Toxicological profile of Doxorubicin

This report gives a brief technical understanding of Doxorubicin, as a chemotherapeutic drug, pharmacological properties, toxicology studies, and potential ways to overcome toxicity.

Mechanism of Action:

Doxorubicin (Dox) is a chemotherapeutic drug belonging to the anthracycline class which is cytotoxic to the tumor cells(Altieri *et al.*, 2016; Bisht *et al.*, 2024).

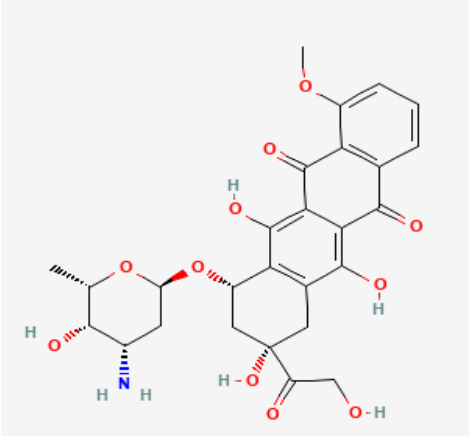

Figure 1: *Structure of Doxorubicin (Adriamycin)* (NCBI, PubChem, 2024)

It is an efficient drug that follows various methods to destroy cancerous cells, which might also be responsible for toxicity in normal cells.

Intercalation of DNA:

Figure 2 shows that Dox is administered intravenously and enters the cytoplasm through passive diffusion or active transport. It has a high affinity for binding with the 26S Proteasome to form a complex and then moves into the cell nucleus. Since doxorubicin has a higher affinity for DNA than the proteasome, it dissociates from the complex and intercalates into the DNA base pairs. This results in damage to the DNA and cell death.(Tacar, Sriamornsak and Dass, 2012)

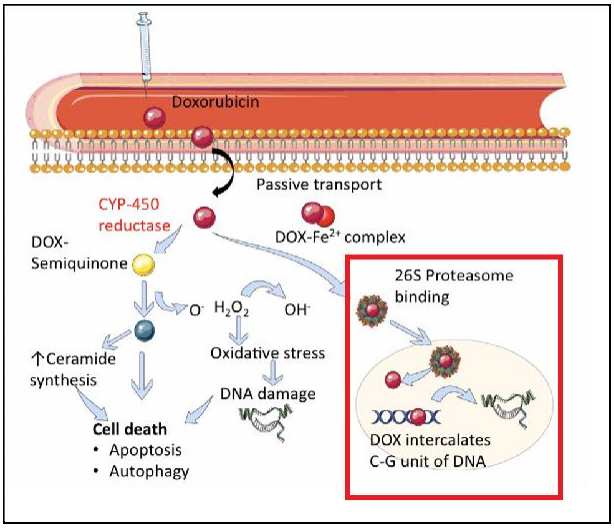

Figure 2: *Intercalation in DNA* (Bisht *et al.*, 2024)

Inhibition of Topoisomerase II:

Due to the intercalation of Dox in DNA, the action of topoisomerase II enzyme in DNA synthesis and RNA transcription is inhibited (Taymaz-Nikerel *et al.*, 2018). The inhibition results in the unwinding of DNA, which enables the signaling pathways responsible for killing the cancerous cells.

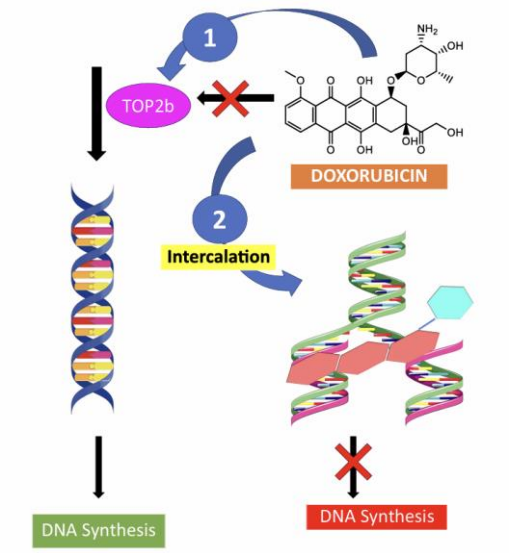

Figure 3: *Inhibition of Topoisomerase II* (Bisht *et al.*, 2024)

Generation of Reactive Oxygen Species (ROS):

Dox in the cytoplasm is converted to unstable semiquinone by enzymes such as NADH dehydrogenase, nitric oxide synthase, and xanthine oxidase. It is then reverted to doxorubicin during which it releases ROS. This causes an imbalance between antioxidants and ROS leading to high levels of ROS in the cytoplasm resulting in oxidative stress, DNA damage, and cell death.(Sritharan and Sivalingam, 2021)

