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# Effect of adenosine therapy at reperfusion on myocardial infarct size in dogs

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**Abstract**

**Objectives:** The concept of lethal reperfusion injury in ischemic myocardium has been the subject of controversy. Adenosine administered during reperfusion has been reported to limit lethal reperfusion injury in several studies. On the contrary, it has been reported that cardioprotection may not be achieved with adenosine alone but may occur if adenosine is co-administered with lidocaine. Still other investigators have reported no beneficial effect of adenosine, given with or without lidocaine. If the positive reports are reproducible, they are important both because they provide evidence for the existence of reperfusion injury and establish a rationale for preventing it. Thus, the present study was done to determine if adenosine could limit lethal reperfusion injury in a canine model of regional myocardial ischemia and reperfusion, carefully controlled for baseline predictors of infarct size. **Methods:** Dogs ($n = 37$) of either sex were subjected to 90 min of coronary occlusion followed by 3 h of reperfusion. Two groups of dogs received adenosine (150 $\mu$g/kg/min) intravenously for 155 min starting 5 min prior to the reperfusion. One treated group received adenosine only and a second group received adenosine plus lidocaine (2 mg/kg). Control dogs received a saline infusion. After 3 h of reflow, hearts were excised and infarct size was measured and expressed as a percentage of the ischemic area at risk (AAR). To control for variation in infarct size due to variation in collateral blood flow (CBF), infarct size among groups was compared using ANCOVA, using CBF as the independent variable and infarct size as the dependent variable. **Results:** Transmural collateral blood flow and AAR were not significantly different between any of the groups. Mean infarct size (adjusted by ANCOVA) in control dogs ($n = 9$) was 38.1 $\pm$ 5.3% of the AAR. Neither adenosine ($n = 9$) nor adenosine plus lidocaine ($n = 7$) significantly limited infarct size (35.6 $\pm$ 5.6% AAR and 38.1 $\pm$ 7.7% AAR, respectively; both $P$ = NS). **Conclusions:** Intravenous adenosine therapy (150 $\mu$g/kg/min) during reperfusion, whether administered alone or in dogs previously treated with lidocaine, did not limit infarct size after 90 min of regional ischemia in canine myocardium.

*Keywords:* Myocardial ischemia; Reperfusion; Myocardial infarct size; Adenosine; Lidocaine

## 1. Introduction

Reperfusion of ischemic myocardium with arterial blood, if achieved in a timely fashion, results in limitation of infarct size. Nevertheless, the concept that reperfusion of ischemic myocardium may have detrimental (termed 'reperfusion injury') as well as beneficial effects has evolved over the past 3 decades. 'Lethal reperfusion injury' is defined as death of myocytes which are still viable at the end of an ischemic period but die as a direct consequence of the restoration of blood flow. The concept is important because, to the extent that infarct size is due to lethal reperfusion injury rather than pre-existing ischemic injury, adjunctive therapy to prevent it should provide more effective limitation of infarct size than does reperfusion alone.

Several mechanisms of lethal reperfusion injury have been proposed. For example, injury from a burst of oxygen-derived free radicals [1–3] produced either in myocytes or by accumulating neutrophils [4,5] have been proposed to cause lethal reperfusion injury. Results of some studies have provided support for this hypothesis

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[5–9]. However, in other studies, therapies directed against neutrophils and/or oxygen-derived free radicals have not been shown to limit infarct size [10–15], casting doubt on the importance of these mechanisms as causes of lethal reperfusion injury. To date, no mechanism has been proven and the very existence of lethal reperfusion injury remains controversial [16].

Several recent studies suggest that adenosine, or adenosine $A_2$-receptor agonists, administered during reperfusion can limit myocardial infarct size in dogs [9,17–20]. However, in a study by Homeister et al (18), adenosine by itself did not limit myocardial infarct size, although the combination of adenosine and lidocaine did. Our own analysis of the data from the study of Homeister et al. suggests that their group treated with adenosine plus lidocaine may have yielded a false-positive result which would disappear if infarct size was controlled for variation in collateral blood flow. Collateral blood flow is a major baseline predictor of infarct size in dogs. Moreover, a study using rabbits showed no effect of adenosine, with or without lidocaine, on myocardial infarct size [21].

If reproducible, the limitation of infarct size by adenosine administered only during reperfusion would provide proof both that lethal reperfusion injury exists and that it can be prevented by therapy. Thus, the present study was undertaken to determine whether treatment during reperfusion, with intravenous adenosine with or without lidocaine, would limit myocardial infarct size in a canine model of ischemia and reperfusion.

