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CARDIOPROTECTIVE EFFECT OF NARINGENIN AGAINST ISOPROTERENOL-INDUCED MYOCARDIAL INJURY IN RATS

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Abstract

Naringenin is a citrus flavanone that has been tested repeatedly in rodent models of myocardial injury, yet the evidence is scattered across studies that differ in dose, timing and endpoints. This review gathers published work on its chemistry, disposition and effects against isoproterenol-induced myocardial injury in rats, the model most often used to screen cardioprotective compounds. Reported benefits include lower cardiac enzyme leakage, better antioxidant defences, reduced lipid peroxidation and less inflammatory signalling. The literature is largely preclinical, rarely blinded, and seldom tests naringenin after injury has started. No new experimental data are reported here; the review ends with the gaps that need closing before translation.

1. INTRODUCTION

Ischaemic heart disease remains the largest single cause of cardiovascular death worldwide, and the burden has kept rising as populations age [1]. Reperfusion therapy has saved many lives, yet a large share of survivors are left with scarred myocardium and a slow slide towards heart failure [2, 3]. That residual damage is why interest in adjunctive, cytoprotective drugs has never faded, even though few have made it from the bench to the bedside [4]. Against that background, a cheap and widely eaten dietary compound with antioxidant activity naturally attracts attention, though attention is not evidence. Heart failure after infarction also carries a heavy economic cost for health systems, which strengthens the case for prevention.

Two features of the problem deserve emphasis from the outset. First, the term cardioprotection is used loosely in the literature. In some papers it means lower enzyme leakage after a single toxic challenge, in others a smaller scar weeks later, and in a few it means better contractile function. These are not the same outcome, and a compound can improve one without touching the others [3, 4]. Second, the evidence for naringenin is entangled with that for naringin, its parent glycoside, because many authors have used the two interchangeably. Throughout this review the two are kept apart wherever the original papers allow it, since they differ in absorption and probably in the form that reaches the myocardium [5, 6].

Plant flavonoids have been a favourite source of candidates. They are abundant in the diet, they scavenge free radicals in simple chemical systems, and they modulate several signalling pathways at once [7, 8]. Naringenin (4',5,7-trihydroxyflavanone) is the aglycone of naringin, the bitter glycoside of grapefruit and other citrus fruits. Interest in it has grown steadily because it is cheap, widely consumed and seems to have a modest toxicity profile in animals [9, 10]. Particular attention is given to study design, because design determines how much a favourable result can really be believed.

Most of what is known about naringenin and the heart comes from rodents. The isoproterenol model, in which a synthetic catecholamine is given in excess to rats, is the workhorse of that literature because it is inexpensive, quick and reproduces many biochemical and histological features of infarction [11, 12]. This review brings together the published studies that used naringenin, or its glycoside naringin, in that model. It asks what the studies agree on, how far the findings can be trusted, and what would be needed to move the compound towards clinical testing. No new animal or laboratory data are presented.

2. NARINGENIN: CHEMISTRY AND DISPOSITION

Naringenin belongs to the flavanone subclass, which lacks the C2–C3 double bond found in flavones and flavonols. The saturated ring gives a non-planar molecule with a chiral centre at C2, and natural sources contain mainly the (2S) form [7]. Its antioxidant behaviour depends on the free hydroxyl groups at positions 4' and 5 and 7, but with only a single hydroxyl on the B ring it is a weaker radical scavenger than catechol-bearing flavonoids such as quercetin [13]. That matters when interpreting claims that rest on direct scavenging alone. Methoxylation or glycosylation of the same skeleton changes both potency and solubility, so structural analogues should not be assumed to behave alike. Chirality has rarely been considered in pharmacological studies, which usually test the racemate or an unspecified preparation.

