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Original Article

Adenosine and lidocaine (AL) combination dilates intimally damaged rat thoracic aortic rings and guinea pig mesenteric arteries: possible significance to cardiac surgery

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Abstract: New pharmacotherapies are required to improve vessel graft protection and prevent vasoconstriction and spasm in CABG surgery. Previously we have studied adenosine (A) and lidocaine (L) relaxation in rat aortic rings, and reported a possible crosstalk between L relaxation and adenosine A_{2a} receptor inhibition. The aim of the present study was to examine the effect of AL combination compared to A and L alone on relaxation in intact and denuded rat aortic rings and in guinea-pig pressurized mesenteric arterial segments. Aortic rings were harvested from Sprague-Dawley rats and equilibrated in an organ bath containing modified Krebs-Henseleit (KH) solution, pH 7.4, 37°C. Rings were pre-contracted sub-maximally with 0.3 μM norepinephrine, and the effects of increasing AL, A or L (up to 1.0 mM) were examined in intact and denuded rings. Mesenteric artery segments were isolated from guinea-pigs and mounted in an arteriograph containing KH solution and pressurised to 60 mmHg. Arteries were preconstricted with 10^{-8} M vasopressin and AL, A, or L was administered luminally or abluminally. Diameters were measured using video-microscopy. We report in intact rat aortic rings, AL increased relaxation from 21 to 100% (0.1-1.0 mM) and relaxation was endothelium-independent. Adenosine alone was also a potent relaxant of aortic rings but, unlike AL relaxation, it was partially endothelium-dependent. In intact mesenteric artery segments, increasing luminal AL produced a potent endothelium-independent dilation (up to 90%). Adenosine dilation was endothelium-independent but not lidocaine, which produced 33% dilation only after endothelial removal. Extra-luminal AL and A led to 76% and 80% dilationin intact segments respectively, whereas L resulted in constriction (10-17%). In conclusion, we show that AL can dilate aortic rings and mesenteric artery segments by up to 90% regardless of whether the endothelium is intact. We discuss the potential translational significance of AL to improve conduit protection in cardiac surgery, and other major surgeries.

Keywords: Rat aorta, mesenteric, artery, adenosine, lidocaine, relaxation, vasodilation, CABG, vasospasm

Introduction

Endothelial damage is common after open surgical or endoscopic conduit harvesting, pressure-testing, storage and implantation in patients undergoing coronary artery bypass graft (CABG) surgery [1-3]. Endothelial dysfunction is a major factor responsible for loss of graft patency after 1 to 5 years [4-6]. Intimal damage is involved in the imbalance between endothelium-dependent and endothelium-independent derived vasodilators and vasoconstrictors that can lead to endothelial-smooth muscle decoupling, vasoconstriction, vasospasm, local inflammation/thrombosis and possible intimal hyperplasia/stenosis [1, 7-10]. The major vasodilators currently include nitric oxide, prostacyclin, bradykinin, adenosine and endothelium-derived hyperpolarising factor, and the major vasoconstrictors include endothelin-1, angiotensin II and reactive oxygen/nitrogen species [6, 10, 11].

An area of ongoing controversy is the role adenosine to regulate vascular tone in a number of vessel types, and their endothelial dependence

[12]. In rat aortic rings, we recently showed that adenosine relaxation was endothelium-dependent above 10 μM, and involved endothelial nitric oxide production but not prostacyclin. Another vasoreactive modulator, lidocaine, has a number of paradoxical properties ranging from smooth muscle relaxation to constriction [13-17]. We recently showed that lidocaine relaxation in rat aortic ringswas significantly enhanced by endothelial removal, which did not appear to be NO or prostacyclin-dependent [18]. In that study, we identified an unknown factor (s) responsible for enhanced lidocainerelaxation, and implicated the involvement of the adenosine A_{2a} receptor [18]. Given the potential crosstalk between A and L, the aim of the present study was to examine the effect of AL combination compared to A and L alone on the relaxation properties of intact and denuded rat thoracic aortic rings and guinea-pig pressurized mesenteric arterial segments. Mesenteric arterial segments were included because mesenteric vasoconstriction and gut ischemia-reperfusion injury during major surgery can be particularly lethal with mortality rates of over 70% [19, 20].

Material and methods

Animals

Adult male Sprague-Dawley rats (300-350 g) and guinea-pigs (250-300 g) were fed *ad libitum* and housed in a 12-hour light/dark cycle. Rats were anaesthetised with Na-thiopentone (100 mg/kg), and adult male guinea-pigs (250-300 g) were sacrificed by stunning and cervical dislocation. Animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). James Cook University ethics approval number was A1535 for the rat study and Flinders University approval number was 734/09 for the guinea-pig study. Adenosine and other drugs and chemicals were purchased from Sigma Aldrich (Castle Hill, NSW). Lidocaine-HCl was sourced as a 2% solution (ilium) from the local Pharmaceutical Suppliers (Lyppard, Queensland).

