

# Another promise against ischemia reperfusion injury: every success raises new questions

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The study by Bian *et al.* (1) raises many important deliberations. Briefly, they found that luteolin (LUT), a flavonoid found in many vegetables, fruits and seeds, inhibits ischemia/reperfusion induced myocardial injury (IRI) in rats.

The first question is: how relevant to the clinical situation are results on IRI alleviation in the experimental setting. The number of successful interventions in animals is legion. However, against these promising results, the very important position paper of the Working Group of Cellular Biology of the Heart of the European Society of Cardiology (2) should be remembered: the experts producing this paper concluded only 3 years ago that there is no effective proven therapy against IRI. It is widely recognized that it is not always possible to translate animal experiments into clinical therapy.

According to Bian *et al.* (1), LUT joins a long list of herbal medicines proposed to have wonder qualities, such as quercetin, curcumin, resveratrol, and many others. LUT is followed by an impressive list of references, supporting it as an antineoplastic and cardioprotective agent. In its latter role LUT has been given only in rodents, mostly on isolated cardiomyocytes (3,4) or hearts under Langendorff perfusion as in the present study (3,5) or both (4,6). Sun *et al.* (7) used the drug in diabetic rats undergoing coronary artery occlusion for 30 minutes followed by 3 hours of reperfusion. The drug was administered via tail vein injection, which makes it a feasible agent for employment in the clinical arena as an adjunct to primary percutaneous coronary intervention.

Many mechanisms have been proposed for the cardioprotective effects of LUT against IRI. Thus in previous studies, Qi *et al.* (8) from the same group found that LUT decreased both necrosis and apoptosis. As

regards apoptosis, they found that LUT upregulated Bcl-2, decreased the ratio of Bax to Bcl-2 and inhibited the activation of caspase-3. These findings are important since IRI engenders both necrosis and apoptosis (9). Xu *et al.* (3) again from the same group in a review recapitulate the action of LUT on the following processes, involved in apoptosis: upregulation of phosphorylated Akt, suppression of NF- $\kappa$ B activation, increase of Bcl-2, inhibition of caspase-8 and -3.

Fang *et al.* (4) additionally reported that LUT increased phosphorylated SERCA-2 and phospholamban as opposed to control, through the p13K/Akt pathway. Also, Wu *et al.* (6) found that it activates pERK and inhibits the JNK pathway. Reduction of JNK, and p38 MAPK are also reported by Cheng *et al.* (10) in rat cortical necrosis.

Sun *et al.* (7) also showed that LUT inhibits LDH release (suggestive of necrosis inhibition) and in addition to its decrease of the Bax to Bcl-2 ratio, it upregulated the anti-apoptotic proteins FGFR2 and LIF and increased BAD phosphorylation. It also inhibited MPO expression and inflammatory cytokine production, including IL-6, IL-1 $\alpha$  and TNF $\alpha$ . They also found that LUT decreased infarct size as measured by Evans Blue/TTC staining, and myocardial apoptosis, as assessed by TUNEL, while improving cardiac function as assessed by echocardiography, and the incidence of arrhythmia.

In the index study, Bian *et al.* (1) studied Langendorff perfused rat hearts, subjected to ischemia for 30 min and reperfused for 120 min, in which cardiac function was studied with a pressure sensor Millar catheter. They found that LUT pretreatment in the perfusate improved cardiac function and also had the following effects on microRNAs

(miRs): it downregulated the expression of 4 miRs, including miR 208b-3p and upregulated the expression of 26 miRs.

Additionally, they transfected the H9c2 cells with a specific miR-208b-3p mimic or a duplex RNA inhibitor to effectively overexpress or knock down miR 208b-3p.

Then after the cells were transfected with a siRNA to knock down Ets-1, they underwent an anoxia/reoxygenation protocol (A/R simulation of I/R).

They showed that in the cell culture LUT-pretreatment protected the cells against A/R injury, reversing the appearance of dead cells, while significantly downregulating miR 208b-3p. They verified that by administering the miR 208b-3p mimic; this miR was very robustly overexpressed with increased cell apoptosis, while it was greatly underexpressed when incubated with its inhibitor, with resulting decreased apoptosis.

Also, with the most effective Ets-1-SiRNA sequence, Ets-1 was strongly underexpressed, with an increase of H9c2 cells in the early phase of apoptosis.

