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Cardiotoxicity of anthracycline: Novel approach through down regulation of TLR-3 via TRAF/MAPK signaling pathway

Guowei Liang[1], David Lazenby[1], Jonathan D Groden[1], Xing Liu[2], Suzan N Pretlow[1], Mark H Mertens[1*]

Abstract

Cardiotoxicity is one of the most important complications doxorubicin (DOX) and its pathomechanisms are not completely elucidated. We hypothesize that signaling via toll-like receptor (TLR)-3, a receptor that is activated upon binding of double-stranded nucleotides, might play a crucial role in the pathogenesis of cardiac-toxicity following DOX treatment. Male adult C57BL6 wild-type mice and TLR-3 knock-out (-/-) mice were subjected to 20 mg/kg; administered intraperitoneally. TLR-3 down-stream signaling was activated in WT mice lead to strong pro-inflammatory response with significant monocyte cells invasion. In contrast, this effect was attenuated in TLR-3-/- mice. Moreover, the TLR-3 activation resulted in cardiac damage that was associated with significantly reduced LV function and increased monocyte chemoattractant protein-1 (MCP)-1 expression in WT mice. This finding was confirmed by increased MAPK and TRIF protein expression in WT mice. This study confirmed that the absence of TLR-3 is associated with lower heart injury and maintained LV function. Thus, we conclude that TLR-3 seems to participate in the pathogenesis of cardiotoxicity of DOX.

Keywords: TLR-3, Cardiotoxicity, Doxorubicin, MAPK, TRIF

*Corresponding author email: MarkH@gmail.com
[1]Department of Cell Biology, Rutgers University, Piscataway, New Jersey
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Introduction

Breast cancer is one of the most common malignant tumors that endanger the health of women [1], with an estimated 252,710 new cases in the United States in 2012 [2] and 26,000 new cases in Canada in 2012. A total of 1 in 8 women in the United States will have breast cancer in their lifetime [3]. Approximately 1.5 million women worldwide develop breast cancer every year, and ~500,000 women succumb to breast cancer.

Early detection and advances in screening have led to a 5-year survival rate approaching 90%, and in the United States, almost 3 million people are living with a prior diagnosis of breast cancer [4]. The treatment options usually include a combination of surgery, cytotoxic chemotherapy, radiation therapy and molecularly targeted endocrine therapy, depending on the type of breast cancer diagnosed [5].

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At present, anthracyclines and taxanes are the two major classes of drugs for breast cancer treatment. Anthracyclines are among the most commonly used and effective drugs in breast cancer treatment.

In the past 30 years, they have become an important component of adjunctive and palliative therapy for breast cancer. Anthracyclines belong to a class of antineoplastic antibiotics, which interfere with cell replication by acting on the DNA at several levels, showing an effect in every phase of the cell cycle [6]. Doxorubicin and epirubicin are commonly used in clinical practice. Administration is only via an intravenous infusion; metabolism is hepatic and excretion via the bile route, while urinary elimination accounts for approximately 1/6 of the total amount. Although anthracyclines exhibit a range of toxic effects, including transient myelosuppression, mucositis and hair loss [7], cardiotoxicity still remains a prominent risk since it may be permanent and progressive, leading to multimorbidity and severely impacting quality of life in patients with breast cancer [8].

Acute cardiotoxicities, as well as the potential effect of cumulative doses, increasing the risk of congestive heart failure, are crucial and should be considered when deciding on a treatment strategy [9]. The present study presents a concise review of the literature, focusing on anthracycline-induced cardiotoxicity, its pathophysiology, prevention, monitoring and outcomes. A comprehensive literature review has been conducted. A bibliographic search was performed in the Cochrane, Medline, PubMed, Scopus, Web of Science and Scielo databases. Databases were searched systematically using the following key words: Anthracyclines, breast cancer, risk factors, prevention and treatment, combined with cardiotoxicity, cardiomyopathy or heart failure [10-12].

