Myricetin as an effective protectant against anthracycline-induced cardiotoxicity

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Abstract

Toxic side effects of anti-cancer drugs has become major concerns for the FDA, patients, and providers in recent years. One of the most critical side effects is cardiotoxicity caused by the use of anthracyclines, a class of chemotherapy drugs that has been on the market for over 50 years as part of combination therapies to treat various types of cancer. Conventional approaches to mitigate the anthracycline-induced cardiotoxicity have included: limiting lifetime cumulative doses, using less cardiotoxic analogs per treatment, and optimizing delivery strategies, all of which are only partial solutions at best and sometimes severely impact clinical outcome. An alternative and more promising therapeutic approach for reducing cardiotoxicity has been to supplement anthracycline with a cardioprotectant. To date, however, there has been only one such clinically available cardioprotectant, dexrazoxane (DEX), which is approved for limited use only in advanced or metastatic breast cancer patients and known to interfere with anticancer activity of anthracyclines. Novel cardioprotectants with improved efficacy and safety profiles not only have the potential to significantly reduce drug-induced cardiotoxicity risk, but could also enable higher anthracycline dosing and expanded usage for patients who are excluded from anthracycline therapy due to pre-existing heart conditions. To this end, we took a data-driven algorithmic approach to identify compounds that can mitigate anthracycline-induced cardiotoxicity and tested the compounds in human iPSC-derived cardiomyocytes. Using this computational approach, we successfully identified members of the flavonoid class of polyphenolic compounds as having varying degrees of cardioprotection capabilities. Among the flavonoids identified, myricetin (MYR) was one of the most effective cardioprotectants that effectively mitigated anthracycline-induced cardiotoxicity without interfering anti-cancer activity of an anthracycline (i.e., doxorubicin). Treatment of myricetin provided protection against doxorubicin-induced cardiomyopathy both in human iPSC-derived cardiomyocytes and a mouse model. Ejection fraction and fractional shortening were rescued by co-dosing myricetin with doxorubicin (DOX). Computational analyses of expression data indicated that myricetin is likely to be in synergy with doxorubicin, reversing expression of genes and pathways that are impacted by doxorubicin in the cardiomyocytes such as hypoxia, oxidative stress and mitochondrial function. We show that myricetin likely exerts its protective capability in a novel way by binding to TOP2B, in contrast to dexrazoxane that depletes the protein level.

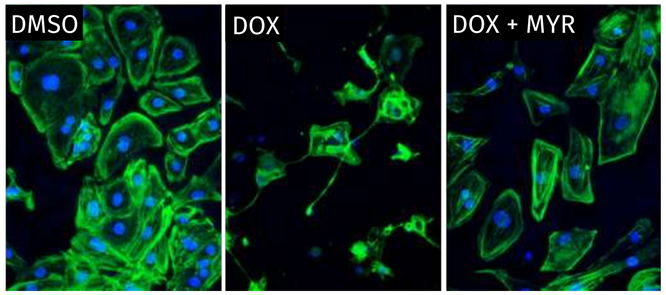
Identification of compounds by the Auransa SMarTR™ Engine

IDz	Compound Name	Max Effect (%)	EC50 (μM)	Toxicity ≤ 100 μM	Rescue
1	3,5,7,3',4',5'-hexahydroxyflavone (myricetin)	78	14.48	-	++++
2	3,7,3',4',5'-pentahydroxyflavone (robinetin)	64	12.62	-	++++
3	5,7,3',4',5'-pentahydroxyflavone (tricetin)	56	17.19	*	+++
4	3,5,7,3',4'-pentahydroxyflavone (quercetin)	58	20.5	*	++
5	3,7,3',4'-tetrahydroxyflavone (fisetin)	36	16.32	*	++
6	7,3',4',5'-tetrahydroxyflavone	71	17.13	-	+++
7	3,5,7,4'-tetrahydroxyflavone (kaempferol)	46	26.01	-	++
8	3',4',5'-trihydroxyflavone	64	43.01	-	+
9	5,7,3',4'-tetrahydroxyflavone (luteolin)	62	9.67	*	+++
10	3,7,4'-trihydroxyflavone (resokaempferol)	27	3.26	*	+
11	7,3',4'-trihydroxyflavone	24	6.25	*	+
12	3,3',4'-trihydroxyflavone	16	6.43	*	+
13	5,7,4'-trihydroxyflavone (apigenin)	†	-	-	-
14	flavone	†	-	*	-
15	chromone	†	-	-	-
16	dihydrorobinetin	53	14.02	-	+++
17	3'-O-methylmyricetin	76	58.7	-	+
18	4'-O-methylmyricetin	68	48.6	-	+
19	3',5'-O-dimethylmyricetin	†	-	*	-
20	3',4',5'-O-trimethylmyricetin	†	-	*	-

