Introduction
1. INTRODUCTION

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1. INTRODUCTION

Aspirations for cancer chemotherapy have grown with appreciation that the majority of patients with malignant disease have tumors that cannot be cured by local therapy alone. In course of its 30 years of development, chemotherapy has acquired a firm place in the treatment of human cancer, in addition to the classical methods of surgery and radiation therapy which have been of considerable benefit in fighting primary tumors.

Newer approaches now under investigation also include anticancer vaccines, agents directed at destroying malignant cells through immunologic mechanisms, and gene therapy. Each form of therapeutic intervention has the potential to produce adverse effects on normal host tissues, and some of these toxicities may be accentuated with combined modality therapy (Grever and Grieshaber, 1997).

1.1 General mechanisms of anticancer drug action

The initial requirement for drug action is adequate drug delivery to the target site. This depends largely on blood flow in the tumor bed and the diffusion characteristics of the drug in tissues. Delivery may also be influenced, however, by the extent of plasma protein binding and for orally administered drugs by absorption and first-pass metabolism in the liver (Fig. 1a). Blood flow across a capillary bed is directly proportional to the arteriovenous pressure difference and inversely proportional to the geometric and viscous resistances. The geometric resistance to blood flow increases with increasing tumor size, a factor that may limit drug and oxygen delivery to large tumors and therapy.
Fig. 1a Schematic representation of pharmacokinetics and pharmacodynamics. Pharmacokinetics represents the distribution, metabolism and elimination of drugs from the body. Pharmacodynamics describes the interaction of drugs with target tissues.

(Adapted from Ratain and Plunkett, 1977)
diminish the effectiveness of treatment with chemotherapy or radiation (Sevick and Jain, 1989).

The most common route of drug administration for both localised and disseminated disease is intravenous infusion, which by definition makes 100% of the drug available in the blood. Drugs may be administered by a number of routes in addition to intravenous infusions to achieve special pharmacologic and therapeutic goals. Regional administration may be employed to more directly target the drug to the principal tumor site and to achieve a higher drug concentration in the vicinity of the tumor.

Intraperitoneal infusion of cisplatin for ovarian cancers, intrapleural administration of interleukin 2 (IL-2) in the treatment of mesothelioma and intrathecal administration of cytarabine (ara-C) for leukemias are examples of intracavitary drug delivery. Alternatively, intravascular administrations such as intra-arterial infusion of fluorodeoxyuridine into the hepatic artery for treatment of liver disease has been used to achieve a pharmacologic advantage. Although oral administration is the most convenient and least expensive route, it is associated with problems of inconsistent drug bioavailability, among and within patients. More consistent pharmacokinetics are achieved with subcutaneous or intramuscular drug injection.

Delivery of the drug to the target cell is also dependent upon the rate of removal from the blood. Excretion either by kidneys or by the biliary route, constitutes a major clearance mechanism. In addition, many drugs are cleared by metabolism to less effective or inactive metabolites as the blood passes
through large organs. Drug binding to plasma proteins can also effectively lower the concentration of free drug available for entry into target cells to a small fraction of the total concentration in blood (Ratain and Plunkett, 1997).

1.2 Anticancer drug targets

While anticancer drugs have traditionally been classified by mechanisms of action or their origins, they can also be grouped by the target of drug action. There are, essentially, four potential targets: nucleic acids, specific enzymes, microtubules, and hormone and/or growth factor receptors. A simplified overview of the sites of action of many of the drugs described is shown in Fig.1b.