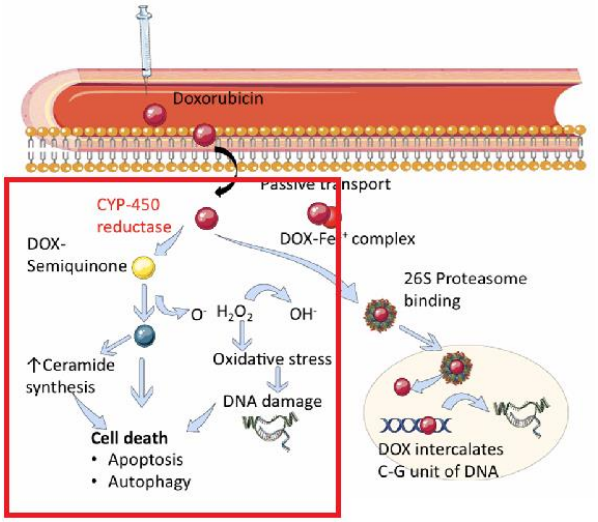

Figure 4: *Generation of reactive oxygen species* (Bisht *et al.*, 2024)

Physicochemical and Pharmacokinetic properties:

Dox can be administered intravenously, intraperitoneally, intra-arterially, intrapleurally, and intravesically. However, it cannot be administered orally since its bioavailability is only 5%(Speth, van Hoesel and Haanen, 1988).

After intravenous administration, Dox is highly available in the plasma immediately and then the concentration of the drug in the plasma decreases upon 50-80% binding to the proteins for distribution(Celio *et al.*, 1983). Dox cannot pass through the blood-brain barrier, so it cannot treat tumors based on the central nervous system.

Dox is metabolized in the liver to form the major metabolite doxorubicinol swiftly. It is then further metabolized into doxorubicinone and 7-deoxydoxorubicinone(Piscitelli *et al.*, 1993) which is not as cytotoxic as Dox and Doxorubicinol.

Excretion occurs majorly through the liver and partially through the kidney(Yesair *et al.*, 1972).

A study has been performed on Walker 256 tumor-induced Athymic nude male rats by administering Dox intravenously to investigate and confirm its properties which is shown in Table 1(Kaminskas *et al.*, 2012).

Table 1: *Physicochemical and Pharmacokinetic properties of Dox*

Properties	Values	References
Solubility	10 mg/mL (Water-soluble)	(NCBI, PubChem, 2024)
Lipophilicity (LogP)	1.27	(NCBI, PubChem, 2024)
Area under the concentration-time curve (AUC)	1834 ± 1007 (ng hr/m1)	(Piscitelli *et al.*, 1993)
Volume of distribution (V_d)	584 mL	(Kaminskas *et al.*, 2012)
Half-maximal Inhibitory concentration (IC_{50})	0.04±0.02 µM	(Kaminskas *et al.*, 2012)
Half-life ($t_{1/2}$)	25.6 ± 16.9 hr	(Piscitelli *et al.*, 1993)
Clearance (CL)	760±163mL/hr	(Kaminskas *et al.*, 2012)

Toxicology:

The potential mechanism of acting on the cancerous cells by causing an imbalance between ROS and antioxidants is the reason for inducing toxicity in normal cells. It has been found to induce programmed cell death in multiple organs including the heart, liver, reproductive system, and lungs upon accumulation of Dox(Pugazhendhi *et al.*, 2018).

Cardiotoxicity:

Many studies suggest that cardiotoxicity results from oxidative stress caused by generating free oxygen radicals during the formation of metabolites such as Doxorubicinol.

Free oxygen radical → H_2O_2

Although free oxygen radicals act on and damage tissues, they are not very reactive. They are then further converted into hydrogen peroxide with the help of the enzyme superoxide dismutase.

H_2O_2 → OH^-

The hydroxyl radicals formed from the hydrogen peroxide are toxic to lipids, proteins, and nucleic acids which utilize sulfhydryl-containing peptides and lipid peroxidation, destroying the genetic material. There are enzymes to react with the free radicals and prevent the damage caused, however, these enzymes are insufficient in the heart rather than in the kidney and liver(Jain, 2000).

There is evidence that Dox binds with the cardiac ryanodine receptor and reduces the availability of thiol groups on the receptors whose function is to prevent oxidative stress. Also, its metabolite doxorubicinol inhibits the uptake of Ca^{2+} into the sarcoplasmic reticulum vesicles affecting the cardiac function(Hanna *et al.*, 2014).