Cardioprotective effect of flavonols

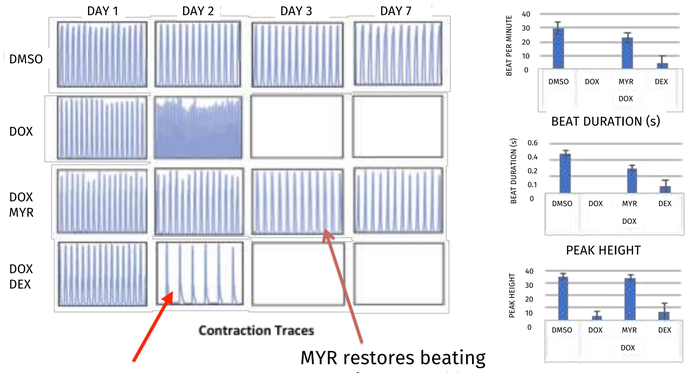
Functional rescue of DOX-induced cardiotoxicity by MYR

MYR prevents sarcomere disruption in cardiomyocytes



Human iPSC-derived cardiomyocytes were immunostained with antibody against Cardiac Troponin T (green) to detect sarcomeric organization

MYR restores normal beating of cardiomyocytes

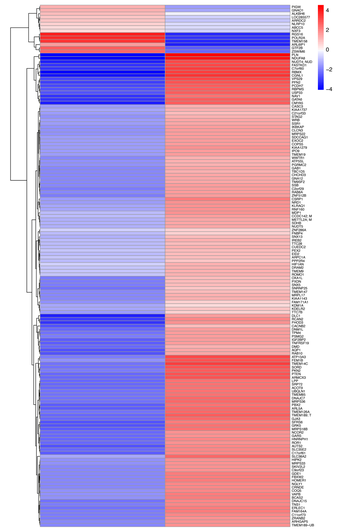


Standard of care, dexrazoxane does not rescue cardiomyocytes

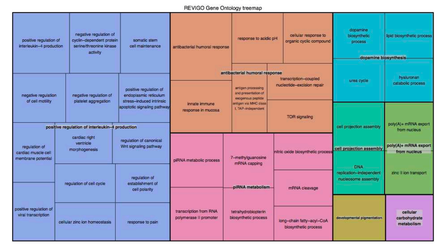
MYR restores beating cessation caused by DOX

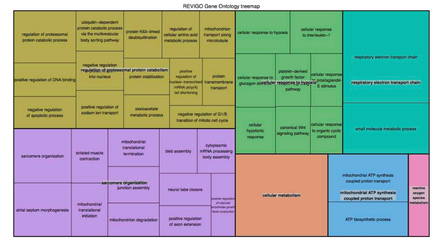
Mechanism of action of cardioprotection by MYR

MYR "reverses" transcriptional footprint of DOX



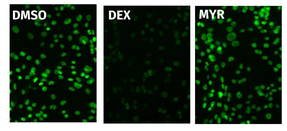
Pathways that are reversed by MYR

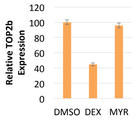




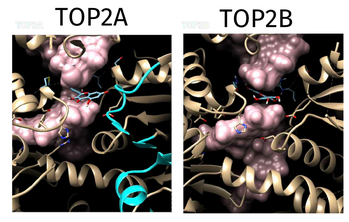
Key pathways restored by MYR

Effect of MYR on topoisomerase 2 (TOP2)

