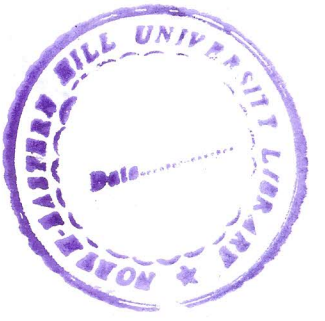
STUDIES ON THE EFFECT OF CISPLATIN ON MITOCHONDRIA IN DALTON'S LYMPHOMA - BEARING MICE



By

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SUBMITTED IN FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

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Declaration

I, Arpaia Kharbangar, do hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other university/Institute.

This is being submitted to the North Eastern Hill University for the degree of **Doctor of Philosophy** in Zoology.

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Akbangr
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(Arpaia Kharbangar)

CONTENTS

ABBREVIATIONS

BSA	Bovine serum albumin
DL cells	Dalton's lymphoma cells
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
EDTA	Ethylene diamine tetraacetic acid
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GST	Glutathione-s-transferase
i.p.	Intraperitoneally
LPO	Lipid peroxidation
MDH	Malate dehydrogenase
Mt	Mitochondrial
NPSH	Non-protein sulfhydryl
O.D.	Optical density
PBS	Phosphate buffer saline
ROS	Reactive oxygen species
SDH	Succinate dehydrogenase
SOD	Superoxide dismutase
TSH	Total sulfhydryl

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INTRODUCTION

INTRODUCTION

i) CANCER

The multiplication of cells in the body is carefully regulated. In an adult, some cell types e.g. nerve cells do not divide while others like skin and progenitors of the blood cells divide throughout the life in order to replace billions of cells that die everyday. Thus, a very carefully controlled programme exists to determine the multiplication of different types of cells in the body.

Eukaryotic cell division occurs in four well defined phases: synthesis or S phase, gap 2 or G2 phase, mitosis or M Phase and gap 1 or G1 phase. After passing through mitosis and into G1, a cell either continues through another division or ceases to divide, entering a quiescent phase (Go) that may last hours, days or the lifetime of the cell. When a cell in Go begins to divide again, it re-enters the division cycle through the G1 (Hunt and Nasmyth, 1997). The cell cycle is controlled by a family of protein kinases that are the heterodimers with a regulatory subunit, cyclin and a catalytic subunit, cyclin-dependent protein kinase (CDK). The cell division is also regulated by a family of extracellular growth factors (Weinberg, 1996).

If occasionally, the exquisite control mechanisms of regulating cell multiplication break down, a cell begins to grow and divide in an uncontrolled manner. Descendants of such cells inherit the propensity to proliferate without responding to regulation and expand indefinitely to develop as a lump, which is commonly referred to as a tumor. Defects in the synthesis, regulation or recognition of growth factors may also be involved in developing a tumor (Rubin, 1985).

Tumors are strictly defined as neoplasm, although the term tumor may be applied to any swelling (Vincent, 1985). The terms neoplasm and tumor are commonly used interchangeably (Friedberg, 1986). Tumors violate the basic homeostatic principle of

the body and ideally fall into one of the two categories, the slowly growing 'benign' and the rapidly growing 'malignant' forms which are invasive, disseminating and show metastasis (Vincent, 1985). The spread of tumor from the primary organ or tissue in which neoplasm initially occurs to secondary sites is called metastasis (Fidler and Hart, 1982).

The development of malignant tumor is a multistep process characterised by a progression of genetic alterations in a single line of cells. Various cancer causing agents are called carcinogens. These carcinogens could be (i) physical agents (ultraviolet rays, γ-rays, X-rays) (ii) chemicals (Benzpyrene, aflatoxin B1, benzanthracene, methylchlolanthrene, lead, carbon tetrachloride, asbestos) and (iii) viral agents (Rous, sarcoma virus, polyoma virus, simian virus 40, adenovirus, Epstein barr virus etc) (Fearon, 1997).

In the carcinogenesis two categories of genes (tumor suppressor genes and oncogenes) may be implicated. Tumor suppressor genes (about 20 in human) normally act as cell's brakes. They encode proteins that restrain cell growth and prevent cells from becoming malignant. The transformation of a normal cell to a cancer cell is accompanied by the loss or decrease of function of one or more tumor suppressor genes. Most of the proteins encoded by tumor suppressor genes act as negative regulators of cells proliferation which may be as transcription factors (p53 and WT1), cell cycle regulators (RB and p16), components regulating signalling pathways (NF1) and components regulating RNA polymerase II elongation (VHL). Thus, their elimination contributes and promotes uncontrolled cell growth (Haber and Harlow, 1997).

