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Limitation of Infarct Size and No-Reflow by Intracoronary Adenosine Depends Critically on Dose and Duration

Tuncay Yetgin, MD,*† André Uitterdijk, MSc,* Maaike te Lintel Hekkert, BASc,* Daphne Merkus, PhD,* Ilona Krabbendam-Peters, BASc,* Heleen M.M. van Beusekom, PhD,* Robert Falotico, PhD,‡ Patrick W. Serruys, MD, PhD,* Olivier C. Manintveld, MD, PhD,* Robert-Jan M. van Geuns, MD, PhD,* Felix Zijlstra, MD, PhD,*† Dirk J. Duncker, MD, PhD*†

ABSTRACT

OBJECTIVES In the absence of effective clinical pharmacotherapy for prevention of reperfusion-mediated injury, this study re-evaluated the effects of intracoronary adenosine on infarct size and no-reflow in a porcine model of acute myocardial infarction using clinical bolus and experimental high-dose infusion regimens.

BACKGROUND Despite the clear cardioprotective effects of adenosine, when administered prior to ischemia, studies on cardioprotection by adenosine when administered at reperfusion have yielded contradictory results in both pre-clinical and clinical settings.

METHODS Swine (54 ± 1 kg) were subjected to a 45-min mid-left anterior descending artery occlusion followed by 2 h of reperfusion. In protocol A, an intracoronary bolus of 3 mg adenosine injected over 1 min (n = 5) or saline (n = 10) was administered at reperfusion. In protocol B, an intracoronary infusion of 50 μg/kg/min adenosine (n = 15) or saline (n = 21) was administered starting 5 min prior to reperfusion and continued throughout the 2-h reperfusion period.

RESULTS In protocol A, area-at-risk, infarct size, and no-reflow were similar between groups. In protocol B, risk zones were similar, but administration of adenosine resulted in significant reductions in infarct size from 59 ± 3% of the area-at-risk in control swine to 46 ± 4% (p = 0.02), and no-reflow from 49 ± 6% of the infarct area to 26 ± 6% (p = 0.03).

CONCLUSIONS During reperfusion, intracoronary adenosine can limit infarct size and no-reflow in a porcine model of acute myocardial infarction. However, protection was only observed when adenosine was administered via prolonged high-dose infusion, and not via short-acting bolus injection. These findings warrant reconsideration of adenosine as an adjuvant therapy during early reperfusion. (J Am Coll Cardiol Intv 2015;8:1990–9) © 2015 by the American College of Cardiology Foundation.

Timely reperfusion remains the single most effective treatment of acute myocardial infarction (AMI) for salvaging ischemic myocardium, leading to improved residual ventricular function and clinical outcome (1). However, reperfusion itself initiates a cascade of harmful events, termed "lethal reperfusion injury," which is characterized by mitochondrial damage and cardiomyocyte death (2,3), and by ultrastructural damage to capillary endothelium, leading to microvascular obstruction, termed "no-reflow" (4). Since lethal reperfusion injury may account for up to 50% of the final myocardial infarct size (3), and because no-reflow is associated with poor clinical prognosis (5), it is clear that reperfusion injury constitutes a key therapeutic target.

From the *Department of Cardiology, Thoraxcenter, Erasmus University Medical Center, Rotterdam, the Netherlands; †Interuniversity Cardiology Institute of the Netherlands, Utrecht, the Netherlands; and the ‡Cordis Corporation, Fremont, California. This work was supported by Cordis Corporation, a Johnson & Johnson Company. Dr. Falotico is an employee of Cordis Corporation, a Johnson & Johnson Company. Dr. van Geuns has received research grants and speakers honoraria from Abbott Vascular and Boston Scientific. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Yetgin and Uitterdijk contributed equally to this work.

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Adenosine exerts a variety of actions that may attenuate many of the proposed mechanisms of reperfusion-mediated injury, including inhibition of neutrophil-mediated vascular damage and preservation of microvascular flow; restoration of calcium homeostasis; inhibition of oxidative stress; and mediation of pre-, post- and remote conditioning (6–9). Yet, attempts to achieve cardioprotection with administration of adenosine at reperfusion have yielded mixed results in both pre-clinical and clinical studies (**Tables 1 and 2**). For example, recent clinical studies using intracoronary adenosine bolus injections in AMI were unable to demonstrate significant reductions in infarct size (10,11). Inconsistent results may be related to several factors, including the availability of adenosine at an optimal concentration at reperfusion and a brief window for therapeutic application. In addition, the optimal dosage for efficacious adenosine treatment in AMI has remained undefined in both experimental (using canine and rabbit models) and clinical settings. Given the present lack of clinically effective adjuvant pharmacotherapy to prevent reperfusion-mediated injury, re-evaluation of adenosine in the setting of AMI would be of great interest, taking aforementioned considerations into account.

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Accordingly, we hypothesized that prolonged local intracoronary delivery of adenosine using an optimal concentration is able to reduce infarct size and no-reflow. To test this hypothesis, we employed a large animal (porcine) model of ischemia-reperfusion to re-evaluate the effects of intracoronary bolus injections of adenosine at reperfusion with doses equivalent to clinical trials. Subsequently, we investigated the cardioprotective effects of a high-dose, prolonged intracoronary infusion of adenosine.

