ORIGINAL RESEARCH

Intracoronary Saline-Induced Hyperemia During Coronary Thermodilution Measurements of Absolute Coronary Blood Flow: An Animal Mechanistic Study

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BACKGROUND: Absolute hyperemic coronary blood flow and microvascular resistances can be measured by continuous thermodilution with a dedicated infusion catheter. We aimed to determine the mechanisms of this hyperemic response in animal.

METHODS AND RESULTS: Twenty open chest pigs were instrumented with flow probes on coronary arteries. The following possible mechanisms of saline-induced hyperemia were explored compared with maximal hyperemia achieve with adenosine by testing: (1) various infusion rates; (2) various infusion content and temperature; (3) NO production inhibition with L-arginine methyl ester and endothelial denudation; (4) effects of vibrations generated by rotational atherectomy and of infusion through one end-hole versus side-holes. Saline infusion rates of 5, 10 and 15 mL/min did not reach maximal hyperemia as compared with adenosine. Percentage of coronary blood flow expressed in percent of the coronary blood flow after adenosine were 48±17% at baseline, 57±18% at 5 mL/min, 65±17% at 10 mL/min, 82±26% at 15 mL/min and 107±18% at 20 mL/min. Maximal hyperemia was observed during infusion of both saline at body temperature and glucose 5%, after endothelial denudation, L-arginine methyl ester administration, and after stent implantation. The activation of a Rota burr in the first millimeters of the epicardial artery also induced maximal hyperemia. Maximal hyperemia was achieved by infusion through lateral side-holes but not through an end-hole catheter.

CONCLUSIONS: Infusion of saline at 20 mL/min through a catheter with side holes in the first millimeters of the epicardial artery induces maximal hyperemia. The data indicate that this vasodilation is related neither to the composition/temperature of the indicator nor is it endothelial mediated. It is suggested that it could be elicited by epicardial wall vibrations.

Key Words: absolute coronary flow ■ adenosine ■ coronary hyperemia ■ coronary thermodilution ■ endothelial shear stress ■ rotational atherectomy

Absolute coronary blood flow can be measured (in mL/min) with a dedicated infusion catheter (RayFlow, HexaCath, Paris, France) using continuous thermodilution technique.[1] This method also allows to quantify the resistance of the microvascular circulation (in Woods Units). In vitro testing demonstrated the accuracy of this continuous thermodilution technique.[2] These measurements are accurate with a good reproducibility in humans.[3] During the first measurements in humans we observed that intracoronary infusion of saline at room temperature at rates of 15 to 20 mL/min through the lateral side-holes of

CLINICAL PERSPECTIVE

What Is New?

- Maximal hyperemic response induced by the infusion through the Rayflow catheter might be related to vibrations of the epicardial wall.

What Are the Clinical Implications?

- In clinical practice absolute coronary blood flow measurements with infusion at 15 to 20 mL/min will induced maximal hyperemia independently from the endothelial function, presence of stent, content or temperature of the infusion.

Nonstandard Abbreviations and Acronyms

CBF   coronary blood flow
LAD   left anterior descending
LOA   limit of agreement

the RayFlow catheter induced maximal hyperemia.[4] In addition, we have shown that this maximal hyperemia induced by saline infusion was selective, involved only the target vessel, that a prolonged infusion of saline at 20 mL/min was not associated with signs of myocardial ischemia, that the infusion of saline at lower rates (5 and 10 mL/min) did not induce hyperemia, and that saline infused at 20 mL/min through an end hole instead of side holes did not induce maximal hyperemia.[4] Thus we hypothesized that the vibration induced by the lateral jets arising from the side holes of the catheter may play a role in the induction of hyperemia. However, the mechanisms of the infusion-induced hyperemia require further investigation.

Accordingly, the goal of the present study was to investigate the mechanisms of the hyperemic response induced by saline infusion during coronary thermodilution in a porcine model. More specifically, we focused on the temperature and composition of the infusion, on the potential role of the epicardial endothelium, and on the potential role of epicardial vibrations.

MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Animal Preparation

Twenty female pigs (Landrace Large White crossed, Lebeau, Gambais, France) were sedated (tiletamine 4 mg/kg and zolazepam 4 mg/kg) and anesthetized with intravenous propofol (3 mg/kg iv bolus followed by infusion at 10 mg/kg per hour) and remifentanil (18 µg/kg per hour). Mechanical ventilation with a mixture of air and oxygen (1:1) at a tidal volume of 8 to 10 mL/kg was adjusted to maintain normocapnia and normoxia. Left thoracotomy was performed to expose the heart and the pericardium was opened. Two coronary blood flow probes (Transonic Systems Inc, Ithaca, NY) were placed around the left anterior descending (LAD) and the left circumflex coronary arteries, respectively. A snare was placed around the proximal LAD to perform coronary artery occlusion. The right carotid artery was surgically exposed to introduce a 6 Fr sheath (Radifocus introducer II, Terumo, Tokyo, Japan). Anticoagulation (Heparin, 10 000 IU) and antiplatelet therapy (acetylsalicylic acid, 250 mg) were administered intravenously at the beginning of the procedure along with 0.1 mg of intracoronary nitrates. Heart rate, a 6-derivations ECG, the LAD, and LCX coronary blood flows (CBF) were continuously recorded using HEM software (Notocord, v3.4, Le Pecq, France). An example of these recordings is shown in Figure 1.

Absolute Coronary Blood Flow Measurements

Through right carotid access, a 6-F guide catheter (EBU 3.0, Medtronic, Minnesota, USA) was positioned in the left main coronary artery. A 0.014-inches diameter pressure/temperature sensor wire (PressureWire X, Abbott, Illinois, USA) was advanced in the distal segment of the artery under study. Over this wire a dedicated coronary infusion catheter (RayFlow HexaCath, Paris, France) was advanced to position the lateral infusion holes in the first 2 centimeters of the coronary artery. A second, 0.014-inchcoronary wire (Runthrough, Terumo, Tokyo, Japan) was placed in the contralateral vessel to stabilize the guiding outside of the left coronary artery. The epicardial quantitative Doppler flow probes on the LAD and the left circumflex were connected to a transonic flowmeter.

Study Protocol

The effect of the various experimental conditions on CBF was defined as the percent increase in CBF above its baseline value. The dose of intracoronary adenosine inducing maximal hyperemia was chosen for each animal and each artery based on dose response curves (25, 50, 100, 150, and 200 µg) and compared with post-occlusional (45 seconds) hyperemia.[5] We demonstrated that an intracoronary bolus of 200 µg of adenosine uniformly induced a similar hyperemic response than post-occlusional hyperemia.[6] This allowed us to use administration of 200 µg of
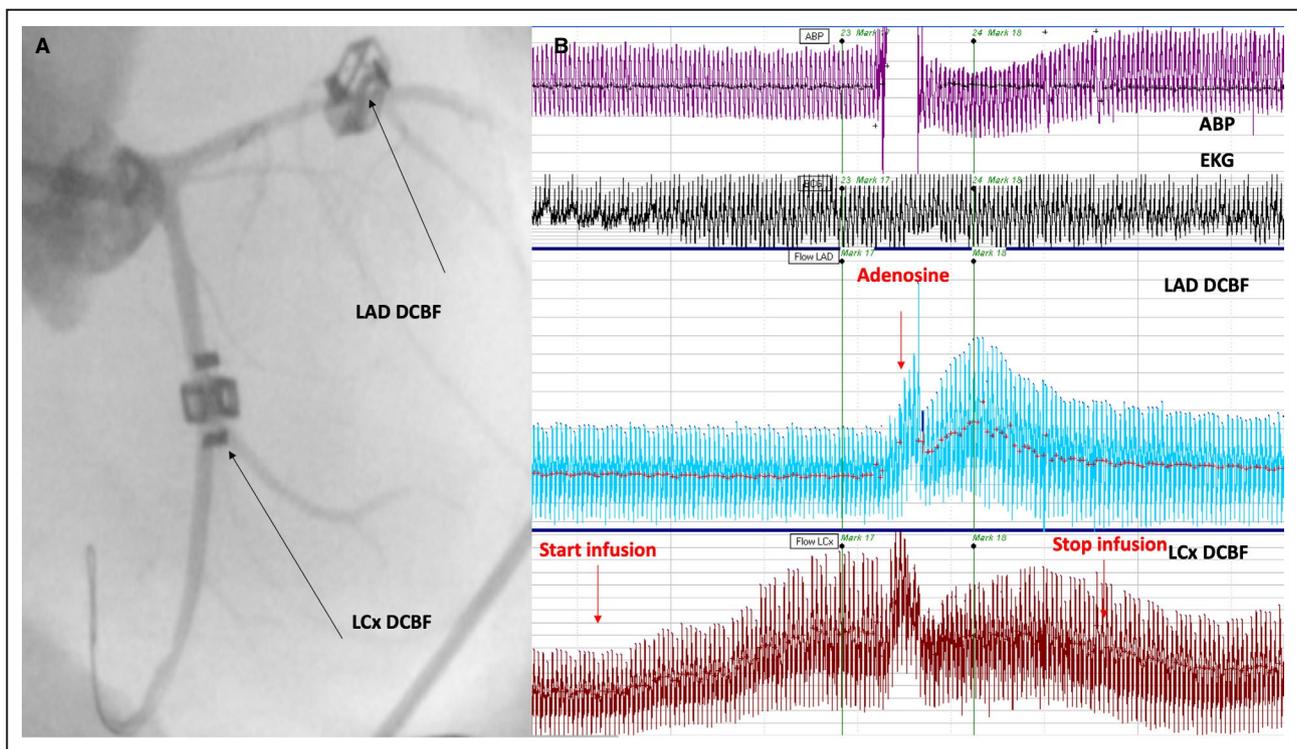
Figure 1. Protocol illustration.
A, Coronary angiogram showing Doppler coronary blood flow rings on the left anterior descending and the left circumflex with wires maintaining the guiding outside of the left coronary artery. **B**, Hemodynamic tracings of ABP in purple, ECG in black, left anterior descending flow measured with Doppler coronary blood flow in blue and left circumflex flow measured with Doppler coronary blood flow in brown. The saline infusion started at 15 mL/min, start coronary blood flows red mark, thereafter increasing blood flow up to a steady state in the left circumflex while left anterior descending flow remained unchanged. Adenosine administration through the guiding catheter was performed at the adenosine red mark which induced maximal hyperemia in the left anterior descending and did not change the hyperemic steady state during coronary blood flows measurement, ABP and ECG remained stable. ABP indicates Aortic blood pressure; DCBF, Doppler coronary blood flow; LAD, left anterior artery; LCD, left coronary artery; and LCx, left circumflex.