In contrast to tumor suppressor genes, oncogenes (Greek; onkos, a tumor) encode proteins that promote the loss of growth control and conversion of a cell to a malignant state. Oncogenes are generally derived from proto-oncogenes which are genes that encode proteins having a function in the normal cell. These oncogenes products act in many

ways, for example, i) as growth factors or their receptors e.g. SIS oncogenes derived growth factor (PDGF), erbB oncogenes which directs the formati receptor, ii) as cytoplasmic protein kinases e.g. RAF that heads the MAP kina the primary growth controlling signalling pathway in cells, iii) as nuclear tr factors e.g. myc oncogene and iv) as the products that inhibits apoptosis oncogene (Hunter, 1997).

Malignant tumors are commonly referred to as cancers. The word ca = crab) suggests its capacity to reach out and cling tenaciously to adjac Cancer is considered to be a dynamic developmental disorder and a disease differentiation (Rubin, 1985). Cancer cells have unlimited life span, require factors and exhibit anchorage independence for growth. Cancer cells ar rounded/convex shape, show reduced adhesion to substratum with the loss inhibition of movement and multilayering in culture. Cancer cells also acquir production of proteolytic enzymes, altered antigenicity, increased negat charge, disorganised cytoskeleton etc (Hynes, 1979).

Cancers are generally classified into three broad groups: carcinomas and leukemia/lymphomas (Cairns, 1986). About 85% of cancers are carc tumors that arise from endodermal or ectodermal tissues such as skin or th lining of internal organs and glands, colon, breast, prostrate, ovary, lungs etc (~5%) arise less frequently and are derived from mesodermal connective such as bone marrow, fat and cartilage. The leukemias and lymphomas (cancers of haematopoietic cells.

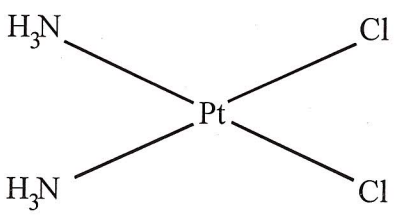
Although the idea about cancer dates back to the late 17th century (Currie, 1982) and in spite of a lot of scientific advancements, cancer unpredictable and fearful disease. Considerable efforts have been directe improving the diagnosis and treatment of cancer. Surgery, chemotherapy and ra

are the three main ways of widely accepted treatment for cancer. In chemotherapy, cisplatin plays a pivotal role and can be used singly or in combination with radiotherapy and/or surgery in the treatment of many cancers.

ii) CISPLATIN

Rosenberg et al. (1965) while studying the effect of electric fields on bacterial (*Escherichia coli*) growth, noted that the bacterial growth continued but cell division was inhibited. The inhibition of cell division was attributed to the formation of amminechloro compounds from platinum electrodes and ammonium chloride in the growth medium and it was subsequently identified as *cis*-diamminedichloroplatinum (II) (Rosenberg et al., 1967). It is now commonly known as cisplatin.

The cell division inhibiting property of cisplatin evoked to study the antitumor activity of cisplatin and it was recognized as a potential antitumor agent (Rosenberg et al., 1969). Now cisplatin has been established to be a potent antitumor drug against a wide spectrum of experimental tumors such as leukemia L1210, DMBA mammary carcinoma, Rous sarcoma, Dunning ascites leukemia, Walker 256 carcinoma (Kociba et al., 1970; Sarna and Sodhi, 1978; Rosenberg, 1985) and also in human malignancies such as ovarian and testicular tumors, bladder carcinoma, head and neck cancer (Pil and Lippard, 1997; Lebwohl and Canetta, 1998).