In the diet, naringenin occurs mostly as naringin, which carries a neohesperidose sugar. Human gut bacteria remove the sugar, so the aglycone appears in plasma largely as glucuronide and sulphate conjugates rather than as free drug [5, 6]. After orange or grapefruit juice, plasma concentrations peak several hours after ingestion and stay in the low micromolar range at most, with free naringenin forming a small fraction of the total [5]. Oral bioavailability is therefore low, a point that is easy to overlook when high doses given to rats are compared with what a person could reach through food. Practically, that means a dose given to a rat is rarely comparable with a glass of juice, and supplement-grade doses sit somewhere in between.

Once absorbed, flavanones meet an extensive conjugation system. Glucuronidation and sulphation occur in the enterocyte and again in the liver, and the conjugates can be pumped back into the gut lumen or into bile, so enterohepatic recycling probably prolongs their presence [6, 14]. Colonic bacteria then break down the ring structure into smaller phenolic acids, some of which are themselves biologically active. For a researcher this complicates the question of which species actually acts on heart tissue. Free naringenin, its conjugates and the ring-fission products may each contribute, and measuring only the parent aglycone in plasma gives a narrow view of exposure [5, 6].

Formulation work has tried to overcome the poor solubility. Complexation with hydroxypropyl-β-cyclodextrin raised the absorption of naringenin in rats several-fold in one often-cited study [15], and similar approaches have since been explored with other carriers. Naringenin also interacts with intestinal and hepatic enzymes. Grapefruit constituents inhibit cytochrome P450 3A4 and intestinal transporters, and this is the basis of well-known juice-drug interactions [14]. Any cardiovascular drug taken alongside a naringenin-rich preparation could therefore be affected, which is relevant to the many patients who already take statins, calcium channel blockers or antiplatelet agents. Such conversions are estimates, not guarantees.

3. ISOPROTERENOL MODEL OF MYOCARDIAL INJURY

Isoproterenol is a non-selective β-adrenoceptor agonist. Rona and colleagues showed in 1959 that large doses cause an infarct-like lesion in the rat heart, and the model has been refined many times since [11, 16]. In many laboratories the drug is given subcutaneously on two consecutive days at a dose of the order of 85 mg/kg, though the schedule varies and some groups use lower doses over longer periods [12, 17]. The choice of schedule influences how much necrosis versus fibrosis is produced, which in turn affects how treatments appear to perform.

The injury has several overlapping drivers. Excessive β1-receptor stimulation raises heart rate and contractility, increasing oxygen demand while diastolic filling time falls and coronary perfusion suffers, so relative ischaemia develops [16, 18]. Intracellular calcium overload follows, mitochondria swell, and auto-oxidation of the catecholamine generates quinones and superoxide, adding oxidative damage [11, 19]. The picture is closer to a catecholamine-driven subendocardial necrosis than to the atherothrombotic occlusion seen in most human infarcts, which is a real limitation when results are extrapolated [4, 12]. Beta-blockers prevent much of the injury in this model, which confirms the receptor origin of the lesion and makes them a sensible positive control for any study of a new protective agent [16, 18].

The time course of the lesion helps to explain why timing of drug administration matters so much. Within minutes of a large catecholamine dose the heart rate climbs and arrhythmias can appear; within hours serum enzymes rise and the first necrotic foci develop in the subendocardium and the apex; over the following days inflammatory cells infiltrate and collagen is laid down [11, 12, 16]. A compound given before the toxin has the opportunity to act on every step in that sequence, whereas a compound given afterwards can only influence the later, slower stages. Interpreting a positive result without knowing which of the two designs was used is therefore risky [4, 17].

Typical readouts include serum creatine kinase-MB, aspartate aminotransferase, lactate dehydrogenase and, increasingly, cardiac troponins, all of which rise when membranes become leaky [20, 21]. Electrocardiographic changes such as ST-segment elevation are recorded in some studies, and histology shows oedema, inflammatory infiltrates and myocyte necrosis. Tissue assays for lipid peroxidation, superoxide dismutase, catalase and glutathione round out the usual panel [22, 23, 24]. Reporting standards have not always kept pace; calls for transparent reporting were made partly to address missing detail on randomisation, blinding and sample size in this type of work [25]. Authors should say so.