1.3 Cisplatin (cis-Diamminedichloroplatinum II; cDDP)

The biological activity of cisplatin was discovered serendipitously in 1965 during studies of the effect of an electric current on Escherichia coli (Rosenberg et al., 1965). Cell division was inhibited not by the electric current but by the production of cis-diamminedichloroplatinum (II) (cis-DDP or cisplatin) from the platinum electrodes. Cisplatin was found to have potent antitumor activity as well and now is widely used for the treatment of many malignancies, including testicular, ovarian, head and neck, bladder, esophageal and small cell lung cancers.
Fig. 1b Summary of the mechanisms and sites of action of chemotherapeutic agents useful in neoplastic disease. (PALA = N-phosphonoacetyl-L-aspartate)

(Adapted from Calabresi and Chabner, 1991)
1.3.1 Structure

Cisplatin is a heavy metal complex containing a central atom of platinum surrounded by two chloride and two ammonia molecules in the cis-position.

\[ \text{cis-Diaminedichloroplatinum II} \]

1.4 The aquation reaction of cisplatin

When cisplatin is dissolved in aqueous solution, chloride ions are displaced to allow the formation of aquated species, which are the reactive forms of the compound as shown in reaction.
The concentration of chloride ions influences the reactivity of cisplatin. After intravenous administration, it is relatively less reactive in the extracellular space where the chloride concentration is ~100 mM, but on crossing the plasma membrane, it is activated in the intracellular space where the chloride concentration drops to ~3 mM. Activated cisplatin is a potent electrophile that will react with any nucleophile including the sulphhydryl groups on proteins and nucleophilic groups on nucleic acids (Chu, 1994).

The mechanism of its action appears to be inhibition of DNA synthesis. This effect seems to be mediated via the guanine, cytosine and adenine bases owing to the formation of inter-intrastrand cross-links.

1.5 Cisplatin cytotoxicity

Chemotherapeutic agents have been important for treatment of several human malignancies (Einhorn and Williams, 1979; Rozencweig et al., 1981). These types of tumours are treated most effectively by using relatively high dose regimens of chemotherapeutic agents. Unfortunately, the importance of adverse effects increases with increased dosage and are dose-limiting. Toxicities associated with cisplatinum includes; cumulative nephrotoxicity (Safirstein et al., 1986; Daugaard et al., 1988), myelosuppression (Gringieri et al., 1988), gastrointestinal toxicity, nausea, ototoxicity, peripheral neuropathies and anaphylactic reactions (Von Hoff et al., 1979).

The search for methods to suppress these toxic reactions has been a continuing one in which many substances have been reported to produce some
benefit. The use of nucleophiles to reduce the nephrotoxicity of platinum complexes with antitumor activity has a considerable history.

1.6 Mechanisms of cisplatin nephrotoxicity

The mechanisms remain unclear and many hypotheses have been suggested. Platinum is concentrated preferentially in the renal corticomedullary junction and maximal tubular necrosis is located in this area. Patients with evidence of renal toxicity had higher renal cortical platinum concentrations than did patients without evidence of kidney damage (Stewart et al., 1985). The mechanisms responsible for the selective accumulation of platinum in corticomedullary tissue are not well identified. It is known that the pars recta is a major site of active tubular secretion of organic anion and it has been suggested that cisplatin or a metabolite is transported by a similar mechanism and is accumulated selectively in the cells of the pars recta. Competition for transport sites between cisplatin and p-aminohippurate has been demonstrated (Goldstein and Mayor, 1983; Guarino et al., 1979); however, this action is not specific.

Cisplatin also inhibits the renal cortical transport of an organic cation, tetraethylammonium, but, since p-aminohippurate and tetraethylammonium are transported by independent systems, competitive inhibition of both transport carries by cisplatin is unlikely. N-methylnicotinamide, an organic cation, did not effect cisplatin clearance, whereas probenecid resulted in a significant increase (Pendyala et al., 1985). Thus, peritubular transport of cisplatin is certainly one route of its excretion in proximal tubule cells; it is
also transported across the luminal membrane. Cisplatin and its metabolites are therefore excreted by the kidney by a complex process which includes glomerular filtration, secretion and active reabsorption.

Biotransformation of cisplatin has been demonstrated in vitro: the chloride ligands of the complex can be labilized in aqueous media. Extrapolating these in vitro data to the in vivo distribution of cisplatin has led to the hypothesis that cisplatin exists as a neutral complex in extracellular fluid, since the chloride concentration is sufficiently high to stabilize the complex and prevent hydrolysis. However, the markedly lower intracellular concentration of chloride facilitates its displacement by water molecules, yielding a positively charged hydrated or hydroxylated complex (Litterst et al., 1977; Leonard et al., 1971).