Aortic ring preparation and organ bath tension measurements

The thoracic aorta was harvested and placed in an ice-cold solution of Krebs-Henseleit (117 mM NaCl, 5.9 mM KCl, 1.2 mM Na_2PO_4, 0.5 mM $MgCl_2$, 1.12 mM $CaCl_2$, 25 mM $NaHCO_3$) pH 7.4 with 11 mM glucose. The aorta was cleaned of surrounding fat and connective tissue and cut into 3-4 mm transverse segments. Aortic rings were equilibrated in a standard 10 ml volume organ bath (Radnotti Glass, AD instruments, NSW, AUS) containing Krebs-Henseleit solution. The organ bath was continuously bubbled with 95% O_2 and 5% CO_2 at 37°C for 15 minutes (zero tension). The rings were vertically mounted on small stainless steel triangles, stirrups and connected to an isometric force transducer (PANLAB, distributed by AD Instruments as MLT 0201/RAD, NSW, AUS) and a computer based data acquisition system (PowerLab, AD Instruments) and data recording software LabChart 7 (AD Instruments Pty Ltd., Castle Hill, Australia).

Ring tension was adjusted to 1.5 g and equilibrated for 60 min. The solution was changed at 15 minutes intervals. Each preparation was sub-maximally contracted using 3 μl of 0.1 mM norepinephrine (NE) (0.3 μM final concentration) [12, 18]. Preliminary studies showed the increase in tension was reached after 10 min and remained at a plateau level for over 60 min, the time course of each experiment. If washed aortic rings failed to contract after NE they were discarded. In all preparations acetylcholine (10 μM final concentration) was applied to confirm the presence or absence of an intact endothelium. If the acetylcholine-mediated relaxation was rapid (>80%) the endothelium was considered intact, and if <10% the endothelium was considered damaged or denuded [21].

After 10 to 15 min stabilization, AL, A and L were added to the organ bath to obtain 1, 5, 10, 50, 100, 500 and 1000 μM concentrations. At the end of each experiment, rings were tested for viability with 100 μM papaverine using the method of Grbović and colleagues [22], and relaxation was expressed as a % maximal relaxation to papaverine.

Guinea-pig mesenteric artery segments

The arterial perfusion system coupled to microscopy and video monitoring is described by Sokoya and colleagues [23]. Briefly, second order mesenteric artery branches were dissected and transferred to ice-cold modified Krebs-

Henseleit solution pH 7.4 with 5.5 mM glucose (95% O_2 and 5% CO_2). Segments were cleaned and at least 1 mm lengths were mounted on transparent micropipette glass cannulaein a custom-made chamber (ChuelTech, Houston, TX, USA) and the Krebs-Henseleit solution was gradually warmed to 37°C. A constant intraluminal pressure of 60 mmHg was maintained using two reservoirs that were mounted above the arteriograph. In-line pressure transducers were connected to two strain gauge panel meters (Omega, Stamford, CT) that provided continual measurement of the intraluminal pressure. One reservoir was filled with oxygenated Krebs-Henseleit and a constant luminal flow rate of 100 µl/min maintained throughout the experiment. Immediately after mounting, arteries were tested for leaks and segments that did not maintain a steady-state pressure were discarded. The outer arterial segment diameter was continuously measured through a NIKON TMS inverted microscopy coupled to a video monitor.

After 30 min equilibration, arterial segments were pre-constricted with 10^{-8} M arginine vasopressin (AVP). Some segments were left intact, and others denuded by pumping air into the vessel lumen at a flow rate 1 ml/min for 10 minutes [23]. Removal of the endothelium was confirmed with 10^{-5} M acetylcholine. After diameter stabilization, the arteries were exposed to AL, A or L luminally or abluminally (1 to 1000 µM) and the change in outer artery diameter was measured. At the end of each experiment, vessels were washed with calcium-free Krebs-Henseleit solution containing 1 mM EGTA to determine the maximal dilation of the vessels [23]. Preliminary experiments found that, unlike aortic rings, 100 µM of papaverine led to inconsistent maximum dilation in mesenteric segments. For mesenteric artery experiments, the effects of A, L and ALM on vasodilation (or relaxation) were expressed % maximal dilation to calcium-free Krebs-Henseleit solution containing 1 mM EGTA. At each concentration of ACh, the percentage dilation was calculated according to the following equation:

$$\text{Percentage Dilation} = (D_{ACh} - D_{base})/(D_{max} - D_{base}) * 100$$

where D_{ACh} is the diameter of the artery after luminal administration of A_{Ch}, D_{base} is the baseline diameter of the artery before addition of ACh, D_{max} is the maximal diameter of the artery in the presence of calcium-free Krebs-Henseleit solution (containing 1 mM EGTA).