4. REPORTED CARDIOPROTECTIVE EFFECTS

The strongest signal across the naringenin and naringin papers is a reduction in biochemical markers of necrosis. Rats pretreated with naringin before isoproterenol showed lower serum levels of creatine kinase-MB, lactate dehydrogenase and transaminases than untreated injured controls, with improved electrocardiographic patterns and less lysosomal enzyme release [26]. The same group reported better lipid profiles and normalised activity of enzymes involved in cardiac lipid metabolism [27]. These findings are summarised with related reports in table 1. Reductions of this kind are usually

expressed as percentage recovery towards control values, and the extent of recovery differs between papers even when the same compound is used.

Table 1: Published findings with naringenin, naringin and related agents in isoproterenol and other myocardial injury models

Agent / model	Principal reported finding	Ref.
Naringin rat, isoproterenol	Lower serum CK-MB, LDH and transaminases; improved ECG pattern and lysosomal enzyme profile	[26]
Naringin rat, isoproterenol	Normalised cardiac lipids, lipoproteins and lipid metabolic enzymes	[27]
Naringin rat, ischaemia-reperfusion	Modulated heat shock proteins, Akt/eNOS and MAPK signalling with less injury	[28]
Lycopene rat, isoproterenol	Raised antioxidant enzymes and lowered lipid peroxidation in injured hearts	[29]
Natural products rat, isoproterenol	Broad review of protection by plant extracts and phytochemicals in this model	[17]
Citrus flavanones review	Link between flavanone intake, endothelial function and cardiovascular risk	[10]
Citrus flavonoids animal studies	Reduced hepatic lipoprotein secretion and atherosclerosis in animals	[30]
Nrf2 pathway general	Controls antioxidant and detoxifying enzyme transcription, a plausible target	[31]
Naringenin human volunteers	Low free plasma levels after juice; mostly conjugated metabolites	[5]
Naringenin rat, cyclodextrin complex	Several-fold higher absorption with hydroxypropyl-β-cyclodextrin	[15]

Antioxidant effects are reported just as consistently. In injured hearts, thiobarbituric acid-reactive substances rise while superoxide dismutase, catalase and reduced glutathione fall, and flavonoid pretreatment moves each of these values back towards control [22, 23, 24, 29]. The mechanism is probably not simple scavenging, given the modest radical-quenching power of naringenin noted above. Induction of endogenous defences through the Nrf2 pathway is more plausible, as Nrf2 controls transcription of many antioxidant and detoxifying enzymes [31, 32]. Direct proof of Nrf2 involvement in this particular model is still thin. Several investigators measured malondialdehyde alongside glutathione, giving a consistent picture of shifted redox balance across tissues [22, 29].

Inflammation is the part of the picture that is least well documented for flavanones in this particular model. Necrotic myocytes release danger signals that recruit neutrophils and macrophages, and the resulting oxidative burst adds to the primary injury [2, 18]. Descriptions of fewer inflammatory cells in histological sections of flavonoid-treated hearts are common, but they are usually secondary findings in papers whose main focus is on antioxidant enzymes [4, 26]. A study designed around inflammation, with cytokine profiling at several time points, would be more informative than the current scattering of single measurements, and would help to show whether any effect is upstream or downstream of oxidative damage. Reviews of cardioprotective natural products make the same point, noting that few include any direct measure of leukocyte activity [4].

Functional and structural endpoints are reported less often, but they give a fuller picture. Hearts from injured rats typically show wall thinning, myocyte swelling and loss of cross-striations, and flavonoid-treated animals are described as having better preserved architecture and less infiltration [17, 26]. Haemodynamic measurements, such as left ventricular developing pressure, are rarer still. The entries gathered in table 1 spread across several themes, among them necrosis markers, lipid metabolism, antioxidant defence and signalling, but none concerns contractile function (fig. 1). That imbalance is itself a finding about the state of the field. Such uncertainty argues for confirming the pathway with a second, independent method, such as gene silencing or a selective inhibitor, in the same model.

