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Review Article

The Interaction of Doxorubicin and Heat Shock Protein Family in the Protection and Drug Resistance

Salih Tunç Kaya[1], Celal Guven[2] and Eylem Taskin[3]*

[1]*Department of Biology, Faculty of Arts and Sciences, Duzce University, Turkey*

[2]*Department of Biophysics, Faculty of Medicine, Adiyaman University, Turkey*

[3]*Department of Physiology, Faculty of Medicine, Adiyaman University, Turkey*

***Corresponding Author:** Eylem Taskin, Department of Physiology, Faculty of Medicine, Adiyaman University, Turkey.

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Abstract

Cancer, affecting more than 6% of the population worldwide, is one of chronic disease characterized by abnormal and aggressive growth of cells and. There is a hope to cure it when it can be diagnosed early stage of it. Four options may be represented to cancer patients. One option is drug treatment. Doxorubicin (DOX) is one of chemotherapeutic drugs which is used in many cancer types. DOX has been efficiently used in the treatment, but it has toxic effects on non-cancerous cells. The undesirable effect has led to its limited clinical use. Up to now, no effective way has been found to abolish its side effect. Therefore, it is very crucial to understand the side-effect mechanism(s). In fact, the cellular mechanism underlying its undesirable side effect remains a mystery today. But there are a few clues to illuminate its toxic pathways. The most accepted idea is that DOX causes oxidative and energy stress in the cell. Recent evidence has suggested that heat shock proteins (HSP) have an essential role in cell stress response, cardio protection and cardiotoxicity induced by DOX. This review will summarize some current knowledge on the interaction between DOX and heat shock protein family (HSP).

Keywords: *Adriamycin; Cancer; Cardiotoxicity; Doxorubicin; Heat Shock Protein; Toxic Effects; Side Effects*

Cancer

Cancer is scaling up every year and the first or second leading cause of death based on World Health Organization (WHO)'s estimation in 2019. Unfortunately, 19.3 million new cases are diagnosed as cancer and 10 million cancer patients dies worldwide in 2020 [1]. Cancer is a curable illness if it is diagnosed early enough. There are some options for treating it. One of the options is surgical removal. The second option is to use some chemotherapeutic drugs, hormones or specific drugs. The third option is radiation. Lastly, the cancer cells shrink and vanish on their own [2]. Another is calorie restriction and/or starvation [3]. Combination therapy is the other option, combining at least two treatments. The combination is suggested to be more effective than monotherapy [4]. Otherwise, cancer is maintaining grow up and eventually dies [2]. Some chemotherapeutics are used in the treatment of cancer patients. One of them is doxorubicin (DOX), also called Adriamycin [5,6].

Doxorubicin

Doxorubicin is one of anticancer drugs, which is potently used in the cure of solid cancers, such as breast cancer [7-9]. Although the strong anticancer activity of the drug is well known, unfortunately, it causes unwanted side effects in many non-cancerous tissues including heart, kidney, brain, pancreas, and liver, etc [5,10-12]. Its molecular mechanism underlying the side-effects have not been fully clarified, however, there is some evidence in the literature, which include oxidative stress [11,13], energy stress [6,14,15], impaired Ca^{2+} handling, mitochondrial dysfunction [7,16], apoptosis [13], and DNA injury [7]. It is well documented that the cardiotoxicity of DOX is related to its cumulative dose [9]. It has been reported that DOX-induced cardiotoxicity has developed in approximately 26% of the patients treated with 550 mg/m^2 [7]. The most sensitive organ to DOX toxicity is the heart for some reason [5,15]. One reason is that heart has less antioxidant capacity and slow turnover of oxidants [5,15], such as catalase detoxifying the H_2O_2 and easily inactivation of selenium dependent GSHPx1 [13] because the heart's need for oxygen is very high due to its continuous work [15]. Therefore, the volume of mitochondria in a cardiomyocyte is high, around 60%. By this way, the production of oxygen species is high; unfortunately, its antioxidant capacity is less, so the heart is exposed to reactive oxygen species (ROS) induced by DOX [15], resulting in mitochondrial dysfunction leading to less ATP production [17]. These effects may trigger cell death pathways, including apoptosis and necrosis [17]. The ROS source in the heart is not only mitochondria, but also xanthine oxidoreductase, nitric oxide synthases, NADPH oxidase, cytochrome P450, and monoamine oxidases [18]. Nevertheless, the electron transport chain (ETC) in the heart mitochondria is considered the main source of ROS production [15]. There are some options to alleviate its undesired effects, including reducing the cumulative dose, changing the route of drug administration, using DOX analogues, cardioprotective and combination therapy with drugs [5]. Therefore, there should be limited clinical use due to the undesirable effect. The clinician may choose to use it at a subclinical dose to eliminate or alleviate its side effect. It should be mentioned that the therapeutic and toxic mechanism of action of DOX is different. Its anticancer effect on solid cancer is related to intercalation to DNA and inhibition of topoisomerase-II [13]. On the other hand, the issue of the mechanism to be resolved is that the toxic effect is mainly multifactorial effects [9,11].