Hydration of cisplatin transforms it into monochloromono-hydrodiammine platinum or dihydrodiammine platinum, which alkylate the purine and pyrimidine bases of nuclear material; there is evidence that the hydrated products of cisplatin induce the nephrotoxic effect of the drug. Litterst et al. (1979) showed that cisplatin at 9 mg/kg body weight, prepared and administered in distilled water, results in acute renal failure and death in 100% of rats so treated. When the same dose was prepared and administered in 0.9% saline, mortality decreased to 66%. The antitumor potency of cisplatin in tumor-bearing rats was not affected by the solvent vehicle.
In another hypothesis, it has become increasingly evident that chemically induced cytotoxicity may be related to the generation of reactive metabolites which bind covalently to tissue macromolecules such as proteins, lipids or nucleic acids. A similar mechanism may mediate the nephrotoxicity of cisplatin. The reactivity of cisplatin in its aquated/hydroxylated form with nuclear DNA has been well documented and may mediate the inhibition of DNA synthesis in tumor tissue (Harder and Rosenberg, 1970). On this basis, it seems reasonable to speculate that such an electrophilic complex may bind to essential macromolecules of the kidney, resulting in nephrotoxicity (Vermeulen and Baldew, 1992; Singh and Manicca-Bozzo, 1990).

1.7 Cisplatin distribution and elimination in tissues

Plasma levels, tissue distribution and the excretion of platinum complexes are similar in mice, rats and dogs. After a single intravenous dose, plasma platinum levels decline biphasically. The initial distribution phase has a half-life of less than 1h and is followed by a slower elimination phase with a half-life of one to several days (Litterst et al., 1979). The principal route of excretion of cisplatin is via the kidney: about 50% of a dose is excreted within 4h and 76% within 48h; 90% of plasma platinum is bound to serum proteins within 2h of cisplatin administration.

Cisplatin undergoes rapid distribution to nearly all organs after intravenous administration, the highest levels appearing in kidney, liver, ovary, uterus, skin and bone (Litterst et al., 1977). Elevated tissue: plasma
ratios are maintained for a long time, and by two to four weeks after treatment significant levels of platinum can still be found in the kidney.

The largest portion of platinum in the kidney (70-80%) was recovered from the cytosolic fraction: about 14% was found in the nuclear fraction and 10-12% in the microsomal fraction. Mitochondrial and plasma membrane fractions, each accounted for 2-4% of renal platinum. Small, dense granules were found only in kidney lysosomes and microanalysis demonstrated the presence of sulphur in the same intralysosomal structure.

1.8 Human nephrotoxicity

Early clinical trails of cisplatin in cancer patients showed a striking incidence of persistent azotaemia and acute renal failure. Rossof et al., (1972) found that doses of less than 50 mg/m² body surface area resulted in a mean blood urea nitrogen concentration of 44 mg/l in 11 of 18 cases; one patient who received 200 mg/m² developed acute, oliguric renal failure.

Lippman et al. (1973) observed an estimated 50% reduction in renal function in each of 16 patients treated with total doses of 120-280 mg/m². Dentino et al. (1978) stated that most patients whose renal function is impaired by cisplatin never regain the pretreatment level of function. It was concluded in the early phase of therapeutic use of cisplatin that, for high doses (> 100 mg/m²), there was a high incidence of nephrotoxicity including irreversible toxicity.
1.9 Experimental nephrotoxicity