The administration of LUT was accompanied by a corresponding decrease of pro-(caspase-3 and Bax) and increase of anti-(Bcl-2) apoptotic agents. The miR 208b-3p mimic decreased Ets-1 protein levels. These levels were however increased with LUT addition and miR-208b-3p inhibition.

These results give some important messages:

First, the decrease of miR-208b-3p expression by LUT is potentially of significance. This miR has been found to be increased after an acute myocardial infarction (11). The novel finding that its inhibition decreases apoptosis can have important clinical consequences. Equally importantly, miR-208b-3p has been associated with post infarct myocardial remodeling (REM), being one of the main miRs associated with this unfavorable course (12).

Thus, an agent, such as LUT could potentially affect a diminution of myocardial death both acutely, signifying cardioprotection, and chronically, signifying cardiopreservation: since LUT is easy to administer orally, a chronic experiment to evaluate its action against REM is in order.

When a miR is found effective in the living organisms, theories about its potential clinical value immediately arise. However, up to now the effectiveness of antagomirs given systematically is less than satisfactory (13); direct local infusion is needed. The same holds true for miR-mimetics, which need to be attached to lipoparticles or to viral vectors (5). Nanoparticle therapy clinically still belongs to

the future.

Thus, the use of a simple substance which can readily and robustly manipulate miR expression is very promising.

A second finding attributed to LUT should not be overlooked. Through its diverse reported actions it has been found to decrease the incidence of myocardial infarction in the Zutphen elderly study (14). If this—as logically expected—could also include re-infarctions, LUT could protect patient populations with coronary artery disease against REM by decreasing re-infarctions, such as Kjekshus (15) has advocated to happen with the use of statins.

The study of Bian *et al.* (1) brings into focus another problem. The authors state that LUT increases Ets1 protein levels. The same result was seen with transfection with a miR-208b-3p inhibitor. Thus they suggest that Ets1 is a target gene of miR-208b-3p. They pertinently state that there exist relatively few reports on the role of Ets-1 on cardiomyocyte apoptosis. However, Wang *et al.* (16) found that in hyperglycemia, HMGB1 induces apoptosis via an ERK/Ets-1 pathway; moreover, caspase is its direct target gene (17).

Ets-1 can induce inflammation and apoptosis in endothelial cells (18,19) but reduce apoptosis in vascular smooth muscle cells (20) which share many properties with cardiomyocytes. Thus, the role of Ets1 on cardiomyocytes needs further study.

Lastly, I must point out another source of perplexity: LUT is advanced as being both cardioprotective through its inhibition of apoptosis of cardiac cells and anti-neoplastic through its promotion of apoptosis and inhibition of angiogenesis (21).

Cai *et al.* (21) showed that in human pancreatic carcinoma cells it increased Bax and caspase-3 and decreased Bcl-2 expression. These are exactly the opposite to what Qi *et al.* (8) and Fang *et al.* (4) have found in the rat heart.

Is the Yin and Yang of Tao philosophy taken too far? Invariably, transcriptional pathways follow similar courses in biological processes, such as cardiac, neoplastic and diabetic system perturbations. Could the very intense proliferation rate of neoplastic cells and the marked quiescence of cardiomyocytes explain this difference? It is interesting that many cancer cells have higher than normal Akt (22) and Bcl-2 levels (23). Obviously these perplexing biological oddities should be studied further. They also often manifest downexpression of the apoptotic phosphatase PTEN (22), an antagonist of pro-survival PI3K which is highly expressed in cardiomyocytes, and inactivation of caspases (24). These differences may play a role.

Here, the author must confess that he is skeptical towards oriental herbal medications, which are advanced as a panacea (an all-curing medicine in ancient and modern Greek). Still one should never dismiss ideas which are not to his preference. It should not be overlooked that very recently, Eggebeen *et al.* (24) in JACC Heart Failure reported that beetroot juice has beneficial effects in older patients with heart failure and preserved ejection fraction. This is American herbal medicine at its best.

Thus, I would conclude that the elegant study of Bian *et al.* (1), a group consistently studying LUT over the years, preempts a host of clinical and theoretical considerations and definitely warrants further study, both acutely and also due to its ease of use against chronic cardiac REM, a subject on which this author has been working for over 20 years (25).

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