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**Combined adrenomedullin and selective A<sub>2a</sub> adenosine agonist treatment in the recovery of coronary function in the infarcted heart**

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Research purpose and background

Prolonged interruption of coronary blood flow to heart muscle causes permanent damage (myocardial infarction, MI), which reduces the ability of the heart to maintain normal heart pumping. Without treatment to restore blood flow the infarcted heart becomes further compromised by haemodynamic stress, spread of necrosis by cytokine production and oxidative stress. These factors contribute to acute mortality as a result of heart failure in the days after a heart attack. Preventing the spread of necrosis of the coronary blood vessels and re-establishing a new microcirculation in the infarcted and adjacent compromised regions of the heart are the two objectives of our research.

Our group is currently investigating whether chronic intermittent stimulation of adenosine A<sub>2a</sub>-receptors in the vasculature and muscle of the heart can improve post MI recovery of function and thereby prevent heart failure. In our 2007B1331 proposal we compared the coronary blood flow regulatory abilities of rat hearts 24h after evoking MI in rats treated with and without a selective A<sub>2a</sub>-receptor agonist CGS-21680 (50 µg/kg twice daily) and vehicle treated or sham-operated animals. Using our coronary contrast angiography approach we established that the coronary perfusion of infarcted and adjacent regions of heart by the left anterior descending coronary artery (LAD) was normal or enhanced in CGS-21680 treated rat hearts (n = 5) but diminished in vehicle treated rats (n = 5). Furthermore, CGS-21680 treated rats had normal coronary vasodilatory responses during a stress test (beta-adrenoceptor stimulation with dobutamine), and both acetylcholine and sodium nitroprusside infusions. We concluded that this selective adenosine A<sub>2a</sub>-receptor agonist treatment prevented acute endothelial dysfunction, most likely by providing increased coronary blood flow and by preventing cardiac inflammation and oxidative stress. Nevertheless, we do not know whether these acute benefits following 24h of treatment can be extended to longer periods as necrosis continues to spread and heart function becomes increasingly impaired by haemodynamic stress (impaired blood ejection and increased vascular resistance to ejection). As cytokine and oxidative stress mediated injury to coronary vessels (and muscle) peaks 24-72h following MI in most species it needs to be determined if (1) the efficacy of CGS-21680 treatment can be extended to 72h post MI and if (2) small coronary vessel loss due to blockage and necrosis in infarcted and adjacent regions can be restored by stimulating angiogenesis with adrenomedullin.

Adrenomedullin (AM) is an endogenous vasoactive peptide released by the endothelium and is another arm of the body's natural response to ischaemia. Recent opinions suggest that adrenomedullin in the heart, like natriuretic peptides is cytoprotective against ischaemia-reperfusion injury [1]. Stem cell therapy is a leading edge approach that is being considered by many research groups as a promising remedy to replace permanently lost cardiac myocytes in the infarcted heart muscle regions. However, poor viability of the damaged tissue region for transplantation has been shown to hinder tissue reconstruction with mesenchymal stem cells [2]. Here it is noteworthy that some research groups at least have shown that improved recovery of function from myocardial infarction can be achieved by combining stem cell therapy with genetic amplification of the AM (and co-related pro-peptide) gene or chronic infusion of AM in animal models. Cytoprotection and increased angiogenesis evoked by expression of adrenomedullin might explain the significant improvement in function [1,3].

