Death, near death, and an antibiotic

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The study by Yamaki et al describes experiments performed in a NZW rabbit model of myocardial ischemia/reperfusion to test their hypothesis that the drug minocycline when administered after onset of the ischemic event limits infarct size by reducing the apoptotic process that leads to necrotic cell death. To demonstrate this therapeutic effect, they compare myocardial uptake of a necrosis avid radiotracer (In-111 labeled GSAO) in the drug-treated rabbits compared to placebo. This manuscript introduces several interesting subjects: the relationship between myocyte apoptosis and necrosis in evolving myocardial infarction, and the pleiotropic cytoprotective properties of a synthetic tetracycline antibiotic patented in 1961.

In animal models of early myocardial infarction and ischemia/reperfusion, as severe hypoxia releases damaging cytokines and free radicals, cells struggling to survive undergo an ordered energy requiring process of cell death called apoptosis. If oxygen is restored, the apoptotic process can reverse and some cells recover but without oxygen cells eventually sustain irreversible damage and die.^{1,2} The quantitative uptake of a radiotracer targeting apoptosis identifies cells that are destined to die plus cells that may recover depending on multiple factors in the local environment and therefore has the potential to over-estimate the extent of ultimate myocardial damage. Annexin A5 is a naturally occurring protein that binds phosphatidylserine, normally retained on the intracellular sarcolemmal membrane, but flipped to the extracellular membrane surface during an early stage in apoptosis. On the outer surface, PS is available

for binding to a radiolabeled annexin V protein. Labeled with ^{99m}Tc via the bifunctional chelator HYNIC (hydrazinonicotinamide), ^{99m}Tc HYNIC-Annexin A5 has proven sensitive for detecting apoptosis in myocardial ischemia and infarction in animal models and in pilot studies in patients.³⁻⁶ Quantitative uptake has correlated well with estimates of ultimate infarct size.⁷ Serial injections and imaging of ^{99m}Tc HYNIC-Annexin A5 can document therapeutic benefits of novel therapies to reduce myocyte apoptosis in acute myocardial infarction.⁸ A novel non-derivatized annexin V protein was developed by investigators at Stanford University to directly labeled with ^{99m}Tc at a single specific site at the N terminus sequence Ala-Gly-Gly-Cys-Gly-His (annexin V-128).^{9,10} This tracer ^{99m}Tc rhAnnexinV-128 is currently in early clinical trials for other applications in several countries (Advanced Accelerator Applications Saint Genis Pouilly, France).

Radiotracers specifically targeting necrosis have been developed including radiolabeled antimyosin antibody, ^{99m}Tc-pyrophosphate, and ^{99m}Tc-glucarate but each is limited by either a long half-life, or narrow window of positivity after the event.¹¹⁻¹⁴ The radiotracer used in this study, ¹¹¹In-GSAO (¹¹¹In 4-(N-(S-glutathionylacetyl) amino)phenylarsonous acid), is a tripeptide with a trivalent arsenic moiety which binds dithiols, molecules normally located intracellularly and absent from extracellular space. A cell membrane transferase upregulated in ischemic stress can transport GSAO metabolite into the cell but modified with a chelator to bind the reporter, the probe cannot cross the cell membrane. When the sarcolemma disrupts during irreversible damage, the tracer binds the dithiols and thereby signals irreversible cell death. In a previous preclinical study, Tahara and co-investigators first describe results of imaging acute myocardial infarction using ¹¹¹In-GSAO.¹⁵ They studied mice and rabbits. In mice, they showed very nicely by dual fluorescence, staining for annexin A5 on the cell membrane and staining for GSAO intracellularly.¹⁵ In rabbits with reperfused myocardial infarctions, they compared uptake of ¹¹¹In-GSAO with ^{99m}Tc-Annexin A5 against

histological staining for apoptosis using both TUNEL and caspase staining and found a good correlation in uptake between the two tracers and with the quantitative immunohistology.

In comparing annexin A5 and GSAO, the question must be asked: how important is it clinically to exclusively target necrosis and not apoptosis which might include some salvageable cells. If the extent of ischemic damage is the endpoint, then it probably does not matter. Other factors to consider are the several photopeaks and longer half-life of ^{111}In limiting dosimetry compared to $^{99\text{m}}\text{Tc}$ with shorter half-life and single photopeak. With these minor drawbacks to both tracers, we can conclude that they have equal applicability as non-invasive tools to follow the efficacy of a novel treatment to limit infarct size.

This brings us to the other interesting subject in this manuscript—the novel drug minocycline. Minocycline synthesized in Lederle Laboratories in 1961 came into commercial use in 1971. While current uses as an antibiotic include skin infections, Lyme disease, and MRSA infection, properties unrelated to its antibiotic function are of greatest interest.¹⁶ These properties include anti-inflammatory and anti-apoptotic properties as well as high lipophilicity allowing it to penetrate into the brain.¹⁶ Its early reported neuroprotective properties led to applications in neurological disorders. There are currently 232 registered clinical trials of minocycline in the US (ClinicalTrials.gov). The majority are in neurological and psychiatric disorders but also in asthma, renal disease, multiple sclerosis, rheumatoid arthritis, and ischemic stroke. Expanding from neuroprotection to possible cardioprotection, several preclinical studies administered minocycline in cardioplegia and in ischemia/reperfusion injury models and found beneficial results.^{17,18} Further studies investigated mechanisms and found that minocycline reduces/inhibits several important mediators of inflammation and apoptosis including high mobility group box 1 protein (HMGB1) an early mediator of inflammation and poly(ADP-ribose) polymerase-1 (PARP-1) extensively activated in ischemic tissue where it releases apoptotic-inducing factors.^{19,20}

In this current study, the investigators used micro-SPECT/CT imaging to demonstrate lower uptake of GSAO in risk regions of minocycline-treated rabbits compared to saline-treated control rabbits. They confirmed their *in vivo* findings with quantitative immunohistology and TUNEL staining on tissue from the risk region and remote myocardium. In comparison to previous preclinical studies of minocycline in myocardial ischemic injury, in the current study investigators administered the drug after the onset of ischemia, making the findings more clinically relevant. It would have been of great interest if the investigators had compared GSAO

and annexin A5 in the same animal model. If the inhibitory effect on apoptosis is the primary mechanism involved in its therapeutic effect in ischemia/reperfusion one would predict that both tracers would show similar results. As pointed out by the investigators under “Limitations,” to confirm cardioprotective effects of GSAO to salvage myocardium in acute MI, it is necessary to perform additional preclinical studies and follow the animals out to the healing phase of infarction, measure ultimate infarct size and LV function. To prove successful myocardial salvage, the minocycline group must have small infarcts and improved function compared to the placebo-treated animals.

Disclosure

The author has no conflicts to disclose.

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