The time course of cisplatin nephropathy is characterised by early degenerative changes in the proximal tubule and consists of cytoplasmic vacuolization, tubular dilatation, pyknotic nuclei and hydropic degeneration (Aggarwal et al., 1980; Dobyan et al., 1980). In rats, pathological changes are most severe by three to five days following cisplatin treatment (6 mg/kg body weight) and are characterised by widespread tubular necrosis of the corticomedullary area, predominantly in the third segment (S₃) or straight portion of the proximal tubule (pars recta). Electron microscopic studies reveal several ultrastructural changes in the pars recta, including severe thinning or focal loss of the brush border, cellular swelling, condensation of nuclear chromatin, cytoplasmic vacuolisation, rounded mitochondria with swollen crystals, dissociation of mitochondria from basal infoldings, loss of basal infoldings, increased number and size of pinocytotic vesicles and lysosomal bodies in the apical region. Renal tubular regeneration, as indicated by enlarged nuclei and mitotic figures, appears on days 15-18. However, this does not appear in all animals and the presence of necrotic debris in the tubular lumen, coupled with persistent tubular damage, following a single administration of cisplatin suggest, incomplete recovery. Chronic treatment with cisplatin may further result in cyst formation, interstitial fibrosis and thickening of tubular basement membranes, including irreversible renal damage.

Dobyan et al. (1980) observed nodular foci of epithelial cells in animals examined six months after treatment with cisplatin: these changes
might be related to some mutagenic effect. In animals, treatment with high
doses of a platinum compound elicits such severe acute toxicity and death or
severe morbidity occurs within three to four days after termination of
treatment.

Goldstein and Meyor, (1983) studying male Fischer 344 rats receiving
a single intravenous dose of 5,10 or 15 mg/kg cisplatin, observed the same
functional renal changes; increased 24h urinary volume, reduced urinary
osmolarity, elevated blood urea nitrogen and decreased concentrations of
insulin and p-aminohippurate; these authors also observed increased 24h
urinary $K^+$ excretion, glucosuria without hyperglycemia (with a dose of
5 mg/kg) or with hyperglycemia (with a dose of 10-15 mg/kg).

The mechanism of action of cisplatin in terms of nephrotoxicity is
multifunctional (Fig.1c), although, its primary action is on mitochondrial
respiration through its hydrolysis products and those of its analogues. The
hydrolysis of cDDP and its analogues can take place only in conditions of low
chloride ion concentrations within the cell. Cisplatin and its various analogues
probably affect the $Ca^{2+}$ receptors on the kidney cells to different degrees,
causing hypocalcemia which, in turn induces cytotoxicity in different organ
systems via inhibition of the release of various neurotransmitters and by
inactivating contractile proteins. Arrest of the mitochondrial respiratory chain
causes efflux of $Ca^{2+}$ which in turn, causes depolymerisation of various
contractile proteins, inhibits ATP synthesis, and affects various ATPases ($Na^+$,
$K^+$-ATPase and $Ca^{2+}/Mg^{+}$-ATPase). Such an action causes ion imbalance in the
cell, leading to stripping of various glycoproteins from the plasma membrane.
Fig. 1c Schematic representation of the mechanism of action of cisplatin in proximal convoluted tubule cells.

(Adapted from Aggarwal, 1993)
The cells respond to the foreign substance by sequestering (Pt drugs) into and increasing the number of lysosomes. However, heavy metals are known to induce leakage of the lysosomes, causing the release of hydrolytic enzymes into the cytosol and leading to eventual cell death. Chlorosis can interrupt the process by reverting the aquated species cis-[(NH\textsubscript{3})\textsubscript{2}Pt(H\textsubscript{2}O)\textsubscript{2}]\textsuperscript{2+} back to the parent compound cis-[(NH\textsubscript{3})\textsubscript{2}Pt Cl\textsubscript{2}] which can be excreted into the urine or sequestered into the lysosomes (Aggarwal, 1993).

1.10 Mitochondrial damage and lipid peroxidation in cisplatin induced nephrotoxicity

Mitochondria seems to play an important role in cisplatin induced nephrotoxicity (Gordon and Gattone, 1986). Oxidative damage has been proposed as a mechanism of cisplatin-induced renal cell death in vitro as well as in vivo (Kameyama and Gemba, 1991; Zhang and Lindup, 1994; Sugihara et al., 1987).