Limiting cumulative dose

Maximum doses of anthracyclines have been implemented. Maximum cumulative doses of 400–550 mg/m^2 doxorubicin and 900 mg/m^2 epirubicin are currently recommended [13].

Prolonging administration time

Cardiac toxicity can be reduced by weekly low doses and a prolonged continuous infusion time (24–96 h). An analysis showed that slow intravenous infusion of doxorubicin (>6 h) over a prolonged period could reduce the risk of clinical heart failure and subclinical myocardial injury [14]. The cardiotoxicity of the weekly treatment regimen was less than that of the usual 3-week treatment regimen (0.8 vs. 2.9%) [15].

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Methods and Material

20 mg/kg; intraperitoneally) (WTDox and TLR3$^{-/-}$Dox) at a dose shown to be cardiotoxic, or with the same volume of saline (WT and TLR3$^{-/-}$). Five days after Dox injection, mice were haemodynamically characterized. Finally, hearts were excised and prepared for molecular, biological and immuno histochemical analyses as described below. To investigate the early regulation of nuclear factor kappa B (NF-kB) in Dox-induced cardiomyopathy, we performed additional experiments (see below) using cardiac tissue from WT and TLR3$^{-/-}$ mice one hour after Dox-injection. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Surgical procedures

Animals were anaesthetized (thiopental 125 µg/g; i.p.), intubated and artificially ventilated. As described previously, a 1.4 F microconductance pressure catheter (ARIA SPR-719; Millar-Instruments, Inc., Texas, USA) was positioned in the LV for continuous registration of LV pressure-volume (PV) loops in a closed-chest model.

Haemodynamic measurements

Systolic function was quantified by LV end-systolic pressure (LVP, mm Hg), and d*P* /d*t* max (mm Hg/s) as an index of LV contractility. Diastolic function was measured by d*P* /d*t* min (mm Hg/s), and the end-diastolic-pressure-volume-relationship (stiffness, mm Hg/εl), determined from an exponential fit to the end-diastolic pressure-volume points. Global cardiac function was quantified by ejection fraction (EF, %), stroke volume (SV, εl), heart rate (HR, beat/min), and cardiac output (CO, ml/min).

Isolation of mouse cardiomyocytes

Hearts of WT, WTDox, TLR3$^{-/-}$ and TLR3$^{-/-}$Dox mice (*n* =8 per group, anaesthetised with Trapanal, 667 mg/kg, i.p.) were excised, mounted on a Langendorff-apparatus and perfused with modified Krebs-Henseleit-buffer containing 110 mM NaCl, 2.6 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 11 mM Glucose, 25 mM Hepes, pH=7.4. For digestion, collagenase type II (Worthington, Lakewood, USA) and 33 mM $CaCl_2$ were added. Perfusion took place over 30 min at a constant pressure of 65 mm Hg. The media were maintained at 37 °C and saturated with oxygen. Ventricles were minced in the same buffer, dispersed for a further 10 min and filtered through a mesh (200 εm) to remove undigested tissue. Viable myocytes were separated by centrifugation in a 4 % bovine serum albumin gradient, immersed into liquid nitrogen, and used for further measurements.

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Quantitative real-time reverse transcriptase PCR (TaqMan®)

For RNA preparation, cardiac tissue samples were homogenized and total RNA was prepared using *TRIZOL* ® reagent (Invitrogen) according to the manufacturer's protocol. Quantitative real-time RT-PCR (qPCR) was performed on an ABI PRISM 7700 sequence detection system (Applied Biosystems). For detection of murine ppET-1, Primer and probe oligonucleotides (forward primer: 5′-TGTTCGTGACTTTCCAAGG-3′; reverse primer: 5′-AGCTCCGGT GCTGAGTTCGG-3′; probe: 5′-6FAM-CTCCAGAAACAGCTGTC-3′) were designed as based on the murine ppET-1 cDNA sequence (accession number U35233). For normalization, 18S rRNA was purchased by a commercial *TaqMan PreDeveloped Assay Reagent* (Applied Biosystems). Measurement of GATA-4 mRNA levels was performed using a commercially available kit (Applied Biosystems).

