was undertaken post termination. No statistical analysis was undertaken.

13.0 RESULTS

A total of nine FFR catheters were included in the study, in seven coronary arteries in three animals. Thirty-nine infusion runs were undertaken with the nine catheters. During the entire study no device failures were identified. The results of the study are further discussed in relation to each of the objectives in turn:

13.1 To demonstrate that the micro-catheter has an atraumatic tip, easily seen under fluoroscopy and can be used without causing damage to the patient's vasculature in an air tight system (to avoid air embolization)

13.1.1 Atraumatic Tip

The tip of the micro-catheter was shown to be atraumatic to the animals' vasculature in three ways:

- during none of the procedures on each animal was any damage visible under fluoroscopy by the surgeon or surgical advisor
- no damage was seen during post-mortem dissection of the test vessels viewed by macroscopic enlargement. Images were examined by the Surgical Advisor (an interventional cardiologist), the two Surgeons (the Lead Surgeon, S Parsapour, a clinical vascular surgeon and the Assistant Surgeon, S Shurey, a research surgeon experienced in vascular surgery) and the Study Director (also experienced in vascular surgery) and no evidence of any traumatic damage was noted
- no damage was seen under microscopic histological examination of the hearts taken post-mortem

Several of the catheters were deliberately and/or accidentally over-advanced into the coronary arteries, mostly over a pressure wire but on some occasions without a wire. Any damage caused during these misuse scenarios would have been evident from the reviews above.

13.1.2 Visibility under Fluoroscopy

Of the nine test catheters used, the tips of eight were easily visible under fluoroscopy whilst one was reported as less easily seen (see Appendix 3). As well as the initial assessment of general catheter tip visibility, a total of 40 repeat visibility tests were performed at various points inside the guide catheter throughout the study with just the one case of compromised visibility. On review with the investigators it was found that the report of compromised visibility was made by the Lead Surgeon, who found it less visible than the devices normally used in non-cardiac procedures. All of the acceptable visibility reports came from the Surgical Advisor who is experienced in coronary procedures.

In the opinion of the Surgical Advisor, under fluoroscopy, the micro-catheter tip was easily visible and could be readily distinguished from the guiding catheter tip. The degree of opacification and visibility of the coated tip seemed equivalent to other commercially available and widely used micro-catheters and devices (see image Appendix 7).

13.1.3 Air Ingress

No evidence of the introduction of air embolism by the use of the FFR catheter was revealed in the catheter or the animals' circulatory system at any time during the study (Appendix 3).

13.2 To demonstrate that it can deliver a hyperaemic agent via continuous infusion at the desired infusion rate without suffering or causing damage or triggering an alarm on the infusion pump.

The micro-catheter was shown to adequately infuse adenosine at the rate selected for use in clinical investigation of 2.4mL/min without triggering the alarm of the infusion pump in any of the 23 runs (Appendix 3).

In incremental challenge testing the infusion rate was increased up to, and beyond, a rate sufficient to trigger the infusion alarm. It was found that the FFR catheter could reliably deliver at a rate of 8.33mL/min (~3.5x the selected rate) whereas at 10 mL/min (>4x the selected rate) the occlusion alarm would be triggered after 1-2 mL had been delivered (Appendix 1 and 3).

There was no evidence of device damage, leak or other malfunction, even under those extremely high infusion rates.

13.3 To show that all components (tip, shaft and valve) function appropriately, with convenient handling and without any failures or unexpected malfunction

None of the devices suffered from any unexpected malfunction or failure. Various answers in the questionnaire summary (Appendix 3) reveal that all of the components of the FFR catheter functioned appropriately throughout the study and there were no reports of malfunction. All of the questions, but two, achieved 100% positive responses for device handling and performance, many with multiple repeats for several catheters. The following is the list of results from questions with 100% positive responses:

- *The Diasolve FFR Catheter can be flushed with a 0.014" pressure wire loaded before insertion into the guide catheter.*
- *The infusion pump can connect to the Diasolve FFR Catheter without issue.*
- *The Diasolve FFR Catheter can infuse adenosine at the recommended clinical rate without triggering the alarm of the infusion pump.*
- *The Diasolve FFR Catheter is compatible for insertion through generic tuohy borst valves (e.g. Merit FLO40), can handle the compression resistance and does not leak.*
- *The Diasolve FFR Catheter can be successfully inserted through the tuohy borst without the need for a needle introducer.*
- *The Diasolve FFR Catheter can be successfully used in conjunction with a 6F guide catheter.*
- *The tuohy borst holds the FFR Catheter satisfactorily in place when sealed.*
- *The hub is familiar, easy and intuitive to use.*
- *The hub is suitable for one-handed use.*
- *The hub is suitable for use in a catheterization lab.*
- *The hub is sufficiently differentiated from the Guide Catheter hub.*
- *The Diasolve FFR Catheter tracks easily through the guide catheter and reaches the target site without damage.*
- *The marker bands are clearly visible against the surgical drape and are in appropriate positions to aid positioning of the tip.*
- *The working length of the catheter is appropriate for easy handling when tip is positioned at GC tip.*
- *The system is free of pressure damping between the Diasolve FFR Catheter and the guide catheter.*
- *The pressure wire can be introduced easily using a standard needle introducer.*
- *The Diasolve FFR Catheter can successfully perform all functions when used with a 0.014" pressure wire (including infusion, tracking, flushing).*

- *The FFR Catheter holds the pressure wire in place satisfactorily during the procedure.*
- *The pressure wire be connected and used as normal.*
- *The Diasolve FFR Catheter, loaded with a 0.014" pressure wire in place, can be inserted into the guide catheter without a needle introducer in place.*
- *The Diasolve FFR Catheter can be retrieved from the system after the procedure is complete.*
- *The Diasolve FFR Catheter can be easily disposed of in waste.*
- *The Diasolve FFR Catheter does not display any physical damage caused by kinking during the procedure.*
- *The Diasolve FFR Catheter doesn't show any signs of leaking at any point in the procedure.*
- *No clinically significant air bubbles were noted to enter the FFR catheter and/or circulatory system.*
- *Sufficient information was supplied in the instructions for use and no errors were noted.*
- *No issues were noted during reinsertion.*

The catheter tip was marked as not clearly visible in only one out of all of the runs of procedures.

13.4 To show that the FFR catheter does not dampen aortic pressure measurements when *in situ*.

In all cases, the test system was demonstrated to be free of pressure damping between the micro-catheter and the guide catheter, as reported in Appendix 3 and summarised in 13.3 above. No material difference was noted by the Surgical Advisor between the shape of recordings on the RADI analyser screen before and after insertion of the FFR Catheter. No trend was seen of narrowing of the gap between systolic and diastolic readings, which would be indicative of a damping of the pressure signals.

Detailed pressure recordings are given in Appendix 1 and the averages summarised below. Of the nine procedures where average blood pressures (BP) were recorded, five increased with the FFR catheter, three decreased and one stayed the same. The mean difference was 3.4mmHg but taking into account the continually fluctuating BP displayed on the analyser this was not considered to represent an effect of the FFR catheter.

Table 4 Average Blood Pressures

	Average Blood Pressure (mmHg)								
Artery/Catheter No.	1/1	3/3	4/4	5/5	6/5	7/6	8/6	10/8	11/9
Average BP at baseline	86	69	82	86	86	63	63	76	92
Average BP with FFR catheter	85	72	90	84	84	72	72	84	92
Difference	*-1*	*+3*	*+8*	*-2*	*-2*	*+9*	*+9*	*+8*	=

14.0 CONCLUSIONS

14.1 The Diasolve FFR micro-catheter has an atraumatic tip when advanced intra-coronary, visible under fluoroscopy and can be used without causing damage to the animal's vasculature in an air tight system. The safety (absence of any damage) to the coronary arteries has been demonstrated by macroscopic and microscopic examination.

14.2 The Diasolve FFR micro-catheter can deliver a hyperaemic agent via continuous infusion at an infusion rate of up to 8.33 mL/min without suffering or causing damage or triggering an alarm on the infusion pump.