Structure of Cisplatin

Cisplatin is a water soluble, square planar coordination complex containing a central platinum atom surrounded by two chloride atoms and two ammonia moieties. The antitumor activity of the complex is much greater when the chloride and ammonia moieties are in the *cis* position as compared to the *trans* position. In an aqueous solution, one or both chloride ions on cisplatin may be replaced by water to produce hydrated intermediate known as an '**aquo**' cisplatin species [$Pt(NH_3)_2Cl_2 + H_2O \rightarrow Pt(NH_3)_2Cl(H_2O)^+ + Cl^-$; $Pt(NH_3)_2Cl(H_2O)^+ + H_2O \rightarrow Pt(NH_3)_2(H_2O)_2^{++} + Cl^-$] and the monochloro monohydroxy platinum species in an alkaline environment [$Pt(NH_3)Cl_2 + OH \rightarrow Pt(NH_3)_2Cl(OH) + Cl^-$] (Rosenberg, 1985).

Isotonic and hypertonic saline solutions as well as human blood plasma have high chloride ion concentrations, which helps to maintain a greater amount of cisplatin as a $Pt(NH_3)_2Cl_2$ species in these fluids. Acidic pH tends to stabilize cisplatin's reactivity towards nucleophiles such as water, sulfate and free thiols (Hausheer et al., 1998). After passive diffusion across the plasma membrane, however, the chloride ion concentration decreases sharply, promoting the hydrolysis of the labile chloride ligands (Chu, 1994).

The major cytotoxic target of cisplatin in the cell is suggested to be DNA (Eastman, 1986), causing inhibition of DNA synthesis by acting on DNA template rather than on DNA polymerase (Howle and Gale, 1970; Pinto and Lippard, 1985). It has been suggested that the *cis* configuration of cisplatin favours the formation of intrastrand cross links in DNA (Roberts and Pascoe, 1972; Roberts, 1974; Roos and Arnold, 1977).

In intact DNA, cisplatin binds preferentially to the N-7 position of guanine and adenine (Pinto and Lippard, 1985). The cytotoxicity of cisplatin against cultured neoplastic cells correlate closely with platinum DNA interstrand cross links and to the formation of intrastrand bifunctional N-7 adducts at d(GPG) and d(APG) (Zwelling et

al., 1979; Plooy et al., 1984; Coste et al., 1999). All bifunctional adducts cause major distorsions of the local DNA structure involving both bending and unwinding of the double helix. Such structural differences between the complexes formed by cisplatin may serve as a molecular basis for their differential biological and chemotherapeutical activity. Intrastrand cross-links strongly inhibit DNA synthesis and its transcription (Heminger et al., 1997; Cullinane et al., 1999). Two classes of proteins HMG1 and HMG2 have recently been identified that bind preferentially to cisplatin-damaged DNA sites. The formation of these specific proteins or damaged DNA complexes may actually function in blocking repair by competing out the binding of the repair-related recognition proteins and hence the cytotoxity of the drug (Zamble and Lippard, 1995; Yaneva et al., 1997; Zlatanova et al., 1998).

Cisplatin has also been reported to interact with the side chains and terminal amino and carboxylate groups of proteins, small peptides and amino acids and negatively charged phospholipids (Taylor et al., 1995; Burger et al., 1999). Prasad and Sodhi (1981, 1982) reported that cisplatin treatment of normal and tumor cells causes the removal of the sialic acid and mucopolysaccharides from the cell surface which may lead to an increase in the antigenicity of tumor cells and render them more immunogenic and accessible to the cells of the immune system. It has also been shown that treatment of tumor cells with cisplatin increases the expression of tumor cell surface associated antigens and thereby permits the immunological recognition and subsequent rejection of cisplatin treated cells (Sarna and Sodhi, 1978). Sodhi and Prasad (1985) reported differential effect of cisplatin on the lectin, concanavalin A (Con A) and wheat germ agglutinin (WGA), agglutinability of splenocytes and Dalton's lymphoma cells. The agglutinability of normal cells (splenocytes) increased and Dalton's lymphoma (tumor) cells decreases after cisplatin treatment. The depolymerization of microfilaments, formation of giant multinucleated cells after cisplatin treatment have also been reported (Sodhi, 1976)

