Abstract

The mode of action of the antincancer drugs anthracycline has been examined extensively over the past 20 years. Different studies have cited the interaction of the inherently reactive drug with DNA, proteins, metal ions, and molecular oxygen, leading to an apparently complex interplay of the mechanism of antitumor action, the major determinants of which may differ according to the properties of target cancer cells (Myers et al., 1988). Although the reactivities of anthracyclines with a range of cellular constituents are well known, but the specific molecular mechanisms involved in the plethora of cascade, which ultimately led to tumor cell death are still to be elucidated. In this regard, the author has tried to investigate the molecular interactions of these drugs with three important targets (i.e. histone, DNA and cellular dynamics), which could impact the eukaryotic transcription.

In cellular environment, eukaryotic genome is condensed within the cell in nucleosomes, the primary structural units of chromatin. Nucleosome consists of an octameric protein core containing two copies each of histones H2A, H2B, H3 and H4 and ~200 bp of DNA. The interaction of anthracycline anticancer drugs epirubicin (EPI) and mitoxantrone (MTX) with histones and its state of acetylation has been extensively studied. We studied the binding of these drugs with core histone and histone H3. The features of drug induced structural perturbation of core histones were studied by circular dichroism (CD) and absorption spectroscopy. Molecular modeling showed that the drugs bind near the N-terminal tail of histone H3. Further analysis of the binding site revealed their mode of interaction. The quantitative
binding parameters of these drugs with histone H3 have been determined for the first time. The mechanism of drug-histone H3 binding was elucidated by steady state fluorescence and static type quenching procedure was suggested for this interaction. The binding parameters for the reaction have been calculated according to Stern-Volmer equation at different temperatures, which suggested one high affinity binding site with the association constants of the order of $10^4$. The stoichiometry of interaction was also evaluated for drug-protein system. The mode of binding was expounded by thermodynamic parameters, which entails that hydrophobic interactions and hydrogen bonding stabilizes the interaction, which also corroborated with the molecular modeling studies. The molecular distance $r$, between donor (histone H3) and acceptor (drug) was estimated according to Förster's theory of non-radiation energy transfer. Further we evaluated the effect of these drugs on the enzymatic machinery involved in histone acetylation. We found that EPI and MTX both reduces histone acetyltransferase (HAT) enzyme activity in a dose- and time-dependent manner, suggestive of the inhibition of histone acetylation and thereby regulated transcription in actively transcribed tumor cell. Interestingly, we also find the slight reduction in Histone deacetylase (HDAC) enzyme activity. Though, HDACs catalyze the removal of acetyl groups from histones and contribute to transcriptional repression but in addition, the HDAC inhibitors induce apoptosis in cancer cells through alterations in histone acetylation and activation of the TRAIL apoptotic pathway. Also, western blot analysis was performed to determine the
drugs. Concentration dependent shift in acetylated state of these lysines were observed for both the drugs. This deranged equilibrium of histone acetylation can lead to alteration in chromatin structure and transcriptional dysregulation of genes that are involved in the control of proliferation, cell-cycle progression, differentiation and or apoptosis.

Depletion of histones after covalent modification from chromatin is the key step in eukaryotic transcription. The complex disassembles nucleosome structure leading to the release of nucleosomal DNA. The majority of anthracyclines administered to sensitive tumor cells is known to rapidly localize in the nucleus. The drug has a high affinity for DNA, thus providing the driving force for further nuclear uptake. It is well known that intercalation is the immediate form of interaction, and there is an extensive body of evidence to show that one of the first cellular responses is the impairment of topoisomerase II activity. Once a DNA-drug adduct is formed, it is widely accepted that the nature of the interaction impedes cellular functions that involve DNA (i.e. replication and transcription). But how this interaction occurs, does it has any specificity and whether sequence specificity of the drugs governs its specific mode of action and interference with specific regulation are still to be expounded. Hence, to get the more insight of the interaction of epirubicin (EPI) and mitoxantrone (MTX), we have evaluated the associated binding interactions of these
anthracyclines with naked DNA. The mechanism of EPI and MTX binding with DNA was elucidated by steady state fluorescence and static type quenching procedure is suggested for this interaction. The binding parameters for the reaction have been calculated according to Stern-Volmer equation at different temperatures, which suggests high affinity binding site with the association constants of the order of $10^5$. The temperature dependence suggests the destabilization of drug-DNA complex. The stoichiometry of interaction was also evaluated. Thermodynamic parameters from van't Hoff plots showed that the interaction of these drugs with DNA is an entropically driven phenomenon. Binding mode was expounded by UV-Vis measurements and competitive binding of known intercalator. Sequence specificity of these drug-DNA complexes were analysed by fourier transform infrared (FTIR) spectroscopy and molecular modeling studies. Circular dichroism (CD) spectroscopy and the plasmid nicking assay showed that the binding of these drugs results in DNA backbone distortions, resulting in structural and conformational transitions. Electrophoretic mobility shift assay (EMSA) was employed to determine whether, these drug-DNA complexes prevent binding of transcription factors to DNA in tumor cells and also the sequence selectivity of these particular drugs will determine which transcription factors are affected, hence, which genes are inhibited. This implicate the net effect of this process is that gene-specific inhibition may occur, depending on the sequence specificity of the particular drug adducts.
To uncover the full spectrum of the pharmacological activities of epirubicin (EPI) and mitoxantrone (MTX), we further analyzed their effect of cellular machinery, which might depict the profound consequences for the interpretation of previous results obtained for these drugs. Also it is a well known fact that administration of antitumour anthracycline led to cell death. But again cell death can be divided into two main categories, necrosis and apoptosis. Whereas necrosis is a catastrophic "death by assault", apoptosis can be thought of as an altruistic death, in which a cell positively executes death. Programmed cell death or apoptosis is an essential homeostatic mechanism in multicellular organisms allowing the elimination of no longer needed or seriously damaged cells by an orderly process of cellular disintegration. Depending on the organism, apoptosis involves a typical set of morphological events including chromatin condensation, DNA and nuclear fragmentation, cell shrinkage, plasma membrane blebbing, increased numbers of cytosol vacuoles and formation of apoptotic bodies followed by phagocytes digestion. Hence in the last section of this study we have tried to address these outcomes for better understanding of epirubicin (EPI) and mitoxantrone (MTX) antineoplastic properties. Inhibition on proliferation by these drugs was detected by evaluating its effect on cell proliferation and growth curve of the cells. EPI and MTX were also found to affect the cell viability and thereby affecting the cell physiology. Typical apoptotic morphology such as condensation of nuclei in apoptotic cells and inhibition of growth at specific point was observed
through confocal laser scanning microscopy (CLSM). The integrity and functionality of the yeast plasma membrane at different levels of drug were assessed using fluorescence spectroscopy, which is supposed to involve in the induction of cell death. As permeabilization of the plasma membranes is the critical event and related to cell death. It may result from lipidic phase transitions in the membrane and from variations in the area-to-volume ratio during the osmotic treatment. Cell cycle distribution was measured by flow cytometric measurements. Compared with the control group, the drug treated group exhibit differential cell distribution. The analysis demonstrated significant cell-cycle arrest with both the drugs.