14.3 All components of the Diasolve FFR micro-catheter (tip, shaft, hub and valve) performed appropriately, with convenient handling and without any failures or unexpected malfunctions.

14.4 The Diasolve FFR micro-catheter does not cause any aortic pressure damping effect on measurements when used in situ.

14.5 The study has demonstrated ease of use, effectiveness and safety of all components of the Diasolve FFR micro-catheter for the desired use and application.

15.0 RECORDS

All measurements, observations and unanticipated events, including adverse events and the protocol amendment were recorded on the appropriate forms and stored in the study file.

16.0 STORAGE OF RECORDS

The study file containing original raw data, the protocol and a copy of the final report will be stored in the archives of NPIMR as described by SOP 1:1:8 for a period of five years, after which, the sponsor will be contacted to ascertain further retention time.

17.0 STUDY IDENTIFICATION

All documents and preparations created during this study were marked with NPIMR study number DIA 276/15.

18.0 RECORDS MAINTAINED

Test item:	Receipt details
Test facility records:	Animal room temperature and humidity
	Diet identification
	Animal identification
Farm records:	Diet identification
In-vivo phase:	Animal reception and disposition
	Surgical procedures and treatments including photographs
	Animal observations
	Animal body weights
Pathology records:	Macroscopic observations
	Pathology
Other:	Routine equipment calibration and/or quality control

All other records that would be required to reconstruct the study and demonstrate adherence to the protocol

19.0 REPORT

19.1 Draft Report

On completion of the experimental work, the draft report was submitted and audited to ensure that the methods, along with the raw data generated, are accurately described. The draft report was also sent to the Sponsor for review and comment. Revisions to the draft were provided to the Sponsor by email.

The draft report included:

Name and address of test facility
Name and address of Sponsor
Identification of test system
Study Director statement of compliance
Quality Assurance Unit statement
Personnel details
Device identification, characterisation and stability details
Archival storage details
Study objective
Study dates
Study design
Study justification
Materials and methods including treatment and animal disposition
Results
Discussion and conclusion
Copy of the protocol amendment
Tables and appendices

19.2 Final Report

The final report, audited by the Quality Assurance Unit, was submitted after the resolution of issues arising from the draft report.

20.0 REPORT DISTRIBUTION

The report was distributed to:

The Sponsor
Study file
Prof. P. Sibbons

21.0 APPENDICES

APPENDIX 1

Data Capture Form Summary

This table was completed once for each artery under study:

	Artery Number/Catheter Number										
	1/1	2/2	3/3	4/4	5/5	6/5	7/6	8/6	9/7	10/8	11/9
Artery under study	RCA	LAD	LAD	LAD	LAD	CIR	LAD	CIR	CIR	LAD	RCA
Base blood pressure transduced by guide (mmHg)	102/71 (86)	83/57 (72)	86/57 (69)	95/76 (82)	96/75 (86)	96/75 (86)	81/51 (63)	81/51 (63)	82/58	91/64 (76)	102/78 (92)
Blood pressure transduced following insertion of the micro-catheter (mmHg)	98/72 (85)		83/59 (72)	98/83 (90)	97/75 (84)	97/75 (84)	85/59 (72)	85/59 (72)	85/63 (72)	94/70 (84)	102/75 (92)
Blood pressure (mmHg) at the point of equalisation (ratio of transduced to pressure wire must = 1.00)	100/73 (87)	88/64 (77)	83/59 (73)				81/58	81/58	84/60 (74)	92/71	102/75
Resting FFR, following advancement of wire to desired location	1.03	0.95	0.90	0.96	0.97	1.02	0.95		0.99	0.98	1.03

This table was completed for every run:

		Run Number/Catheter Number									
		1/1	2/1	3/1	4/1	5/1	6/2	7/2	8/2	9/2	10/3
Mode and dose and duration of adenosine infusion (IV or IC).	Mode:	IC	IC	IC	IC	IV	IV	IC	IC	IC	IV
	Dose (µg/min):	Disregarded due to mis-dose	360	360	360	9100	9100	360	360	360	9100
	Duration (min):		2	2	2	2	2.5	2	2	2	2.5
Peak hyperaemia (stress FFR) value.			1.00	1.00	1.00	1.00	0.94	0.95	0.92	0.92	0.88
FFR recorded when wire withdrawn again to guide tip.			0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96
Value after equalization (if <0.995 or >1.05 before)			1.02								1.0
Duration/delay before starting the next run (min).			>2	2	5		>5	>5	2		3