## 2. Methods

### 2.1. Experimental design

Thirty-seven (37) adult mongrel dogs of either sex were entered into the study. All animals used had packed hematocrits of $> 35\%$ and were free of clinically evident disease. The experimental protocol consisted of 3 separate groups and is summarized in Fig. 1. All dogs were subjected to 90 min of regional ischemia followed by arterial reperfusion for either 4 days ($n = 3$) or 3 h ($n = 34$). Dogs treated with adenosine alone received an intravenous adenosine infusion (150 $\mu$g/kg/min) starting at 85 min of occlusion (5 min prior to arterial reperfusion) and continuing for 150 min into reperfusion (matching the protocol of [19]). Because a previous study suggested that prevention of lethal reperfusion injury with adenosine was dependent on concomitant treatment with lidocaine [18], a second group of dogs was treated with the same infusion of adenosine but also received two intravenous injections of lidocaine (2 mg/kg each), given over a 15-s interval, one just after the onset of coronary occlusion and one just before reperfusion. Control dogs received an intravenous infusion of sterile saline throughout the experiment.

Adenosine was obtained from Sigma Company (St. Louis, MO, USA) and lidocaine was from Abbott Laboratories, North Chicago, IL, USA). Adenosine was dissolved in sterile 0.9% saline at a concentration of 1 mg/ml.

### 2.2. Surgical preparation

Dogs were anesthetized with sodium pentobarbital (approximately 40 mg/kg i.v.), following which they were immediately intubated and mechanically ventilated using room air supplemented with oxygen. Dogs were placed on a thermal blanket (#50-7079 Harvard Homeothermic Blanket System, South Natick, MA) and a thermistor probe was placed in the rectum to maintain core temperature at 37°C. A femoral cutdown was performed and the femoral artery and vein were cannulated. The arterial catheter was used to measure blood pressure, to obtain blood samples for measurement of arterial blood gases, and to obtain reference samples for measurement of regional myocardial blood flow by microspheres. The venous catheter was used for administration of drug(s), normal saline, and additional anesthetic as indicated. Arterial blood gases were checked periodically and ventilation settings were adjusted if necessary to maintain the blood gases within the physiological range. A thoracotomy was performed in the 4th intercostal space and the heart was suspended in a pericardial cradle. The left circumflex coronary artery was isolated beneath the atrial appendage, proximal to its first large marginal branch. Following isolation, a strip of moistened umbilical tape was passed around the vessel for later occlusion. Occlusion was accomplished by snaring the artery into a small plastic tube. Catheters were inserted into the left atrium for microsphere injection and measurement of atrial pressure. Tween 80 (0.6 ml, 0.05%) was given to desensitize the animal before

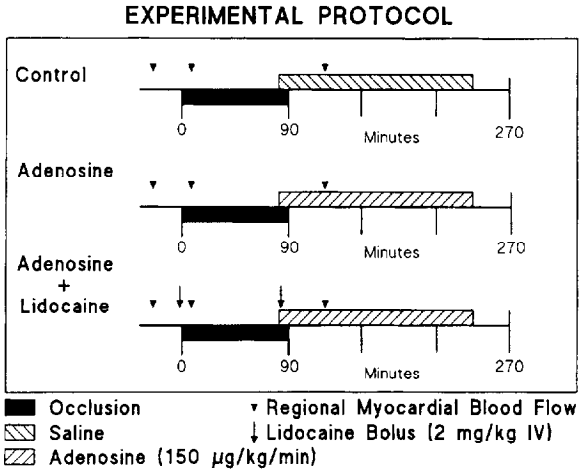


Fig. 1. Experimental design. Dogs underwent a 90-min period of occlusion of the circumflex coronary artery followed by 3 h of arterial reperfusion. Control dogs received only saline during the experiment. Adenosine-treated dogs received a 155-min infusion of adenosine beginning 5 min prior to reperfusion and continuing for 150 min into the reperfusion period. Adenosine plus lidocaine-treated dogs received two separate doses of lidocaine (2 mg/kg) at the indicated times.

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injection of microsphere suspensions containing this detergent. (Although transient hypotension has been previously reported following the initial administration of Tween 80, no dog had any detectable hemodynamic reaction to Tween 80 in the present study.) A 30-min equilibration period was permitted after completion of surgical preparation to assure hemodynamic stability before the onset of coronary occlusion. Left atrial pressure, arterial pressure, lead II of the electrocardiogram, and pericardial temperature were monitored throughout the experiment. At the completion of the experimental protocol, the heart was excised and processed for post-mortem analysis.

If a dog suffered ventricular fibrillation during the protocol (occlusion or reperfusion), the heart was immediately countershocked with internal paddles using the lowest energy necessary to resuscitate the dog, starting with 5 joules and never exceeding 20 joules. Any dog that could not be successfully resuscitated was excluded from the study. Also, if defibrillation produces epicardial necrosis that compromises accurate measurement of infarct size, the animal is excluded even if defibrillation was achieved. However, in the present study, the latter situation did not occur.