Overall, the cardiotoxicity is due to the combination of the generation of free radicals and Dox-iron complex formation which are highly toxic to intracellular proteins and lipids(Jain, 2000).

Hepatotoxicity:

The liver plays a vital role in metabolizing dox like any other drug, it accumulates most of the injected drug and undergoes detoxification activities. This results in the overproduction of ROS and activates the enzyme IkB kinase, which catalyzes the phosphorylation of IkK inhibitors thus reducing the ATP molecules in the liver(Tacar, Sriamornsak and Dass, 2013).

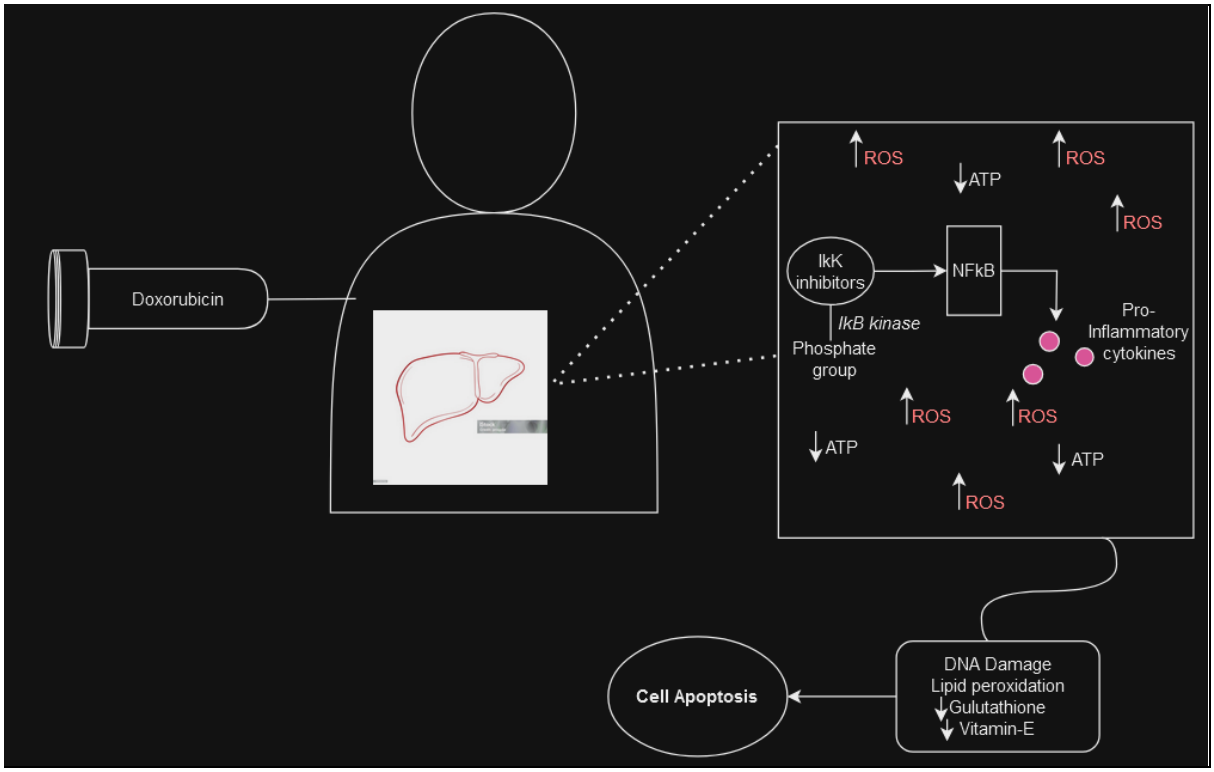

Figure 5: *Toxicity in hepatocytes (Drawn using draw.io)*

Figure 5 shows the abundance of ROS formed and depletes all the ATP, ADP, and AMP molecules for phosphorylating the inhibitors. They are involved in activating nuclear factor kappa B (NFkB). NFkB then triggers the pro-inflammatory cytokines, causes DNA damage, produces lipid peroxidation, and reduces vitamin E levels and glutathione.

Due to less availability of ATPs, there is no energy fuel to maintain homeostasis and prevent the accumulation of Dox. This is also one of the reasons, the patients undergoing chemotherapy become weak and take longer to heal(Tacar, Sriamornsak and Dass, 2013).

From this, we conclude that the liver is also a common site similar to that of the heart where Dox accumulation leads to toxicity.

