Silibinin protects against cisplatin-induced nephrotoxicity without compromising cisplatin or ifosfamide anti-tumour activity

C Bokemeyer1, LM Fels2, T Dunn3, W Voigt1, J Gaedeke1, H-J Schmoll4, H Stolte1 and H Lentzen5

1Department of Internal Medicine II, University of Tübingen, Otfrid-Müller-Str. 10, D-72076 Tübingen; Departments of 2Experimental Nephrology and 3Hematology/Oncology, Hannover University Medical School, 30623 Hanover; 4Martin-Luther University Halle, Department of Hematology/Oncology, 06120 Halle; 5MADUS AG, 51109 Cologne, Germany.

Summary Cisplatin is one of the most active cytotoxic agents in the treatment of testicular cancer, but its clinical use is associated with side-effects such as ototoxicity, neurotoxicity and nephrotoxicity. Long-term kidney damage from cisplatin particularly affects the proximal tubular apparatus and can be detected by increased urinary excretion of brush-border enzymes, such as l- 

Correspondence: C Bokemeyer
Received 22 April 1996; revised 1 July 1996; accepted 8 July 1996

With the introduction of cisplatin-based combination chemotherapy, testicular cancer has become a highly curable malignancy even in patients with metastatic disease (Williams et al., 1987; Einhorn et al., 1977). Approximately 75–80% of all patients can expect to be cured by standard combination chemotherapy, such as PEB (cisplatin, etoposide and bleomycin) or PEI (cisplatin, etoposide and ifosfamide). Based on the advances achieved by the use of cisplatin-based chemotherapy, interest has now also focused on treatment-related toxicity. Cisplatin (DDP) represents one of the most active cytotoxic agents in the treatment of testicular cancer, but its clinical use is associated with particular side-effects, such as ototoxicity, neurotoxicity and nephrotoxicity (Bitran et al., 1982; Hacke et al., 1983; Werner-Hansen et al., 1988; Schilsky et al., 1982). The risk of DDP-associated nephrotoxicity has been reduced by the use of hyperhydration and forced diuresis, but persistent kidney damage is still found in some patients (Daugaard et al., 1988a). The effects of DDP on renal function have been extensively studied in animal models. In a rat model the type of DDP nephrotoxicity seems similar to humans, affecting different segments of the nephron, such as the tubular apparatus and the glomerulus (Jones et al., 1985; Safirstein et al., 1981). Impaired transport processes occur at the luminal and to a lesser degree at the contra-luminal side of the proximal tubular membrane and morphological examinations have revealed necrotic cells in the proximal tubules (Ammer et al., 1993; Field et al., 1989). The mechanisms of DDP nephrotoxicity are still not fully understood. However, the generation of free oxygen radicals in tubular cells has been proposed as an important pathogenic process (Ishikawa et al., 1990; Hannemann et al., 1988). Further evidence points to the inhibition of protein synthesis in tubular cells by DDP (Tay et al., 1988).

The management of nephrotoxicity requires either that DDP is discontinued or that doses are reduced. However, this may result in inferior anti-tumour activity. Cytoprotective agents have been developed to ameliorate a variety of functional disorders (Munshi et al., 1992; Anderson et al., 1990). Silibinin (Figure 1) is one of three isomers constituting silymarin, a flavonoid extracted from Silybum marianum, the milk thistle, that has long been known as a medicinal plant (Wagner et al., 1974; Hahn and Mayer, 1981). Silibinin has been successfully used as a protective agent in clinical and in experimental work in in vivo and in vitro models of liver toxicity (Valenzuela and Guerra, 1985; Valenzuela et al., 1985). Silibinin possesses membrane-stabilising, anti-inflammatory, antioxidant and RNA and protein synthesis-stimulating properties (Faulstich et al., 1980; Middleton et al., 1992; Sonnenbichler and Zetl, 1987; Mira et al., 1994). However, before the clinical use of new cytoprotective agents, not only protection from toxicity, but also the absence of an interference of the agent with the anti-tumour activity of the cytotoxic agents used, have to be demonstrated (Bokemeyer et al., 1994).