Heat shock protein family

Stress response proteins are conserved and abundantly expressed from Drosophila to mammals as well as viruses [19,20]. It is well known that the stress response is crucial survival processes for body physiology and the pathophysiology of some disease as well [21]. One of these responses is heat shock protein family (HSPs). HSPs can be stimulated under certain stress conditions such as fever, lack of nutrient, oxidative- and toxic stress and exposure to inflammatory cytokines [22], radiation, infectious agents and heavy metal toxicity [23].

HSPs were first reported to be discovered in 1962 [24]. In addition, it was noted that chaperone activity was first described in bovine eyes [25]. The family can be divided into some subgroups based on molecular weight. Large subgroups with high molecular weight include HSP100s, HSP90s, HSP70s, HSP60s, HSP40s although small groups (sHSPs) have been reported to include fly Hsp22, mammalian αA-crystal and αB-crystal, mouse Hsp25, human Hsp27 and Hsp22 [22,25]. sHSPs are supposed to have not only the function of reducing protein aggregation independent of ATP, stabilizing stress damage cell membrane, cell differentiation, apoptosis and longevity, but also include many pathologies, e.g. cardiovascular diseases, tumor, neuron diseases etc [25].

sHSPs have been reported to have many substrate proteins such as malate dehydrogenase, alcohol dehydrogenase, β-galactosidase, elastase, luciferase, xylose reductase, Abrin, β-actin, β-tubulin, titin from different organisms, and also some peptides, including insulin β chain and melittin [25]. It has been suggested that HSPs are multifunctional such as modulation of protein complex assembly, protein refolding and trafficking of denatured proteins, mitochondrial protein folding and assembly, misfolded protein degradation, and blocking apoptosis [26]. It is therefore recognized that regulation of the expression of HSP is crucial for some physiological processes such as de-

velopment and growth [19]. The family is involved in the cell survival process under stress conditions [27]. It has been declared that HSPs support tumor growth and development by regulating apoptosis, necroptosis, autophagy, tumor cell root structure, epithelial to mesenchymal transition, lipid metabolism, angiogenesis, and tumor immunity. Moreover, HSPs are located in some cell compartments such as the cytosol, endoplasmic reticulum (ER), and mitochondria, having compartment-specific cellular functions. For example, they participate in cancer aggression and anticancer drug resistance when they are in the extracellular fluid. In addition, HSPs with compartment-specific roles are essential for the regulation of immune responses [23].