intracoronary adenosine as the maximal hyperemia indicator, instead of a transient coronary occlusion to induce repeated maximal hyperemia. Various experimental conditions were created to explore possible mechanisms of the coronary hyperemic response observed during infusion of saline through the RayFlow catheter:

1. Effect of different infusion rates on CBF: 5, 10, 15, and 20 mL/min compared with adenosine in 10 pigs. For these infusions, at room temperature saline was used.
2. Effect of different temperatures of saline and of the content of the infusion. Namely, saline at room temperature, saline heated at 37°C, and glucose 5% solution given at a rate of 20 mL/min through the RayFlow catheter compared with adenosine in 7 pigs.
3. Effect of the endothelium status on the hyperemic effect of saline infusion at a rate of 20 mL/min through the RayFlow catheter. This was achieved after inhibition of the NO pathway by intravenous administration of L-arginine methyl ester 100 mg over 10 minutes (n=8), after endothelial injury by repeated semi-compliant coronary angioplasty balloon inflations at the site of infusion (n=9),and by the placement of drug eluting stents in 3 pigs (Xience, Abbott, Illinois, USA) and covered stents in 2 pigs (Graftmaster, Abbott, Illinois, USA) at the site of the infusion. The effect on CBF of each of these maneuvers was compared with the one observed with adenosine.

4. Effect of vibrations on the arterial wall was tested by using a 1,25 mm rotational atherectomy burr (Rotablator, Boston Scientific, Marlborough, Massachusetts, United States) introduced in the proximal part of the artery over a dedicated 0.009-inch diameter wire and activated at 200 000 rounds per minute or at 80 000 rounds per minute. The effect on CBF of this maneuver was compared with CBF achieved with adenosine in 5 pigs. In addition, the infusion of saline at room temperature through using a catheter without side holes compared with adenosine in 4 pigs.

The same sequence was followed for each series of measurements: first, baseline recording, second,

Table. **Coronary Flow in the Study Vessel and Control Vessel in mL/min and Percentage of the Ratio of the Coronary Flow With Flow With Adenosine**