APPENDIX 1 (continued)

Data Capture Form Summary (continued)

		Run Number/Catheter Number									
		11/3	12/3	13/3	14/3	15/4	16/4	17/4	18/4	19/5	20/5
Mode and dose and duration of adenosine infusion (IV or IC).	Mode:	IC	IC	IC	Misuse Scenarios IC 600 mL/hr - 4mL then alarm 500mL/hr 5mL, no alarm then advanced 5 times into artery	IV	IC	IC	IC	IV	Abandoned
	Dose (µg/min):	360	360	360		9100	360	360	360	9100	
	Duration (min):	2	2	2		3	2			3	
Peak hyperaemia (stress FFR) value.		0.92	0.9	0.91		0.93	0.95	0.96	0.95	0.93	
FFR recorded when wire withdrawn again to guide tip.		0.99	1.0	1.0		0.98	1.0	1.0	1.0	0.98	
Value after equalization (if <0.995 or >1.05 before)		1.0	0.95			1.0				1.0	
Duration/delay before starting the next run (min).		2	3			2	2	2		2	

		Run Number/Catheter Number									
		21/5	22/5	23/5	24/5	25/6	26/6	27/6	28/6	29/6	30/7
Mode and dose and duration of adenosine infusion (IV or IC).	Mode:	IV	IC	Misuse scenario Overadvancement x 8 into Left Main Stem	Misuse scenario 600mL/hr 1 mL then alarm 500mL/hr for 6 mL	IV	IC	Abandoned	IC	IC	IV
	Dose (µg/min):	9100	360			9100	360		360	360	9100
	Duration (min):	2.4	2			2.5	2		2	2	2.5
Peak hyperaemia (stress FFR) value.		1.01	1.0			0.76	0.53		0.98	0.99	0.98
FFR recorded when wire withdrawn again to guide tip.						1.0	1.0		0.99	1.0	1.0
Value after equalization (if <0.995 or >1.05 before)		1.0							1.0		
Duration/delay before starting the next run (min).						3			2		2

APPENDIX 1 (continued)

Data Capture Form Summary (continued)

		Run Number/Catheter Number									
		31/7	32/8	33/8	34/8	35/9	36/9	37/9	38/9	39/9	
Mode and dose and duration of adenosine infusion (IV or IC).	Mode:	IC	IC	IV	IC	IV	IC	IC	IC	Misuse scenario 500mL/hr; 5 mL then800 mL/hr for 1.5mL then alarm Advanced ~8cm over wire into artery, then removed & reinserted and finally advanced without wire.	
	Dose (µg/min):	360	360	9100	360	9100	360	360	360		
	Duration (min):	2	2	3		2.5	2	2	2		
Peak hyperaemia (stress FFR) value.		0.96	0.97	0.71	0.73	1.03	1.0	0.99	1.0		
FFR recorded when wire withdrawn again to guide tip.		1.0	1.0	No read-ing	1.0	1.0	1.0	0.99	0.99		
Value after equalization (if <0.995 or >1.05 before)				1.0				1.0			
Duration/delay before starting the next run (min).			2			2	2	2			

NB. The blanks in the Data Capture Forms are due to the human guide catheters used in the study not being specifically designed for the porcine anatomy. During the study it was sometimes challenging to locate them and/or get them to remain in the target coronary artery. Therefore it was not always possible to record every measurement in every artery. In addition, where FFR values did not materially change from the previous one, then they were not always called out by the Surgical Advisor or recorded.