Statistics

Values are expressed as mean ± SEM. The number of rats and guinea-pigs were selected from a priori G-power analysis to achieve a level of 1.0. All data was tested for normality using *Kolmogorov-Smirnov* test. Relaxation responses to adenosine, lidocaine and AL were analysed for homogeneity of variances followed by two-way repeated measures ANOVA coupled with the *Bonferroni* post-hoc test for individual data point comparisons. The alpha level of significance for all experiments was set at $P<0.05$.

Results

Effect of increasing AL on relaxationin rat aortic rings

AL produced a dose-dependent relationship in intact and denuded rat aortic rings (**Figure 1A**). In intact rings, AL had little effect below 100 µM (<5% relaxation) and increased relaxation to 76% above 100 µM. Curiously, when the experiment was repeated but started at 100 µM (**Figure 1B**), rather than 1 µM, (**Figure 1A**), relaxation was higher (21% vs. 3.7%), and maximum relaxation after 1000 µM was 98% compared to 76% after 1 to 1000 µM serial dilution (**Figure 1A**). The data also show that serial additions of AL from 1 to 1000 µM vs. 100 to 1000 µM lead to different maximum relaxations at 100 µM (4 vs. 14% relaxation) and 1000 µm (76 versus 98% dilation), indicating a tachyphylaxis-like response in the first experiment (**Figure 1A**) but not in the latter (**Figure 1B**). Similar relaxation profiles were found in rings with the endothelium removed. Another interesting feature of **Figure 1B** was that at the AL-induced relaxation at the cardioplegic concentrations (0.25 to 1 mM AL) (**Figure 1B**), showed a slight curvilinear effect that was more pronounced after removing the endothelium (greater dilation at a given AL concentration), but this was not significant.

Effect of A and L alone on relaxation in rat aortic rings

Increasing adenosine from 100 to 1000 µM significantly increased relaxation by 82% in

