Reducing Infarct Size Beyond That From Platelet Inhibitors Alone

Michael V Cohen, Diego Alvarez, Jonathon Audia, Xi-Ming Yang and James M Downey

University of South Alabama, Mobile, AL, USA

ABSTRACT

Today $P2Y_{12}$ receptor inhibitors (**PI**) are given to all patients presenting with acute myocardial infarction. If given prior to primary angioplasty, they greatly reduce morbidity and mortality. The assumption has been that they protect by preventing formation of recurrent thrombi in the coronary vasculature. However, we observed that PI (cangrelor, ticagrelor or clopidogrel) were all very cardioprotective in animals if present at the time of reperfusion, and that the same signaling inhibitors that block postconditioning also block protection from PI. None of these blockers interfered with PI's anti-platelet effect indicating that PI were actually protecting by conditioning the heart. Neither pre- nor postconditioning (**IPOC**) could offer any additional protection to that from PI in animals presumably because the PI had already conditioned their hearts. Not surprisingly, IPOC stopped being effective in all clinical trials conducted after PI became the standard of care. In order to find a clinically effective cardioprotectant we have screened potential therapies in animals receiving a PI. Recent findings suggest that fragments of mitochondrial (**mt**) DNA released from injured myocardium are very pro-inflammatory and kill neighboring cells by pyroptosis (death by inflammation). An important source of inflammation in the heart is the TLR9 / NLRP3 inflammasome. Mitochondrial injury during ischemia/reperfusion causes the release of oxidized fragments of mtDNA which activate this pathway and cause release of cytotoxic cytokines that can attack surviving heart cells. Caspase-1 is a key step in this pathway and we find that inhibiting it with VX765 reduces infarction when the drug is given to rats just before reperfusion. Importantly protection from VX765 adds to that from the PI cangrelor. The combination therapy reduced infarct size in rats from 75% to just 15% of the risk zone following 60 min of coronary artery occlusion when treatment was started just prior to reperfusion.

Loading with a $P2Y_{12}$ inhibitor prior to PCI protects the heart

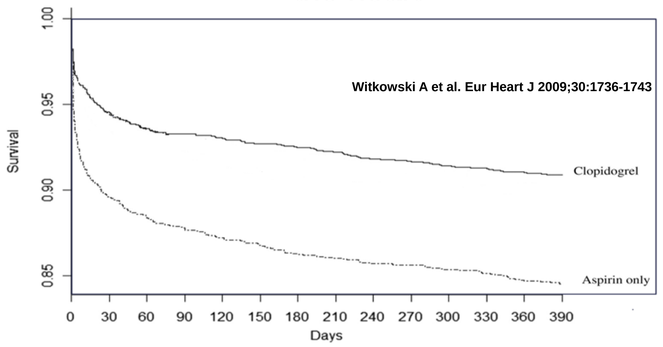
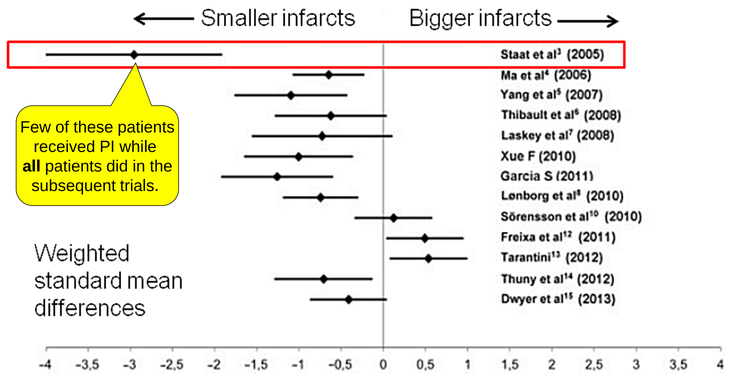


FIG 1. In this large scale clinical trial (~2000 patients per group) it was found that a loading dose of clopidogrel, a $P2Y_{12}$ inhibitor, prior to reperfusing an acutely occluded coronary artery with percutaneous coronary intervention (PCI) cut the 1-year mortality rate by ~45% over that seen in patients treated only with aspirin. Giving clopidogrel prior to PCI was clearly very cardioprotective and loading with a PI is now standard of care.