### 2.3. Regional myocardial blood flow

Regional myocardial blood flow was measured using radioactive microspheres before coronary occlusion, 10 min after the onset of occlusion, and 35 min after the onset of reperfusion. Two to three million microspheres ($10 \pm 1$ $\mu$m; New England Nuclear, Boston, MA, USA) labeled with $^{46}$Sc, $^{113}$Sn or $^{141}$Ce were injected through a left atrial catheter. Reference arterial blood samples were withdrawn from the femoral artery at a rate of 7.75 ml/min beginning just before and continuing for 2.5 min after injection.

### 2.4. Postmortem studies

#### 2.4.1. Area at risk

To determine the anatomical boundaries of the previously ischemic and non-ischemic vascular beds, triphenyltetrazolium chloride (TTC) (1%, Sigma Chemical Co.) and monastral blue (4%, Sigma Chemical Co.) were injected simultaneously at 37°C under 120–140 mmHg pressure into the previously occluded left circumflex coronary artery and the left main coronary artery, respectively. The heart was then fixed by coronary perfusion and subsequent immersion in phosphate-buffered 3.7% formalin. The fixed hearts were cut into 8 transverse slices which were weighed and their apical surfaces photographed. The area at risk and area of infarction were identified and traced from an enlarged projection (magnification $\times 8$) of the color slide of each ventricular slice. The area at risk and area of necrosis were quantitated using a digitizing tablet interfaced to an IBM-compatible personal computer.

We have shown previously that TTC macrochemistry provides a valid measure of infarct size compared to microscopic evaluation ($r = 0.98$) when done following 3 or more hours of reperfusion [15,22]. However, to ensure that tissue not staining with TTC was indeed necrotic, tissue blocks representing the area at risk were cut and weighed and two microscopic slides were prepared from each tissue sample. In any case where tissue staining with TTC was ambiguous, microscopic slides were examined to distinguish necrotic from non-necrotic myocardium.

#### 2.4.2. Regional myocardial blood flow

Slices were divided into non-ischemic and central ischemic regions for blood flow analysis. The samples were further divided into sub-epicardial, mid-myocardial, and sub-endocardial thirds. Tissue and reference blood radioactivity was counted in a Packard model 5912 gamma counter. Counts were corrected for overlap of isotope spectra and myocardial blood flow was calculated as (tissue counts) $\times$ (reference flow)/(reference counts) and expressed as ml/min/g wet weight.

### 2.5. Statistical analysis

Data are expressed as the group mean $\pm$ s.e.m. The effects of treatment on hemodynamic parameters and pre-occlusion coronary blood flow at different times in the same animals were analyzed with Student's paired $t$-test. Analysis of variance (ANOVA) was applied to test for possible differences among groups in hemodynamic parameters, area at risk, and collateral blood flow. To test for differences in the relationship between infarct size and collateral blood flow, analysis of covariance (ANCOVA) was performed, using infarct size as the dependent variable and collateral blood flow as the independent covariate. Adjusted group means generated by the ANCOVA program (e.g., mean infarct size, adjusted for any intergroup variation in collateral blood flow) were compared using Student's $t$-test. In all analyses, a $P$-value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. Mortality and animal exclusions

The numbers of animals enrolled in each group and the exclusions are summarized in Table 1. Thirty-seven (37)

Table 1
Mortality and exclusions

| Group | Number entered | VF/Death | | Final number entered |
|---|---|---|---|---|
| | | In occl. | In reflow | |
| Control | 10 | 1/1 | 3/0 | 9 |
| Adenosine | 12 | – | 3/3 | 9 |
| ADO + LIDO | 15 | 8/7 | 2/0 | 8 |

VF = ventricular fibrillation; occl. = occlusion; ADO + LIDO = adenosine plus lidocaine.

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Table 2
Hemodynamic variables at baseline (before treatment) and after 45 min of coronary artery occlusion

| Parameter | Group | Baseline | Occlusion |
|---|---|---|---|
| HR | | | |
| | Control | 155 ±3 | 158 ±3 |
| | Adenosine | 151 ±3 | 154 ±4 |
| | ADO+LIDO | 174 ±7 *† | 169 ±6 |
| SBP | | | |
| | Control | 163 ±4 | 149 ±4 |
| | Adenosine | 178 ±9 | 158 ±7 |
| | ADO+LIDO | 194 ±8 * | 173 ±7 * |
| RPP/1000 | | | |
| | Control | 25.3±0.7 | 23.5±0.8 |
| | Adenosine | 26.9±1.5 | 24.4±1.3 |
| | ADO+LIDO | 34.2±2.4 * | 29.4±1.7 * |
| DBP | | | |
| | Control | 119 ±2 | 112±3 |
| | Adenosine | 124 ±4 | 112 ±4 |
| | ADO+LIDO | 143 ±5 *† | 133 ±5 *† |