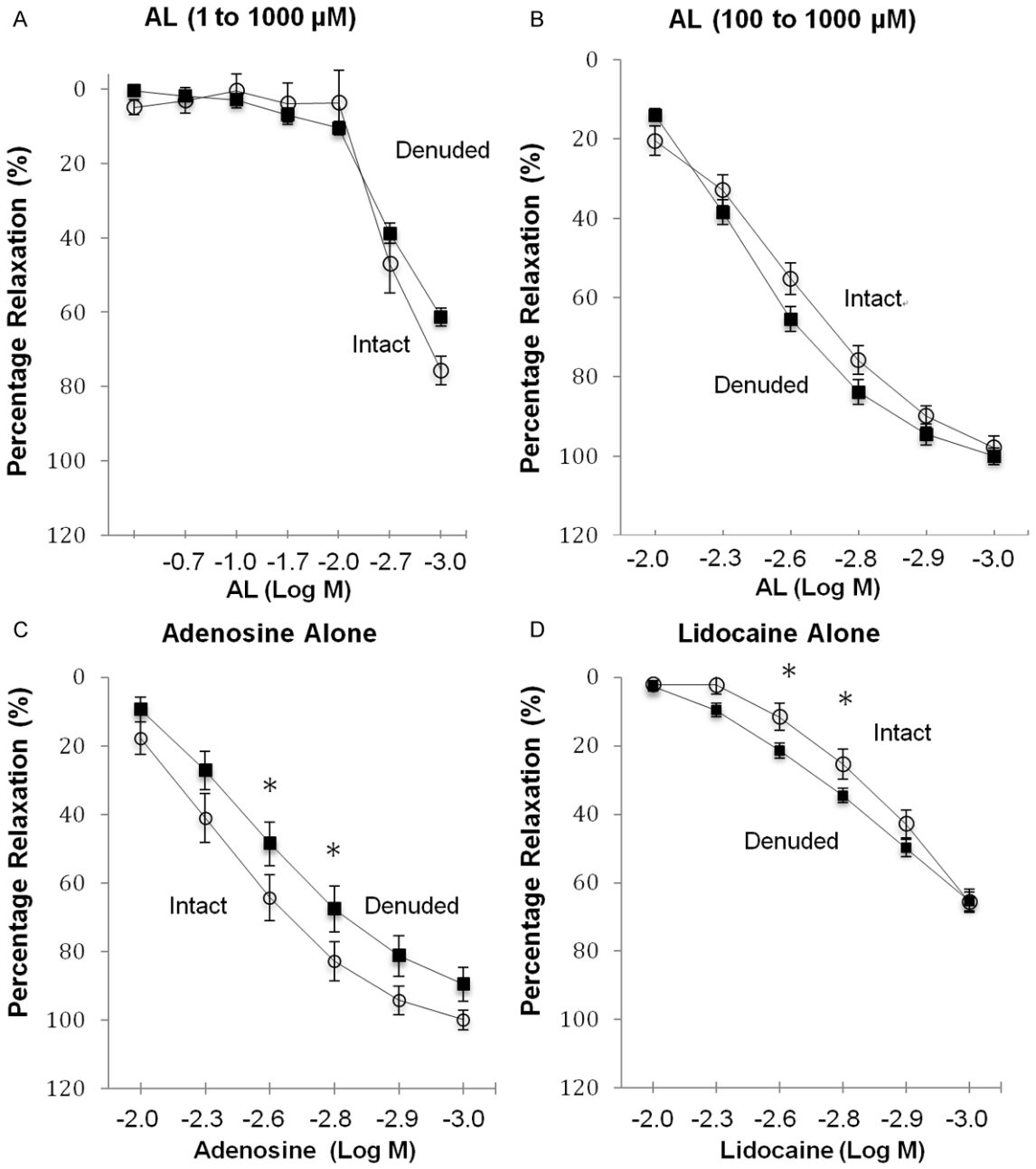

Figure 1. Dose-response curves of adenosine-lidocaine (AL), adenosine (A) and lidocaine (L) in isolated intact and denuded rat thoracic aortic rings. (A) AL (1-1000 μM, log scale). (B) AL (100-1000 μM, log scale). (C) Adenosine (100-1000 μM) and (D) Lidocaine (100-1000 μM). Values are mean ± S.E.M for aortic rings from 8 animals. *P<0.05 adenosine endothelium intact and denuded groups.

intact rings, and by 81% in denuded rings from baseline (**Figure 1C**). The effect of removing the endothelium significantly reduced relaxation by 23 to 18% at 400 and 600 μM respectively (**Figure 1C**). Lidocaine alone increased relaxation by 63% in intact and denuded rings, and endothelial removal significantly enhanced dilation 1.5 times at 400 μM and 1.3 times at 600 μM lidocaine compared to intact rings (**Figure 1D**). **Figure 2A** and **2B** represents the combined data in intact and denuded rat aortic rings. In intact rings, AL relaxation was not significantly different from adenosine, and both were significantly higher than lidocaine alone. In denuded rings, adenosine relaxation was significantly lower for a given concentration, lido-

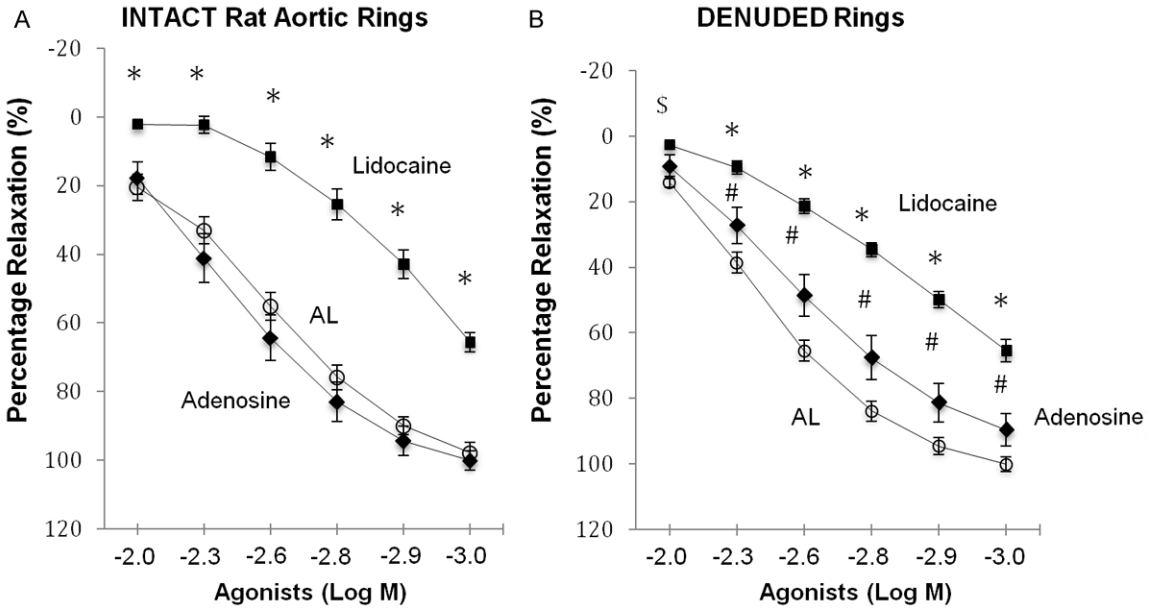

Figure 2. Comparison of dose-response curves to AL, Adenosine and Lidocaine in isolated intact (A) and denuded (B) rat thoracic aortic rings. Valuesare mean ± S.E.M for aortic rings from 8 animals. *P<0.05 lidocaine group and other groups. #P<0.05 adenosine group and AL group.