The first aim of the preclinical study presented here was to evaluate in vivo the protective effects of the flavonoid silibinin on acute DDP nephrotoxicity in an established rat nephrotoxicity model. Serum and urinary parameters specifically detecting glomerular and tubular damage were therefore studied following treatment with DDP and/or silibinin. The second aim was then to evaluate in vitro, in three human non-seminomatous germ cell tumour cell lines, whether silibinin interferes with the cytotoxicity of DDP and also ifosfamide, which in many standard combination chemotherapies is applied together with DDP.

Materials and methods
In vivo studies on a rat model of DDP nephrotoxicity
Female Wistar rats with an initial body weight between 170 and 230 g were used. Animals, housed 3–4 per cage under standardised laboratory conditions with controlled light-
dark cycle, room temperature (21°C) and moisture, had free access to tap water and pelleted diet (Altromin R, Lage, Germany).

Treatment of animals A total of 45 rats were randomised into four groups. One group received cisplatin (DDP) \((n=12)\), one group silibinin and DDP \((n=11)\), one group silibinin alone \((n=10)\) and one group the vehicle isoton saline \((n=12)\). The animals only received one injection of the compounds.

DDP (Medac, Hamburg, Germany) dissolved in saline was given at a concentration of 5 mg kg\(^{-1}\) body weight (b.w.). Silibinin \((C_25H_{22}O_{12}, FW 482.45)\) was given as silibinin-C-2,3-dihydrogen-succinate, disodium salt (MADAUS AG, Cologne, Germany). The compound was dissolved in saline, the animals received about 0.4 mmol silibinin kg\(^{-1}\) body weight \((=0.2\, g\, \text{silibinin kg}^{-1}\, \text{b.w.})\). This dose is below the oral maximum tolerated dose (MTD) that was \(\geq 1000\, \text{mg kg}^{-1}\) body weight (U Mengs, MADAUS AG, Cologne, personal communication). In the group with combined treatment silibinin was given 1 h before the injection of DDP: studies on humans had shown an elimination half-life of 6.3 h (Lorenz et al., 1984). All injections were given i.v. into the tail vein.

Sample collection Urine and plasma samples for an assessment of kidney function were collected during a control phase before treatment (day \(-1\)) and on days 1, 3 and 7 following treatment. For sample collection, animals were housed in individual metabolic cages, which allowed collection of urine samples without food or faecal contamination. Urine was collected overnight under paraffin oil to avoid evaporation. After each collection, a venous blood sample was drawn from the orbital plexus under light ether anaesthesia. Urine samples were supplemented with 0.01% sodium nitrite. Urine and serum aliquots were stored at \(-20°C\).

Analysis of urine and plasma samples For all animals, body weight was recorded every 2 days and 24 h urinary volume and total urinary protein were assessed as general parameters of renal function. Total protein was measured with the Coomassie blue binding method (Bradford, 1976). Changes in urinary L-alanine-aminopeptidase (AAP) activity and urinary magnesium were followed and served as parameters of kidney tubular function. L-alanine-aminopeptidase (AAP, EC 3.4.11) was measured by kinetic determination at 25°C, pH 7.6, using L-alanine-4-nitro-anilide hydrochloride as a substrate (Matzenheimer et al., 1992). Magnesium was determined with the xylidil blue method (Magnesium test kit, Merck, Darmstadt, Germany). Serum and urinary creatinine were measured using a Beckman creatinine analyser and reagents supplied by the manufacturer (Creatinine analyser 2 Reagents, Beckman, Munich, Germany) and served as kidney glomerular function parameters. Blood urea and nitrogen levels were measured with a test kit (Harnstoff Test-Kit, Boehringer, Mannheim, Germany).

Calculations and statistics Data are expressed as mean \(\pm\) standard deviation (s.d.). Excretion rates were related to body weight. Changes in excretion rates or serum levels of analytes following treatment were assessed with ANOVA procedures. Differences between groups on specific days were evaluated with the \(t\)-test for independent data. The level of significance was defined as \(P<0.01\). Statistical analysis was performed with SPSS 4.1 (SPSS, Chicago, IL, USA).