APPENDIX 2

ACTIVATED CLOTTING TIMES (ACT)

Study time	**ACT (secs)**		
	ANIMAL NUMBER		
	474	**475**	**476**
Pre-op	*Out of Range HIGH*	*Out of Range HIGH*	*Out of Range HIGH*
30mins	*469*	*282*	*217*
60mins	*154*	*187*	*176*
90mins	*358*	*197*	*172*
120mins	*197*	*216*	
150mins	*Out of Range HIGH*		
180mins	*169*		
210mins	*206*		
240mins	*219*		

APPENDIX 3

QUESTIONNAIRE SUMMARY

TEST	CHALLENGE	YES/NO									#REPEATS								
		Catheter Number									Catheter Number								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Flushable	Can the Diasolve FFR Catheter be flushed with a 0.014" pressure wire loaded before insertion into the guide catheter?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
Infusion Pump & Adenosine Compatibility	Can the infusion pump connect to the Diasolve FFR Catheter without issue?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
	Can the Diasolve FFR Catheter infuse adenosine at the recommended clinical rate without triggering the alarm of the infusion pump?	Y	Y	Y	Y	Y	Y	Y	Y	Y	3	3	6	3	8				
Tuohy Borst Compatibility	Is the Diasolve FFR catheter compatible for insertion through generic tuohy borst valves (e.g. Merit FLO40)? This includes compression resistance and leak check.	Y	Y	Y	Y	Y	Y	Y	Y	Y	2	2		3	1				
	Can the Diasolve FFR catheter be successfully inserted through the tuohy borst without the need for a needle introducer?	Y	Y	Y	Y	Y	Y	Y	Y	Y	3	2		3	2				
6F Guide Catheter Compatibility	Can the Diasolve FFR Catheter be successfully used in conjunction with a 6F guide catheter?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
	Does the tuohy borst hold the FFR Catheter satisfactorily in place when sealed?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
Form Factor	Is the hub familiar, easy and intuitive to use?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
	Is the hub suitable for one-handed use?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
	Is the hub suitable for use in a catheterization lab?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
	Is the hub sufficiently differentiated from the Guide Catheter hub?	Y	Y	Y	Y	Y	Y	Y	Y	Y	10	6		10					
Pushability/ Torquability	Does the Diasolve FFR Catheter track through the guide catheter and reach the target site without damage?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
Radiopaque Tip	Is the radiopaque tip clearly visible under fluoroscopy at multiple points inside the guide catheter?	Y	Y	N *	Y	Y	Y	Y	Y	Y	15	10		10	5				
Proximal Marker Bands	Are the marker bands clearly visible against the surgical drape?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
	Are they in the most appropriate position to aid positioning of the tip?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
Catheter Length	Is the working length of the catheter appropriate for easy handling when tip is positioned at GC tip?	Y	Y	Y	Y	Y	Y	Y	Y	Y									

***N** Not very well

APPENDIX 3 (continued)

QUESTIONNAIRE SUMMARY (continued)

TEST	CHALLENGE	YES/NO									#REPEATS								
		Catheter No.									Catheter No.								
		1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
Pressure Damping	Is the system free of pressure damping between the Diasolve FFR Catheter and the guide catheter?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
0.014" Pressure Wire Compatibility	Can the pressure wire be introduced easily using a standard needle introducer?	Y	Y	Y	Y	Y	Y	Y	Y	Y					3				
	Can the Diasolve FFR Catheter successfully perform all functions when used with a 0.014" pressure wire? (functions include infusion, tracking, flushing).	Y	Y	Y	Y	Y	Y	Y	Y	Y									
	Does the FFR Catheter hold the pressure wire in place satisfactorily during the procedure?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
	Can the pressure wire be connected and used as normal?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
	Can the Diasolve FFR Catheter, with 0.014" pressure wire in place, be inserted into the guide catheter without a needle introducer in place?	Y	Y	Y	Y	Y	Y	Y	Y	Y					2				
Retrievability	Can the Diasolve FFR Catheter be retrieved from the system after procedure is complete?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
Disposability	Can the Diasolve FFR Catheter be easily disposed of in waste?	Y	Y	Y	Y	Y	Y	Y	Y	Y									
Kink Resistance	Does the Diasolve FFR Catheter display any physical damage caused by kinking during the procedure?	N	N	N	N	N	N	N	N	N									
Leak Resistance (radial force)	Does the Diasolve FFR Catheter present any sign of leaking at any point in the procedure which cannot be resolved by tightening the valve?	N	N	N	N	N	N	N	N	N									
Air ingress	Were any clinically significant air bubbles noted to enter the FFR catheter and/or circulatory system?	N	N	N	N	N	N	N	N	N									
	Were any symptoms indicative of air embolism noted?	N	N	N	N	N	N	N	N	N									
Damage to Pressure wire	Does the Pressure wire show any signs of damage due to the FFR catheter?	N	N	N	N	N	N	Y	N	Y							1		2
Packaging*	Is there all information required? Do any changes need to be made?	N	N	N	N	N	N	N	N	N									
Reinsertion	Were any issues noted during reinsertion?	N	N	N	N	N	N	N	N	N									

