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M2-like Macrophages Exert Cardioprotective Effects in Doxorubicin-induced Cardiotoxicity

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Background

- Cardiotoxicity, a form of severe heart damage, can result from chemotherapy such as Doxorubicin, an anthracycline drug that is most effective for breast cancer.
- Autophagy is a highly regulated intracellular metabolic process and has recently emerged as a potential protective mechanism against cardiotoxicity and a possible therapeutic target to reduce Doxorubicin-induced cardiotoxicity (PMID: 35817962).
- Previous literature further suggests that M2 macrophages have a cardioprotective effect (PMID: 34760943) or direct improvement in energetics (PMID: 35410296).

Research Objectives

The purpose of our study was to investigate the effect of M2 macrophages on autophagic flux in the setting of in-vitro Doxorubicin-induced H9C2 cardiomyocyte damage.

We hypothesize that M2 macrophages can exert cardioprotective effects in the heart through modulating autophagy.

Methods

We induced the growth of M2-like macrophages through application of macrophage colony-stimulating factor (M-CSF) and cytokine interleukin 4 (IL-4) to bone marrow collected from female Balb/C mice over a culture period of 7 days. After 7 days of culture, the macrophages were collected, pelleted, and readjusted to a concentration of 1 x 10^6 cells/mL. Cells were immediately used for assays or cryopreserved in complete growth media containing 10% of DMSO. Differences in the collection method resulted in different viability levels of cells (Table 1).
The M2-like macrophages were co-cultured for 24 hours with H9C2 cells, that had either no Doxorubicin exposure, 30-minute, or 24-hour Doxorubicin exposure.

Table 1. Viability of M2 Macrophage Cells Cultures

	Mean % Viable	Mean % Viable ± SD
Culture #1	60.6%	60.6% ± 3.5%
Culture #2	82.6%	82.6% ± 6.1%

After 24 hours of co-culture, cells were treated with autophagy-detecting nanoparticles (ADN) manufactured in-lab and Cy5.5, Annexin V , and Sytox Blue. FACS was performed on the Cytek® Aurora. FACS analysis was performed using FlowJo™ software.

Figure 1. H9C2 cells with AnnexinV fluorescence quantifying apoptotic signals from H9C2 cells

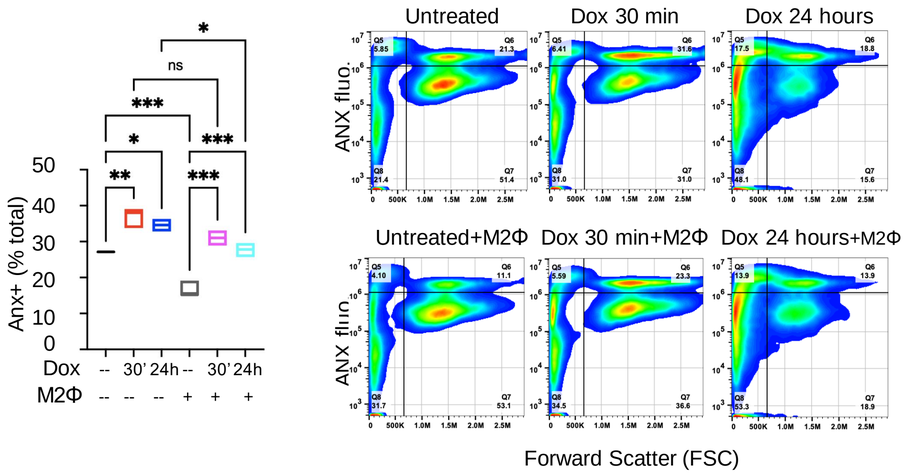

Figure 1. H9C2 cells with AnnexinV fluorescence quantifying apoptotic signals from H9C2 cells.