Inflammation and cell death pathways have also been examined. Naringin and related flavanones reduced the activation of mitogen-activated protein kinases and heat

shock protein changes in ischaemia-reperfusion hearts, and altered Akt and endothelial nitric oxide synthase signalling [28]. Reviews of citrus flavanones link these effects to lower expression of pro-inflammatory cytokines and to preserved endothelial function [10, 33]. Whether the same pathways operate when injury is caused by a catecholamine rather than by occlusion is assumed more often than demonstrated, and few papers use inhibitors or knockout animals to test it.

Lipid-lowering and vascular actions may add to direct myocardial effects. Citrus flavonoids regulate hepatic lipoprotein secretion and have been linked to reduced atherosclerosis in animal models [30]. Since isoproterenol-treated rats often show disturbed serum and tissue lipids, part of the benefit seen with naringin could come from this systemic route rather than from a purely cardiac action [27, 30]. Separating the two is difficult without dedicated experiments. Separating the two would need dedicated experiments, for example comparing hearts from animals with matched lipid levels, or isolated perfused hearts exposed to the compound directly.

Dose and duration receive less attention than they should. Many papers test a single dose of naringin or naringenin, so a dose-response relationship cannot be drawn, and the doses differ between laboratories by an order of magnitude [26, 27, 34]. Pretreatment periods range from a few days to several weeks. Longer pretreatment may allow adaptive induction of endogenous antioxidant enzymes, which would change the interpretation of the result, because the protection then reflects a primed tissue rather than an acute pharmacological action [29, 31]. Without direct comparisons within a single study, these possibilities cannot be separated. Direct comparison studies are needed, because the effects of the two routes are likely to differ between compounds.

A recurring design feature is worth stating plainly. Almost every study gives the flavonoid before and during the isoproterenol challenge, a preventive design. That is informative about mechanism, but it says little about treating an infarct that has already begun, which is the clinically relevant question [4, 35]. Studies that start treatment after the insult are scarce, and those that exist tend to show smaller effects. A sensible reader will therefore ask of each paper when the first dose was given, relative to the toxin, before accepting claims about treatment.

5. TRANSLATIONAL CONSIDERATIONS

Several reviews of naringenin have noted that human data are sparse. Clinical studies have mainly measured pharmacokinetics, tolerability and surrogate markers such as endothelial function or lipids, and sample sizes have been small [9, 10]. No trial has tested whether naringenin lowers infarct size or improves outcomes in patients, so the preclinical signal cannot be taken as proof of benefit. The gap between rat and person is wide for flavonoids, where metabolism, dose and exposure all differ [5, 36]. Any clinical programme would also have to define which formulation, dose and duration were being tested.

Pharmacists are also asked about citrus products by patients who read that grapefruit is good for the heart. The honest answer is mixed. Epidemiological studies of flavonoid intake suggest modest associations with lower cardiovascular risk, but these are observational and open to confounding by overall diet and lifestyle [8, 10]. The experimental work reviewed here does not alter that position, since doses, timing and species differ so widely from habitual intake. Counselling should therefore focus on the proven interaction hazard of grapefruit with some cardiovascular drugs, not on unproven protective claims [9, 14].

Safety questions are mostly about interactions. Because naringenin and related constituents inhibit CYP3A4 and drug transporters, co-administration with narrow-therapeutic-index drugs or with common cardiovascular medicines may alter exposure [14]. Supplement-grade products vary in content, and nobody has defined a safe upper intake for isolated naringenin. Pharmacists advising patients with cardiac disease should treat concentrated citrus flavonoid supplements with the same caution

as grapefruit juice until better data exist. Labelling and quality control of such products is not consistent between manufacturers, which complicates any advice given in the pharmacy. Such caution is routine in pharmacy practice and costs little.