METHODS

We followed the ARRIVE (Animals in Research: Reporting In Vivo Experiments) guidelines for reporting animal research (12). Experiments were performed in Yorkshire × Landrace swine of either sex weighing 54 ± 1 kg. All procedures were performed in compliance with the "Guiding Principles in the Care and Use of Animals" as approved by the Council of the American Physiological Society and under the regulations of the Animal Care Committee of the Erasmus University Rotterdam.

EXPERIMENTAL PROTOCOL. Animals were subjected to regional ischemia by occluding the mid-left anterior descending coronary artery (LAD) for 45 min followed by 2 h of reperfusion. In protocol A, animals received an intracoronary adenosine bolus injection at reperfusion (3 mg in 1 ml injected over 1 min) or an equivalent intracoronary bolus of saline. The dose per kilogram of bodyweight and timing of injection approximated or was similar to that employed in clinical studies (**Table 2**). In protocol B, animals received an intracoronary adenosine infusion of 50 μg/kg/min starting at 40 min of occlusion (5 min prior to reperfusion) and continuing until the end of reperfusion (infusion rate: 0.67 ml/min) or an equivalent intracoronary infusion of saline. The dose of 50 μg/kg/min adenosine was determined in dose-finding studies available in the Online Appendix. Protocols A and B were performed consecutively and animals were allocated to either control or adenosine treatment by weighted randomization.

SURGICAL PREPARATION AND PROCEDURES. Swine were sedated with ketamine (20 mg/kg, intramuscularly) and midazolam (1 mg/kg, intramuscularly), anesthetized with sodium pentobarbital (15 mg/kg, intravenously), intubated, and placed on a positive-pressure ventilator (O_2:N_2 = 1:3 volume:volume). Electrocardiographic electrodes for the limb leads were placed subcutaneously. Catheters were inserted into the right external jugular vein for infusion of saline, drugs, and sodium pentobarbital (10 to 15 mg/kg/h) to maintain anesthesia. A micromanometer-tipped catheter (Millar Instruments, Houston, Texas) was advanced into the left ventricle (LV) via the right external carotid artery to measure LV pressure and its first derivative over time (dP/dt). A fluid-filled catheter was inserted via the left femoral artery into the aorta to measure arterial pressure and to obtain blood samples for measurement of arterial blood gases. A Swan-Ganz catheter was advanced into the pulmonary artery via the left femoral vein to measure pulmonary artery pressure and to monitor body core temperature. Arterial blood gases were checked periodically, and ventilation settings were adjusted as necessary to maintain blood gases within the physiological range.

A median sternotomy was performed and the pericardium was opened. An electromagnetic flow probe was placed around the ascending aorta for measurement of cardiac output. A segment (2 to 3 cm) of the LAD was isolated just distal to the first diagonal branch. Following isolation, the LAD was instrumented from proximal to distal with a surgical monofilament ligature around the vessel for later

ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

CBF = coronary blood flow

dP/dt = first derivative of pressure measured over time

LAD = left anterior descending coronary artery

LV = left ventricle

PCI = percutaneous coronary intervention

SEM = standard error of the mean

TIMI = Thrombolysis In Myocardial Infarction

TABLE 1 Pre-Clinical Studies Investigating the Effects of Adenosine Infusion on Infarct Size and No-Reflow

First Author (Year) (Ref. #)	Species	Ischemic Time (min)	Reperfusion Period (h)	Dose of Administration	Infusion Time (min)	Start Adenosine Administration	Concomitant Lidocaine	Determination of Optimal Dose for Infusion	Infarct Size Reduction, %	No-Reflow Reduction, %
Left atrium infusion										
Zhao et al. (1999) (33)	Dog	60	6	140 μg/kg/min	125	5 min before R	No	No	Yes, 48	Yes (↓PMN), 58
Intravenous infusion										
Goto et al. (1991) (34)	Rabbit	30	3, 72	150, 370 μg/kg/min	60	5 min before R	Yes/no	No	No (with or without L)	–
Norton et al. (1991) (35)	Rabbit	30	48	0.1, 0.3, 0.55 mg/min	65	5 min before R	Yes	No	Yes (all doses), 32–40	–
Pitarys et al. (1991) (36)	Dog	90	72	150 μg/kg/min	155	5 min before R	Yes	No	Yes, 52	Yes (↓NP; p = NS)
Norton et al. (1992) (37)	Rabbit	30	48	0.001, 0.01, 0.1 mg/min	65	5 min before R	Yes	No	Yes (all 3 doses), 31–53	–
Vander Heide et al. (1996) (38)	Dog	90	3	150 μg/kg/min	155	5 min before R	Yes/no	No	No (with or without L)	–
Budde et al. (2000) (39)	Dog	60	24	140 μg/kg/min	120	5 min before R	No	No	No	No (↔PMN)
Budde et al. (2004) (40)	Dog	60	6, 24, 48	140 μg/kg/min	120*	5 min before R	No	No	Yes, 50†	Yes (↓PMN)†
Intracoronary infusion										
Olafsson et al. (1987) (41)	Dog	90	24	3.75 mg/min	60	At R	Yes	No	Yes, 76	Yes (↓NP; p = NS)
Babbitt et al. (1990) (42)	Dog	180	72	3.75 mg/min	60	At R	Yes	No	No	No (↔NI)
Homeister et al. (1990) (43)	Dog	90	6	150 μg/kg/min	60	At R	Yes	No	Yes (only with L), 56	–
Velasco et al. (1991) (44)	Dog	40	72	3.75 mg/min	60	At R	Yes	No	Yes, 63	–
Forman et al. (1993) (45)	Dog	120	24	3.75 mg/min	60	At R	Yes	No	Yes, 75	–