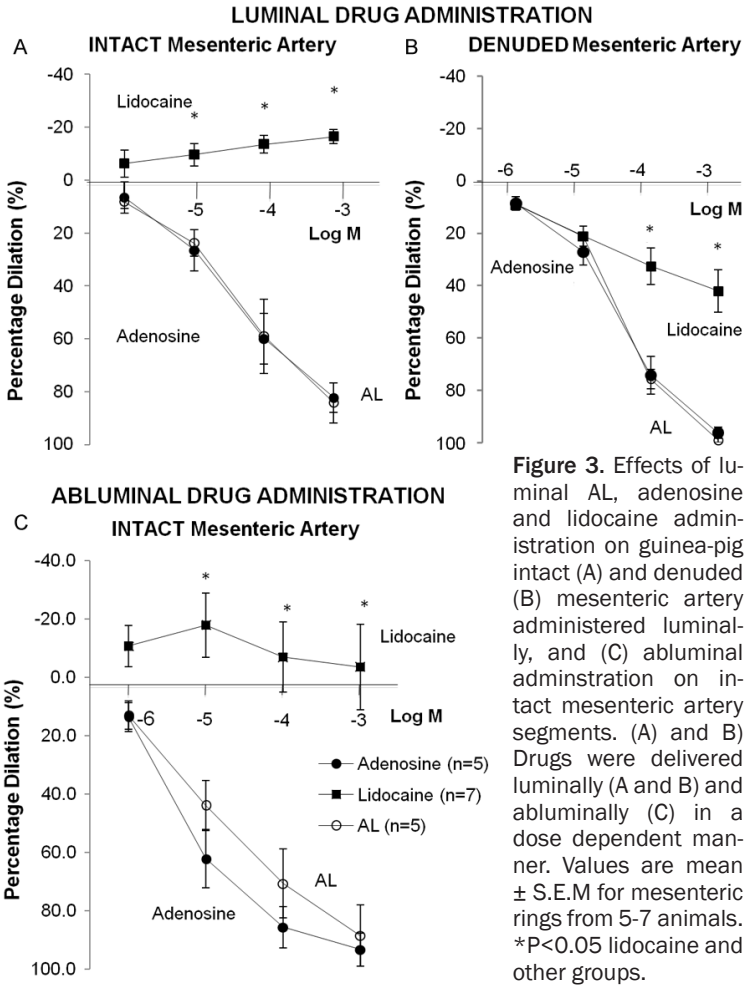

Figure 3. Effects of luminal AL, adenosine and lidocaine administration on guinea-pig intact (A) and denuded (B) mesenteric artery administered luminally, and (C) abluminal adminstration on intact mesenteric artery segments. (A) and B) Drugs were delivered luminally (A and B) and abluminally (C) in a dose dependent manner. Values are mean ± S.E.M for mesenteric rings from 5-7 animals. *P<0.05 lidocaine and other groups.

caine relaxation was significantly higher, and there was no change in AL relaxation after endothelial removal.

Guinea-pig mesenteric artery

Effect of luminal AL, A and L on endothelium intact and denuded segments: For allmesenteric artery experiments, there was no significant difference in AVP-stimulated constriction between the groups in either intact or denuded segments. In intact arterial segments, increasing luminal AL or adenosine led to over a 10-fold increase in dilation from 8% to 84% (**Figure 3A**). The AL or A treatments were not significantly different from each other. In contrast, increasing intraluminal lidocaine concentration produced a 2.6 fold constriction, and was significantly different from all other groups (**Figure 3A**).

In denuded segments, increasing luminal AL and adenosine followed a similar pattern to