In vitro cytotoxicity studies on germ cell cancer cell lines Drugs DDP was obtained from Medac (Hamburg, Germany). A prodrug of the active metabolite of ifosfamide (4-hydroxypropiolofosamide) was kindly supplied by ASTA Medica AG (Frankfurt, Germany), since tumour cells cannot metabolise ifosfamide. The product spontaneously gives rise to the active in vivo metabolite (4-OH-Ifo) in solution, which then further degrades to active derivatives. Stock solutions were therefore prepared immediately before use. The solid powder was stored at \(-20°C\) and 8 mg weighed out on the day of the experiment and dissolved in phosphate-buffered saline (PBS) (approximate pH 7) to a final concentration of 2 mg ml\(^{-1}\) (6.82 mm). Stock solutions of silibinin (MADAUS AG, Cologne) were freshly prepared for each experiment by dissolving 10 mg powder per ml of culture medium and filter sterilising. Silibinin was used at final concentrations from 3.62 \(\times\) 10\(^{-3}\) to 3.62 \(\times\) 10\(^{-2}\) mol l\(^{-1}\).

Non-seminomatous germ cell cancer cell lines Three human testicular germ cell tumour cell lines were used for the in vitro experiments. The origin and histology of the initial tumour and of the heterotransplanted nude mouse tumour is shown in Table I (Casper et al., 1987). The cell lines were grown as continuous monolayer cultures in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) (Biochrom, Berlin, Germany), penicillin 2 IU ml\(^{-1}\), streptomycin 2 \(\mu\)g ml\(^{-1}\) and L-glutamine 0.04 mmol l\(^{-1}\). For the experiments, cells from passages 70 to 80 of the three cell lines were used.

Treatment of cell lines Initially, all cell lines were exposed to silibinin at concentrations ranging from 3.62 \(\times\) 10\(^{-7}\) up to 3.62 \(\times\) 10\(^{-1}\) mol l\(^{-1}\); nine different concentrations of silibinin were tested and each experiment was performed twice. The rationale for the silibinin concentrations used was based on the experience in animals. Most of the silibinin is excreted via the bile, only a small percentage via the kidney. Pharmacokinetic studies on animals showed a first-pass effect (Bülles et al., 1975), therefore silibinin blood levels following oral administration remain low. In the in vivo studies each animal (body weight \(\approx 200\) g) received about 0.08 mm silibinin. The initial plasma concentrations must have been \(\leq 0.01\, \text{mmol l}^{-1}\) \((=1 \times 10^{-3}\, \text{M l}^{-1})\). The concentrations tested in the cell cultures were chosen based on this calculation.

DDP was used at concentrations ranging from 3 to 30 000 mmol either applied alone or in combination with 0.05 mg ml\(^{-1}\) or 0.005 mg ml\(^{-1}\) of silibinin. Measurements were calculated as means with standard deviation from three

---

Table I Characteristics of three human testicular cancer cell lines giving the histology of the primary tumour and histology of xenografted nude mouse tumours

| Cell line | Histology of the patient tumour | Histology of the nude mouse tumour |
|-----------|---------------------------------|-----------------------------------|
| H12.1     | S.T,CC,EC                       | EC,STGC,T                         |
| 577LM     | TC,YS                           | EC,T                              |
| TC-1777NR CI-A | TC, teratocarcinoma | TC, teratocarcinoma; YS, yolk sac tumour; T, teratoma; EC, embryonal carcinoma; CC, choriocarcinoma; S, seminoma; STGC, syncytiothrophoblastic giant cells. |
separate experiments. 4-Hydroperoxy-ifosfamide was tested in cell lines 1777 NR-CLA and H 12.1 using a concentration range from 30 to 10 000 nmol. 4-Hydroperoxy-ifosfamide was given either alone or in combination with 7.25 x 10^-4, 7.25 x 10^-3 and 3 x 10^-4 mol l^-1 of silibinin.

Cytotoxicity assay To assess the cytotoxic effect of DDP and ifosfamide either alone or in combination with silibinin, a sulphorhodamine-B assay was used as described by Skeehan et al. (1990). In brief, cells were seeded into 96-well microtitre plates at cell densities previously determined to give exponential growth during the period of the experiment. Cell survival relative to non-treated controls was then quantified on day 5 after 96 h of drug exposure. Medium was carefully removed and the cells were fixed with 100 µl of 10% trichloroacetic acid overnight. After washing, the plates were stained with 0.4% sulphorhodamine-B in 1% acetic acid for 30 min and, after additional washing and drying, the dye was solubilised in 100 µl TRIS-base (10 mmol, pH 8.5). The absorbance was read in an automatic plate reader at a wavelength of 570 nm. Eight separate wells were used for one drug concentration and all experiments were performed in triplicate. The concentration that inhibited tumour cell growth by 50% (IC50) was obtained graphically from semi-logarithmic dose–response plots.