*Same study dose readministered at 2, 6, 12, and 18 h of reperfusion in groups with 24-h and 48-h reperfusion periods; †Significant infarct size and polymorphonuclear neutrophil reduction with single-dose adenosine in the 6-h reperfusion group versus control swine in the 6-h reperfusion group. Significant infarct size and polymorphonuclear neutrophil reduction with multidose adenosine in the 24-h and 48-h reperfusion groups (vs. control swine in the 6-h and 24-h reperfusion groups).

↓ = reduced; ↔ = unchanged; L = lidocaine; NI = neutrophil infiltration; NP = neutrophil plugging of capillaries; PMN = polymorphonuclear neutrophils; R = reperfusion.

occlusion; a transit-time flow probe (Transonic Systems, Ithaca, New York) for coronary blood flow (CBF) measurements; and a 22-gauge, nonobstructing, intracoronary catheter for administration of adenosine or saline. The anterior interventricular vein was cannulated with a 20-gauge catheter for coronary venous blood sampling. For measurement of atrial pressure, a catheter was inserted into the left atrial appendage. For measurement of regional contractile function, 2 pairs of ultrasonic crystals (Triton Technology Inc., San Diego, California) were implanted in the mid-myocardium of the area-at-risk and the remote myocardium (13).

After completion of surgical instrumentation, a stabilization period of 30 min was permitted to ensure hemodynamic stability before the onset of coronary occlusion. Systemic and coronary hemodynamics, regional contractile function, electrocardiographic changes, and body core temperature were monitored and recorded throughout the experiment. Arterial and coronary venous blood samples were obtained serially at several time points. Anticoagulation was ensured using heparin (5,000 IU/h, intravenously). Animals that developed ventricular fibrillation during the protocol were defibrillated using internal paddles (30 to 50 J, direct current).

MYOCARDIAL OXYGEN BALANCE. Measurements of partial pressure of oxygen (mm Hg), partial pressure of carbon dioxide (mm Hg), pH, oxygen saturation, and hemoglobin concentration (g/100 ml) were performed with a blood gas analyzer (ABL 800, Radiometer, Copenhagen, Denmark). Blood oxygen content, myocardial oxygen delivery, myocardial oxygen consumption in the LAD region, and myocardial oxygen extraction were computed as previously described (14).

AREA-AT-RISK, INFARCT SIZE, AND NO-REFLOW. At the end of the 2-h reperfusion period, the LAD was perfused with 5 ml of 4% thioflavin-S (Sigma, Zwijndrecht, the Netherlands) for determination of the no-reflow-area (15). Hereafter, the LAD was reoccluded and 40 ml of 16% Evans Blue (Sigma) was infused intra-atrially for area-at-risk determination (13). The heart was then excised and the LV was isolated and cut into 5 transversal slices of equal thickness parallel to the atrioventricular groove from apex to base. The area-at-risk and no-reflow-area (using ultraviolet light) of each slice were demarcated on an acetate sheet (15). The slices were incubated for 15 min in 3% buffered triphenyltetrazolium chloride (Sigma) at 37°C for determination of the infarct area (13,15). Myocardial infarct size was defined as the ratio of the summed infarct areas and summed areas-at-risk and

TABLE 2 Randomized Clinical Studies Investigating the Effects of Intracoronary Adenosine in Patients With AMI Undergoing Primary PCI