	No. of Pigs	Study Vessel (mL/min)	Study Vessel Flow/Flow Adenosine (%)	Control Vessel (mL/min)	Study Vessel Flow/Flow Adenosine (%)	Mean Aortic Pressure (mm Hg)	Heart Rate (beats per minute)
Baseline	10	20±11	48±17	20±7	37±22	66±7	75±12
Saline 5 mL/min	10	24±10	57±18	20±7	40±11	69±8	75±12
Saline 10 mL/min	10	31±12	65±17	19±7	39±12	71±10	77±12
Saline 15 mL/min	10	41±18	82±26	18±6	38±14	67±11	73±8
Saline 20 mL/min	8	50±28	107±18	20±7	41±19	61±15	72±11
Baseline	7	25±11	55±14	20±11	40±28	63±3	67±15
Saline 20 mL/min at 37°C	7	47±27	99±21	20±9	31±11	64±5	60±3
Glucose 5% 20 mL/min	7	57±21	106±18	20±10	34±12	69±16	58±9
Baseline	9	21±15	41±14	21±7	28±13	65±4	70±20
Saline 20 mL/min after endothelial denudation	9	46±19	113±24	21±8	39±12	68±8	65±14
Saline 20 mL/min after LNAME	9	55±40	98±32	15±7	21±10	67±14	77±30
Saline 20 mL/min within stent	5	41±15	119±17	26±8	52±18	66±15	67±14
Baseline	5	18±3	54±17	23±10	54±16	55±11	66±15
Rotational atherectomy low speed	5	20±6	76±10	22±10	59±15	56±14	60±26
Rotational atherectomy high speed	5	31±7	92±22	25±11	59±30	53±4	60±21
Baseline	4	13±10	57±32	20±3	53±28	57±13	55±11
End-hole saline 20 mL/min	4	19±13	54±27	20±3	48±28	56±2	56±14
Saline 20 mL/min	4	36±7	103±29	17±5	51±16	51±25	53±4

LNAME indicates L-arginine methyl ester.

infusion/intervention period, and third, return to baseline after stopping the infusion/intervention. A pig could have several experimentations if the hemodynamics completely returned to baseline values without any coronary damage assessed with the coronary angiogram.

All experiments were approved by the local animal ethical committee (ComEth AFSSA-ENVA-UPEC agreement # E-94-046-2).

Statistical Analysis

Values with normal distribution are expressed as mean±SD, and non-normally distributed variable as median value (interquartile range). Categorical variables are shown as percentages. Hyperemia is defined as the coronary blood flow during the interventions as a percent of coronary blood flow observed with adenosine. The agreement between the various experimental conditions and intracoronary adenosine on the increase in CBF achieved was assessed by the Bland-Altman method. Mean difference between the experimental condition and intracoronary adenosine is presented as percentage above the baseline

and 95% limit of agreement (LOA). Systematic and proportional differences between the abovementioned experimental condition with respect to the administration of intracoronary adenosine were assessed using Passing-Bablok regression.[7] For the Passing-Bablok regression analysis, the α and β coefficients assess for systematic and proportional differences. If 0 is in the CI of α, and 1 is in the CI of β, the 2 methods are comparable. If 0 is not in the CI of α there is a systematic difference and if 1 is not in the CI of β then there is a proportional difference between the 2 methods. The procedure is symmetrical and is robust in the presence of one or few outliers. All statistical analyses were performed using R Studio version (R Foundation) and GraphPad Prism version 7.0.

RESULTS

Effect of Different Rates of Saline Infusion

The effects on CBF of incremental rates 5, 10, 15, and 20 mL/min of saline infusion at room temperature through the RayFlow catheter were measured in

10 pigs. Mean pigs weight were 29.8±3.5 kg (range 25–34 kg). In 2 pigs, saline infusion at 20 mL/min could not be performed due to coronary spasm during the measurements. The results are shown in Table, in Figure 2 and Figure S1. Mean arterial blood pressure and heart rates remained unchanged throughout all the experimental conditions. Saline infusion rates of 5, 10, and 15 mL/min did not reach maximal hyperemia as compared with adenosine. CBF expressed as a percent of the CBF with adenosine were 48±17% t baseline, 57±18% at 5 mL/min, 65±17% at 10 mL/min, 82±26% at 15 mL/min and 107±18% at 20 mL/min. The observed mean difference in CBF between saline infusion rates of 5, 10, 15 and 20 mL/min with respect to adenosine were –125% (LOA [–241; –9]), –103% (LOA [–206; 1]), –60% (LOA [–176; 57]), and –3% (LOA [–126; 121]), respectively, ie, the increase in CBF with saline infusion at 20 mL/min was similar to the one observed with adenosine. No significant difference in flow was observed according to pigs weight.

Effect of Temperature and Composition of the Infusion

The effect of saline heated at body temperature (36.7±1.7°C) infused at a rate of 20 mL/min with the RayFlow catheter was performed and compared with adenosine. The observed mean difference in CBF between heated saline and adenosine was –17% (LOA [–105; 70]). The effect of glucose 5% solution at room temperature infused at a rate of 20 mL/min with the RayFlow catheter was performed and compared with adenosine. The observed mean difference in CBF between glucose 5% solution and adenosine was 9% (LOA [–68; 87]). The results are shown in Table and Figure 3.