Results
In vivo studies on a rat model of DDP nephrotoxicity
Cisplatin led to a decline in kidney function. Silibinin administered alone did not affect any of the investigated parameters of renal function (data not shown).

Animals treated with DDP alone showed a significant reduction in creatinine clearance, which was most pronounced on day 3 following treatment. This indicates glomerular damage. No such changes could be observed in the group treated with silibinin and DDP (Table II). Plasma levels of urea were concomitantly elevated in the group given DDP, but not in the group that was pretreated with silibinin (Figure 2).

Tubular function was affected by DDP treatment, indicated by a significant increase in the excretion of AAP. This increase was significantly less pronounced in animals pretreated with silibinin (Figure 3). Mean fractional magnesium excretion ranged from 10–15% of the filtered magnesium load in the animal group studied during the control phase (day –1). Following cisplatin, an approximately 2.5-fold increase in magnesium excretion was seen, resulting in reduced serum magnesium levels at day 7 in animals receiving DDP alone (0.32 ± 0.05 mmol l^-1 on day –1, 0.62 ± 0.13 mmol l^-1 on day 7, P < 0.01, t-test). No significant alteration of urinary magnesium excretion and of magnesium serum levels were found when DDP was given after pretreatment with silibinin.

In vitro cytotoxic activity assays on germ cell cancer cell lines
Silibinin cytotoxicity Figure 4 shows the dose response curves of the cell lines to silibinin alone. The response of the cell lines was rather variable, but the IC50 of silibinin in the cell lines investigated was approximately 1.45 x 10^-4 mol l^-1 for cell line 577LM and > 1.45 x 10^-4 mol l^-1 for the other two cell lines. Two relatively non-cytotoxic concentrations of silibinin (7.25 x 10^-6 and 7.25 x 10^-5 mol l^-1) and two relatively toxic concentrations (1.45 x 10^-4 and 7.25 x 10^-4 mol l^-1) were therefore chosen for further studies with the combination of either DDP or ifosfamide, over a 9-fold log concentration.

Dose–response of cell lines to cisplatin with or without silibinin The dose–response curves to DDP combined with non-toxic silibinin doses (see above) did not deviate significantly (tested with ANOVA procedures and t-test for IC50 values) from those of DDP alone in any of the three cell lines tested in vitro, indicating that silibinin at these concentrations, has no effect on the cytotoxicity of DDP. As an example, data for cell line 1777 NR CL-A are shown in Figure 5.

![Figure 2](image-url) Changes in urea plasma levels in female Wistar rats treated with cisplatin (DDP), silibinin and DDP, silibinin or sodium chloride before treatment (day –1) and on days 3 and 7 following treatment. (*P < 0.01 against day –1 of this group; for dosage of drugs see Materials and methods section).

![Figure 3](image-url) Changes in the urinary activity of AAP in animals treated with DDP (n = 12), silibinin and DDP (n = 11), silibinin (n = 10) or sodium chloride (n = 12) before treatment (day –1) and on days 3 and 7 following treatment. (*P < 0.01 against day –1 of this group, ANOVA; *P < 0.01 between corresponding days of the group given DDP or silibinin and DDP, t-test). For dosage of drugs see Materials and methods section.

Table II Changes in creatinine following administration of DDP and/or silibinin in a rat animal model of DDP nephrotoxicity

| Group             | Day –1     | Day 3       | Day 7       |
|-------------------|------------|-------------|-------------|
| Sodium chloride   | 0.47 ± 0.03 | 0.49 ± 0.06 | 0.51 ± 0.05 |
| Silibinin         | 0.59 ± 0.08 | 0.51 ± 0.08 | 0.53 ± 0.09 |
| DDP               | 0.54 ± 0.09 | 0.15 ± 0.04* | 0.42 ± 0.06* |
| Silibinin + DDP   | 0.32 ± 0.06 | 0.38 ± 0.09 | 0.44 ± 0.08 |