Lipid peroxidation in cardiomyocytes and the left ventricle

Cardiomyocyte and left ventricular lipid peroxidation were measured using the commercially available colorimetric assay kit Bioxytech® LPO-586 (Oxis International). Briefly, 150 εl of protein extracts were used for measurement of malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) considered as indicators of lipid peroxidation as described in the manufacturer's directions for use.

Western blot analysis

Western blot analyses were performed using primary antibodies raised against Bax (Santa Cruz, diluted 1:500), Bcl-2 (Santa Cruz, diluted 1:500) and GATA-4 (Santa Cruz 1:1000). GAPDH (Biodesign International, diluted 1:1500) served as loading control. Detection of the signals was performed using the *LumiPhos* ™ reagent (Pierce) and chemiluminescence was detected using x-ray films.

Statistical analysis

Student's *t*-test was used to analyze data for significant differences. Values of $P < 0.05$ were regarded as significant.

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Results

LV dysfunction is a hallmark of acute Dox-induced cardiotoxicity. We measured LV function by assessing pressure-volume loops using a microconductance catheter. Haemodynamic data are shown in Table 1. No parameter of systolic, diastolic, and global LV function was found to differ between WT and TLR3$^{-/-}$ mice. Five days after Dox injection, WTDox mice displayed significantly impaired systolic (LVP −31%, d*P* /d*t* max −45%, *P* <0.01), diastolic (d*P* /dtmin −41%, stiffness +300%; *P* <0.05) and global (EF −27%, SV −39%, HR −18%, CO −54%; *P* <0.05) LV function. In contrast, among TLR3$^{-/-}$Dox mice, parameters of systolic (LVP +27%, d*P* /d*t* max +44%, *P* <0.05), diastolic (d*P* /d*t* min +30%, stiffness −63%; *P* <0.05), and global (EF +29%, SV +57%, Co +84%; *P* <0.05) LV function were significantly improved compared to the WTDox mice.

Table 1.
Haemodynamic parameters

	WT	TLR3-/-	*P* *	WTDox	*P* #	TLR3-/- Dox	*P* 0
Heart rate (bpm)	290±35	326±76	1.0	238±31	<0.05	246±65	1.0
LV pressure (mm Hg)	91±7	83±6	0.22	67±8	<0.001	85±10	<0.05
d*P* /dt max (mm Hg/s)	5795±1102	5207±982	1.0	3163±596	<0.001	4546±959	<0.05
Stroke volume (ml)	38±3	37±6	1.0	17±6	<0.001	36±7	<0.05
Cardiac output (ml/min)	11.0±1.6	11.9±±4.6	1.0	5.1±1.7	<0.001	9.4±3.6	<0.05
Ejection fraction (%)	56±9	53±12	0.65	44±15	<0.05	53±15	1.0
LV end-diastolic pressure (mm Hg)	7±2	5±1	1.0	8±4	1.0	10±7	1.0
d*P* /dt min (mm Hg/s)	−4945±728	−4441±504	0.8	−2757±601	<0.001	−3594±847	<0.05
Stiffness (in mm Hg/ml)	0.04±0.005	0.05±0.003	0.9	0.16±0.027	<0.001	0.056±0.013	<0.05

TRAF/MAPK protein expression and cardiac cell infiltration

To characterize the cardiac inflammatory response, we determined cardiac TRAF/MAPK expression and cell infiltration of CD3-, CD4- and CD11b-positive cells; this did not differ between untreated WT and TLR3$^{-/-}$ mice. Dox injection led to an increased (1.9-fold; *P* <0.05) expression of TRAF/MAPK expression in WT mice compared to untreated controls. TLR3$^{-/-}$Dox mice did not display a significant difference in comparison to untreated TLR3$^{-/-}$ mice (Fig. 1). In addition, there was a significant correlation between decreased parameters of systolic LV function and cardiac TRAF/MAPK expression (d*p* /d*t* max: *P* =0.0046, LVP: *P* =0.0052).