Role of the Endothelium

After endothelial denudation, the hyperemic effect of the infusion of saline at a rate of 20 mL/min with the RayFlow catheter compared with adenosine had an observed mean difference of 41% (LOA [–82; 165]). After infusion of L-arginine methyl ester the observed mean difference was –40% (LOA [–372; 292]) and after stent implantation the observed mean difference was 6% (LOA [–57; 69]) compared with the increase in CBF than that observed after adenosine. The results are shown in Table and Figure 4.

Effect of Vibrations on the Arterial Wall

To induce vibrations into the coronary arterial wall, a 1.25 mm diameter burr of rotational atherectomy was advanced in the proximal part of the target vessel. Low rotational speed (79 400±3400 rounds per minute) was not associated with a significant
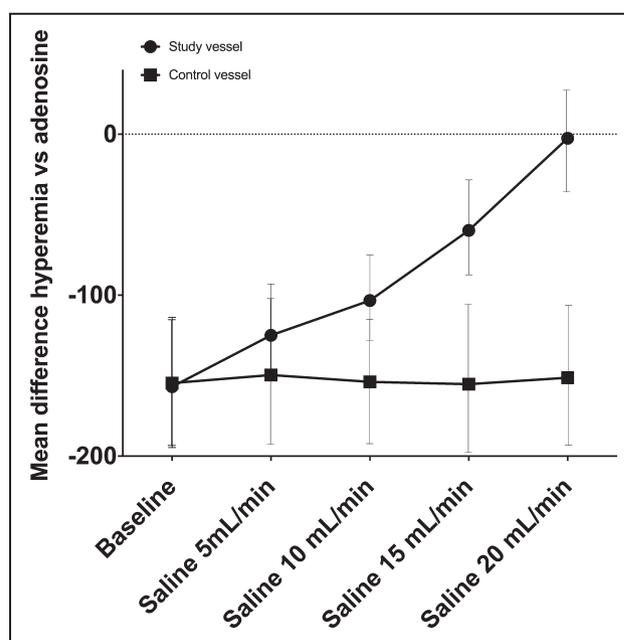
Figure 2. Hyperemic effect of saline infusion rates of 5, 10, 15, and 20 mL/min through the RayFlow catheter expressed in mean difference of percentage of change in absolute blood flow above the baseline as compared with hyperemia achieve with adenosine in the study vessel and in the control vessel.

increase in CBF as compared with baseline but inferior to maximal hyperemia. High rotational speed (193 000±9300 rounds per minute) was associated with an increase in CBF which was similar to that observed with adenosine (–9% (LOA [–67; 49]). This hyperemic response was not observed in the contralateral artery Figure 5.

Effect of Side Holes on the Arterial Wall

To test the effect of the lateral turbulent jets of saline against the vascular wall, a modified catheter without side holes but with only one end-hole was used in 4 pigs. The infusion of saline at room temperature at a rate of 20 mL/min did not induce any significant changes in CBF with an observed mean difference compared with adenosine of –96% (LOA [–280; 89]) while the observed mean difference was –10% (LOA [–135; 155]) with the Rayflow catheter compared with adenosine (Figure 5).
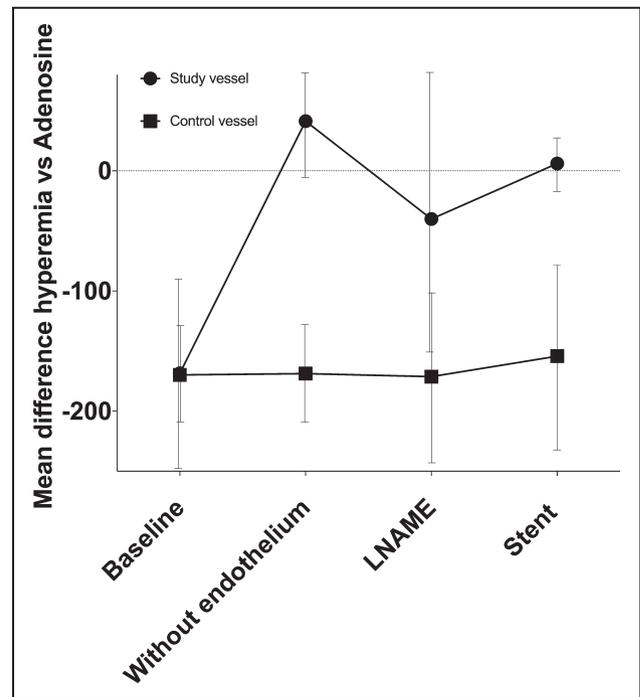
DISCUSSION