*N in the answer boxes means that all the required information was there and that no changes need be made

APPENDIX 4

TRANSCRIPTION OF THE POST-MORTEM EXAMINATION OF THE CORONARY VESSELS

Animal Number	Observations
474	*LMS + LAD both exposed & examined – No evidence of dissection or damage*
475	*LMS + LAD + circumflex arteries exposed and examined macroscopically. No evidence of damage or dissection*
476	*LAD & RCA exposed & examined macroscopically. No evidence of damage or dissection.*

Key to Abbreviations

Abbreviation	Meaning
LMS	Left Main Stem
LAD	Left Artery Descending
RCA	Right Coronary Artery

APPENDIX 5

HISTOPATHOLOGY REPORT OF STUDY NO. DIA 276/15

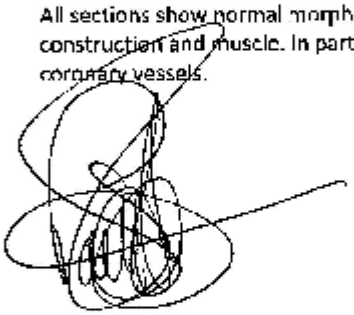
Histopathology of study DIA/15

Subjects
This study consists of three porcine subjects numbered 474, 475 and 476. These subjects were allocated histology numbers P8807, P8808 and P8809 respectively.

Samples
Samples for analysis are presented as 5 µm histological sections stained with haematoxylin and eosin. There are three sections for each subject and the sections are taken from lower ventricular myocardium, mid ventricular myocardium and atrium. The myocardial sections have been prepared to include coronary arteries and veins.

Analyses
Sections were examined using standard transmitted light microscopy.

Results
All sections show normal morphology with regard to cellular type and distribution, tissue construction and muscle. In particular, there were no abnormalities noticed in any of the coronary vessels.



Prof Paul Sibbons. FIBMS, FRMS, PhD, FRCPath.

CPS
14.4.15

APPENDIX 6

AMENDMENT DIA 276/15/01

PU 167

PROTOCOL AMENDMENT

Study Number: DIA 276/15 **Amendment Number:** DIA 276/15/01

Title: SAFETY AND PERFORMANCE OF THE DIASOLVE FER MICRO-CATHETER IN A PORCINE MODEL.

Date: 03.03.2015

Amendment(s):

An insertion into section 8.5.29 as follows:

8.5.29 The catheter and wire will be withdrawn *then washed, flushed and reinserted before being withdrawn again* and the animal killed by an intravenous overdose of sodium pentobarbitone (Lethobarb).

An addition to the Questionnaire (Appendix 8) as follows:

Test	Challenge	Yes/No	#Repeats	Comment
Reinsertion	Were any issues noted during reinsertion?			

THIS AMENDMENT WILL NOT IMPACT UPON THE COMPLIANCE STATUS OF THE STUDY.

