Activation of invariant natural killer T cells ameliorates doxorubicin-induced cardiotoxicity in mice

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Objective: Doxorubicin (DOX) is one of the most important anticancer agents and widely used to treat cancers but clinical utility of DOX is limited for its dose-dependent cardiotoxicity. The precise mechanism of DOX-induced cardiotoxicity is still not fully understood but it has been reported that cardiac inflammation is involved in the cardiotoxicity. Invariant natural killer T (iNKT) cells, a unique subset of T lymphocytes that recognize glycolipid antigens and secrete a large amount of both Th1 and Th2 cytokines on activation, have been shown to play crucial roles in the regulation of immune responses. However, it remains unclear whether iNKT cells are involved in DOX-induced cardiotoxicity.

Methods and results: Male C57BL/6J mice were administered DOX (20mg/kg body weight; n=28) or vehicle (Vehicle; n=6). DOX-administered mice were further divided into 2 groups; those treated with α-galactosylceramide (αGC, 0.1μg/g body weight; DOX-αGC; n=14), which specifically activates iNKT cells, or those treated with PBS (DOX-PBS; n=14) by intraperitoneal injections (twice; 4 days before and 3 days after DOX administration).An echocardiography conducted at 14 days after DOX/Vehicle administration revealed that LV fractional shortening was significantly reduced in the DOX-PBS compared to the Vehicle (49.3±0.8% vs. 59.2±1.7%, $P<0.05$), and this decrease was completely attenuated in the DOX-αGC (57.7±1.3%, $P<0.05$ vs. DOX-PBS)without affecting LV end-diastolic diameter. Flow cytometric analysis revealed that the ratio of iNKT cells to mononuclear cells infiltrated into the heart tissue was significantly increased in the DOX+αGC compared to the Vehicle and the DOX+PBS (1.00±0.09% vs. 0.54±0.09% and 0.71±0.07%, $P<0.05$). Immuno-histochemistry revealed that the infiltration number of Iba1+macrophages in the heart tissue was significantly elevated in the DOX+αGC compared to the Vehicle and the DOX+PBS (55.4±3.2 cells/mm^2 vs. 21.7±2.0 cells/mm^2 and 37.5±5.9 cells/mm^2, $P<0.05$) The ratio of fibrosis area to the heart tissue was markedly higher in the DOX-PBS than in Vehicle (4.3±0.5% vs. 2.2±0.1%, $P<0.05$), and this increase was completely attenuated in the DOX-αGC (2.8±0.1%, $P<0.05$ vs.DOX-PBS).Real-time PCR analysis revealed that mRNA expressions of M2 macrophage markers (Arginase 1 and Retnla) and IL-4 were significantly enhanced in the DOX+αGC compared to the DOX+PBS (Arginase 1: 2.5±0.4 vs. 1.6±0.3 [relative ratio to the Vehicle], P=0.08; Retnla: 2.4±0.5 vs. 1.1±0.2 [relative ratio to the Vehicle], $P<0.05$; IL-4: 1.0±0.3 vs. 8.94±2.8 [relative ratio to the DOX+PBS], $P<0.05$), while those of M1 macrophage markers (iNOS and MCP-1) did not change among all groups.

Conclusions: Activation of iNKT cells ameliorates DOX-induced cardiotoxicity in mice via enhanced M2 macrophage polarization with the upregulation of IL-4 and reducing cardiac fibrosis. iNKT cell activation can be a novel preventive strategy against DOX-induced cardiotoxicity.