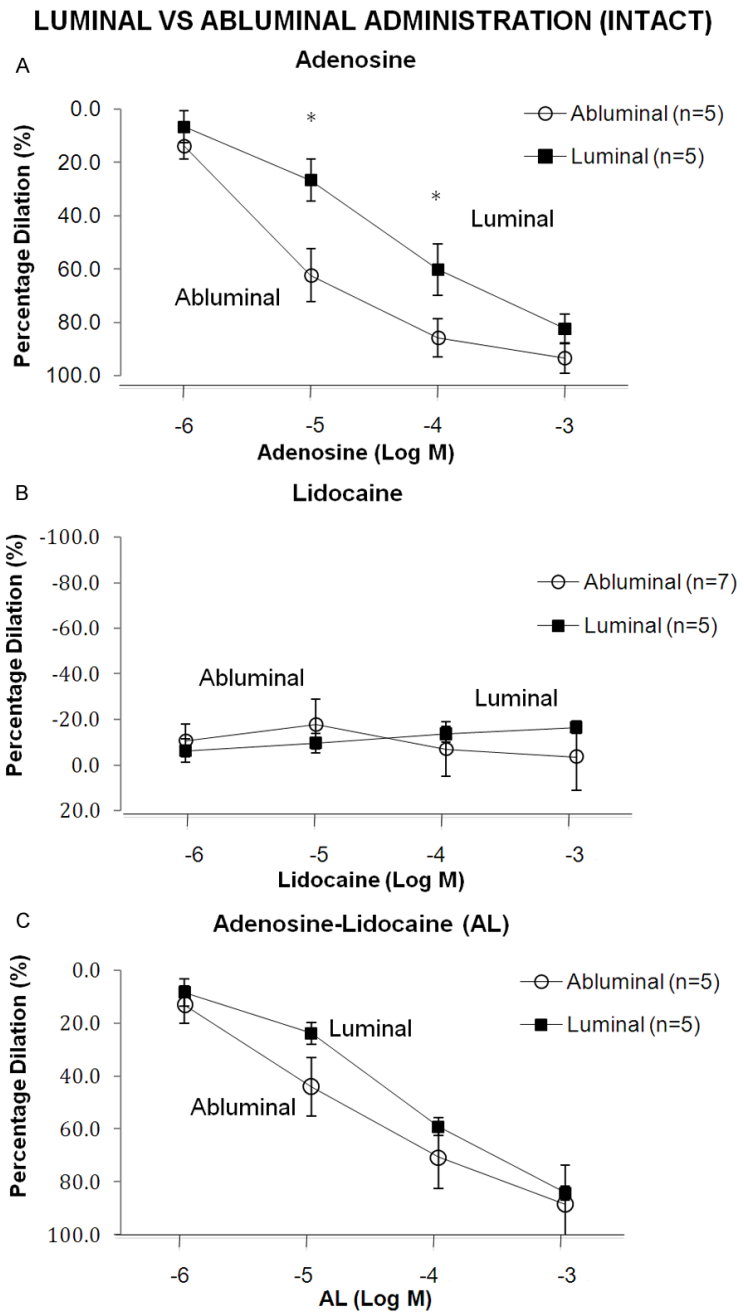

Figure 4. Luminal versus Abluminal Comparison between adenosine (A), lidocaine (B) and AL (C) in intact guinea-pig mesenteric artery. Values represent mean ± S.E.M of mesenteric rings from 5-7 animals. *P<0.05 statistical difference in responses between luminal and abluminal adenosine administration.

the intact artery with 90% dilation from 1 to 1000 μM (**Figure 3B**). Removal of the endothelium led to a dramatic and significant change in mesenteric arterial reactivity to lidocaine. Instead of modest constriction in intact segments, increasing lidocaine led to a 4.7-fold increase in dilation from 1 to 1000 μM (**Figure 3B**). Lidocaine's maximum dilation response after denudation was 42% at 1000 μM compared to 9% at 1.0 μM (**Figure 3B**).

Effects of abluminal AL, A and L on intact mesenteric segments

The effect of increasing abluminal AL produced 76% dilation from 1 to 1000 μM (**Figure 3C**). Similarly, adenosine led to a significant increase in dilation of 80% (**Figure 3C**). The differences between AL and adenosine were not significant. Increasing lidocaine from 1 to 10 μM produced an increase in constriction from 10% to 17% with vessel diameter slowly dilating to baseline (**Figure 3C**). The comparative effects of AL, adenosine or lidocaineon luminal verses abluminal administration in intact mesenteric segments are summarized in **Figure 4A** and **4C**. The data show that adenosine relaxation is significantly enhanced when administered abluminally (**Figure 4A**), while no differences were found in lidocaine administration (**Figure 4B**). Abluminal versus luminal AL administration, like adenosine, produced up to 45% more dilation over a range of AL concentrations, however, the differences were not significant (**Figure 4C**).

Discussion

New pharmacological strategies are required to improve autologous supplementary arterial and venous graft protection in CABG surgery [3]. In our study, we report the following: 1) In rat aortic rings, AL combined produced a strong relaxation response that was endothelium-independent: 2) Adenosine relaxation in intact rings was similar to AL, but differed by being partially endothelium-dependent: 3) Lidocaine relaxation was significantly

less than AL and adenosine, but like adenosine was endothelium-dependent: 4) In intact mesenteric arterial segments, luminal administration of AL or adenosine produced a strong vasodilation (up to 90%) and was endothelium-independent, where as lidocainedilation was significantly less and occurred only after endothelial removal, and 5) Abluminal AL and adenosine produced 80% dilation, and lidocaine produced 10 to 17% constriction. These differences will now be discussed.