First Author (Year) (Ref. #)	n	Eligibility	Ischemia Time (min)*	Type of Administration	Dose of Administration	Administration Time (min)	Start and Site of Adenosine Administration	Infarct Size	Reperfusion Markers
Marzilli et al. (2000) (24)	54	SO <3 h, pre-TIMI 0–2	106	Bolus	4 mg in 2 ml	1	After wire crossing and balloon inflation distal to PCI site	↓ peak CK/CK-MB (p = NS)	↓ No-reflow (↓ ≥1 TIMI grades final angio relative to post-PCI angio)
Hendler et al. (2006) (17)	20	SO <12 h, post-TIMI 3 with MBG 0/1	120	Bolus	60–120 μg	NA	Catheter site	–	↔ STR, TMPG
Stoel et al. (2008) (25)	49	STR <70% post-PCI	196	Infusion	6 mg/ml	5–10	>10 min after last balloon inflation at catheter site	Trend ↓ peak CK-MB (p = 0.08)	↑ STR >70%, MBG, ↓TFC
Fokkema et al. (2009) (11)	448	SO <12 h, pre-TIMI 0–3	180	Bolus (2×)	2 × 120 μg in 20 ml	NA	1st bolus after TA, 2nd after stenting in IRA	↔ peak CK/CK-MB	↔ ST-deviation, STR, MBG, post-TIMI flow
Desmet et al. (2011) (10)	110	SO <12 h, pre-TIMI 0–3	215	Bolus	4 mg in 5 ml	1	After wire crossing distal to target lesion site	↔ AUC CK/CK-MB/TnI, MSI or IS MRI 4 months	↔ STR, MBG, TFC, post-TIMI flow or MVO MRI 2–3 days
Grygier et al. (2011) (16)	70	SO <6 h, pre-TIMI 0–2	273	Bolus (2×)	2 × (1 mg for RCA and 2 mg for LCA) in 10 ml	NA	1st bolus after wire crossing, 2nd after balloon inflation at occlusion site	↔ peak CK/CK-MB/TnI	↑ STR >50%, corrected TFC, MBG 3, post-TIMI 3 flow
Niccoli et al. (2013) (26)	160	SO <12 h, pre-TIMI 0/1	277	Bolus + (brief) infusion	120 μg + 2 mg in 33 ml	1st bolus fast + 2	After wire crossing and TA beyond occlusion site	↓ peak CK-MB/TnT	↑ STR >70%, ↓ MVO (TFG ≤2 or MBG <2)
Garcia-Dorado et al. (2013) (46)	201	SO <6 h, persistent TIMI 0/1 after wire crossing	NA	(Brief) infusion	4 mg	2	Immediately before reperfusion mostly with TA and direct stenting distal to culprit lesion	↔ IS MRI 2–7 days or 6 months, ↓ IS in pts with SO <200 min (n = 84)	↔ MVO MRI 2–7 days or 6 months

*Ischemia time in the adenosine groups.

↓ = reduced; ↑ = improved; ↔ = unchanged; AMI = acute myocardial infarction; angio = angiogram; AUC = area under the curve; CK = creatine kinase; CK-MB = creatine kinase-myocardial band; IRA = infarct-related artery; IS = infarct size; LCA = left coronary artery; MBG = myocardial blush grade; MRI = magnetic resonance imaging; MSI = myocardial salvage index; MVO = microvascular obstruction; NA = not available; PCI = percutaneous coronary intervention; pts = patients; RCA = right coronary artery; SO = symptom onset; STR = ST-segment resolution; TA = thrombus aspiration; TFC = TIMI frame count; TFG = TIMI flow grade; TIMI = Thrombolysis In Myocardial Infarction; TMPG = TIMI myocardial perfusion grade; TnI = troponin I; TnT = troponin T.

TABLE 3 Enrollment Summary and Baseline Data

	Bolus Injection		Prolonged Infusion	
	Control	Adenosine	Control	Adenosine
Enrollment summary				
Animals entered	11	11	26	22
Exclusions				
Technical failure*	0	3	1	1
Staining failure†	1	0	0	1
Death				
Nonconvertible ventricular fibrillation during occlusion‡	0	3	2	4
Acute pump failure	0	0	2	1
Final number entered in analysis	10	5	21	15
Baseline data				
Animal weight, kg	56 ± 1	58 ± 2	53 ± 1	51 ± 1
Male/female	3/7	3/2	12/9	7/8
Myocardial masses, g				
Left ventricle	118 ± 5	130 ± 4	125 ± 3	119 ± 3
Area-at-risk	30 ± 1	38 ± 4	33 ± 1	28 ± 2

Values are n or mean ± SEM. *Technical difficulties resulting in failure to complete the experimental protocol. †Staining difficulties compromising accurate measurement of infarct size and/or no-reflow. ‡Occurring during the first 40 min of occlusion, that is, prior to adenosine treatment; none of the animals developed nonconvertible ventricular fibrillation during reperfusion.

expressed as a percentage. No-reflow was defined as the ratio of the summed no-reflow areas and summed infarct areas and expressed as a percentage.

NEUTROPHILS. Sections of infarct area, with either reflow or no-reflow, and remote non-area-at-risk (posterior wall) LV tissue were fixed in 4% buffered formaldehyde for at least 24 h, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. Then, 4-μm sections were stained to identify acute influx of neutrophils (azurocidin, mouse antihuman, 1:100, Abnova, Heidelberg, Germany) following antigen retrieval (10-min citrate buffer boil [pH 6]). Rabbit antimouse secondary antibodies were used (1:100, horseradish peroxidase label, DAKO, Heverlee, Belgium) with diaminobenzidine as the chromogen. Primary antibodies were omitted as a negative control. Three randomly selected high power fields (90.000 μm^2/field) per section were morphometrically quantified by a blinded technician (Clemex Vision PE, version 6.0.010A, Clemex Technologies Inc., Longueuil, Canada).