In the antitumor studies against murine ascites Dalton's lymphoma, Prasad and Giri (1994) showed that during tumor regression ascites fluid volume decreases sharply and there is an increase in carbohydrate and decrease in protein concentration in the ascites supernatants with the infiltration of leukocytes towards the tumor cells. Membrane vesicles and vacuoles were also formed before the disintegration and lysis of tumor cells. Prasad and Giri (1999) reported that cisplatin treatment of mice results in a decrease in the lactate dehydrogenase (LDH) activity. Isozyme pattern of LDH revealed the presence of five isozymes along with the tissue specificity of different isozymes with only LDH-5 in tumor cells and appearance of some specific isozyme variant LDH-T in the serum of tumor-bearing host. Prasad et al. (1999) reported a decrease in the activities of enzymes such as Na^+K^+-ATPase, 5'-nucleotidase, arginase and lactate dehydrogenase in tumor cells and tissues of tumor-bearing mice after cisplatin treatment. Nicol and Prasad (2002) reported that cisplatin treatment of tumor-bearing mice resulted in an overall decrease of sialic acid contents in the DL cells as well as other tissues such as liver, kidney and testes which may help in tumor regression. Based on the various findings on the effect of cisplatin in the cells particularly involving the cellular components other than DNA, it has been suggested that these may play an additional significant role in the anticancer activity of cisplatin and this led to propose the involvement of multistep and multilevel effects of cisplatin in the tumor cells or host (Giri, 1995).

a) Cisplatin and side effects

Although cisplatin is one of the most widely used chemotherapeutic agents, human tumors exhibit a spectrum of response to cisplatin according to their histology, with tumors of germ cell origin the most sensitive and those arising from the breast, pancreas and gastro intestinal tract the most resistant (Kaye et al., 1992).

The therapeutic efficacy of cisplatin is limited due to its side effects which include

nephrotoxicity, neurotoxicity, gastrointestinal toxicity, ototoxicity, embryotoxicity and mutagenicity (Prestayko et al., 1979; Keller and Aggarwal, 1983; Roberts et al., 1988; Giri et al., 1998). Cisplatin-induced nephrotoxicity shows i) an increase in blood urea nitrogen, ii) a decrease in glomerular filtration rate, iii) impaired renal concentrating capacity, iv) non-specific tubular necrosis with tubule dilatation and cast formation, and v) specific tubular lesions characterised by renal potassium and magnesium wasting (Lippman et al., 1973; Gonzales-Vitale et al., 1977; Dentino et al., 1978; Schilsky and Anderson, 1979; Safirstein et al., 1981; Blachley and Hill, 1981). A variety of strategies have been proposed to protect the kidney and neurological functions following cisplatin treatment. It has been shown that the administration of glutathione (GSH) provides protection against cisplatin-induced nephrotoxicity without reducing the antitumor activity of the cytotoxic agent (Zunino et al., 1998).

In an attempt to overcome nephrotoxicity the use of cisplatin in combination with other agents have also been tried with different degrees of success (Treskes and Van der Vijgh, 1993; Giri et al., 1998). Vitamin C has been reported to be effective as a protectant against a variety of toxic chemical agents including heavy metals (Holloway and Peterson, 1984). The protective role of vitamin C on cisplatin- induced nephrotoxicity and mutagenicity have been observed (Giri and Prasad, 1996; Giri et al., 1998).

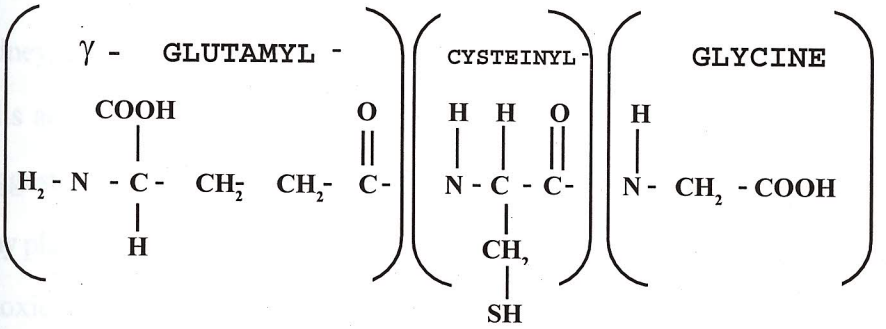
The mutagenic potential of cisplatin reported in bacteria (Cross et al., 1996) as well as in mammalian system (Khynriam, 2001) raises concern that its use in cancer chemotherapy may have carcinogenic risk with the development of secondary malignancies.