AL relaxation was endothelial-independent

Two interesting results of the present study were: 1) AL was a powerful dilator in isolated rat thoracic aortic rings and pressurized guinea-pig mesenteric segments, and 2) Relaxation/dilation was endothelium-independent. The finding that AL was a strong relaxant in rat aorta, regardless of whether the intima was damaged or not, suggests that AL may find utility in harvesting and perfusing conduits for revascularisation. Harvest, storage and implantation are times when the conduit vessel is highly vulnerable to intimal damage and potential failure [24-26]. Thus maintaining optimal vascular tone in harvested vessels may lead to improved perfusion and reduce myocardial dysfunction.

Interestingly, the relaxation effect of AL in aortic rings and mesenteric artery segments was not a synergistic effect of adenosine and lidocaine, but was dominated by adenosine and a complex dilation-contraction behavior from lidocaine (**Figures 2** and **3**). Currently, we do not know the underlying mechanisms of AL's action. It is important to recognise that the aortic ring experiments and pressurized artery experiments were different with regards to drug application. In the rings, drugs have access to both the endothelium and smooth muscle whereas in the pressurized vessels, we applied them to either the endothelium or smooth muscle. This may be particularly important for AL combination because it appears that there are receptors for both adenosine and lidocaine on both cell types (see below).

Rat thoracic aortic rings

In rat thoracic aorta, adenosine relaxation has been reported in the literature to be either fully endothelium dependent, partially dependent or have no dependency see [12]. Adenosine vasodilation is believed to involve a complex interplay between endothelial A_{2a} subtype receptor activation, nitric oxide (NO), prostanoids (e.g Prostaglandins and thromboxane A_2), hyperpolarising factors, and voltage-dependent K^+ channels [27]. We recently revisited the question and found that relaxation in isolated rat thoracic aortic rings was endothelium-dependent, and involved activation of smooth muscle A_{2a}, endothelial NO production, and voltage-dependent K_v, K_{ATP} channels (sarcoplasmic and mitochondrial), but not prostanoid production [12]. The present study confirmed adenosine's partial endothelium-dependency, but here we focused on the higher concentrations that appear in AL cardioplegia.

Lidocaine reactivity in isolated rat thoracic rings is also controversial [18]. In the present study, we showed that by removing the endothelium it led to significant enhancement of lidocainedilation (**Figure 1D**). In a previous study, we proposed aputative relaxation factor(s) to explain this anomalous effect, and showed it was not NO- or prostacyclin-dependent [18]. However, dilation was significantly reduced in the presence of K_v and MitoK_{ATP} inhibition in denuded rings [18], and interestingly by adenosine A_{2a} subtype antagonism (above 100 μM lidocaine) in intact and denuded rings, indicating a potential role for voltage-dependent K^+ channels and crosstalk with adenosine receptor activation [18]. Further studies are required to identify and understand the nature of this putative lidocaine relaxation factor, which appears to be activated upon denudation (**Figure 1D**).

Mesenteric artery segments

In contrast to rat aortic rings, luminal adenosine relaxation was endothelium-independent in mesenteric arterial segments (**Figure 3A** and **3B**). A possible reason for the difference is because adenosine in the isolated rat aortic rings was reaching receptors on both the endothelium and smooth muscle but not in the pressurized arteries. That luminal infusion of adenosine elicits an endothelium-independent dilation indicates the presence of smooth muscle adenosine receptors. This curious result supports the 2005 study of Radenković and colleagues in the rat mesenteric artery [28], who reported that dilation was partly induced by activation of smooth muscle A_{2a} receptors and

mediated via the opening a mixed population of smooth muscle K^+ channels, and possibly the Na^+/K^+-ATP ase pump [28]. Unfortunately, there appear to be only a handful of studies on the effect of adenosine on mesenteric artery vaso reactivity.

In our study, increasing luminal lidocaine concentration also resulted in complex behaviour (**Figure 3A** and **3B**). In intact mesenteric artery segments, lidocaine produced a constriction (~10%), and denudation produced an opposite effect i.e. a significant dilation (42% relaxation at 1000 μM) (**Figure 3B**), which was similar to what we found in rat aortic rings. It appears that the lidocaine-induced 'constricting' factor(s) originated from the intact endothelium, and the 'dilation' factor(s) from vascular smooth muscle. In the abluminal lidocaine experiments, it is also possible that lipid-soluble lidocaine diffuses through to the endothelium, and the only way to remove the 'constricting' factor(s) is to remove the endothelium. Further, the addition of AL suggests that the effect of the lidocaine-mediated 'constricting' factor is overcome by the adenosine-mediated dilation.