STATISTICAL CONSIDERATIONS. In protocol A, the sample size for the primary endpoint was based on an expected infarct size (as percentage of area-at-risk) of 60% in the control group with an equal standard deviation of 11% (based on previous data from our laboratory) and assuming a (clinically) relevant 25% relative reduction in the experimental group. With a 2-sided 5% alpha level, 80% power, and 20% attrition rate, ~11 animals in each group were needed. In protocol B, we expected an infarct size of 60% in the control group and assumed a 25% relative reduction in the experimental group, but, as the standard deviations in protocol A were higher than originally expected, with an equal standard deviation of 17%. With a 2-sided 5% alpha level, 80% power, and 20% attrition rate, ~26 animals in each group were needed.

Data are presented as mean ± standard error of the mean (SEM). Hemodynamic variables, myocardial metabolism, global and regional ventricular function, and neutrophil infiltration were analyzed with a repeated-measures 2-way analysis of variance (time × treatment or treatment × location) followed by the Student-Newman-Keuls post-hoc test. Infarct and no-reflow data were compared with unpaired Student *t* test. Control swine were time-matched to the adenosine swine within each protocol. Computations were performed with SigmaPlot (version 12.5, Systat Software Inc., San Jose, California), with statistical significance set at $p < 0.05$ (2-tailed).

RESULTS

Numbers of animals enrolled in each group and the exclusions are summarized in **Table 3**. A total of 70 swine were enrolled, of which 51 swine were included in the final analysis. An overview of arrhythmias during the protocols is provided in Online Table 1. Regional myocardial function and myocardial metabolism of animals are available in Online Tables 2 and 3, respectively.

SYSTEMIC HEMODYNAMICS. Hemodynamic data for heart rate, mean aortic pressure, cardiac output, systemic vascular resistance, $LVdP/dt_{P40}$ (rate of rise of LV pressure at 40 mm Hg) and LV end-diastolic pressure in protocols A and B are summarized in Online Table 4. Coronary occlusion was associated with similar increases in heart rate and LV end-diastolic pressure, and similar decreases in mean aortic pressure, cardiac output and $LVdP/dt_{P40}$ in the adenosine and control groups in both protocols. Treatment with adenosine during reperfusion did not affect any of the systemic hemodynamic variables compared to the respective control group in either protocol A or B.

CORONARY HEMODYNAMICS. Release of the coronary ligature resulted in reactive hyperemia reflected by increases in CBF and coronary vascular conductance in both groups (**Table 4**, **Figure 1**). In protocol A, adenosine did not increase CBF beyond the reactive

TABLE 4 Coronary Hemodynamics

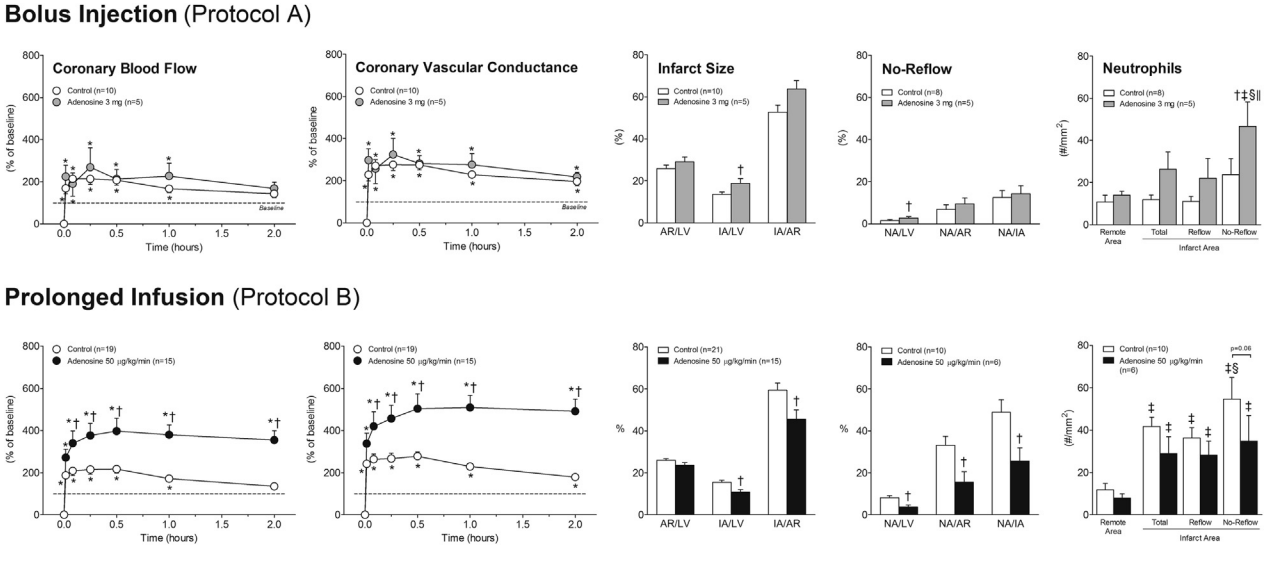
			Reperfusion				
		Baseline	1-Min	5-Min	15-Min	60-Min	120-Min
Bolus injection							
CBF, ml/min	Control	15 ± 1	24 ± 2*	31 ± 3*	31 ± 4*	25 ± 3*	21 ± 3
	Adenosine	15 ± 5	28 ± 7*	25 ± 7	33 ± 9*	30 ± 7*	24 ± 8
CVC, ml/min/mm Hg	Control	0.18 ± 0.02	0.37 ± 0.03*	0.46 ± 0.05*	0.48 ± 0.05*	0.42 ± 0.05*	0.34 ± 0.05*
	Adenosine	0.16 ± 0.05	0.44 ± 0.09*	0.37 ± 0.10*	0.46 ± 0.10*	0.43 ± 0.12*	0.34 ± 0.10*
Prolonged infusion							
CBF, ml/min	Control	17 ± 2	29 ± 3*	31 ± 3*	32 ± 4	27 ± 3*	21 ± 2*
	Adenosine	11 ± 1	27 ± 4*	32 ± 3*	37 ± 3*	38 ± 4*†	35 ± 3*†
CVC, ml/min/mm Hg	Control	0.19 ± 0.03	0.41 ± 0.03*	0.43 ± 0.04*	0.43 ± 0.04*	0.39 ± 0.04*	0.31 ± 0.03*
	Adenosine	0.13 ± 0.01	0.38 ± 0.05*	0.45 ± 0.03*	0.51 ± 0.04*	0.58 ± 0.05*†	0.55 ± 0.04*†