*P < 0.01 against control phase (day –1), ANOVA.
The combined dose–response curves to DDP for cell line 1777 NRCL-A in the presence of $1.45 \times 10^{-4}$ or $7.25 \times 10^{-4}$ mol l$^{-1}$ silibinin were also not significantly different from those of DDP alone. However, a slightly antagonistic interaction between DDP and silibinin at higher doses ($7.25 \times 10^{-4}$ mol l$^{-1}$) was observed. The highest end of the concentration range of cisplatin studied (10 μmol), where antagonism appears to be strongest, represents the peak plasma concentration expected following an intravenous dose of DDP of 100 mg m$^{-2}$. The in vitro relevance of silibinin concentrations of $7.25 \times 10^{-4}$ mol l$^{-1}$ is not known and, therefore, the potential clinical implications are difficult to assess. For cell lines 577 LM and H12.1, no effect of silibinin on DDP cytotoxicity was observed. Evaluation of the effect of $7.25 \times 10^{-4}$ mol l$^{-1}$ silibinin on DDP cytotoxicity was not reliable for these two cell lines, since silibinin alone at this concentration gave only 40% relative cell survival.

**Discussion**

The nephrotoxicity of cisplatin (DDP) has already been recognised during early phase I trials and Hayes et al. (1977) were able to demonstrate that the renal toxicity of high-dose bolus cisplatin (>100 mg m$^{-2}$) could be ameliorated by forced diuresis and hydration (Higby et al., 1974). In order to use DDP in germ cell cancer patients in combination chemotherapy regimens without severe nephrotoxicity, the dose of DDP is usually split to 20 mg m$^{-2}$ x 5 days. This schedule has been incorporated into the formerly used regimen of platin, vinblastine and bleomycin (PVB) and into the current standard PEB regimen (Einhorn et al., 1977; Williams et al., 1987). Since the nephrotoxicity of DDP seems to be related to peak serum-free platinum levels, DDP bolus doses >100 mg m$^{-2}$ would be predicted to be more toxic than smaller daily doses such as 20 mg m$^{-2} \times 5$ days (Reece et al., 1987). On the other hand, reducing DDP to cumulative doses lower than 75 mg m$^{-2}$ at 3 week intervals results in inferior survival in patients with metastatic germ cell cancer (Samson et al., 1984). Thus, adequate platinum dosing appears to be relevant for maintaining cure rates but may also be associated with a higher incidence of acute and late nephrotoxicity (Osanto et al., 1992). Vigorous hydration using at least 3 l of normal saline per 24 h before and during cisplatin application, in combination with forced diureses by mannitol or furosemide are standard precautions taken to prevent DDP nephrotoxicity.

Cytotoxic protective agents, particularly sulphhydril-containing drugs, have been investigated as cytotoxicants against nephrotoxicity (Anderson et al., 1990). The current study shows that the flavonoid agent silibinin has a protective effect on renal function. Creatinine clearance and plasma levels of urea were taken as indicators of glomerular function and excretion of a brush-border enzyme and magnesium as indicators of tubular damage.

The changes in renal function observed in the rat model as described above correlate well with the nephrotoxic effects of DDP observed in man (Daugard et al., 1988b). Alterations in creatinine clearance and urea serum levels observed following treatment with DDP, but not following treatment with silibinin and DDP (Table II, Figure 1), are taken as indications of an altered glomerular function. Creatinine is filtered in the glomerulus, but tubular backleak of creatinine, following, for example, tubular obstruction, can also occur. However, backleak does not play a role in this early stage of nephropathy in the animal model studied (Jones et al., 1985). Excretion of the tubular enzyme alanine-aminopeptidase (AAP) served as an indicator of proximal tubular function (Pfleiderer et al., 1980; Fels et al., 1994). Urinary activity of this brush-border enzyme was significantly elevated following treatment of Wistar rats with DDP. This increase was significantly less pronounced in animals that had received silibinin before DDP (Figure 2).

The aetiology of cisplatin nephrotoxicity is still not completely solved. It has been demonstrated that the final common pathway for DDP nephrotoxicity is damage to the proximal tubular epithelial cell, resulting in magnesium-wasting nephropathy (Mavichak et al., 1985; Schilsky et al., 1988).
Silibinin for prevention of cisplatin nephrotoxicity
C Bokemeyer et al.