Recently, a new method to quantify absolute coronary blood flow and microvascular resistances measurements by continuous coronary thermodilution was described.[1–4] When saline at room temperature is infused at 20 mL/min the proximal part of the vessel, the extent to which the blood temperature decreases in the distal
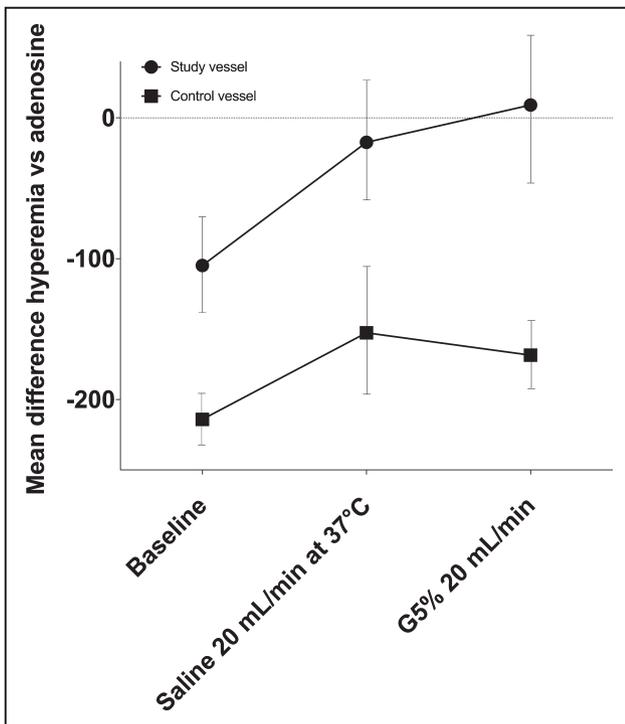
Figure 3. Hyperemic effect of saline infusion at 37°C and glucose 5% solution at room temperature through the RayFlow catheter expressed in mean difference of percentage of change in absolute blood flow above the baseline compared with hyperemia achieve with adenosine in the study vessel and control vessel.

Figure 4. Hyperemic effect of saline infusion at a rate of 20 mL/min through the RayFlow under different conditions expressed in mean difference of percentage of change in absolute blood flow above the baseline compared with hyperemia achieve with adenosine in the study vessel and control vessel.
Without endothelium, after L-arginine methyl ester administration, within implanted coronary stent. LNAME indicates L-arginine methyl ester.

part of the artery can be used to calculate instantaneous absolute coronary blood flow. In addition, we have shown that continuous saline infusion at a rate of 20 mL/min through a dedicated catheter with side holes positioned in the proximal part of the epicardial artery induced maximal microvascular dilation. Recent data have validated the accuracy of this method in humans by comparison with PET-derived assessment of myocardial perfusion.[8] It is not yet clear whether resting flow can be measured by continuous thermodilution. The reasons of this hyperemic response are unknown. The present animal study aimed at exploring some possible mechanisms of this crosstalk between epicardial and microvascular coronary compartments.

The main findings of the present study can be summarized as following: (1) intracoronary infusion of saline at room temperature at 20 mL/min through a dedicated catheter induces within 10 to 15 seconds a hyperemic state similar to the one observed with adenosine; This hyperemic response is not observed with 5 and 10 mL/min, and observed inconsistently with 15 mL/min. These findings actually confirm what was found in humans. (2) this hyperemic response depends neither on the temperature or on the composition of the infusion, nor on the presence and function of the epicardial endothelium; (3) vibrations of the epicardial wall seem to

trigger microvascular dilation specifically in the territory depending on the stimulated epicardial artery.

Several mechanisms can be considered to explain the fact that the infusion of saline in the conductance arteries is associated with a vasodilation of the resistance arteries located downstream, the segment where the infusion takes place.

First, myocardial ischemia related to partial replacement of blood by saline could play a role in the observed vasodilation. Coronary occlusion and its associated "post-occlusion hyperemia" is generally considered the most potent vasodilator.[9,10] However, in the present experiment, the amount of saline that induces hyperemia (20 mL/min) is small as compared with normal resting coronary flow (≈60 mL/min in the left anterior descending coronary artery), and even more so when compared with hyperemic flow (>300 mL/min). In addition, in humans no signs of ischemia have been detected during a 2-minutes infusion of 20 mL/min in the LAD, while the hyperemic response takes place within 10 to 15 seconds after the start of the infusion.

