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Combined adrenomedullin and selective A_{2a} adenosine agonist treatment in the recovery of coronary function in the infarcted heart

Project Leader James Pearson, Department of Physiology, Monash University
Team: Keiji Umetani , Mikiyasu Shirai, Mat Jenkins
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Research purpose and background

Our group is currently investigating possible therapies for myocardial infarction using synchrotron angiography. In our recent experiments we have shown that chronic intermittent stimulation of adenosine A_{2a}-receptors in the vasculature and muscle of the heart can improve recovery of function following ischemia-reperfusion injury and might thereby prevent the development of heart failure. This proposal was a continuation of our proposal 2008A1875 (Bio Med EX). The aim of these proposals was to determine 1) whether combined treatment of an adenosine A_{2a}-receptor agonist and adrenomedullin (AM), a cardioprotective peptide with potent angiogenic properties, can produce greater benefits in the prevention of reperfusion injury to the heart than either treatment when given individually, and 2) whether the benefits we observed for adenosine A_{2a}-receptor are sustained following a single treatment at the time of reperfusion.

Experimental / analytical method

We compared the vascular calibre (internal diameter) of the coronary arterial circulation in closed-chest rats 3 days after inducing a myocardial infarction under anaesthesia. Animals were treated with either vehicle, CGS (50 μg/kg once only), AM (0.05 microgram/kg/min, continuous subcutaneous infusion by minipump) or a combination of CGS (once) and AM from the time of reperfusion immediately following a 45-min ischaemia period (coronary artery ligation). On the experiment day, male rats were anaesthetized with pentobarbital sodium (50 mg/kg i.p.) and artificially ventilated. A jugular vein was cannulated for a maintenance infusion. The right carotid artery was cannulated for high speed injection of contrast agent and the left femoral artery for recording of arterial pressure. Contrast medium was injected as small boli at 15 min intervals (~0.3 ml bolus, pure Iomeron 350, Bracco-Eisai). The same imaging and high-speed shutter system as described in earlier studies (2003A0464-NL2-np, 2003B0090-NL3-np) was used here. Images (1024 x 1024 pixels) were stored in 10-bit format. In all images a 50 micron tungsten wire was included for calibration. The input field of the SATICON camera was 6.9 mm x 6.9 mm. Shutter open time was 1.5-2.5 ms. Monochromatic X-ray energy was adjusted to 33.3 keV, just above the iodine K-edge energy for maximal contrast.

After recording baseline vessel calibre of the closed chest models we then performed imaging during a dobutamine stress test (i.v. 4 μg/kg/min), acetylcholine and sodium nitroprusside (2-3 μg/kg/min) to evaluate endothelium dependent and independent vasodilatory ability respectively.

Research results

Combined treatment with CGS and AM significantly improved cardiac function after reperfusion injury, at least to the same extent as intermittent treatment with CGS both in terms of coronary function (Fig.1) and cardiac haemodynamic function (data not shown). However, the vasodilatory capacity and baseline calibers of coronary vessels in animals treated with a single dose of CGS were intermediate between vehicle and chronic CGS treated rats (Fig.1 b &e). Notably, in the latter group small arteries (~50 μm) became constricted during acetylcholine administration, suggesting that a single dose of CGS was insufficient to prevent endothelial dysfunction completely over the 3 day recovery period.