2040

1982). However, further tubular functions seem also to be affected (Daugaard et al., 1988a). A functional study, such as the one presented here, can only give limited information on the mechanisms of DDP kidney damage or protection. The involvement of free radicals in DDP nephrotoxicity has been discussed (Ishikawa et al., 1990; Sadzuka et al., 1992).

Silibinin possesses antioxidant and membrane-stabilising properties that have already been evaluated in hepatocytes challenged with a variety of radical-generating drugs (Valenzuela and Guerra, 1985; Valenzuela et al., 1985; Soose, 1994). Another mechanism of renal toxicity of DDP may be the depression of DNA, RNA and protein synthesis as demonstrated in studies, in vitro (Tay et al., 1988). Silibinin is known to up-regulate the function of a DNA-dependent RNA polymerase I in liver cells (Sonnenbichler et al., 1976; Sonnenbichler and Zetl, 1987) and may thereby counteract the decrease in synthetic activity of the kidney. Thus, the therapeutic activities of silibinin are based on a variety of potentially protective effects. The phenolic structure, for example, makes silibinin a radical scavenger, although it also has membrane-stabilising and regenerative properties (Faulstich et al., 1980; Ferenci et al., 1989; Sonnenbichler and Zetl, 1986). It is currently difficult to assess which of the properties of the flavonoid are responsible for the protection observed in this study. However, the number of potential mechanisms of action may make silibinin advantageous compared with intracellular radical scavengers, such as sodium thiosulphate.

The in vitro studies showed that silibinin at least partly counteracts the nephrotoxic side-effects of DDP. In in vitro studies on human cancer cell lines, it could then be shown that the application of silibinin does not decrease the anti-tumour activity of either DDP or ifosfamide. Although some cell line-specific differences may exist, the available in vitro data do not indicate a significant interaction of clinically relevant levels of silibinin and the cytotoxic activity of these two major drugs used in testicular cancer. Based on pharmacokinetic studies in patients receiving oral silibinin, the therapeutic plasma levels have reached a maximum of 7.25 × 10⁻¹⁵ m⁻¹ h⁻¹, a range that has been tested in our experiments (Löppky et al., 1984). These levels did not interfere with the anti-tumour activity of cisplatin in vitro. However, adverse pharmacokinetic interactions in vivo had not been addressed in our experiments. Since many attempts to reduce DDP toxicity, e.g. the substitution of DDP by the less nephrotoxic compound carboplatin, have resulted in inferior clinical anti-tumour activity in non-germinomatous germ cell cancer patients, cisplatin still remains the most important drug in the treatment of this disease (Bajorin et al., 1993). The current data support silibinin as a potentially useful selective cytoprotective agent, which may prevent nephrotoxicity without decreasing cisplatin or ifosfamide anti-tumour activity. Since certain acute renal tubular alterations, such as the elevated excretion of tubular brush-border enzymes, recognised in testicular cancer patients treated with DDP may also be ameliorated by the application of silibinin, it might be speculated that long-term kidney side-effects may also be avoidable. The current data form the basis for a clinical study using cisplatin-based combination chemotherapy including silibinin, in patients with testicular cancer in order to reduce the acute and long-term nephrotoxic potential of cisplatin. A randomised clinical study on kidney function in patients treated for testicular cancer has been initiated.