Nephrotoxicity:

Dox interrupts the activity of mitochondria by reducing the activity of its complexes which elevates the levels of triglycerides, superoxides, and citrate synthases. Also, there is a fall in the levels of vitamin E and antioxidants due to lipid peroxidation. This results in damaging the glomeruli and causes diseases associated with affecting it such as hypertension, proteinuria, and resistance to steroids ultimately resulting in kidney failure(Tacar, Sriamornsak and Dass, 2013).

Chronic Toxicity:

A study has been performed to understand whether dox is responsible for the reactions associated with ROS production, including lipid peroxidation. The investigation was made on dox-treated rats chronically by analyzing their tissues upon performing various assays to determine the levels of GSH, ROS, etc(Pryor, 1976).

Table 2: Effect of chronic dox treatment on fluorescent lipid peroxidation production in the rat's organs(Thayer, 1988)

Organ	Fluorescence intensity of Control (relative units/ g wet weight)	Fluorescence intensity of Dox-treated (relative units/ g wet weight)
Heart	29.2 ± 10.6	42.1 ± 15.6
Kidney	18.3 ± 2 4.7	54.2 ± 22.1
Liver	203.0 ± 2 14.4	230.6 ± 2 19.0

Table 2 shows the intensity of fluorescence, which is directly proportional to products obtained during the lipid peroxidation reactions, ultimately one of the mechanisms of cell apoptosis. From the data, we can extract that there is a 40% increase in heart and just a 10% increase in liver, whereas it is three times greater in Kidney after treating the rats with dox compared to before treatment.

Table 3: Effect of chronic dox treatment on glutathione in the rat's organs(Thayer, 1988)

Organ	GSH of Control (pmol/g wet weight)	GSH of Dox-treated (pmol/g wet weight)
Heart	1.41 ± 0.23	1.72 ± 0.26
Kidney	0.74 ± 0.21	1.12 ± 0.30
Liver	5.46 ± 0.65	4.35 ± 1.09

Table 3 shows the levels of GSH, determined by an enzymatic assay involving glutathione-S-transferase. From the data, we can conclude that there is a positive effect on the heart after chronic treatment of Dox. The 20% increase in GSH levels in the heart and a 2-fold increase in the kidney, promote the capability to protect the cells from oxidative stress by utilizing the excess free oxygen

radicals in the system. In contrast, there is a 20% decline in GSH levels in the liver, causing oxidative stress.

Sensitization:

Dosage-dependent cardiotoxicity:

An investigation was conducted to compare the levels of cardiotoxicity as a major side effect when Dox is used for treatment. It was revealed to reduce the possibility of causing dysfunction in the left ventricle when Dox was administered continuously for at least 6 hours to a relatively shorter period without any effect on its potency(van Dalen, van der Pal and Kremer, 2004).

The cumulative dose of Dox tends to damage the heart at the rate of 550 mg/m^2 and the ideal dose limit of the drug is determined to be 450 mg/m^2(van Dalen, van der Pal and Kremer, 2004).

Dosage-dependent Reproductive toxicity:

An investigation was performed in mature Wistar rats to compare the effect of single-dose and repeated doses of Dox treatment. There was no change in the body weight of Dox-treated rats when a single dose of 7.5 mg/kg was administered. However, a notable reduction in the body weight and testes weight of rats was observed due to the prolonged toxicity of Dox(Silva *et al.*, 2018).

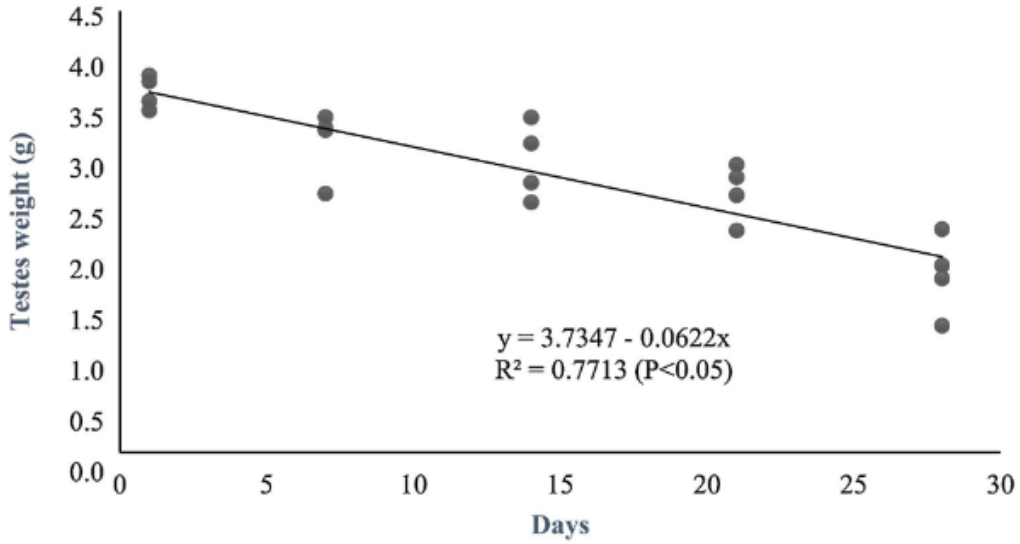

Figure 6: *Effect of Dox on the weight of testes* - Short-term Vs Long term(Silva *et al.*, 2018)