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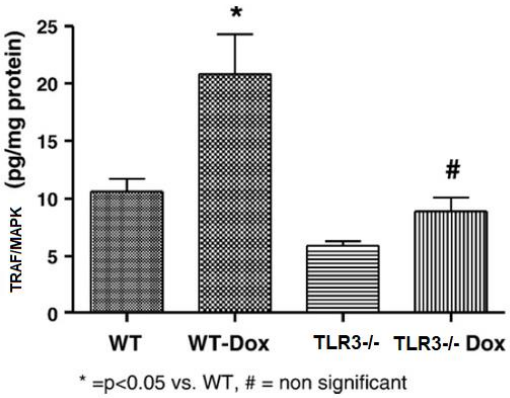

Figure 1.

Cardiac protein expression of TNF-α in doxorubicin-induced cardiomyopathy. Protein expression was measured using ELISA. Data are expressed as mean±SEM. * = P <0.05; # = non-significant vs. TLR3$^{-/-}$ (n =8 per group). WT, wild-type; Dox, doxorubicin; TLR3, Toll-like receptor 3.

Endothelin-1 expression

LV dysfunction leads to neurohumoral activation. Hence, we measured cardiac ET-1 mRNA- and protein expression five days after Dox injection using molecular biological techniques. Five days after Dox administration, WT mice displayed a significant increase in ET-1 mRNA (5.2-fold; P <0.01) and protein (2.1-fold; P =0.02) content when compared to WT controls. In comparison with untreated TLR4$^{-/-}$ mice, Dox administration into TLR3$^{-/-}$ mice did not result in any significant enhancement.

Discussion

Here we show for the first time that TLR3 contributes to cardiac inflammation, oxidative stress, and apoptosis as well as LV function in experimental, Dox-induced cardiomyopathy.

In agreement with others, WTDox mice were found to display severe systolic and diastolic LV dysfunction, resulting in impaired cardiac output as measured by the assessment of pressure-volume loops in vivo [16].

In TLR3$^{-/-}$Dox mice, global LV function as indexed by stroke volume and cardiac output was improved as a result of enhanced systolic and diastolic performance. This was associated with a reduction in neurohumoral activation, with a reduction in cardiac ET-1 which is known to contribute to the development of heart failure [17]. Thus, we conclude that TLR3 plays a pivotal role in LV dysfunction due to Dox-induced cardiomyopathy [18]. To further analyze the mechanisms involved, we characterized cardiac oxidative stress, inflammatory response, and apoptosis, which are all known to be relevant in this disease.

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Oxidative stress can directly damage cells, trigger cytokine expression, increase leukocyte chemotaxis, and initiate complement activation [19] In agreement with others 20], we found that lipid peroxidation and protein expression of nitrotyrosine as an index of oxidative stress were markedly increased in cardiac tissue of WTDox mice when compared to that of WT controls.

Since lipid peroxidation is active in a variety of cell types possibly present in the LV, we measured lipid peroxidation activity in myocytes isolated from WT and TLR3$^{-/-}$ mice which were treated with doxorubicin in order to identify cardiac specific stress. In line with our findings from LV tissue, lipid peroxidation activity was also found to be enhanced in myocytes isolated from WTDox mice in comparison with myocytes from WT mice. In contrast, in both LV tissue and isolated myocytes, no significant regulation in TLR3$^{-/-}$Dox mice was seen when compared to TLR3$^{-/-}$ mice [22].