References

AAMDAL S, FODSTA O AND PIHL A. (1987). Some procedures to reduce the drug toxicity reduce antitumour activity. Cancer Treat. Rev., 14, 389 – 395.
AMMER U, NATOCHIN Y, DAVID C, RUMRICH G AND ULLRICH KJ. (1993). Site of functional disturbance and correlation to loss of bodyweight. Renal Physiol. Biochem., 16, 131 – 145.
ANDERSON ME, NAGANUMBA A AND MEISTER A. (1990). Protection against cisplatin toxicity by administration of glutathione ester. FASEB J., 4, 3251 – 3255.
BAJORIN DF, SAROSDY MF, PFISTER DG, MAZUMDAR M, MOTZER RJ, SCHER HI, GELLER M, FAIR WR, HERR H, SOGANI P, SHEINFELD J, RUSSO P, VLAMIS V, CAREY R, VOGEZELANG NJ, CRAWFORD ED AND BOSLI GJ. (1993). A randomized trial of etoposide and cisplatin versus etoposide and carboplatin in patients with good risk germ cell tumors. J. Clin. Oncol., 11, 598 – 606.
BITRAN JD, DESSER RK, BILLINGS AA, KOZLOFF MF AND SHAPIRO CM. (1982). Acute nephrotoxicity following cis-dichlorodi-amine-platinum. Cancer, 49, 1784 – 1788.
BOKEMEYER C, SCHMOLL HJ, LUDWIG E, HARSTICK A, DUNN T AND CASPER I. (1994). The anti-tumour activity of ifosfamide on heterotransplanted testicular cancer cell lines remains unaltered by the uroproctector mesna. Br. J. Cancer, 69, 863 – 867.
BRADFORD MM. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72, 248 – 254.
BÜLLES H, BÜLLES J, KRUMBIEGEL G, MENNICKE WH AND NITZ D. (1975). Untersuchungen zur Verstoffwechslung und zur Ausscheidung von Silybin bei der Ratte. Arzneim.-Forsch./Drug Res., 25, 902 – 905.
CASPER J, SCHMOLL H-J, SCHNAIDT U AND FONATSCHE C. (1987). Cell lines of human germlinal cancer. Int. J. Androl., 10, 105 – 113.
DAUGAARD G, ABILGAARD U, HOLSTEIN-RATHLOU NH, BRUUNSHUUS I, BUCHER D AND LEYSAC PP. (1988a). Renal tubular function in patients treated with high-dose cisplatin. Clin. Pharmacol. Ther., 44, 164 – 172.
DAUGAARD G, HOLSTEIN-RATHLOU NH, AND LEYSAC PP. (1988a). Effect of cisplatin on proximal convoluted and straight segments of the rat kidney. J. Pharmacol. Exp. Ther., 244, 1081 – 1085.
ISHIKAWA M, TAKAYANAGI Y, SASAKI KI. (1990). Enhancement of cisplatin toxicity by buthionine sulfoximine, a glutathione-depleting agent, in mice. Res. Comm. Chem. Pathol. Pharmacol., 67, 131 – 141.

JONES TW, CHOPRA S, KAUFMANN JS, FLAMENTBAUM W, TRUMP BF. (1985). cis-diaminedichloroplatinum (II)-induced acute renal failure in the rat. Lab. Invest., 5, 363 – 374.

LORENZ D, LÜCKER PW, MENNICKE WH AND WETZELSBERGER N. (1984). Pharmacokinetic studies with silymarin in human serum and bile. Meth. Find. Res. Clin. Pharmacol., 6, 655 – 661.

MATTENHEIMER H, JUNG K, GRÖTSCH H. (1992). Membrane-bound enzymes; Alanine Aminopeptidase. In Urinary Enzymes in Clinical and Experimental Medicine. Jung K, Mattenheimer H, Buchardt U, (eds.) Chap. 8.I. Springer Verlag: Berlin.

MAVICHAK M, SILVA L, SILVA M, TAKAYANAGI, S, SASAKI KI. (1985). Studies on the pathogenesis of cisplatin-induced hypomagnesaemia in rats. Kidney Int., 28, 914 – 921.

MIDDLETON E AND KANDASWAMI C. (1992). Effects of flavonoids on immune and inflammatory cell function. Biochem. Pharmacol., 43, 1167 – 1179.

MIRA L, SILVA M AND MANSO CF. (1994). Scavenging of reactive oxygen species by siliobindihemisuccinate. Biochem. Pharmacol. 48, 753 – 759.

MUNSH NC, LOEHRER PJ, WILLIAMS SD, LANGEFELD C, SLEDGE G, NICHOLS VR, ROTH BJ, NEUMAN A AND WALSH WB. (1992). Comparison of N-acetylcycteine and mesna as uroprotectors with ifosfamide combination chemotherapy in refractory germ cell tumours. Invest. New Drugs, 10, 159 – 163.

OSANTO S, BIKMAN A, VAN HOEK F, STERPH PJ, DELAAT JA AND HERMANS J. (1992). Long term effect of chemotherapy in patients with testicular cancer. J. Clin. Oncol., 10, 574 – 579.

PFEIFERER G, BAER M, MONDORF AW, STEFANEXCU T, SCHEBERBICHJE AND MÜLLER H. (1980). Change in alkaline phosphatase isozyme pattern in urine as possible marker of renal disease. Kidney Int., 17, 242 – 249.