Complex lidocaine vasoreactivity has also been reported in human internal mammary artery (IMA), radial artery (RA) and saphenous vein segments [29]. Gur and colleagues reported that lidocaine at low concentrations (10^{-9} to $10^{-7.5}$ M resulted in ~40% dilation in IMA and RA segments isolated from 20 patients, and constriction at higher concentrations (>$10^{-7.5}$ M) [29]. In saphenous vein segments, the group also reported a 24% dilation at 10^{-9} to 10^{-7} M lidocaine, and a dose dependent constriction above 10^{-7} M [29]. Unfortunately, there are only a few studies on the effect of lidocaine on the mesenteric artery. In 1975, Sovac and colleagues found that intra-arterial injection of lidocaine was a poor mesenteric dilator in dogs [30], which is consistent with our data (**Figure 3A**). Lastly, abluminal AL and A administration produced greater dilation than luminal delivery (**Figure 4A**, **4C**) with no change in lidocaine reactivity (**Figure 4B**). Unfortunately, we did not perform this experiment on denuded mesenteric segments.

Possible clinical translational significance

The findings of the present study have the potential to translate and improve graft protection in patients undergoing CABG surgery [9, 26, 31, 32]. AL solution at cardioplegia concentrations (0.25 to 1 mM) may further improve protection of the heart and conduits during reperfusion when vessels are susceptible tospasm, particularly radial arterial conduits [4]. Translation may be achievable because the AL cardioplegia is in clinical use in the USA and Italy, and two prospective randomized trials have shown superiority over high potassium solutions [33, 34]. The uniqueness of the AL cardioplegia resides in arresting the heart at its natural or resting membrane potential (5 mM K^+), not unnatural depolarizing potentials from high potassium solutions (>15 mM K^+) [2]. Thus, the AL cardioplegic solution may not only arrest and protect the heart but may protect the newly implanted grafts with the potential to improve graft patency.

AL solution may also find clinical utility as a topical (abluminal) or intravenous antispasmodic agent for the internal mammary artery (IMA), which is commonly used *in situ* for CABG surgery [6, 35]. Similar techniques may also apply to the *in situ* protection of the gastro-epiploic artery (a branch of celiac trunk), which is less commonly used today because it is particularly prone to vasospasm [6]. AL topical use could also be used in neurosurgery where cerebral arterial spasm can lead to ischemic neurological deficits. Lastly, our finding that AL dilates mesenteric artery segments helps explain our recent trauma work which showed that small-volume intravenous infusion of AL and magnesium (ALM) led to increased blood flow and local pO_2 to the gut after non-compressible haemorrhage and shock in the rat [36, 37]. Protecting the gut is important during trauma or major surgery because it is the "motor of multiple organ failure" and responsible for triggering, heightening, and perpetuating the systemic inflammatory response [38, 39].

Limitations and future studies

A major limitation of the present study was that the arterial rings and mesenteric segments were from healthy rats and guinea-pigs, and it would be clinically important to investigate segments from human conduit arteries and saphenous veins used in cardiac surgery, and the underlying pathogenesis of vasospasm. Another limitation is that we used rat thoracic aorta, which is a large, highly elastic compliance vessel that normally offers little resistance to flow in contrast to smaller peripheral

and coronary arterioles [12]. From a mechanistic viewpoint, studies are required to investigate the possible cross-talk between A and L that leads to relaxation in the presence and absence of an intact endothelium, and include the effect of key modulators, ion channel activators and inhibitors and protein/mRNA analysis of candidate receptors. Since the net effect of this dynamic system is to regulate vascular reactivity via intracellular Ca^{2+}, A and L cross-talk may be revealed from electrophysiological, immune-histochemical and qRT-PCR techniques targeting microdomain Ca^{2+} signalling receptor sites and pathways in denuded and intact arterial segments [40]. It would also be important to investigate the effect of AL on changing the membrane potential of endothelial and smooth muscle cells during relaxation because the cardioplegia confers its superior protection by keeping the heart at its resting membrane potential [2, 41].

Conclusions

We conclude that AL solution above 100 μM produced a concentration-dependent relaxation/dilation in rat aorta and guinea-pig mesenteric artery segments with or without an intact endothelium. AL may find translational utility to improve conduit protection in CABG surgery, and other major surgeries, where varying degrees of endothelial damage, vasoconstriction or vasospasm are known to occur.

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Disclosure of conflict of interest

None.

Authors' contribution

All authors contributed substantially to the conception, design, implementation, data acquisition and analysis, writing and final approval of the manuscript.

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