Values are mean ± SEM. *p < 0.05 versus corresponding baseline. †p < 0.05 versus change from baseline in control group.
CBF = coronary blood flow; CVC = coronary vascular conductance.

hyperemia produced by the ischemic period. In protocol B, adenosine infusion enhanced reactive hyperemia, reaching significance at 5 min of reperfusion, which was maintained throughout the remainder of the 2-h reperfusion period, with a maximum 4-fold increase relative to baseline at 30 min of reperfusion (Figure 1).

AREA-AT-RISK, INFARCT SIZE, NO-REFLOW. The area-at-risk, infarct size, and extent of no-reflow are shown in Figure 1. Area-at-risk was similar between the adenosine and control groups in both protocols. Bolus injection of adenosine did not reduce infarct size (64 ± 4% vs. 53 ± 3% in control swine; p = 0.07) or no-reflow (14 ± 4% vs. 12 ± 3% in control swine; p = 0.74). In contrast, infusion of adenosine during reperfusion significantly reduced infarct size (46 ± 4% vs. 59 ± 3% in control swine; p = 0.02) as well as no-reflow (26 ± 6% vs. 49 ± 6% in control swine; p = 0.03).

FIGURE 1 CBF, CVC, Infarct Size, No-Reflow, and Neutrophils



Compared with control group results, intracoronary adenosine infusion significantly increased coronary blood flow (CBF) and coronary vascular conductance (CVC) throughout the 2-h reperfusion period, whereas bolus injections did not. Risk zone (area-at-risk/left ventricle [LV]) was similar between groups in both protocols. Compared with control group results, intracoronary adenosine infusion significantly reduced infarct size (infarct area/area-at-risk), no-reflow (no-reflow area/infarct area) and neutrophil influx in both infarct and no-reflow areas, whereas bolus injections did not. Values are group means with SEM. *p < 0.05 versus corresponding baseline. †p < 0.05 versus corresponding control. ‡p < 0.05 versus remote. §p < 0.05 versus reflow infarct area. ||p < 0.05 versus total infarct area. AR = area-at-risk; IA = infarct area; NA = no-reflow area.

NEUTROPHILS. Neutrophil influx in remote and infarct areas in both protocols are shown in **Figure 1**. Adenosine bolus did not attenuate neutrophil influx into the no-reflow area, whereas adenosine infusion demonstrated a trend ($p = 0.06$) of reduced neutrophil influx in the no-reflow area.

DISCUSSION

In the present study, an intracoronary infusion of high-dose adenosine (50 μg/kg/min) initiated shortly before the onset of reperfusion and maintained throughout the 2-h reperfusion period resulted in a significant decrease in both infarct size and no-reflow in an open-chest porcine model subjected to 45 min of coronary artery occlusion and 2 h of reperfusion. In contrast, a single bolus of adenosine (3 mg over 1 min) during the first minute of reperfusion was ineffective. This is, to our knowledge, the first study to directly compare the cardioprotective effect of adenosine using a clinical bolus regimen versus prolonged infusion, against infarction and no-reflow in a large animal model of regional ischemia-reperfusion.