Data represent all surviving dogs ($n = 26$); SBP = systolic arterial blood pressure (mmHg); DBP = diastolic arterial blood pressure (mmHg); HR = heart rate; RPP = rate pressure product (mmHg·min$^{-1}$). * $P < 0.05$ compared to control group at the same time. † $P < 0.05$ compared to value in the adenosine group at the same time.

dogs were entered into the study. Among control dogs ($n = 10$), one dog developed lethal ventricular fibrillation (VF) during occlusion. Three dogs developed VF during reflow and all 3 were successfully defibrillated. In the adenosine-treated group ($n = 12$) 3 dogs developed VF during reflow and none of these dogs could be defibrillated. In the adenosine plus lidocaine (A + L) group ($n = 15$), 8 dogs developed occlusion VF of which only one was successfully defibrillated. Two (2) dogs developed reflow VF and both were successfully defibrillated. Thus, 26 dogs were included in the final analysis.

### 3.2. Predictors of myocardial infarct size

#### 3.2.1. Effect of adenosine and/or lidocaine on hemodynamic parameters

Hemodynamic data obtained at baseline and midway during the 90-min occlusion are reported in Table 2. At baseline, the ADO + LIDO-treated dogs had slightly higher HR, SBP, and DBP compared to control dogs and higher HR and DBP compared to adenosine-treated dogs. During occlusion, the ADO + LIDO-treated dogs had higher SBP compared to control and higher DBP compared to adenosine-treated dogs. Corresponding rate pressure products were also slightly elevated in the ADO + LIDO-treated dogs (see table). Because of the unexpectedly larger number of dogs with occlusion VF in the ADO + LIDO group, we compared the hemodynamic status of the dogs which died versus those which lived. No significant baseline difference in heart rate, systolic or diastolic blood pressure, or rate pressure product were found.

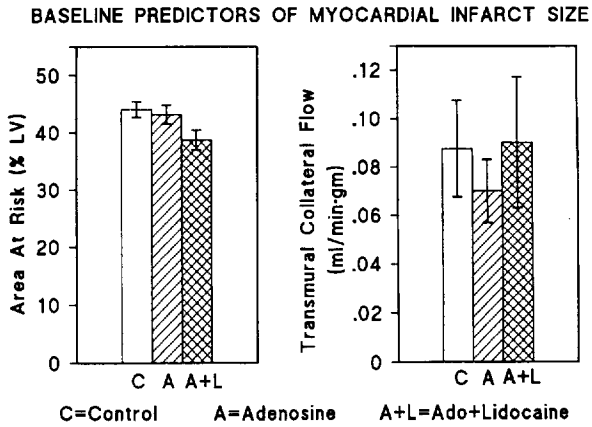


Fig. 2. The size of the ischemic area at risk and transmural collateral blood flow measured within this area at risk are shown. These two parameters, which are major baseline predictors of myocardial infarct size in dogs, did not differ among the 3 experimental groups.

#### 3.2.2. Area at risk and collateral blood flow

The size of the ischemic area at risk and mean transmural collateral blood flow within the ischemic region for each of the 3 groups are shown in Fig. 2. There was no significant difference among the 3 groups in either area at risk or collateral blood flow measured at 10 min of the test occlusion. Thus, the two major predictors of myocardial infarct size were comparable among groups.

#### 3.2.3. Myocardial temperature

Recent data indicate that myocardial temperature during coronary occlusion is an independent risk factor for infarct size in rabbit and dog myocardium. Pericardial temperature, measured at 3 times during coronary occlusion was stable over time within individual dogs and mean tempera-

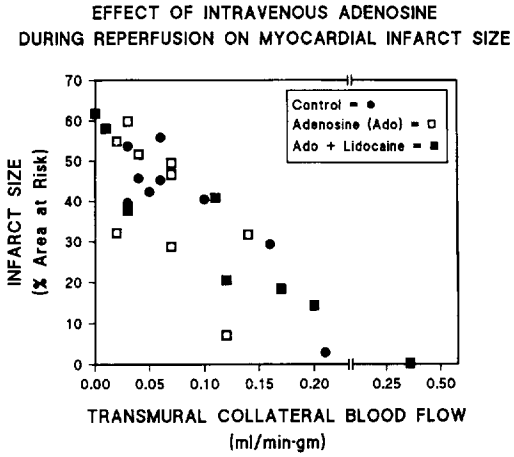


Fig. 3. Infarct size (percent of area at risk; AAR) versus collateral blood flow in control and treated groups. In control animals infarct size was inversely related to collateral blood flow. Neither adenosine alone nor adenosine with lidocaine significantly shifted the relationship downward at any given level of collateral blood flow, indicating that neither treatment had a significant protective effect on infarct size.