Sadzuka et al. (1991) found that cisplatin did not increase enzymatic or non-enzymatic lipid peroxidation in vitro, which action is generally considered to be the cause of the increased lipid peroxides induced by antitumor drugs in vivo (Goodman and Hochstein, 1977). Therefore, it is possible that the increase in lipid peroxides seen in the kidneys of rats treated with cisplatin is attributable to a decrease in the renal activity of lipid peroxidation protecting enzymes.
1.11 Electrolyte disturbances associated with cisplatin treatment

1.11.1 The effect of cisplatin on sodium excretion

Both proximal and distal sodium reabsorption rates are affected by cisplatin administration (Daugaard et al., 1987). In human studies, renal sodium loss has been shown to be dose-dependent and has only been observed in patients treated with high-dose cisplatin (i.e., 40 mg/m²), in whom the increase in sodium clearance persisted for at least 6 months after the termination of treatment (Daugaard et al., 1987). In the literature, severe hyponatremia, secondary to renal tubular sodium wasting has been reported in only one case in association with cisplatin therapy (Lammers et al., 1984).

1.11.2 The effect of cisplatin on potassium excretion

An increase in potassium clearance was observed both shortly and 48-72 hours after the administration of cisplatin to dogs (Daugaard et al., 1986). Likewise a tendency towards an increased potassium clearance was noted in patients (Daugaard et al., 1988). It seems likely that the decrease in proximal reabsorption caused an increased delivery of sodium, potassium and water to the more distal segments of the nephron, resulting in a sodiumload-dependent potassium-secretion (Giebisch and Windhager, 1973). Hypokalemia has been reported in some patients during cisplatin treatment (Hill and Russo, 1981).

1.11.3 The effect of cisplatin on magnesium excretion

Hypomagnesemia is a common complication of cisplatin administration in humans (Schilsky et al., 1982). Persistent, increased
excretion of magnesium in the presence of severe hypomagnesemia suggests that, the hypomagnesemia is due to a defect in renal magnesium reabsorption (Schilsky and Anderson, 1979).

1.12 Heat-shock proteins

Heat-shock proteins (HSPs) are highly conserved proteins through evolution and are rapidly synthesised in cells in response to a variety of stresses, such as elevated temperatures, anoxia and toxic agents (Lindquist, 1986). HSPs are therefore considered to have essential protective functions in cells. HSPs are generally classified into three families by their functions or molecular sizes: the HSP90 family, the HSP70 family, and the low molecular weight HSP family (Itoh and Tashima, 1991).

These properties of HSP90 suggest its various essential functions in cells. In normal rat kidney, HSP90 distribute mainly in the cytoplasm of tubular epithelial cells and is considered to have some role in maintaining tubular functions in the kidney under physiological conditions (Matsubara et al., 1990). Satoh et al. (1994) observed the induction and altered localisation of HSP90 in rat kidneys with cisplatin induced acute tubular injury.

1.13 Mechanisms and preventative measures of cisplatin renal toxicity

An understanding of the mechanisms of cisplatin toxicity is inextricably entwined with attempts to prevent it, whether in experimental animals or in humans.
Since, the therapeutic efficacy of cisplatin seems to be proportional to the delivered dose (Sobrero et al., 1990), there has been a continuous search for biological and pharmacological strategies to protect the renal function and thus permit the administration of high quantities of the drug: these strategies include modification of administration modes, development of new galenic forms, and use of chemoprotectors among others.

The cisplatin induced nephrotoxicity is modulated by some strategies like glutathione and glutathione reducing agents, N-acetylcysteine and selenium are replenishing sulphydryl groups and sequester cisplatin in a nontoxic form, whereas, diethyldithiocarbamate removes the platinum from monoguanine adducts.

The use of antidotes against cisplatin induced renal toxicity represents a highly interesting concept. Directly inhibiting the platinum uptake into tubular cells could contribute to a limitation of the cumulative toxicity. Current study lies in using thiol compounds as nephroprotectant.