Figure 2. H9C2 cells with (ADN) fluorescence indicating autophagic signals from H9C2 cells

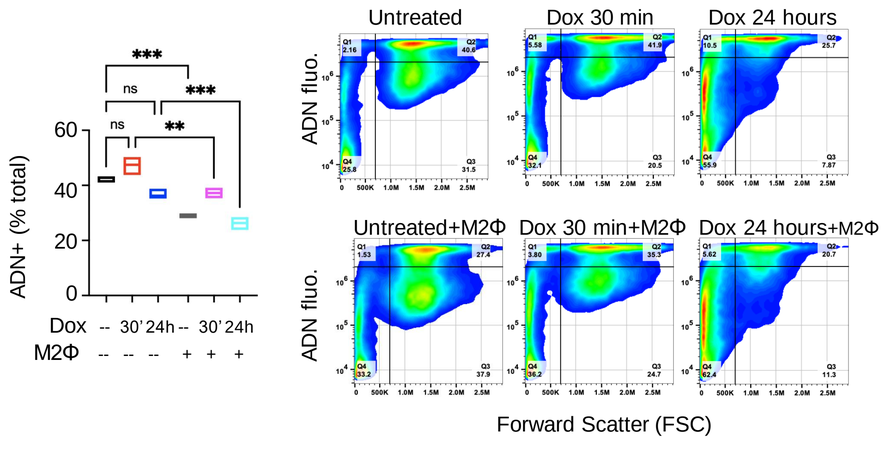

Figure 2. H9C2 cells with Autophagy-detecting nanoparticle (ADN) fluorescence indicating autophagic signals from H9C2 cells.

Figure 3. Doxorubicin (Dox) uptake by H9C2 cells

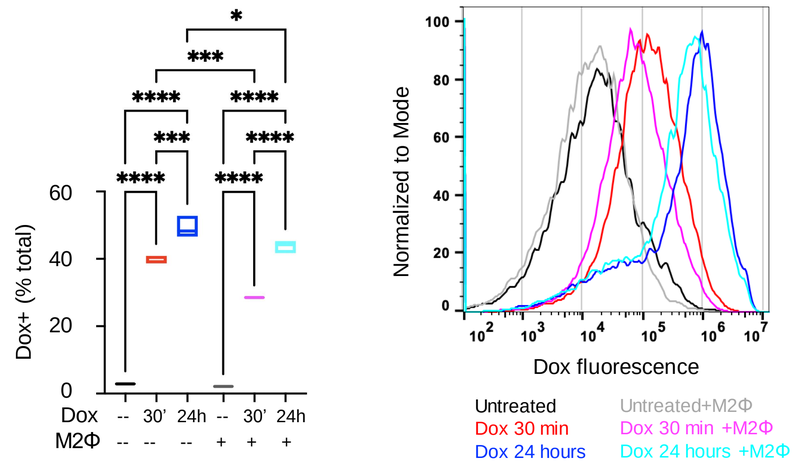

Figure 3. Doxorubicin (Dox) fluorescence in co-cultured and non co-cultured samples at various time points to quantify Doxorubicin uptake by H9C2 cells.

Results and Conclusions

- M2 macrophage co-culture significantly decreased apoptosis and autophagy in cardiomyocytes treated with Dox for 30' or 24 hours.
- Dox reduced cell size in all samples treated with Dox, which is not reversed by M2 macrophages.
- The presence of M2-like macrophages significantly reduced Dox uptake and retention in all the samples, presumably through previously published Doxorubicin-efflux mechanism via intracellular myoglobins.
- M2-like macrophages may interact with cellular components to decrease uptake and retention of Doxorubicin in H9C2 cardiomyocytes as a protective mechanism (PMID: 34760943).

Future Directions

- FACS to confirm M2 differentiation using anti-CCR2-APC and anti-CD206-PE markers and dual-staining for necrotic death and apoptosis.
- Repeat experiments with just the conditioned media -> if soluble factors can replicate the protective effects of the macrophages.
- Assess if results are translatable in-vivo using Doxorubicin-injected tumor mice.

Acknowledgements

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