Second, given the different effects on endothelial functions of warm and cold cardioplegia,[11,12] different temperatures of the saline infusion were tested. No

Figure 5. Effect of rotational atherectomy on coronary blood flow.
A, Effect of rotational atherectomy expressed in mean difference of percentage of change in absolute blood flow above the baseline compared with hyperemia achie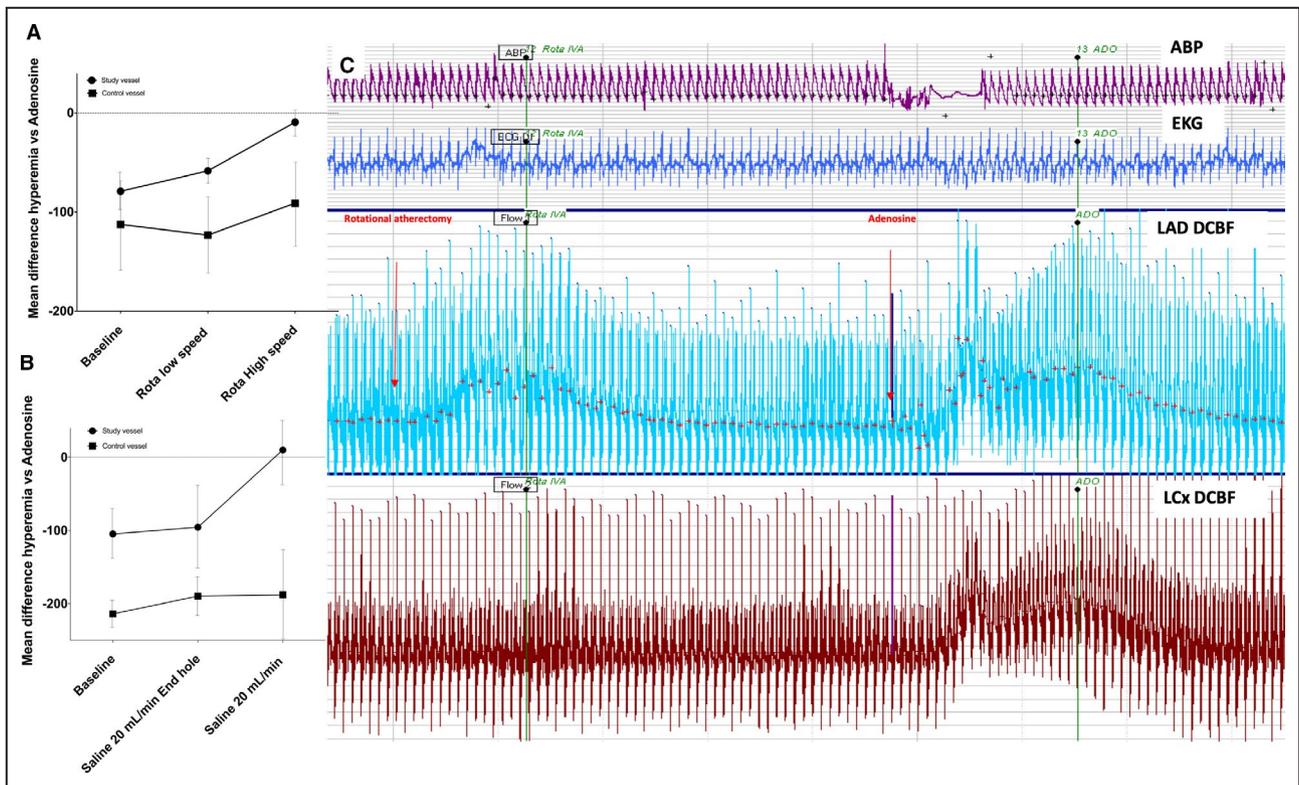ve with adenosine in the study vessel and control vessel. Rota low speed (rotational atherectomy at 80 000 rounds per minute) and Rota high speed (rotational atherectomy at 190 000 rounds per minute). **B**, Hyperemic effect of saline infusion at a rate of 20 mL/min through a modified end hole RayFlow (without side holes) and through a RayFlow catheter expressed in mean difference of percentage of change in absolute blood flow above the baseline compared with hyperemia achieve with adenosine in the study vessel and control vessel. **C**, Hemodynamic tracings of coronary blood flows in purple, ECG in black, left anterior descending flow measured with coronary blood flow in blue and left circumflex flow measured with Doppler coronary blood flow in brown. The saline infusion started at 20 mL/min, start rotational atherectomy red mark, thereafter increasing blood flow up to a steady state in the left anterior descending while left circumflex flow remain unchanged. Adenosine administration through the guiding catheter was performed at the adenosine red mark which induced maximal hyperemia in the left anterior descending at the same level that the one induced with rotational atherectomy and maximal hyperemia in the left circumflex, ABP and EKG remained stable. DCBF indicates Doppler coronary blood flow; G5%, glucose 5% solution; LAD, left anterior artery; and LCx, left circumflex.

difference in hyperemic response was observed when the temperature of the saline infusion was brought to 37°C rather than when saline at room temperature (22°C) was used. Similarly, the infusion of a glucose 5% solution at room temperature was associated with the same hyperemic effect than a saline infusion. The infusion of arterial blood has not been tested.