1.14 Glutathione

Hopkins (1921) observed that *philothion* (former name of glutathione discovered by De Rey-Pailhade in 1888) in muscle, liver and yeast could be extracted with water and he suspected that *philothion* was a dipeptide containing glutamate and cysteine. In 1921, Hopkins renamed *philothion* as glutathione and he demonstrated that actually glutathione contained sulphur. Hopkins, (1929) and Kendall et al. (1929) independently discovered that
glutathione was actually a tripeptide and that the peptide contained glycine (Glu-Cys-Gly). Since, the discovery of the tripeptide glutathione, research has been very popular in all times especially because of the apparent ubiquity and multifaceted functions of the low molecular weight thiol. Much of the current knowledge in glutathione biochemistry has been contributed by Meister and associates who have pioneered the concept of the \( \gamma \)-glutamyl cycle (Meister and Anderson, 1983; Mesiter, 1995).

Glutathione (GSH), a major non-protein cellular thiol, participates in numerous cellular functions including protection from free radical damage and detoxification of xenobiotics (Russo et al., 1986; Meister, 1983).

The biochemical pathways of the synthesis of intracellular GSH have been extensively reviewed and are summarised (Fig. 1d).

1.14.1 GSH as an intracellular reductant

It has long been known that GSH participates in transhydrogenation reactions. Thiol-disulfide transhydrogenations involving GSH were first observed with homocysteine and cysteine and later with compounds such as CoA and proteins. Mixed disulfides between GSH and other thiols, including proteins, have also been observed. GSH functions to form or maintain protein thiol groups which may be required for catalysis, and involved in protein assembly and degradation. It also provides reducing capacity for other reactions, eg. formation of deoxyribonucleotides by ribonucleotide reductase; reduced glutaredoxin, formed by reaction with glutaredoxin with GSH,
Fig. 1d Overall scheme of GSH metabolism and function. Enzymes: (1) γ-glutamyl transpeptidase; (2) γ-glutamyl cyclotransferase; (3) 5-oxoprolinase; ATP; (4) γ-glutamylcysteine synthetase; ATP; (5) GSH synthetase; ATP; (6) dipeptidase; AA, amino acids. X, compounds that react with GSH to yield adducts involved in the formation of mercapturic acids, leukotrienes and melanin and in the metabolism of estrogens, prostaglandins and other compounds.

(Adapted from Meister, 1981)
interacts with ribonucleoside diphosphate to form deoxyribonucleoside diphosphate and glutaredoxin.

GSH protects proteins and cell membranes against peroxides and free radicals and GSH peroxidase catalyses the reaction of GSH with $\text{H}_2\text{O}_2$ and organic peroxides to yield glutathione disulfide (GSSG). Reduction of GSSG to GSH is catalysed by the widely distributed enzyme GSH reductase, which uses NADPH. Normally, the intracellular concentration of GSH far exceeds that of GSSG. In the erythrocyte, oxidation of glucose-6-phosphate and 6-phosphogluconate provides NADPH. In diseases characterised by glucose-6-phosphate dehydrogenase deficiency, inefficient reduction of GSSG is associated with the denaturation of hemoglobin and the destruction of erythrocyte membranes (Meister, 1981).

1.15 Antioxidant defense network

Apart from functions such as the GSH peroxidase-dependent metabolism of hydroperoxides and direct scavenging of reactive oxygen species, GSH may contribute to antioxidant defense by networking with other major antioxidants such as vitamin E and C (Sen and Hanninen, 1994; Packer et al., 1995; Sen, 1997). GSH plays a critical central role in regenerating vitamins C and E from their oxidised byproducts. In this context, it should be noted that the antioxidant activity of selenium and vitamin B$_6$ is also GSH dependent. Selenium functions as a cofactor of glutathione peroxidase (Burk, 1983). Vitamin B6 facilitates the availability of selenium for glutathione peroxidase activity (Yin et al., 1991).
To obtain best therapeutic results, antioxidant supplementation protocols should be considered for the requirement of all components of the antioxidant defense network (Sen, 1995). For example, excess vitamin E in the absence of adequate amounts of regenerating agents will fail to provide full strength antioxidant protection and accumulation of oxidised vitamin E (\(\alpha\)-tocopheroxyl radical) may even lead to the initiation of pathophysiological processes (Bowry et al., 1992; Kuzuya et al., 1989). Glutathione and vitamin E appear to be interdependent on each other with respect to the circumvention of oxidative stress induced cytotoxicity (Pascoe and Reed, 1989).