HSP27 is another HSP involved in anti-apoptotic protein and increased cellular resistance to stress, including heat, toxic or oxidative [28]. Another HSP is HSP60, known as known as HSPD1, located in the matrix of mitochondria. Its function is to protect the mitochondria against proteotoxicity under stress condition. Moreover, HSP60 is essential for mitochondrial antioxidant enzyme identified as manganese superoxide dismutase. It is stated that elimination of HSP60 in the heart results in the dissipation of mitochondria membrane potential and respiratory enzyme activity, resulting in decreased ATP production that causes energy stress and increases ROS [29]. In addition, HSP70 is also another well-know HSP. It is suggested that HSP70 modulates apoptotic, inflammation, cell survival and immunity by reduction of nuclear factor kB (NF-κB) activation by binding to IKKγ [21]. HSP70 has also role in averting the irreversible protein denaturation and cell death. Moreover, its overexpression has been noted to modulate the immune response [27]. Indeed, HSP70 has a dual effect on the immune response-dependent cell compartment. When HSP70 is in the cytosol, it can inhibit the immune system despite potentially activating the immune system in extracellular fluids [30].

The intercommunication between doxorubicin and HSPs

DOX is shown to give rise to metabolic stress in the heart by decreasing RNA and protein synthesis and oxidative stress elevation, thereby enabling the activation of heat-shock or stress proteins (HSP). Activation of HSP triggers the immune system via T lymphocytes, particularly cytolytic T lymphocytes, resulting in the onset of cardiomyocyte damage [14]. HSP family is divided into some subfamily based on their molecular weight. One of HSPs is HSP90, which is expressed in all organism by about 1 - 2% of all cellular proteins [30]. However, under stress conditions, such as heat, hypoxia, heavy metal [30], ischemia, oxidative and osmotic stress ischemia, oxidative and osmotic stress [19], HSPs expression was indicated to reach 15 - 20% in cellular protein content [19], and also HSP90 expression is suggested to elevate 10 times [30]. So, stress is the main factor for initiating HSPs.

HSP20 is a member of the HSP whose role in the cardiotoxicity of DOX has been investigated. The author reported that HSP20 has a protective effect against cardiotoxicity of DOX by reducing apoptotic cell death via Akt pathway when higher expression is present [31]. One study also reported that pretreatment of lipopolysaccharide (LPS) abolished ischemia/reperfusion-induced kidney injury through HSP27 upregulation by reducing inflammatory reaction, oxidative stress, and apoptosis [32]. In addition, it has been reported that HSP70 takes a part in renal ischemia/reperfusion injury through the regulation of Akt and glycogen synthase kinase-3β, which play a role in the regulation of apoptosis through Bax, one of the proapoptotic proteins. [26]. MAPK also participates in the interaction. It is stated that MAPK-activated protein-2 (MAPKAP-2) activates HSPs and causes a decrease in apoptosis. Moreover, P38 can phosphorylate HPS27, thereby reducing DOX-cardiotoxicity by increasing SOD activity [13]. One of the previous studies reported that DOX induces apoptosis in fetal kidney tissue by HSP70 and is reversed by nitric oxide (NO, L-arginine) [33]. Therefore, several previous studies have demonstrated the contribution of HSPs to cardiotoxicity and/or cardio protection against DOX. But many researchers have still tried to find ways to eliminate its cardiotoxicity (Figure 1).

Heat shock genes are mainly regulated by heat shock transcription factor (HSF1), which is found in an inactive form in the cytoplasm under normal physiological conditions, by binding to a multi-chaperone complex containing HSP90 [30]. After the complex is dispersed, HSF1 becomes activated and translocate to the nucleus and binds to the heat shock element in the promoters of HSP after being hyperphosphorylated [19,30]. Under the stress condition, the misfolded protein increases, then HSF1 can stimulate genes.