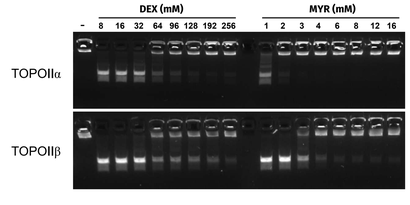




Unlike DEX, MYR does not decrease TOP2B protein levels

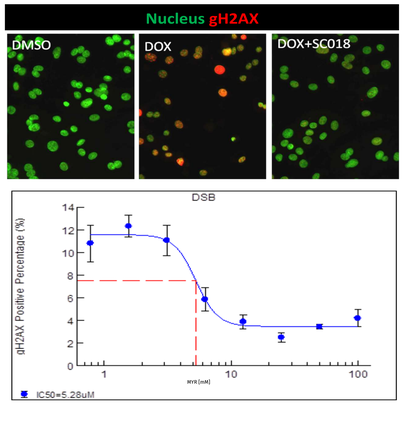


Molecular docking of MYR

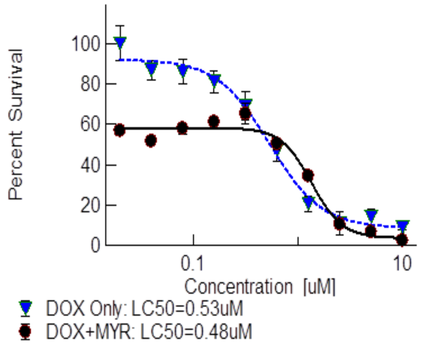


Catalytic inhibition of TOP2A and TOP2B by DEX or MYR

DNA damage (γ-H2AX) induced by doxorubicin is prevented by MYR

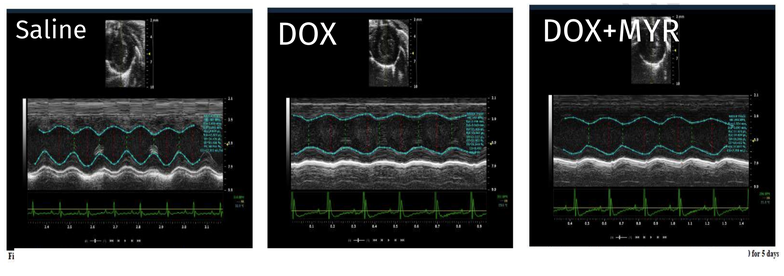


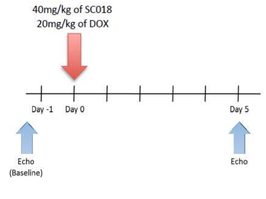
MYR does not interfere with anti-tumor activity *in vitro*

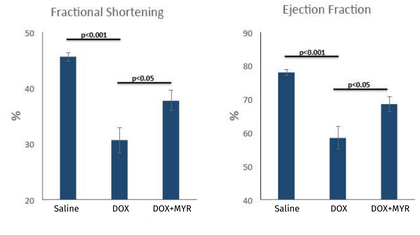


*Breast Cancer Cell Line

MYR improves DOX-induced heart dysfunction in mice







CONCLUSIONS

1. A family of flavonoids have varying degrees of cardioprotection against doxorubicin-induced cardiotoxicity.
2. The most potent of the flavonoids is Myricetin (MYR) as shown by *in vitro* and *in vivo* rescue studies.
3. MYR does not reduce the toxicity of doxorubicin in cancer cell lines.
4. MYR reverses the cardiac toxicity molecular signature in the heart.
5. Pathways that are "corrected" by MYR include oxidative and cellular stress, mitochondrial function and RNA splicing.
6. We hypothesize that MYR acts by binding to the TOP2B protein, a topoisomerase that is highly expressed in the heart.