It has been shown that ascorbate may spare glutathione consumption by minimising the glutathione dependent reduction of dehydroascorbate to ascorbate and by providing an alternative cellular reducing agent (Martensson and Meister, 1992).

### 1.16 Biological activity of GS-platinum complex

Sharma and Edwards, (1983) reported the high accumulation of platinum in the cytosol of rat kidney. They pointed out that localisation of cellular platinum in the cytosol and the presence of a high proportion as non-protein bound species (molecular weight <1,000) may be an important factor in relation to renal toxicity of cisplatin. Their suggestion would become even more important if the GS-platinum complex is identified in the kidney and its biological activity in the tissue is characterised. Using several resistant and sensitive variant cells derived from the L1210 cell line, Richon et al. (1987) indicated that both decreased cellular accumulation of platinum and increased
cellular GSH level are important in the development of resistance to cisplatin. Based on those studies, Ishikawa and Ali-Osman, (1993) suggest that the formation of the chelate complex between platinum and GSH and the subsequent elimination of this complex from cells would be of biological importance (Fig.1e).

1.17 Efflux of GS-Platinum complex from cells

Many metals are eliminated into bile and urine (Gregus and Klaassen, 1986). The complex formation of these metals with GSH and the subsequent active membrane transport of the glutathione-metal complexes is assumed to be the underlying mechanism for their elimination (Igwe, 1986). Recent studies have shown that chromium (VI), which induces DNA damage, forms a thiolate complex with GSH (Brauer and Wetterhahn, 1991) and arsenic, a cytotoxic metal ion, is excreted into bile as a GSH complex (Gyurasics et al., 1991). Evidence that some glutathione drug conjugates have biological activity (Kauffmann, 1987) strongly suggests that the elimination of such complexes could be a critical determinant of cellular response to the drugs.

1.18 Glutathione ester (Glutathione monoisopropyl ester)

\[
\text{Esterified} \quad \downarrow \\
\text{CH}_2 \quad \text{NH}_3^+ \\
\text{OOC} - \text{CH}_2 - \text{NH} - \text{CO} - \text{CH} - \text{NH} - \text{CO} - \text{CH}_2 - \text{CH}_2 - \text{CH} - \text{COO}'
\]

(GSH monoisopropyl ester)
DNA + cisplatin monoadducts

Inhibitory Effect on Protein Synthesis

GS-X Pump

Fig.1e  Schematic illustration for intracellular reactions of cisplatin with GSH and DNA as well as for the elimination of GS-Platinum complex. The antitumor activity of cisplatin is attributed primarily to the formation of DNA-platinum-DNA cross-links. GSH in the nucleus can quench DNA-platinum monoadducts before their conversion to the cross-links. In the cytoplasm, and probably in the nucleus as well, GSH reacts with cisplatin to form the GS-platinum chelate complex which is potentially active in the inhibition of protein synthesis. The GS-X pump localized in the plasma membrane eliminates the GS-platinum complex from the cell by an ATP-dependent manner.

(Adapted from Ishikawa and Ali-Osman, 1993)
Monoesters of glutathione in which the glycine carboxyl group of this tripeptide is esterified are effective delivery agents for glutathione (Anderson et al., 1985; Martensson and Meister, 1989; Puri and Meister, 1983). Such esters are readily transported into cells and split, presumably by intracellular esterases to glutathione and the corresponding alcohol. In contrast, glutathione itself is not effectively transported into cells.

The effective transport of glutathione monoesters has led to their use in the protection of cells against radiation (Wellner et al., 1984; Astor et al., 1988) and various toxic compounds such as acetaminophen (Puri and Meister, 1983), heavy metal ions (Singhal et al., 1987) and certain anticancer agents (eg., melphelan, cis-platinum, cyclophosphamide) (Anderson et al., 1990; Babu et al., 1995). Administration of glutathione monoester also protects against cellular damage (eg. mitochondrial degeneration, cataracts (Martensson et al., 1989a; Martensson et al., 1998b)) that is produced by administration of buthionine sulfoximine, a selective inhibitor of glutathione synthesis (Griffith et al., 1979).