Another main limitation for the full clinical evaluation of cisplatin, is the development of drug resistance (Dabholkar and Reed, 1996) by the cancer cells and it may be multifactorial involving reduced drug uptake, enhanced DNA repair mechanisms, enhanced cellular GSH levels and impaired influx through the cell membrane or enhance efflux (Andrews and Howell, 1990).

b) Cisplatin and mitochondria

Cisplatin has been shown to bind preferentially to mitochondrial DNA (mtDNA) as compared to nuclear DNA (nDNA) (Olivero et al., 1995,1997). As compared to nDNA, cisplatin adduction in mtDNA is reported to be 4-fold to 8-fold higher (Olivero et al., 1995,1997). This preferential binding of cisplatin could be explained by the naked structure of mtDNA, which makes it highly accessible to damaging agents (Salazar et al., 1982). The increased susceptibility of mtDNA to damaging agents could be due to the existence of a non-nucleoprotein structure, the lipophilic nature of the mitochondrial membrane and prevalent oxidative environment in the mitochondria (Sawyer and Van Houten, 1999). mtDNA encodes proteins which participate in the electron transport chain and a damage to mtDNA will diminish electron transport function resulting in a fall in cell bioenergetics and subsequent cellular dysfunction. (Olivero et al., 1997). Mitochondrial dysfunction have been reported in hepatotoxicity (Pessayre et al., 1999) and cisplatin-induced nephrotoxicity (Kruidering et al., 1997).

c) Cisplatin and Glutathione



Structure of Glutathione

Glutathione, a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine), is an important antioxidant. In the cells under normal physiological conditions more than 98% of glutathione exists in reduced form (GSH) (Arrick and Nathan, 1984; Wang and Ballatori, 1998). Cellular GSH is involved in a variety of important physiological and metabolic

functions including the synthesis of proteins and DNA, transport, enzyme activity and protection of cells (Meister, 1988; Deleve and Kaplowitz, 1991; Wang and Ballatori, 1998). It inhibits free radical mediated injury by eliminating toxic peroxides and protects protein sulfhydryl groups from oxidation (Meister, 1988). GSH has also become the focus of intensive interest in cancer chemotherapy and also shown to be implicated in the metabolism of cisplatin causing alterations in the rate of drug uptake. GSH may also be involved in the detoxification of cisplatin (Li et al., 1997). It has been observed that cisplatin treatment of tumor bearing mice causes a decrease of GSH levels in blood, ascites supernatants and tumor cells. Blood haemoglobin, erythrocytes, packed cell volume (PCV) and leukocytes were also decreased along with the development of various morphological abnormalities in erythrocytes after the treatment. It was suggested that blood glutathione level and development of haematological abnormalities are inversely related (Khynriam and Prasad, 2001). In the studies involving GSH-related enzymes, Khynriam and Prasad (2002) have reported that the activity of glutathione related enzymes is variable in different tissues. In liver, glutathione-S-transferase (GST), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activity decreased while glutathione reductase (GR) increased after cisplatin treatment. In kidney, catalase and GST activity decreased while GR, GPx and SOD increased. In DL cells activities of GST, GPx, catalase and GR decreased but SOD increased. It was suggested that these changes could affect cellular antioxidant defense potential which may play an important contributory role in cisplatin-mediated toxicity particularly nephrotoxicity and anticancer activity in the host.

iii) Mitochondria

a) General features

Mitochondria (Gr, mito = thread; chondrion = granule) are considered as the direct descendants of a bacterial endosymbiont representative that became established at an early stage in a nucleus containing host cell (Gray et al., 1999). Mitochondria consist of two membranes and two compartments. The outer membrane limits the intermembranous space and inner membrane surrounds the mitochondrial matrix and itself projects as infoldings, called mitochondrial cristae (Detailed review, Fromenty and Pessayre, 1995). The outer membrane contains majority of integral protein, called porins, which form small aqueous channels so that outer membrane is freely permeable to most small molecules with the molecular weights in the range of 4000-5000. The inner membrane is highly specialized with a variety of transport proteins and contains high proportion of double phospholipid, cardiolipin (Melnick and Parker, 1971).