Study design for any future clinical test would need careful thought. Early-phase work might focus on healthy volunteers receiving defined naringenin doses, measuring exposure to the aglycone and conjugates and any change in oxidative stress markers or endothelial function [9, 10]. Only after that would it make sense to test patients, perhaps those undergoing elective percutaneous coronary intervention, where a predictable and modest troponin rise offers a surrogate endpoint for periprocedural injury [3, 20]. Such a design is feasible and relatively inexpensive, which makes the absence of such trials somewhat surprising given the volume of laboratory work.

Methodological progress would help more than additional positive studies. Dose-response designs with at least three doses, a positive control such as a β-blocker, blinded histology, and outcome measures beyond serum enzymes would all strengthen the case [25, 34]. Delayed-treatment arms and chronic models, such as pressure overload or coronary ligation, would test whether the findings generalise. Improved delivery systems could close part of the exposure gap noted earlier, and are best evaluated by measuring tissue levels of the aglycone and its conjugates in the heart itself [15, 36]. Clinical pharmacists are well placed to help.

Finally, publication bias is hard to rule out. Nearly every published rodent study of a natural product in this model reports a favourable result, which is statistically unlikely if the true effect is modest [4, 12]. Registering animal protocols and publishing neutral findings would make the field more credible. Funders could reasonably require this for animal work on natural products. Journals could encourage this by accepting well-conducted neutral studies, and funders could ask that animal protocols be lodged in a public register before work starts. Registries for animal studies already exist in some countries.

6. LIMITATIONS AND FUTURE DIRECTIONS

This review is narrative and was not designed as a systematic synthesis, so the selection of papers may favour well-known groups and positive findings. Some of the most-cited work concerns naringin instead of naringenin, and the two are not interchangeable pharmacokinetically. Where the glycoside was used, this has been stated, and conclusions about the aglycone have been drawn cautiously [5, 26, 27]. The intention is to give an honest map of the evidence, with its soft spots marked. Several of the best-known papers concern naringin, which behaves differently in the gut. Readers may wish to treat the numerical details of individual studies as indicative only.

Reporting is the other area where improvement is straightforward. The survey of animal studies by Kilkenny and colleagues found that many papers omitted basic details such as the number of animals used, how they were assigned and whether assessors were blinded [25, 34]. Cardioprotection papers are no exception. Adoption of a checklist at submission, along with sharing of raw data, would let readers judge whether a reported effect is robust, and would make later meta-analysis possible for the flavonoid literature as a whole [4, 25]. Taken as a whole, the evidence would be strengthened mainly by better trial-style rigour in the animal laboratory. Transparent reporting of negative or neutral results would help considerably.

7. CONCLUSION

Published rat studies consistently show that naringin and naringenin soften the biochemical, oxidative and histological damage caused by isoproterenol. The most reliable findings are lower leakage of cardiac enzymes and a shift towards better antioxidant status, with signs that Nrf2, MAPK and Akt signalling may be involved [26, 28, 29, 31]. Yet most designs are preventive, mechanistic proof is limited, and reporting quality is uneven [25]. Taken at face value, the findings fit a consistent biological story, though a story is not the same as a demonstration.

For now, naringenin should be regarded as a promising experimental cardioprotectant rather than a therapy. Low oral bioavailability, interactions with drug-metabolising enzymes and the absence of human outcome data are the main obstacles [5, 9, 14]. Better-designed animal work, formulation research and carefully controlled early clinical studies are the sensible next steps, and pharmacists should advise patients with heart disease against assuming that high-dose citrus flavonoid products are harmless. Until such data exist, the compound is best described as a hypothesis worth testing.

8. ABBREVIATIONS

CK-MB-Creatine kinase-MB isoenzyme, CYP3A4-Cytochrome P450 3A4, ECG-Electrocardiogram, eNOS-Endothelial nitric oxide synthase, LDH-Lactate dehydrogenase, MAPK-Mitogen-activated protein kinase, Nrf2-Nuclear factor erythroid 2-related factor 2, ARRIVE-Animal Research: Reporting of In Vivo Experiments

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10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

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