Study Director: CDS

Print Name: Colin Shurey

Date: 4.3.15

Sponsor:

Print Name: Paul Weinberger

Date: 04.03.15

Approved by QA: JR Mallard

Print Name: Jim Mallard

Date: 5 March 2015

APPENDIX 7

IMAGE SHOWING GUIDE CATHETER, MICROCATHETER TIP AND PRESSURE WIRE

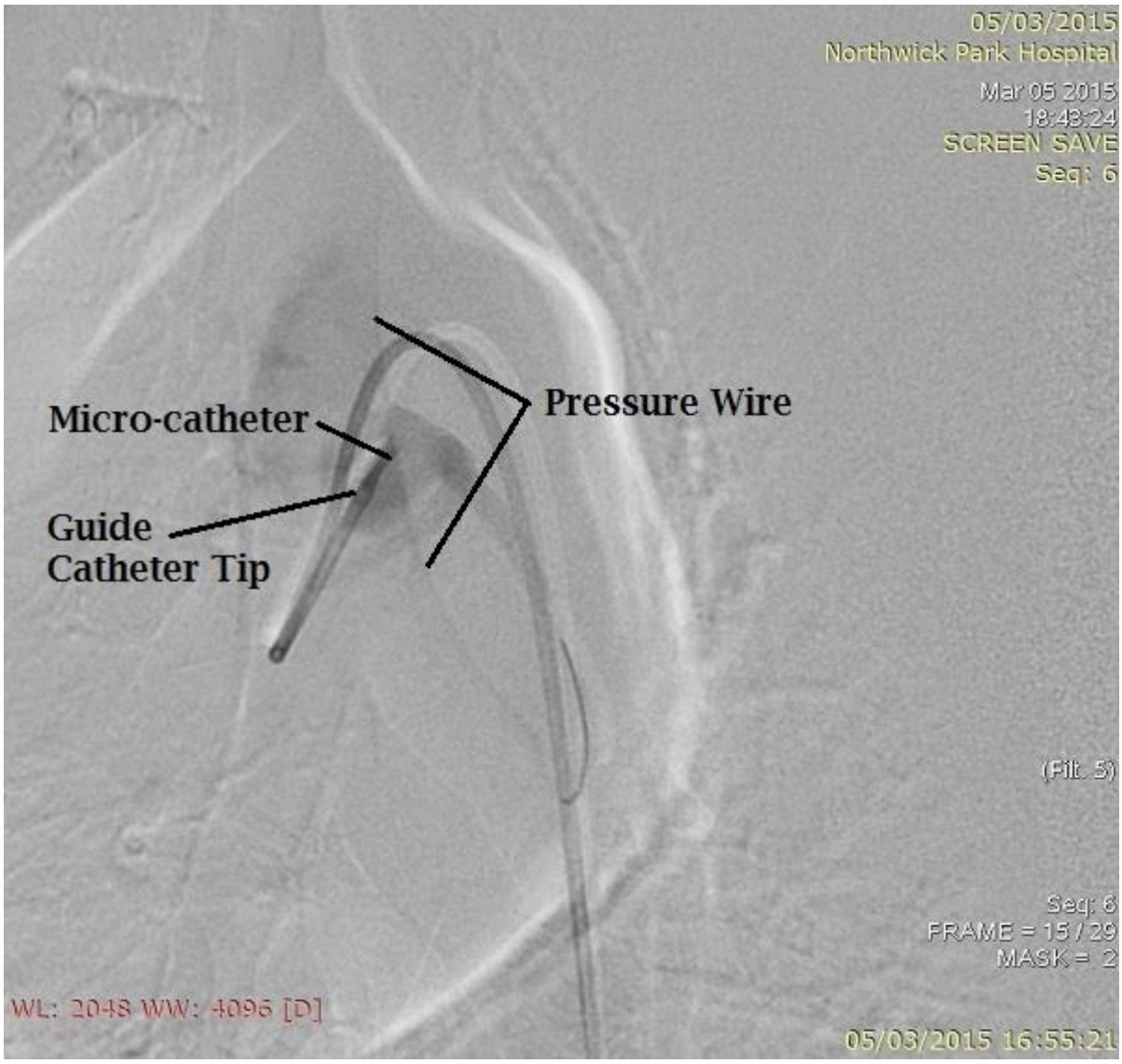


Fig. 1 Image Showing Guide Catheter, Micro-catheter and Pressure Wire

This image is Frame 15 of 29 from fluoroscopy Sequence No. 6 of Animal No. 476