#### Experimental / analytical method

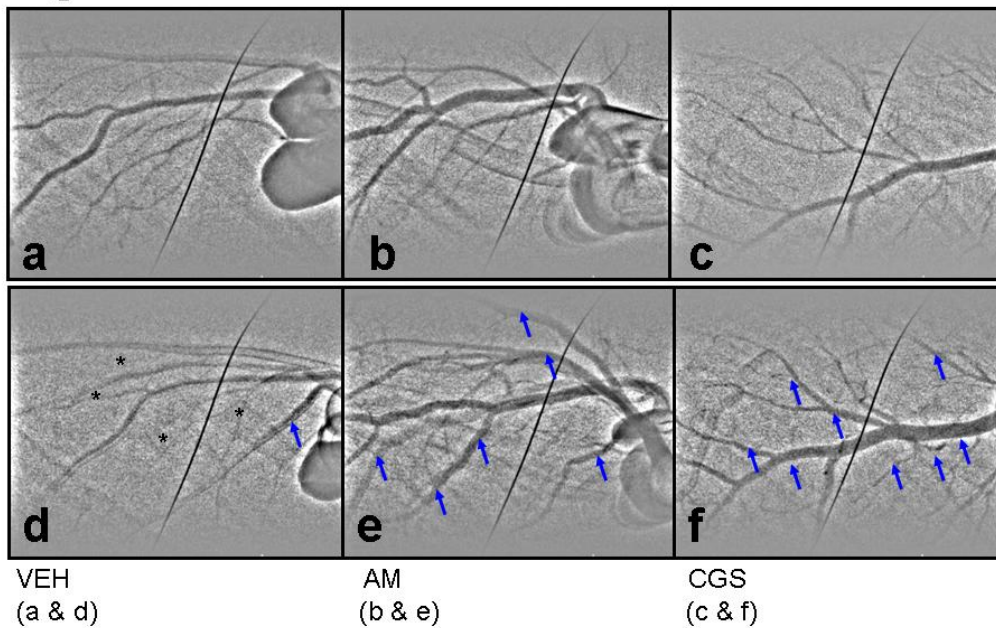
We compared the vascular calibre (internal diameter) of the coronary arterial circulation in closed-chest rats 3 days after inducing a myocardial infarction under anaesthesia. Animals were treated with either vehicle, CGS (50 µg/kg twice daily) or Adrenomedullin (0.05 microgram/kg/min, continuous subcutaneous infusion by osmotic minipump) 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 a small bolus 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 (intravenous 4 µg /kg/min), acetylcholine and sodium nitroprusside (3 µg /kg/min) to evaluate endothelium dependent and independent vasodilation respectively.

#### Research results

We found that baseline vessel calibre of first, second and third order branches of the left anterior descending coronary artery were most variable in vehicle treated rats. Furthermore, blood flow was less pulsatile through vessels within infarcted regions and contrast transit times were prolonged in vehicle treated rats (4 out of 5). In one such example (Fig. 1a & b) of a vehicle treated rat heart, only one vessel segment was observed to dilate during acetylcholine infusion (Fig.1b) or dobutamine infusion (not shown), suggesting the presence of endothelial dysfunction. In contrast, in the example of a CGS treated rat heart blood flow was pulsatile (Fig. 1e & f), and significant increases in blood flow were found during the cardiac stress test and normal endothelial-mediated dilation was evident from the increase in microvessel number and diameter during acetylcholine (indicated by arrows). A similar pattern was recorded in all CGS treated rats (n = 4). A similar pattern of coronary flow improvement was seen in AM treated rats (4).

**Figure1.**



**Figure1** shows images of the circulation in the MI region of three rat hearts, a vehicle treated rat (a & d), AM treated rat (b & e) and a CGS-21680 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 (Vehicle rat). A 50 µm diameter tungsten wire is visible in the centre of each image for vessel calibration purposes.

#### Current and future issues / challenges

Twice daily administration of CGS-21680 or AM infusion prevented the irreversible damage to the endothelium following myocardial infarction and hence, treated animals showed near normal control of coronary blood flow. However, in the limited time available it was not possible to investigate MI rats treated with both AM and CGS-21680 and this remains the goal of a future investigation to establish if combined therapy provides additive benefits.

#### References

- [1] Burley, Hamid and Baxter. Heart Failure Reviews 12:279-91, 2007.
- [2] Jo, Nagaya et al. Tissue Engineering 13:313-22, 2007.
- [3] Fujii, Nagaya et al. Am J Physiol 288:H1444-50, 2005.

#### Status of publication and patent

Additional experiments were required in 2008B to complete this study. 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).