The total number of different proteins making up a mitochondrion is approximated to be more than 1000 (Bauer et al., 1999). Most of these proteins are encoded by the nuclear genome and synthesized in the form of precursor proteins and imported into mitochondria in a multi-step process which is facilitated by the coordinated action of independent translocation systems in the mitochondrial outer and inner membranes. In the yeast, *sacharomyces cerevisiae*, three district translocases have been described to mediate mitochondrial protein import: one translocase complex in the outer membrane (TOM complex) and two translocase complexes in the inner membrane (TIM 23.17 and TIM 22.54). For import of preproteins into the matrix and into the inner membrane, the TOM complex cooperates with two distinct translocation machineries in the inner membrane, (Neupert, 1997; Rassow et al., 1999). The TIM 23.17 complex mediates the import of precursor proteins which carry on N-terminal presequence into mitochondrial matrix in an ATP - and membrane potential dependent manner (Bauer et al., 1996). The membrane potential $\Delta\Psi$ is required for translocation of the presequence across the

inner membrane and ATP is needed to complete translocation of the mature portion of a percursor. TIM 17 and TIM 23 are parts of a protein conducting channel across the inner membrane that can only accomodate unfolded proteins. The preproteins are not tightly bound within the translocation channel (Berthold et al., 1995). When the presequence emerges from the matrix side of the translocation channel it is bound by mt-Hsp70 which together with its co-chaperone Mge 1p and TIM 44 forms an import motor which then drives further translocation (Review, Neupert, 1997). For import and membrane insertion of a class of hydrophobic carrier proteins such as ADP/ATP carrier into the inner membrane, the TOM complex cooperates with the TIM 22.54 complex via additional soluble components located in the intermembrane space (Sirrenberg et al., 1998; Koehler et al., 1998). Upon entering the intermembrane space, the hydrophobic proteins first bind to the small proteins TIM 9 and TIM 10 and are then handed over to TIM 12. The insertion of the hydrophobic preproteins into the inner membrane is then mediated by TIM 22, which has been shown to interact with TIM 9-10-12 proteins (Sirrenberg et al., 1998). It has now been shown that TOM and TIM complexes of yeast and mammals are conserved and apparently composed of homologous components (Bauer et al., 1999).

Mitochondrial enzymes are highly compartmentalized. The outer membrane is characterised by the presence of monoamine oxidase while the inner membrane contains succinate dehydrogenase (SDH). Malate dehydrogenase (MDH) is the enzyme of the matrix and is present in two forms, cytosolic and mitochondrial (Lehninger et al., 1993).

Within all mammalian cells there are two distinct genomes, one located in the nucleus and the other in the mitochondria. mtDNA encodes for only 13 proteins of the respiratory chain, 22 tRNA and 2 rRNA species. The remaining proteins (around 80) needed in the respiratory chain are encoded by nuclear DNA. Respiratory chain

polypeptides are located in the inner membrane although cytochrome C is loosely associated with this membrane, in the intermembranous space (Fromenty and Pessayre, 1995; Saraste, 1999).

mtDNA is a circular double stranded molecule and it is extremely sensitive to oxidative damage owing to its proximity to the inner membrane, the absence of protective histones and incomplete repair mechanisms in mitochondria (Bogenhagen, 1999). The mitochondrial genome contains very few non-coding sequences, no introns and is not protected by histones and the damage to mitochondrial DNA can be expected to have a significant impact on cell functions. Therefore, as compared to nuclear DNA, mtDNA is more sensitive to be affected under different pathological conditions. Interest in mtDNA damage has risen with the discovery that defects in the mitochondrial genome are associated with several human hereditary diseases such as Kearns-Sayre syndrome, Lebers hereditary optic neuropathy, Pearson's syndrome and some cases of chronic progressive external opthalmoplegia (Wallace, 1992;Taylor, 1992). Additionally accumulations of mutations and deletions in mtDNA with their associated defects in oxidative phosphorylation have been implicated in diabetes, ischemic heart disease, Parkinson's disease, demyelinating polyneuropathy, cancer and aging (Ikebe et al., 1990; Wallace, 1992; Taylor, 1992; Ballinger et al., 1992).

b) Mitochondria and apoptosis

Apoptosis or programmed cell death is an active physiological cell death that controls cell populations during embryogenesis, immune response, hormone regulation and normal tissue homeostasis. Changes in the mechanism of apoptosis are also associated with the pathophysiology of cancer, AIDS or neurodegenerative diseases. The process of apoptosis involves a cascade of biochemical events which in the late stages of the process, leads to activation of specific cysteine proteases called caspases, and finally to characteristic changes in nuclear morphology and DNA fragmentation (Green, 1998).