Third, a direct stimulation of the epicardial segment appears to be involved, although the nature of this stimulus and the mode of transmission towards the microvascular compartment remain elusive. The stimulation of the epicardial endothelium has been shown to induce a dilatation of the epicardial segments through paracrine NO pathways[13–15] in the experimental setting. Also in humans, selective microvascular vasodilation have been shown to be associated with an epicardial vasodilation.[16] Yet, the reverse, namely the potential vasodilation of the microvasculature by stimulation of the

epicardial segment has not been explored. Our results plea against a significant contribution of the epicardial endothelium. After administration of L-arginine methyl ester, after endothelial denudation, and even after implantation of metallic stents, the infusion of 20 mL/min through the RayFlow catheter was still able to induce maximal hyperemia. It can therefore be speculated that other component of the epicardial artery[17] (smooth muscle cells, extracellular matrix, nerves) are involved in sensing and transducing various stimuli. The fact that the activation of a Rotablator burr in the proximal epicardial artery is immediately followed by a maximal hyperemic response and the fact that infusion of saline through an end hole does not induce hyperemia while the infusion through side holes do, may suggest that microvibrations of the epicardial wall may be that stimulus. The transmission of this information may involve nerve fibers, vascular smooth muscle cells, gap junctions in

the vessel wall, or convection of signal substances with the flowing blood.[18] Vibrations are a well-described but poorly understood stimulus in nature.[19] Several experimentations could be performed to closely explore this mechanism:

1. Coronary venous blood sampled could be performed to examine the free hemoglobin for hemolysis.
2. A methylxanthine test could be used to determine if it blocks the hyperemic effect of the absolute coronary flow measurements for adenosine release mechanism.
3. Evaluate the separation of combined P1 (adenosine and adenosine monophosphate) and P2 (adenosine diphosphate and adenosine triphosphate) receptor blockade to explore the adenosine and the adenosine triphosphate hypotheses.[20]
4. Glibencamide could be used to block K-adenosine triphosphate channels should give insight into the final molecular target of the resistance vessel dilation.[21]

A number of limitations should be acknowledged. First, the number of observations is relatively small especially the number of animals in which the effect on flow of Rotablation could be tested. Nevertheless, this response was reproducible, "dose-dependent," and not observed in any of the contralateral arteries. Second, the presence of the Rotablation burr could have been responsible for some degree of ischemia as can be suspected from a more limited increase in flow that was observed with both adenosine and during high speed rotation. Yet, this decreased coronary flow reserve should not have led to myocardial ischemia in the absence of increased cardiac work. Third, it should be acknowledged that the degree of NO blockade could be suboptimal in our experimental conditions. As our goal was to avoid major change in hemodynamic, we lowered to ≈5 mg/kg the dose of L-arginine methyl ester and therefore, NO production might not have been total. Finally, the present study did not explore the potential contribution of hemolysis in the observed hyperemic response. The release of ATP by red blood cells is an important signaling event in the microvasculature that contributes to control local blood flow. Several external stimuli have been shown to trigger adenosine triphosphate release from red blood cells, including, hypoxia, changes in pH, but also centrifugation. It is therefore possible that the jets of saline perpendicular to the direction of the flow—as well as the activation of a Rotablator burr—might induce a breakdown of the red blood cells and a secondary local increase in plasmatic adenosine concentration. Yet, preliminary data showing a renal hyperemic effect during saline infusion at 20 mL/min tend to contradict this hypothesis as adenosine is known to induce a potent renal vasoconstriction while the infusion of saline induced a renal vasodilation.[7]

CONCLUSIONS

The present experimental study confirms that a saline infusion rate of 20 mL/min through a dedicated catheter for coronary thermodilution induces maximal hyperemia, irrespective of the temperature and the composition of the infusion, and regardless of the presence/absence of normally functioning epicardial endothelium. These data suggest a role for vibrations of the epicardial segment. The way this information is transmitted toward the microvasculature remains to be established.

Affiliations

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SUPPLEMENTAL MATERIAL

Figure S1. Bland and Altman plots of the hyperemic effect of saline infusion rates of 5,10,15 and 20mL/min through the RayFlow catheter expressed in mean difference of percentage of change in absolute blood flow above the baseline as compared to hyperemia achieve with adenosine in the study vessel.