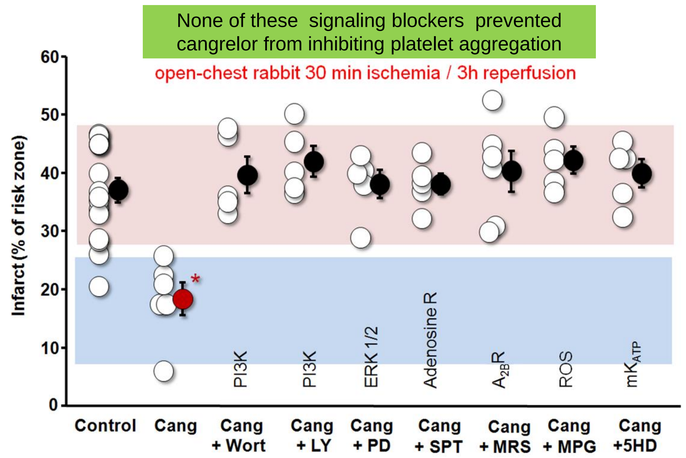
The magnitude of protection in the original ischemic postconditioning trial could not be reproduced



Favaretto et al. , Am J Cardiol. 2014; 114:946-52

FIG 2. In 2005 Staat et al. (red box) found that postconditioning patients with 4 short inflations and deflations of the angioplasty balloon at the onset of reperfusion caused a dramatic reduction of infarct size. Yet 12 subsequent studies including one from Staat's lab, failed to obtain a similar degree of protection. Less than a quarter of the patients in the study by Staat et al. had proper treatment with a PI while all of the subsequent studies had 100% PI coverage. We asked: Were **these disappointing results observed because the PI had already postconditioned the patients?**

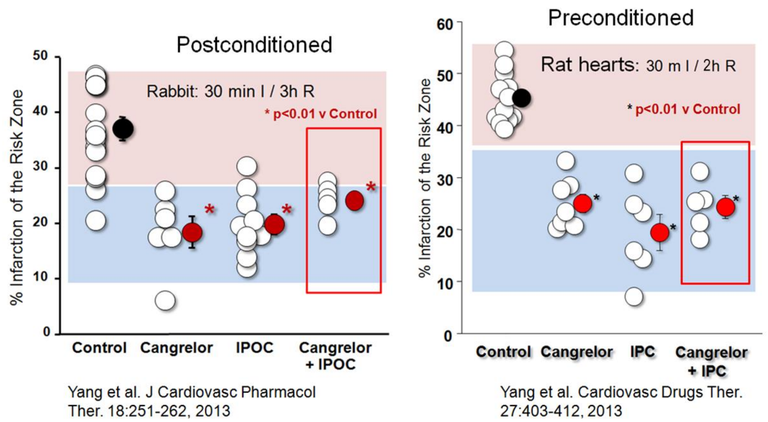
Cangrelor's protection depends on the same signaling components as ischemic postconditioning.



Yang et al. J Cardiovasc Pharmacol Ther. 18:251-62, 2013

FIG 3. When the PI cangrelor (Cang) was given iv 5 min prior to reperfusion, it cut infarct size in half. When we combined cangrelor with signaling inhibitors that block IPOC's protection, protection from cangrelor also disappeared. Similar behavior was seen with clopidogrel and ticagrelor. **We concluded that PI protect by activating conditioning's signaling pathway rather than by preventing thrombosis**.

Combining pre- or postconditioning with cangrelor offers no additional protection because all three use the same mechanism



Yang et al. J Cardiovasc Pharmacol Ther. 18:251-262, 2013

Yang et al. Cardiovasc Drugs Ther. 27:403-412, 2013

FIG 4. As we suspected, combining cangrelor with ischemic pre- or postconditioning offered no additional protection, presumably because all three protect through the same signal transduction mechanism. While PI loading has greatly decreased mortality and heart failure, it has hardly eliminated it. **Currently, even with PI treatment, 25% of patients with an LAD thrombus will die or develop heart failure in the subsequent year*. More protection is needed**.