Although apoptosis is a well-defined morphological process, the biochemical mechanisms involved in it, remain under investigation. It is well known that cellular redox status modulates various aspects of cellular function. Kane et al. (1993) suggested that proto-oncogene Bcl-2; an inhibitor of apoptosis exerts its action by reducing the production of reactive oxygen intermediates (ROI), thus, working as an antioxidant in neurons. Recent reports have emphasised the role of oxidative stress and nuclear DNA damage in apoptosis (Polyak et al., 1997; Vanden Dobbelsteen et al., 1996). In addition, antioxidants have been shown to protect against apoptosis in different experimental models. However, normal apoptosis occurs in very low oxygen environments (Jacobson and Raff, 1995). It has been shown that apoptosis is closely related to mitochondrial impairment also (Zamzami et al., 1996). Although, the possible effect of apoptosis on mtDNA is not known, it has been reported that during the apoptotic process, the following events may take place in mitochondria: mtDNA oxidation, increased mitochondrial peroxide production and cytosolic peroxide levels, early oxidation of the mitochondrial and cytosolic GSSG/GSH couple and decreased mitochondrial membrane potential (Esteve et al., 1999).

c) Mitochondria and aging

Mitochondria has been an adversely affected organelle during aging. The electron transport chain is known to be the major intracellular site for the generation of 0_2 and H_2O_2 and subsequently other potentially deleterious reactive oxygen species (ROS) as well as for ATP production via oxidative phosphorylation (Chance et al., 1979). There is a large body of evidence indicating an age-related increase in the rate of mitochondrial O_2 and H_2O_2 generation and the amounts of oxidative damage to mitochondrial proteins and DNA (Sohal, 1997; Martinez et al., 1996; Shigenega et al., 1994). Oxidative damage to mitochondria has been experimentally demonstrated to cause both an elevation in the rate of mitochondrial ROS, H_2O_2 generation and a derangement of

mitochondrial respiratory activity (Sohal and Dubey, 1994). Furthermore, the rate of mtDNA transcription, as indicated by the levels of cytochrome C oxidase RNA relative to mt DNA also seems to decline in human skeletal muscle during aging (Barrientos et al., 1997) which indicates that mitochondria become progressively more damaged during senescence (Yan et al., 1997). Nevertheless, whether activities of mitochondrial electron transport complexes uniformly decline during aging remains controversial. In human skeletal muscles, Cooper et al. (1992) have reported an age-associated decline in the activities of complex I and IV, whereas Barrientos et al. (1996) found no correlation between specific activities of electron transport complexes and age. Furthermore, the pattern of age related changes in the enzymatic activities of the complexes, observed in the liver mitochondria (Miquel et al., 1995), was dissimilar from that observed in mitochondria isolated from skeletal muscle, heart and brain (Desai et al., 1996; Guerrieri et al., 1993, Bowling et al., 1993; Fernandiz et al., 1994).The mitochondrial theory of aging postulates that organisms age due to the accumulation of DNA damage and mutations in the multiple mitochondrial genomes, leading to mitochondrial dysfunctions. Among the many types of DNA damage, 8-oxodG has received the most attention due to its mutagenicity and because of the possible correlation between its accumulation and pathological processes like cancer, degenerative diseases and aging (Croteau et al., 1999).

d) Mitochondria and calcium regulation

Since calcium ions (Ca^{++}) function as important cellular signals regulating many physiological processes, the cytosolic concentration of Ca^{++} is maintained at very low levels (approximately 10^{-7}M) with the concentration gradient between extra and intracellular environments of the order of 10,000. Cells use multiple mechanisms to tran-

siently increase the Ca^{++} concentration in the cytoplasm, in response to appropriate stimuli (Clapham, 1995; Berridge, 1997). Mitochondria, in addition to their function as cellular power plants, have been recognised to play a central role in Ca^{++} homeostasis and cellular Ca^{++} signalling (Thomas et al., 1996; Simpson and Russell, 1998).