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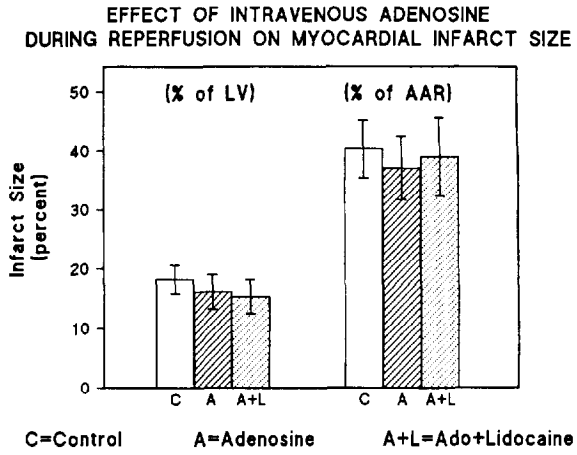


Fig. 4. Effect of adenosine and adenosine plus lidocaine on myocardial infarct size. Adjusted mean infarct size (IS) is expressed as percent of left ventricle and as percent of area at risk (AAR). Neither adenosine alone nor adenosine plus lidocaine significantly limited IS compared to control hearts.

ture was similar among the 3 groups, being 37.2, 37.1 and 37.6°C for control, adenosine, and adenosine plus lidocaine groups, respectively ($P$ = NS).

### 3.3. Myocardial infarct size

The regression of infarct size versus transmural collateral blood flow for individual dogs is shown in Fig. 3. In control animals there was an inverse relationship between infarct size and collateral blood flow. Neither adenosine nor adenosine plus lidocaine resulted in a significant downward shift in this relationship between infarct size and collateral blood flow ($P$ = NS based on ANCOVA).

Adjusted mean infarct size derived from the ANCOVA analysis is shown in Fig. 4. In control hearts the adjusted mean infarct size was 38.1 ± 5.3% of the area at risk (AAR). Neither adenosine nor adenosine plus lidocaine significantly limited infarct size compared to control (35.6 ± 5.6% adenosine; 38.1 ± 7.7% adenosine plus lidocaine; $P$ = NS vs. control).

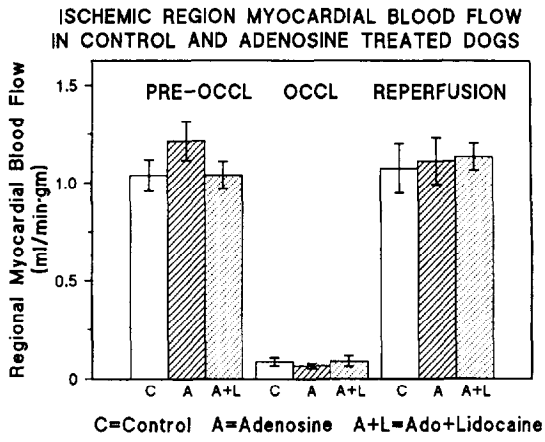


Fig. 5. Effect of adenosine on myocardial blood flow. Coronary blood flow (CBF) was measured with radioactive microspheres prior to ischemia, midway through the 90-min episode of ischemia, and 35 min after initiation of reperfusion (40 min after onset of drug treatment). Transmural myocardial blood flow did not differ among any group at any of these measurement times. Moreover, flow during reperfusion was not different from flow before occlusion in any group.

### 3.4. Effect of adenosine on myocardial blood flow during reperfusion

Comparison of regional transmural myocardial blood flow (ischemic circumflex bed) prior to ischemia, during ischemia, and during reperfusion is shown in Fig. 5. Neither adenosine nor adenosine plus lidocaine significantly affected transmural regional myocardial blood flow when compared to control hearts. Furthermore, adenosine, with or without lidocaine, did not affect transmural regional myocardial blood flow compared to pre-ischemic flows from the same vascular territory.

## 4. Discussion

The purpose of this study was to determine if adenosine reduced lethal reperfusion injury in an open chest model of canine model of regional ischemia and reperfusion. The results demonstrate that adenosine, administered to provide protection during reperfusion, did not limit infarct size in canine hearts following 90 min of regional ischemia and reperfusion.

### 4.1. Comparison with previous studies of adenosine in reperfusion injury

These results differ from those of several similar studies reported previously [9,17–20]. Therefore, the results of the present study must be considered in light of these previous positive studies.