Finally, the level of exposure to the drug shows there is a variation in response and impact on various body cells clearly implying sensitization.

Genotoxicity:

Dox is confirmed to be a mutagen in somatic(Smith, 2003) and sperm cells(Sjöblom, West and Lähdetie, 1998), inducing DNA damage and causing testicular apoptosis paving the way for genotoxic damage(Habas, Anderson and Brinkworth, 2017).

A study has confirmed that Dox treatment has altered the morphology of Sertoli cells, reducing the nourishment for immature sperm cells. Sperm cells undergo mitotic division at a high rate allowing Dox to increase the risk of mutagenesis and cytotoxicity in germ cells(Brilhante *et al.*, 2012).

Altogether, Dox has an impact on the reproductive system such as degenerating the spermatocytes, minimizing sperm count, and its ability to be mobile either temporarily or permanently(Brilhante *et al.*, 2012).

Tumor targeted delivery:

To improve the specificity of the drug upon targeting it to the tumor through two main approaches, include:

1) Dox encapsulation in liposomes:
   Phospholipid bilayer vesicles act as drug carriers by encapsulating Dox in them thus influencing the pharmacokinetics and bioavailability of Dox. The encapsulation has resulted in a reduced volume of distribution henceforth concentrating the dose only on tumor cells(Harris *et al.*, 2002).

2) Dox conjugation to molecules:
   The drug is conjugated to any peptides, ligands, antibodies, or nanoparticle forming a complex to enhance the therapeutic efficacy and selectivity.
   The crucial enzymes involved in activating Dox by the cleavage from conjugate are alkaline phosphatase, β-glucuronidase, β-lactamase, and penicillin amidase(Hanušová, Boušová and Skálová, 2011).

Prodrug Approach:

IBPA-Dox is a prodrug developed, that binds to Human Serum Albumin (HSA) in the plasma which helps in elevating the therapeutic function of the drug by preferentially targeting it to Cav-1 positive cells thus reducing the cytotoxic effects on cancer-free cells(Liu *et al.*, 2024).

Table 4: Various conjugates for tumor-specific delivery of Dox(Hanušová, Boušová and Skálová, 2011)

Compound	Mechanisms of action	*In vitro* model	Confirmation *in vivo*	Reference
Cell-penetrating peptides (pene, TAT)	Accumulation in perinuclear region or cytoplasm of cell and limit DOX efflux by transport proteins	Breast cancer MCF-7 sensitive, MCF-7 resistant, MDA-MB-231,Adult malignant rat prostate tumor AT3B-1,Chinese hamster ovarian cells CHO, Human Umbilical Vein Endothelial Cells HUVEC	No	Liang and Yang, 2005; Aroui et al., 2009a; Aroui et al., 2009b; Aroui et al., 2010
HPMA copolymer	VEGF gene overexpression, increased vascular permeability and drug accumulation	Mouse lymphocytic Leukemia L1210Melanoma cells B16F10Ovarian reticular cell sarcoma M5076	Yes	Reviewed in Kratz 2008
HPMA copolymer-AGM (aminoglutethimide)	Inhibition of aromatase	Breast cancer MCF-7, MCF-7ca (aromatase transfected cell line)	No	Greco et al., 2005; Greco et al., 2007
TPGS (D-α-tocopheryl glycol 1000 succinate) and TPGS-FOL (TPGS and folic acid)	DOX delivery to intracellular compartments, prolonged circulation	Breast cancer MCF-7, Glioma cells C6	Yes	Cao and Feng, 2008; Anbharasi et al., 2009

These are the advanced research methodologies that successfully promote the use of Dox despite its toxicity.

Conclusion:

To conclude, Doxorubicin is a key anticancer drug that can be tailored to reduce toxicity by improving its selectivity. In the future, combination therapies to overcome drug resistance, nano-encapsulation

for targeted delivery, and cardioprotective agents to reduce cardiotoxicity, a wide range of Dox can be studied to enhance its therapeutic uses.

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