It seems of importance to learn whether administration of glutathione monoesters may protect against other types of toxicity and also to examine their potential value in ameliorating a variety of conditions thought to be produced by free radicals and reactive oxygen compounds (Meister, 1988). In addition, the facile transport of glutathione monoesters into cells offers a variety of experimental approaches for the studies on cellular and subcellular glutathione transport phenomena.
Nucleophilic thiol reagents have a potential for reacting with and inactivating toxic cisplatin metabolites, their action being based upon the affinity of sulphur containing ligands for platinum (II) complexes (Dedon and Borch, 1987). Exogenous glutathione has been proposed for use against cisplatin induced toxicity and the antitumor efficacy of cisplatin does not seem to be impaired (Zunino et al., 1989). Oral administration of glutathione monoesters to mice was reported to increase glutathione levels in liver and kidney (Anderson et al., 1985).

1.19 AIM AND SCOPE OF THE PRESENT INVESTIGATION

Chemotherapeutic agents are effective in combating disseminated cancer. The drugs to treat neoplastic diseases have a complex biopharmacology and extremely narrow margin of safety.

Cisplatin is one of the most active antitumor agents available in medical oncology. Its clinical use was rapidly limited due to unexpected very severe renal toxicity and other toxicities. A acute and cumulative renal toxicity associated with histological damage has been shown in both animal and human studies. Knowing the need for situation, the therapeutic efficacy of cisplatin seems to be proportional to the delivered dose, there has been a continuous search for biological and pharmacological administration of drug: these strategies include modification of administration modes, development of new galenic forms, and the use of chemoprotector against cisplatin induced nephrotoxicity.
Many compounds are known to reduce the side effects of the administered drug. Earlier reports by different authors, showing a decrease in cisplatin nephrotoxicity are at the experimental levels and are mostly attempts to reduce cisplatin induced nephrotoxicity that have been centered on decreasing the exposure of the kidney to active cisplatin.

The current toxicity-modulating strategies to date have been most effective against acute cisplatin induced nephrotoxicity. Current interest lies in using thiol compounds as nephroprotectant. Glutathione ester is an effective delivery agent of glutathione. The effective transport of glutathione protects cells in biological functions, including detoxification of xenobiotics and scavenging of free radicals in cisplatin induced nephrotoxicity.

The present study mainly focusses the therapeutic efficacy of glutathione ester as a nephroprotectant on the oncolytic agent cisplatin induced nephrotoxicity in experimental rats.

The biochemical investigations carried out in blood, plasma, serum, urine, liver, and kidney comprise of:

1. Blood glucose
2. Non-protein nitrogen compounds
3. Lysosomal and transaminases (alkaline and acid phosphatases; aspartate and alanine aminotransferases)
4. Albumin/globulin ratio and total protein
5. Non-enzymic antioxidants (reduced glutathione, vitamin C, vitamin E, sulphhydryl groups and ceruloplasmin)

6. Enzymic antioxidants (superoxide dismutase, catalase and glutathione peroxidase)

7. Lipid peroxidation (basal, ferrous sulphate and ascorbate induced)

8. Glutathione metabolising enzymes

9. Key enzymes of glycolytic and gluconeogenic pathways

10. Tricarboxylic acid (TCA) cycle enzymes

11. Renal lysosomal enzymes

12. Adenosine triphosphatases (Na\(^+\)K\(^+\)-ATPase, Mg\(^{2+}\)-ATPase and Ca\(^{2+}\)-ATPase)

13. Platinum concentration and minerals (selenium, copper, magnesium, calcium, sodium and potassium)

14. Urinary marker enzymes and urinary constituents

15. Histopathology of liver and kidney and

16. Immunohistochemical localisation of HSP90 in kidney.

The results obtained in control and experimental animals were compared accordingly and discussed in the light of relevant literature.