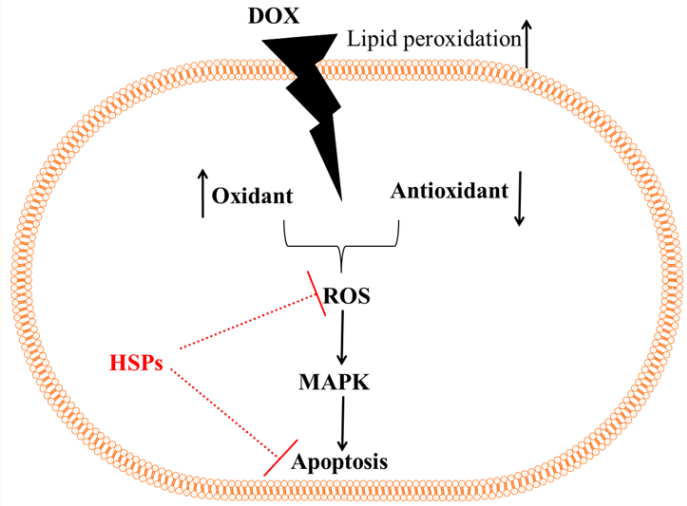

Figure 1: *The three-modal interaction of doxorubicin with HPS and MAPK. DOX: doxorubicin, HSPs: Heat Shock Proteins, MAPK: Mitogen Activated Kinases Family.*

Nonpharmacological strategy in the interaction

Low-intensity exercise training during doxorubicin treatment has been shown to prevent DOX-cardiotoxicity by increasing protein expression of SOD isoforms or HSP72, resulting in inhibition of apoptotic cell death by abolishing caspase-3 activity [8]. The importance of SOD lies in the first line of defense against oxidative stress induced by DOX through superoxide radical scavenging. Interestingly, myocardial SOD isoform activity and expression has been suggested to be more expressed in male than female rat heart after chronic forced treadmill running [7]. Other voluntary acute and chronic exercise training has also been shown to avoid cardiotoxicity of acute DOX via lipid peroxidation and HSP72 (enhancement rate approximately 78%) [7]. Aerobic exercise is also investigated for cardio protection against the cardiotoxicity of DOX. It is well known that exercise ameliorates systolic and diastolic function and reduces pathological left ventricular remodeling, thereby boosting exercise tolerance and improving fatigue in the heart failure patient. How aerobic exercise can alleviate DOX-cardiotoxicity is that it depresses mitochondrial ROS production and elevate some antioxidant enzyme in heart such as glutathione peroxidase 1, catalase, and manganese superoxide dismutase. In addition, the HSP60 and HSP72 can be upgraded and contribute to cardio protection. The exact mechanism of HSPs in cardiotoxicity has not been clearly elucidated, but it has shown some evidence of their function, including blunting the damage protein in cells [9]. However, more studies are needed to investigate the communication between DOX-cardiotoxicity and HSPs in different exercises during and after DOX treatment in both animal and human studies.

The interaction in the drug resistance

It is not ruled out that HSPs has played a role in drug resistance such as in the treatment with DOX. Overexpression of HSP27 is suggested to be involved in the resistance of DOX, although its protein level is normally low in human breast cancer cells (MCF-7) [34]. Moreover, another study reported that HSP70 rendered the DOX's cardiotoxicity. Furthermore, the authors suggested that heat preconditioning could reverse the cardiotoxicity of DOX by HSP70 in heart muscle cells *in vitro*. The authors speculated that HSP70 could inhibit the apoptotic cardiac cells death through SAPK/JNK inhibition [35]. HSPs involved in drug toxicity rely on heat shock factor 1 (HSF1), one of the transcription factors involved in some stress (heat shock, ischemia, and aging)-related gene expressions. The most important role

against multidrug resistance in cancer therapy has been the well-known multidrug resistance gene 1 (MDR-1) when it is overexpressed in cells and/or its product, P-glycoprotein (P-gp), an energy-dependent drug efflux pump. It is recognized that HSPs can increase P-gp expression, as HSP1 can induce the MDR-1 gene due to the presence of the heat shock element (HSE) in the MDR-1 promoter [36] However, both interactions are still controversial. Therefore, more research is needed to evaluate the relationship (Figure 2).