The present study confirms recent clinical studies that have failed to demonstrate any significant advantage on either infarct size (10,11,16) or no-reflow (10,11,17), using intracoronary adenosine bolus injections. Considering the extremely short half-life of adenosine (<15 s), the bolus injections probably were inadequate to reach therapeutic concentrations, as reflected by unaltered coronary hemodynamics in protocol A. In this regard, prolonged intracoronary delivery initiated just before reperfusion may increase local drug concentration several-fold and may achieve adequate concentrations in the target microvascular bed, thereby improving therapeutic efficacy. Consequently, in protocol B, intracoronary infusion of adenosine was initiated 5 min before the onset of reperfusion, enabling therapeutic drug levels at reperfusion, and thereby attenuating lethal reperfusion injury by inhibiting detrimental events in the early minutes of reperfusion as reflected by significant infarct size reduction. The beneficial effects observed in the current study with prolonged infusion of adenosine also suggest that maintaining a high therapeutic drug level in the coronary microcirculation is necessary to afford protection against reperfusion-mediated injury. Indeed, compared with control group results, intracoronary adenosine produced a 3- to 4-fold increase in CBF relative to baseline and remained stably elevated throughout the 2 h infusion period, affording adequate concentrations in the target microvascular bed. Notably, although the heterogeneity of CBF responses among animals in the dose-response study might impact the selected dose of 50 μg/kg/min in facilitating maximal hyperemia in the ischemia-reperfusion study, it appeared that a plateau was already reached with the dose of 20 μg/kg/min. Thus, CBF increased in most animals by less than an additional 10% upon increasing the dose to 50 μg/kg/min in most animals (Online Appendix). Interestingly, there was much less heterogeneity in the CBF responses to adenosine among animals in the ischemia-reperfusion study compared with the animals in the dose-response study, which may have been due to the larger sample size.

Prolonged infusion of adenosine resulted in a trend towards reduced neutrophil influx into the infarct area, particularly in the no-reflow area. These observations suggest that adenosine attenuated no-reflow, at least in part, through vasodilation of coronary arterioles and reduction of neutrophil activation. These actions in turn likely contributed to decreased neutrophil adherence to endothelial cells, thereby leading to preserved capillary endothelial patency, as evidenced by thioflavin-S staining. Interestingly, in protocol A, compared with saline, bolus injections of intracoronary adenosine increased neutrophil influx into the no-reflow area. Although these observations are not readily explained, the modulation of neutrophil movement by adenosine might play a role. Adenosine can stimulate or inhibit neutrophil function depending on its concentration in the microenvironment. Lower local concentrations of adenosine have been noted to promote neutrophil recruitment (via the A_1 and A_3 adenosine receptor subtypes), whereas high concentrations of adenosine limited neutrophil recruitment (via A_{2A} and A_{2B} receptors) (18).

The current results in swine are in agreement with earlier studies in rabbits and dogs demonstrating infarct size reduction with adenosine infusions mostly with concomitant lidocaine at reperfusion using somewhat similar adenosine doses (**Table 1**). Unlike the current study, it is not clear whether the cardioprotection observed in those studies is entirely attributable to adenosine alone. In contrast, not all pre-clinical studies have shown cardioprotective effects. Inspection of **Table 1** does not readily reveal a methodological explanation for these equivocal results. Thus, differences in species, duration of ischemia and reperfusion, and varying routes and timing and duration of administration of adenosine do not appear to separate positive from negative studies in rabbits and dogs. Interestingly, none of the aforementioned negative (and positive) studies specifically assessed the optimal dose for infusion of adenosine. Thus, it cannot be excluded that in

several studies, the dose employed may have been insufficient to afford myocardial protection for a given duration of ischemia and reperfusion. This is particularly true in the case of intravenous adenosine administration, as maximal doses are difficult to achieve in view of the marked systemic hypotension that is associated with higher adenosine doses. That cardioprotection was still observed in some of the intravenous studies may have been the result of stimulation of remote pathways of cardioprotection (19,20). Whether maximal adenosine dosages were achieved in clinical studies using intravenous adenosine is also unclear. The AMISTAD (Acute Myocardial Infarction Study of Adenosine) I tested a 3 h intravenous adenosine infusion (10 to 70 μg/kg/min) in patients receiving thrombolysis and demonstrated adenosine to be effective in reducing infarct size in the subgroup with anterior infarction only (21). In AMISTAD II, adenosine infusions (50 or 70 μg/kg/min) were used for anterior infarction prior to revascularization with infarct size reduction observed only in the high-dose group (22) and a reduction in major clinical endpoints observed only in patients receiving early reperfusion (within 3.2 h of symptom onset) (23).