#### 4.1.1. Differences in species, duration of coronary occlusion or duration of arterial reflow

All of the aforementioned studies were conducted in canine myocardium and therefore species differences cannot account for the observed differences. Two studies [9,20] utilized 60 min of coronary occlusion while the remaining three used 90 min [17–19]. The duration of reperfusion varied from 1 to 72 h, after which time infarct size was measured and expressed as a percentage of the area at risk. Thus, neither differences in duration of ischemia nor duration of reperfusion appear to explain the conflicting findings regarding adenosine's efficacy.

#### 4.1.2. Role of lidocaine in an adenosine-mediated effect

Four previous studies of the influence of adenosine treatment during reperfusion on myocardial infarct size included lidocaine in the experimental protocol [9,17–19]. In three of these studies, lidocaine was administered to all

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animals and the necessity of including lidocaine to achieve cardioprotection was not assessed. In our study and all but one of the cited studies, lidocaine was administered in a 2 mg/kg bolus immediately before coronary occlusion and this bolus was repeated just before reperfusion. In one previous study [9], lidocaine was administered before reperfusion but not before occlusion. In our study, the presence of lidocaine during coronary occlusion was associated with a high incidence of ventricular fibrillation (see below). Whether a similar effect occurred in the other studies is unknown because these data were not reported.

Homeister et al. [18] specifically investigated the effect of adenosine on myocardial infarct size, with or without concomitant lidocaine administration. Adenosine limited infarct size only when lidocaine also was administered. The investigators hypothesized that the protective effect of adenosine requires a synergistic interaction with lidocaine. Our present results do not support this hypothesis since adenosine did not limit infarct size when administered either with or without lidocaine. Our results are consistent with those of Goto et al. [21] who reported that lidocaine plus low-dose or high-dose adenosine did not limit infarct size in rabbit myocardium subjected to 30 min of in vivo myocardial ischemia and reperfusion. The explanation for the difference between the present data and the study by Homeister et al. [18] may lie in the individual collateral flow data for each dog. Inspection of their flow data reveals that the dogs in the adenosine plus lidocaine group often had relatively high collateral blood flow compared with that present in dogs of the other groups. Indeed, when we analyzed their infarct and flow data using ANCOVA, adenosine plus lidocaine did not show significant protection. We consider it possible that dogs with low collateral flow (and associated large infarcts) may have not survived the occlusion in the Homesiter et al. study, thereby contributing to a false-positive protective effect.

#### *4.1.3. Influence of dose and route of administration of adenosine*

The studies investigating the effect of adenosine on infarct size in canine myocardium have used varying doses and routes of administration of adenosine which might explain some of the differences among various studies. However, the present study was designed to deliver adenosine at the same dose and in the same manner as that described in the first study in which markedly positive results were obtained using intravenous adenosine therapy [19]. Therefore, no difference in drug dosage or timing of administration can explain the differences in efficacy between the latter study and ours. In the other negative study, by Goto et al. [21], two different doses of intravenous adenosine (one of which caused significant hemodynamic effects) was tested and neither limited infarct size.

The dogs in this study had somewhat higher heart rates and lower collateral blood flows compared to those in the study of Pitarys et al. [19]. It is hypothetically possible that because of this difference we did not achieve an equivalent concentration of adenosine in the ischemic bed prior to the onset of reperfusion. However, evaluation of the individual dogs in our study shows no evidence for infarct limitation even in the dogs with higher levels of collateral blood flow (Fig. 4). Thus, it is unlikely that small differences in collateral blood flow between studies explain the lack of cardioprotection seen in the present study.

#### *4.1.4. Baseline predictors of infarct size*

Other possible variables among studies are the methods used to analyze infarct size or baseline predictors of infarct size. It is well known that area at risk and the degree of innate collateral blood flow present during coronary occlusion are the two most important determinants of infarct size in canine myocardium. To control for these factors in our studies, infarct size is measured as a fraction of the area at risk and is assessed with respect to collateral blood flow using ANCOVA, in which infarct size is the dependent variable and collateral blood flow is the independent covariate. Other studies have not included such analysis [9,18]. Failure to control for variation in baseline predictors of infarct size among animals may lead to false-positive conclusions.

In addition, recent data have shown that myocardial temperature is an independent determinant of myocardial infarct size in both rabbits and dogs [23,24]. Temperature was carefully controlled throughout our studies to prevent temperature-induced variation in infarct size and pericardial temperature was measured. We found that temperature was stable over time within individual dogs and that mean temperature did not differ among groups (data not shown). No previous studies have assessed this important variable.

In the present study, there was a significant difference in rate pressure product (RPP) between control and the ADO + LIDO groups. However, analysis of the dogs in the ADO + LIDO group showed no significant correlation between RPP and infarct size and the dogs in this group with the smallest RPP's showed no evidence of cardioprotection. Thus, it is unlikely that the overall higher RPP in the ADO + LIDO masked a cardioprotective effect.