*Chung et al. N Engl J Med 2015;373:1021-31

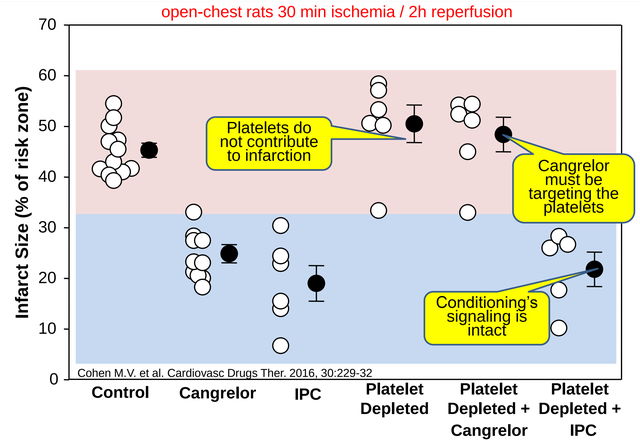


FIG 5. We depleted platelets in rats by >95% with an anti-platelet antibody. Platelet depletion did not reduce infarct size indicating that platelets do not contribute to infarction. Cangrelor no longer protected hearts indicating that the target $P2Y_{12}$ receptors must be on the platelet. Conditioning's signaling was intact as ischemic preconditioning could still protect the hearts of the platelet-depleted rats.

Can we find something that can add to the protection from PI?

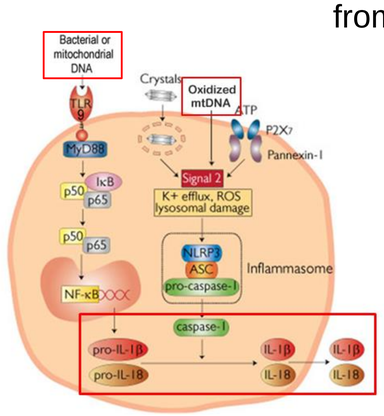


FIG 6. Conditioning reduces but does not abolish infarction. We propose that some of the cells that conditioning does not save are being killed by inflammatory pathways in the ventricular wall. We have focused on the TLR9 / NLRP 3 inflammasome pathway that is present in both cardiac muscle and fibroblasts. mtDNA released from damaged mitochondria causes production of cytotoxic interleukins. Caspase-1 plays a key role in their production. We propose that the interleukins kill the cell through pyroptosis causing them to release more mtDNA into the interstitial space where it can trigger lethal inflammation in neighboring cells.

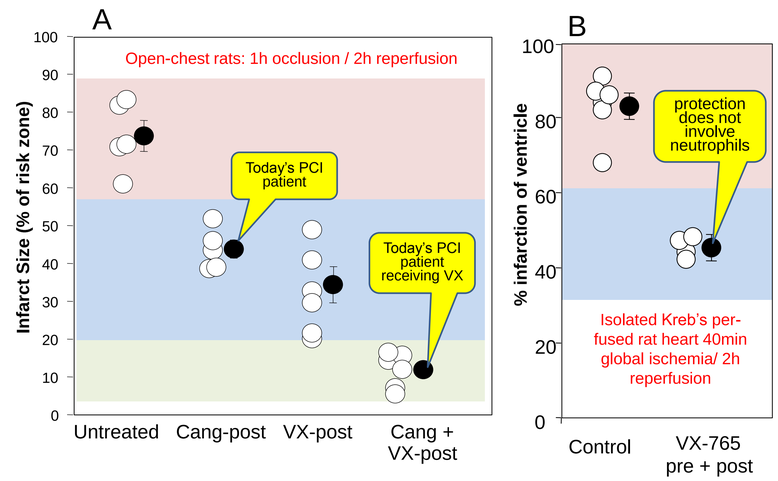


FIG 7. **A:The caspase-1 inhibitor VX765 given at reperfusion does provide protection that is additive to that from the PI cangrelor in a rat heart.** This suggests that caspase -1 activates pro-inflammatory cytokines involved in pyroptosis during reperfusion. B: Note that VX765 also protects the blood-free isolated heart indicating that neutrophils are not the source of these cytokines. VX765 has been approved for human trials

printed by MegaPrint Inc. www.postersession.com