In line with these findings, cardiac expression of nitrotyrosine was significantly reduced in TLR3$^{-/-}$Dox mice compared to WTDox mice. Our data suggest - the other way round - that TLR3 deficiency is able to attenuate the generation of oxidative stress in the heart implying that TLR3 is not only able to be activated by oxidative stress [23], but also contributes to its development as has also recently been demonstrated in an animal model of cardiac ischaemia/reperfusion [24]. The precise mechanisms involved in the interaction between TLRs and oxidative stress have not yet been completely defined. In murine leukocytes, oxidative stress is involved in TLR3-mediated intracellular pro-inflammatory gene activation in response to lipopolysaccharide [25-31]. This is in agreement with our findings showing that Dox did not lead to any significant TNF-α up-regulation in TLR3$^{-/-}$Dox mice in comparison with TLR3$^{-/-}$ mice. Concordant with these findings, cardiac infiltration of activated lymphocytes, monocytes and macrophages were significantly attenuated in TLR3$^{-/-}$Dox mice when compared to WTDox mice as indicated by decreased expressions of CD3-, CD8a-, and CD11b-positive cells [32].

Oxidative stress and inflammation might be associated with an induction of apoptosis, as is also known for Dox-induced cardiomyopathy [33].

In agreement with in vitro and in vivo studies using myocytes, we found a marked up-regulation of the pro-apoptotic protein Bax five days after Dox administration in our model [34]. This effect was blunted in TLR3$^{-/-}$Dox mice, suggesting that TLR3 contributes to pro-apoptotic activation in Dox-induced cardiomyopathy. The activation of this typical pro-apoptotic protein was associated with a significant increase of cardiac TUNEL-positive apoptotic cells five days after Dox injection in WT mice as previously demonstrated in this model [35], which was significantly attenuated in TLR3$^{-/-}$ mice .

However, the amount of these cells was moderate, which might be explained by the early time point of investigation in our model. To investigate possible mechanisms, which may lead to attenuated apoptosis in Dox-treated TLR3$^{-/-}$ mice, we measured the anti-apoptotic protein Bcl-2, which is a pivotal regulator of mitochondrial apoptosis [36-39]. Although others have found a reduction in the anti-apoptotic protein Bcl-2 in chronic Dox-induced cardiomyopathy [41], we

found no regulation of this protein in our acute model. Interestingly, despite the non-regulation of Bcl-2 due to Dox in WT mice, the Bcl-2 content of TLR3$^{-/-}$Dox mice were increased more than 6-fold when compared to TLR3$^{-/-}$ controls (P <0.01) suggesting anti-apoptotic protection due to TLR3 deficiency. Very recent investigations support an emerging role for Bcl-2 in protecting cardiac cells against death, including apoptosis and non-apoptotic cell death, depending on autophagy genes [40].

To get more insights into the intracellular mechanisms induced by any TLR3 deficiency possibly involved, we determined cardiac NFκB and GATA-4 regulation in our model [41]. Specifically, a down-regulation of GATA-4 in Dox-induced cardiomyopathy was identified as belonging to a disease-aggravating mechanism. We showed that TLR3 deficiency prevents GATA-4 down-regulation, suggesting that direct or indirect effects of TLR3 signalling are involved in the regulation of this transcription factor. In contrast, NFκB, which is known not be a classical down-stream target for TLR3 signalling was not regulated in WT or TLR3$^{-/-}$ mice, indicating that NFκB regulation is, at least during the investigated time frame, also not under the control of TLR3 in our model [42].

Conclusion

We conclude that TLR-3 seems to participate in the pathogenesis of cardiotoxicity of DOX.

Competing interests

The authors declare that they have no competing interests.