### *4.2. Role of lidocaine in morbidity and mortality*

In the present study, we included a lidocaine plus adenosine group to determine if the presence of lidocaine was necessary to observe a cardioprotective effect of adenosine in this model system. The dosing regimen used in this study was the same as that used by Homeister et al. [18] who showed that rapid bolus infusion of lidocaine resulted in substantial plasma lidocaine levels measured 15 min after occlusion and 45 min of reperfusion. Although the inclusion of lidocaine with adenosine did not alter infarct size, the incidence of ventricular fibrillation refractory to cardioversion was unexpectedly increased. Seven of the 15 dogs in the lidocaine-plus-adenosine group suffered a lethal episode of ventricular fibrillation within 2–4 min

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of coronary occlusion. Of the remaining dogs (control and control plus adenosine-alone groups; total $n = 22$), only two suffered lethal occlusion VF, and in both of those dogs the onset was at approximately 15–20 min after occlusion. The previous studies using adenosine plus lidocaine [18,19] did not report any increased incidence of dysrhythmias or ventricular fibrillation.

The reason for the increased incidence of occlusion VF observed in this study is not clear. The slightly higher mean heart rate and systolic blood pressure in the adenosine plus lidocaine group hypothetically may have contributed to the increased incidence of VF. However, we have observed no relationship between baseline hemodynamic parameters and ventricular fibrillation in this or any other experimental group. As noted above, there was no significant difference in heart rate, systolic or diastolic blood pressure, or rate–pressure product between the ADO + LIDO subgroup which lived versus the subgroup which developed VF and died.

Even though lidocaine usually acts as an anti-arrhythmic drug, there have been several reports in the literature of pro-arrhythmic effects. For example, Anderson et al. reported that sustained polymorphic ventricular tachycardia (VT) occurred in 6 of 16 lidocaine-treated dogs subjected to rapid pacing, but no dogs suffered VT with either lidocaine alone or rapid pacing alone [25]. Patterson et al. showed, in conscious and anesthesized dogs 4–7 days post-myocardial-infarction, that bolus intravenous administration of methyl lidocaine may actually promote the formation of new re-entry pathways [26]. In addition, the same investigators have suggested that lidocaine may play a proarrhythmic role in animal models of acute as well as long-term ischemia [27–29].

### *4.3. Summary and conclusions*

The present study showed that intravenous adenosine treatment during reperfusion (150 $\mu$g/kg/min) did not significantly limit infarct size following 90 min of regional ischemia in canine myocardium. Furthermore, administration of adenosine in lidocaine-treated dogs also did not limit infarct size. Finally, the addition of lidocaine resulted in much higher mortality than control and adenosine-alone treated groups.

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## References

[1] Zweier JL, Flaherty JT, Weisfeldt ML. Direct measurement of free radical generation following reperfusion of ischemic myocardium. Proc Natl Acad Sci USA 1987; 84:1404–1407.

[2] Zweier JL, Rayburn BK, Flaherty JT, Weisfeldt ML. Recombinant superoxide dismutase reduces oxygen free radical concentrations in reperfused myocardium. J Clin Invest 1987;80:1728–1734.

[3] Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB. Demonstration of free radical generation in 'stunned' myocardium of intact dogs with the use of the spin trap alpha-phenyl *N-tert-butyl* nitrone. J Clin Invest 1988;82:476–485.

[4] Lucchesi BR, Mullane KM. Leukocytes and ischemia-induced injury. Ann Rev Pharmacol Toxicol 1986;26:201–224.

[5] Simpson PJ, Todd RF III, Mickelson JK, et al. Sustained limitation of myocardial reperfusion injury by a monoclonal antibody that alters leukocyte function. Circulation 1990;81:226–237.

[6] Simpson PJ, Mickelson JK, Fantone JC, Gallagher KP, Lucchesi BR. Iloprost inhibits neutrophil function in vitro and in vivo and limits experimental infarct size in canine heart. Circ Res 1987;69:666–673.

[7] Simpson PJ, Todd RF III, Fantone JC, Mickelson JK, Griffin JD, Lucchesi BR. Reduction of experimental canine myocardial reperfusion injury by a monoclonal antibody (anti Mol, anti CD11b) that inhibits leukocyte adhesion. J Clin Invest 1988;81:624–629.

[8] Simpson PJ, Mickelson JK, Fantone JC, Gallagher KP, Lucchesi BR. Reduction of experimental canine myocardial infarct size with prostaglandin E1. Inhibition of neutrophil migration and activation. J Pharmacol Exp Ther 1988;244:619–624.

[9] Schlack W, Schäfer M, Uebing A, Schäfer S, Borchard U, Thämer V. Adenosine $A_2$-receptor activation at reperfusion reduces infarct size and improves myocardial wall function in dog heart. J Cardiovasc Pharmacol 1993;22:89–96.