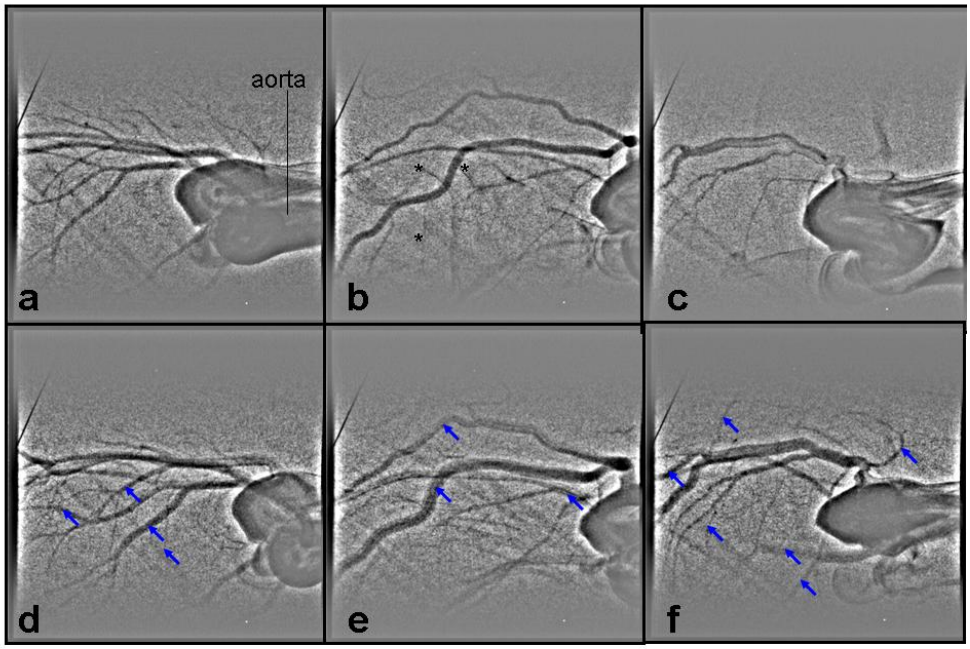

Figure1 shows images of the circulation in the MI region of three rat hearts, a chronic CGS treated rat (twice daily, a & d), single dose CGS treated rat (b & e) and a combined CGS+AM treated rat (c &f). Repeated recordings were made on the same hearts under baseline condition and during infusion of the vasodilator acetylcholine 3 days after MI surgery. Arrows indicate vessels in the anterior wall of the heart showing obvious dilation (>30% of baseline) during subsequent stimulation in the AM and CGS treated animals. Asterisks indicate vessel segments that constricted (single dose CGS rat). A 50 μm diameter tungsten wire is visible in the left corner of each image for vessel calibration purposes.

Current and future issues / challenges

This suggests to us that acute adenosine A_{2a} agonist treatment at reperfusion prevents cardiac inflammation and initial oxidative stress, based on the findings from the group of rats treated with a single dose of CGS. However, sustained benefits were only obtained following chronic intermittent treatment (twice daily) with CGS, suggesting that adenosine A_{2a} stimulation also reduces the impact of other mediators of reperfusion injury that contribute to long term dysfunction and heart failure. AM treatment and combined therapy also provided sustained benefits in all the animals examined. Detailed analysis of vasodilatory capacity are now required to determine if combined therapy provides greater improvement over chronic treatment with AM or CGS alone.

In future experiments we need to determine if these treatments during the first days following myocardial infarction can prevent cardiac remodelling and deterioration of contractile function in the long term. We next plan to investigate whether long term benefits of these treatments include an increase in re-vascularisation of the damaged heart. Preclinical research has now established that cardioprotective roles for adenosine A_1 and A_3 receptor subtype agonists are evident in pre-conditioning settings [1]. However, it is not known whether stimulation of these receptor subtypes can provide the same benefits described here in the setting of post-ischemic treatment of myocardial infarction. Our synchrotron microangiography approach provides the only means of directly determining whether cardioprotective therapy candidates can restore and improve coronary vascular function in the intact organism.

References

[1] Hausenloy D, Yellon D. New directions for protecting the heart against ischaemia-reperfusion

injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway. Cardiovascular Research 61:448-460, 2004.

Status of publication and patent

Experiments in this first study of combined therapy against ischemia-reperfusion injury were completed. The changes in vessel calibre are now being quantitatively analysed for manuscript submission in the very near future.

Keywords and annotations

contrast angiography – method of enhancing visualization of the structure and function of the vasculature by increasing blood X-ray absorption by infusion of contrast agents.

vasodilation – increase in blood supply to an organ as a result of vessel relaxation (calibre increase).