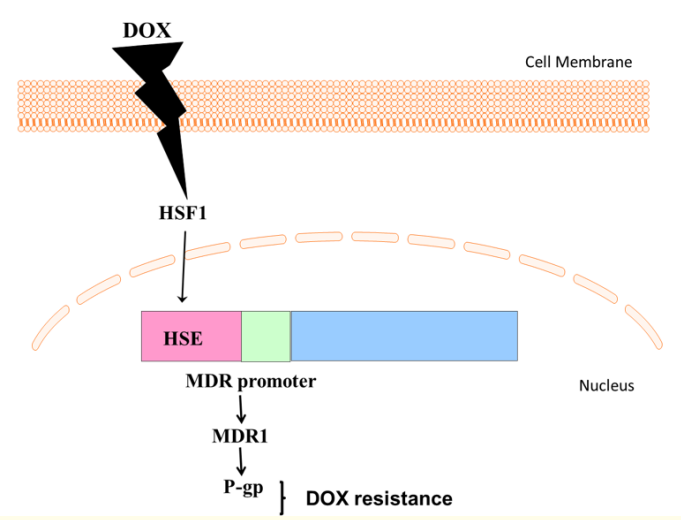

Figure 2: *The graphical abstract on DOX's resistance involving HSF1 and HSE AND MDR promoter. DOX: Doxorubicin, HSE: Heat Shock Element, HSF1: Heat Shock Transcription Factor 1, P-gp: P-Glycoprotein.*

It is well documented that HSP90 is one of the important chaperone proteins, particularly in cell growth, cell proliferation, and cell differentiation. Although HSP90 may be part of the stress response as well as mutant protein stabilization, it may not exclude the development of certain cancers, such as prostate [37], multiple myeloma (MM) [38]. HSP90 is also suggested to be involved in drug resistance to DOX in the small cell lung cancer line (SCLC) through Ak-strain transformation (AKT) and activation of β-catenin signaling and inhibition of glycogen synthase kinase 3β (GSK3β) [37]. Its blockage activated mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase/ERK signaling pathway, and inhibited E-cadherin expression in prostate [37] as well as inhibited RAS-RAF-MEK-ERK signaling pathway in MM [38]. Based on this knowledge, it can be concluded that attenuation of HSP90 level may therefore be one of therapeutic targets against cancer therapy [37]. In contrast, one of the previous studies reported that HSP might contribute to myocardial tolerance to DOX-cardiotoxicity. HSP60, mainly expressed in mitochondria, has been shown to preserve the cardiotoxicity of DOX when endurance exercise is performed, possibly through mitochondrial adaptations [15]. In another study, it was stated that DOX caused HSP72 expression in the heart [39]. A previous study evaluated the relationship between thermotolerance and HSP72 accumulation after DOX-treatment. Interestingly, combination treatment with DOX and hyperthermia was found to result in accumulated HSP72 with greater therapeutic gain of the drug, not only DOX treatment in Chinese hamster V-79 cells [40].

Conclusion

DOX is a great drug for solid cancer therapy, but its clinical utility is limited due to its undesired effect on non-cancerous tissue. The molecular mechanism of its undesired effect is still unclear. That is why there is no exact therapy to get rid of its side effect. The heat shock protein family is a conservative protein family that play a role in the fight to stress conditions. HSP might decrease DOX's toxicity through

alleviation of ROS production and suppression of apoptotic cell death as well. There are some clues on not only the therapy of DOX's toxicity but also in drug resistance. However, both interactions are still unclear. Therefore, more studies are needed to investigate the interaction before and after DOX treatment in both animal and human studies.

Bibliography

1. Sung H., *et al.* "Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries". *CA: A Cancer Journal for Clinicians* 71.3 (2021): 209-249.

2. Roy PS and Saikia BJ. "Cancer and cure: A critical analysis". *Indian Journal of Cancer* 53.3 (2016): 441-442.

3. Cangemi A., *et al.* "Dietary restriction: could it be considered as speed bump on tumor progression road?". *Tumor Biology* 37.6 (2016): 7109-7118.