[10] Reimer KA, Jennings RB. Failure of the xanthine oxidase inhibitor allopurinol to limit infarct size after ischemia and reperfusion in dogs. Circulation 1985;71:1069–1075.

[11] Uraizee A, Reimer KA, Murry CE, Jennings RB. Failure of superoxide dismutase to limit size of myocardial infarction after 40 minutes of ischemia and 4 days of reperfusion in dogs. Circulation 1987;75:1237–1248.

[12] Nejima J, Knight DR, Fallon JT, et al. Superoxide dismutase reduces reperfusion arrhythmias but fails to salvage regional function of myocardium at risk in conscious dogs. Circulation 1989;79:143–153.

[13] Patel BS, Jeroudi MO, O'Neill PG, Roberts R, Bolli R. Human superoxide dismutase fails to limit infarct size after 2-h ischemia and reperfusion. Circulation 1988;78:II-373 (Abstract).

[14] Go LO, Murry CE, Richard VJ, Jennings RB, Reimer KA. Neutrophil accumulation during early myocardial reperfusion: 40 vs. 90 minutes of ischemia. Circulation 1987;76(4):202 (Abstract).

[15] Tanaka M, Brooks SE, Richard VJ, et al. Effect of anti-CD18 antibody on myocardial neutrophil accumulation and infarct size after 90 minutes of ischemia and 3 hours of reperfusion in dogs. Circulation 1993;87:526–535.

[16] Ganz W, Watanabe I, Kanamasa K, Yano J, Han D-S, Fishbein MC. Does reperfusion extend necrosis? A study in a single territory of myocardial ischemia—half reperfused and half not reperfused. Circulation 1990;82:1020–1033

[17] Olafsson B, Forman MB, Puett DW, et al. Reduction of reperfusion injury in the canine preparation by intracoronary adenosine: importance of the endothelium and no-reflow phenomenon. Circulation 1987;76:1135–1145.

[18] Homeister JW, Hoff PT, Fletcher DD, Lucchesi BR. Combined adenosine and lidocaine administration limits myocardial reperfusion injury. Circulation 1990;82:595–608.

[19] Pitarys CJ, Virmani R, Vildibill HD Jr, Jackson EK, Forman MB. Reduction of myocardial reperfusion injury by intravenous adenosine administered during the early reperfusion period. Circulation 1991;83:237–247.

[20] Nichols WW, Nicolini FA, Yang BC, Henson K, Stechmiller JK, Mehta JL. Adenosine protects against attenuation of flow reserve and myocardial function after coronary occlusion and reperfusion. Am Heart J 1994;127:1201–1211.

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[21] Goto M, Miura T, Iliodoromitis K, et al. Adenosine infusion during early reperfusion failed to limit myocardial infarct size in a collateral deficient species. Cardiovasc Res 1991;25:943–949.

[22] Tanaka M, Richard VJ, Murry CE, Jennings RB, Reimer KA. Superoxide dismutase plus catalase therapy delays neither cell death nor the loss of the TTC reaction in experimental myocardial infarction in dogs. J Mol Cell Cardiol 1993;25:367–378.

[23] Chien GL, Wolff RA, Davis RF, Van Winkle DM: Normothermic range temperature affects myocardial infarct size. Cardiovasc Res 1994;28:1014–1017.

[24] Verbinski SG, Schwartz LM, Vander Heide RS, Reimer KA. Minor variation in pericardial temperature is a major predictor of infarct size in dogs. Circulation 1995;92:1–457 (Abstract).

[25] Anderson K, Walker R, Lux R, et al. Conduction velocity depression and drug-induced ventricular tachyarrhythmias. Effect of lidocaine in the intact canine heart. Circulation 1990;81:1024–1038.

[26] Patterson E, Gibson J, Lucchesi B. Electrophysiologic actions of lidocaine in a canine model of chronic myocardial ischemic damage — arrhythmogenic actions of lidocaine. J Cardiovasc Pharmacol 1982;4:925–934.

[27] Patterson E, Gibson J, Lucchesi B. Antiarrhythmic and arrhythmogenic actions of methyl lidocaine during the recovery phase after canine myocardial infarction. Pharmacology 1988;36:73–83.

[28] Carson D, Cardinal R, Savard P, et al. Relationship between arrhythmogenic action of lidocaine and its effects on excitation patterns in acutely ischemic porcine myocardium. J Cardiovasc Pharmacol 1986;8:126–136.

[29] Bergey J, Nocella K, McCallum J. Acute coronary artery occlusion-reperfusion-induced arrhythmias in rats, dogs and pigs: antiarrhythmic evaluation of quinidine, procainamide and lidocaine. Eur J Pharmacol 1982;81:205–216.

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