References

1. Choi EH, Chang H-J, Cho JY, Chun HS. Cytoprotective effect of anthocyanins against doxorubicin-induced toxicity in H9c2 cardiomyocytes in relation to their antioxidant activities. Food Chem Toxicol 2007; 45:1873-1881. https://doi.org/10.1016/j.fct.2007.04.003
2. Teraoka K, Hirano M, Yamaguchi K, Yamashina A. Progressive cardiac dysfunction in adriamycin-induced cardiomyopathy rats. Eur J Heart Fail 2000;2(4):373-378. https://doi.org/10.1016/S1388-9842(00)00111-2
3. Mogensen TH, Paludan SR. Reading the viral signature by Toll-like receptors and other pattern recognition receptors. J Mol Med 2005;83:180-192. https://doi.org/10.1007/s00109-004-0620-6
4. Wang XY, Yang CT, Zheng D, et al. Hydrogen sulfide protects H9c2 cells against doxorubicin-induced cardiotoxicity through inhibition of endoplasmic reticulum stress. Mol Cell Biochem 2012;363(1-2):419-26. https://doi.org/10.1007/s11010-011-1194-6
5. Wang XY, Yang CT, Zheng D, et al. Hydrogen sulfide protects H9c2 cells against doxorubicin-induced cardiotoxicity through inhibition of endoplasmic reticulum

stress. Mol Cell Biochem 2012;363(1-2):419-26.
https://doi.org/10.1007/s11010-011-1194-6

6. Muzio M, Bosisio D, Polentarutti N, et al. Differential expression and regulation of Toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. J Immunol 2000;164:5998-6004.
https://doi.org/10.4049/jimmunol.164.11.5998
7. Vanhoutte F, Paget C, Breuilh L, et al. Toll-like receptor (TLR)2 and TLR3 synergy and cross-inhibition in murine myeloid dendritic cells. Immunology letters 2008;116: 86-94.
https://doi.org/10.1016/j.imlet.2007.11.014
8. Iarussi D, Indolfi P, Casale F, et al. Recent advances in the prevention of anthracycline cardiotoxicity in childhood. Curr Med Chem 2001;8:1649-60.
https://doi.org/10.2174/0929867013371888
9. Schroder M, Bowie AG. TLR3 in antiviral immunity: key player or bystander? Trends Immunol 2005; 26:462-468.
https://doi.org/10.1016/j.it.2005.07.002
10. Doyle S, Vaidya S, O'Connell R, et al. IRF3 mediates a TLR3/TLR4-specific antiviral gene program. Immunity 2002;17:251-263.
https://doi.org/10.1016/S1074-7613(02)00390-4
11. Doyle S, Vaidya S, O'Connell R, et al. IRF3 mediates a TLR3/TLR4-specific antiviral gene program. Immunity 2002;17:251-263.
https://doi.org/10.1016/S1074-7613(02)00390-4
12. Kanter PM, Bullard GA, Ginsberg RA, et al. Comparison of the cardiotoxic effects of liposomal doxorubicin (TLC D-99) versus free doxorubicin in beagle dogs. In Vivo. 1993;7:17-26.
13. Lipshultz SE. Exposure to anthracyclines during childhood causes cardiac injury. Semin Oncol 2006;33:S8-14.
https://doi.org/10.1053/j.seminoncol.2006.04.019
14. Vanhoutte F, Paget C, Breuilh L, et al. Toll-like receptor (TLR)2 and TLR3 synergy and cross-inhibition in murine myeloid dendritic cells. Immunology letters 2008;116: 86-94.
https://doi.org/10.1016/j.imlet.2007.11.014
15. Vanhoutte F, Paget C, Breuilh L, et al. Toll-like receptor (TLR)2 and TLR3 synergy and cross-inhibition in murine myeloid dendritic cells. Immunology letters 2008;116: 86-94.
https://doi.org/10.1016/j.imlet.2007.11.014
16. Vanhoutte F, Paget C, Breuilh L, et al. Toll-like receptor (TLR)2 and TLR3 synergy and cross-inhibition in murine myeloid dendritic cells. Immunology letters 2008;116: 86-94.
https://doi.org/10.1016/j.imlet.2007.11.014
17. Lyass O, Uziely B, Ben-Yosef R, et al. Correlation of toxicity with pharmacokinetics of pegylated liposomal doxorubicin (Doxil) in meta-static breast carcinoma. Cancer 2000;89:1037-47.
https://doi.org/10.1002/1097-0142(20000901)89:5<1037::AID-CNCR13>3.0.CO;2-Z
18. Mrozek E, Rhoades CA, Allen J, et al. Phase I trial of liposomal encapsulated doxorubicin (Myocet; D-99) and weekly docetaxel in advanced breast cancer