The cell achieves low cytosolic Ca^{++} concentration by actively expelling Ca^{++} ions to the exterior and by judicious compartmentalization of Ca^{++} into specialised cellular stores (Clapham, 1995; Berridge, 1997). Plasma membrane Na^{+}-Ca^{++} exchanger (PMNCE) and a Ca^{++} ATPase pump (PMCA) move Ca^{++} across the plasma membrane to the cell exterior and the sarcoendoplasmic reticulum Ca^{++} pump known as SERCA which moves Ca^{++} ions into ER Ca^{++} stores. In addition, mitochondria have long been recognised to be important in removing cytoplasmic Ca^{++} particularly when the Ca^{++} levels reach above tens of micromolar in concentration in the space around mitochondria (Rizzuto et al., 1993). Thus, mitochondria can act as low-affinity, but high capacity cytosolic Ca^{++} sponges. Furthermore, a number of Ca^{++} binding proteins present in the cytoplasm act as local buffers of Ca^{++} and help to remove signalling Ca^{++} away from its targets. These proteins together with the mitochondria and ER Ca^{++} transport systems function to restrict rapid diffusion of Ca^{++} in the cytosol and contribute to discrete local signals (Simpson and Russell, 1998).

e) Mitochondria and Lipid peroxidation

Lipid peroxidation is a complex process known to occur in both plants and animals. It involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids producing a variety of breakdown products. Lipid peroxidation usually begins with the obstruction of a hydrogen atom from an unsatur-

ated fatty acid resulting in the formation of a lipid radical. An attack by molecular oxygen produces a lipid peroxy radical, which can abstract a hydrogen atom from an adjacent lipid to form a lipid endoperoxide, which can lead to the formation of malondialdehyde as a breakdown product. Malondialdehyde formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for determining the extent of the peroxidation reaction. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid, which in total is referred to as thiobarbituric acid reacting substance (TBARS) (Buege and Aust, 1978).

The polyunsaturated fatty acids located in the mitochondrial membranes are excellent targets for lipid peroxidations. Conversely, it is known that the lipid environment can affect membrane function (Pamplona et al., 1996), including mitochondrial electron transport which could influence ROS production. Microsomes (Catala et al., 1994) as well as mitochondria are susceptible to lipid peroxidation (Wright et al., 1979 ; Vladimirov et al., 1980) and the measurement of lipid peroxidation has become one of the most commonly used parameter for radical-induced damage (Svingen et al., 1979; Sevanian and Hochstein, 1985).

f) Mitochondria and tumorigenicity

Various studies have suggested a role of cytoplasmic components, including mitochondria, in maintaining the tumorigenic phenotype (Cavalli and Liang, 1998). Because the mitochondrion has the distinctive property of harbouring its own DNA, studies have indicated that the mitochondria may play a direct role in the tumorigenic phenotype. The studies of cytoplasmic fusion of cancer and normal cells have suggested extra-nuclear cellular components can maintain the tumorigenic phenotype (Welter et al., 1989). Creation of mtDNA-less yeast cells can mimic cell surface changes similar to vertebrate malignancy, and in mtDNA-depleted hamster cells, membrane glycopep-

tide profiles have been found to be similar to transformed tumorigenic cells (Wilkie et al., 1983). Other studies have suggested that mutations in the nuclear p53 and *bcl-2* gene are involved in the induction of anchorage-independent growth *in vitro* (Nikiforov et al., 1996; Sierra et al., 1995). However, some evidence has also been provided that mtDNA-less tumor cells withour p53 mutation and *bcl-2* overexpression can loose the anchorage-independent phenotype (Cavalli and Liang, 1998).

The biochemical and ultrastructural events in mitochondrial that may eventuate cisplatin-induced effects in the tumor cells/ host remains to be investigated in detail. In the present piece of work involving the effect of cisplatin in the cells/host, mitochondria have been chosen as cellular targets because i) their highly specialized membrane-linked functions are very sensitive to drugs, ii) mitochondria play a central role in cellular homeostasis and the study of the effects of cisplatin might shed light on the importance of mitochondria in cellular toxic effects, and iii) platinum (II) complexes interact with energy-dependent functions in the cells (Beltrame et al., 1984). Thus, in this study investigations were undertaken to look into the changes in mitochondrial protein, glutathione, lipid peroxidation, succinate dehydrogenase activity, malate dehydrogenase activity, oxygen consumption and ultrastructure in relation to tumorous condition and cisplatin treatment. This may help to understand further the significance of mitochondria in the effectiveness of cisplatin in toxicity/cytotoxicity in tumor-bearing mice.