4. Bayat Mokhtari R., *et al.* "Combination therapy in combating cancer". *Oncotarget* 8.23 (2017): 38022-38043.

5. Shirinbayan V and Roshan VD. "Pretreatment effect of running exercise on HSP70 and DOX-induced cardiotoxicity". *The Asian Pacific Journal of Cancer Prevention* 13.11 (2012): 5849-5855.

6. Taskin E., *et al.* "Silencing HMGB1 expression inhibits adriamycin's heart toxicity via TLR4 dependent manner through MAPK signal transduction". *Journal of BUON* 25.1 (2020): 554-565.

7. Chicco AJ., *et al.* "Voluntary exercise protects against acute doxorubicin cardiotoxicity in the isolated perfused rat heart". *The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 289.2 (2005): R424-R431.

8. Chicco AJ., *et al.* "Low-intensity exercise training during doxorubicin treatment protects against cardiotoxicity". *Journal of Applied Physiology* 100.2 (2006): 519-527.

9. Scott JM., *et al.* "Modulation of anthracycline-induced cardiotoxicity by aerobic exercise in breast cancer: current evidence and underlying mechanisms". *Circulation* 124.5 (2011): 642-650.

10. Taskin E., *et al.* "The role of toll-like receptors in the protective effect of melatonin against doxorubicin-induced pancreatic beta cell toxicity". *Life Sciences* 233 (2019): 116704.

11. Taskin E., *et al.* "Acute adriamycin-induced cardiotoxicity is exacerbated by angiotension II". *Cytotechnology* 68.1 (2016): 33-43.

12. Taskin E., *et al.* "The restoration of kidney mitochondria function by inhibition of angiotensin-II production in rats with acute adriamycin-induced nephrotoxicity". *Renal Failure* 36.4 (2014): 606-12.

13. Turakhia S., *et al.* "Doxorubicin-induced cardiotoxicity: direct correlation of cardiac fibroblast and H9c2 cell survival and aconitase activity with heat shock protein 27". *The American Journal of Physiology-Heart and Circulatory Physiology* 293.5 (2007): H3111-3121.

14. Huber SA. "Heat-shock protein induction in adriamycin and picornavirus-infected cardiocytes". *Laboratory Investigation* 67.2 (1992): 218-224.

15. Ascensao A., *et al.* "Endurance training attenuates doxorubicin-induced cardiac oxidative damage in mice". *International Journal of Cardiology* 100.3 (2005): 451-460.

16. Dursun N., *et al.* "Selenium-mediated cardioprotection against adriamycin-induced mitochondrial damage". *Drug and Chemical Toxicology* 34.2 (2011): 199-207.

17. Dikalov SI and Nazarewicz RR. "Angiotensin II-induced production of mitochondrial reactive oxygen species: potential mechanisms and relevance for cardiovascular disease". *Antioxidants and Redox Signaling* 19.10 (2013): 1085-1094.

18. D'Oria R., *et al.* "The Role of Oxidative Stress in Cardiac Disease: From Physiological Response to Injury Factor". *Oxidative Medicine and Cellular Longevity* 2020 (2020): 5732956.

19. Kelly KJ. "Heat shock (stress response) proteins and renal ischemia/reperfusion injury". *Contributions to Nephrology* 148 (2005): 86-106.

20. Jacob P., *et al.* "The heat-shock protein/chaperone network and multiple stress resistance". *Plant Biotechnology Journal* 15.4 (2017): 405-414.

21. Pal G., *et al.* "Effects of nitric oxide modulators and antioxidants on endocrine and cellular markers of acute stress in rats". *Biochemical and Biophysical Research Communications* 589 (2022): 234-239.

22. Wieten L., *et al.* "Cell stress induced HSP are targets of regulatory T cells: a role for HSP inducing compounds as anti-inflammatory immuno-modulators?". *FEBS Letters* 581.19 (2007): 3716-3722.