patients. Ann Oncol 2005;16:1087-93.
https://doi.org/10.1093/annonc/mdi209
19. Minotti G, Cairo G, Monti E. Role of iron in anthracycline cardiotoxicity: new tunes for an old song? FASEB J 1999;13:199-212.
https://doi.org/10.1096/fasebj.13.2.199
20. Spallarossa P, Garibaldi S, Altieri P, Fabbi P, Manca V, Nasti S, et al. Carvedilol prevents doxorubicin-induced free radical release and apoptosis in cardiomyocytes in vitro. J Mol Cell Cardiol 2004;37(4):837-846.
https://doi.org/10.1016/j.yjmcc.2004.05.024
21. Weinstein DM, Mihm MJ, Bauer JA. Cardiac peroxynitrite formation and left ventricular dysfunction following doxorubicin treatment in mice. J Pharmacol Exp Ther 2000; 294(1):396-401.
22. Minotti G, Ronchi R, Salvatorelli E, et al. Doxorubicin irreversibly inactivates iron regulatory proteins 1 and 2 in cardiomyocytes: evidence for distinct metabolic pathways and implications for iron-mediated cardiotoxicity of antitumor therapy. Cancer Res 2001;61:8422-8.
23. Mayhew E, Cimino M, Klemperer J, et al. Free and liposomal doxorubicin treatment of intraperitoneal colon 26 tumor: therapeutic and pharmacologic studies. Sel Cancer Ther 1990; 6:193-209.
https://doi.org/10.1089/sct.1990.6.193
24. Matsumoto M, Funami K, Tanabe M, et al. Subcellular localization of Toll-like receptor 3 in human dendritic cells. J Immunol 2003; 171: 3154-3162.
https://doi.org/10.4049/jimmunol.171.6.3154
25. Bien S, Riad A, Ritter CA, et al. The endothelin receptor blocker bosentan inhibits doxorubicin-induced cardiomyopathy. Cancer Res 2007; 67(21):10428-10435.
https://doi.org/10.1158/0008-5472.CAN-07-1344
26. Jiang B, Xu S, Hou X, Pimentel DR, Brecher P, and Cohen RA. Temporal control of NF-kappaB activation by ERK differentially regulates interleukin-1beta-induced gene expression. J Biol Chem 2004; 279:1323-1329.
https://doi.org/10.1074/jbc.M307521200
27. Jiang B, Xu S, Hou X, Pimentel DR, Brecher P, Cohen RA. Temporal control of NF-kappaB activation by ERK differentially regulates interleukin-1beta-induced gene expression. J Biol Chem 2004; 279:1323-1329.
https://doi.org/10.1074/jbc.M307521200
28. Honda K, Sakaguchi S, Nakajima C, et al. Selective contribution of IFN-α/β signaling to the maturation of dendritic cells induced by double stranded RNA or viral infection. Proc Natl Acad Sci USA 2003;100: 10872-10877.
https://doi.org/10.1073/pnas.1934678100
29. Hause L, Al-Salleeh FM, Petro TM. Expression of IL-27 p28 by Theiler's virus-infected macrophages depends on TLR3 and TLR7 activation of JNK-MAP-kinases. Antiviral research 2007; 76: 159-167.
https://doi.org/10.1016/j.antiviral.2007.06.013
30. Hou L, Sasaki H, Stashenko P. Toll-like receptor 4-deficient mice have reduced bone destruction following mixed anaerobic infection. Infection and immunity 2000; 68: 4681-4687.
https://doi.org/10.1128/IAI.68.8.4681-4687.2000
31. Sabbatucci M, Purificato C, Fantuzzi L, Gessani S. Toll-like receptor cross-talk in human monocytes regulates CC-chemokine production, antigen uptake and

immune cell recruitment. Immunobiology 2011; 216: 1135-1142. https://doi.org/10.1016/j.imbio.2011.04.005