In contrast, the intracoronary route allows administration of much higher adenosine doses without direct systemic adverse effects. However, in the clinical setting, the administration of adenosine via the intracoronary route has been studied mainly using bolus injections (**Table 2**). One could assume that the very short biological half-life of adenosine (<15 s) makes a bolus injection unlikely to be effective. Indeed, in the present study, mimicking a typical clinical protocol of intracoronary adenosine bolus administration, we failed to observe any additional increase in CBF or coronary vascular conductance beyond the reactive hyperemia. This lack of effect on the coronary microvasculature may explain that intracoronary bolus injections of adenosine failed to reduce either infarct size (10,11,16) or no-reflow (10,11,17). Another important issue in the clinical context is patient selection. Despite the failure to significantly reduce infarct size, adenosine bolus regimens have been found to improve various indices of reperfusion in patients presenting with TIMI (Thrombolysis In Myocardial Infarction) flow grade ≤2 (16,24), whereas, trials also recruiting patients with presenting TIMI flow grade 3 (i.e., patients experiencing spontaneous reperfusion) were unable to demonstrate any advantage of intracoronary bolus injections of adenosine on infarct size or reperfusion markers (10,11). These data suggest that adenosine should be administered before or, at least, at the very onset of reperfusion and highlight the brief window of opportunity. The only clinical trial that did use a continuous intracoronary infusion regimen (albeit for only 5 to 10 min) showed accelerated recovery of microvascular perfusion in cases of persistent ST-segment elevation after percutaneous coronary intervention (PCI) (25). This suggests that adenosine can still be effective even after suboptimal myocardial reperfusion, provided that adequate dosing and duration of administration is used, a finding consistent with the present results. Interestingly, recent data point to an increased beneficial effect of a combined adenosine bolus (120 μg) and infusion (2 mg in 2 min) regimen as evidenced by a reduced infarct size and an improved microvascular perfusion (26), which translated into an improvement of LV remodeling at 1-year clinical follow-up (27).

BARRIERS TO CLINICAL TRANSLATION. Rationale for the use of adenosine as a cardioprotective agent following reperfusion on the ischemic myocardium arose from its ability to inhibit mechanisms involved in reperfusion injury, as also evidenced by the current 14% absolute and 23% relative infarct size reduction of percentage of area-at-risk (corresponding to a 7% absolute and 30% relative infarct size reduction percentage of LV). Considering this potential amount of infarct size reduction, adenosine holds promise as an adjunct to primary PCI when compared with other therapies for acute AMI, such as, for instance, stem cell therapies demonstrating absolute infarct size reductions of ~3% (28). However, the obvious differences between the experimental setting and the clinical reality constitute important barriers to efficacious clinical translation. Patient comorbidities and concurrent use of medication with cardioprotective properties may be potential confounding factors blunting the benefits reported in the pre-clinical setting (9). Perhaps more importantly, therapeutic optima for adenosine treatment at reperfusion in animal models of ischemia reperfusion have not been established. Therefore, it is not surprising that results obtained from clinical studies are inconclusive, as optimal conditions for efficacious adenosine administration remain undefined. Successful clinical application of adenosine in AMI will depend on enhancing our understanding of the optimal dose of adenosine and the optimal onset and duration of adenosine infusion. Thus, exploring the response curves to both dose and duration of adenosine administration will be essential when designing a clinical trial. Notwithstanding these issues, pre-clinical and clinical data suggest that only (sustained) high doses of adenosine,

reaching the coronary microcirculation immediately before or at the onset of reflow, are able to afford protection against reperfusion-mediated injury at a time when the amount of potentially salvageable myocardium is maximal.

STUDY LIMITATIONS. First, we employed healthy juvenile animals in our experiments without comorbidities as encountered in patients. Second, the exact mechanisms of adenosine-mediated protection were not assessed. Nonetheless, there is abundant data regarding the mechanisms through which adenosine is effective in ameliorating reperfusion-mediated injury (6). Third, likely as a result of seasonal variation (unpublished observations), the no-reflow area was smaller in control swine in protocol A than in control swine in protocol B. Indeed, there is evidence of seasonal variation of myocardial infarct size (29) and of circadian rhythms in sensitivity to ischemia-reperfusion injury (30). After initial demonstration in mice (30), this phenomenon has also been observed in patients (31,32). Importantly, control swine were time-matched to the adenosine swine within each protocol.

CONCLUSIONS

Prolonged high-dose intracoronary infusion of adenosine starting just prior to reperfusion, but not a single bolus of adenosine administered during early reperfusion, significantly reduced infarct size and no-reflow in a porcine model of AMI. Considering that there is currently no successful clinical pharmacological treatment for prevention of reperfusion injury, the findings in the present study warrant further clinical studies in patients with AMI, using prolonged high-dose intracoronary adenosine infusion.

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REPRINT REQUESTS AND CORRESPONDENCE: Dr. Dirk J. Duncker, Department of Cardiology, Thoraxcenter, Room Ee-2351 A, Erasmus University Medical Center, P.O. Box 2040, 3000 CA Rotterdam, the Netherlands. E-mail: d.duncker@erasmusmc.nl.

PERSPECTIVES

WHAT IS KNOWN? Attempts to achieve cardioprotection with administration of adenosine at reperfusion have yielded mixed results in both pre-clinical and clinical studies.

WHAT IS NEW? Whereas a high-dose bolus injection at reperfusion did not afford protection, a high-dose prolonged intracoronary infusion of adenosine did limit infarct size and no-reflow.

WHAT IS NEXT? Successful clinical application of adenosine in ST-segment elevation myocardial infarction will depend on enhancing our understanding of the optimal dose of adenosine and the optimal duration of adenosine infusion. Thus, exploring the response curves to both dose and duration of adenosine administration will be vital when designing a clinical trial.

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APPENDIX For supplemental tables and additional material, please see the online version of this article.