REECE PA, STAFFORD I, RUSSELL K, KHAN M AND GILL PG. (1987). Creatinine clearance as a predictor of ultrafiltrable platinum disposition in cancer patients treated with cisplatin: relationship between peak ultrafiltrable platinum levels and nephrotoxicity. J. Clin. Oncol., 5, 304 – 309.

SAADZUKA Y, SHOJ T, TAKINO A. (1992). Effects of cisplatin on the activity of enzymes which protect against lipid peroxidation. Biochem. Pharmacol., 43, 1872 – 1875.

SAFIRSTEIN R, MILLER P, DIKMAN S, LYMAN N AND SHAPIRO C. (1981). Cisplatin nephrotoxicity in rats: defects in papillary hypertonocity. Am. J. Physiol., 241, F175 – F185.

SAMSON MK, RIVKIN SE, JONES SE, COSTANZI JJ, LOBUGLIO AF, STEPHENS RC, GEHAN EA AND CUMMING GD. (1984). Dose response and dose survival advantage for high versus low-dose cisplatin combined with vinblastine and bleomycin in disseminated testicular cancer. A Southwest Oncology Group Study. Cancer, 53, 1029 – 1035.

SCHILSKY RL, BARLOCK A AND OZOLS RF. (1982). Persistent hypomagnesaemia following cisplatin chemotherapy for testicular cancer. Cancer Treat. Rep., 66, 1767 – 1769.

SKEEPAH P, STORENG R, SCUDIERO D, MONKS A, MCMAHON J, VISTICA D, WARREN JT, BOKESCH H,KENNEY S AND BOYD MR. (1990). New colorimetric cytotoxicity assay for anticancer drug screening. J. Natl Cancer Inst., 82, 1107 – 1112.

SONNENBICHLER J AND ZETL1. (1986). Biochemical effects of the flavonign silybinin on RNA, protein and DNA synthesis in rat livers. In Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure Activity Relationships. Cody V, Middleton E, Harborn JB (eds.). pp. 319 – 331. Alan R. Liss Inc.: New York.

SONNENBICHLER J AND ZETL1. (1987). Stimulating influence of a flavonign derivative on proliferation, RNA synthesis and protein synthesis in liver cells. In Assessment and Management of Hepatobiliary Disease. Okolicsanyi I, Cosmos G, Crepadi G (eds.) pp. 265 – 272. Springer Verlag: Berlin.

SOOSE M. (1994). Properties of silybin and of antioxidants against adriamycin cytotoxicity in an unicellular eukaryote, Tetrahymana thermophila. Eur. J. Protistol, 30, 394 – 403.

TAY LK, BREGMAN CL, MASTERS BA AND WILLIAMS PD. (1988). Effect of cis-diaminedichloro-platinum (II) on rabbit kidney in vivo and on rabbit renal proximal tubule cells in culture. Cancer Res., 48, 2538 – 2543.

VALENZUELA A AND GUERRA R. (1985). Protective effects of the flavonoid silyban dehemisuccinate on the toxicity of phenylhydrazine on rat liver. FEBS Lett., 81, 291 – 294.

VALENZUELA A, LAGOS C, SCHMIDT K AND VIDE LA L. (1985). Sylmarin protects against high hepatic lipid peroxidation induced by acute ethanol intoxication in rats. Biochem. Pharmacol., 34, 2209 – 2212.

WAGNER J, DIESEL P AND SEITZ M. (1974). Zur Chemie und Analytik von Silymarin aus Silybum marianum Gaertn. Arzneim. Forsch./Drug Res., 24, 466 – 471.

WERNER-HANSEN S, GROTH S, DAUGAARD G, ROSSING N AND RORTH M. (1988). Long-term effects on renal function and blood pressure of treatment with cisplatin, vinblastine and bleomycin in patients with germ cell cancer. J. Clin. Oncol., 6, 1728 – 1731.

WILLIAMS SD, BIRCH R, EINHORN LH, IRWIN L, GRECO FA AND LOEHRER PJ. (1987). Treatment of disseminated germ cell tumors with cisplatin, bleomycin, and either vinblastine or etoposide. N. Engl. J. Med., 316, 1435 – 1440.