32. Hoebe K, Du x Georgel P, Janssen E, et al. Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. Nature 2003; 424: 743-748. https://doi.org/10.1038/nature01889
33. Lyass O, Uziely B, Ben-Yosef R, et al. Correlation of toxicity with pharmacokinetics of pegylated liposomal doxorubicin (Doxil) in meta-static breast carcinoma. Cancer 2000;89:1037-47. https://doi.org/10.1002/1097-0142(20000901)89:5<1037::AID-CNCR13>3.0.CO;2-Z
34. Lipshultz SE, Colan SD, Gelber RD, et al. Late cardiac effects of doxorubicin therapy for acute lymphoblastic leukemia in childhood. N Engl J Med 1991;324:808-15. https://doi.org/10.1056/NEJM199103213241205
35. Zhang R, Singh S, Ha X, Cowsik G, Lavezzi O, Caudell H, Ohura R. TLR3 exaggerated sepsis induced cardiac dysfunction via activation of TLR4-mediated NF-κB and TRIF/IRF signaling pathways. American journal of BioMedicine 2014;2(1):80-93. https://doi.org/10.18081/2333-5106/014-01/125-136
36. Tabeta K, Georgel P, Janssen E, et al. Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. Proc Natl Acad Sci USA 2004;101: 3516-3521. https://doi.org/10.1073/pnas.0400525101
37. So EY, Kang MH, Kim BS. Induction of chemokine and cytokine genes in astrocytes following infection with Theiler's murine encephalomyelitis virus is mediated by the Toll-like receptor 3. Glia 2006; 53: 858-867. https://doi.org/10.1002/glia.20346
38. Bjorkbacka H, Kunjathoor VV, Moore KJ, et al. Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. Nat Med 2004;10:416-421. https://doi.org/10.1038/nm1008
39. Cario E and Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000;68:7010-7017. https://doi.org/10.1128/IAI.68.12.7010-7017.2000
40. Yousif NG, Al-amran FG. Novel Toll-like receptor-4 deficiency attenuates trastuzumab (Herceptin) induced cardiac injury in mice. BMC cardiovascular disorders 2011;11(1):62. https://doi.org/10.1186/1471-2261-11-62
41. Yamamoto M, Sato S, Hemmi H, Hoshino K, et al. Role of adaptor TRIF in the MyD88-independent Toll-like receptor signaling pathway. Science 2003;301:640-643. https://doi.org/10.1126/science.1087262
42. Tissari J, Siren J, Meri S, Julkunen I, Matikainen S. IFN-alpha enhances TLR3-mediated antiviral cytokine expression in human endothelial and epithelial cells by up-regulating TLR3 expression. J Immunol 2005;174:4289-4294. https://doi.org/10.4049/jimmunol.174.7.4289

43. Yang X, Coriolan D, Schultz K, Golenbock DT, and Beasley D. Toll-like receptor 2 mediates persistent chemokine release by Chlamydia pneumoniae-infected vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2005;25:2308-2314. https://doi.org/10.1161/01.ATV.0000187468.00675.a3
44. Yang R, Mark MR, Gray A, Huang A, Xie M, Zhang M, Goddard A, Wood WI, Gurney AL, and Godowski PJ. Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signaling. Nature 1998;395:284-288. https://doi.org/10.1038/26239
45. Austin EW, Ao L, Fullerton DA, Meng X. Ghrelin reduces myocardial injury following global ischemia and reperfusion via suppression of myocardial inflammatory response. American journal of BioMedicine 2013;1(2):33-47. https://doi.org/10.18081/ajbm/2333-5106-013-12/38-48

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