23. Albakova Z and Mangasarova Y. "The HSP Immune Network in Cancer". *Frontiers in Immunology* 12 (2021): 796493.

24. Prangenberg J., *et al.* "Mini Review: The Forensic Value of Heat Shock Proteins". *Frontiers in Medicine* 8 (2021): 800100.

25. Fu X. "Chaperone function and mechanism of small heat-shock proteins". *Acta Biochimica et Biophysica Sinica* 46.5 (2014): 347-356.

26. O'Neill S., *et al.* "Heat-shock proteins and acute ischaemic kidney injury". *Nephron Experimental Nephrology* 126.4 (2014): 167-174.

27. Yener AU., *et al.* "Protective role of heparin in the injury of the liver and kidney on the experimental model of ischemia/reperfusion". *Journal of Cardiothoracic Surgery* 9 (2014): 35.

28. Xiong B., *et al.* "A1AR-mediated renal protection against ischemia/reperfusion injury is dependent on HSP27 induction". *International Urology and Nephrology* 50.7 (2018): 1355-1363.

29. Huang YH., *et al.* "Heat Shock Protein 60 Restricts Release of Mitochondrial dsRNA to Suppress Hepatic Inflammation and Ameliorate Non-Alcoholic Fatty Liver Disease in Mice". *International Journal of Molecular Sciences* 23.1 (2022).

30. O'Neill S., *et al.* "The role of heat shock protein 90 in modulating ischemia-reperfusion injury in the kidney". *Expert Opinion on Investigational Drugs* 21.10 (2012): 1535-1548.

31. Fan GC., *et al.* "Heat shock protein 20 interacting with phosphorylated Akt reduces doxorubicin-triggered oxidative stress and cardiotoxicity". *Circulation Research* 103.11 (2008): 1270-1279.

32. He K., *et al.* "LPS ameliorates renal ischemia/reperfusion injury via Hsp27 up-regulation". *International Urology and Nephrology* 50.3 (2018): 571-580.

33. Pedrycz A and Brzeski Z. "L-arginine decreases heat shock protein 70 (marker of environmental stress) expression in kidney cells of rat fetuses during apoptosis--Late effect of Adriamycin action". *Annals of Agricultural and Environmental Medicine* 13.1 (2006): 129-132.

34. Oesterreich S., *et al.* "The small heat shock protein hsp27 is correlated with growth and drug resistance in human breast cancer cell lines". *Cancer Research* 53.19 (1993): 4443-4448.

35. Ito H., *et al.* "Thermal preconditioning protects rat cardiac muscle cells from doxorubicin-induced apoptosis". *Life Sciences* 64.9 (1999): 755-761.

36. Tchenio T., *et al.* "Heat shock-independent induction of multidrug resistance by heat shock factor 1". *Molecular and Cellular Biology* 26.2 (2006): 580-591.

37. Du Y., *et al.* "Secreted Heat Shock Protein 90alpha Attenuated the Effect of Anticancer Drugs in Small-Cell Lung Cancer Cells Through AKT/GSK3beta/beta-Catenin Signaling". *Cancer Control* 25.1 (2018): 1073274818804489.

38. Suzuki R., *et al.* "Combination of a Selective HSP90alpha/beta Inhibitor and a RAS-RAF-MEK-ERK Signaling Pathway Inhibitor Triggers Synergistic Cytotoxicity in Multiple Myeloma Cells". *PLoS One* 10.12 (2015): e0143847.

39. Chen H., *et al.* "Protective effect of doxorubicin induced heat shock protein 72 on cold preservation injury of rat livers". *World Journal of Gastroenterology* 10.9 (2004): 1375-1378.

40. Ohtsuboa T., *et al.* "Enhancement of heat-induced heat shock protein (hsp)72 accumulation by doxorubicin (Dox) in vitro". *Cancer Letters* 159.1 (2000): 49-55.

Volume 10 Issue 10 October 2022